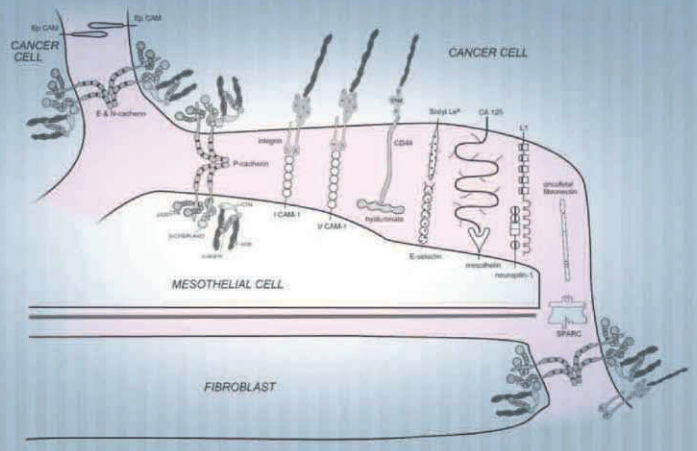
Cancer Treatment and Research
Steven T. Rosen, M.D., Series Editor
Robert H. Lurie Comprehensive Cancer Center
Northwestern University Medical School

Peritoneal Carcinomatosis

A Multidisciplinary Approach



edited by
Wim P. Ceelen

Springer

PERITONEAL CARCINOMATOSIS:

A MULTIDISCIPLINARY APPROACH

PERITONEAL CARCINOMATOSIS:

A MULTIDISCIPLINARY APPROACH

edited by

Wim P. Ceelen, MD
Surgical Oncology
University Hospital
Ghent, Belgium

Springer

Wim P. Ceelen, MD
Department of Surgery
University Hospital
B-9000 Ghent, Belgium

PERITONEAL CARCINOMATOSIS: A MULTIDISCIPLINARY APPROACH

Library of Congress Control Number: 2006935881

ISBN-13: 978-0-387-48991-9 e-ISBN-13: 978-0-387-48993-3
ISBN-10: 0-387-48991-6 e-ISBN-10: 0-387-48993-2

Printed on acid-free paper.

9 8 7 6 5 4 3 2 1

springer.com

Foreword

Progression of gastrointestinal or ovarian cancer to the peritoneal surfaces remains a dreaded clinical condition. It remains a perplexing challenge with pitfalls in both diagnosis and treatment that continue to vex the oncologist. Recently, pharmacologic studies, an aggressive surgical approach, and concentration of patients in peritoneal surface malignancy treatment centers have begun to generate a small optimism regarding the management of these patients.

A problem in the past now finding a partial solution is definitive diagnosis and lack of delay in treatment. Laparoscopy has greatly facilitated prompt diagnosis. Our interpretation of abdominal and pelvic CT scans has improved allowing characterization of this condition in order to select patients for elective treatment. Also, more accurate staging histopathologically shows promise. Systems of prognostic indicators are being tested in order to properly select patients for the broad array of treatment possibilities that exist. Standardization of these assessments between institutions will be difficult but is necessary. A molecular diagnosis for this disease may be evident within the near future.

Another development promoting limited optimism in dealing with carcinomatosis concerns the concentration of patients in peritoneal surface malignancy treatment centers whereby natural history data plus clinical, radiological and histopathological correlates become evident. A well maintained database and sufficient patient accrual can provide the necessary information for progress in patient management to occur.

A great value for these patients in going to a treatment center concerns the increased knowledge and skill of the caregivers at these centers. No longer are patients being cared for by physicians treating only one or two patients a year. Treatment centers are now regularly managing a patient a week. Patients managed in an anecdotal fashion are unlikely to have optimal management and no knowledge is gained. With referral centers this disease can be managed more efficiently than at the community hospital level.

As awareness of the unique features of carcinomatosis becomes more evident, an increased number of global conferences devoted to the new and more aggressive treatment strategies bring the designated treatment centers together. Carcinomatosis has become a condition that “stands alone” and deserves to have its own basic science, radiologic, medical, and surgical cadre and support staff from nurses and other caregivers. Having these peritoneal surface oncologists get together on a regular basis and share their new data has led to an escalation of progress with this condition.

This book on peritoneal carcinomatosis attests to the fact that cooperative efforts in behalf of these patients are occurring. Assembling and disseminating the available information and the identification of “breakthroughs” in understanding this problem can occur. This book should act as an authoritative guide to medical

oncologists, radiation oncologists, surgical oncologists, gastroenterologists, and basic science individuals who wish to focus on a particular aspect of this disease or to broaden their knowledge concerning its many and varied complexities. These efforts should be taking place in the United States and Europe and need to be carried to the underdeveloped nations. This book can be of help in that regard. Much progress has been made and much more is required.

Paul H. Sugarbaker, MD, FACS, FRCS
Director, Program in Peritoneal Surface Malignancy
Washington Cancer Institute
Washington, DC, USA

Preface

Peritoneal carcinomatosis (PC) represents a fitting illustration of the complexities faced by modern medicine. On the one hand, recent data suggest that in a well selected group of patients with PC, extensive surgery with intraperitoneal (ip) chemotherapy can extend survival beyond five years, a figure rarely achieved even with modern palliative chemotherapy and attested by a growing number of expert centres offering this demanding therapy.

On the other hand, however, most of these patients will not be cured and careful weighing of the possible benefits against the risks and quality of life consequences of extensive surgery should be the effort of a multidisciplinary team. Individual patient care decisions are fraught with a lack of a high quality evidence base regarding essential treatment components such as patient selection, timing and extent of surgery, and the relative contribution of chemotherapy and hyperthermia. National guidelines therefore recommend to treat all PC patients in the context of clinical trials [1,2]. Moreover, in contrast to the exponential growth in basic science literature concerned with cancer growth, angiogenesis and systemic metastasis, surprisingly little is known about the molecular mechanisms at the origin of PC.

The aim of the present volume was to bring together leading basic science and clinical investigators in the field of PC in order to provide a multidisciplinary 'status praesens' of current knowledge. It is hoped that their efforts will not only assist in individual patient care, but also facilitate consensus among surgical and medical oncologists, define future areas of basic research and help to overcome the major challenge in the field of PC: to extend the limited and indeed often anecdotal evidence supporting the various therapeutic options by well designed multicenter clinical studies.

The Editor

Ghent, 2006

References

1. Guide sur le traitement de la carcinomatose péritonéale par cytoréduction chirurgicale et chimiothérapie hyperthermique intrapéritonéale peropératoire. Comité de l'évolution des pratiques en oncologie (CÉPO), Direction de la lutte contre le cancer (Québec), February 2006. Available at: www.msss.gouv.qc.ca/sujets/prob_sante/cancer/download.php?id=584134,214,2 (Accessed October 4, 2006)

2. National Institute for Clinical Excellence (NICE) Interventional Procedures Programme. Interventional procedures overview of complete cytoreduction and heated intraoperative intraperitoneal chemotherapy (Sugarbaker technique) in patients with peritoneal carcinomatosis, July 2004. Available at: www.nice.org.uk/download.aspx?o=ip256overview
(Accessed October 4, 2006)

Contents

Part 1. Peritoneal Carcinomatosis: Basic Concepts

List of Contributors

H. Richard Alexander, MD
Department of Surgery
University of Maryland Medical Center
22 S. Greene Street S4B05A
Baltimore, Md 21201, USA
e-mail: HRAlexander@smail.umaryland.edu

Frederic Amant, MD, PhD
Division of Gynaecological Oncology
Department of Obstetrics and Gynaecology
University Hospitals, Katholieke Universiteit
Herestraat 49, B-3000 Leuven, Belgium

Eturou Bando
Peritoneal Dissemination Program
Shizuoka Cancer Center, Sunto-gun 411-8777, Japan

Dario Baratti
Department of Surgery, National Cancer Institute
via Venezian n.1, 20133 Milano, Italy

Annie Claude Beaujard, MD
Department of Anesthesiology and Intensive Care
Lyon 1 University
Centre Hopitalo-Universitaire Lyon Sud
69495, Pierre Bénite cedex, France

Gerhild Becker, MD MSc
Department of Internal Medicine II
University Hospital Freiburg
Hugstetter Str. 55, D-79106 Freiburg i. Br., Germany
e-mail: becker@medizin.ukl.uni-freiburg.de

Patrick Berteloot, MD
Division of Gynaecological Oncology
Department of Obstetrics and Gynaecology
University Hospitals, Katholieke Universiteit
Herestraat 49, B-3000 Leuven, Belgium

Tom Boterberg MD, PhD
Department of Radiotherapy
University Hospital
De Pintelaan 185, B-9000 Ghent, Belgium
e-mail: tom@krtkg1.ugent.be

Marc Bracke
Laboratory of Experimental Cancerology
University Hospital
De Pintelaan 185, B-9000 Ghent, Belgium
e-mail: brackemarc@hotmail.com

Wim P. Ceelen, MD
Department of Surgery
University Hospital
De Pintelaan 185, B-9000 Ghent, Belgium
e-mail: wim.ceelen@ugent.be

Keith A. Cengel
Department of Radiation Oncology
University of Pennsylvania School of Medicine
3400 Spruce Street, Philadelphia, PA 19104, USA

Eddy Cotte, MD
Lyon 1 University, Surgical department
Centre Hopitalo-Universitaire Lyon Sud
69495, Pierre Bénite cedex, France

Marc H. Dahlke
Department of Surgery
University of Regensburg
93042 Regensburg, Germany

Wilfried De Neve MD, PhD
Department of Radiotherapy
University Hospital
De Pintelaan 185, B-9000 Ghent, Belgium

Hannelore Denys, MD, PhD
Department of Medical Oncology
University Hospital
De Pintelaan 185, B-9000 Ghent, Belgium

Marcello Deraco
Department of Surgery, National Cancer Institute
via Venezian n.1, 20133 Milano, Italy
e-mail: marcello.deraco@istitutotumori.mi.it

Wim Duthoy, MD, PhD
Department of Radiotherapy
University Hospital
De Pintelaan 185, B-9000 Ghent, Belgium

Dominique Elias, MD, PhD
Chief of the Department of Surgical Oncology
Institut Gustave Roussy, Département de Chirurgie
39 Rue Camille Desmoulins, 94805 Villejuif, France
e-mail: elias@igr.fr

Yoshio Endo
Department of Experimental Therapeutics
Shizuoka Cancer Center, Sunto-gun 411-8777, Japan

Michael F. Flessner, MD, PhD
Department of Medicine/Nephrology
University of Mississippi Medical Center
2500 North State Street
Jackson, MS 39216-4505, USA
e-mail: mflessner@medicine.umsmed.edi

Gunnar Folprecht
University hospital "Carl Gustav Carus"
Medical Dep. I, Fetscherstr. 74, 01307 Dresden, Germany

Trivadi S Ganesan, MD, PhD, MNAMS, FRCP
Chairman of Cancer Institute
& Institute of Molecular Medicine
Amrita Institute of Medical Sciences
Kochi, Kerala, 682 026, India
e-mail: tsganesan@aims.amrita.edu

Olivier Glehen, MD
Lyon 1 University, Surgical department
Centre Hopitalo-Universitaire Lyon Sud
69495, Pierre Bénite cedex, France

François Noël Gilly, MD, PhD
Professor of Surgery
Lyon 1 University, Surgical department
Centre Hopitalo-Universitaire Lyon Sud
69495, Pierre Bénite cedex, France
e-mail : francogi@lyon-sud.univ-lyon1.fr

Eli Glatstein
Department of Radiation Oncology
University of Pennsylvania School of Medicine
3400 Spruce Street, Philadelphia, PA 19104, USA

Diane Goere
Institut Gustave Roussy,
Département de Chirurgie,
39 Rue Camille Desmoulins, 94805 Villejuif, France

Claudia Hadtstein
Junior scientist, Institute of Applied Pharmacy (IFAP), Cologne
Bitburgerstrasse 4, 54668 Echternacherbrück, Germany

Stephen M. Hahn
Henry K. Pancoast Professor and Chair
Department of Radiation Oncology
University of Pennsylvania School of Medicine
3400 Spruce Street, Philadelphia, PA 19104, USA
e-mail: hahn@xrt.upenn.edu

Nader Hanna, MD
Department of Surgery
University of Maryland Medical Center
22 S. Greene Street S4B05A
Baltimore, Md 21201, USA

Markus M. Heiss
Department of Surgery
Merheim Medical Center
University of Witten/Herdecke
Ostmerheimer-Strasse 200, D-51109 Koln-Merheim, Germany

Bert Hildebrandt
Charité-Centrum Tumormedizin
Charité Universitätsmedizin Berlin
Augustenburger Platz 1, D-13353 Berlin, Germany
e-mail: hyperthermia@charite.de

Hiroaki Ito
Peritoneal Dissemination Program
Shizuoka Cancer Center, Sunto-gun 411-8777, Japan

Joachim Jähne, MD, PhD
Clinic for General and Visceral Surgery
Center for Endocrine and Oncological Surgery, Henriettenstiftung Hannover
Marienstrasse 72 – 90, D - 30171 Hannover, Germany
e-mail: avg.chirurgie@henriettenstiftung.de

Mr David Jayne
Senior Lecturer in Surgery
Academic Surgical Unit
St. James's University Hospital
Leeds LS9 7TF, United Kingdom
e-mail: david.jayne@leedsth.nhs.uk

Taiich Kawamura
Peritoneal Dissemination Program
Shizuoka Cancer Center
Sunto-gun 411-8777, Japan

Thekla Kiffmeyer
Head of Department. of Environmental Medicine
Institute of Energy and Environmental Technology (IUTA)
Bliersheimer Straße 60, D-47229 Duisburg, Germany
e-mail: kiffmeyer@iuta.de

Kouichi Kiyosaki
Department of Surgery
Jichi Medical School Oomiya
Saitama 330-8503, Japan

Claus-Henning Köhne
Klinikum Oldenburg gGmbH
Dept. of Oncology and Hematology
Dr.Eden Str.10, 26133 Oldenburg, Germany

Stefan Kübler, MD
Clinic for General and Visceral Surgery
Center for Endocrine and Oncological Surgery, Henriettenstiftung Hannover
Marienstrasse 72 – 90, D - 30171 Hannover, Germany
e-mail: stefan.kuebler@henriettenstiftung.de

Shigeki Kusamura
Department of Surgery
National Cancer Institute
via Venezian n.1, 20133 Milano, Italy

Joon-Mo Lee, MD
Department of Obstetrics & Gynecology
The Catholic University of Korea
Kangnam St. Mary's Hospital, Seocho-go
Banpo-dong, 137-701 Seoul, South Korea
e-mail : leejm@catholic.ac.kr

Karin Leunen, MD
Division of Gynaecological Oncology
Department of Obstetrics and Gynaecology
University Hospitals, Katholieke Universiteit
Herestraat 49, B-3000 Leuven, Belgium

Edward A. Levine, MD
Professor of Surgery
Chief, Surgical Oncology
Wake Forest University, Winston-Salem, NC 27106, USA
e-mail: elevine@wfubmc.edu

Jean Christophe Lifante, MD
Lyon 1 University, Surgical department
Centre Hopitalo-Universitaire Lyon Sud
69495, Pierre Bénite cedex, France

Matthias Löhr
Dept. of Medicine II
University Hospital Mannheim, University of Heidelberg,
D-68135 Mannheim, Germany

Teri A. Longacre, MD
Department of Pathology
Stanford University School of Medicine
Room L235, 300 Pasteur Drive
Stanford, CA, 94305, USA
e-mail: longacre@stanford.edu

Nicholas J. Lutton
North Hampshire Hospital
Aldermaston Road
Basingstoke, Hampshire, RG24 9NA United Kingdom

Manfred P. Lutz
Caritasklinik St. Theresia
Rheinstrasse 2, 66113 Saarbrücken, Germany
e-mail: m.lutz@caritasklinik.de

Srinivasan Madhusudan
Cancer Research UK Medical Oncology Unit
The Churchill Hospital
Oxford OX3 7LJ, United Kingdom

Haile Mahteme, MD, PhD
Department of surgical sciences
Section of Surgery
University hospital of Uppsala
Akademiska sjukhuset, Uppsala, Sweden
e-mail: haile.mahteme@surgsci.uu.se

Maurie Markman, MD
University of Texas M.D. Anderson Cancer Center
Mail Box #121, 1515 Holcombe Boulevard,
Houston, Texas 77005, USA
e-mail: mmarkman@mdanderson.org

Thomas J. Miner, MD, FACS
Assistant Professor of Surgery
Director of Surgical Oncology
Department of Surgery
Brown Medical School
593 Eddy St, APC 439, Providence, RI, 02903, USA
e-mail: TMiner@usasurg.org

Masahiro Miura
Department of Anatomy
Oita University
School of Medicine
Oita, Japan

Mr. Brendan J. Moran, MCh, FRCS
Director, Pseudomyxoma Peritonei Centre
North Hampshire Hospital
Aldermaston Road
Basingstoke, Hampshire, RG24 9NA United Kingdom
e-mail: Brendan.Moran@nhht.nhs.uk

Sophie Morris
Research Fellow
Academic Surgical Unit
10th Floor QEQM Wing, St Mary's Hospital
London W2 1NY, United Kingdom

Steven E. Mutsaers, PhD
Adjunct Associate Professor
Centre for Asthma, Allergy and Respiratory Medicine
University of Western Australia and
Senior Research Scientist
Asthma & Allergy Research Institute
4th Floor G Block, Sir Charles Gairdner Hospital
Hospital Avenue, Nedlands WA 6009, Australia
e-mail: mutsaers@aari.uwa.edu.au

Patrick Neven, MD, PhD
Division of Gynaecological Oncology
Department of Obstetrics and Gynaecology
University Hospitals, Katholieke Universiteit
Herestraat 49, B-3000 Leuven, Belgium

Lars Påhlman
Department of surgical sciences
Section of Surgery
University hospital of Uppsala
Akademiska sjukhuset
Uppsala, Sweden

Reetesh K. Pai, MD
Department of Pathology
Stanford University School of Medicine
Room L235, 300 Pasteur Drive, Stanford, CA, 94305, USA

Mr Paraskevas Paraskeva, PhD, FRCS
Senior Lecturer Academic
Academic Surgical Unit
10th Floor QEQM Wing, St Mary's Hospital
London W2 1NY, United Kingdom

Piet Pattyn, MD, PhD
Department of Surgery
University Hospital
De Pintelaan 185, B-9000 Ghent, Belgium
e-mail: piet.pattyn@ugent.be

Marc Peeters, MD, PhD
Department of Gastroenterology
University Hospital
De Pintelaan 185, B-9000 Ghent, Belgium
e-mail: marc.peeters@ugent.be

James F. Pingpank, MD
Department of Surgery
University of Maryland Medical Center
22 S. Greene Street S4B05A
Baltimore, MD 21201, USA

Pompiliu Piso
Department of Surgery
University of Regensburg
93042 Regensburg, Germany
e-mail: pompiliu.piso@klinik.uni-regensburg.de

Marc Pocard
Institut Gustave Roussy,
Département de Chirurgie,
39 Rue Camille Desmoulins
94805 Villejuif, France

Stephan Samel, MD
University of Göttingen
Waldweg 1, D-37073 Göttingen, Germany
e-mail: Stephan.samel@web.de

Takuma Sasaki
Laboratory of Bio-organic Chemistry
School of Pharmacy
Aichi Gakuin University
Nagoya 464-8650, Japan

Hans J. Schlitt
Department of Surgery
University of Regensburg
93042 Regensburg, Germany

Perry Shen, MD
Surgical Oncology
Wake Forest University
Winston-Salem, NC 27106, USA

John H. Stewart, IV, MD
Surgical Oncology
Wake Forest University
Winston-Salem, NC 27106, USA

Michael A Ströhlein
Department of Surgery
Merheim Medical Center
University of Witten/Herdecke
Ostmerheimer-Strasse 200, D-51109 Koln-Merheim, Germany
e-mail: StroehleinM@kliniken-koeln.de

Paul H. Sugarbaker, MD
Washington Cancer Institute
106 Irving St., NW
Suite 3900, Washington, DC 20010, USA
e-mail: Paul.Sugarbaker@medstar.net

Jouke T. Tamsma, MD, PhD
Leiden University Medical Center (LUMC)
Vascular Medicine, Dept. of Endo & Gen.Int.Med, C4-R66
PO Box 9600, 2300 RC Leiden, The Netherlands
e-mail: jttamsma@lumc.nl

Toon Van Gorp, MD
Division of Gynaecological Oncology
Department of Obstetrics and Gynaecology
University Hospitals, Katholieke Universiteit
Herestraat 49, B-3000 Leuven, Belgium

Sybile Van Lierde, MD
Department of gastroenterology
University Hospital
De Pintelaan 185, B-9000 Ghent, Belgium
e-mail: sybvanlierde@hotmail.com

Ignace Vergote, MD, PhD
Division of Gynaecological Oncology
Department of Obstetrics and Gynaecology
University Hospitals, Katholieke Universiteit
Herestraat 49, B-3000 Leuven, Belgium
Ignace.Vergote@uz.kuleuven.ac.be

Vic J Verwaal, MD, PhD
Department of Surgery
The Netherlands Cancer Institute
Plesmanlaan 121
NL-1066 CX Amsterdam
The Netherlands
e-mail: v.verwaal@nki.nl

Sylwia Wilkosz
Lung Institute of Western Australia and
Centre for Asthma, Allergy and Respiratory Research
University of Western Australia
Sir Charles Gairdner Hospital,
Nedlands, WA 6009, Australia

Peter Wust
Charité-Centrum Tumormedizin
Charité Campus Virchow Klinikum
Charité Universitätsmedizin Berlin
Augustenburger Platz 1
D-13353 Berlin, Germany
e-mail: hyperthermia@charite.de

Yutaka Yonemura
Peritoneal Dissemination Program
Shizuoka Cancer Center
Sunto-gun 411-8777, Japan
e-mail: y.yonemura@scchr.jp

Structure and Function of Mesothelial Cells

SE Mutsaers, S Wilkosz

Introduction

Mesothelial cells are specialised cells that line the entire surface of the three serosal cavities (pleural, pericardial and peritoneal) and in the male, the sac which surrounds the testes. This layer of cells is termed 'mesothelium' with the visceral mesothelium lining the internal organs and the parietal mesothelium lining the body wall. The mesothelium was first described by Bichat in 1827 but it was not until 1890 that Minot proposed the term 'mesothelium' to reflect the epithelial-like nature of the cells lining the mammalian mesodermic cavities [1]. Despite the early discovery and description of the mesothelium, it has only been in recent years that its importance both in health and disease has been realised.

Structure of Mesothelial Cells

Mesothelial cells are predominantly flattened, squamous-like cells, approximately 25 μm in diameter, with the cytoplasm raised over a central round or oval nucleus. The cells contain microtubules and microfilaments, glycogen, few mitochondria, a poorly developed Golgi apparatus and little rough endoplastic reticulum (RER). The luminal surface has a well developed microvillous border with microvilli varying in length, shape and density, which can change under different physiological conditions reflecting functional adaptation [2]. Cilia are also present on some resting mesothelial cells but are more abundant on biosynthetically active cells. These cilia may be motile but the evidence is not conclusive. However, it has been proposed that they may be part of a sophisticated surveillance system that may respond to elicit discrete cellular responses [3].

Mesothelial cells have a well developed system of vesicles and vacuoles; most are micropinocytic but multivesicular bodies and large vacuoles can be found. These vesicles reflect the biosynthetic potential of the mesothelium and are involved in transport of fluids and particulates across the serosal surface [4]. The

boundaries between mesothelial cells are tortuous, with adjacent cells often overlapping. They have well developed cell-cell junctional complexes including tight junctions, adherens junctions, gap junctions and desmosomes [5]. Apical junctional complexes are crucial for the development of cell surface polarity and the establishment and maintenance of a semi-permeable diffusion barrier.

Although the mesothelium is composed predominantly of squamous-like cells, cuboidal mesothelial cells can be found in various areas including the septal folds of the mediastinal pleura, the parenchymal organs (liver, spleen), the "milky spots" of the omentum and the peritoneal side of the diaphragm overlying the lymphatic lacunae. Cells which are morphologically similar to these cuboidal mesothelial cells can also be identified after injury or stimulation of the serosal surfaces. These cells are larger and contain a prominent nucleolus. They have abundant mitrochondria and RER, a well developed Golgi apparatus, microtubules and a comparatively greater number of microfilaments, suggesting a more metabolically activate state [6].

Mesothelial cells rest on a thin basement membrane supported by connective tissue stroma, which varies in quantity depending on site and species, containing blood vessels, lymphatics, resident macrophages, lymphocytes and fibroblast-like cells [7]. Mesothelial cells can also line "stomata", cavities at the junction of two or more mesothelial cells. Stomatal openings are 3-12 μm in diameter and are generally found in regions where cuboidal mesothelial cells are present such as in the milky spots of the omentum. These openings provide a direct access to the underlying submesothelial lymphatic system allowing rapid removal of fluid, cells, bacteria and particles from the serosal cavities [8].

Mesothelial cells are unique as although they are derived from the mesoderm and express the mesenchymal intermediate filaments vimentin and desmin, they also express cytokeratins which are intermediate filaments characteristic of epithelial cells [9]. When appropriately stimulated these cells can undergo an epithelial to mesenchymal transition (EMT) response, losing their epithelial characteristics and adopting a more fibroblast-like phenotype. This has been observed in cells from patients who have undergone continuous ambulatory peritoneal dialysis (CAPD) or following exposure of cultured mesothelial cells to transforming growth factor beta (TGF-β). These cells show down regulation of cytokeratins and E-cadherin and an increase in alpha smooth muscle actin, type I collagen and the transcription factor snail, characteristic of EMT [10,11]. Recently, mesothelial cells were also shown to be the precursor cells for endothelium and smooth muscle of developing vasculature in the gut and heart of mice [12]. These findings show that the mesothelium is a source of mesenchymal cells which apart from being important in development and serosal repair, may also play a role in serosal fibrosis and adhesion formation. Although there is a lack of information regarding the differentiation potential of mesothelial cells, these cells have been used for over a century to repair damaged tissues and organs, as well as being employed in tissue engineering applications including vascular and nerve grafts (reviewed by Herrick and Mutsaers) [13].

Mesothelial Cell Functions

Slippery Protective Layer

The mesothelial cell has many diverse functions which are important in maintaining serosal homeostasis (Fig. 1).

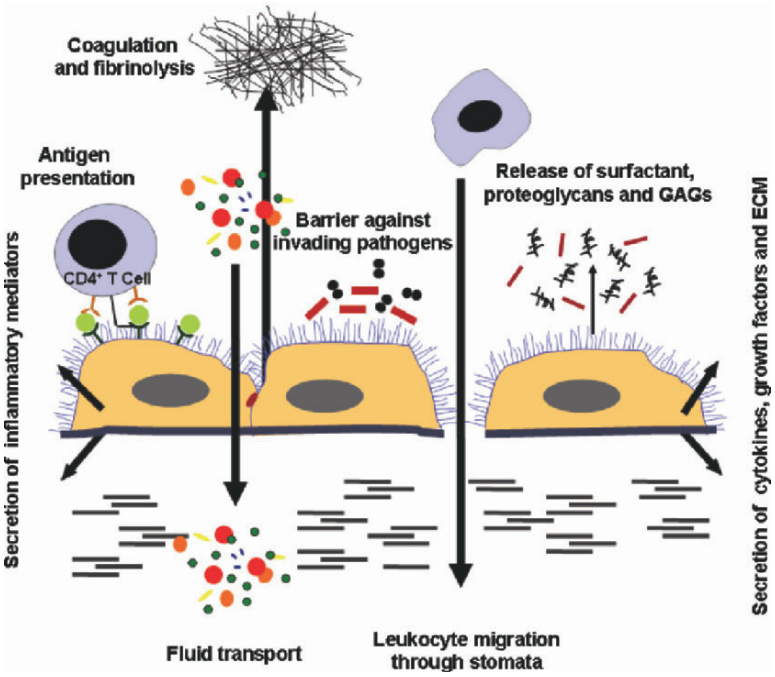


Figure 1. Functions of mesothelial cells. Mesothelial cells provide a protective barrier against abrasion and invading pathogens and secrete surfactant, proteoglycans and glycosaminoglycans to provide a slippery, non-adhesive surface to allow intracoelomic movement. They facilitate transport of fluid and cells across the serosal cavities, present antigen to T cells and participate in the induction and resolution of inflammation and tissue repair by secreting cytokines, growth factors, ECM, proteases and other biological mediators. They are also the major source of PAs in serosal fluid which is important in fibrinolysis. Modified from Mutsaers 2004 [100]

Mesothelial cells synthesise and actively secrete large amounts of phosphatidylcholine, the major constituent of lamella bodies and pulmonary surfactant, which lubricates the internal organs providing a slippery, non adhesive surface to facilitate intracoelomic movement [14,15]. They also reduce friction through expression of numerous microvilli that facilitate surfactant distribution [16].

Like other epithelial layers, the mesothelium is the first line of defense against invading organisms, acting as a physical barrier and initiating inflammatory and immune responses. Adjacent cells are held together by interdigitations of cytoplasmic membrane and a complex arrangement of intercellular junctions including tight junctions and desmosomes which ensures integrity of the mesothelium. In addition, it has been proposed that glycosaminoglycans, predominantly hyaluronan, secreted by mesothelial cells and assembled into hyaluronan-containing pericellular matrixes "coats" protects the cells from viral infections and the cytotoxic effects of lymphocytes [17] and possibly tumour cell adhesion and growth (discussed later). Although the mesothelium is a barrier to invading organisms and particulates, it is a semi-permeable membrane and actively transports fluid and particulate matter across the serosal cavities through micropinocytic vesicles [4]. Again the microvilli play an important role by increasing the surface area of the cell and binding fluids in its glycosaminoglycan rich glycocalyx, aiding adsorption [18].

Inflammation and Immune Response

In addition to its structural function, the mesothelial cell clearly plays an important role in serosal inflammation and immune responses [19]. To mount an effective immune response against invading pathogens, a large number of leukocytes are recruited from the vascular compartment into the serosal space. Serosal inflammation is likely to be activated on the surface of the mesothelial cell with release of chemokines including interleukin (IL)-8, growth-related oncogene-α (GRO-α), interferon-gamma-inducible protein-10 (IP-10), monocyte chemoattractant protein-1 (MCP-1), RANTES [20,21], eotaxin [22] and stromal cell-derived factor-1 (SDF-1) [23]. SDF-1 stimulates the growth of B lymphocyte precursors (B1a) *in vitro*, therefore SDF-1 production by mesothelial cells may account for the selective accumulation of B1 lymphocytes in body cavities. Chemokine secretion is polarised toward the cell apical surface, creating a chemotactic gradient from the basolateral to the apical side of the mesothelial cell promoting directed transmesothelial migration of inflammatory cells [24].

Movement of leukocytes from the circulation to the site of inflammation is facilitated by the expression of integrins and adhesion molecules including intercellular adhesion molecule (ICAM-1), vascular cellular adhesion molecule (VCAM-1), E-cadherin, N-cadherin and various alpha and beta integrin chains [20,25,26]. Leukocytes express the β_2 integrin family members, lymphocyte function-associated antigen-1 (LFA-1) (CD11a/CD18) and Mac-1 (CD11b/CD18) on their surface, which are counter receptors for ICAM-1. Interaction between LFA-

1/Mac-1 and ICAM-1 leads to cell-cell adherence and results in transmigration of leukocytes across mesothelial cell monolayers. Interestingly, ICAM-1 and VCAM-1 were only expressed on the microvilli of mesothelial cells with VCAM-1 less numerous and on less microvilli (24%) than ICAM-1 (90%) [27]. This suggests that leukocytes might not crawl on the cell surface, but to and from microvilli.

A great deal is known about leukocyte influx into an inflamed site but the subsequent events are less clear. It is likely that leukocyte clearance from serosal cavities is via stomata and the draining lymphatics [8] in contrast to influx directly across the mesothelium from the vasculature. Kinetic studies suggest that resident and inflammatory macrophages are cleared from the peritoneum at different rates [28]. We recently showed that macrophage clearance is controlled through integrin-mediated regulation of macrophage-mesothelial cell interactions involving very late antigen (VLA) 4 and VLA5 [29]. Similar mechanisms may be involved in clearance of other inflammatory cell types.

There is growing support for an antigen presenting role by the mesothelial cell. Valle et al. [30] demonstrated that human peritoneal mesothelial cells express major histocompatibility complex class II molecules and are able to present tetanus toxoid and *C. albicans* bodies to peripheral blood mononuclear cells and cloned T cells. In a similar study, Hausmann and colleagues demonstrated that in the absence of professional antigen presenting cells, human peritoneal mesothelial cells stimulated by interferon-γ (IFN-γ) induced $CD4^+$ T cell proliferation in the presence of antigen and secreted interleukin (IL)-15, a T-cell growth factor and activator [31]. More recently it was shown that IFN-γ-induced IL-15 production was mediated via ligation of CD40, a key molecule for antigen presentation, which is expressed on mesothelial cells. When cells were stimulated with both IFN-γ and tumour necrosis factor-α (TNF-α), IL-15 production increased more than 3 fold. In addition, CD40 ligation was strongly synergistic with IFN-γ in inducing RANTES production by mesothelial cells which was shown to be important for mononuclear cell infiltration during peritonitis [32,33].

Mesothelial cells also secrete cytokines including IL-6, heat shock proteins (HSP)-72/73, granulocyte-colony stimulating factor (G-CSF), granulocyte-macrophage-CSF (GM-CSF) and IL-1, which are upregulated in response to mediators such as bacterial lipopolysaccharide (LPS), IL-1β, TNF-α and epidermal growth factor (EGF) [20,34,35]. IL-6 is often induced together with pro-inflammatory cytokines IL-1 and TNF-α and circulating IL-6 plays an important role in the induction of acute phase reactions. Endogenous IL-6 plays a crucial anti-inflammatory role in both local and systemic acute inflammatory responses by controlling the levels of pro-inflammatory cytokines [36]. Mesothelial cells also produce reactive nitrogen and oxygen species in vitro in response to cytokines, bacterial product and asbestos [37]. To counteract the effect of these reactive species, mesothelial cells contain significant quantities of antioxidants. As

well as proinflammatory molecules, mesothelial cells can regulate the inflammatory response by releasing anti-inflammatory prostaglandins and prostacyclin both constitutively and following induction by inflammatory mediators [38].

Tissue Repair

Mesothelial cells play an important role in tissue repair by releasing growth factors which initiate cell proliferation, differentiation and migration of mesothelial and submesothelial cells surrounding a lesion. TGF-β, platelet-derived growth factor (PDGF), fibroblast growth factor (FGF), hepatocyte growth factor (HGF), keratinocyte growth factor (KGF) and members of the EGF family (EGF, heparin binding EGF (HB-EGF) and vascular EGF (VEGF) are some of the factors likely to regulate these processes [39,40].

Mesothelial cells synthesise a variety of ECM molecules which are important for cell function and repair of serosal membranes. Cultured mesothelial cells produce collagen types I, III and IV, elastin, fibronectin and laminin [41,42] which can be further stimulated when exposed to peritoneal effluents from patients with acute peritonitis [43] or various cytokines and growth factors such as IL-1β, TNF-α, EGF, PDGF and TGF-β [42]. They can also organise these components into complex structures that resembled components of the ECM in vivo (thick collagen fibres, the amorphous components of elastic fibres and basement membrane-like structures) [41]. Over-expression of TGF-β in serosal tissues has also been shown to lead to fibrosis and adhesion formation [44]. Mesothelial cells are likely to regulate ECM turnover by secreting proteases and antiproteases such as metalloproteinases and tissue inhibitors of metalloproteinases and molecules such as decorin and biglycan which inhibit TGF-β activity [45,46]. In addition, they can regulate their interaction with the ECM through altering their integrin expression. Mesothelium-ECM interactions are likely to have roles in embryonic development, maintenance of tissue architecture, inflammatory response, tissue repair and tumour metastases [47], although more studies are required to elucidate the exact mechanisms regulating these processes.

Fibrin Regulation

Mesothelial cells also play an important role in local fibrin deposition and clearance within serosal cavities. The procoagulant activity is due to secretion of tissue factor, the main cellular initiator of the extrinsic coagulation cascade [48]. However, mesothelial cells can also regulate local expression of protein C, part of an anticoagulant pathway which controls thrombin generation [49]. Their fibrinolytic activity is mainly through secretion of plasminogen activators (PA); tissue-PA (tPA) and urokinase-PA (uPA). The PAs convert the inactive zymogen plasminogen into active plasmin which in turn enzymatically breaks down fibrin. Mesothelial cells regulate their fibrinolytic activity through secretion of plasminogen

activator inhibitors (PAI), PAI-1 and PAI-2 [50]. Both pro- and anti-fibrinolytic mediators are regulated by inflammatory factors including LPS, TNF-α and IL-1 and fibrogenic mediators such as TGF-β and thrombin [51]. Fibrogenic mediators reduce production of tPA by mesothelial cells while increasing synthesis of PAI-1 causing a significant delay in fibrinolysis.

There is a fine balance between fibrin deposition and breakdown in serosal cavities, which if inappropriately regulated can cause reduced fibrin clearance and result in adhesion formation [52]. Adhesions are a common complication of abdominal surgery and infection and may lead to intestinal obstruction, chronic pelvic pain and infertility in women. Readdressing this imbalance by blocking PAIs or the mediators upregulating their synthesis may be a way of preventing adhesions [52] as current barrier approaches using membranes and gels have had limited success.

Role of Mesothelial Cells in Tumour Dissemination

Dissemination of peritoneal tumours requires four basic steps: 1) detachment of cancer cells from the primary tumour, 2) attachment to distant peritoneum, 3) invasion into subperitoneal space, and 4) proliferation and vascular neogenesis [53]. Although there are many studies examining peritoneal carcinomatosis there have been few studies specifically examining the role mesothelial cells play in promoting or inhibiting tumour cell attachment, invasion and growth within the peritoneal cavity. Most studies have concentrated on examining tumour recurrence following surgery, showing that traumatised mesothelial surfaces are privileged sites for tumour cell adhesion [54].

Cell Adhesion

The most likely mechanisms for tumour cell attachment is through entrapment of tumour cells within fibrinous exudate which is deposited following trauma, and attachment of these cells to exposed submesothelial connective tissue via integrins [55]. However, experimental studies have demonstrated that following surgical trauma, tumour growth is also enhanced at sites distal to the injury [56] suggesting that the mesothelial cell may be directly involved in carcinomatosis through direct mesothelial-tumour cell adhesion, implantation, invasion and subsequent growth (Fig. 2).

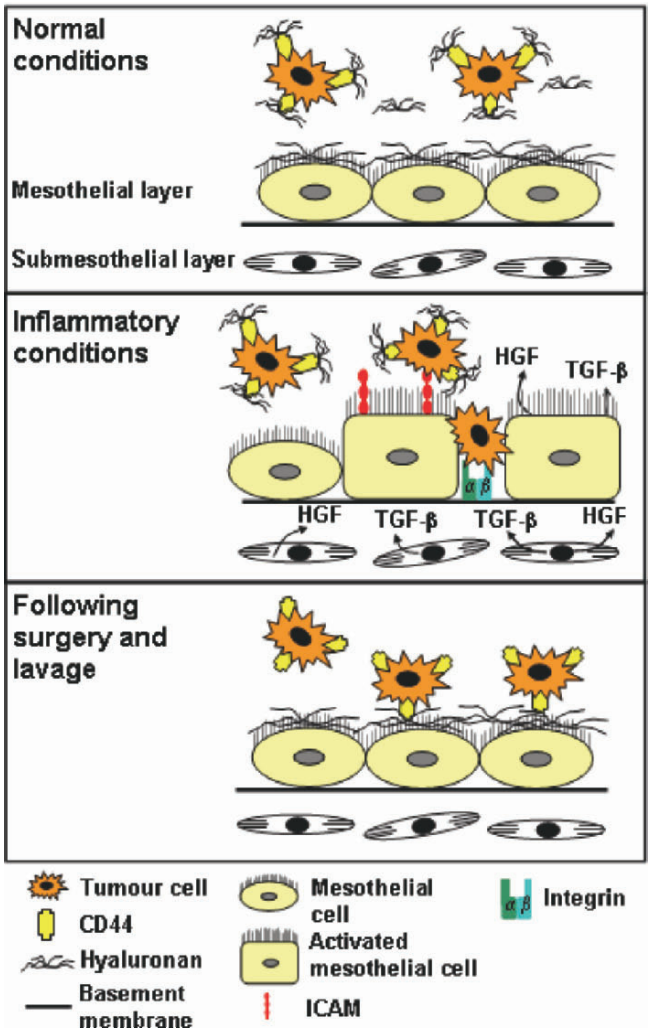


Figure 2. Possible sequence of events that lead to peritoneal dissemination of tumours. Under normal conditions free hyaluronan in the serosal fluid binds to tumour cells reducing tumour binding to hyaluronan on the surface of mesothelial cells. During inflammatory conditions, mediators including HGF and TGF-β are produced by activated mesothelial cells and submesothelial fibroblasts, which leads to rounding up of mesothelial cells and exposure of the ECM. Tumour cells may attach to ICAMs on activated mesothelial cells and to exposed ECM via integrins. Following surgery and peritoneal lavage, the free hyaluronan in the serosal fluid is removed enabling tumour cells to bind to the mesothelial hyaluronan pericellular coat

A number of sites within the peritoneal cavity show increased tumour cell implantation. One of the most common sites is the greater omentum, in particular the milky spots [57]. This is not surprising given that this is the main site for clearance of cells and particulate matter from the peritoneal cavity. Whether this is due to specific interactions between tumour cells and omental mesothelial cells or because the milky spots do not have a continuous mesothelial layer, is not clear.

Van del Wal and colleagues [58] suggested that tumour cells can attach to mesothelium due to upregulation of adhesion molecules on mesothelial cells in response to inflammatory mediators. Indeed, IL-1β, TNF-α, IL-6 and IFN-γ upregulate ICAM-1 expression on mesothelial cells and IL-1β, TNF-α and EGF increase tumour cell adhesion to cultured mesothelial cells [59-61]. ICAM-1 binds to tumours expressing CD43 [61,62]. Ziprin and colleagues were able to successfully attenuate tumour cell adhesion to mesothelial cells in vitro using blocking antibodies against ICAM-1 and CD43 [61,62]. In addition, they showed that heparin downregulated ICAM-1 expression on mesothelial cells in vitro and prevented tumour growth when administered to rats [63] suggesting that heparin may have therapeutic antimetastatic potential.

The role of integrins in peritoneal tumour dissemination has been the focus of several studies. It was demonstrated that adhesion of an ovarian carcinoma cell line and ovarian carcinoma spheroids (aggregated tumour cells) to a confluent mesothelial cell monolayer was attenuated by addition of monoclonal antibodies against β1 integrin subunit. The β1 integrin is common to many integrin molecules and can bind a variety of ECM proteins, which are also synthesised by mesothelial cells [64,65]. Furthermore, migration of ovarian carcinoma cell lines towards fibronectin, type IV collagen and laminin was blocked by antibodies against α5β1, α2β1 and α6β1 respectively [66,67]. Similarly, adhesion of gastric carcinoma cell lines to mesothelial cells was inhibited by antibodies against β1, α2 and α3 subunits [68], indicating that α2β1 and α3β1 integrins play a role in gastric carcinoma cell adhesion to peritoneum. Expression of these two integrins also correlates with increased metastatic potential [69]. Although these studies clearly show a role for integrins in peritoneal carcinomatosis, not all tumour cell adhesion to mesothelial cells can be explained by integrin binding. Several studies have shown that ovarian carcinoma cell adhesion and migration is regulated by both integrin-dependent mechanisms and an integrin-independent mechanism that involves the interaction of CD44 and hyaluronan [65,67] (Fig. 2).

Numerous studies have demonstrated that adhesion of tumour cells to the hyaluronan pericellular coat of mesothelial cells is an important step in peritoneal spread of ovarian, colon and colorectal cancer [64,65,67]. A wide variety of malignancies of epithelial and mesenchymal origin express high levels of the hyaluronan receptor, CD44 [70], although the degree of adhesion does not necessarily relate to the amount of CD44 expressed [71,72]. This may be due to differences in CD44 glycosylation [72] or variant forms of CD44 [70]. Indeed, Kayastha et al.

[73] found that expression of the standard form of CD44 (CD44S) was significantly associated with poorer disease free survival of ovarian cancer patients. Blocking interaction of CD44 and hyaluronan using antisense CD44 cDNA [74], monoclonal antibodies that block the hyaluronan-binding site of CD44 [65,67,72], intact hyaluronan and hyaluronan oligomers [64,67], reduced cell adhesion and inhibited cell migration.

Although mesothelial cells either directly or indirectly appear to promote tumour dissemination and growth, intact hyaluronan inhibits the adhesion of tumour cells to mesothelium [67]. Indeed, conditioned medium from a confluent mesothelial cell culture containing high amounts of hyaluronan successfully prevented tumour cell adhesion [75]. It is likely that free hyaluronan binds to CD44 molecules on the tumour cells, thus preventing their binding to the mesothelial hyaluronan pericellular coat. Removal of free hyaluronan may explain why tumour cells adhered to mesothelial cells in other studies. Therefore, under normal physiological conditions, secretion of hyaluronan by mesothelial cells into the serosal fluid may protect the serosal surface from tumour implantation (Fig. 2). Peritoneal lavage following surgery is a procedure favoured by many surgeons. However, it is possible that such practice may be detrimental to cancer patients, as it provides an ideal environment for tumour cell attachment. Furthermore, peritoneal lavage may also cause damage to the delicate mesothelial cell layer leading to exposure of ECM, increasing the likelihood of peritoneal tumour dissemination [76,77]. Replacement of hyaluronan removed through peritoneal lavage should be considered as a possible preventative measure, however further studies are necessary to determine the efficacy of such treatment.

Cell Invasion

Once the tumour cells have adhered to the serosal surface, mesothelial cells may also play a role in cell invasion. Mesothelial cells produce lysophosphatidic acid (LPA), which is a biologically active lipid able to stimulate adhesion, migration and invasion of ovarian cancer cells [78]. Ren and colleagues [79] demonstrated that LPA produced by cultured mesothelial cells induced ovarian cancer cell migration, cell adhesion to collagen type I and cell invasion across a mesothelial monolayer, with two out of three LPA receptors, LPA_1 and LPA_2 being involved. LPA also stimulates VEGF production by mesothelial cells which may play a significant role in tumour angiogenesis [80].

For tumour cells to invade the interstitial tissue various proteases such as matrix metalloproteinases (MMPs) are needed. Indeed, it has been suggested that MMP-1, MMP-2 and MMP-7 play a role in the progression of gastric cancer [53,81]. MMP-2 is known to break down collagen type IV, laminin and fibronectin (all components of basement membrane) whilst MMP-1 acts on collagens type I and III. In vitro studies have shown that mesothelial cells spontaneously express MMP-1 and MMP-2 when in contact with tumour cells, which leads

to enhanced tumour invasion [53]. Furthermore, Burleson and colleagues [82] were able to inhibit tumour invasion by addition of broad-scale MMP inhibitor, highlighting the importance of proteases in tumour dissemination.

The protease inhibitor plasminogen activator inhibitor-I (PAI-1), has also been implicated in peritoneal tumour cell invasion and metastasis [83]. Mesothelial cells are a recognised source of PAI-1 within the peritoneal cavity [84]. In vitro studies have shown that mesothelial cells upregulate PAI-1 expression in response to growth factors such as TGF-β1 secreted directly by tumour cells, which then facilitates tumour cell adhesion, local invasion and peritoneal dissemination [83].

It is recognised that cell attachment and migration is highly dependent on the cytoskeletal system. Studies have shown that a cytoskeletal-like protein, calponin, may also play an important role in peritoneal tumour dissemination [85]. Mice deficient in calponin h1 have been reported to have fragile blood vessels and peritoneum [86]. Therefore Hashimoto and colleagues [87] hypothesized that calponin may protect mesothelial cells, which express calponin h1, against tumour invasion. The authors demonstrated that when exposed to tumour cells, cultured mesothelial cells isolated from calponin knock out mice retracted, while the wild-type mesothelial cells resisted cell invasion. Furthermore, viral gene transfer of calponin h1 resulted in suppression of tumour cell invasion both in vitro and in vivo. The results of this study suggest that the interaction of mesothelial cells with tumour cells may result in downregulation of calponin h1, subsequently weakening the integrity of the mesothelial cell layer. This process is likely to involve various growth factors such as TGF-β, bFGF and PDGF, which have been reported to decrease calponin expression [88].

Tumour Growth

Various growth factors produced by mesothelial cells are increased within the peritoneal fluid following surgery and may enhance local and distant tumour growth [89-91]. For example, HGF has a potent mitogenic and motogenic effect on a wide range of cells including ovarian, gastric, pancreatic and colorectal cancer [92]. In addition, the HGF receptor, c-Met, is overexpressed in several peritoneal cancers including gastric and ovarian cancer [93,94]. As well as directly stimulating tumour cell responses, HGF also induces mesothelial cells to break their cell-to-cell junctions, round up exposing underlying ECM, migrate and proliferate [40,95]. IL-1β and TNF-α also stimulate similar responses in mesothelial cells [59] but they may act through HGF as both IL-1β and TNF-α upregulate HGF and c-met expression in cells [96,97]. Interestingly, Fujiwara et al. [98] suppressed gastric cancer dissemination and increased survival time in mice following intraperitoneal injection of an adenovirus vector encoding the NK4 gene, a competitive antagonist for HGF.

Summary and Conclusions

The mesothelium was first described about 180 years ago but only in the last twenty years have we begun to appreciate the roles that mesothelial cells play in maintaining normal serosal membrane integrity and function. Mesothelial cells are sentinel cells that can sense and respond to signals within their microenvironment. They secrete glycosaminoglycans and surfactant to allow the parietal and visceral serosa to slide over each other. They actively transport fluids, cells and particulates across the serosal membrane and between serosal cavities. They synthesise and secrete a diverse array of mediators in response to external signals which play important roles in regulating inflammatory, immune and tissue repair responses. In addition, they are likely to protect from peritoneal dissemination of tumours until the integrity of the mesothelium is breached.

Although the importance of the mesothelial cell is being realised, we still do not understand the mechanisms regulating many of their functions. How the cells communicate with each other and surrounding cells, whether mesothelial cells differentiate into different cell types or if a mesothelial stem cell exists, the mechanisms regulating mesothelial repair and the role mesothelial cells play in serosal pathologies, all need further study. Although it has long been accepted that mesothelial cells are similar irrespective of site or species, apart from morphology, few studies have truly compared biochemical and functional characteristics of these cells between species and within different anatomical sites. In a recent study examining the effect of aging on human peritoneal mesothelial cells, there was a positive correlation between the age of the donor's cells and the proinflammatory profile [99]. Although mesothelial cells share many similarities, it is likely that functional and physiological adaptation will alter these cells. Addressing these questions are paramount if we hope to find better ways to protect serosal integrity and prevent peritoneal dissemination of tumours.

References

1. Whitaker D, Papadimitriou JM, Walters MN (1982) The mesothelium and its reactions: a review. Crit Rev Toxicol 10(2):81-144
2. Mutsaers SE, Whitaker D, Papadimitriou JM (1996) Changes in the concentration of microvilli on the free surface of healing mesothelium are associated with alterations in surface membrane charge. J Pathol 180(3):333-339
3. Bird SD (2004) Mesothelial primary cilia of peritoneal and other serosal surfaces. Cell Biol Int 28(2):151-159
4. Fedorko ME, Hirsch JG (1971) Studies on transport of macromolecules and small particles across mesothelial cells of the mouse omentum. I. Morphologic aspects. Exp Cell Res 69(1):113-127

5. Kluge T, Hovig T (1967) The ultrastructure of human and rat pericardium. II. Intercellular spaces and junctions. Acta Pathol Microbiol Scand 71(4):547-563
6. Mutsaers SE, Whitaker D, Papadimitriou JM (2002) Stimulation of mesothelial cell proliferation by exudate macrophages enhances serosal wound healing in a murine model. Am J Pathol 160(2):681-692
7. Albertine KH, Wiener-Kronish JP, Roos PJ, Staub NC (1982) Structure, blood supply, and lymphatic vessels of the sheep's visceral pleura. Am J Anat 165(3):277-294
8. Nakatani T, Ohtani O, Tanaka S (1996) Lymphatic stomata in the murine diaphragmatic peritoneum: the timing of their appearance and a map of their distribution. Anat Rec 244(4):529-539
9. LaRocca PJ, Rheinwald JG (1984) Coexpression of simple epithelial keratins and vimentin by human mesothelium and mesothelioma in vivo and in culture. Cancer Res 44(7):2991-2999
10. Yanez-Mo M, Lara-Pezzi E, Selgas R, Ramirez-Huesca M, Dominguez-Jimenez C, Jimenez-Heffernan JA, et al (2003) Peritoneal dialysis and epithelial-to-mesenchymal transition of mesothelial cells. N Engl J Med 348(5): 403-413
11. Yang AH, Chen JY, Lin JK (2003) Myofibroblastic conversion of mesothelial cells. Kidney Int 63(4):1530-1539
12. Wilm B, Ipenberg A, Hastie ND, Burch JB, Bader DM (2005) The serosal mesothelium is a major source of smooth muscle cells of the gut vasculature. Development 132(23):5317-5328
13. Herrick SE, Mutsaers SE (2004) Mesothelial progenitor cells and their potential in tissue engineering. Int J Biochem Cell Biol 36(4):621-642
14. Hills BA (2000) Role of surfactant in peritoneal dialysis. Perit Dial Int 20(5):503-515
15. Michailova KN (2004) Mesothelial lamellar bodies in norm and experimental conditions. Transmission and scanning electron microscopic observations on the peritoneum, pleura and pericardium. Anat Embryol (Berl) 208(4):301-309
16. Andrews PM, Porter KR (1973) The ultrastructural morphology and possible functional significance of mesothelial microvilli. Anat Rec 177(3):409-426
17. Heldin P, Pertoft H (1993) Synthesis and assembly of the hyaluronan-containing coats around normal human mesothelial cells. Exp Cell Res 208(2):422-429
18. Wang NS (1974) The regional difference of pleural mesothelial cells in rabbits. Am Rev Respir Dis 110(5):623-633
19. Topley N (1995) The host's initial response to peritoneal infection: the pivotal role of the mesothelial cell. Perit Dial Int 15(2):116-117
20. Jonjic N, Peri G, Bernasconi S, Sciacca FL, Colotta F, Pelicci P, et al (1992) Expression of adhesion molecules and chemotactic cytokines in cultured human mesothelial cells. J Exp Med 176(4):1165-1174
21. Visser CE, Tekstra J, Brouwer-Steenbergen JJ, Tuk CW, Boorsma DM, Sampat-Sardjoepersad SC, et al (1998) Chemokines produced by mesothelial

cells: huGRO-alpha, IP-10, MCP-1 and RANTES. Clin Exp Immunol 112(2):270-275
22. Katayama H, Yokoyama A, Kohno N, Sakai K, Hiwada K, Yamada H, et al (2002) Production of eosinophilic chemokines by normal pleural mesothelial cells. Am J Respir Cell Mol Biol 26(4):398-403
23. Foussat A, Balabanian K, Amara A, Bouchet-Delbos L, Durand-Gasselin I, Baleux F, et al (2001) Production of stromal cell-derived factor 1 by mesothelial cells and effects of this chemokine on peritoneal B lymphocytes. Eur J Immunol 31(2):350-359
24. Nasreen N, Mohammed KA, Hardwick J, Van Horn RD, Sanders KL, Doerschuk CM, et al (2001) Polar production of interleukin-8 by mesothelial cells promotes the transmesothelial migration of neutrophils: role of intercellular adhesion molecule-1. J Infect Dis 183(11):1638-1645
25. Ross JA, Ansell I, Hjelle JT, Anderson JD, Miller-Hjelle MA, Dobbie JW (1998) Phenotypic mapping of human mesothelial cells. Adv Perit Dial 14:25-30
26. Simsir A, Fetsch P, Mehta D, Zakowski M, Abati A (1999) E-cadherin, N-cadherin, and calretinin in pleural effusions: the good, the bad, the worthless. Diagn Cytopathol 20(3):125-130
27. Liang Y, Sasaki K (2000) Expression of adhesion molecules relevant to leukocyte migration on the microvilli of liver peritoneal mesothelial cells. Anat Rec 258(1):39-46
28. Bellingan GJ, Caldwell H, Howie SE, Dransfield I, Haslett C (1996) In vivo fate of the inflammatory macrophage during the resolution of inflammation: inflammatory macrophages do not die locally, but emigrate to the draining lymph nodes. J Immunol 157(6):2577-2585
29. Bellingan GJ, Xu P, Cooksley H, Cauldwell H, Shock A, Bottoms S, et al (2002) Adhesion molecule-dependent mechanisms regulate the rate of macrophage clearance during the resolution of peritoneal inflammation. J Exp Med 196(11):1515-1521
30. Valle MT, Degl'Innocenti ML, Bertelli R, Facchetti P, Perfumo F, Fenoglio D, et al (1995) Antigen-presenting function of human peritoneum mesothelial cells. Clin Exp Immunol 101(1):172-176
31. Hausmann MJ, Rogachev B, Weiler M, Chaimovitz C, Douvdevani A (2000) Accessory role of human peritoneal mesothelial cells in antigen presentation and T-cell growth. Kidney Int 57(2):476-486
32. Basok A, Shnaider A, Man L, Chaimovitz C, Douvdevani A (2001) CD40 is expressed on human peritoneal mesothelial cells and upregulates the production of interleukin-15 and RANTES. J Am Soc Nephrol 12(4):695-702
33. Mazar J, Agur T, Rogachev B, Ziv NY, Zlotnik M, Chaimovitz C, et al (2005) CD40 ligand (CD154) takes part in regulation of the transition to mononuclear cell dominance during peritonitis. Kidney Int 67(4):1340-1349
34. Lanfrancone L, Boraschi D, Ghiara P, Falini B, Grignani F, Peri G, et al (1992) Human peritoneal mesothelial cells produce many cytokines (granulo-(granulacyte colony-stimulating factor [CSF], granulocyte-monocyte-CSF,

macrophage- CSF, interleukin-1 [IL-1], and IL-6) and are activated and stimulated to grow by IL-1. Blood 80(11):2835-2842
35. Topley N, Jorres A, Luttmann W, Petersen MM, Lang MJ, Thierauch KH, et al (1993) Human peritoneal mesothelial cells synthesize interleukin-6: induction by IL-1 beta and TNF alpha. Kidney Int 43(1):226-233
36. Xing Z, Gauldie J, Cox G, Baumann H, Jordana M, Lei XF, et al (1998) IL-6 is an antiinflammatory cytokine required for controlling local or systemic acute inflammatory responses. J Clin Invest 101(2):311-320
37. Choe N, Tanaka S, Kagan E (1998) Asbestos fibers and interleukin-1 upregulate the formation of reactive nitrogen species in rat pleural mesothelial cells. Am J Respir Cell Mol Biol 19(2):226-236
38. Hott JW, Godbey SW, Antony VB (1994) Mesothelial cell modulation of pleural repair: thrombin stimulated mesothelial cells release prostaglandin E2. Prostaglandins Leukot Essent Fatty Acids 51(5):329-335
39. Mutsaers SE, Bishop JE, McGrouther G, Laurent GJ (1997) Mechanisms of tissue repair: from wound healing to fibrosis. Int J Biochem Cell Biol 29(1): 5-17
40. Warn R, Harvey P, Warn A, Foley-Comer A, Heldin P, Versnel M, et al (2001) HGF/SF induces mesothelial cell migration and proliferation by autocrine and paracrine pathways. Exp Cell Res 267(2):258-266
41. Rennard SI, Jaurand MC, Bignon J, Kawanami O, Ferrans VJ, Davidson J, et al (1984) Role of pleural mesothelial cells in the production of the submesothelial connective tissue matrix of lung. Am Rev Respir Dis 130(2):267-274
42. Saed GM, Zhang W, Chegini N, Holmdahl L, Diamond MP (1999) Alteration of type I and III collagen expression in human peritoneal mesothelial cells in response to hypoxia and transforming growth factor- beta1. Wound Repair Regen 7(6):504-510
43. Perfumo F, Altieri P, Degl'Innocenti ML, Ghiggeri GM, Caridi G, Trivelli A, et al (1996) Effects of peritoneal effluents on mesothelial cells in culture: cell proliferation and extracellular matrix regulation. Nephrol Dial Transplant 11(9):1803-1809
44. Margetts PJ, Kolb M, Galt T, Hoff CM, Shockley TR, Gauldie J (2001) Gene transfer of transforming growth factor-beta1 to the rat peritoneum: effects on membrane function. J Am Soc Nephrol 12(10):2029-2039
45. Ma C, Tarnuzzer RW, Chegini N (1999) Expression of matrix metalloproteinases and tissue inhibitor of matrix metalloproteinases in mesothelial cells and their regulation by transforming growth factor-beta1. Wound Repair Regen 7(6):477-485
46. Yung S, Thomas GJ, Stylianou E, Williams JD, Coles GA, Davies M (1995) Source of peritoneal proteoglycans. Human peritoneal mesothelial cells synthesize and secrete mainly small dermatan sulfate proteoglycans. Am J Pathol 146(2):520-529
47. Howe A, Aplin AE, Alahari SK, Juliano RL (1998) Integrin signaling and cell growth control. Curr Opin Cell Biol 10(2):220-231
48. Morrissey JH, Gregory SA, Mackman N, Edgington TS (1989) Tissue factor regulation and gene organization. Oxf Surv Eukaryot Genes 6:67-84

49. Iakhiaev A, Idell S (2006) Activation and degradation of protein C by primary rabbit pleural mesothelial cells. Lung 184(2):81-88
50. Ivarsson ML, Holmdahl L, Falk P, Molne J, Risberg B (1998) Characterization and fibrinolytic properties of mesothelial cells isolated from peritoneal lavage. Scand J Clin Lab Invest 58(3):195-203
51. Tietze L, Elbrecht A, Schauerte C, Klosterhalfen B, Amo-Takyi B, Gehlen J, et al (1998) Modulation of pro- and antifibrinolytic properties of human peritoneal mesothelial cells by transforming growth factor beta1 (TGF-beta1), tumor necrosis factor alpha (TNF-alpha) and interleukin 1beta (IL- 1beta). Thromb Haemost 79(2):362-370
52. Falk K, Bjorquist P, Stromqvist M, Holmdahl L (2001) Reduction of experimental adhesion formation by inhibition of plasminogen activator inhibitor type 1. Br J Surg 88(2):286-289
53. Yonemura Y, Endou Y, Fujita H, Fushida S, Bandou E, Taniguchi K, et al (2000) Role of MMP-7 in the formation of peritoneal dissemination in gastric cancer. Gastric Cancer 3(2):63-70
54. Cunliffe WJ, Sugarbaker PH (1989) Gastrointestinal malignancy: rationale for adjuvant therapy using early postoperative intraperitoneal chemotherapy. Br J Surg 76(10):1082-1090
55. Sugarbaker PH (1991) A perspective on clinical research strategies in carcinoma of the large bowel. World J Surg 15(5):609-916
56. van den Tol PM, van Rossen EE, van Eijck CH, Bonthuis F, Marquet RL, Jeekel H (1998) Reduction of peritoneal trauma by using nonsurgical gauze leads to less implantation metastasis of spilled tumor cells. Ann Surg 227(2):242-248
57. Tsujimoto H, Hagiwara A, Shimotsuma M, Sakakura C, Osaki K, Sasaki S, et al (1996) Role of milky spots as selective implantation sites for malignant cells in peritoneal dissemination in mice. J Cancer Res Clin Oncol 122(10):590-595
58. van der Wal BC, Hofland LJ, Marquet RL, van Koetsveld PM, van Rossen ME, van Eijck CH (1997) Paracrine interactions between mesothelial and colon-carcinoma cells in a rat model. Int J Cancer 73(6):885-890
59. van Grevenstein WM, Hofland LJ, Jeekel J, van Eijck CH (2006) The expression of adhesion molecules and the influence of inflammatory cytokines on the adhesion of human pancreatic carcinoma cells to mesothelial monolayers. Pancreas 32(4):396-402
60. van Rossen ME, Hofland LJ, van den Tol MP, van Koetsveld PM, Jeekel J, Marquet RL, et al (2001) Effect of inflammatory cytokines and growth factors on tumour cell adhesion to the peritoneum. J Pathol 193(4):530-537
61. Ziprin P, Ridgway PF, Pfistermuller KL, Peck DH, Darzi AW (2003) ICAM-1 mediated tumor-mesothelial cell adhesion is modulated by IL-6 and TNF-alpha: a potential mechanism by which surgical trauma increases peritoneal metastases. Cell Commun Adhes 10(3):141-154
62. Ziprin P, Alkhamesi NA, Ridgway PF, Peck DH, Darzi AW (2004) Tumour-expressed CD43 (sialophorin) mediates tumour-mesothelial cell adhesion. Biol Chem 385(8):755-761

63. Alkhamesi NA, Ziprin P, Pfistermuller K, Peck DH, Darzi AW (2005) ICAM-1 mediated peritoneal carcinomatosis, a target for therapeutic intervention. Clin Exp Metastasis 22(6):449-459
64. Burleson KM, Casey RC, Skubitz KM, Pambuccian SE, Oegema TR, Jr., Skubitz AP (2004) Ovarian carcinoma ascites spheroids adhere to extracellular matrix components and mesothelial cell monolayers. Gynecol Oncol 93(1):170-181
65. Lessan K, Aguiar DJ, Oegema T, Siebenson L, Skubitz AP (1999) CD44 and beta1 integrin mediate ovarian carcinoma cell adhesion to peritoneal mesothelial cells. Am J Pathol 154(5):1525-1537
66. Ahmed N, Riley C, Rice G, Quinn M (2005) Role of integrin receptors for fibronectin, collagen and laminin in the regulation of ovarian carcinoma functions in response to a matrix microenvironment. Clin Exp Metastasis 22(5):391-402
67. Casey RC, Skubitz AP (2000) CD44 and beta1 integrins mediate ovarian carcinoma cell migration toward extracellular matrix proteins. Clin Exp Metastasis 18(1):67-75
68. Takatsuki H, Komatsu S, Sano R, Takada Y, Tsuji T (2004) Adhesion of gastric carcinoma cells to peritoneum mediated by alpha3beta1 integrin (VLA-3). Cancer Res 64(17):6065-6070
69. Kawamura T, Endo Y, Yonemura Y, Nojima N, Fujita H, Fujimura T, et al (2001) Significance of integrin alpha2/beta1 in peritoneal dissemination of a human gastric cancer xenograft model. Int J Oncol 18(4):809-815
70. Zeng C, Toole BP, Kinney SD, Kuo JW, Stamenkovic I (1998) Inhibition of tumor growth in vivo by hyaluronan oligomers. Int J Cancer 77(3):396-401
71. Catterall JB, Gardner MJ, Jones LM, Turner GA (1997) Binding of ovarian cancer cells to immobilized hyaluronic acid. Glycoconj J 14(5):647-649
72. Catterall JB, Jones LM, Turner GA (1999) Membrane protein glycosylation and CD44 content in the adhesion of human ovarian cancer cells to hyaluronan. Clin Exp Metastasis 17(7):583-591
73. Kayastha S, Freedman AN, Piver MS, Mukkamalla J, Romero-Guittierez M, Werness BA (1999) Expression of the hyaluronan receptor, CD44S, in epithelial ovarian cancer is an independent predictor of survival. Clin Cancer Res 5(5):1073-1076
74. Harada N, Mizoi T, Kinouchi M, Hoshi K, Ishii S, Shiiba K, et al (2001) Introduction of antisense CD44S CDNA down-regulates expression of overall CD44 isoforms and inhibits tumor growth and metastasis in highly metastatic colon carcinoma cells. Int J Cancer 91(1):67-75
75. Jones LM, Gardner MJ, Catterall JB, Turner GA (1995) Hyaluronic acid secreted by mesothelial cells: a natural barrier to ovarian cancer cell adhesion. Clin Exp Metastasis 13(5):373-380
76. Breborowicz A, Oreopoulos DG (2005) Is normal saline harmful to the peritoneum? Perit Dial Int 25 Suppl 4:S67-70
77. Yao V, Platell C, Hall JC (2005) Lavage enhances the production of proinflammatory mediators by peritoneal mesothelial cells in an experimental model. Dis Colon Rectum 48(3):560-566

78. Symowicz J, Adley BP, Woo MM, Auersperg N, Hudson LG, Stack MS (2005) Cyclooxygenase-2 functions as a downstream mediator of lysophosphatidic acid to promote aggressive behavior in ovarian carcinoma cells. Cancer Res 65(6):2234-2242
79. Ren J, Xiao YJ, Singh LS, Zhao X, Zhao Z, Feng L, et al (2006) Lysophosphatidic acid is constitutively produced by human peritoneal mesothelial cells and enhances adhesion, migration, and invasion of ovarian cancer cells. Cancer Res 66(6):3006-3014
80. Sako A, Kitayama J, Shida D, Suzuki R, Sakai T, Ohta H, et al (2006) Lysophosphatidic acid (LPA)-induced vascular endothelial growth factor (VEGF) by mesothelial cells and quantification of host-derived VEGF in malignant ascites. J Surg Res 130(1):94-101
81. Mizutani K, Kofuji K, Shirouzu K (2000) The significance of MMP-1 and MMP-2 in peritoneal disseminated metastasis of gastric cancer. Surg Today 30(7):614-621
82. Burleson KM, Hansen LK, Skubitz AP (2004) Ovarian carcinoma spheroids disaggregate on type I collagen and invade live human mesothelial cell monolayers. Clin Exp Metastasis 21(8):685-697
83. Hirashima Y, Kobayashi H, Suzuki M, Tanaka Y, Kanayama N, Terao T (2003) Transforming growth factor-beta1 produced by ovarian cancer cell line HRA stimulates attachment and invasion through an up-regulation of plasminogen activator inhibitor type-1 in human peritoneal mesothelial cells. J Biol Chem 278(29):26793-26802
84. Falk P, Ma C, Chegini N, Holmdahl L (2000) Differential regulation of mesothelial cell fibrinolysis by transforming growth factor beta 1. Scand J Clin Lab Invest 60(6):439-447
85. Taniguchi S (2005) Suppression of cancer phenotypes through a multifunctional actin-binding protein, calponin, that attacks cancer cells and simultaneously protects the host from invasion. Cancer Sci 96(11):738-746
86. Taniguchi S, Takeoka M, Ehara T, Hashimoto S, Shibuki H, Yoshimura N, et al (2001) Structural fragility of blood vessels and peritoneum in calponin h1-deficient mice, resulting in an increase in hematogenous metastasis and peritoneal dissemination of malignant tumor cells. Cancer Res 61(20):7627-7634
87. Hashimoto S, Takeoka M, Taniguchi S (2003) Suppression of peritoneal dissemination through protecting mesothelial cells from retraction by cancer cells. Int J Cancer 107(4):557-563
88. Hayashi K, Saga H, Chimori Y, Kimura K, Yamanaka Y, Sobue K (1998) Differentiated phenotype of smooth muscle cells depends on signaling pathways through insulin-like growth factors and phosphatidylinositol 3-kinase. J Biol Chem 273(44):28860-28867
89. Baker EA, Gaddal SE, Aitken DG, Leaper DJ (2003) Growth factor profiles in intraperitoneal drainage fluid following colorectal surgery: relationship to wound healing and surgery. Wound Repair Regen 11(4):261-267
90. Hofer SO, Shrayer D, Reichner JS, Hoekstra HJ, Wanebo HJ (1998) Wound-induced tumor progression: a probable role in recurrence after tumor resection. Arch Surg 133(4):383-389

91. Whitworth MK, Sheen A, Rosa DD, Duff SE, Ryder D, Burumdayal A, et al (2006) Impact of laparotomy and liver resection on the peritoneal concentrations of fibroblast growth factor 2, vascular endothelial growth factor and hepatocyte growth factor. J Cancer Res Clin Oncol 132(1):41-44
92. Jiang W, Hiscox S, Matsumoto K, Nakamura T (1999) Hepatocyte growth factor/scatter factor, its molecular, cellular and clinical implications in cancer. Crit Rev Oncol Hematol 29(3):209-248
93. Ayhan A, Ertunc D, Tok EC, Ayhan A (2005) Expression of the c-Met in advanced epithelial ovarian cancer and its prognostic significance. Int J Gynecol Cancer 15(4):618-623
94. Kaji M, Yonemura Y, Harada S, Liu X, Terada I, Yamamoto H (1996) Participation of c-met in the progression of human gastric cancers: anti-c-met oligonucleotides inhibit proliferation or invasiveness of gastric cancer cells. Cancer Gene Ther 3(6):393-404
95. Yashiro M, Chung YS, Inoue T, Nishimura S, Matsuoka T, Fujihara T, et al (1996) Hepatocyte growth factor (HGF) produced by peritoneal fibroblasts may affect mesothelial cell morphology and promote peritoneal dissemination. Int J Cancer 67(2):289-293
96. Khan KN, Masuzaki H, Fujishita A, Kitajima M, Hiraki K, Sekine I, et al (2005) Interleukin-6- and tumour necrosis factor alpha-mediated expression of hepatocyte growth factor by stromal cells and its involvement in the growth of endometriosis. Hum Reprod 20(10):2715-2723
97. Weng J, Mohan RR, Li Q, Wilson SE (1997) IL-1 upregulates keratinocyte growth factor and hepatocyte growth factor mRNA and protein production by cultured stromal fibroblast cells: interleukin-1 beta expression in the cornea. Cornea 16(4):465-471
98. Fujiwara H, Kubota T, Amaike H, Inada S, Takashima K, Atsuji K, et al (2005) Suppression of peritoneal implantation of gastric cancer cells by adenovirus vector-mediated NK4 expression. Cancer Gene Ther 12(2):206-216
99. Nevado J, Vallejo S, El-Assar M, Peiro C, Sanchez-Ferrer CF, Rodriguez-Manas L (2006) Changes in the human peritoneal mesothelial cells during aging. Kidney Int 69(2):313-322
100. Mutsaers SE (2004) The mesothelial cell. Int J Biochem Cell Biol 36(1):9-16

Molecular Biology of Peritoneal Carcinomatosis

D Jayne

Introduction

Peritoneal carcinomatosis refers to the complex sequence of events by which tumour cells disseminate from their primary organ of origin to establish independent metastatic deposits on the visceral and parietal peritoneal lining of the abdominal cavity. With few exceptions, once peritoneal dissemination occurs the malignant process is deemed non-curative as it is seldom amenable to surgical resection and current chemotherapeutic regimens are merely palliative. An understanding of the molecular events involved in peritoneal carcinomatosis is therefore of paramount importance if we are to advance therapeutic strategies for this devastating form of cancer progression.

In order to better understand the events involved in peritoneal carcinomatosis it is necessary to break the process down into a series of steps known as the "peritoneal metastatic cascade". Although this subdivision is analytically useful, it is important to realise that each step in the metastatic cascade does not necessarily occur in isolation, but represents a continuous and interdependent process.

Firstly, individual or clumps of tumour cells must break free of the primary tumour mass and gain access to the peritoneal cavity. They are then free to disseminate around the peritoneal cavity, with their ultimate destination being determined by many factors, including gravity, the movement of the abdominal viscera, and the flow of ascitic fluid. The first surface that free tumour cells encounter is the innermost layer of the peritoneum, the mesothelium. The mesothelium forms a cellular monolayer supported by a basement membrane. Adherence of tumour cells to the mesothelium is the second step in the metastatic cascade, which temporarily arrests the tumour cells to their eventual site of metastasis. The third step involves the penetration of the mesothelial monolayer and its basement membrane giving tumour cells access to the submesothelial connective tissue. Invasion of the underlying connective tissue, the fourth step, provides the necessary scaffold for tumour proliferation, and provided tumour-stromal interaction is compatible results in the establishment of a discrete metastatic tumour deposit. The final step involves the induction of angiogenesis to sustain tumour proliferation and enable further metastatic growth.

The aim of this chapter is to sequentially review each step of the peritoneal metastatic cascade and to highlight the molecular mediators that may be involved.

Peritoneal Tumour Dissemination

Dissemination of tumour cells from the primary cancer may occur by one of several mechanisms. Probably the most important mechanism in gastrointestinal cancers is the spontaneous exfoliation of tumour cells from cancers that have invaded through the full thickness of the bowel wall and its investing serosa. This process may be aided by the down-regulation of intercellular adhesion molecules on the tumour cell surface [1].

It is well recognised that viable tumour cells can be isolated from ascitic fluid or by direct contact with the tumour at the time of surgery and their presence has been linked with poor prognosis [2,3]. In a similar manner, perforation of the primary cancer, which may either be spontaneous or occur inadvertently during surgery, increases the rate of local recurrence and reduces survival [4,5]. Alternatively, tumour cells may be inadvertently liberated from transected lymphatics and blood vessels during the course of surgical resection. Whatever the mechanism of spillage, once liberated from their normal tissue constraints, the tumour cells are free to be disseminated around the peritoneal cavity.

Mesothelial Adhesion

Adherence of liberated tumour cells to the mesothelium is the second step in the peritoneal metastatic cascade. Several candidate adhesions molecules have been implicated in this process, including the lymphocyte-homing molecule, CD44, members of the integrin superfamily, the Selectins, and a variety of other leukocyte associated adhesion molecules.

Much of the original work on tumour-mesothelial interactions was based on studies of peritoneal sepsis. Parallels were drawn between the mesothelial cell and the endothelial cell, in that both cell types form monolayers that regulate the passage of leukocytes between serosal cavities. The endothelial adhesion molecules involved in leukocyte trafficking have been well characterised, and a search for related molecules on mesothelium revealed an overlapping, yet distinct, pattern of expression. Mesothelial cells were shown to express adhesion molecules belonging to the Immunoglobulin Superfamily (Intercellular Adhesion Molecule-1 (ICAM-1), Vascular Adhesion Molecule-1 (VCAM-1), Platelet-Endothelial Cell Adhesion Molecule-1 (PECAM-1)) [6], the Selectin Family (Platelet (P) - and Endothelial (E) - Selectin) [7] and the lymphocyte-homing receptor, CD44 [8]. Whilst ICAM-1 and PECAM-1, are constitutively expressed by quiescent mesothelium, VCAM-1 and E-Selectin require mesothelial activation by pro-inflammatory cytokines (Il-1β, TNF-α, IFN-γ) to induce their expression. Meso-

thelial expression of these adhesion molecules has subsequently been shown to play an important part in lymphocyte trafficking during peritoneal inflammation and it is proposed that the same adhesion molecules may be "hijacked" by invading tumour cells. In this way, Alkhamesi et al. have shown that mesothelial ICAM-1 may be involved in tumour-mesothelial adhesion and its downregulation by exogenous heparin may have a beneficial effect [9].

Schlaeppi et al. studied the mechanisms involved in the adhesion of four colorectal cancer cell lines to mesothelial cell monolayers and to various extracellular matrix proteins [10]. All cell lines adhered rapidly to the mesothelial monolayer, but this adhesive event was not inhibited by either anti-integrin antibodies or antibodies against CD44. In contrast, cell adhesion to the extracellular matrix components was completely integrin-dependent, and could be inhibited by anti-β1 integrin antibodies. The authors concluded "initial colorectal tumour cell-mesothelial cell interaction occurs through an integrin-independent mechanism while adhesion to matrix proteins are integrin-dependent events."

Kotanagi et al. studied the growth of colorectal cancer cells in the peritoneal cavity of mice [11]. They established two cancer cell lines from a patient with colon cancer: AKT-CC-K-LM cells from liver metastatic nodules and AKT-CC-K-PC cells from peritoneal nodules. They found that the two cell lines differed in their morphology in vitro, and in their expression of cell surface adhesion molecules. The expression of carcinoembryonic antigen (CEA), E-cadherin and sialyl-Lewis antigens was significantly higher in AKT-CC-K-LM cells. The expression of CD44v6 was significantly higher in AKT-CC-K-PC cells. After injection of AKT-CC-K-LM cells into the spleen or peritoneal cavity of mice, metastatic nodules were observed only in the liver. In contrast, the injection of AKT-CC-K-PC cells into the spleen or peritoneal cavity yielded metastatic nodules only in the peritoneal cavity. Thus, contrary to the findings of Schlaeppi [10], these authors suggested that the adhesion molecule CD44 was involved in tumour-peritoneal adhesion and might account for the site-specific nature of peritoneal tumour metastasis.

Similar evidence for the involvement of CD44 in tumour adhesion to the peritoneum has been found in models of ovarian and gastric cancer models [11,12]. Cannistra et al. studied the expression of adhesion molecules on ovarian cancer cells and their role in tumour-mesothelial adherence [13]. They showed that both ovarian cell lines and fresh ovarian cancer specimens exhibited CD44 expression. Tumour-mesothelial adhesion was partly inhibited by anti-CD44 antibodies. Similar studies, by the same authors, have subsequently identified a role for the β1-integrins in ovarian-mesothelial adhesion. They have demonstrated an additive inhibitory effect when β1-integrin blocking antibodies are combined with anti-CD44 antibodies in tumour-mesothelial adhesion studies. The inhibitory effect of anti-β1 antibody was attributed to the disruption of tumour β1-integrin interactions with its ligand, fibronectin, on the mesothelial cell surface. A similar inhibitory effect could be reproduced with the use of anti-fibronectin blocking antibodies or the peptidomimetic RGD molecules, which competitively block integrin-fibronectin interactions.

Other leukocyte associated adhesion molecules that have been implicated in peritoneal tumour metastasis include the Very Late Antigens (VLA-2 and VLA-3) and the Leukocyte Functioning Antigen (LFA-3). Mayer et al. performed a histological study to examine the expression of leukocyte cell adhesion molecules in gastric cancer [14]. They found that both primary tumours and lymph node metastases expressed LFA-3. Positive LFA-3 expression was associated with a poorer outcome and correlated with vessel invasion, tumour recurrence and decreased survival time.

Mesothelial Invasion

Before invading tumour cells can gain firm adherence to the submesothelial connective tissue, they must penetrate the mesothelial monolayer. Two possible mechanisms exist: either tumour cells invade the intercellular spaces between adjacent mesothelial cells; or they must destroy the mesothelial monolayer.

Akedo et al. observed three patterns of tumour growth when rat ascites hepatoma cells where co-cultured with mesothelial monolayers [15]. Tumour cells either formed "piled-up" nests upon the mesothelial monolayer, exhibited invasive growth between adjacent mesothelial cells, or failed to attach and grew in suspension. The implication was that intercellular invasion was the predominant mechanism for tumour-mesothelial invasion.

However, other researchers have commented on a change in mesothelial morphology that occurs in areas of tumour cell invasion [16,17]. Mesothelial cells take up a characteristic "rounded" morphology with separation of cell-cell contacts to expose the submesothelial basement membrane. Yonemura et al. explored this observation further using a mouse model and the gastric cell line, MKN-45-P [18]. Intraperitoneal inoculation of MKN-45-P resulted in mesothelial contraction and eventual exfoliation. Similar effects could be induced in vivo by intra-peritoneal injection of IL-6, TNF-α and IL-8, and in-vitro by cytokine stimulation of mesothelial monolayers. It was postulated that tumour-derived cytokines were responsible for disruption of the mesothelial barrier, exposing the submesothelial basement membrane, and facilitating tumour adhesion.

The author's research would favour mesothelial destruction to be the predominant mechanism underlying tumour-mesothelial invasion. Using a threedimensional in vitro model of the human peritoneum [19], it was found that colorectal cancer cell lines adhered rapidly to the outer mesothelial monolayer. Whilst the majority of adherent cells showed proliferative growth on the mesothelial surface without invasion, a proportion invaded between adjacent mesothelial cells. Closer inspection revealed that invasion of the mesothelium was frequently accompanied by changes in mesothelial cell morphology in keeping with apoptosis, namely membrane blebbing, cell shrinkage, and nuclear fragmentation (Fig. 1).

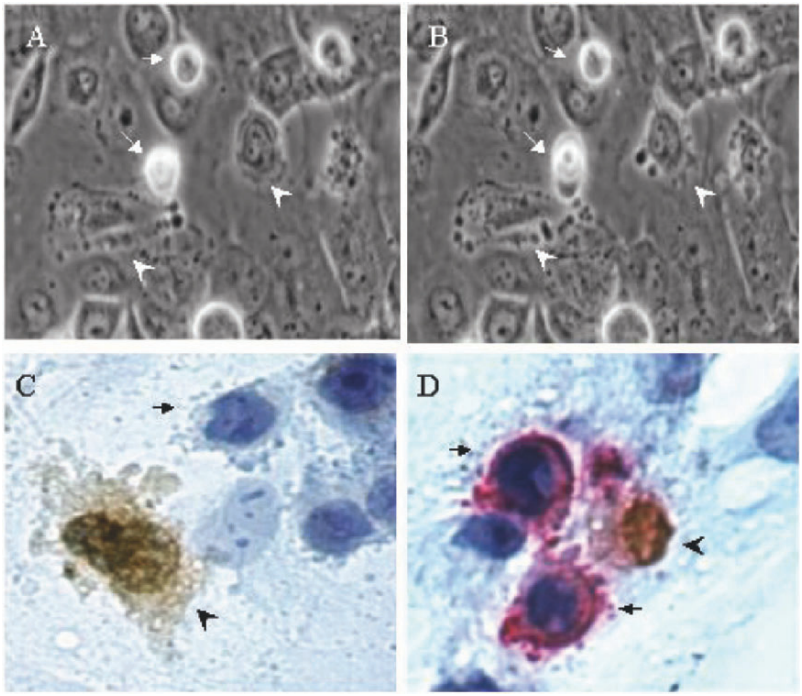


Figure 1. Mesothelial-SW480 co-cultures. A and B: Phase contrast photomicrographs illustrating the cellular changes of apoptosis (membrane blebbing, cell shrinkage, and nuclear fragmentation) observed in mesothelial cells (arrowheads) adjacent to adherent SW480 tumour cells (arrows). Original magnification x200. C and D: Immunocytochemistry of mesothelial-SW480 co-cultures. Mesothelial cells (arrowheads) adjacent to SW480 tumour cells (arrows) show apoptotic changes with characteristic nuclear fragmentation. Original magnification x200. Reproduced with permission from Ann Acad Med Singapore 2003;32:219-225

The presence of mesothelial apoptosis upon co-culture with colorectal cancer cell lines was confirmed by DNA fragmentation assays and immunocytochemistry.

In an attempt to explore the molecular mediators involved in tumour-induced mesothelial apoptosis the potential role of the Fas/FasL apoptotic death signalling was investigated [20]. Human mesothelial cells and SW480 colorectal tumour cells constitutively expressed Fas and FasL mRNA and protein as determined by RT-PCR and confocal fluorescent microscopy. Stimulation of human mesothelial cells with anti-Fas mAb or crosslinked sFasL induced apoptosis, confirming the functional status of the Fas receptor. Pretreatment of SW480 cells with a blocking recombinant anti-FasL monoclonal antibody significantly reduced mesothelial apoptosis (Fig. 2). Thus it would appear that tumour-induced mesothelial apoptosis may, at least in part, be mediated via a Fas-dependent mechanism. These

finding require further investigation in animal models as well investigation of other apoptotic mediators such as TRAIL receptor signalling.

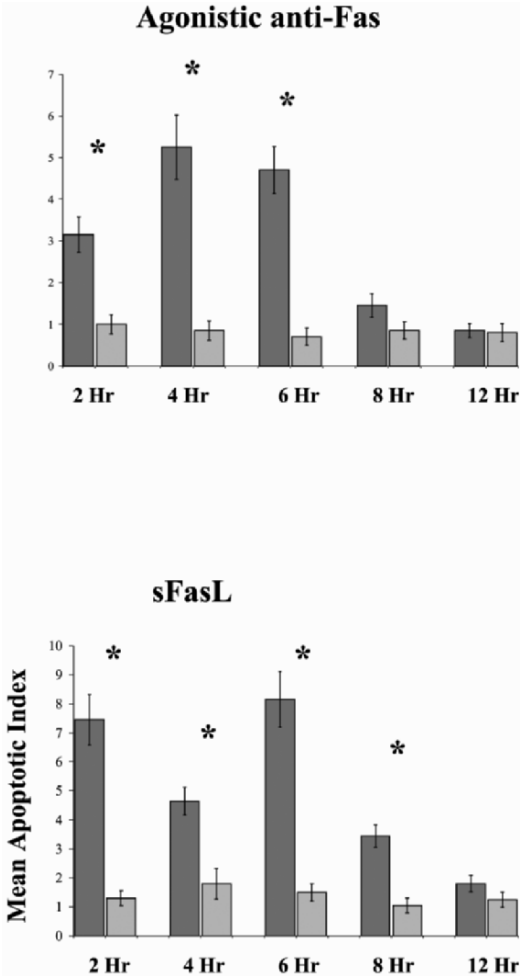


Figure 2. Assessment of the functionality of human mesothelial Fas. Mesothelial monolayers were incubated with an agonistic anti-Fas mAb or stimulating crosslinked sFasL (dark columns). Controls were untreated mesothelial monolayers (light columns). Mesothelial apoptosis was detected using a TUNEL assay. Results are expressed as mean apoptotic index of triplicate experiments ± SD. * $P < 0.05$, Mann-Whitney U Test. Reproduced with permission from Br J Cancer 2004;90(7):1437-1442

Stromal Invasion and Proliferation

Having attached to the peritoneum and penetrated the mesothelial barrier, tumour cells must next gain stable adherence to the submesothelial connective tissue before they can invade and proliferate.

Current evidence suggests that adherence to the submesothelial connective tissue is orchestrated via integrin-ligand interactions. Schlaeppi et al. found that adhesion of colorectal cell lines to extracellular matrix components was completely integrin dependent [10]. These findings are supported by the work of Yonemura et al. [21] who used a gastric cell line, MKN-45, to establish a highly metastatic variant, MKN-45-P, by serial peritoneal passages in a mouse model. The differential expression of various metastasis-related genes (integrin subunits, motility factors, proteases, growth factors) between MKN-45 and MKN-45-P were examined by RT-PCR. Integrin $\alpha 2$ and $\alpha 3$ subunits were significantly elevated in MKN-45-P compared to MKN-45. These α-integrins dimerise with $\beta 1$-subunits to form adhesion molecules for various basement membrane proteins, including fibronectin, laminin, and collagen IV, which are secreted by human mesothelium [71]. Treatment with anti-$\beta 1$-integrin antibodies significantly inhibited the adherence of MKN-45-P in an ex-vivo peritoneal model, suggesting a role for $\beta 1$-mediated integrin adhesion to the submesothelial basement membrane.

Thus it would appear that integrin mediated adherence is involved in stabilisation of invading tumour cells to the submesothelial connective tissue. Activation of tumour integrin receptors would also serve to facilitate tumour proliferation and motility through well established $\beta 1$ integrin-mediated cell signalling pathways.

Further proliferation and survival of the adherent tumour cells requires a compatible interaction between the invading cells and the peritoneal stroma. Although the consequences of tumour-stromal interaction have been much studied in other metastatic systems, this interaction has received little attention with respect to peritoneal metastasis development.

Davies et al. showed that epidermal growth factor (EGF) enhanced the invasive potential of mammary carcinoma cells when injected into the peritoneal cavities of rats [22] and that this growth promoting effect was due to the production of EGF by the peritoneal host tissue. Injection of a murine mammary carcinoma cell line, which was negative for EGF production, resulted in the production of multiple small peritoneal deposits, which could be abolished by simultaneous injection of anti-EGF antibodies. No such effect was seen with subcutaneous tumour growth, suggesting a site-specific requirement for EGF in peritoneal metastases.

Using an in vitro Transwell system, van der Wal et al. found that mesothelial cells inhibited the growth of CC531 colon carcinoma cells, whilst CC531 cells stimulated mesothelial cell growth [23]. Both cell types produced insulin growth factor-1 (IGF-1), and possessed IGF-receptors. In co-culture, IGF-1 potentiates the inhibitory effect of mesothelial cells on CC531 proliferation, whilst enhancing mesothelial proliferation. It was postulated that the inhibitory effects of IGF-1 like molecules might explain why tumour cells grow poorly in a surgically uncompromised abdomen.

The role of chemokines in the development of peritoneal carcinomatosis was studied in a murine model of peritoneal carcinomatosis by Yasumoto et al. [24]. They found that the CXCL12 chemokine enhanced proliferation of the NUGC4 gastric tumour cell line and that specific inhibition of its receptor, CXCR4, effectively reduced tumour growth and ascites formation. CXCR4 expression in primary gastric cancers also significantly correlated with the clinical development of peritoneal disease.

Said et al. have shown the importance of the extracellular glycoprotein SPARC (secreted protein acidic and rich in cysteine) in a murine model of ovarian peritoneal carcinomatosis [25]. Compared to wild-type mice, SPARC-null mice were found to have significantly shorter survival and more extensive nodular peritoneal dissemination when inoculated with a syngeneic ovarian cancer cell line. Immunohistochemical analysis of tumour nodules from SPARC-null mice revealed higher proliferation and lower apoptotic indices.

The author has previously been interested in the potential role of the heparin-binding growth factors (HBGF's) in stimulating peritoneal carcinomatosis. This diverse group of growth factors, which includes vascular endothelial growth factor (VEGF), heparin-binding epidermal growth factor (HB-EGF), basic fibroblast growth factor (bFGF), platelet derived growth factor (PDGF), and interleukin-8 (IL-8), shares the common ability to bind exogenous heparin-derived molecules. This heparin binding capacity enhances growth factor – ligand interaction.

HBGF's are involved in normal wound healing, where their expression is upregulated by the early inflammatory cytokines IL-1β and TNF-α, which are also produced by peritoneal mesothelial cells in response to injury or surgical trauma. In healing wounds HBGF's stimulate fibroblast and epithelial proliferation, and due to their additional angiogenic properties also probably contribute to wound vascularisation. Using in vitro human mesothelial monolayers, it was shown that peritoneal mesothelial cells constitutively expressed bFGF, HB-EGF and two spliced variants of VEGF ($VEGF_{121}$ and $VEGF_{165}$) [26]. Stimulation with exogenous IL-1β and TNF-α upregulated mesothelial production of HB-EGF and VEGF, whereas IL-6 had no detectable effect, and IL-2 suppressed mesothelial HB-EGF and bFGF. Many gastrointestinal cancers are known to express receptors and to be responsive to the HBGF's. In addition to stimulating tumour cell proliferation, HBGF's upregulate tumour expression of the integrin and immunoglobulin superfamily of adhesion molecules. Thus the production of HBGF's by the activated peritoneum may facilitate tumour cell adhesion, proliferation and invasion. The significance of this finding lies in the ability to inhibit the actions of HBGF's with exogenous heparin-like molecules and therefore suppress peritoneal metastasis deveolment [9].

Assuming tumour cells successfully attach to the submesothelial connective tissue and have encountered a favourable host response, it is then necessary for them to invade the extracellular matrix. The matrix metalloproteinases (MMPs) may play a central role in stromal invasion. Yonemura et al. studied the role of MMP-7 in a mouse model of peritoneal carcinomatosis [27]. Specific antisense oligonucleotides inhibited the expression of MMP-7 by the highly metastatic gastric cell line, MKN-45-P, and suppressed invasion without altering cell proliferation. In

addition, survival of MKN-45-P bearing mice, which had been pre-treated with antisense oligonucleotides, was significantly better than that of control mice. Aparicio et al. found that the MMP inhibitor, batimastat, significantly reduced metastasis formation and prolonged survival in a rat model of peritoneal carcinomatosis [28]. However, batimastat treatment was associated with marked peritoneal inflammation and ascites, raising concerns about its potential as an anti-metastatic agent in humans.

Other potential mediators of peritoneal stromal invasion include the urokinase-plasminogen activating (UPA) system. UPA has been widely implicated in many cancer systems. In our laboratory, we have shown that tumour expression of the UPA receptor (UPAR) and its ligand is upregulated in vitro by inflammatory cytokines or postoperative peritoneal drain fluid, suggesting enhanced activation of this system in the early postoperative period. Pre-treatment of colorectal tumour cells with anti-UPAR antibody significantly reduced invasion in a Matrigel invasion assay (Fig. 3).

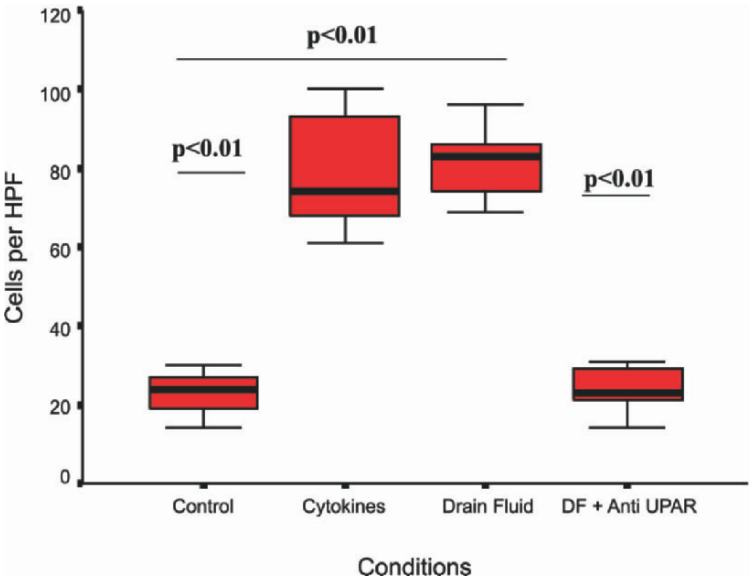


Figure 3. Incubation of HT29 colon cancer cell lines with physiological doses of TNF-α or 20% v/v postoperative drain fluid significantly increased cellular invasion in a Matrigel invasion assay. Pre-treatment of HT-29 cells with anti-UPAR antibody inhibited drain fluid induced cellular invasion. Control experiments used HT-29 cells with neither cytokine, drain fluid, nor anti-UPAR treatment

Furthermore, the urokinase receptor is known to interact with $\alpha_v\beta_5$ integrin, a receptor for the extracellular matrix protein vitronectin, leading to enhanced tumour cell migration and invasion [29]. The protease inhibitor Bikunin (bik) inhibits tumour cell invasion and metastasis through suppression of UPA mRNA

expression. Tansfection of the human ovarian carcinoma cell line HRA with a vector harbouring a cDNA encoding for bik resulted in reduced invasion, but not proliferation, adhesion, or migration relative to the parenteral cells [30]. Inoculation of bik (+) clones into animal models resulted in reduced peritoneal dissemination and long term survival.

Tumour-Peritoneal Angiogenesis

As the peritoneal metastatic deposit grows it needs to develop a blood supply to meet its increasing metabolic demands. The deeper layer of the peritoneum contains a rich capillary network and is ideally suited to this function. Surprisingly little is known about the mediators of peritoneal angiogenesis. The potential role of HBGF's in tumour-stromal interactions has been described and it should be recognised that many of these growth factors, which include VEGF and IL-8, also possess angiogenic properties. Fan et al. showed that the angiogenesis inhibitor, TNP-470, reduced growth and dissemination of a colorectal cancer cell line in a nude mouse model compared to sham treated animals [31]. Furthermore, the mean survival time was significantly longer in the TNP-470 treated group.

Suganuma et al. investigated the role of the renin-angiotensin system in peritoneal carcinomatosis from ovarian cancer [32]. The angiotensin II type I receptor (AT1R) was highly expressed in malignant ovarian adenocarcinomas and its expression showed a positive correlation with VEGF and microvessel density. In a mouse model of ovarian peritoneal carcinomatosis, the administration of the AT1R blocker, candesartan, resulted in the reduction of peritoneal dissemination, decreased ascitic VEGF concentration, and suppression of tumour angiogenesis. Stoeltzing et al. used a human colon cancer cell line transfected with a vector containing angiopoietin-1 in a mice model of peritoneal carcinomatosis [33]. Thirty days following tumour cell inoculation, a significant reduction in the number of peritoneal metastases, tumour volume, vessel counts, and tumour cell proliferation were observed in the animals inoculated with angiopoietin-1 over-expressing tumours as compared to control animals. Other animal studies utilising adenoviral vector mediated anti-angiogenic therapy have shown similar results with down-regulation of ascites formation, tumour growth, vascularity, and prolonged animal survival, underlining the importance of angiogenesis in the peritoneal metastatic cascade [34].

Summary

Peritoneal carcinomatosis can be thought of as a sequence of events that together form a peritoneal metastatic cascade. Presently our understanding of the molecular mediators that orchestrate this cascade is ill-understood. Initial tumour-mesothelial interaction appears to involve several adhesion molecules, including CD44, the

Selectins, and various leukocyte associated antigens. The exact molecules involved are probably determined by the nature of the metastatic tumour cell. Invasion of the mesothelial monolayer appears to occur by tumour-induced mesothelial apoptosis, at least in part via the Fas/FasL system, although invasion between intercellular spaces may also play a role. Adhesion to the submesothelial connective tissue is mediated by tumour integrin binding. The peritoneal stromal tissue appears to be a favourable host for tumour proliferation, providing a rich source of growth factors and chemokines known to be involved in tumour metastasis. Angiogenesis is vital to peritoneal tumour growth and although the peritoneum has a well developed blood supply the angiogenic events specific to peritoneal tumour metastasis remain to be elucidated. Further investigation is required to unravel the complexities of the peritoneal metastatic cascade and this will inevitably open up many avenues for novel therapeutic manipulation and disease modulation.

References

1. Yonemura Y, Nojima N, Kaji M et al (1995) E-cadherin and urokinase-type plasminogen activator tissue status in gastric carcinoma. Cancer 76:941-953
2. Wisbeck WM, Becher EM, Russel AH (1986) Adenocarcinoma of the stomach: autopsy observations with therapeutic implications for the radiation oncologist. Radiother Oncol 7:13-18
3. Sperti C, Pasquali C, Piccoli A et al (1997) Recurrence after resection for ductal adenocarcinoma of the pancreas. World J Surg 21:195-200
4. Ranbarger KR, Johnston WD, Chang JC (1982) Prognostic significance of surgical perforation of the rectum during abdominoperineal resection for rectal cancer. Am J Surg 143:186-188
5. Slanetz CAJR (1984) The effect of inadvertant intraoperative perforation on survival and recurrence in colorectal cancer. Dis Colon Rectum 27:792-797
6. Jonjic N, Peri G, Bernasconi S et al (1992) Expression of adhesion molecules and chemotactic cytokines in cultured human mesothelial cells. J Exp Med 176:1165-1174
7. Klein CL, Bittinger F, Skarke CC et al (1995) Effects of cytokines on the expression of cell adhesion molecules by cultured human omental mesothelial cells. Pathobiology 63:204-212
8. Muller J, Yoshida T (1995) Interaction of murine peritoneal leukocytes and mesothelial cells: in vitro model system to survey cellular events on serosal membranes during inflammation. Clin Immunol Immunopathol 75:231-238
9. Alkhamesi NA, Ziprin P, Pfistermuller K et al (2005) ICAM-1 mediated peritoneal carcinomatosis, a target for therapeutic intervention. Clin Exper Metastasis 22(6):449-459
10. Schlaeppi M, Ruegg C, Tran-Thang C et al (1997) Role of integrins and evidence for two distinct mechanisms mediating human colorectal carcinoma cell interaction with peritoneal mesothelial cells and extracellular matrix. Cell Adhes Commun 4:439-455

11. Kotanagi H, Saito Y, Yoshioka T et al (1998) Characteristics of two cancer cell lines derived from metastatic foci in liver and peritoneum of a patient with colon cancer. J Gastroenterol 33:842-849
12. Nishimori H, Yasoshima T, Denno R et al (2000) A novel experimental mouse model of peritoneal dissemination of human gastric cancer cells: different mechanisms in peritoneal dissemination and hematogenous metastasis. Jpn J Cancer Res 91:715-722
13. Cannistra SA, Kansas GS, Niloff J et al (1993) Binding of ovarian cancer cells to peritoneal mesothelium in vitro is partly mediated by CD44H. Cancer Res 53:3830-3838
14. Mayer B, Lorenz C, Babic R et al (1995) Expression of leukocyte cell adhesion molecules on gastric carcinomas: possible involvement of LFA-3 expression in the development of distant metastases. Int J Cancer 64:415-423
15. Akedo H, Shinkai K, Mukai M et al (1986) Interaction of rat ascites hepatoma cells with cultured mesothelial cell layers: a model for tumour invasion. Cancer Res 46:2416-2422
16. Kiyasu Y, Kaneshima S, Koga S (1981) Morphogenesis of peritoneal metastasis in human gastric cancer. Cancer Res 41:1236-1239
17. Kimura A, Koga S, Kudoh H et al (1985) Peritoneal mesothelial cell injury factors in rat cancerous ascites. Cancer Res 45:4330-4333
18. Yonemura Y, Endou Y, Nojima N et al (1997) A possible role of cytokines in the formation of peritoneal dissemination. Int J Oncol 11:349-358
19. Jayne DG, O'Leary R, Gill A et al (1999) A three-dimensional in-vitro model for the study of peritoneal tumour metastasis. Clin Exper Metastasis 17(6):515-523
20. Heath RM, Jayne DG, O'Leary R et al (2004) Tumour-induced apoptosis in human mesothelial cells: a mechanism of peritoneal invasion by Fas Ligand/Fas interaction. Br J Cancer 90(7):1437-1442
21. Yonemura Y, Endou Y, Yamaguchi T et al (1996) Roles of VLA-2 and VLA-3 on the formation of peritoneal dissemination in gastric cancer. Int J Oncol 8:925-931
22. Davies DE, Farmer S, White J et al (1994) Contribution of host-derived growth factors to in vivo growth of a transplantble murine mammary carcinoma. Br J cancer 70:263-269
23. van der Wal BCH, Hofland LJ, Marquet RL et al (1997) Paracrine interactions between mesothelial and colon-carcinoma cells in a rat model. Int J Cancer 73:885-890
24. Yasumoto K, Koizumi K, Kawashima A et al (2006) Role of the CXCL12/CXCR4 axis in peritoneal carcinomatosis of gastric cancer. Cancer Res 66(4):2181-2187
25. Said N, Motamed K (2005) Absence of host-secreted protein acidic and rich in cysteine (SPARC) augments peritoneal ovarian carcinomatosis. Am J Pathol 167(6):1739-1752
26. Jayne DG, Perry SL, Morrison E et al (2000) Activated mesothelial cells produce heparin-binding growth factors: implications for tumour metastases. Br J Cancer 82(6):1233-1238

27. Yonemura Y, Endo Y, Fujita H, Kimura K, Sugiyama K, Momiyama N (2001) Inhibition of peritoneal dissemination in human gastric cancer by MMP-7-specific antisense oligonucleotide. J Exp Clin Cancer Res 20:205-212
28. Aparicio T, Kermorgant S, Dessirier V, Lewin MJ, Lehy T (1999) Matrix metalloproteinase inhibition prevents colon cancer peritoneal carcinomatosis development and prolongs survival. Carcinogenesis 20:1445-1451
29. Silvestri I, Longanesi C, I, Franco P et al (2002) Engaged urokinase receptors enhance tumor breast cell migration and invasion by upregulating alpha(v)beta5 vitronectin receptor cell surface expression. Int J Cancer 102(6):562-571
30. Suzuki M, Kobayashi H, Tanaka Y et al (2003) Suppression of invasion and peritoneal carcinomatosis of ovarian cancer cell line by overexpression of bikunin. Int J Cancer 104(3):289-302
31. Fan YF, Huang ZH (2002) Angiogenesis inhibitor TNP-470 suppresses growth of peritoneal disseminating foci of human colon cancer line Lovo. World J Gastroenterol 8(5):853-856
32. Suganuma T, Ino K, Shibata K et al (2005) Functional expression of the angiotensin II type 1 receptor in human ovarian carcinoma cells and its blockade therapy resulting in suppression of tumor invasion, angiogenesis, and peritoneal dissemination. Clin Cancer Res 11(7):2686-2694
33. Stoeltzing O, Ahmad SA, Liu W et al (2002) Angiopoietin-1 inhibits tumour growth and ascites formation in a murine model of peritoneal carcinomatosis. Br J Cancer 87(10):1182-1187
34. Hampl M, Tanaka T, Albert PS et al (2001) Therapeutic effects of viral vector-mediated antiangiogenic gene transfer in malignant ascites. Hum Gene Ther 12(14):1713-1729

Role of Adhesion Molecules in Locoregional Cancer Spread

ME Bracke

The Micro-ecosystem of Peritoneal Carcinomatosis

Peritoneal carcinomatosis (PC) is the result of a molecular crosstalk between cancer cells and host elements. Only when this dialogue can be established successfully, a micro-ecosystem is created which is favourable for the development of a new secondary peritoneal tumour [1]. In fact, PC is a confirmation of the old "seed and soil" hypothesis by Paget, who launched that the metastatic capability of tumour cells (the "seeds") is dependent on the finding of a suitable implantation environment (the "soil"). As a result, metastasis formation is a relatively inefficient process, because it requires the constellation of a number of cancer and host cell activities. Invasion at the implantation site is probably the first and the most crucial activity, but subsequent ectopic survival ("escape from anoikis") and cell proliferation are important as well. Invasion is regulated by promoter and suppressor gene expression [2]. Invasion promoter gene products have been identified on the one hand, and include cell-matrix adhesion molecules (*e.g.* integrins), extracellular proteinases (*e.g.* matrix metalloproteinases and plasminogen activators) and directional migration actors (*e.g.* cytoplasmic microtubles, F-actin and semaphorins). Invasion suppressor molecules, on the other hand, were found among proteinase inhibitors (*e.g.* tissue inhibitors of metalloproteinases and plasminogen activator inhibitors) and cell-cell adhesion molecules (*e.g.* E-cadherin, a calcium-dependent adhesion molecule). Invasion occurs as the result of a disturbed equilibrium between the activities of the invasion promoters and suppressors. This is for instance the case when an increased production of matrix metalloproteinases (MMP's) by the cancer cells outbalances the production of tissue inhibitors of metalloproteinases (TIMP's) by the host fibroblasts, or when E-cadherin in the cancer cells is downregulated by stromal factors. Clearly, the micro-ecosystem concept in PC is complex, because it takes into account the contributions of both cancer cells and host cells, and because it considers the different cell activities within the context of a balance. Complexity is still added to this by the fact that invasion is not the monopoly of the cancer cells: macrophages, polymorphonuclear leukocytes (PMN's), lymphocytes, myofibroblasts and endothelial cells migrate and occupy

their surroundings during the immunological and angiogenic host responses to the presence of cancer cells [3]. These host cells can become intermingled with the cancer cells, and some immunologists have formulated a "countercurrent" hypothesis for invasion [4], while pathologists paraphrased the phenomenon by questioning "who invades who?". A final element to consider, when adhesion within the PC micro-ecosystem is studied, concerns its dual role in invasion. Adhesion of cancer cells to neighbouring cancer cells ("homotypic" cell-cell interactions) or to the basement membrane (BM) of the peritoneum can function as an anchor that restricts further cancer cell motility and hence invasion [5]. We [6] and others [7] have for instance described "stop signal" molecules in the BM, for which cancer cells may or may not be perceptive. Other adhesions, however, such as to the apical side of the mesothelial cells ("heterotypic" cell-cell interactions) or to the extracellular matrix (ECM) components of the interstitial stroma (like collagen fibers), are conceived as grips for moving cells to promote invasion.

It is useful to dissect the different compartments of the PC micro-ecosystem, and define the resident players with their adhesive contributions (Fig. 1).

1. The peritoneal cavity. Here cancer cells, either isolated or as aggregates, become detached from existing tumour deposits, and are transported passively with the peritoneal fluid. This fluid also contains macrophages, and a few PMN's, natural killer (NK) cells and lymphocytes, which all secrete cytokines, chemokines and growth factors.
2. The mesothelium: these epithelioid cells show homotypic and heterotypic cell-cell adhesions. They cover a BM, and prevent in this way direct cell-matrix adhesion by the cancer cells. In some physiological (at the so-called "milky spots") [8] and pathological (apoptosis and retraction of the mesothelial cells induced by contact with cancer cells) circumstances the BM is denuded, and offers a favourable soil for PC. The apical side of the mesothelium is covered with a layer of hyaluronate, a glycosaminoglycan presenting a high net negative charge to the cells from the peritoneal cavity, and making adhesion and implantation as such unfavourable [9]. In PC, however, the fibrinolytic cascade can be interrupted, which leads to the formation of fibrin deposits on the mesothelial cells, and this phenomenon is thought to promote adhesion of cancer cells [10].
3. The stroma: The interstitial matrix of the stroma contains collagen fibres, mainly of type I, fibronectin and proteoglycans. Like the peritoneal fluid, this matrix is a source of cytokines, chemokines and growth factors secreted by resident fibroblasts, myofibroblasts and adipocytes. The difference with the fluid is that these factors are mainly attached to the structural matrix molecules, and hence transiently inactive: their activation requires enzymatic proteinase or glycanase activity.
4. The vessels. The peritoneal stroma contains lymph vessels (some enlarged regions are called "lacunae") and blood vessels. Their lining endothelium can become activated during PC formation, and promote adhesion of blood cells: monocytes, PMN's, lymphocytes, platelets and probably circulating stem cells. These blood cells then become part of the micro-ecosystem, and

pass the endothelial BM. As a result of tumour-induced angiogenesis, both the lymph and the blood vessel compartments are enlarged as compared to normal peritoneum, and the contribution of each compartment depends on the type of vascular endothelial growth factor (VEGF) available [11]. The enlargement of the vascular bed in PC should theoretically also facilitate lymphogenic and angiogenic metastasis formation.

After this schematic description of the scenery and the players in the PC micro-ecosystem, the next chapters will describe the major classes of adhesion molecules involved.

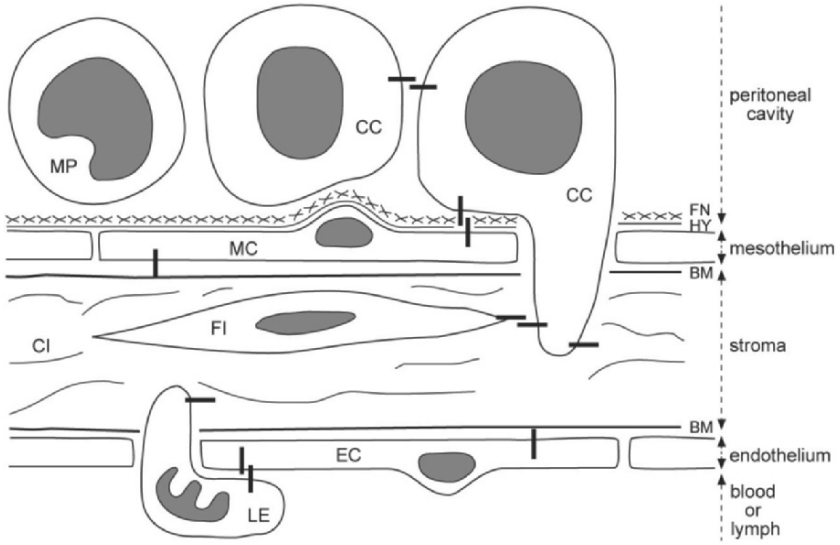


Figure 1. Schematic representation of the micro-ecosystem of peritoneal metastases. From top to bottom four compartments can be distinguished: (1) the peritoneal cavity, containing fluid and cancer cells (*CC*) and immune cells (such as macrophages, MP), (2) the mesothelium formed by mesothelial cells (*MC*), and lined at the apical side by a coat of hyaluronate (*HY*) and possibly fibrin (*FN*), and at the basal side by a basement membrane (*BM*), (3) the stroma, containing the interstitial extracellular matrix with type I collagen fibres (*CI*) and stromal cells such as (myo)fibroblasts (*FI*), and (4) the blood and lymph vessels, lined by a basement membrane (*BM*) and endothelial cells (*EC*). From the blood, leukocytes (*LE*) can enter the micro-ecosystem

Homotypic Cell-Cell Adhesion by the Cadherins

Cadherins are transmembrane glycoproteins with an extracellular part, a membrane-spanning domain and a cytoplasmic tail. They form a family with currently

about 80 members, but information related to PC is now restricted to the subfamily of "classical" (or type I) cadherins. As shown schematically in Fig 2, the extracellular part of these cadherins is built up by five similar domains, the most distant one (EC1) containing a histidine-alanine-valine (HAV) sequence. The HAV sequence is involved in the homophilic recognition of another classical cadherin molecule [12], and the flanking sequences restrict the interaction to cadherins of the same type: epithelial (E-), neural (N-) or placental (P-) cadherin [13]. The cytoplasmic tail of the classical cadherins is decorated by catenin molecules: p120, α-, β- and γ-catenin (plakoglobin). This cadherin/catenin complex is connected with the actin cytoskeleton in a non-covalent way, and allows in- and outward signal transduction.

Experimental and clinical evidence has now accumulated to consider the E-cadherin/catenin complex as a potent invasion suppressor. In epithelioid tumors the expression or the function of E-cadherin is downregulated, and this has also been confirmed for colorectal [14], gastric [15] and ovarian [16] cancers with PC. This downregulation can have etiologies at numerous levels [17]. Germ-line and somatic mutations in the E-cadherin gene have been described, but are relatively uncommon, and the somatic mutations are mainly found in lobular breast carcinoma and in undifferentiated gastric cancers. Mutations in the α- and β-catenin genes were detected in invasive prostate, lung and colon carcinomas: they lead to reduced cell-cell adhesion and invasiveness as well. More frequently the downregulation is at the level of the E-cadherin promoter, and is regulated by negative transcription factors (Slug, Snail, SIP-1). Posttranslational functional downregulation of the E-cadherin function can also be the result of tyrosine phosphorylation of β-catenin, endocytosis of the E-cadherin/catenin complex or enzymatic cleavage of the extracellular part of E-cadherin. Importantly, in many cancer cells the downregulation of E-cadherin is reversible, which opens avenues for future treatments [5]. In experimental conditions in vitro some compounds have been found to restore the adhesive function of the E-cadherin/catenin complex, and to inhibit tumour cell invasion: insulin-like growth factor I, insulin [18], retinoic acid (vitamin A analogue), tamoxifen (selective estrogen receptor modulator) [19], tangeretin (methoxyflavone derived from citrus peel oil) and xanthohumol (prenylated chalcone from hops) [20]. The reversibility of the E-cadherin downregulation in invasive tumours sometimes explains its transient nature: in both distant and locoregional metastasis (such as in PC) re-expression of E-cadherin is often noted, and a number of reports indicate that the immunosignal for E-cadherin in histological sections is higher in distant metastases and in secondary peritoneal tumor deposits than in the primary tumour [21]. For the development of PC, E-cadherin is important for the detachment of cancer cells from the primary tumor into the peritoneal fluid, and for the possible aggregate formation in this fluid. It is unlikely that the molecule is involved in the cancer cell adhesion to the mesothelium, since no convincing reports on E-cadherin expression in mesothelial cells are available up to now.

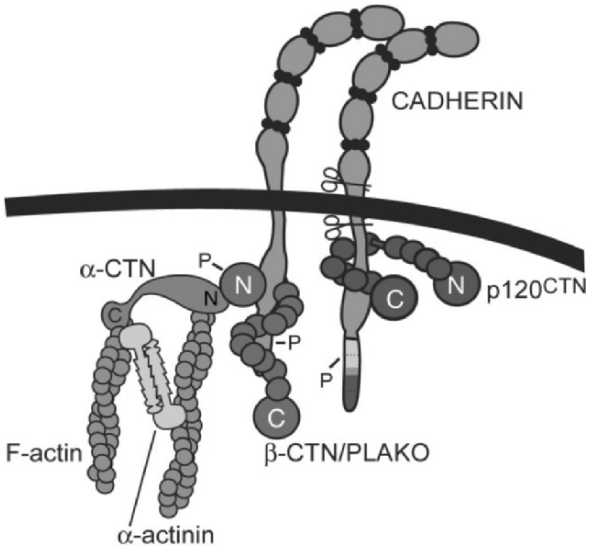


Figure 2. Schematic representation of the cadherin/catenin complex. The classical (or type I) cadherins are transmembrane glycoproteins that show homophilic doublet interactions with a neighbouring molecule, and homophilic intractions with molecules from a neighbouring cell (not shown). The extracellular part contains five protomers (or extracellular domains, EC), while the cytoplasmic tail interacts directly with p120-, α-, β- and γ-catenin (CTN). Plakoglobin (*PLAKO*) is a synonym of γ-catenin. α-Catenin and α-actinin link the complex to the F-actin microfilament cytoskeleton. *N* and *C* indicate amino- and carboxy terminal ends respectively

When during tumor progression cells become invasive, they use to undergo an epithelial to mesenchyme transition (EMT). This transition is not only associated with downregulation of E-cadherin, but also often with upregulation of N-cadherin. The latter cadherin is only a poor adhesion molecule, but rather activates cancer cell motility and angiogenesis [22]. The extracellular part ("ectodomain") of N-cadherin can be cleaved off by proteinases (*e.g.* ADAM10), and assessed in biological fluids including blood with an ELISA. In contrast to E-cadherin, upregulation of N-cadherin is expected to promote shedding of cancer cells into the peritoneal fluid, but no homophilic counterpart is present on the mesothelial cells. In the stromal and the vessel compartment of the PC micro-ecosystem, however, N-cadherin presenting cells are present, such as myofibroblasts and endothelial cells. Some authors believe that these cells offer a grip to invading cancer cells

[23]. The recent observation that the soluble ectodomain can act as a potent angiogenesis inducer, may be relevant in PC development.

The only classical cadherin expressed by mesothelial cells is P-cadherin, so this molecule could serve homophilic heterotypic adhesion with cancer cells from the peritoneal fluid. This phenomenon may be of particular interest for the laproscopic surgeon, since it was reported that P-cadherin in the mesothelium was upregulated as a result of CO_2 insufflation [24].

Apart from the mesothelium, only few normal tissues express P-cadherin: basal keratinocytes and hair follicles of the skin, and myo-epithelial cells of the mammary glands. The effect of overexpression in cancers on their invasiveness depends on the cellular context: in human MCF-7 breast carcinoma cells induction of P-cadherin has a clear pro-invasive effect [26], while in human BLM melanoma cells, P-cadherin transfection blocks invasion in a reconstructed skin model in organ culture [27]. Heterotypic cell-cell adhesion is not a frequent phenomenon with the classical cadherins: one example is the E-cadherin interaction between melanocytes and skin keratinocytes. So, the role of P-cadherin in PC cancer-mesothelium interactions deserves now full attention.

Heterotypic Cell-Cell and Cell-Matrix Adhesion by the Integrins

Integrins share functional similarities with the cadherins: as calcium/magnesium-dependent transmembrane glycoproteins, they serve adhesion and allow inside-out/-outside-in signal transduction to the actin cytoskeleton via a complex of cytoplasmic proteins (Fig. 3). The main difference with the cadherins is the type of external ligand molecules they are receptive for and adhesive to. Heterophilic interactions with the structural components of the ECM, such as laminin, fibronectin, vitronectin, tenascin and a number of collagen types, are the best known functions of the integrins. Heterophilic heterotypic interactions with other cells, however, are also possible, and particularly apply to cell adhesion to mesothelial and endothelial cells. Integrins are heterodimers consisting of an α- and a β-subunit, and bind to their ligands along the low affinity/high avidity principle. This means that the strength of each molecular intraction is low, while the number of interactions (integrins) per cell is high, and a comparison with Velcro is often made. This principle allows the cell to rapidly activate its interactions for instance by clustering the integrins to form focal contacts or by inducing an activated integrin conformation. This plasticity requires signalling, which is dependent on the interaction of the cytoplasmic integrin tail with a complex of proteins, such as talin, α-actinin, filamin, paxillin, and recruitment of focal adhesion kinase and Src.

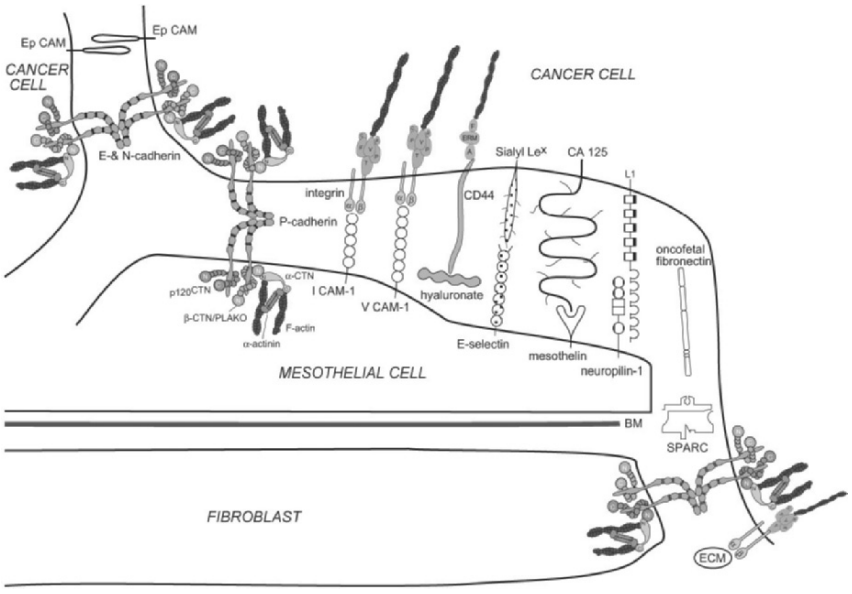


Figure 3. Schematic representation of adhesion molecules involved in the pathogenesis of peritoneal carcinomatosis. The figure shows a detail of the peritoneal cavity (up), the mesothelium and the stroma (bottom). Cell-cell adhesion can be homotypic between the cancer cells via E- and N-cadherin, and epithelial cell adhesion molecule (*EpCAM*), or heterotypic between cancer cells and mesothelial cells via P-cadherin, integrins, intercellular adhesion molecule 1 (*ICAM-1*), vascular cell adhesion molecule 1 (*VCAM-1*), CD44 (the hyaluronate receptor), sialyl Lewis$^{x \text{ or } a}$, E-selectin, CA 125, mesothelin, L1 and neuropilin-1, or heterotypic between cancer cells and (myo)fibroblasts via N-cadherin. Adhesion with the basement membrane (*BM*) and interstitial extracellular matrix (*ECM*) involves integrins and matrix components such as laminin, nidogen, (oncofetal) fibronectin, several collagen types, proteoglycans, tenascin C and the so-called secreted protein, acidic and rich in cystein (*SPARC*)

Integrins appear to be important for the formation of PC at many levels. First, the interaction of cancer cells, macrophages, NK cells, PMN's and lymphocytes from the peritoneal cacity on the one hand with mesothelial cells on the other is dependent on the expression of two immunoglobulin-like adhesion molecules on the mesothelium. Intercellular adhesion molecule 1 (ICAM-1, CD56) on the mesothelial cells interacts with $\alpha_L\beta_2$ (LFA-1α, CD11a) and $\alpha_M\beta_2$ (CD11b) on the tumor or immune cells [27-29]. Interestingly, the ICAM-1 expression is upregulated by tumor necrosis factor α (TNFα) and interleukin 1β, and downregulated by heparin, which is reflected in the strength of the heterotypic cell-cell adhesive forces [30]. Vascular cell adhesion molecule 1 (VCAM-1, CD106) on the mesothelial cells binds to $\alpha_4\beta_1$ (VLA-4) or $\alpha_4\beta_7$ on the tumor or immune cells. Again

the expression of VCAM-1 is sensitive to upregulation by TNFα and IL-1β, so mesothelial adhesion can be facilitated by local inflammation.

Second, migration into the ECM of the stroma of the peritoneum is integrin-mediated. Peritoneal cancer cells were indeed found to express the following integrins: $\alpha_2\beta_1$ (laminin and collagen receptor), $\alpha_3\beta_1$ (laminin, fibronectin and collagen receptor), $\alpha_v\beta_5$ (fibronectin, vitronectin and fibrinogen receptor) and $\alpha_6\beta_4$ (hemidesmosome receptor not connected to actin microfilaments but to intermediate filaments) and the 67 kD laminin receptor (strictly spoken not belonging to the integrin family) [32-34].

Third, integrins are implicated in the extravasation of leukocytes. After the process of slowing down the passage of leukocytes over activated endothelium sites ("rolling") of the peritoneal vessels, integrins are crucial for stopping the leukocyte at the extravasation site, shape change and its subsequent migration through the vessel wall [35]. Stopping is the result of the interaction of integrins on the leukocytes (β_2 integrins, $\alpha_4\beta_1$ orVLA-4 and $\alpha_4\beta_7$) and immunoglobulin-like adhesion molecules (ICAM-1 and VCAM-1). Then, aggregation and shape changing involve the integrins $\alpha_L\beta_2$ (LFA-1α, CD11a) and $\alpha_M\beta_2$ (CD11b) on the leukocytes, interacting with P-selectin on the endothelial cells. Next, for migration the interaction between β_2 integrins on the leukocytes and immunoglobulin-like adhesion molecules on the endothelium is required (ICAM-1, VCAM-1 and platelet/endothelial adhesion molecule 1 (PECAM-1, CD31)).

Although the cadherin and integrin adhesion systems have been presented here separately for didactic reasons, this should not suggest that they act independently. Both systems interact with each other by their connections with the actin cytotoskeleton, and some components from their cytoplasmic complexes, such as α-actinin, are shared and can be interchanged.

Other Adhesion Compounds relevant for Peritoneal Carcinomatosis

Apart from cadherins and integrins some other adhesion compounds have been described on the peritoneal mesothelium.

CD44 and Hyaluronate

CD44 is a transmembrane glycoprotein with a postulated role in matrix adhesion, lymphocyte activation and lymph node homing. The molecule is expressed on many normal and tumoral cell types in an inactive form, and activation is required for its biological activities [36]. It is expressed as a family of molecular isoforms generated from alternative RNA splicing and posttranslational modifications. Certain CD44 isoforms that regulate activation and migration of lymphocytes and

macrophages may also enhance local growth and metastatic spread of tumor cells [37]. One ligand of CD44 is the glycosaminoglycan hyaluronic acid (hyaluronate) [38], binding of which to the extracellular part of CD44 enhances cellular aggregation and tumour cell growth. CD44v6 and v7 splice variants are expressed by some gastro-intestinal cancers, and are markers for metastatic capability [39]. The v6 variant was in this context indeed shown to mediate cancer cell adhesion to peritoneal mesothelium. Furthermore, adhesion of cancer cells to the hyaluronate coat of peritoneal mesothelial cells appears not to be affected by RNA splicing, but rather depends on intact glycosylation [40]. Relevant for the pathogenesis of PC in gastric carcinoma may also be the observation that transforming growth factor β (TGF-β) from the intratumoral fibroblasts upregulates CD44 expression and increases cancer cell adhesion from the peritoneal cavity to the mesothelium [41]. It is another striking example of the PC micro-ecosystem. Expression of CD44 by peritoneal mesothelial cells [42] also seems to contibute to heterotypic cell adhesion by pancreatic cancer cells in vitro, and upregulation by TNFα and IL-1β was possible, indicating again that mesothelial adhesion can be facilitated by local inflammation.

Sialyl Lewis and E-selectin

Lewis^a and sialyl Lewis^a are carbohydrate structures present on cancer cells, mainly of gastro-intestinal origin, and their detection is an indicator of metastatic potential [43-45]. The monoclonal antibody CA 19-9 is a useful and popular tool to assess circulating sialyl Lewis^a epitopes in the blood of cancer patients. A similar structure coined sialyl Lewis^x is present on leukocytes, and was shown to interact with P-cadherin expressed by activated vascular endothelia. The high avidity/low affinity interaction is considered as the initial force implicated in the "rolling" of extravasating leukocytes. Recently it was found that sialyl Lewis^a on cancer cells serves a similar interaction with E-selectin on the endothelial cells. In fact this rolling phenomenon induced by sialyl Lewis^a and E-selectin is the initial step that can lead to metastasis formation eventually. Selectins are also found on peritoneal mesothelium (both E- and P-selectin), and it is tempting to speculate that recognition of the sialyl Lewis^x epitopes on peritoneal leukocytes and of the sialyl Lewis^a epitopes on the cancer cells is an early pathophysiological event in PC as well. It is noteworthy that the final and specific biochemical step in the synthesis of the Lewis epitopes is a fucosyl transferase activity [46]. Inhibition of this enzyme may be a target for possible anti-invasive rationales in future prevention of PC.

CA 125 and Mesothelin

CA 125 is a large cell surface mucin-like glycoprotein expressed in mesothelial cells and upregulated in malignant ovarian tumours. It is considered as a relatively specific circulating tumour marker in ovarian cancer patients, and, due to its inca-

pability to pass intact basement membranes (as in benign ovarian cysts), its presence in blood indicates basement membrane breakdown and hence invasion.

Mesothelin is another protein expressed by the normal mesothelium, and soluble mesothelin is used to detect the overexpressed protein as a circulating tumour marker for mesothelioma. As in the case of CA 125, upregulation is also found in cancer cells, particularly in ovarian carcinoma. Recent studies have shown the interaction between CA 125 and mesothelin, and their role as adhesion molecules between ovarian carcinoma cells and mesothelial cells in the formation of PC [47].

L1 and Neuropilin-1

The binding of L1, an adhesion molecule present on the surface of some cancer cell types, to neuropilin-1, a VEGF receptor on endothelial and mesothelial has been brought in relation to PC [48,49]. L1, which belongs to the immunoglobulin superfamily, is – like N-cadherin – a substrate for ADAM10, and can yield a motogenic soluble L1. Probably L1 is active at different levels: it increases the adhesion of peritoneal cancer cells to the lining mesothelium, and stimulates their migration through the stroma.

Other Adhesion Molecules in the Peritoneum

A number of adhesion molecules have recently been brought in relation with the pathogenesis of PC, but the reports are still isolated, and their potential relevance will have to be confirmed. Some of these molecules are: SPARC (a host-secreted protein, acidic and rich in cysteine, which in fact is an anti-adhesion molecule in the peritoneal stroma) [50], EpCAM (an epithelial cell adhesion molecule, which increases the homotypic cell-cell adhesion between cancer cells) [51] and oncofetal fibronectin (detected in ascitic fluid of patients with advanced ovarian cancer, and localized in the primary sites and the metastatic implants) [52].

Conclusion and Future Perspectives

Within the micro-ecosystem of PC, adhesion is involved in a number of cell-cell and cell-matrix interactions. Molecular cell-cell interactions can be homophilic or heterophilic, homotypic or heterotypic, and can result in cancer cell arrest or movement. A major part of our knowledge about the role of cell adhesion and PC stems from endothelial cell interactions, which are studied extensively in the context of leukocyte diapedesis and cancer cell extravasation in hematogenous metastasis formation. We hope these research fields will continue to inseminate each other. Notably some new concepts about systemic metastasis formation seem to become new breakthroughs. First, the concept of early metastasis gene activation in the primary tumour seems to allow to predict whether and where a primary

tumour has the tendency to metastasize to [53,54]. In practice this would mean that PCR on a selected set of metastasis (often adhesion) genes in the primary tumour should check their activation status, and indicate whether the tumour will develop peritoneal metastases or not. Second, the role of circulating stem cells as conditioners of many organs to form niches that facilitate cancer metastasis formation [55], has recently led to interesting speculations on (pre)treatment of the cancer patient. One problem to be solved in the near future is evidently the possible commitment of stem cells in PC.

Acknowledgements

Parts of this work were supported by the FWO, the Stichting tegen Kanker and the Centrum voor Gezwelziekten (Belgium).

References

1. Mareel M, Van Roy F, and De Baetselier P (1990) The invasive phenotypes. Cancer Metastasis Rev 9:45-62
2. Mareel M, Bracke M, and Van Roy F (1994) Invasion promoter versus invasion suppressor molecules: the paradigm of E-cadherin. Mol Biol Rep 19: 45-67
3. Derycke L, Van Marck V, Depypere H, and Bracke M (2005) Molecular targets of growth, differentiation, tissue integrity, and ectopic cell death in cancer cells. Cancer Biother Radiopharm 20:579-588
4. Opdenakker G, and Van Damme J (2004) The countercurrent principle in invasion and metastasis of cancer cells. Recent insights on the roles of chemokines. Int J Dev Biol 48:519-527
5. Bracke ME, Van Roy FM, and Mareel MM (1996) The E-cadherin/catenin complex in invasion and metastasis. In: Attempts to Understand Metastasis Formation I, pp 123-161 U Günthert and W Birchmeier, Eds, Springer, Berlin
6. Coopman PJ, Bracke ME, Lissitzky JC, De Bruyne GK, Van Roy FM, Foidart J-M, and Mareel MM (1991) Influence of basement membrane molecules on directional migration of human breast cell lines *in vitro*. J Cell Sci 98:395-401
7. Porter BE, Weis J, and Sanes JR (1995) A motoneuron-selective stop signal in the synaptic protein S-laminin. Neuron 14:549-559
8. Mironov VA, Gusev SA, and Baradi AF (1979) Mesothelial stomata overlying omental milky spots: scanning electron microscopic study. Cell Tissue Res 201:327-330
9. Jones LM, Gardner MJ, Catterall JB, and Turner GA (1995) Hyaluronic acid secreted by mesothelial cells: a natural barrier to ovarian cancer cell adhesion. Clin Exp Metastasis 13:373-380

10. Thompson JN, Paterson-Brown S, Harbourne T, Whawell SA, Kalodiki E, and Dudley HA (1989) Reduced human peritoneal plasminogen activating activity: possible mechanism of adhesion formation. Br J Surg 76:382-384
11. Scavelli C, Vacca A, Di Pietro G, Dammacco F, and Ribatti D (2004) Crosstalk between angiogenesis and lymphangiogenesis in tumor progression. Leukemia 18:1054-1058
12. Blaschuk OW, Sullivan R, David S, and Pouliot Y (1990) Identification of a cadherin cell adhesion recognition sequence. Dev Biol 139:227-229
13. Noë V, Willems J, Vandekerckhove J, Van Roy F, Bruyneel E, and Mareel M (1999) Inhibition of adhesion and induction of epithelial cell invasion by HAV-containing E-cadherin-specific peptides. J Cell Sci 112:127-135
14. Pocard M, Debruyne P, Bras-Gonçalves R, Mareel M, Dutrillaux B, and Poupon M-F (2001) Single alteration of *p53* or E-cadherin genes can alter the surgical resection benefit in an experimental model of colon cancer. Dis Colon Rectum 44:1106-1112
15. Hippo Y, Yashiro M, Ishii M, Taniguchi H, Tsutsumi S, Hirakawa K, Kodama T, and Aburatani H (2001) Differential gene expression profiles of scirrhous gastric cancer cells with high metastatic potential to peritoneum or lymph nodes. Cancer Res 61:889-895
16. Elloul S, Elstrand MB, Nesland JM, Trope CG, Kvalheim G, Goldberg I, Reich R, and Davidson B (2005) Snail, Slug, and Smad-interacting protein 1 as novel parameters of disease aggressiveness in metastatic ovarian and breast carcinoma. Cancer 103:1631-1643
17. Nollet F, Berx G, and Van Roy F (1999) The role of the E-cadherin/catenin adhesion complex in the development and progression of cancer. Mol Cell Biol Res Commun 2:77-85
18. Bracke ME, Vyncke BM, Bruyneel EA, Vermeulen SJ, De Bruyne GK, Van Larebeke NA, Vleminckx K, Van Roy FM, and Mareel MM (1993) Insulin-like growth factor I activates the invasion suppressor function of E-cadherin in MCF-7 human mammary carcinoma cells *in vitro*. Br J Cancer 68:282-289
19. Bracke ME, Charlier C, Bruyneel EA, Labit C, Mareel MM, and Castronovo V (1994) Tamoxifen restores the E-cadherin function in human breast cancer MCF-7/6 cells and suppresses their invasive phenotype. Cancer Res 54:4607-4609
20. Vanhoecke B, Derycke L, Van Marck V, Depypere H, De Keukeleire D, and Bracke M (2005) Antiinvasive effect of xanthohumol, a prenylated chalcone present in hops (*Humulus lupulus* L.) and beer. Int J Cancer 117:889-895
21. Imai T, Horiuchi A, Shiozawa T, Osada R, Kikuchi N, Ohira S, Oka K, and Konishi I (2004) Elevated expression of E-cadherin and alpha-, beta-, and gamma-catenins in metastatic lesions compared with primary epithelial ovarian carcinomas. Hum Pathol 35:1469-1476
22. Derycke LDM, and Bracke ME (2004) N-cadherin in the spotlight of cell-cell adhesion, differentiation, embryogenesis, invasion and signalling. Int J Dev Biol 48:463-476
23. De Wever O, Westbroek W, Verloes A, Bloemen N, Bracke M, Gespach C, Bruyneel E, and Mareel M (2004) Critical role of N-cadherin in myofibro-

blast invasion and migration in vitro stimulated by colon-cancer-cell-derived TGF-ß or wounding. J Cell Sci 117:4691-4703
24. Tahara K, Fujii K, Yamaguchi K, Suematsu T, Shiraishi N, and Kitano S (2001) Increased expression of P-cadherin mRNA in the mouse peritoneum after carbon dioxide insufflation. Surg Endosc 15:946-949
25. Paredes J, Albergaria A, Oliveira JT, Jeronimo C, Milanezi F, and Schmitt FC (2005) P-cadherin overexpression is an indicator of clinical outcome in invasive breast carcinomas and is associated with CDH3 promoter hypomethylation. Clin Cancer Res 11:5869-5877
26. Van Marck V, Stove C, Van Den Bossche K, Stove V, Paredes J, Vander Haeghen Y, and Bracke M (2005) P-cadherin promotes cell-cell adhesion and counteracts invasion in human melanoma. Cancer Res 65:8774-8783
27. Koyama S, Ebihara T, and Fukao K (1992) Expression of intercellular adhesion molecule 1 (ICAM-1) during the development of invasion and/or metastasis of gastric carcinoma. J Cancer Res Clin Oncol 118:609-614
28. Alkhamesi NA, Ziprin P, Pfistermuller K, Peck DH, and Darzi AW (2005) ICAM-1 mediated peritoneal carcinomatosis, a target for therapeutic intervention. Clin Exp Metastasis 22:449-459.
29. Ziprin P, Ridgway PF, Peck DH, and Darzi AW (2003) Laparoscopic enhancement of tumour cell binding to the peritoneum is inhibited by anti-intercellular adhesion molecule-1 monoclonal antibody. Surg Endosc 17: 1812-1817
30. van Grevenstein WM, Hofland LJ, Jeekel J, and van Eijck CH (2006) The expression of adhesion molecules and the influence of inflammatory cytokines on the adhesion of human pancreatic carcinoma cells to mesothelial monolayers. Pancreas 32:396-402
31. Schlaeppi M, Rüegg C, Trân-Thang C, Chapuis G, Tevaearai H, Lahm H, and Sordat B (1997) Role of integrins and evidence for two distinct mechanisms mediating human colorectal carcinoma cell interaction with peritoneal mesothelial cells and extracellular matrix. Cell Adhesion Commun 4:439-455
32. Kawamura T, Endo Y, Yonemura Y, Nojima N, Fujita H, Fujimura T, Obata T, Yamaguchi T, and Sasaki T (2001) Significance of integrin alpha2/beta1 in peritoneal dissemination of a human gastric cancer xenograft model. Int J Oncol 18:809-815
33. Nishimura S, Chung YS, Yashiro M, Inoue T, and Sowa M (1996) Role of alpha 2 beta 1- and alpha 3 beta 1-integrin in the peritoneal implantation of scirrhous gastric carcinoma. Br J Cancer 74:1406-1412
34. Fujita S, Suzuki H, Kinoshita M, and Hirohashi S (1992) Inhibition of cell attachment, invasion and metastasis of human carcinoma cells by anti-integrin beta 1 subunit antibody. Jpn J Cancer Res 83:1317-1326
35. Dejana E, Breviario F, and Caveda L (1994) Leukocyte-endothelial cell adhesive receptors. Clin Exp Rheumatol 12 (Suppl 10):S25-S28
36. Lessan K, Aguiar DJ, Oegema T, Siebenson L, and Skubitz AP (1999) CD44 and beta1 integrin mediate ovarian carcinoma cell adhesion to peritoneal mesothelial cells. Am J Pathol 154:1525-1537

37. Nishimura S, Chung YS, Yashiro M, Inoue T, and Sowa M (1996) CD44H plays an important role in peritoneal dissemination of scirrhous gastric cancer cells. Jpn J Cancer Res 87:1235-1244
38. Catterall JB, Jones LM, and Turner GA (1999) Membrane protein glycosylation and CD44 content in the adhesion of human ovarian cancer cells to hyaluronan. Clin Exp Metastasis 17:583-591
39. Harada N, Mizoi T, Kinouchi M, Hoshi K, Ishii S, Shiiba K, Sasaki I, and Matsuno S (2001) Introduction of antisense CD44S CDNA down-regulates expression of overall CD44 isoforms and inhibits tumor growth and metastasis in highly metastatic colon carcinoma cells. Int J Cancer 91:67-75
40. Kayastha S, Freedman AN, Piver MS, Mukkamalla J, Romero-Guittierez M, and Werness BA (1999) Expression of the hyaluronan receptor, CD44S, in epithelial ovarian cancer is an independent predictor of survival. Clin Cancer Res 5:1073-1076
41. Koyama T, Yashiro M, Inoue T, Nishimura S, and Hirakawa-YS Chung K (2000) TGF-beta1 secreted by gastric fibroblasts up-regulates CD44H expression and stimulates the peritoneal metastatic ability of scirrhous gastric cancer cells. Int J Oncol 16:355-362
42. Chhieng DC, Yee H, Cangiarella JF, Symmans WF, and Cohen JM (2000) Use of E-cadherin and CD44 aids in the differentiation between reactive mesothelial cells and carcinoma cells in pelvic washings. Cancer 90:299-306
43. Thurin M, and Kieber-Emmons T (2002) SA-Lea and tumor metastasis: the old prediction and recent findings. Hybrid Hybridomics 21:111-116
44. Futamura N, Nakamura S, Tatematsu M, Yamamura Y, Kannagi R, and Hirose H (2000) Clinicopathologic significance of sialyl Le(x) expression in advanced gastric carcinoma. Br J Cancer 83:1681-1687
45. Nakamori S, Furukawa H, Hiratsuka M, Iwanaga T, Imaoka S, Ishikawa O, Kabuto T, Sasaki Y, Kameyama M, Ishiguro S, and Irimura T (1997) Expression of carbohydrate antigen sialyl Le(a): a new functional prognostic factor in gastric cancer. J Clin Oncol 15:816-825
46. Asao T, Nagamachi Y, Morinaga N, Shitara Y, Takenoshita S, and Yazawa S (1995) Fucosyltransferase of the peritoneum contributed to the adhesion of cancer cells to the mesothelium. Cancer 75(Suppl 6):1539-1544
47. Scholler N, Garvik B, Hayden-Ledbette M, Kline T, and Urban N (2006) Development of a CA125-mesothelin cell adhesion assay as a screening tool for biologics discovery. Cancer Lett May 3 [Epub ahead of print]
48. Stoeck A, Schlich S, Issa Y, Gschwend V, Wenger T, Herr I, Marme A, Bourbie S, Altevogt P, and Gutwein P (2005) L1 on ovarian carcinoma cells is a binding partner for Neuropilin-1 on mesothelial cells. Cancer Lett Dec 21 [Epub ahead of print]
49. Arlt MJ, Novak-Hofer I, Gast D, Gschwend V, Moldenhauer G, Grunberg J, Honer M, Schubiger PA, Altevogt P, and Kruger A (2006) Efficient inhibition of intra-peritoneal tumor growth and dissemination of human ovarian carcinoma cells in nude mice by anti-L1-cell adhesion molecule monoclonal antibody treatment. Cancer Res 66:936-943

50. Said N, and Motamed K (2005) Absence of host-secreted protein acidic and rich in cysteine (SPARC) augments peritoneal ovarian carcinomatosis. Am J Pathol 167:1739-1752
51. Mayer B, Klement G, Kaneko M, Man S, Jothy S, Rak J, and Kerbel RS (2001) Multicellular gastric cancer spheroids recapitulate growth pattern and differentiation phenotype of human gastric carcinomas. Gastroenterology 121:839-852
52. Menzin AW, Loret de Mola JR, Bilker WB, Wheeler JE, Rubin SC, and Feinberg RF (1998) Identification of oncofetal fibronectin in patients with advanced epithelial ovarian cancer: detection in ascitic fluid and localization to primary sites and metastatic implants. Cancer 82:152-158
53. Tarbe N, Evtimova V, Burtscher H, Jarsch M, Alves F, and Weidle UH (2001) Transcriptional profiling of cell lines derived from an orthotopic pancreatic tumor model reveals metastasis-associated genes. Anticancer Res 21:3221-3228
54. Minn AJ, Kang Y, Serganova I, Gupta GP, Giri DD, Doubrovin M, Ponomarev V, Gerald WL, Blasberg R, and Massague J (2005) Distinct organ-specific metastatic potential of individual breast cancer cells and primary tumors. J Clin Invest 115:44-55
55. Kaplan RN, Riba RD, Zacharoulis S, Bramley AH, Vincent L, Costa C, MacDonald DD, Jin DK, Shido K, Kerns SA, Zhu Z, Hicklin D, Wu Y, Port JL, Altorki N, Port ER, Ruggero D, Shmelkov SV, Jensen KK, Rafii S, and Lyden D (2005) VEGFR1-positive haematopoietic bone marrow progenitors initiate the pre-metastatic niche. Nature 438:820-827

Surgical Trauma, Minimal Residual Disease and Locoregional Cancer Recurrence

WP Ceelen, S Morris, P Paraskeva, P Pattyn

Introduction

Surgery is the mainstay of therapy in solid cancer, and complete (R0) resection represents the single most important determinant of cure. Recent data, however, suggest that surgical removal may in itself be associated with enhanced or accelerated growth of microscopic or macroscopic residual tumour [1]. This phenomenon was already described in the nineteenth century by Stephen Paget, who observed that survival of breast cancer patients who underwent surgery was not always better than those treated conservatively [2]. Recently, this finding was confirmed by the observation that mammography screening programs resulted in a paradoxically higher mortality in operated women aged 40-49 years [3].

This chapter provides an overview of the underlying mechanisms giving rise to accelerated tumour growth in the presence of microscopic residual disease, with an emphasis on peritoneal cancer recurrence. First, we describe the various mechanisms known to cause minimal residual disease (MRD) after open and laparoscopic surgery. Secondly, an overview is provided of the evidence supporting the hypothesis that (surgical) cancer removal creates a permissive environment enhancing residual tumour growth. Finally, potential therapeutic approaches are highlighted.

Minimal Residual Disease following Surgery

Residual disease can occur systemically (micrometastases) or locally (in the surgical field). Several factors have been identified giving rise to residual and eventually locally recurrent disease involving the peritoneal surfaces. These can be related to the properties of the tumour, to technical circumstances during surgery or to postoperative events.

Tumour Associated Factors

Residual peritoneal disease can be caused by direct invasion, perforation, shedding of loose cells, or a systemic route.

Several clinical studies have demonstrated that tumours penetrating the entire bowel wall (T_3) and those infiltrating the abdominal wall or adjacent organs (T_4) are associated with both a worse prognosis and an increased risk of peritoneal recurrence [4-6]. Lennon et al. found, in a cohort of stage II colon cancer patients, that peritoneal involvement on histological slides was a powerful predictor of adverse outcome [7]. Interestingly, the survival of stage pT_4N_0 (stage IIb) patients was found to be worse than that of patients with stage IIIa ($pT_{1,2}N_1$) disease, representing a formulated critique on the sixth edition of the AJCC/IUCC TNM staging system [8].

Perforation of bowel cancer with intraperitoneal spill and peritonitis represents a well known adverse risk factor for postoperative outcome and survival [9-11].

Loose tumour cells can spontaneously exfoliate from the main mass by a shedding or bursting mechanism. The occurrence of free cancer cells as determined by cytological analysis represents a risk factor for peritoneal recurrence in most gastrointestinal cancers as well as in ovarian cancer [12-17]. Viability studies have suggested that, in contrast to circulating tumour cells in blood, bone marrow or liver, the metastatic efficiency of loose intraperitoneal cells is outspoken [18-21]. However, the population of loose intraperitoneal cancer cells is likely heterogeneous with both invasive cells possessing a metastatic phenotype and noninvasive cells that are merely transported by the physiological lymph flow. Once liberated in the peritoneal cavity, loose cancer cells are transported along with the physiological peritoneal lymph flow. The lymph flow is directed towards the right diaphragm since the intraabdominal pressure tends to be the lowest beneath the right diaphragm during inspiration. Since resorption of particulate matter also occurs through the diaphragmatic surfaces, they represent a frequent location of peritoneal metastasis. Similarly, the greater omentum is nearly always involved in peritoneal carcinomatosis patients despite the presence of numerous macrophages in the omental milky spots [22,23]. Possibly, adhesion of cancer cells is facilitated by the reduced flow and shear forces along the irregular omental surface [24].

In rare cases, hematogenous spread from lobular breast cancer or melanoma can be at the origin of peritoneal metastases [25].

Surgery Related Factors

Technical circumstances can give rise to a peritoneal recurrence. Obviously, this will be the case when an R1 or R2 resection is performed or when the tumour is inadvertently ruptured, opened or cut into. This is well illustrated in rectal cancer surgery, where a clear relationship exists between incomplete resection (positive circumferential resection margin) and the development of a local recurrence [26,27].

Theoretically, tumour spill could also arise from section of blood or lymph vessels with subsequent leakage. This concept was proven by Hansen et al., who detected tumour cells in the blood shed during oncologic surgery in 57 out of 61 patients undergoing cancer surgery [28]. Importantly, the identified cancer cells demonstrated proliferation capacity, invasiveness, and tumorigenicity.

Interestingly, leakage of bile during surgery for cholangiocarcinoma has also been noted to be associated with peritoneal recurrence [29].

During and after colorectal surgery, circulating levels of lipopolysaccharides (LPS) are increased as a result of bacterial translocation. Recent experimental data have shown that LPS can enhance residual tumour growth by promoting the metastatic phenotype. Wang et al. showed that endotoxin/LPS activates NF-kappa B through the Toll like receptor and enhances tumour cell adhesion and invasion through an β_1 integrin-dependent mechanism [30].

Tumour Seeding during Laparoscopy

Local recurrence situated at port site or extraction site skin incisions has been a concern since the introduction of laparoscopic techniques in malignant disease [31,32] . The risk of port site metastasis (PSM) depends on surgical technique, instrumentation and technology, and tumour biology.

Grasping and manipulating the tumour with laparoscopic instruments is associated with tumour cell contamination of both the instruments and the trocars [33,34]. Also, aerosolization of particles and viable cells can occur during laparoscopy [35,36]. Champault et al. passed the gas escaping from pneumoperitoneum through a filter in nine patients undergoing various laparoscopic procedures for both benign and malignant disease [37]. The filters and tubing were subsequently washed or examined by electron microscopy, and in six of nine samples viable cells (although no cancer cells) were identified. Aerosolization of tumour cells could in turn cause PSM by the so called 'chimney effect', when the insufflation gas is allowed to escape through a skin incision or along a trocar [38,39]. However, Wittich et al. showed in a rat colorectal cancer model that the tumour load in the gas flow required to cause PSM is very high and therefore the clinical relevance of this mechanism is probably limited [40].

The increase in intraperitoneal pressure associated with laparoscopy has been shown to promote tumour growth and invasiveness in a number of preclinical studies [41-43]. Paraskeva et al. found that exposure of a human colon cancer cell line to a laparoscopic environment significantly enhanced production of the proteases matrix metalloproteinase (MMP)-2, MMP-9 and urokinase-type plasminogen activator (uPA); at the same time invasive capacity as measured with a Matrigel assay was also enhanced [44]. Similarly, Basson et al. noted that even a moderate increase in pressure stimulated malignant colonocyte adhesion by a cation-dependent β_1-integrin-mediated mechanism [45]. The same group showed that increased extracellular pressure in general stimulates colon cancer cell adhesion by activating focal adhesion kinase (FAK) and Src [46,47].

The increased intraabdominal pressure may also alter the functional integrity of the mesothelial lining. In an animal model, Volz et al. performed scanning electron microscopy of the peritoneum after intraperitoneal injection of 200.000 cells of a malignant melanoma followed by CO_2 pneumoperitoneum for 30 minutes [48]. In the group that underwent pneumoperitoneum, pronounced alterations of the peritoneum were evident and parts of the underlying basal lamina were laid bare; tumour cells were noted to attach to the free basal lamina. Similar ultrastructural alterations of the peritoneum were noted by Rosario et al. and were more pronounced after CO_2 pneumoperitoneum compared to air insufflation [49].

The type of insufflation gas has also been the subject of scrutiny in relation to tumour growth and laparoscopy. Ridgway et al. exposed colon carcinoma cells to an in vitro pneumoperitoneum of CO_2 or He at 3 mmHg and found an increase in tumour cell invasiveness abolished by the presence of a known inhibitor of matrix metalloproteinases (MMPs), suggesting that MMPs have an important role in the metastatic potential of tumours exposed to a hypoxic environment related to pneumoperitoneum [50].

Jacobi et al. found that growth of a colon cancer cell line was significantly increased both in vitro and in vivo following CO_2 insufflation compared to helium insufflation and controls [51]. Similarly, in a rat adenocarcinoma model examining the effects of different insufflation gases, Neuhaus et al. found a significantly lower incidence of PSM with helium insufflation compared to air, CO_2 or N_2O [52].

Others, however, did not note any difference in tumour growth between helium and carbon dioxide in a similar rat model [53]. At present, therefore, it is unclear whether the type of insufflation gas is an important variable in the mechanisms giving rise to PSM. It is clear, however, that CO_2 pneumoperitoneum induces more pronounced physiological changes such as peritoneal acidosis which seems to be independent from systemic pH and may well alter the microenvironment of the cancer cell-mesothelial interaction [54-55].

Despite the concerns raised by preclinical models, the incidence of PSM has been noted to decrease with appropriate protective measures such as fixation of the trocars, wound protection, and avoidance of desufflation through a skin incision. In colorectal cancer, the results of recently completed large randomized trials comparing open with laparoscopic colectomy demonstrated that the rate of wound recurrence is low (<1%) and not different between the open and laparoscopic technique [56,57].

Postoperative Factors

Clinical studies have shown that the development of an anastomotic leak following colonic surgery is associated with an increased likelihood of local recurrence and a significantly worse survival [58,59]. The underlying mechanisms are at present unclear. Possibly, at least part of this effect is explained by the fact that patients who leaked probably had a more difficult procedure due to larger or more

advanced cancers. On the other hand, there is evidence that viable cancer cells may be present at the site of the anastomosis at the time of surgery [60]. Moreover, the additional both local and systemic inflammation associated with an anastomotic leak could affect the growth of residual cancer cells that otherwise would not have clinically appeared [61-63].

Entrapment of malignant cells by exudated fibrin(ogen) has been proposed as a mechanism of tumour growth on surgical wounds including peritonectomized surfaces [21]. Indirect evidence supporting this hypothesis is the demonstrated ability of fibrin and fibrin matrices to bind to a variety of normal and cancer cell types via cell surface integrin and non integrin (VE-Cadherin, ICAM-1, P-selectin) receptors [64]. Moreover, other plasma proteins present in wound surface exudate such as fibronectin and vitronectin may act as bridging molecules between endothelial cells, smooth muscle cells and cancer cells via the $\alpha_5\beta_1$ and $\alpha_v\beta_3$ receptors [65].

The Link between Residual Tumour Growth and Surgery

Removal of a primary cancer by surgery, radiotherapy or other means can enhance the growth of residual tumour by two general mechanisms. Firstly, the inflammatory process associated with a (surgical) wound enhances tumour growth. Secondly, the primary cancer produces a number of anti-angiogenic and anti-proliferative stimuli that keep secondary cancer foci in a state of dormancy; removal of the primary cancer will therefore reactivate growth and invasiveness.

The importance of Inflammation

The acute inflammatory response and healing processes observed at the site of surgical injury are important not only in relation to the formation of postoperative adhesions but also in the enhancement of tumour growth. The specific mesothelial repair mechanisms and their relation with tumour cell adhesion and invasion are discussed in chapter 1.

The general link between cellular or wound fluid components and tumour growth in general has been established. Hofer et al. found that the growth of subcutaneously injected melanoma cells was significantly enhanced when coinjected with wound fluid or isolated TGF-β and bFGF [66]. In breast cancer, wound drainage fluid and postsurgical serum samples from patients were found to stimulate in-vitro growth of HER2-overexpressing breast carcinoma cells [67].

In preclinical models, intraperitoneal tumour growth has been shown to be related to the presence and extent of peritoneal trauma [68,69]. Also, growth of intraperitoneally administered colon carcinoma cells was enhanced when they were injected together with lavage fluid from intraabdominally traumatized animals [69]. The importance of timing of peritoneal wounding versus tumour injection was illustrated by a paper of Zeamari et al., who found that tumour growth in an artificially induced peritoneal wound was much less pronounced when cells were

injected 10 days after the wounding versus injection after 8 hours to three days [70]. Adhesion of tumour cells to the peritoneum has been linked to inflammatory mediators such as interleukin (IL) -1 beta, IL-6, TNF-α and epidermal growth factor (EGF)[71,72]. Interestingly, use of an inhibitory monoclonal antibody against ICAM-1 attenuated the enhanced mesothelial adhesion mediated by IL-6 or TNF-alpha in an in vitro model [71]. Taken together, these findings illustrate the long established link between wound healing - associated inflammation and cancer [73].

One of the chief inflammation effectors present in the healing wound are the *macrophages*. Traditionally, infiltration of tumour by leucocytes has been associated with a better outcome. Macrophages, however, produce an array of mediators that have been shown to potentially and in certain circumstances enhance tumour growth [74,75] (Table 1).

Table 1. Inflammatory mediators and growth factors released by macrophages and their relation to tumour growth

Mediator	Result	Effects on Tumor phenotype
ROS		Direct DNA damage
H_2O_2	Activates NF-κB	Inhibits apoptosis
MIF	Suppression of p53	Inhibits apoptosis
TNF-α, IL-1, IL-6,IL-8, IFN-γ	Induction of VEGF, NO	Promote tumour adhesion, growth and invasion
Proteases, MMP-9		Promote invasion
IL-10		Suppress host immunity
EGF		Increases invasiveness
TGF-α		Stimulates growth and angiogenesis
TGF-β		Stimulates growth and metastatic potential
bFGF		Stimulates growth and angiogenesis

ROS, reactive oxygen species; MIF, migration inhibitory factor, TNF, tumour necrosis factor; MMP, matrix metalloproteinase; IL, interleukin; IFN, interferon; VEGF, vascular endothelial growth factor; NO, nitric oxide; EGF, epidermal growth factor; TGF, transforming growth factor; FGF, fibroblast growth factor

Whether macrophages have a stimulatory or inhibitory effect on tumour growth clearly depends on the tumour microenvironment and the stroma involved. It has been suggested that macrophages present in the tumour nodules (TAM, tumour associated macrophages) are associated with a better survival, whereas macrophages present in the (submesothelial) stroma appear to enhance tumour progression [76].

Recently, stromal *fibroblasts* have been shown to enhance tumour growth by their production of growth factors, chemokines and extracellular matrix facilitating the angiogenic recruitment of endothelial cells and pericytes. A subpopulation of myofibroblasts has been identified that secrete elevated levels of stromal cell-

derived factor 1 (SDF-1), also called CXCL12, which plays a central role in the promotion of tumour growth and angiogenis [77-79].

The abundance of common mechanisms such as the NF-κB pathway shared between inflammatory processes and tumour growth illustrates the concept that the gene expression profile related to wound healing is frequently activated in tumours. This was recently shown in breast cancer patients, in whom survival was significantly related to the expression of a 'wound response signature' by the tumour [80].

Recent data indicate that accelerated postoperative tumour growth is associated with reduced tumor cell apoptosis [81]. In a recent animal model, recurrent tumours following cytoreductive surgery were characterized by accelerated growth and a anti-apoptotic phenotype [82]. When gene expression profiles of primary and recurrent tumours were compared, recurrent tumours showed changes in the expression of genes encoding phosphatidylinositol 3-kinase (PI3K), a key enzyme in the balance between proapoptotic and antiapoptotic signals.

Surgery and Tumour Dormancy

A second mechanism that can enhance residual tumour growth following surgical removal of a primary cancer is related to the concept of tumour dormancy. It is known that many of the tumour cells that reach the peritoneal surfaces or the systemic circulation and invade distant organs will never develop into a clinical metastasis. This phenomenon is termed 'metastatic inefficiency', and results from tumour dormancy characterized by prolonged survival without DNA replication (G0 arrest; Ki67 negative) [83]. As a result, these cells are resistant to therapy with cytotoxic drugs.

Tumour dormancy is the result of a balance between proliferation and apoptosis [84]. Recent data indicate that primary tumours induce apoptosis in micrometastatic foci by the production of anti-angiogenic agents such as thrombospondin-1, endostatin and angiostatin [85-89]. Guba et al. showed in a mouse colon carcinoma model that the presence of a primary tumour significantly inhibited the development of liver metastasis by interfering with angiogenesis [90]. Conversely, removal of the primary tumour by surgery or irradiation resulted in activation and growth of dormant residual cancer by turning on the 'angiogenic switch' in animal models [91,92]. Clinically, activation of dormant metastatic deposits was proposed as the underlying mechanism to explain the existence of an early (after 18 months) peak in relapse frequency in breast cancer patients treated with surgery only [93].

Prevention and Treatment of Residual Tumour Growth

Prevention of Surgical Trauma

Physical measures to prevent tumour growth include the prevention or reduction of surgical trauma. Animal models have shown a reduction in tumour implantation with the use of atraumatic gauze or non powdered gloves [69,94].

Laparoscopic surgery was similarly associated with less tumour growth compared to open surgery in several animal models [95,96]. The beneficial effect of a laparoscopic approach was shown to extend to systemic disease by Carter et al., who found significantly less pulmonary metastases upon tail injection of cancer cells after laparoscopic than after open cecectomy [97].

Nonspecific Intraperitoneal Therapy

Once isolated tumour cells are present in the peritoneal cavity, efforts to mechanically or chemically remove them have been described in animal models by instillation of a suitable solution in the abdominal cavity. Basha et al. showed that instillation of Povidone-iodine was effective in preventing tumour take only when a limited tumour inoculum was used; moreover local toxicity was problematic in this rat model [98]. In an animal model of laparoscopy assisted tumour splenectomy, abdominal irrigation with dilute povidone-iodine solution significantly reduced the number of animals with PSM [99]. It should be noted, however, that many of the commonly used antiseptics are known to be inactivated by the presence of blood [100].

In another study, distilled water was used to achieve osmotic lysis of cancer cells [101]. Complete lysis took significantly longer (more than 30 minutes) in vivo compared to in vitro instillation.

In a small non randomized clinical study, Yamamoto et al. used EIPL (‘extensive intraoperative peritoneal lavage’) after curative resection of pancreatic cancer and found that local recurrence was significantly lower in the (small) group of patients who received this therapy [102].

Intraperitoneal Chemotherapy

Experimentally, intraperitoneal instillation of chemotherapy was shown to be effective in preventing cancer implantation in animal models of tumour spill [103,104].

The noted experimental efficacy of ip chemotherapy to prevent residual tumour growth has not been confirmed in clinical trials. Nordlinger et al. reported a randomized trial in which 753 patients with stage II-III colorectal cancer were assigned to systemic chemotherapy alone or immediate postoperative regional

chemotherapy (5FU intraperitoneal or intraportal according to treatment center) followed by systemic chemotherapy [105]. No differences were observed in overall or disease free survival, although details concerning the incidence of peritoneal recurrence are lacking.

In patients with peritoneal carcinomatosis, cytoreduction followed by hyperthermic intraperitoneal chemoperfusion might offer a survival advantage. The reader is referred to the relevant chapters discussing this novel therapy.

Inhibition of the Angiogenic Switch and Reversal of Tumour Dormancy

Since dormant cells are resistant to cytotoxic drugs, therapeutic efforts should be directed towards inhibiting angiogenesis and activation of dormant cancer populations possibly starting already before surgery. In preclinical models, maintenance drugs shown to suppress the metastatic phenotype include histone deacetylase inhibitors, NFκB inhibitors, MMP modifiers, or growth factor antagonists [106-109]. Coffey et al. showed that in vitro targeting of PI3K using LY294002 restored sensitivity to TNF related apoptosis inducing legend (TRAIL) in recurrent tumour epithelia and greatly enhanced apoptosis levels [82].

Inhibition of the Inflammatory Response

Inhibition or modulation of the immune response could be of value in preventing surgery related stimulation of tumour growth. In murine cancer models, Roh et al. found that celecoxib had a significant inhibitory effect on tumour growth in the surgical wound that was most obvious when administered daily from 1 day before surgical wounding and tumour implantation [110,111]. Connolly et al. showed a significant inhibition of growth and metastasis of mouse mammary tumours after administration of either a selective or a non selective COX inhibitor [112].

In line with the observed effects of bacterial LPS on tumour apoptosis, anti-LPS therapy using taurolidine abrogated the effects of surgical trauma on primary and metastatic tumour growth in a mouse melanoma model [113].

In colorectal cancer patients, preoperative administration of IL-2 significantly reduced postoperative VEGF production and at the same time inhibited the decline of the anti-angiogenic cytokine IL-12 [114].

Helguera et al. studied the effects of cytokines fused to antibodies in mice receiving intraperitoneal HER2/neu expressing tumours, and found that combined administration of 1. anti-HER2/neu fused with IL-2 and 2. anti-HER2/neu fused with granulocyte macrophage colony-stimulating factor (GM-CSF) prevented tumour growth in 100% of animals [115].

Inhibition of Adhesion of Free Intraperitoneal Cancer Cells

Specific therapy targeting the various mechanisms of tumour-mesothelial interaction has been addressed in preclinical studies. One approach has been directed towards the binding sites of the extracellular matrix (ECM). Alkhamesi et al. showed that intraperitoneal application of heparin caused a significant decrease in tumour cell adhesion accompanied by a decrease in ICAM-1 expression [116].

Similarly, low molecular weight heparin significantly reduced tumour growth following laparoscopy in a rat colorectal cancer model [117]. Covering the ECM binding sites with a phospholipid emulsion also reduced tumour-mesothelial cell adhesion [118].

Targeting of specific adhesion molecules such as integrins [119,120], L1 cell adhesion molecule [121] and JAM-C [122] with monoclonal antibodies has shown to be effective in preventing tumour adhesion and/or growth in preclinical models and may represent a future clinical therapeutic tool.

Summary and Conclusion

The persistence of residual tumour is associated with the histology and stage of the primary cancer, the completeness and quality of surgery, and postoperative events such as anastomotic leakage or entrapment of cells in exudating wound surfaces. At present, there is no clinical evidence that the use of laparoscopic techniques adversely influences the risk of residual disease.

The inflammatory process associated with surgery shares a number of central mediators and pathways with tumour growth and invasiveness. Both cellular components (mainly macrophages and fibroblasts) and humoral factors associated with inflammation have been shown to enhance tumour growth in numerous preclinical studies.

Tumour foci at a distance from the main cancer are kept in a dormant state by a range of anti-angiogenic mediators produced by the main cancer. Preclinical studies have shown that removal of the primary cancer reactivates proliferative and metastatic pathways in the residual tumour. Clinically, this phenomenon has been proposed as underlying the observed rapid systemic relapse after surgery in young node positive breast cancer patients.

Strategies proposed to prevent residual disease encompass avoidance of tumour spill and minimization of surgical trauma and related inflammation. Efforts to remove or kill free intraperitoneal cells by local antiseptic or cytotoxic regimens have met only limited clinical success. Specific targeted therapy aimed at inhibiting the inflammatory response, tumour cell adhesion, or the metastatic phenotype of dormant cells appears promising in preclinical models and needs to be addressed in future clinical trials.

References

1. Coffey JC, Wang JH, Smith MJF, Bouchier-Hayes D, Cotter TG, Redmond HP. (2003) Excisional surgery for cancer cure: therapy at a cost. Lancet Oncol 4(12):760-768
2. Paget S. (1889) The Distribution Of Secondary Growths In Cancer Of The Breast. Lancet 133(3421):571-573
3. Baines CJ. (2005) Are there downsides to mammography screening? Breast Journal 11(2):S7-S10
4. Lennon AM, Mulcahy HE, Hyland J, Lowry C, White A, Fennelly D, Murphy JJ, O'Donoghue D, Sheahan K. (2002) Peritoneal involvement is a powerful prognostic indicator in Stage II colonic cancer. Gastroenterology 122(4):A41-A41
5. Shepherd NA, Baxter KJ, Love SB. (1997) The prognostic importance of peritoneal involvement in colonic cancer: A prospective evaluation. Gastroenterology 112(4):1096-1102
6. Ludeman L, Shepherd NA. (2005) Serosal involvement in gastrointestinal cancer: its assessment and significance. Histopathology 47(2):123-131
7. Lennon AM, Mulcahy HE, Hyland JMP, Lowry C, White A, Fennelly D, Murphy JJ, O'Donoghue DP, Sheahan K. (2003) Peritoneal involvement in stage II colon cancer. Am J Clin Pathol 119(1):108-113
8. O'Connell JB, Maggard MA, Ko CY. (2004) Colon cancer survival rates with the new American Joint Committee on cancer sixth edition staging. J Natl Cancer Inst 96(19):1420-1425
9. McArdle CS, McMillan DC, Hole DJ. (2006) The impact of blood loss, obstruction and perforation on survival in patients undergoing curative resection for colon cancer. Br J Surg 93(4):483-488
10. Komatsu S, Shimomatsuya T, Nakajima M, Amaya H, Kobuchi T, Shiraishi S, Konishi S, Ono S, Maruhashi K. (2005) Prognostic factors and scoring system for survival in colonic perforation. Hepatogastroenterology 52(63):761-764
11. Chen HS, Sheen-Chen SM. (2000) Obstruction and perforation in colorectal adenocarcinoma: An analysis of prognosis and current trends. Surgery 127(4):370-376
12. Rosenberg R, Nekarda H, Bauer P, Schenck U, Hoefler H, Siewert JR. (2006) Free peritoneal tumour cells are an independent prognostic factor in curatively resected stage IB gastric carcinoma. Br J Surg 93(3):325-331
13. Ribeiro U, Safatle-Ribeiro AV, Zilberstein N, Mucerino D, Yagi OK, Bresciani CC, Jacob CE, Iryia K, Gama-Rodrigues J. (2006) Does the intraoperative peritoneal lavage cytology add prognostic information in patients with potentially curative gastric resection? J Gastrointest Surg 10(2):170-176
14. Meszoely IM, Lee JS, Watson JC, Meyers M, Wang H, Hoffman JP. (2004) Peritoneal cytology in patients with potentially resectable adenocarcinoma of the pancreas. Am Surgeon 70(3):208-213

15. Kanellos I, Demetriades H, Zintzaras E, Mandrali A, Mantzoros I, Betsis D. (2003) Incidence and prognostic value of positive peritoneal cytology in colorectal cancer. Dis Colon Rectum 46(4):535-539
16. Simojoki M, Santala M, Vuopala S, Kauppila A. (1999) The prognostic value of peritoneal cytology in ovarian cancer. Eur J Gynaecol Oncol 20(5-6):357-360
17. Schott A, Vogel I, Krueger U, Kalthoff H, Schreiber HW, Schmiegel W, Henne-Bruns D, Kremer B, Juhl H. (1998) Isolated tumor cells are frequently detectable in the peritoneal cavity of gastric and colorectal cancer patients and serve as a new prognostic marker. Ann Surg 227(3):372-379
18. Patel H, Le Marer N, Wharton RQ, Khan ZAJ, Araia R, Glover C, Henry MM, Allen-Marsh TG. (2002) Clearance of circulating tumor cells after excision of primary colorectal cancer. Ann Surg 235(2):226-231
19. Tanida O, Kaneshima S, Iitsuka Y, Kuda H, Kiyasu Y, Koga S. (1982) Viability of Intraperitoneal Free Cancer-Cells in Patients with Gastric-Cancer. Acta Cytol 26(5):681-687
20. Kodera Y, Yamamura Y, Shimizu Y, Torii A, Hirai T, Yasui K, Morimoto T, Kato T. (1999) Peritoneal washing cytology: Prognostic value of positive findings in patients with gastric carcinoma undergoing a potentially curative resection. J Surg Oncol 72(2):60-64
21. Sugarbaker PH. (2005) Strategies for the prevention and treatment of peritoneal carcinomatosis from gastrointestinal cancer. Cancer Invest 23(2):155-172
22. Meyers MA. (1973) Distribution of Intraabdominal Malignant Seeding - Dependency on Dynamics of Flow of Ascitic Fluid. Am J Roentgenol 119(1):198-206
23. Oosterling SJ, van der Bij GJ, Bogels M, van der Sijp JRM, Beelen RHJ, Meijer S, van Egmond M. (2006) Insufficient ability of omental milky spots to prevent peritoneal tumor outgrowth supports omentectomy in minimal residual disease. Cancer Immunol Immunother 55(9):1043-1051
24. De Wever I. Surgery for advanced ovarian cancer. (1991) A critical analysis of its contribution to combined treatment. PhD thesis. Leuven: Katholieke Universiteit Leuven
25. Mylonas L, Janni W, Friese K, Gerber B. (2004) Unexpected metastatic lobular carcinoma of the breast with intraabdominal spread and subsequent port-site metastasis after diagnostic laparoscopy for exclusion of ovarian cancer. Gynecol Oncol 95(2):405-408
26. Adam IJ, Mohamdee MO, Martin IG, Scott N, Finan PJ, Johnston D, Dixon MF, Quirke P. (1994) Role of Circumferential Margin Involvement in the Local Recurrence of Rectal-Cancer. Lancet 344(8924):707-711
27. Nagtegaal ID, Marijnen CAA, Kranenbarg EK, van de Velde CJH, van Krieken J. (2002) Circumferential margin involvement is still an important predictor of local recurrence in rectal carcinoma - Not one millimeter but two millimeters is the limit. Am J Surg Pathol 26(3):350-357

28. Hansen E, Wolff N, Knuechel R, Ruschoff J, Hofstaedter F, Taeger K. (1995) Tumor-Cells in Blood Shed from the Surgical Field. Arch Surg 130(4):387-393
29. Verbeek PCM, Vanderheyde MN, Ramsoekh T, Bosma A. (1990) Clinical-Significance of Implantation Metastases after Surgical-Treatment of Cholangiocarcinoma. Sem Liver Dis 10(2):142-144
30. Wang JH, Manning BJ, Wu QD, Blankson S, Bouchier-Hayes D, Redmond HP. (2003) Endotoxin/lipopolysaccharide activates NF-kappa B and enhances tumor cell adhesion and invasion through a beta(1) integrin-dependent mechanism. J Immunol 170(2):795-804
31. Savalgi RS. (1998) Port-site metastasis in the abdominal wall: Fact or fiction? Sem Surg Oncol 15(3):189-193
32. Ouellette JR, Ko AS, Lefor AT. (2005) The physiologic effects of laparoscopy: Applications in oncology. Cancer Journal 11(1):2-9
33. Hewett PJ, Thomas WM, King G, Eaton M. (1996) Intraperitoneal cell movement during abdominal carbon dioxide insufflation and laparoscopy - An in vivo model. Dis Colon Rectum 39(10):S62-S66
34. Reymond MA, Wittekind C, Jung A, Hohenberger W, Kirchner T, Kockerling F. (1997) The incidence of port-site metastases might be reduced. Surg Endoscopy 11(9):902-906
35. Mathew G, Watson DI, Ellis T, DeYoung N, Rofe AM, Jamieson GG. (1997) The effect of laparoscopy on the movement of tumor cells and metastasis to surgical wounds. Surg Endoscopy 11(12):1163-1166
36. Champault G, Catheline JM, Taffinder N, Ziol M. (1997) Laparoscopic surgery: Can smoke particles carry cells? Ann Chir 51(2):140-143
37. Champault G, Taffinder N, Ziol M, Riskalla H, Catheline JMC. (1997) Cells are present in the smoke created during laparoscopic surgery. Br J Surg 84(7):993-995
38. Tseng LNL, Berends FJ, Wittich P, Bouvy ND, Marquet RL, Kazemier G, Bonjer HJ. (1998) Port-site metastases - Impact of local tissue trauma and gas leakage. Surg Endoscopy 12(12):1377-1380
39. Ikramuddin S, Ellison EC, Schirmer WJ, Lucas J, Melvin WS. (1997) The detection of aerosolized cells during laparoscopy. Gastroenterology 112(4):A1450-A1450
40. Wittich P, Marquet RL, Kazemier G, Bonjer HJ. (2000) Port-site metastases after CO2 laparoscopy - Is aerosolization of tumor cells a pivotal factor? Surg Endoscopy 14(2):189-192
41. Jacobi CA, Wenger FA, Ordemann J, Gutt C, Sabat R, Muller JM. (1998) Experimental study of the effect of intra-abdominal pressure during laparoscopy on tumour growth and port site metastasis. Br J Surg 85(10):1419-1422
42. Gutt CN, Kim ZG, Hollander D, Bruttel T, Lorenz M. (2001) CO2 environment influences the growth of cultured human cancer cells dependent on insufflation pressure. Surg Endoscopy 15(3):314-318
43. Wittich P, Steyerberg EW, Simons SHP, Marquet RL, Bonjer HJ. (2000) intraperitoneal tumor growth is influenced by pressure of carbon dioxide pneumoperitoneum. Surg Endoscopy 14(9):817-819

44. Paraskeva PA, Ridgway PF, Jones T, Smith A, Peck DH, Darzi AW. (2005) Laparoscopic environmental changes during surgery enhance the invasive potential of tumours. Tumor Biol 26(2):94-102
45. Basson MD, Yu CF, Herden-Kirchoff O, Ellermeier M, Sanders MA, Merrell RC, Sumpio BE. (2000) Effects of increased ambient pressure on colon cancer cell adhesion. J Cell Biochem 78(1):47-61
46. Thamilselvan V, Basson MD. (2004) Pressure activates colon cancer cell adhesion by inside-out focal adhesion complex and actin cytoskeletal signaling. Gastroenterology 126(1):8-18
47. Thamilselvan V, Basson MD. (2005) The role of the cytoskeleton in differentially regulating pressure-mediated effects on malignant colonocyte focal adhesion signaling and cell adhesion. Carcinogenesis 26(10):1687-1697
48. Volz J, Koster S, Spacek Z, Paweletz N. (1999) The influence of pneumoperitoneum used in laparoscopic surgery on an intraabdominal tumor growth. Cancer 86(5):770-774
49. Rosario MTA, Ribeiro U, Corbett CEP, Ozaki AC, Bresciani CC, Zilberstein B, Gama-Rodrigues JJ. (2006) Does CO2 pneumoperitoneum alter the ultrastructuture of the mesothelium? J Surg Res 133(2):84-88
50. Ridgway PF, Smith A, Ziprin P, Jones TL, Paraskeva PA, Peck DH, Darzi AW. (2002) Pneumoperitoneum augmented tumor invasiveness is abolished by matrix metalloproteinase blockade. Surg Endoscopy 16(3):533-536
51. Jacobi CA, Sabat R, Bohm B, Zieren HU, Volk HD, Muller JM. (1997) Pneumoperitoneum with carbon dioxide stimulates growth of malignant colonic cells. Surgery 121(1):72-78
52. Neuhaus SJ, Watson DI, Ellis T, Rowland R, Rofe AM, Pike GK, Mathew G, Jamieson GG. (1998) Wound metastasis after laparoscopy with different insufflation gases. Surgery 123(5):579-583
53. Ludemann R, Watson DI, Smith E, Ellis T, Jamieson GG. (2003) Tumor implantation during laparoscopy using different insufflation gases - an experimental study using cultured cancer cells. Min Invas Ther All Technol 12(6):310-314
54. Hanly EJ, Aurora AR, Fuentes JM, Shih SP, Marohn MR, De Maio A, Talamini MA. (2005) Abdominal insufflation with CO2 causes peritoneal acidosis independent of systemic pH. J Gastrointest Surg 9(9):1245-1251
55. Mynbaev OA, Molinas CR, Adamyan LV, Vanacker B, Koninckx PR. (2002) Pathogenesis of CO2 pneumoperitoneum-induced metabolic hypoxemia in a rabbit model. J Am Assoc Gynecol Laparosc 9(3):306-314
56. Lacy AM, Garcia-Valdecasas JC, Delgado S, Castells A, Taura P, Pique JM, Visa J. (2002) Laparoscopy-assisted colectomy versus open colectomy for treatment of non-metastatic colon cancer: a randomised trial. Lancet 359(9325):2224-2229
57. Nelson H, Sargent D, Wieand HS, Fleshman J, Anvari M, Stryker SJ, Beart RW, Hellinger M, Flanagan R, Peters W, Ota D, Hellinger M. (2004) A comparison of laparoscopically assisted and open colectomy for colon cancer. N Engl J Med 350(20):2050-2059

58. Bell SW, Walker KG, Rickard M, Sinclair G, Dent OF, Chapuis PH, Bokey EL. (2003) Anastomotic leakage after curative anterior resection results in a higher prevalence of local recurrence. Br J Surg 90(10):1261-1266
59. McArdle CS, McMillan DC, Hole DJ. (2005) Impact of anastomotic leakage on long-term survival of patients undergoing curative resection for colorectal cancer. Br J Surg 92(9):1150-1154
60. Fermor B, Umpleby HC, Lever JV, Symes MO, Williamson RCN. (1986) Proliferative and Metastatic Potential of Exfoliated Colorectal-Cancer Cells. J Natl Cancer Inst 76(2):347-349
61. Balkwill F, Mantovani A. (2001) Inflammation and cancer: back to Virchow? Lancet 357(9255):539-545
62. Abramovitch R, Marikovsky M, Meir G, Neeman M. (1999) Stimulation of tumour growth by wound-derived growth factors. Br J Cancer 79(9-10):1392-1398
63. Hilmy M, Bartlett JMS, Underwood MA, McMillan DC. (2005) The relationship between the systemic inflammatory response and survival in patients with transitional cell carcinoma of the urinary bladder. Br J Cancer 92(4):625-627
64. Laurens N, Koolwijk P, De Maat MPM. (2006) Fibrin structure and wound healing. J Thromb Haemost 4(5):932-939
65. Ikari Y, Yee KO, Schwartz SM. (2000) Role of alpha 5 beta 1 and alpha v beta 3 integrins on smooth muscle cell spreading and migration in fibrin gels. Thromb Haemost 84(4):701-705
66. Hofer SOP, Shrayer D, Reichner JS, Hoekstra HJ, Wanebo HJ. (1998) Wound-induced tumor progression - A probable role in recurrence after tumor resection. Arch Surg 133(4):383-388
67. Tagliabue E, Agresti R, Carcangiu ML, Ghirelli C, Morelli D, Campiglio M, Martel M, Giovanazzi R, Greco M, Balsari A, Menard S. (2003) Role of HER2 in wound-induced breast carcinoma proliferation. Lancet 362(9383):527-533
68. Eggermont AMM, Steller EP, Sugarbaker PH. (1987) Laparotomy Enhances Intraperitoneal Tumor-Growth and Abrogates the Antitumor Effects of Interleukin-2 and Lymphokine-Activated Killer-Cells. Surgery 102(1):71-78
69. van den Tol RM, van Rossen EME, van Eijck CHJ, Bonthuis F, Marquet RL, Jeekel H. (1998) Reduction of peritoneal trauma by using nonsurgical gauze leads to less implantation metastasis of spilled tumor cells. Ann Surg 227(2):242-248
70. Zeamari S, Roos E, Stewart FA. (2004) Tumour seeding in peritoneal wound sites in relation to growth-factor expression in early granulation tissue. Eur J Cancer 40(9):1431-1440
71. Ziprin P, Ridgway PF, Pfistermuller KLM, Peck DH, Darzi AW. (2003) ICAM-1 mediated tumor-mesothelial cell adhesion is modulated by IL-6 and TNF-alpha: A potential mechanism by which surgical trauma increases peritoneal metastases. Cell Commun Adhes 10(3):141-154
72. van Rossen MEE, Hofland LJ, van den Tol MP, van Koetsveld PM, Jeekel J, Marquet RL, van Eijck CHJ. (2001) Effect of inflammatory cytokines and

growth factors on tumour cell adhesion to the peritoneum. J Pathol 193(4):530-537
73. Rowley DR. (1998) What might a stromal response mean to prostate cancer progression? Cancer Metastasis Rev 17(4):411-419
74. Hagemann T, Wilson J, Kulbe H, Li NFF, Leinster DA, Charles K, Klemm F, Pukrop T, Binder C, Balkwill FR. (2005) Macrophages induce invasiveness of epithelial cancer cells via NF-kappa B and JNK. J Immunol 175(2):1197-1205
75. Chen JJW, Lin YC, Yao PL, Yuan A, Chen HY, Shun CT, Tsai MF, Chen CH, Yang PC. (2005) Tumor-associated macrophages: The double-edged sword in cancer progression. J Clin Oncol 23(5):953-964
76. Dalgleish AG, O'Byrne K. (2006) Inflammation and Cancer: the role of the immune response and angiogenesis. In: The link between Inflammation and Cancer. Wounds that do not heal. Springer: New York, 11
77. Bhowmick NA, Neilson EG, Moses HL. (2004) Stromal fibroblasts in cancer initiation and progression. Nature 432(7015):332-337
78. Orimo A, Gupta PB, Sgroi DC, Arenzana-Seisdedos F, Delaunay T, Naeem R, Carey VJ, Richardson AL, Weinberg RA. (2005) Stromal fibroblasts present in invasive human breast carcinomas promote tumor growth and angiogenesis through elevated SDF-1/CXCL12 secretion. Cell 121(3):335-348
79. Kalluri R, Zeisberg M. (2006) Fibroblasts in cancer. Nat Rev Cancer 6(5):392-401
80. Chang HY, Nuyten DSA, Sneddon JB, Hastie T, Tibshirani R, Sorlie T, Dai HY, He YDD, van't Veer LJ, Bartelink H, van de Rijn M, Brown PO, van de Vijver MJ. (2005) Robustness, scalability, and integration of a wound-response gene expression signature in predicting breast cancer survival. Proc Natl Acad Sci U S A 102(10):3738-3743
81. Li TS, Kaneda Y, Ueda K, Hamano K, Zempo N, Esato K. (2001) The influence of tumour resection on angiostatin levels and tumour growth - an experimental study in tumour-bearing mice. Eur J Cancer 37(17):2283-2288
82. Coffey JC, Wang JH, Smith MJF, Laing A, Bouchier-Hayes D, Cotter TG, Redmond HP. (2005) Phosphoinositide 3-kinase accelerates postoperative tumor growth by inhibiting apoptosis and enhancing resistance to chemotherapy-induced apoptosis. J Biol Chem 280(22):20968-20977
83. Luzzi KJ, MacDonald IC, Schmidt EE, Kerkvliet N, Morris VL, Chambers AF, Groom AC. (1998) Multistep nature of metastatic inefficiency - Dormancy of solitary cells after successful extravasation and limited survival of early micrometastases. Am J Pathol 153(3):865-873
84. Wong CW, Lee A, Shientag L, Yu J, Dong Y, Kao G, Al-Mehdi AB, Bernhard EJ, Muschel RJ. (2001) Apoptosis: An early event in metastatic inefficiency. Cancer Res 61(1):333-338
85. Oreilly MS, Holmgren L, Shing Y, Chen C, Rosenthal RA, Moses M, Lane WS, Cao YH, Sage EH, Folkman J. (1994) Angiostatin - a Novel Angiogenesis Inhibitor That Mediates the Suppression of Metastases by a Lewis Lung-Carcinoma. Cell 79(2):315-328

86. Holmgren L, Oreilly MS, Folkman J. (1995) Dormancy of Micrometastases - Balanced Proliferation and Apoptosis in the Presence of Angiogenesis Suppression. Nat Med 1(2):149-153
87. Cao YH, O'Reilly MS, Marshall B, Flynn E, Ji RW, Folkman J. (1998) Expression of angiostatin cDNA in a murine fibrosarcoma suppresses primary tumor growth and produces long-term dormancy of metastases. J Clin Invest 101(5):1055-1063
88. Naumov GN, Bender E, Zurakowski D, Kang SY, Sampson D, Flynn E, Watnick RS, Straume O, Akslen LA, Folkman J, Almog N. (2006) A model of human tumor dormancy: An angiogenic switch from the nonangiogenic phenotype. J Natl Cancer Inst 98(5):316-325
89. Indraccolo S, Stievano L, Minuzzo S, Tosello V, Esposito G, Piovan E, Zamarchi R, Chieco-Bianchi L, Amadori A. (2006) Interruption of tumor dormancy by a transient angiogenic burst within the tumor microenvironment. Proc Natl Acad Sci U S A 103(11):4216-4221
90. Guba M, Cernaianu G, Koehl G, Geissler EK, Jauch KW, Anthuber M, Falk W, Steinbauer M. (2001) A primary tumor promotes dormancy of solitary tumor cells before inhibiting angiogenesis. Cancer Res 61(14):5575-5579
91. Retsky M, Bonadonna G, Demicheli R, Folkman J, Hrushesky W, Valagussa P. (2004) Hypothesis: Induced angiogenesis after surgery in premenopausal node-positive breast cancer patients is a major underlying reason why adjuvant chemotherapy works particularly well for those patients. Breast Cancer Res 6(4):R372-R374
92. Camphausen K, Moses MA, Beecken WD, Khan MK, Folkman J, O'Reilly MS. (2001) Radiation therapy to a primary tumor accelerates metastatic growth in mice. Cancer Res 61(5):2207-2211
93. Baum M, Demicheli R, Hrushesky W, Retsky M. (2005) Does surgery unfavourably perturb the "natural history" of early breast cancer by accelerating the appearance of distant metastases? Eur J Cancer 41(4):508-515
94. van den Tol MP, Haverlag R, van Rossen MEE, Bonthuis F, Marquet RL, Jeekel J. (2001) Glove powder promotes adhesion formation and facilitates tumour cell adhesion and growth. Br J Surg 88(9):1258-1263
95. Allendorf JDF, Bessler M, Horvath KD, Marvin MR, Laird DA, Whelan RL. (1998) Increased tumor establishment and growth after open vs laparoscopic bowel resection in mice. Surg Endoscopy 12(8):1035-1038
96. Allendorf JDF, Bessler M, Horvath KD, Marvin MR, Laird DA, Whelan RL. (1999) Increased tumor establishment and growth after open vs laparoscopic surgery in mice may be related to differences in postoperative T-cell function. Surg Endoscopy 13(3):233-235
97. Carter JJ, Feingold DL, Kirman I, Oh A, Wildbrett P, Asi Z, Fowler R, Huang E, Whelan RL. (2003) Laparoscopic-assisted cecectomy is associated with decreased formation of postoperative pulmonary metastases compared with open cecectomy in a murine model. Surgery 134(3):432-436
98. Basha G, Ghirardi M, Geboes K, Yap SH, Penninckx F. (2000) Limitations of peritoneal lavage with antiseptics in prevention of recurrent colorectal cancer

caused by tumor-cell seeding - Experimental study in rats. Dis Colon Rectum 43(12):1713-1718
99. Lee SW, Gleason NR, Bessler M, Whelan RL. (1999) Peritoneal irrigation with povidone-iodine solution after laparoscopic-assisted splenectomy significantly decreases port-tumor recurrence in a murine model. Dis Colon Rectum 42(3):319-326
100. Docherty JG, McGregor JR, Purdie CA, Galloway DJ, Odwyer PJ. (1995) Efficacy of Tumoricidal Agents in-Vitro and in-Vivo. Br J Surg 82(8):1050-1052
101. Huguet EL, Keeling NJ. (2004) Distilled water peritoneal lavage after colorectal cancer surgery. Dis Colon Rectum 47(12):2114-2119
102. Yamamoto K, Shimada S, Hirota M, Yagi Y, Matsuda M, Baba H. (2005) EIPL (extensive intraoperative peritoneal lavage) therapy significantly reduces peritoneal recurrence after pancreatectomy in patients with pancreatic cancer. Int J Oncol 27(5):1321-1328
103. Hribaschek A, Kuhn R, Pross M, Meyer F, Fahlke J, Ridwelski K, Boltze C, Lippert H. (2006) Intraperitoneal versus intravenous CPT-11 given intra- and postoperatively for peritoneal carcinomatosis in a rat model. Surg Today 36(1):57-62
104. Abaza R, Keck RW, Selman SH. (2006) Intraperitoneal chemotherapy for the prevention of transitional cell carcinoma implantation. J Urol 175(6):2317-2322
105. Nordlinger B, Rougier P, Arnaud JP, Debois M, Wils J, Ollier JC, Grobost O, Lasser P, Wals J, Lacourt J, Seitz JF, dos Santos JG, Bleiberg H, Mackiewickz R, Conroy T, Bouche O, Morin T, Baila L, van Cutsem E, Bedenne L. (2005) Adjuvant regional chemotherapy and systemic chemotherapy versus systemic chemotherapy alone in patients with stage II-III colorectal cancer: a multicentre randomised controlled phase III trial. Lancet Oncol 6(7):459-468
106. Chiba T, Yokosuka O, Fukai K, Kojima H, Tada M, Arai M, Imazeki F, Saisho H. (2004) Cell growth inhibition and gene expression induced by the histone deacetylase inhibitor, trichostatin A, on human hepatoma cells. Oncology 66(6):481-491
107. Greten FR, Eckmann L, Greten TF, Park JM, Li ZW, Egan LJ, Kagnoff MF, Karin M. (2004) IKK beta links inflammation and tumorigenesis in a mouse model of colitis-associated cancer. Cell 118(3):285-296
108. Montel V, Kleeman J, Agarwal D, Spinella D, Kawai K, Tarin D. (2004) Altered metastatic behavior of human breast cancer cells after experimental manipulation of matrix metalloproteinase 8 gene expression. Cancer Res 64(5):1687-1694
109. Weber KL, Doucet M, Price JE, Baker C, Kim SJ, Fidler IJ. (2003) Blockade of epidermal growth factor receptor signaling leads to inhibition of renal cell carcinoma growth in the bone of nude mice. Cancer Res 63(11):2940-2947
110. Roh JL, Sung MW, Park SW, Heo DS, Lee DW, Kim KH. (2004) Celecoxib can prevent tumor growth and distant metastasis in postoperative setting. Cancer Res 64(9):3230-3235

111. Roh JL, Sung MW, Kim KH. (2005) Suppression of accelerated tumor growth in surgical wounds by celecoxib and indomethacin. Head Neck 27(4):326-332
112. Connolly EM, Harmey JH, O'Grady T, Foley D, Roche-Nagle G, Kay E, Bouchier-Hayes DJ. (2002) Cyclo-oxygenase inhibition reduces tumour growth and metastasis in an orthotopic model of breast cancer. Br J Cancer 87(2):231-237
113. Da Costa ML, Redmond HP, Bouchier-Hayes DJ. (2001) Taurolidine improves survival by abrogating the accelerated development and proliferation of solid tumors and development of organ metastases from circulating tumor cells released following surgery. J Surg Res 101(2):111-119
114. Brivio F, Lissoni P, Rovelli F, Nespoli A, Uggeri F, Fumagalli L, Gardani G. (2002) Effects of IL-2 preoperative immunotherapy on surgery-induced changes in angiogenic regulation and its prevention of VEGF increase and IL-12 decline. Hepatogastroenterol 49(44):385-387
115. Helguera G, Rodriguez JA, Penichet ML. (2006) Cytokines fused to antibodies and their combinations as therapeutic agents against different peritoneal HER2/neu expressing tumors. Mol Cancer Ther 5(4):1029-1040
116. Alkhamesi NA, Ziprin P, Pfistermuller K, Peck DH, Darzi AW. (2005) ICAM-1 mediated peritoneal carcinomatosis, a target for therapeutic intervention. Clin Exp Metastasis 22(6):449-459
117. Pross M, Lippert H, Misselwitz F, Nestler G, Kruger S, Langer H, Halangk W, Schulz HU. (2003) Low-molecular-weight heparin (reviparin) diminishes tumor cell adhesion and invasion in vitro, and decreases intraperitoneal growth of colonadeno-carcinoma cells in rats after laparoscopy. Thrombosis Res 110(4):215-220
118. Jansen M, Jansen PL, Otto J, Kirtil T, Neuss S, Treutner KH, Schumpelick V. (2006) The inhibition of tumor cell adhesion on human mesothelial cells (HOMC) by phospholipids in vitro. Langenbecks Arch Surg 391(2):96-101
119. Takatsuki H, Komatsu S, Sano R, Takada Y, Tsuji T. (2004) Adhesion of gastric carcinoma cells to peritoneum mediated by alpha 3 beta 1 integrin (VLA-3). Cancer Res 64(17):6065-6070
120. Heyder C, Gloria-Maercker E, Hatzmann W, Niggemann B, Zanker KS, Dittmar T. (2005) Role of the beta(1)-integrin subunit in the adhesion, extravasation and migration of T24 human bladder carcinoma cells. Clin Exper Metastasis 22(2):99-106
121. Arlt MJE, Novak-Hofer I, Gast D, Gschwend V, Moldenhauer G, Grunberg J, Honer M, Schubiger PA, Altevogt P, Kruger A. (2006) Efficient inhibition of intra-peritoneal tumor growth and dissemination of human ovarian carcinoma cells in nude mice by anti-L1-cell adhesion molecule monoclonal antibody treatment. Cancer Res 66(2):936-943
122. Lamagna C, Hodivala-Dilke KM, Imhof BA, Aurrand-Lions M. (2005) Antibody against junctional adhesion molecule-C inhibits angiogenesis and tumor growth. Cancer Res 1;65(13):5703-5710

Pseudomyxoma Peritonei Syndrome: Classification of Appendiceal Mucinous Tumours

RK Pai, TA Longacre

"Mucocele of the appendix is an uncommon but mysterious condition about which a large volume of literature has accumulated without much clarification of the underlying pathology….The mystery deepens when 'pseudomyxoma peritonei', which is a complication of mucocele of the appendix, is considered" [1].

Introduction

Pseudomyxoma peritonei is an overutilized and underspecified condition that has garnered much attention in the historic literature. In recent years, this condition has been convincingly linked to appendiceal mucinous neoplasms, yet there has been insufficient and often conflicting attention to the histologic characteristics and classification of these neoplasms. This chapter provides a coherent approach to the diagnosis and classification of appendiceal mucinous tumours and the peritoneal implants associated with the pseudomyxoma peritonei syndrome. Guidelines for the diagnosis and management of patients with these conditions that more closely reflect and predict their biologic behavior are proposed [2].

'Mucocele'

Appendiceal mucoceles are uncommon entities, arising in association with a variety of underlying pathologic processes, only a subset of which are associated with the subsequent development of pseudomyxoma peritonei. Although there are a variety of historical connotations associated with the appendiceal mucocele, strictly speaking 'mucocele' of the appendix denotes a dilatation of the appendiceal lumen, with or without overt obstruction, due to abnormal accumulation of mucus, which may be related to a variety of neoplastic and non-neoplastic epithelial and non-epithelial processes. The term is a gross or macroscopic description, not a histopathologic diagnosis and although of value as a descriptor, it is of limited diagnostic value. Since, like a lot of terminology in common usage in medicine, it is not likely to disappear anytime soon, the term 'mucocele' should be reserved for

gross, clinical or macroscopic descriptive purposes only, and always with the understanding that it is the pathologist's task to determine the underlying cause or process associated with the development of the mucocele, because it is that process which constitutes the pathologic diagnosis.

The epithelial processes most commonly associated with mucoceles include: mucinous hyperplasia (hyperplastic polyp), serrated adenoma, mucinous adenoma or cystadenoma, mucinous neoplasm of uncertain malignant potential, mucinous neoplasm of low malignant potential (the epithelial neoplasm most commonly associated with pseudomyxoma peritonei), and mucinous adenocarcinoma (less commonly associated with pseudomyxoma peritonei, this epithelial neoplasm most closely resembles mucinous carcinoma of the colon, not otherwise specified). Non-epithelial processes are relatively uncommon causes of 'mucocele' and comprise a variety of inflammatory, post-inflammatory and/or obstructive lesions, which chiefly include appendicitis and fecaliths [3-5]. In most mucoceles associated with inflammatory or obstructive processes, the appendiceal mucosa is markedly thinned and denuded with apparent crypt atrophy and the appendix is usually only minimally dilated (usually < 2.0 cm). There are no hyperplastic or neoplastic changes in the residual epithelium or on extensive sectioning of areas removed from the area of dilatation. Although there may be mucin dissection into the appendiceal wall or even focally present along the serosal surface, there are no epithelial cells associated with the mucin dissection. In most instances, the mucin is microscopic, but occasionally it may form a localized collection in the right lower quadrant.

Mucosal Hyperplasia and Hyperplastic Polyp

- Uncommon cause of 'mucocele'*
- Sessile = mucosal hyperplasia; polypoid = hyperplastic polyp
- Cytoarchitectural features similar to hyperplastic polyps elsewhere in GI tract
- Asymptomatic; often incidental finding
- Appendix minimally dilated; may have mucin extravasation
- Clinically benign

*Mucocele with or without overt obstruction, due to dilatation of the appendix, with or without overt obstruction. As such, mucocele may occur secondary to neoplastic or non-neoplastic processes, which may be epithelial or non-epithelial.

Mucosal hyperplasia and hyperplastic polyps are an extremely uncommon cause of appendiceal 'mucocele' are usually asymptomatic and are typically detected as incidental findings in appendices removed during hemicolectomy procedures for cecal adenocarcinoma or during unrelated gynecological surgical procedures in women [6-9]. When hyperplastic proliferations are associated with a so-called 'mucocele', the appendix is generally only minimally dilated. Hyperplastic proliferations may be sessile (mucosal hyperplasia) or polypoid (hyperplastic

polyp). Microscopically, mucosal hyperplasia and hyperplastic polyps appear similar to hyperplastic polyps encountered elsewhere in the large intestine, but they typically have a more serrated appearance (Fig. 1). A connection between appendiceal mucosal hyperplasia/hyperplastic polyps and mixed hyperplastic/-adenomatous polyps or serrated adenomas has been suggested [10]. Because of their overlapping histologic features, the distinction between hyperplastic polyp on the one hand, and serrated adenomas and mucinous adenomas on the other can be especially difficult [3,11]. Indeed, the early literature describing 'mucoceles' of the appendix contains many examples of mucinous neoplasms, which were initially reported as mucinous hyperplasia. Because of the problems associated with this histologic overlap and the potential risk of developing pseudomyxoma peritonei in association with appendiceal mucinous neoplasms, the diagnosis of a hyperplastic polyp (or mucosal hyperplasia) in the appendix should be strictly limited to unequivocally benign, hyperplastic processes.

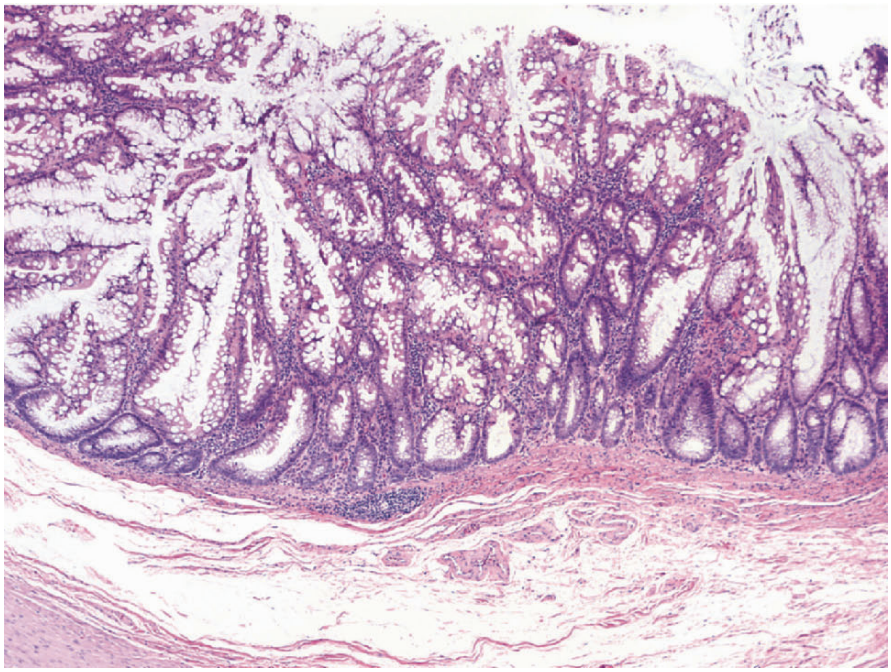


Figure 1. Mucosal hyperplasia/hyperplastic polyp in the appendix. The superficial mucosa exhibits serrated architecture and distended goblet cells with minimal or no cytologic atypia, similar to hyperplastic polyps in the colon

Only focal proliferations with small, basally located, uniformly bland nuclei without hyperchromasia or stratification, should be classified as "hyperplastic" when they occur in the appendix.

If these changes are present in a diffuse fashion or are associated with a villiform or papillary architecture or the presence of even minimal cytologic atypia,

consideration should be given to the diagnosis of a mucinous neoplasm (i.e., mucinous adenoma, mucinous neoplasm of uncertain malignant potential, or mucinous neoplasm of low malignant potential) or serrated adenoma. Coexistence of hyperplastic-type mucosa with mucinous adenomatous mucosa is not infrequent in the appendix and all appendices harboring a hyperplastic mucosa should be carefully examined in order to exclude the presence of an adenomatous component [7,12,13].

Serrated Adenoma (Mixed Hyperplastic-Adenomatous Polyp)

There is a relatively high proportion of polyps in the appendix and right colon with hybrid hyperplastic-adenomatous features, many of which conform to the diagnostic criteria for serrated adenomas [14]. These lesions are characterized by serrated architecture on low magnification, often with a villous surface configuration, and cells with eosinophilic cytoplasm with prominent interspersed goblet cells, some of which may be dystrophic. In contrast to hyperplastic polyps, mitoses may be present in the upper zones of the crypts. Although cytologic atypia may be minimal, in most cases, elongation of the nuclei with at least focal pseudostratification is present, allowing distinction from mucosal hyperplasia and hyperplastic polyps. These lesions are dysplastic and precancerous, like the more common adenomas encountered in the gastrointestinal tract.

The distinction between serrated adenomas and mucinous neoplasms of low malignant potential (see below) may be difficult as both lesions may have a villiform architecture with luminal serration. The diagnosis of a serrated adenoma implies a benign neoplasm with no risk of recurrence following complete excision while the diagnosis of a mucinous neoplasm of low malignant potential implies a neoplasm with a definite risk of recurrence. In our opinion, the term “serrated adenoma” should be reserved for those tumours with prominent glandular serration identified at low power that are clearly confined to the appendix and are completely excised with an uninvolved proximal margin. At present, there is no known association of serrated adenomas with pseudomyxoma peritonei; however, this requires further investigation. A recent study identified 10 serrated adenomas in the appendix, defined in this series solely by the presence of greater than 50% of dysplastic epithelium containing a saw-tooth pattern, among 38 non-carcinoid polyps of the appendix [15]. Of the 10 serrated adenomas in this series, four were associated with invasive carcinoma highlighting the neoplastic nature of these lesions. Jass and colleagues have suggested a serrated pathway of colonic carcinogenesis with a large proportion of neoplastic serrated lesions showing microsatellite instability due to defective DNA mismatch repair [16,17]. However, to our knowledge, serrated adenomas of the appendix have not been evaluated for microsatellite instability. Regardless, non-invasive lesions can be cured by complete removal with clear margins. As with mucinous adenomas, it is important that the pathologist

section the entire appendix to ensure that there are no undetected areas of histologic malignancy.

Mucinous Adenoma (Mucinous Cystadenoma)

- Common cause of 'mucocele'*
- Simple or focally stratified columnar epithelium with goblet cells (may be cuboidal or flat due to compression). Mild to moderate cytologic atypia. Mitotic figures present, but not atypical.
- Epithelium does not penetrate muscular wall and not present in extra-appendiceal mucin
- Sessile, circumferential involvement of mucosa
- Appendix often dilated
- Perforation associated with mucin extravasation
- Clinically benign, does not recur after complete excision

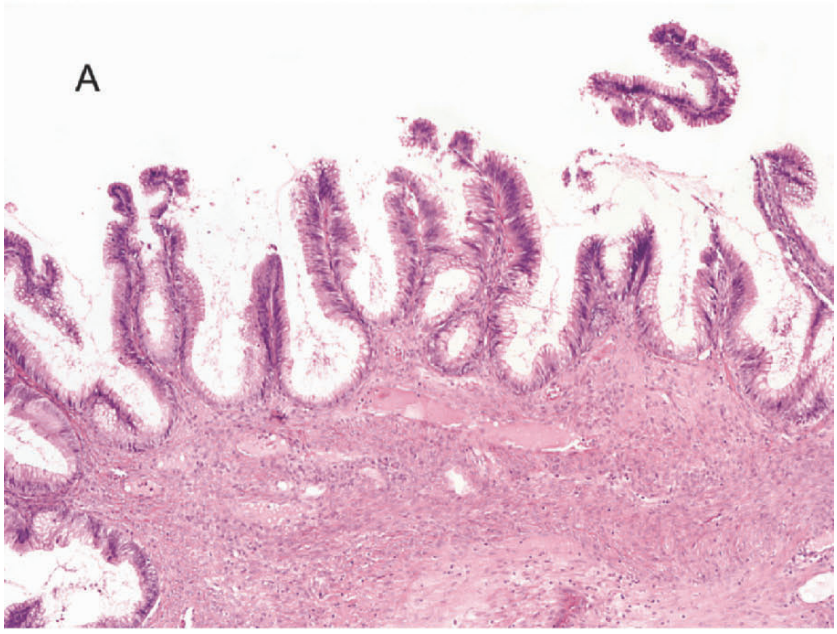
Note: When these criteria are utilized to classify appendiceal epithelial neoplasms, adenomas of colonic type are very uncommon. *Mucocele with or without overt obstruction, due to dilatation of the appendix, with or without overt obstruction. As such, mucocele may occur secondary to neoplastic or non-neoplastic processes, which may be epithelial or non-epithelial.

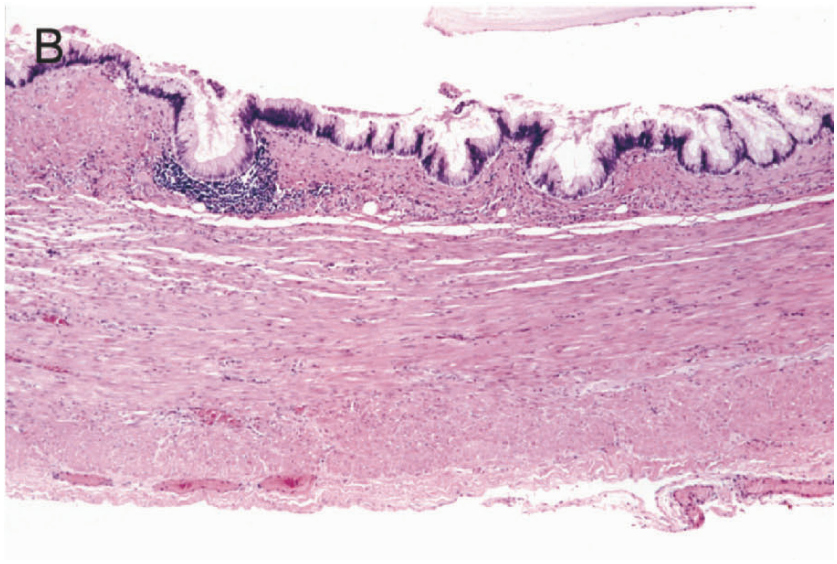
Mucinous cystadenomas have been historically described as neoplastic lesions analogous to adenomas occurring elsewhere in the gastrointestinal tract, but possessing a different appearance due to unique growth constraints in the appendix. However, with accumulating experience, it has become apparent that mucinous adenomas of appendiceal origin are unique not only by virtue of their site of origin, but by virtue of a variety of immunophenotypic and molecular genetic features [18-21]. Although the terms adenoma and cystadenoma (these two terms are interchangeable) are used in different ways in the gastrointestinal and gynecologic pathology literature, they are strictly utilized here to refer to a neoplastic process which, once completely excised, is benign and does not recur.

Symptoms are absent or nonspecific in 25-50% of cases, but patients with mucinous adenomas may present with acute appendicitis, a palpable mass, torsion, acute or chronic right lower quadrant abdominal pain, or intussusception. The lesions may be initially identified as a filling defect in the caecum with non-visualization of the appendix [22]. When present, focal calcification forming a rim around the area of the dilated appendix is frequently noted on routine radiologic imaging studies. Occasionally, aggregates of opaque pearl-like globules consisting of acellular laminated mucin surrounding an amorphous granular and mucinous core may be identified in the appendiceal lumen or peri-appendiceal tissue at surgery, a condition descriptively referred to as myxoglobulosis [23].

Microscopically, mucinous adenomas are typically sessile lesions, which circumferentially involve the appendiceal mucosal surface and are composed of mucin-rich epithelium demonstrating an undulating or villous growth pattern (Fig. 2). Luminal dilatation may extend to 6.0 cm and perforation is present in approximately 20% of cases. Cytologic atypia is mild to moderate at most and there may be focal stratification of nuclei, but unlike typical adenomas in the colon, this is often not a prominent feature. The villous lesions characteristically demonstrate maturation at the tips of the villi, similar to that seen in mucinous tumours of the ovary. Although the epithelium is usually columnar, the extensive mucin accumulation can create pressure atrophy and the cells may be cuboidal or even extensively flattened (Fig. 2). Perforation is usually associated with mucin dissection and localized collections of mucus attached to the serosa or lying free within the peritoneal cavity. The extravasated mucin often incites an inflammatory reaction with fibrosis, granulation tissue, chronic inflammation and dystrophic calcification. By definition, the epithelium does not infiltrate or invade into the muscular wall; nor is it present on the appendiceal serosa or in the extra-appendiceal mucin. In up to one-half of cystadenomas, the muscularis mucosa may be markedly attenuated or even absent due to apparent compression and fibrosis of the submucosa, making it difficult to determine with confidence whether or not there is mural invasion. Loss of the normal complement of tissue is also a common feature and this can create further distortion of normal structures. In these instances, it is important to section the entire appendix to ensure that there are no undetected areas of histologic malignancy (diagnosed on the basis of cytologic or architectural features or the presence of invasion) or epithelium in the extra-appendiceal mucin. The presence of either of these features warrants a diagnosis of mucinous neoplasm of at least low malignant potential.

Mucinous adenomas of the appendix, so defined, are cured by appendectomy, provided the resection margin at the base of the appendix is free of involvement [9,22]. Patients with appendiceal adenoma or cystadenoma should be evaluated for the presence of lesions elsewhere in the colon, due to the strong association with synchronous or metachronous colorectal adenoma or carcinoma [22,24]. Because of the observed occurrence of synchronous appendiceal and ovarian mucinous tumours, consideration should also be given to an evaluation of the ovaries in women who present with appendiceal adenomas [25,26].


A


B

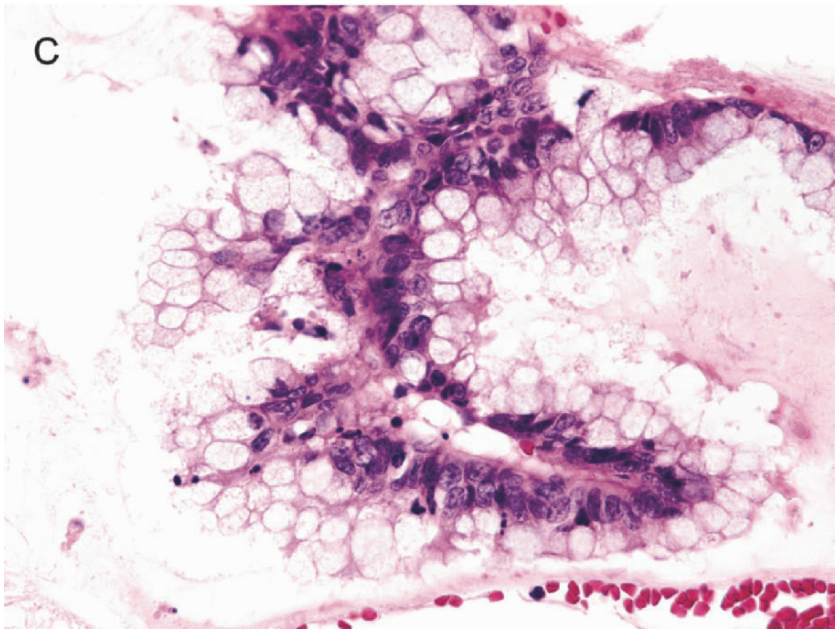


Figure 2. Mucinous adenoma of the appendix. The mucosa is often villous (A), but may be flattened and attenuated secondary to intraluminal pressure (B). The mucosal villi are lined by tall columnar epithelium with prominent intracytoplasmic mucin and minimal to moderate nuclear atypia (C)

Sometimes, the distinction between a mucinous adenoma and a mucinous neoplasm of low malignant potential is not straightforward. Since the diagnosis of a mucinous adenoma implies a benign neoplasm with no risk of recurrence following complete excision and the diagnosis of a mucinous neoplasm of low malignant potential implies a neoplasm with a definite risk of recurrence despite attempted complete excision, this differential diagnostic problem is non-trivial. In our opinion, the term adenoma should be strictly reserved for those cytologically bland mucinous tumours that are clearly confined to the appendix and are completely excised (i.e., the proximal margin is uninvolved by the neoplastic process and there is no involvement of the appendiceal serosa or peritoneal contents by neoplastic epithelium). This would include cases in which acellular mucin extravasation has occurred, but there is no invasion or infiltration into the appendiceal wall, provided there is no significant cytologic atypia or complex glandular structures associated with the appendiceal adenoma. Given the prognostic implications of the margin status of appendectomy specimens, the complete surgical margin of the appendix should be evaluated for the presence of neoplastic epithelium. In addition, if a tumour has features of a low-grade mucinous neoplasm, the presence of invasion of neoplastic epithelium or carcinoma-like areas affects the diagnosis and has significant prognostic implications. Therefore, we recommend that appendices containing mucinous neoplasms should be entirely submitted for histologic

examination. In those cases in which the appendix has been obliterated by a mucinous mass, it is also appropriate to entirely submit the specimen for histologic examination to evaluate for the presence of neoplastic epithelium

In some instances, the adenomatous epithelium is extensively flattened or atrophic in appearance due to the pressure of the accumulated mucin and in these instances, the adenoma may go undetected. This is of no significant clinical consequence as long as the entire lesion has been surgically excised and it meets all the criteria enumerated above. However, the diagnosis of mucinous adenoma should be avoided in cases in which the proximal margin is involved or in which there is mucin with epithelium within the appendiceal wall or when it is unclear whether there is epithelium in the extravasated mucin, despite the presence of innocuous-appearing epithelium lining the appendiceal mucosa. In all cases in which the adequacy of excision is undetermined, the term uncertain malignant potential should be utilized and whenever possible, the surgeon should be urged to attempt a complete excision, even if this requires additional surgery.

Mucinous Neoplasm of Uncertain Malignant Potential (M-UMP)

- Cytoarchitectural features of mucinous adenoma, but
- Proximal margin of appendectomy specimen involved, or
- Mucin with epithelium within the appendiceal wall, but not clearly invasive, or
- Any uncertainty exists whether there is epithelium within extra-appendiceal mucin
- Clinical behavior uncertain: small percentage go on to develop recurrences

If, after complete evaluation of the appendectomy specimen, the biologic potential of a mucinous neoplasm is difficult to predict, the term “mucinous neoplasm of uncertain malignant potential” is an appropriate diagnosis. This designation should only be used for extremely well differentiated (cytologically bland) mucinous neoplasms that extend through the appendiceal wall but are not clearly associated with infiltrative and destructive invasion; the term reflects the difficulty in determining invasion in this setting (Fig. 3). The appendiceal wall is frequently distorted by mucinous distention in adenomas as well as in low malignant potential neoplasms, and mucinous epithelium in both lesions can be found pushing into the wall, often herniating along sites of apparent diverticula [27]. Since both lesions typically contain cytologically bland epithelium and a simple villous or flattened architecture, the degree of dysplasia or architectural complexity is often not a helpful distinguishing criterion. In all such difficult cases, extensive sampling and microscopic examination of the appendiceal neoplasm is essential.

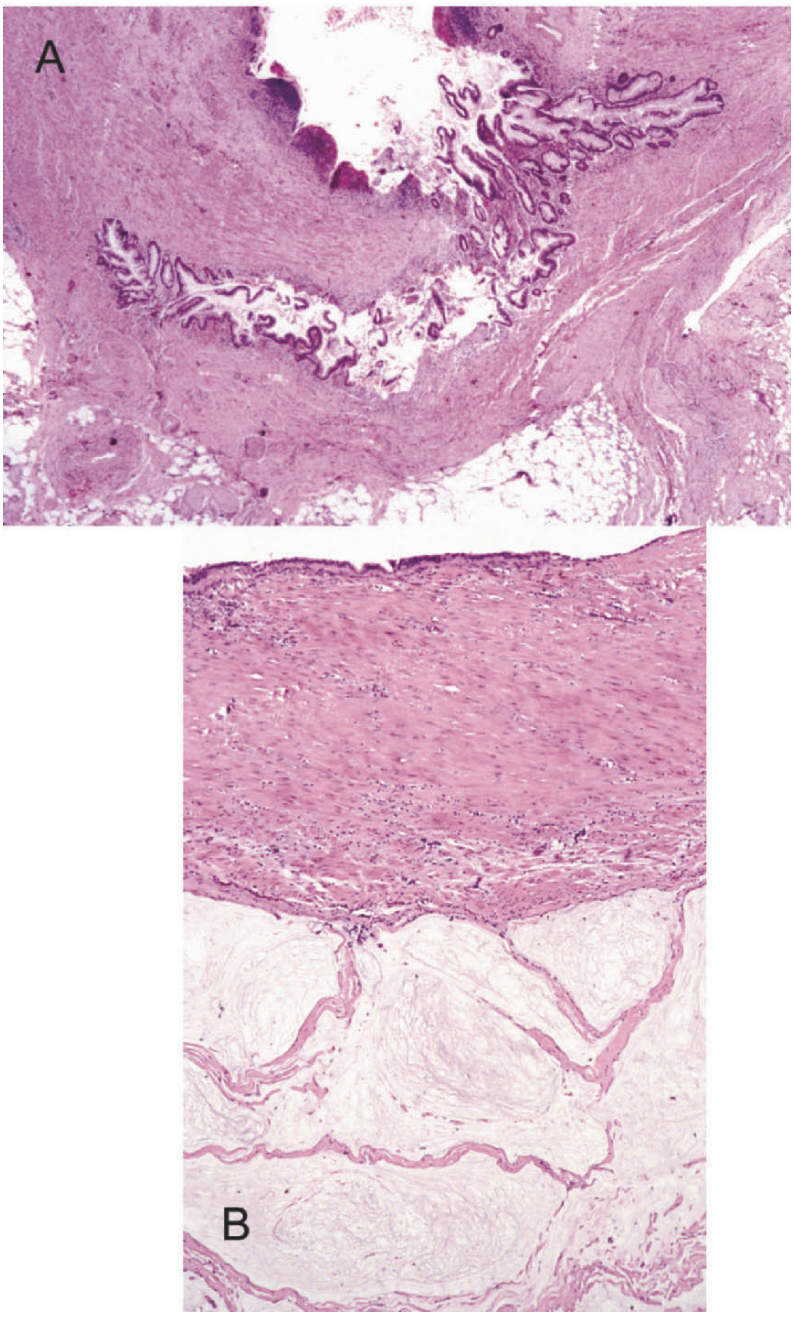


Figure 3. Mucinous tumour of uncertain malignant potential in the appendix (M-UMP). (A) This appendiceal epithelial neoplasm exhibits the cytologic and architectural characteristics of mucinous adenoma but features intramural glandular epithelium. (B) Mucinous

The presence or absence of mucin on the external surface of the appendix should be documented, because of the risk for pseudomyxoma peritonei, should there be undetected epithelial cells present.

Although the diagnosis of mucinous tumour of uncertain malignant potential was associated with an exceptionally good prognosis in one series, follow-up was relatively limited [3]. In our experience, several patients with appendiceal lesions meeting the criteria for mucinous tumours of uncertain malignant potential have developed late recurrences which eventually progressed to extensive intraperitoneal disease. For this reason, we err on the side of caution in the interpretation and recommended treatment of these more problematic cases. Despite the reported improved survival rates of patients with pseudomyxoma peritonei compared to those with peritoneal carcinomatosis, early diagnosis and aggressive surgery prior to development of the full-blown pseudomyxoma peritonei syndrome continue to offer the best chance for survival for these patients.

Mucinous Neoplasm of Low Malignant Potential (M-LMP)

- Most common cause of 'pseudomyxoma peritonei'
- Cytoarchitectural features similar to mucinous adenoma, but
- Neoplastic cells penetrate appendiceal wall and are present in peritoneal implants
- Implants secrete voluminous, thick mucinous material
- Extensive peritoneal disease may be present, but
- No lymph node, lung or liver metastases
- Protracted clinical course, with multiple recurrences; prognosis improved by surgical debulking

These neoplasms are responsible for the majority of cases of pseudomyxoma peritonei. Macroscopically, they are indistinguishable from mucinous adenomas; when localized, they present with signs and symptoms of a dilated appendix and may be adherent to surrounding structures. Microscopically, the individual cells do not differ significantly from those of the mucinous adenoma, but in the neoplasm of low malignant potential, neoplastic cells penetrate the appendiceal wall and spread beyond the appendix in the form of peritoneal implants and ovarian involvement. Although invasion through the wall with reactive desmoplasia has been offered as a diagnostic feature for mucinous carcinoma in the appendix, we agree with Carr and coworkers that the presence of a desmoplastic response is not a very useful feature in the evaluation of appendiceal mucinous neoplasms [3,28].

adenomas with prominent extra-appendiceal mucin are also candidates for this diagnosis, especially when it is uncertain whether or not the lesion and the mucin accumulation are completely excised

The key finding is the presence of epithelium outside the appendix and in association with peritoneal implants (Fig. 4).

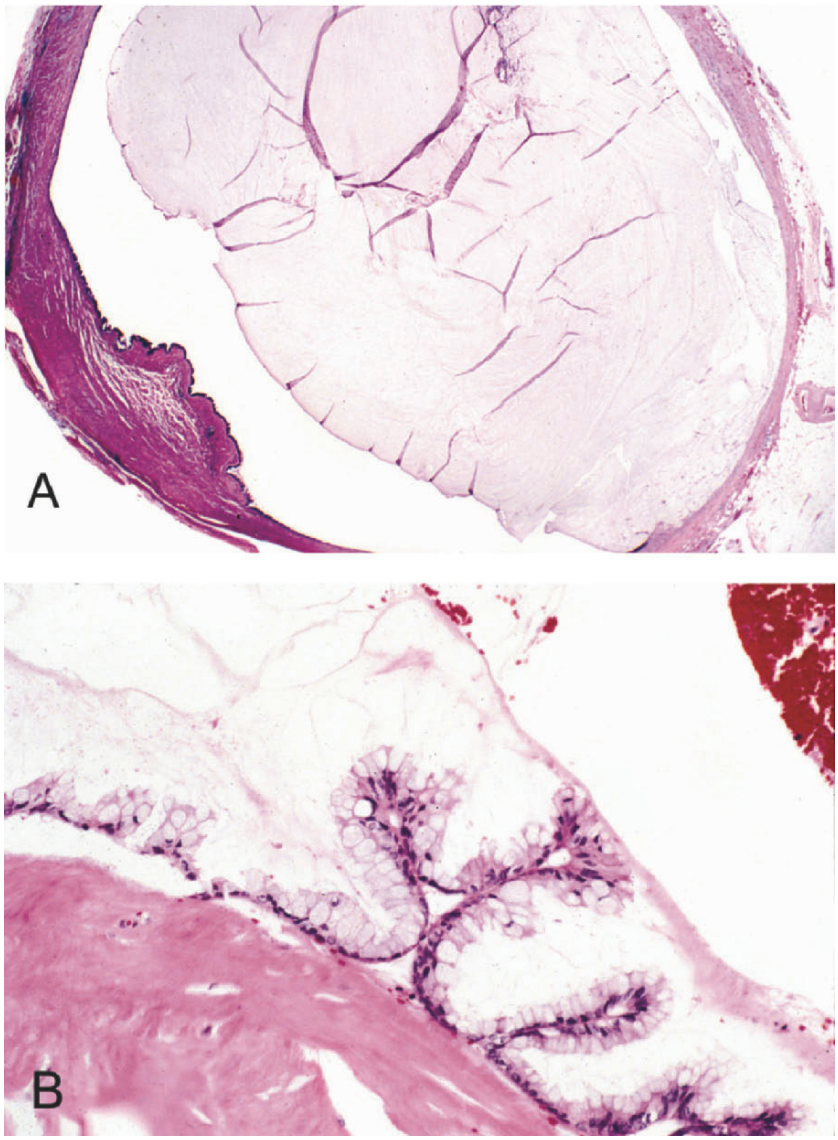


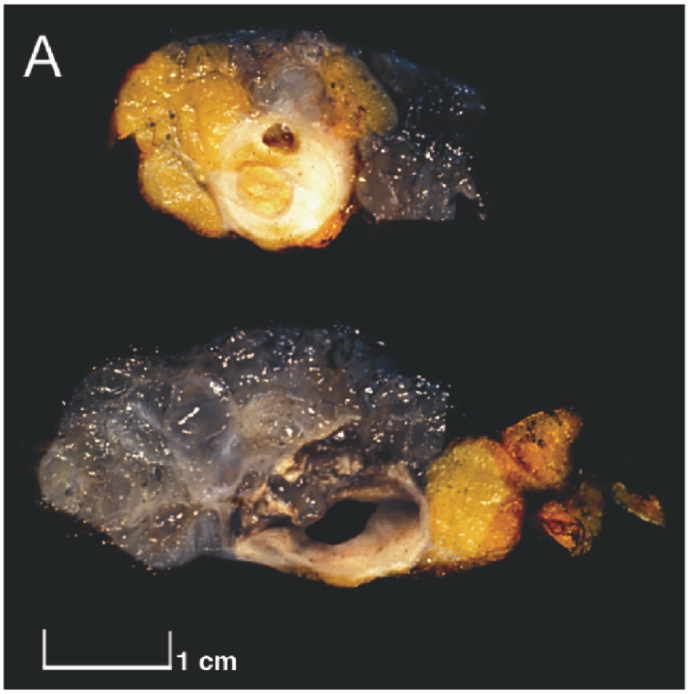
Figure 4. Mucinous neoplasm of low malignant potential (M-LMP). (A) The lumen is dilated and there is extensive denudation of the epithelium due to accumulation of mucinous material. The residual mucosa is flattened, but there is mucinous dissection through the appendiceal wall. (B) In this case, the presence of epithelium in the extra-appendiceal mucin is diagnostic of a neoplasm of low malignant potential (low-grade appendiceal mucinous neoplasm)

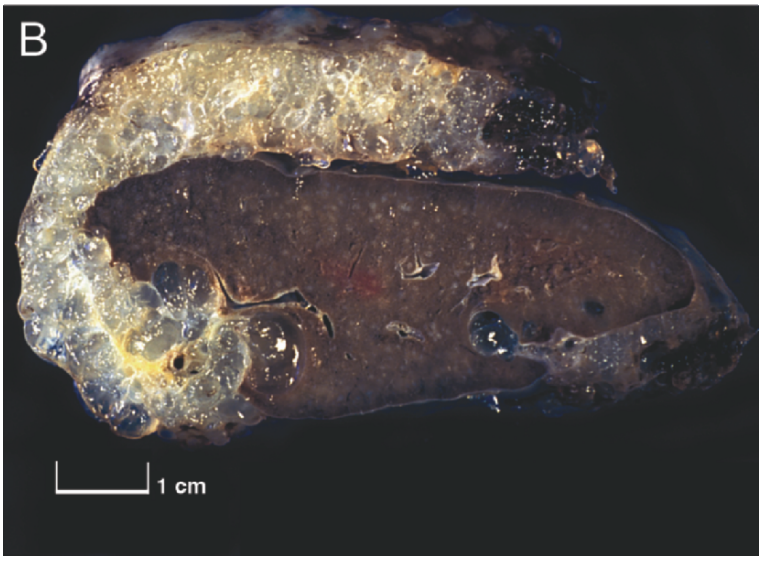
The implants remain, for the most part, intraperitoneal. In the fully developed state, these implants secrete voluminous, thick mucinous material and the abdomen becomes distended, creating the clinical condition known as pseudomyxoma peritonei. Surface implants occur on the spleen, liver and in women, the ovaries; a substantial number of female patients present with large mucinous ovarian cysts, which for many years were interpreted as independent primary tumours or even the primary tumours themselves (Fig. 5).

Spread to the uterus and fallopian tube with partial replacement of the tubal mucosa may also occur. Although some debate continues concerning the site of origin [29] and a field abnormality is not completely without merit [30], an appendiceal lesion should always be excluded in any woman presenting with a mucinous ovarian tumour or with the clinical picture of pseudomyxoma peritonei. Similarly, a cystic ovarian tumour should always be carefully excluded in women with an appendiceal adenoma or neoplasm of low malignant potential.

The clinical presentation of mucinous neoplasms of low malignant potential (M-LMP) is similar to that of the mucinous adenoma and is not distinctive; patients most frequently present with acute appendicitis or a vague abdominal mass. The correct diagnosis is almost never made pre-operatively and the diagnosis is established intra-operatively in less than one-third of the cases. M-LMP of the appendix often have a protracted clinical course, with death from disease occurring many years after initial diagnosis. In one series, just over half of patients died of disease after 10 years [31]. Death results because of extensive peritoneal disease with fibrosis and progressive loss of intestinal function and obstruction, not as a result of lymph node, liver or lung metastases. This clinical behavior is distinct from that which is typically associated with adenomas and for this reason, this terminology should be avoided whenever an appendiceal tumour is encountered that is likely to behave in such a fashion. In absence of overt cytoarchitectural features of carcinoma, the term mucinous carcinoma should also be avoided in order to distinguish these low grade lesions from mucinous carcinomas that arise elsewhere in the gastrointestinal tract and are associated with a much more aggressive clinical course, often with lung, liver and lymph node metastases. This distinction is extremely important, since the surgery and therapy are significantly different for these two processes.

Some have proposed the term low-grade appendiceal mucinous neoplasm (LAMN) for tumours that demonstrate low-grade cytologic atypia and minimal architectural complexity regardless of whether the tumour is confined to the mucosa of the appendix (which we would term mucinous adenoma) or has extra-appendiceal spread of neoplastic epithelium (which we term mucinous neoplasm of low malignant potential) [31]. Although we agree that tumours confined to the mucosa and those tumours with extra-appendiceal spread are histologically identical, mucinous neoplasms with extra-appendiceal spread of neoplastic epithelium are associated with considerable morbidity and mortality in contrast to those tumours that are organ-confined.


A
1 cm


B
1 cm

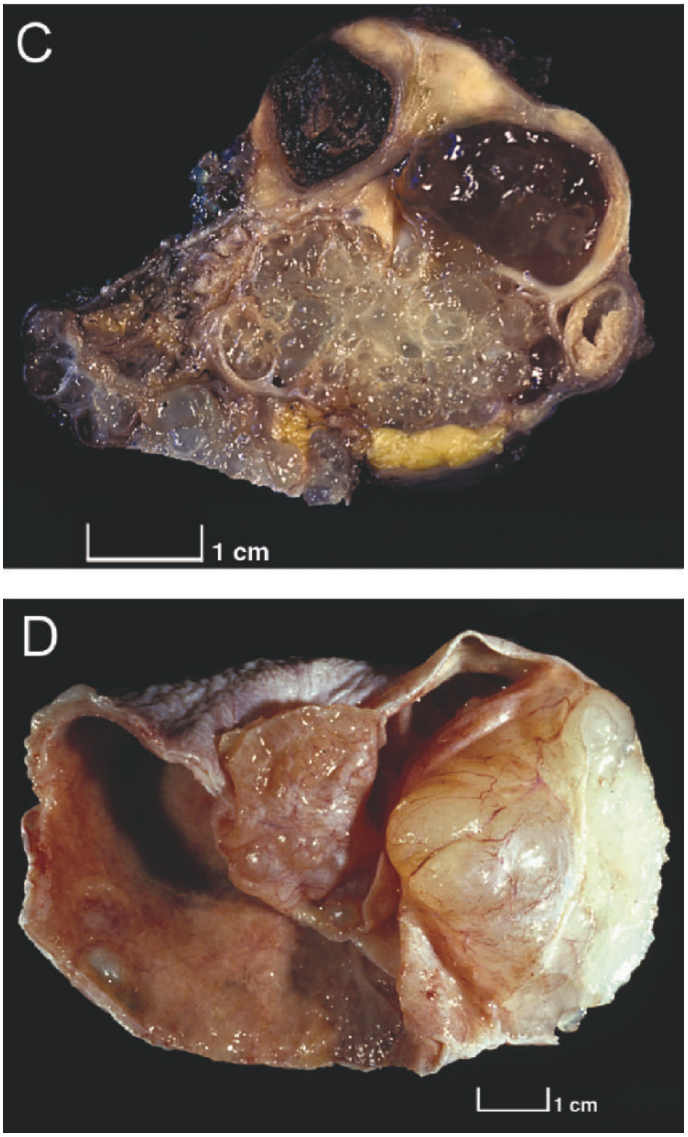


Figure 5. M-LMP in the appendix with mucinous ascites and ovarian and perisplenic implants (pseudomyxoma peritonei syndrome). (A) The appendix is only minimally dilated. Thick viscous, mucinous material is present within the lumen and periappendiceal tissue. (B) The spleen is surrounded and compressed by loculated mucinous material, but there is no parenchymal invasion. (C) The ovary is partially replaced by mucin. Preserved parenchyma contains a small corpus luteum. (D) Ovary is massively replaced by multiloculated mucinous cysts simulating a primary ovarian neoplasm. (Photomicrographs A-C courtesy of Dr. Christina Kong)

For this reason, we advocate separating low-grade mucinous neoplasms into two distinct pathologic entities, mucinous adenoma and mucinous neoplasms of low malignant potential (M-LMP). We believe this distinction has prognostic significance given the drastically different long-term prognosis of these two neoplasms. We also retain the term mucinous neoplasm of uncertain malignant potential when there is any uncertainty about the presence of invasion or the presence of epithelium within extra-appendiceal mucin. Either way, these low-grade neoplasms should always be distinguished from adenocarcinomas, not otherwise specified, due to the differences is spread, treatment, and prognosis.

Regardless of the terminology employed the importance of removing as much of the mucinous cystic masses in M-LMP, whether they occur in the appendix, in the ovaries or elsewhere in the abdomen cannot be overemphasized, in view of the reports of improved survival with aggressive cytoreduction for these tumours. Although there is uniform agreement concerning the critical role for surgery in the treatment for mucinous neoplasms of uncertain or low malignant potential, controversy exists concerning the optimum surgical procedure. The older literature maintains that appendectomy is sufficient, but others have advocated a more aggressive approach to the management of these unpredictable lesions with right hemicolectomy, debulking and complete removal of all mucinous material and implants, even if it requires a second procedure [32,33]. Still others recommend removal of the caecum or right colon only for patients with involvement of the base of the appendix [34].

Adenocarcinoma (Mucinous, Intestinal, and Signet Ring Types)

- Uncommon, infrequently associated with clinical scenario of 'pseudomyxoma peritonei'
- Cytoarchitectural features of frank carcinoma: mucinous type requires > 50% mucin; intestinal type same as usual colonic carcinoma; signet ring should be distinguished from goblet cell carcinoid
- Lymph node, liver and lung metastases present
- When associated with intra-abdominal mucin, best referred to as peritoneal carcinomatosis
- Clinically malignant with poor prognosis; clinical course may not be altered by extensive debulking

Invasive adenocarcinoma of the appendix is rare and significantly less common than mucinous adenomas and M-LMP (Fig. 6). Histologically, adenocarcinomas are classified as mucinous, intestinal, or signet ring types. Mucinous adenocarcinomas of the appendix, which account for approximately 40% of appendiceal adenocarcinomas, are relatively uncommon and very infrequently associated with the development of pseudomyxoma peritonei syndrome [35]. By definition, these

neoplasms contain > 50% mucin, appear histologically identical to mucinous adenocarcinomas encountered elsewhere in the gastrointestinal tract, and exhibit destructive invasion of the appendiceal wall with architectural complexity and high-grade cytologic atypia with marked nuclear pleomorphism and brisk mitotic activity.

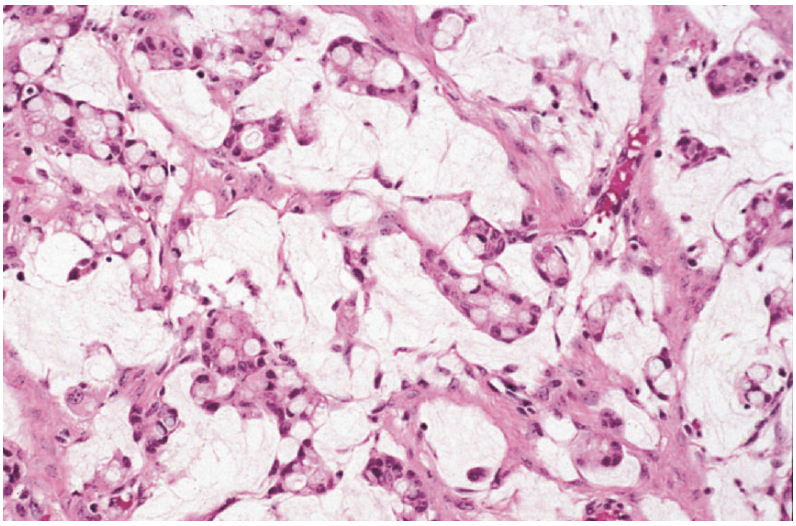


Figure 6. Mucinous adenocarcinoma in appendix. Invasive mucinous adenocarcinoma features clusters of fused malignant cribriform glands floating in pools of mucin. The glandular configuration and degree of cytologic atypia exceeds that which is allowable in mucinous adenoma or mucinous LMP

Based on one recent series, some authors require only high-grade cytologic atypia with or without the presence destructive invasion for the diagnosis of mucinous adenocarcinoma [31]. However, in all of the reported cases without invasion, the appendix was not entirely submitted for histologic evaluation. We feel that if adequately sampled, destructive invasion of the appendiceal wall can be identified in most, if not all, cases of mucinous adenocarcinoma and its presence should be reported. Even less commonly, the appendix develops adenocarcinomas of the usual colonic type [12]. Both types of adenocarcinoma are staged and treated in a similar fashion to caecal adenocarcinomas and appear to have the same prognosis, although numbers are limited.

Signet ring carcinoma is rare in the appendix (Fig. 7), accounting for approximately 5% of appendiceal adenocarcinomas, and has a very poor prognosis due to frequent extension of tumour to adjacent organs and metastasis [12,35]. These tumours frequently metastasize to the ovary [36].

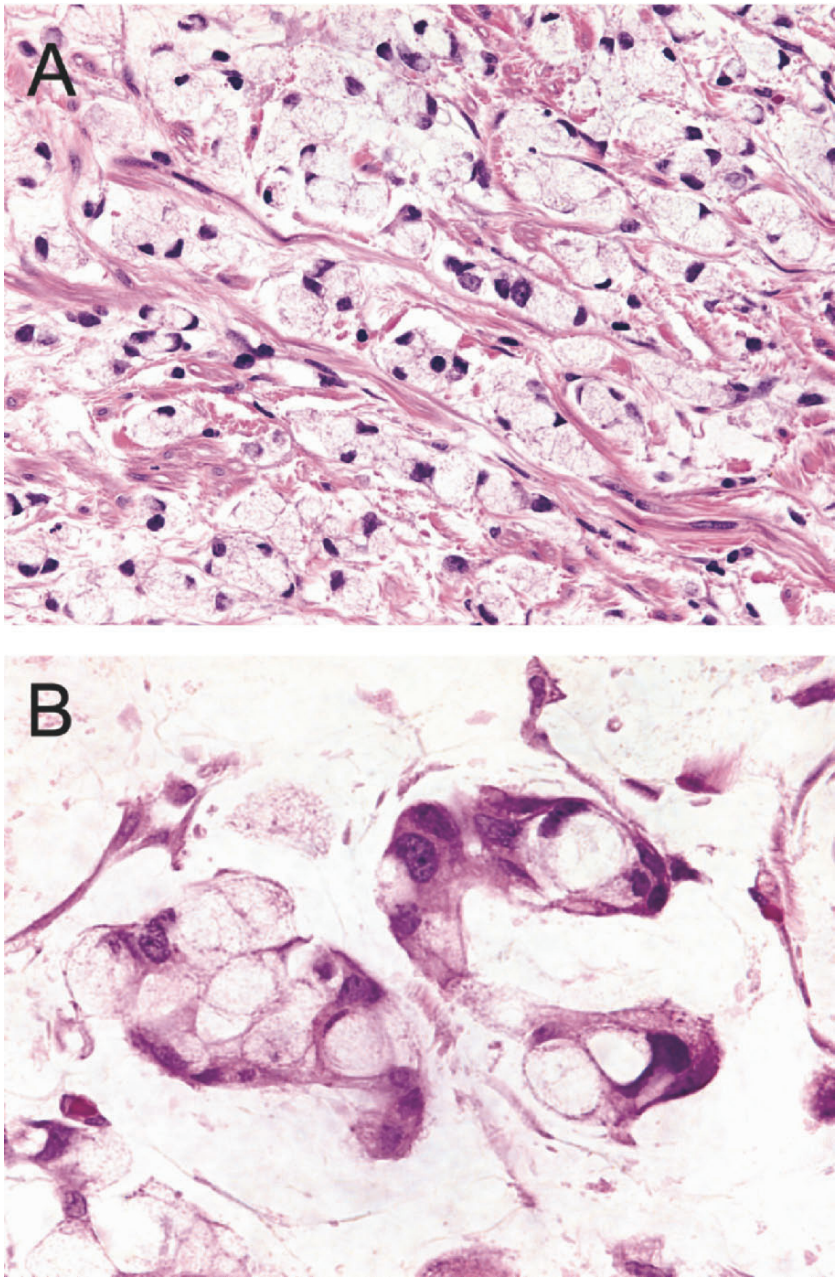


Figure 7. Signet-ring adenocarcinoma of the appendix. (A) Diffuse infiltration by individual cells and cell clusters containing prominent intracytoplasmic mucin vacuoles. (B) The compressed nuclei are hyperchromatic and enlarged

The appropriate treatment for all of these tumour types is right hemicolectomy, especially if the disease appears to be confined to the appendix, since complete excision with removal of potentially involved lymph nodes improves the potential for cure [32]. When associated with intraperitoneal dissemination and accumulation of mucinous implants, the prognosis is similar to that of peritoneal carcinomatosis.

Goblet Cell and Tubular Carcinoids

Appendiceal neuroendocrine tumours with mucinous differentiation have distinct morphologic and clinical features compared to neuroendocrine tumours seen elsewhere in the gastrointestinal tract. In addition, these tumours have engendered considerable confusion in the literature regarding their classification, and they have been described under several different names, including adenocarcinoid, goblet cell carcinoid, crypt cell carcinoma, and mucinous carcinoid. We prefer the WHO-recommended classification of these tumours as goblet cell carcinoid (Fig. 8) and tubular carcinoid.

Like most other appendiceal neoplasms, goblet cell carcinoids (Fig. 8) are often diagnosed initially on microscopic examination as these tumours typically diffusely infiltrate into the wall of the appendix and a mass is often not seen grossly [37,38]. The neoplastic cells are characterized by a concentric infiltration of submucosa, muscularis propria and serosa by small clusters and nests of Paneth cells, endocrine cells, and goblet cells, which may have distinct signet ring morphology [37,38]. Tubular structures with well-formed lumens are typically absent. However, if tubular structures are identified, the lining cells are composed of goblet cells with intracytoplasmic mucin in contrast to tubular carcinoids, which have mucin, restricted to the lumens of tubules [38]. Vascular and perineural invasion is often found [37,39]. Lakes of mucin may be seen surrounding nests of tumour cells or in the stroma adjacent to the neoplastic cells. However, unlike mucinous epithelial neoplasms, the tumour cells within the mucin form well-defined glands with central lumens [38]. Endocrine cells, best highlighted by chromogranin or synaptophysin stains, are present to a variable degree and may be rare or in some cases, absent [38, 40]. Rare cases of simultaneous goblet cell carcinoid tumours and mucinous epithelial neoplasms of the appendix have been reported in the literature [6,41]. This unusual occurrence most likely represents two independent, separate tumours (collision tumours) with no histogenetic relationship [6].

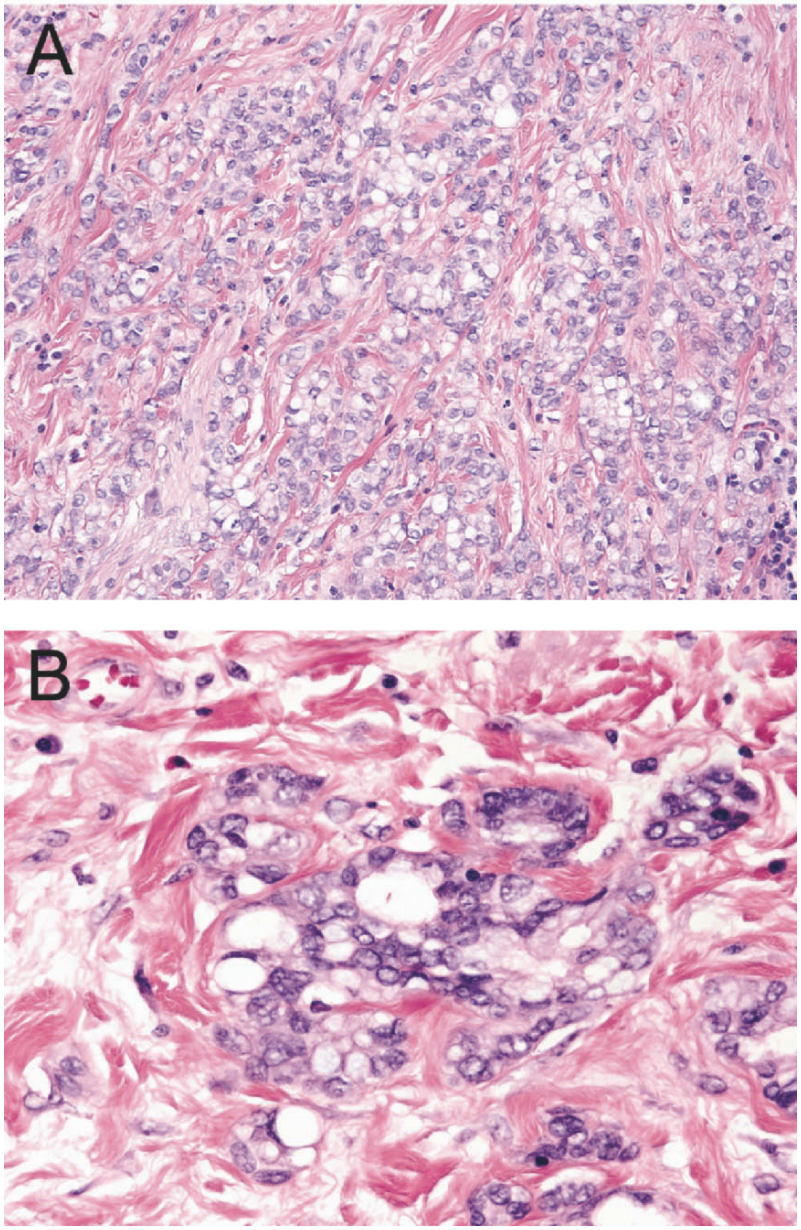


Figure 8. Goblet cell carcinoid. (A) Goblet cell carcinoids appear to arise from the basal mucosa and circumferentially extend throughout the appendiceal wall with preservation of the mucosa. This tumour is composed of well-formed nests of goblet cells and endocrine cells with mild to moderate nuclear atypia. (B) The tight cell clusters, presence of the eosinophilic neuroendocrine cells and the distinct absence of significant cytologic atypia help distinguish this tumour from signet-ring cell adenocarcinoma (Fig. 7)

Thought to develop from a pluripotent stem cell with divergent mucinous epithelial and neuroendocrine differentiation, these tumours are characterized by unpredictable behavior with delayed local peritoneal recurrences and eventually, lung metastases [37,42,43]. Spread to the ovary is especially common and many are initially diagnosed as ovarian masses [39,44]. One study identified a set of histological features that predicted malignant behavior, but in our opinion, all goblet cell carcinoids should be regarded as of at least low malignant potential, particularly whenever the distinction between goblet cell carcinoid and signet-ring carcinoma is problematic (a not uncommon occurrence in these authors' experience) [38]. We feel that the presence of frank adenocarcinoma, either mucinous or non-mucinous, warrants the diagnosis of adenocarcinoma and treatment as a gastrointestinal adenocarcinoma, although the term mixed carcinoid-adenocarcinoma has been proposed [38]. Although a goblet cell carcinoid–signet ring cell spectrum has been proposed by some authors, in our opinion, goblet cell carcinoid should be distinguished from primary signet ring carcinoma, which behaves as a poorly differentiated carcinoma similar to those occurring elsewhere in the gastrointestinal tract [6,35] Histologically, primary signet-ring cell carcinomas of the appendix form complex, cribriform nests and solid sheets and diffusely infiltrating signet-ring cells with single-file arrangements compared to the typical rounded aggregates of tumour cells seen in goblet cell carcinoids [38]. In addition, the presence of Paneth cells in goblet cell carcinoids is a helpful distinguishing feature from signet-ring cell carcinoma [37,45]. On occasion, both signet ring carcinomas and goblet cell carcinoid tumours may be associated with extra-cellular mucin production and the intra-abdominal accumulation of mucinous material [36].

Treatment for goblet cell carcinoid tumours is not well established in the literature. Some authors recommend right hemicolectomy and bilateral oophorectomy for tumours with diffuse involvement of the appendix [46,47]. However, this approach is not universally accepted and many believe that tumours localized to the appendix may be treated with appendectomy provided a clear margin of resection is obtained [48]. If it cannot be confirmed that the base of the appendix is free of tumour, a right hemicolectomy may be indicated [42]. Regardless of the surgical approach, the main predictor of poor prognosis appears to be the presence of tumour spread beyond the appendix [37,42].

Tubular carcinoids are typically small, often involve younger individuals, and are characterized by small discrete acinar or gland-like structures lined a single layer of uniform cells [37,38]. Some of the tubular gland-like structures contain inspissated mucin in their lumens and large pools of mucin may be seen in the muscularis propria [37,38]. Mucin stains will highlight the presence of intraluminal inspissated mucus, but the lining cells themselves are negative, in contrast to goblet cell carcinoid. The neoplastic cells are often concentrated around mucosal crypts and extension into the muscular wall of the appendix in uncommon. Like goblet cell carcinoids, a discrete mass is often not found grossly. These tumours often show no apparent connection to mucosa and may be initially misdiagnosed as metastatic carcinoma, especially when encountered in women due to their resemblance to metastatic breast carcinoma. Unlike other carcinoid tumours, immunohistochemical studies for chromogranin are often negative in tubular car-

cinoids, although they often are strongly positive for glucagon [28,38,42]. Tubular carcinoids do not metastasize and may be treated in the same manner as classical carcinoid tumours of the appendix [37,38].

'Pseudomyxoma Peritonei Syndrome'

Pseudomyxoma peritonei is a clinical condition that has been characterized as a localized or generalized accumulation of thick, gelatinous material in the abdominal and/or pelvic peritoneal cavity. Because of the variety of connotations that have been attached to this term in the medical literature, 'pseudomyxoma peritonei' (like 'mucocele') is best used as a clinical, radiologic or even syndromic descriptor and not as a histopathologic diagnosis. The occurrence of this syndrome in women in association with ovarian mucinous tumours has created considerable controversy regarding the pathology and site of origin of pseudomyxoma peritonei and its ultimate relationship to the appendiceal mucinous tumours [9,21,25,26]. However, it is now commonly accepted that the vast majority of cases of classic pseudomyxoma peritonei develop as a result of a mucinous neoplasm of low malignant potential arising in the appendix with spontaneous or intraoperative rupture into the surrounding peritoneum. Although the tumour spreads throughout the peritoneal cavity often with ovarian involvement, invasion into visceral organs is exceedingly rare and metastatic spread via lymphatics or hematogenous spread does not occur. Extra-peritoneal spread of disease (via pleural extension), is very unusual and is typically iatrogenic (secondary to aggressive cytoreductive therapy) or very rarely, due to the presence of a congenital pleuroperitoneal communication and/or direct extension through the diaphragm [49]. Regardless of the cause, the prognosis of full-blown pseudomyxoma peritonei is poor and the condition ultimately leads to death due to obstruction of intra-abdominal organs. With repeated laparotomy and aggressive surgical debulking with evacuation of the ascitic fluid, survival ranges from 75 to 85% at five years, and decreases to between 45 to 68% at ten years [26,31,50]. Pseudomxyoma peritonei is 2-3 times more common in females than in males. Because it occurs in only 2 out of 10,000 laparotomies, it is often an unexpected finding [51]. Patients may first come to clinical attention due to symptoms related to abdominal distension or generalized abdominal pain. Not uncommonly, patients initially present with a mucin-containing scrotal or hernia mass [52,53]. Many cases are not detected until the time of surgery for an ovarian mass or acute appendicitis. CT scans (Fig. 9) are quite useful in suggesting the diagnosis due to a characteristic scalloping of the hepatic and splenic margins from compression by loculated spaces containing the gelatinous material and compression of abdominal viscera without direct invasion [54]. The mucin-secreting epithelium tends to accumulate in the regions of the right sub-diaphragm and subhepatic space, the left abdominal gutter, greater omentum, and pelvis (the 'redistribution' phenomenon) [55].

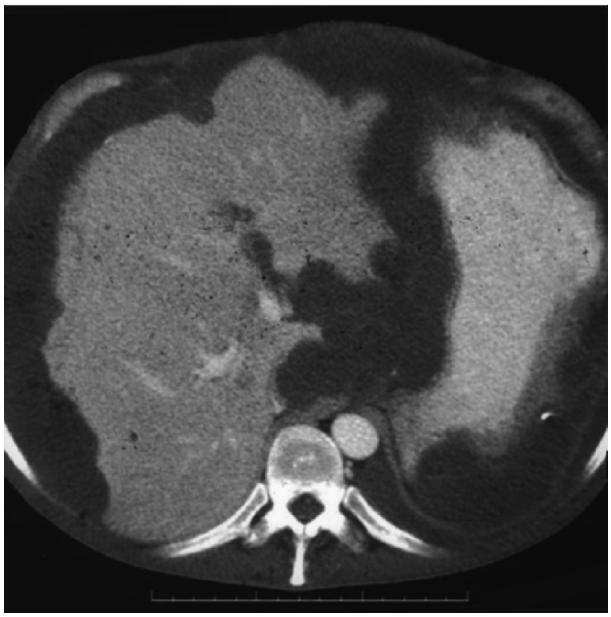
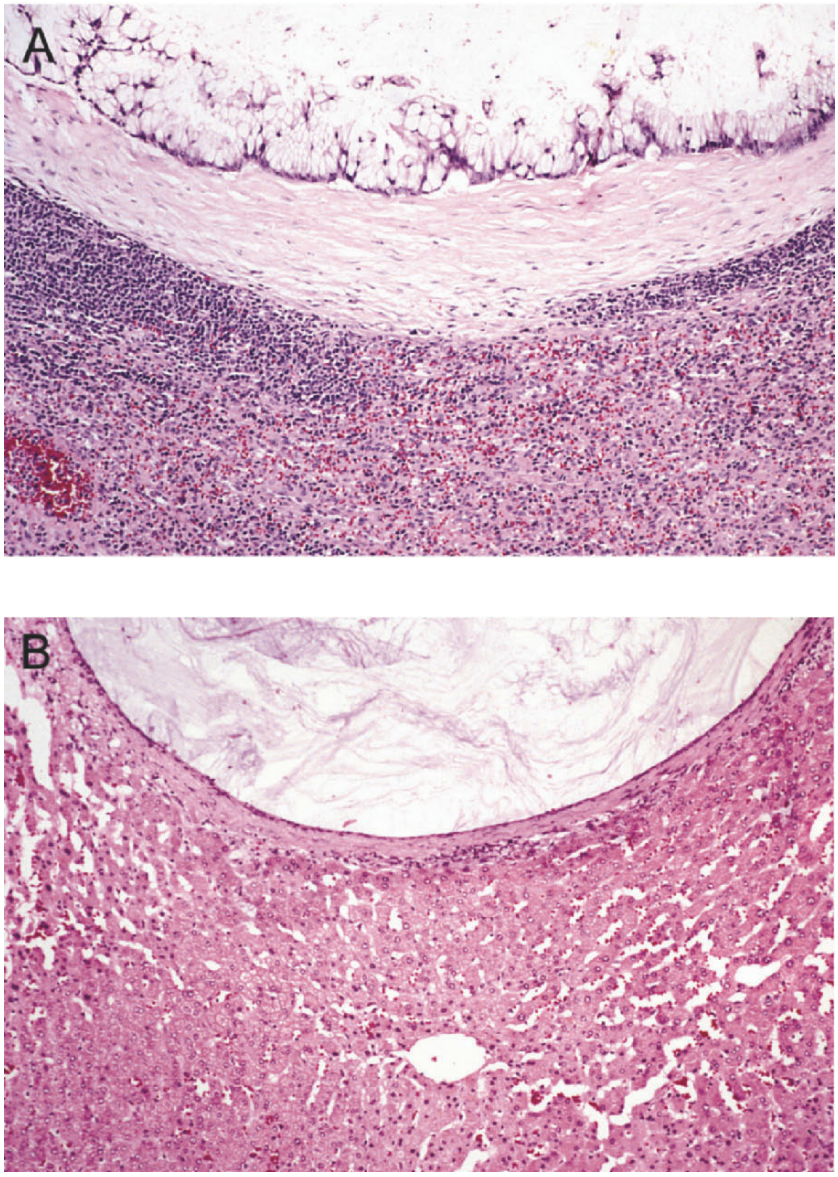


Figure 9. CT scan depicts characteristic scalloping effect on surfaces of visceral organs in pseudomyxoma peritonei due compression by viscous mucinous secretions and organizing fibrosis

On histologic examination, the mucinous implants consist of amorphous mucinous material, fibrous tissue and strips of cytologically bland, non-invasive mucin-secreting epithelium (Fig.10). In general, most cases are CK20-positive and CK7-negative, although expression of CK7 is observed in up to 30% of cases and the use of this panel may not be useful in determining the site of origin in problematic cases. In most cases studied, the mucinous ovarian neoplasm and the associated appendiceal neoplasm demonstrate an identical pattern of immunoreactivity. These neoplasms also express CDX2 and the accumulation of extracellular mucin has been linked to increased numbers of MUC2-secreting goblet cells (Fig.11) [19].

The degree of intraperitoneal mucinous epithelial cellularity may affect prognosis [26]. Pseudomyxoma peritonei without epithelial cells appears to have a much better prognosis than pseudomyxoma peritonei with epithelial cells, but if peritoneal implants are extensively sampled, epithelial cells are detected in most cases [26]. In all cases, the degree of cytologic atypia of the intraperitoneal mucinous epithelial implants should be reported, especially in those cases in which a primary tumour has not been identified, since this feature often reflects the grade of the primary tumour (Fig.12).


A
B

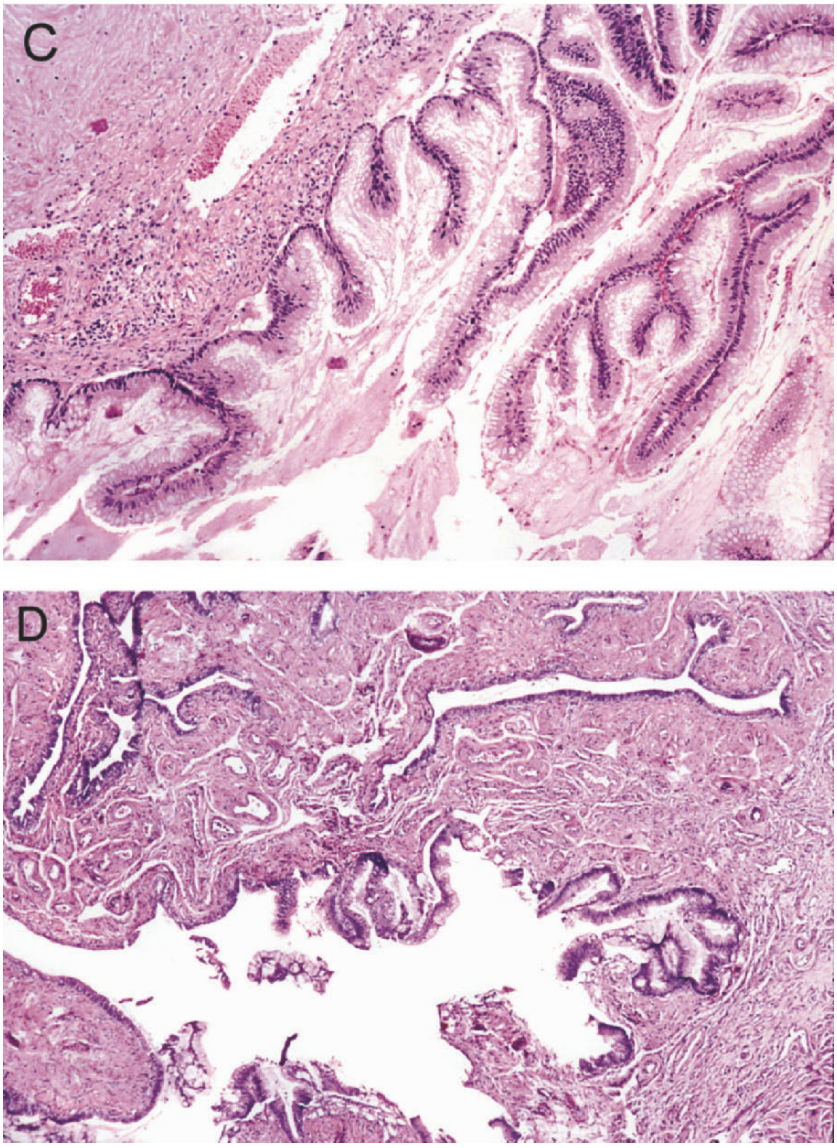


Figure 10. Pseudomyxoma peritonei syndrome features mucinous implants that compress spleen (A) and liver (B), but do not invade the parenchyma in a destructive fashion. In women, ovarian involvement (C), tubal serosal and mucosal involvement (D) and even endometrial involvement may also occur

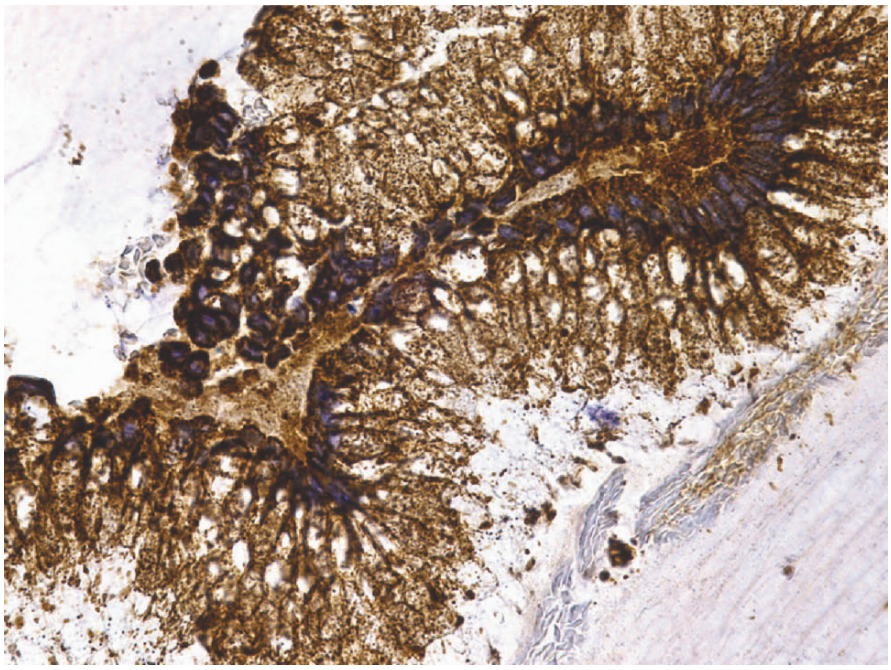
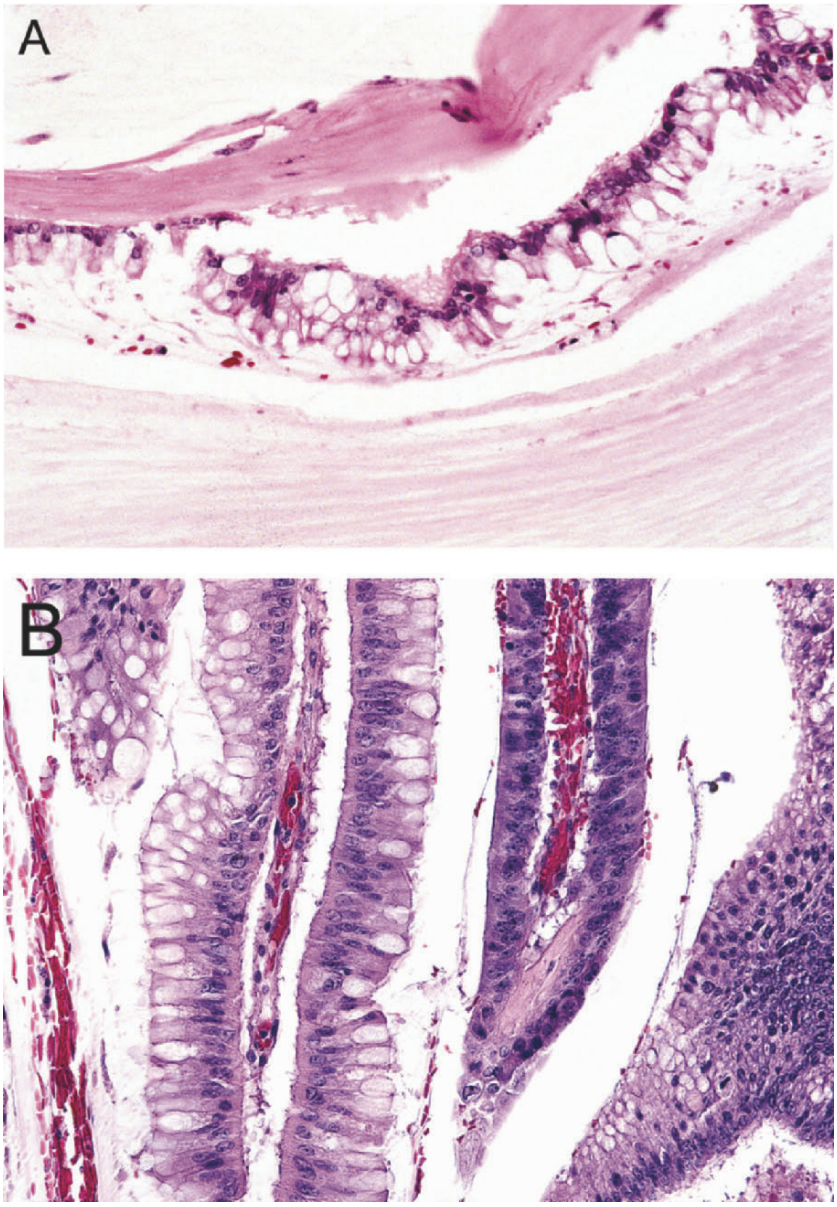


Figure 11. The accumulation of intraperitoneal mucin in pseudomyxoma peritonei secondary to appendiceal M-LMP has been attributed to the presence of increased numbers of MUC2-secreting goblet cells, however increased expression of MUC2 is not specific for this syndrome and should not be relied upon in order to determine the primary site of origin in the setting of disseminated peritoneal mucinous epithelial implants

Morbidity and mortality are significantly worse for peritoneal carcinomatosis with mucinous ascites secondary to mucinous carcinomas (which may present with a clinical scenario similar to pseudomyxoma peritonei) than pseudomyxoma peritonei secondary to mucinous neoplasms of low malignant potential (Table 1). The five-year survival for mucinous adenocarcinoma with peritoneal carcinomatosis is between 10 to 15%, whereas the five-year survival with mucinous tumours of low malignant potential and pseudomyxoma peritonei is closer to 75 to 85% [26]. In most cases, the cytologic features of the primary neoplasm and the implants are similar, but occasionally discordant features are present. Although experience is limited with these types of cases, an intermediate prognosis has been reported by some [26,50]. However, others have found no difference in survival between those cases of pseudomyxoma peritonei with low-grade and intermediate peritoneal mucinous epithelium [56]. The degree of cytologic atypia present in the implants of recurrent pseudomyxoma should also be assessed, because some patients appear to experience a more aggressive disease course concomitant with histologic progression, although in most instances, the cytologic features do not appear to change significantly over time [57].


A
B

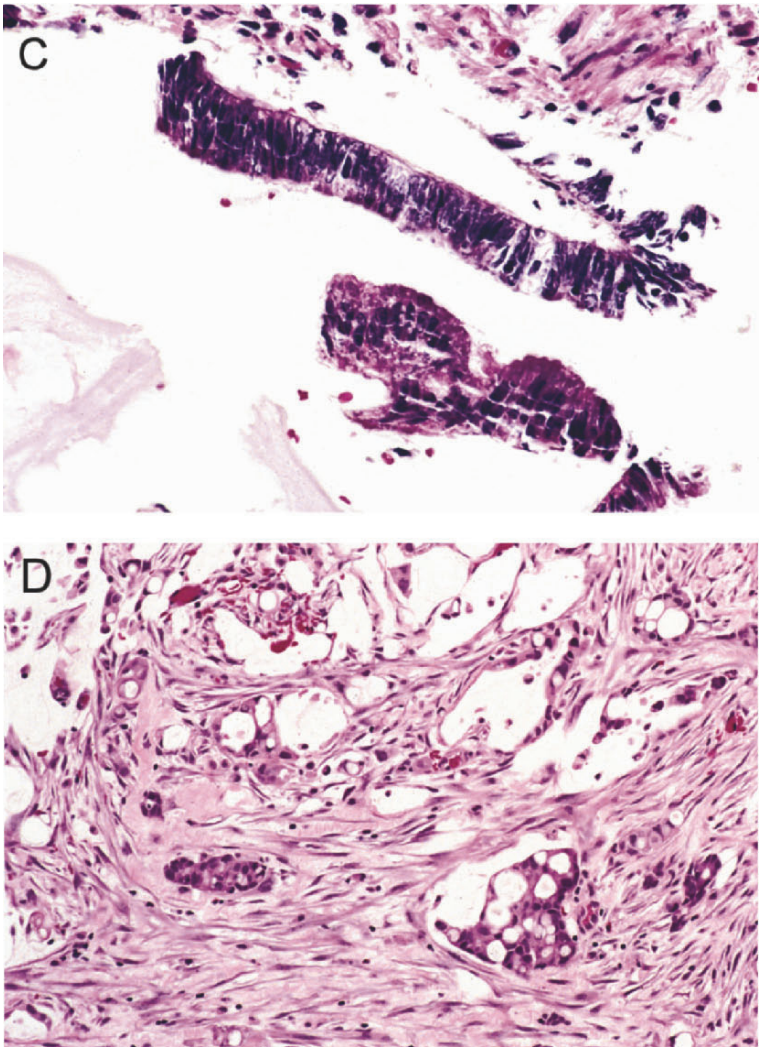


Figure 12. Implants of epithelium in pseudomyxoma peritonei (PMP) typically exhibit cytoarchitecturally bland features (A), but may show transition (B) or over time, transformation (C) to more dysplastic and architecturally complex epithelium. Implants with more dysplastic epithelium (B, C) have been associated with a poorer prognosis than implants solely composed of cytologically bland epithelium in some series. (D) Mucinous ascites secondary to carcinomatosis (colloid carcinoma) exhibits complex, cribriform glands with enlarged hyperchromatic nuclei, with coarsened chromatin and frequent mitotic figures. Peritoneal carcinomatosis may occasionally present with clinical and radiologic features that are similar to PMP and is distinguished chiefly by the cytoarchitectural features. Site of origin is usually elsewhere in the GI tract, although rarely, appendiceal carcinomas may give rise to gelatinous ascites (Table 7)

Table 1. Differential Diagnosis of Appendiceal Mucinous Neoplasms

Feature	Mucinous Adenoma	Mucinous Neoplasm UMP	Mucinous Neoplasm LMP	Mucinous Carcinoma
Architecture	Flat or villiform	Flat or villiform	Flat or villiform	Complex papillary fronds with cribriforming
Cytomorphology	Low-grade	Low-grade	Low-grade	High-grade
Resection margin	Not involved	May be involved	May be involved	May be involved
Appendiceal wall with epithelium	No	May be seen, but not clearly invasive	Yes	Yes
Peritoneal implants	No	No	Yes; noninvasive, paucicellular	Yes; invasive, highly cellular
Metastasis to lymph nodes or distant sites	No	No	No	Often present
Treatment	Resection curative	Complete resection with clear margin	Complete resection and surgical debulking	Surgical debulking has little impact on survival
Clinical Behavior	Cured by resection	Low potential for recurrence	Recurs often with 50% 5-year survival	Poor prognosis with <10% 5-year survival

UMP, uncertain malignant potential; LMP, low malignant potential

Cytologic examination of aspirated mucus is usually not reliable in predicting whether an individual case is a carcinoma or a tumour of low malignant potential, but a relatively high degree of concordance between the degree of cytologic atypia detected on peritoneal washings and implant histology has been reported [58].

Since the prognosis for patients with frank adenocarcinoma, either in the initial primary tumour or in the recurrent tumour implants is extremely poor and these patients do not respond to extensive peritoneal debulking, it is important that the pathologist make an attempt to determine whether the primary and/or implant histology is compatible with a mucinous neoplasm of low malignant potential or a mucinous adenocarcinoma of the usual colonic type (Table 2).

Table 2. Differential Diagnosis of Mucinous Ascites

Feature	Pseudomyxoma peritonei	Peritoneal carcinomatosis
Primary site	Appendix	Colon, appendix, stomach
Primary diagnosis	Mucinous neoplasm of low malignant potential	Mucinous adenocarcinoma
Peritoneal implants	Noninvasive, surface implants; often paucicellular	Invasive implants; glands and cells easily found
Cytoarchitectural features	Simple or focal proliferative epithelium with minimal to moderate atypia. Mitotic figures sparse, nonatypical.	Cribriform and/or tubular structures and/or signet ring cells with enlarged nuclei, prominent nucleoli. Frequent mitotic figures, which may be atypical.
Lymph node, liver and lung metastases	Practically never	Often present
Treatment	Surgical debulking improves prognosis	Surgical debulking has little impact on survival
Clinical behavior	Slowly progressive with 50% five year survival	Less than 10% five year survival

Cribriform structures, signet ring cells and severe cytologic atypia in the form of enlarged nuclei with prominent nucleoli strongly favour a colonic-type mucinous adenocarcinoma and proliferations with these features should be diagnosed as adenocarcinoma with mucinous ascites (i.e., peritoneal carcinomatosis). These neoplasms behave in a manner that is analogous to the usual widely metastatic mucinous carcinoma of the gastrointestinal tract and should be treated as such. Those cases in which mucinous implants are composed of nonstratified, simple or focally proliferative columnar and cuboidal epithelium containing uniform, cytologically bland nuclei, and few mitotic figures strongly favour a low grade neoplasm and proliferations with these features should be diagnosed as mucinous neoplasms of low malignant potential in order to distinguish them from the usual colonic-type mucinous adenocarcinoma with peritoneal carcinomatosis (Fig.12). These latter lesions, unlike the low malignant potential lesions, show no significant response or improved survival to aggressive surgery with extensive debulking.

In lieu of the absence of tissue invasion and parenchymal metastases, pseudomyxoma peritonei associated with cytologically bland mucinous epithelial implants has been referred to as “disseminated peritoneal adenomucinosis”, but this terminology has not been widely adopted and we think that any reference to an “adenomatous” process in this setting is deceptive and potentially confusing. Similarly, we think it is inaccurate to diagnose cytologically and histologically bland neoplastic proliferations as mucinous adenocarcinoma, not otherwise specified on the sole basis of their extension to the peritoneal cavity, especially since these proliferations do not metastasize to lymph nodes or visceral organs, as do most mucinous adenocarcinomas. Nor are they treated in the same fashion as the

usual colonic-type mucinous adenocarcinoma. For these reasons, we prefer alternative designations, such as "mucinous neoplasm of low malignant potential" or "low grade appendiceal mucinous neoplasm" with a comment in the pathology report that specifically defines what is meant by this terminology. We often use a combination of terms when rendering diagnoses, labeling these tumours as mucinous neoplasm of low malignant potential (low grade appendiceal mucinous neoplasm) with or without peritoneal and/or ovarian involvement by appendiceal mucinous neoplasm of low malignant potential (pseudomyxoma peritonei). The strong resemblance between the appendiceal tumours and those occurring in the ovary and designated at that site as "low malignant potential" or "borderline" is a strong argument in favour of such terminology. Indeed, in our opinion, the similarity between ovarian mucinous neoplasms with an intestinal phenotype and appendiceal mucinous tumours is significantly more striking than that between appendiceal mucinous tumours and the usual colonic mucinous neoplasms.

Regardless of the terminology used, the important information to be utilized in the management of the patient is the type of appendiceal neoplasm; the presence or absence of mucin dissection through the appendiceal wall; the extent of the extra-appendiceal mucin (e.g., confined to the right lower quadrant, ovarian surface, or elsewhere in the peritoneal cavity); the presence of epithelium in the mucin or elsewhere in the peritoneum; and the degree of cytologic atypia of the epithelium in the extra-appendiceal mucin or peritoneal implants. Although mucin outside the right lower quadrant is an unfavourable prognostic feature, the occurrence of mucin in the appendiceal wall or in the immediate vicinity of the appendix is not, unless it is associated with epithelial cells [3].

The surgical treatment for pseudomyxoma peritonei is appendectomy or caecectomy/right hemicolectomy to obtain clear margins, and thorough surgical debulking with omentectomy. Some surgeons favour right hemicolectomy. However, right hemicolectomy may not confer any additional survival benefit over appendectomy in patients with pseudomyxoma peritonei if a clear margin of resection is achieved with appendectomy [59]. As far as is possible, all gross disease should be removed. Following excision of the appendix, the entire specimen should be examined microscopically if a lesion is not evident macroscopically [60]. Many authors advocate bilateral oophorectomy even if the ovaries appear macroscopically uninvolved, due to the frequent involvement of these organs. Sugarbaker advocates a complete peritonectomy followed by adjuvant intraperitoneal chemotherapy, but the efficacy of ultra-radical surgery and intraperitoneal chemotherapy, mucolytic therapy, or even radiotherapy remains uncertain [51,61]. In most centers, treatment is symptomatic, involving multiple partial resections, evacuation of the mucoid material, drainage of fistula tracts, and repeated debulking.

There are sporadic case reports of pseudomyxoma peritonei developing in association with other tumours of the appendix (see goblet cell carcinoids, above) as well as with tumours of the colon and rectum, gallbladder and bile ducts, pancreas, lung, breast, fallopian tubes, small intestine and urinary bladder [62-69]. Most, if not all of these cases probably represent peritoneal carcinomatosis in association with metastatic mucinous adenocarcinoma. Gelatinous ascites may occur in

association with mucinous tumours arising in gonadal and extra-gonadal teratomas, some of which are high grade carcinomas, while others are histologically indistinguishable from the low grade (low malignant potential) mucinous tumours that arise in the appendix [70, 71].

The differential diagnosis of pseudomyxoma peritonei includes mucinous (colloid) adenocarcinoma with mucinous ascites (peritoneal carcinomatosis), endometriosis with myxoid change, and mucinous extravasation secondary to inflammatory, non-neoplastic processes [72]. The importance of distinguishing peritoneal carcinomatosis due to metastatic mucinous adenocarcinoma from pseudomyxoma peritonei due to mucinous neoplasm of low malignant potential has already been discussed (Table 2). Pools of mucin may be found in association with foci of endometriosis, a condition that may simulate localized pseudomyxoma peritonei [5]. Distinction between endometriosis with secondary irritation of the appendiceal mucosa from a mucinous neoplasm can be extraordinarily difficult in this setting and the pathologist should exercise caution in the interpretation of these lesions. Acute appendicitis may present with mucinous extravasation. If the mucinous material is small in amount, limited in extent, without epithelial cells and is related to an inflamed perforated appendix or viscus that, despite extensive sectioning (in the case of the appendix, the entire organ should be sampled) shows no evidence of neoplastic change, the mucin may represent simple extravasation.

Although the controversies surrounding pseudomyxoma peritonei have not been completely resolved, cases in which gelatinous ascites is associated with an ovarian mucinous neoplasm and in which an appendiceal or other intestinal neoplasm is present or has not been conclusively excluded, should be considered to be an entity distinct from ovarian mucinous surface epithelial neoplasms of the usual sort and should not be classified as Stage II or III ovarian mucinous carcinomas or tumours of low malignant potential. The clinically relevant information is whether or not there are cells within the mucinous ascites and whether or not the cells are dysplastic, since it appears that the degree of cytologic atypia may have prognostic significance over and above the presence of the ascitic cells. When pseudomyxoma peritonei and a mucinous ovarian tumour is present, the appendix should always be removed even if it is grossly normal and the appendix should be extensively sectioned by the pathologist, since small neoplasms may be missed on a cursory examination.

Conclusion

The classification of mucinous tumours of the appendix can be confusing due to the variety of diagnostic terms that have been historically applied to these neoplasms. Both “adenoma” and “carcinoma” have been utilized to designate histologically identical low grade appendiceal mucinous proliferations that lack the cytoarchitectural features of malignancy, but can, over time, spread throughout the peritoneum, creating abundant gelatinous mucinous ascites. The epithelial implants associated with this condition are characteristically bland and non-invasive,

but tend to persist over time despite repeated debulking, eventually leading to re-accumulation of the mucinous material with resultant peritoneal fibrosis. Ovarian involvement is common, presumably due to repeated peritoneal exposure during ovulatory cycles. Occasionally, the epithelial implants transform to more dysplastic or malignant epithelium and this is usually associated with increased tempo of disease. The rather unique clinical and radiologic features of this condition, have led to the designation of pseudomyxoma peritonei syndrome. Since this syndrome, as classically defined, is associated with a natural history, prognosis and treatment that is distinctly different from peritoneal mucinous carcinomatosis (peritoneal spread due to a high grade appendiceal mucinous proliferations, i.e., mucinous adenocarcinoma), it is important that physicians be cognizant of appropriate descriptors to be used for primary appendiceal neoplasms and their associated implants. Due to significant similarities to ovarian serous tumours of low malignant potential (e.g., atypical, but not malignant cytoarchitectural features; propensity for noninvasive peritoneal spread; characteristically indolent but progressive clinical course; and potential to transform to carcinoma), these tumours and their peritoneal implants should be classified as low malignant potential or, in the setting of malignant transformation, as adenocarcinoma, based on the degree of cytologic atypia. Alternative terms, such as borderline mucinous tumour of the appendix or low-grade mucinous appendiceal neoplasm have also been proposed and are equally appropriate provided all those involved in the care of these patients understand one another. The plethora of descriptors that have been created to describe this condition reflects the inaccurate and counterproductive reliance on standard terminology to classify these tumours and the "need to introduce new terms that do not carry the same connotations as "adenoma" and "carcinoma" [28].

References

1. Gibbs NM. (1973) Mucinous cystadenoma and cystadenocarcinoma of the vermiform appendix with particular reference to mucocele and pseudomyxoma peritonei. J Clin Pathol 26:413-421
2. Pai RK, Longacre TA. (2005) Appendiceal mucinous tumours and pseudomyxoma peritonei: histologic features, diagnostic problems, and proposed classification. Adv Anat Pathol 12:291-311
3. Carr NJ, McCarthy WF, Sobin LH. (1995) Epithelial noncarcinoid tumours and tumour-like lesions of the appendix. A clinicopathologic study of 184 patients with a multivariate analysis of prognostic factors. Cancer 75:757-768
4. Nopajaroonsri C, Mreyoud N. (1994) Retention mucocele of appendix due to endometriosis. South Med J 87:833-835
5. Driman DK, Melega DE, Vilos GA, et al. (2000) Mucocele of the appendix secondary to endometriosis. Report of two cases, one with localized pseudomyxoma peritonei. Am J Clin Pathol 113:860-864
6. Carr NJ, Remotti H, Sobin LH. (1995) Dual carcinoid/epithelial neoplasia of the appendix. Histopathology 27:557-562

7. Qizilbash AG. (1974) Hyperplastic (metaplastic) polyps of the appendix: report of 19 cases. Arch Pathol 97:385-388
8. MacGillivray JB. (1972) Mucosal metaplasia in the appendix. J Clin Pathol 25:809-811
9. Higa E, Rosai J, Pizzimbono CA, et al. (1973) Mucosal hyperplasia, mucinous cystadenoma, and mucinous cystadenocarcinoma of the appendix. A re-evaluation of appendiceal "mucocele". Cancer 32:1525-1541
10. Williams GR, du Boulay CE, Roche WR. (1992) Benign epithelial neoplasms of the appendix: classification and clinical associations. Histopathology 21:447-451
11. Carr NJ, Sobin LH. (1995) Epithelial noncarcinoid tumours and tumour-like lesions of the appendix. Cancer 76:2383-4
12. Qizilbash AH. (1975) Primary adenocarcinoma of the appendix. A clinicopathological study of 11 cases. Arch Pathol 99:556-562
13. Qizilbash AH. (1975) Mucoceles of the appendix. Their relationship to hyperplastic polyps, mucinous cystadenomas, and cystadenocarcinomas. Arch Pathol 99:548-555
14. Longacre TA, Fenoglio-Preiser CM. (1990) Mixed hyperplastic adenomatous polyps/serrated adenomas. A distinct form of colorectal neoplasia. Am J Surg Pathol 14:524-537
15. Rubio CA. (2004) Serrated adenomas of the appendix. J Clin Pathol 57:946-949
16. Jass JR, Whitehall VL, Young J, et al. (2002) Emerging concepts in colorectal neoplasia. Gastroenterology 123:862-876
17. Iino H, Jass JR, Simms LA, et al. (1999) DNA microsatellite instability in hyperplastic polyps, serrated adenomas, and mixed polyps: a mild mutator pathway for colorectal cancer? J Clin Pathol 52:5-9
18. Shih IM, Yan H, Speyrer D, et al. (2001) Molecular genetic analysis of appendiceal mucinous adenomas in identical twins, including one with pseudomyxoma peritonei. Am J Surg Pathol 25:1095-1099
19. O'Connell JT, Tomlinson JS, Roberts AA, et al. (2002) Pseudomyxoma peritonei is a disease of MUC2-expressing goblet cells. Am J Pathol 161:551-564
20. Kabbani W, Houlihan PS, Luthra R, et al. (2002) Mucinous and nonmucinous appendiceal adenocarcinomas: different clinicopathological features but similar genetic alterations. Mod Pathol 15:599-605
21. Carr NJ, Emory TS, Sobin LH. (2002) Epithelial neoplasms of the appendix and colorectum: an analysis of cell proliferation, apoptosis, and expression of p53, CD44, bcl-2. Arch Pathol Lab Med 126:837-841
22. Deans GT, Spence RA. (1995) Neoplastic lesions of the appendix. Br J Surg 82:299-306
23. Gonzalez JE, Hann SE,Trujillo YP. (1988) Myxoglobulosis of the appendix. Am J Surg Pathol 12:962-966
24. Wolff M, Ahmed N. (1976) Epithelial neoplasms of the vermiform appendix (exclusive of carcinoid). II. Cystadenomas, papillary adenomas, and adenomatous polyps of the appendix. Cancer 37:2511-2522

25. Young RH, Gilks CB, Scully RE. (1991) Mucinous tumours of the appendix associated with mucinous tumours of the ovary and pseudomyxoma peritonei. A clinicopathological analysis of 22 cases supporting an origin in the appendix. Am J Surg Pathol 15:415-429
26. Ronnett BM, Zahn CM, Kurman RJ, et al. (1995) Disseminated peritoneal adenomucinosis and peritoneal mucinous carcinomatosis. A clinicopathologic analysis of 109 cases with emphasis on distinguishing pathologic features, site of origin, prognosis, and relationship to "pseudomyxoma peritonei". Am J Surg Pathol 19:1390-1408
27. Lamps LW, Gray GF, Jr., Dilday BR, et al. (2000) The coexistence of low-grade mucinous neoplasms of the appendix and appendiceal diverticula: a possible role in the pathogenesis of pseudomyxoma peritonei. Mod Pathol 13:495-501
28. Carr NJ, Sobin LH. (1996) Unusual tumours of the appendix and pseudomyxoma peritonei. Semin Diagn Pathol 13:314-325
29. Seidman JD, Elsayed AM, Sobin LH, et al. (1993) Association of mucinous tumours of the ovary and appendix. A clinicopathologic study of 25 cases. Am J Surg Pathol 17:22-34
30. Sumithran E, Susil BJ. (1992) Concomitant mucinous tumours of appendix and ovary. Result of a neoplastic field change? Cancer 70:2980-2983
31. Misdraji J, Yantiss RK, Graeme-Cook FM, et al. (2003) Appendiceal mucinous neoplasms: a clinicopathologic analysis of 107 cases. Am J Surg Pathol 27:1089-1103
32. Cortina R, McCormick J, Kolm P, et al. (1995) Management and prognosis of adenocarcinoma of the appendix. Dis Colon Rectum 38:848-852
33. Nitecki SS, Wolff BG, Schlinkert R, et al. (1994) The natural history of surgically treated primary adenocarcinoma of the appendix. Ann Surg 219:51-57
34. Mann WJ Jr., Wagner J, Chumas J, et al. (1990) The management of pseudomyxoma peritonei. Cancer 66:1636-1640
35. McCusker ME, Cote TR, Clegg LX, et al. (2002) Primary malignant neoplasms of the appendix: a population-based study from the surveillance, epidemiology and end-results program, 1973-1998. Cancer 94:3307-3312
36. Ronnett BM, Kurman RJ, Shmookler BM, et al. (1997) The morphologic spectrum of ovarian metastases of appendiceal adenocarcinomas: a clinicopathologic and immunohistochemical analysis of tumours often misinterpreted as primary ovarian tumours or metastatic tumours from other gastrointestinal sites. Am J Surg Pathol 21:1144-1155
37. Warkel RL, Cooper PH, Helwig EB. (1978) Adenocarcinoid, a mucin-producing carcinoid tumour of the appendix: a study of 39 cases. Cancer 42:2781-2793
38. Burke AP, Sobin LH, Federspiel BH, et al. (1990) Goblet cell carcinoids and related tumours of the vermiform appendix. Am J Clin Pathol 94:27-35
39. Edmonds P, Merino MJ, LiVolsi VA, et al. (1984) Adenocarcinoid (mucinous carcinoid) of the appendix. Gastroenterology 86:302-309

40. Ramnani DM, Wistuba II, Behrens C, et al. (1999) K-ras and p53 mutations in the pathogenesis of classical and goblet cell carcinoids of the appendix. Cancer 86:14-21
41. al-Talib RK, Mason CH, Theaker JM. (1995) Combined goblet cell carcinoid and mucinous cystadenoma of the appendix. J Clin Pathol 48:869-870
42. Anderson NH, Somerville JE, Johnston CF, et al. (1991) Appendiceal goblet cell carcinoids: a clinicopathological and immunohistochemical study. Histopathology 18:61-65
43. Kanthan R, Saxena A, Kanthan SC. (2001) Goblet cell carcinoids of the appendix: immunophenotype and ultrastructural study. Arch Pathol Lab Med 125:386-390
44. Butler JA, Houshiar A, Lin F, et al. (1994) Goblet cell carcinoid of the appendix. Am J Surg 168:685-687
45. Subbuswamy SG, Gibbs NM, Ross CF, et al. (1974) Goblet cell carcinoid of the appendix. Cancer 34:338-344
46. Doede T, Foss HD, Waldschmidt J. (2000) Carcinoid tumours of the appendix in children--epidemiology, clinical aspects and procedure. Eur J Pediatr Surg 10:372-377
47. Burke AP, Thomas RM, Elsayed AM, et al. (1997) Carcinoids of the jejunum and ileum: an immunohistochemical and clinicopathologic study of 167 cases. Cancer 79:1086-1093
48. Varisco B, McAlvin B, Dias J, et al. (2004) Adenocarcinoid of the appendix: is right hemicolectomy necessary? A meta-analysis of retrospective chart reviews. Am Surg 70:593-599
49. Pestieau SR, Esquivel J, Sugarbaker PH. (2000) Pleural extension of mucinous tumour in patients with pseudomyxoma peritonei syndrome. Ann Surg Oncol 7:199-203
50. Ronnett BM, Yan H, Kurman RJ, et al. (2001) Patients with pseudomyxoma peritonei associated with disseminated peritoneal adenomucinosis have a significantly more favourable prognosis than patients with peritoneal mucinous carcinomatosis. Cancer 92:85-91
51. Hinson FL, Ambrose NS. (1998) Pseudomyxoma peritonei. Br J Surg 85:1332-1339
52. Esquivel J, Sugarbaker PH. (2001) Pseudomyxoma peritonei in a hernia sac: analysis of 20 patients in whom mucoid fluid was found during a hernia repair. Eur J Surg Oncol 27:54-58
53. Young RH, Rosenberg AE, Clement PB. (1997) Mucin deposits within inguinal hernia sacs: a presenting finding of low-grade mucinous cystic tumours of the appendix. A report of two cases and a review of the literature. Mod Pathol 10:1228-1232
54. Zissin R, Gayer G, Kots E, et al. (1999) Imaging of mucocoele of the appendix with emphasis on the CT findings: a report of 10 cases. Clin Radiol 54:826-832
55. Sugarbaker PH. (1994) Pseudomyxoma peritonei. A cancer whose biology is characterized by a redistribution phenomenon. Ann Surg 219:109-111

56. Bradley RF, Stewart JH, Russell GB, et al. (2006) Pseudomyxoma peritonei of appendiceal origin: a clinicopathologic analysis of 101 patients uniformly treated at a single institution, with literature review. Am J Surg Pathol 30:551-559
57. Yan H, Pestieau SR, Shmookler BM, et al. (2001) Histopathologic analysis in 46 patients with pseudomyxoma peritonei syndrome: failure versus success with a second-look operation. Mod Pathol 14:164-171
58. Jackson SL, Fleming RA, Loggie BW, et al. (2001) Gelatinous ascites: a cytohistologic study of pseudomyxoma peritonei in 67 patients. Mod Pathol 14:664-671
59. Gonzalez-Moreno S, Sugarbaker PH. (2004) Right hemicolectomy does not confer a survival advantage in patients with mucinous carcinoma of the appendix and peritoneal seeding. Br J Surg 91:304-311
60. Prayson RA, Hart WR, Petras RE. (1994) Pseudomyxoma peritonei. A clinicopathologic study of 19 cases with emphasis on site of origin and nature of associated ovarian tumours. Am J Surg Pathol 18:591-603
61. Bryant J, Clegg AJ, Sidhu MK, et al. (2005) Systematic review of the Sugarbaker procedure for pseudomyxoma peritonei. Br J Surg 92:153-158
62. Stenhouse G, McRae D, Pollock AM. (2003) Urachal adenocarcinoma in situ with pseudomyxoma peritonei: a case report. J Clin Pathol 56:152-153
63. Young RH, Scully RE. (1990) Ovarian metastases from carcinoma of the gallbladder and extrahepatic bile ducts simulating primary tumours of the ovary. A report of six cases. Int J Gynecol Pathol 9:60-72
64. McCarthy JH, Aga R. (1988) A fallopian tube lesion of borderline malignancy associated with pseudo-myxoma peritonei. Histopathology 13:223-225
65. Ikejiri K, Anai H, Kitamura K, et al. (1996) Pseudomyxoma peritonei concomitant with early gastric cancer: report of a case. Surg Today 26:923-925
66. Chejfec G, Rieker WJ, Jablokow VR, et al. (1986) Pseudomyxoma peritonei associated with colloid carcinoma of the pancreas. Gastroenterology 90:202-205
67. Costa MJ. (1994) Pseudomyxoma peritonei. Histologic predictors of patient survival. Arch Pathol Lab Med 118:1215-1219
68. Hawes D, Robinson R, Wira R. (1991) Pseudomyxoma peritonei from metastatic colloid carcinoma of the breast. Gastrointest Radiol 16:80-82
69. Kurita M, Komatsu H, Hata Y, et al. (1994) Pseudomyxoma peritonei due to adenocarcinoma of the lung: case report. J Gastroenterol 29:344-348
70. Ronnett BM, Seidman JD. (2003) Mucinous tumours arising in ovarian mature cystic teratomas: relationship to the clinical syndrome of pseudomyxoma peritonei. Am J Surg Pathol 27:650-657
71. McKenney JK, R.A. S ,T.A. L. (2004) Mucinous neoplasms arising in mature teratomas: A clinicopathologic study of ovarian sacrococcygeal tumours. Mod Pathol 17:206A
72. Hameed A, Jafri N, Copeland LJ, et al. (1996) Endometriosis with myxoid change simulating mucinous adenocarcinoma and pseudomyxoma peritonei. Gynecol Oncol 62:317-319

The Pathogenesis of Malignant Ascites

J Tamsma

Introduction

Peritonitis carcinomatosa (PC) indicating the presence of malignant cells in the peritoneal cavity, is a well known complication of malignant disease. As a result, so-called malignant ascites develops. In this chapter, we will address the subject from a pathophysiologic perspective. First, we will review the complex microscopic anatomy and physiology of the normal peritoneal membrane. Secondly, characteristics of malignant ascites and its pathophysiology will be reviewed using Starling's equation of capillary forces.

Anatomical and Physiological Considerations

Anatomy of the Peritoneal Membrane

The microscopic anatomy of the peritoneal membrane reveals, apart from the capillary endothelium and basement membrane, three distinct barriers to prevent the loss of proteins into the peritoneal cavity: the interstitial stroma, the mesothelial basement membrane and the mesothelial cells lining the peritoneum [1]. Following the route from the intravascular to the intraperitoneal space, the endothelial cells are the first barrier encountered. Endothelial cells have an extracellular glycocalix with fixed anionic charges that is difficult to pass for anionic macromolecules such as albumin, an important contributor to plasma oncotic pressure [2].

Peritoneal endothelial cells are linked with tight junctions, and as a result transport is transendothelial using intracellular pores [3,4]. Endothelial cells are separated from the interstitial space by the endothelial basement membrane. The general plan for basement membranes is a core of collagen to which several different kinds of macromolecules are anchored. Proteoglycans present in the basement

membrane may constitute a net negative charge, which forms a selective barrier for anionic proteins.

The interstitial space consists of loose connective tissue composed of fibroblasts, collagen, hyaluronic acid and negatively charged macromolecules. Hyualuronic acid is able to bind a considerable amount of water, for instance edema during peritonitis. The interstitial space acts as a filter with significant resistance against diffusion of macromolecules.

The submesothelial basement membrane normally appears as a continuous layer at the interstitial site of the mesothelial cells. Evidence is available that negatively charged glycosaminoglycans are also present at this site. Mesothelial cells are the last barrier to be passed. The mesothelium consists of a monolayer of flat cells with a total estimated surface of about 2 square meters. The mesothelial cells show some functional similarity to endothelial cells. They have a glycocalix containing anionic charges and transcellular channels for macromolecular transport [5].

In short, the presence of tight junctions between the endothelial cells in the peritoneal capillaries and the presence of negatively charged macromolecules at several extracellular sites produce an effective barrier against leakage of negatively charged molecules such as albumin from plasma to the peritoneal cavity. Thus, the anatomy of the peritoneal membrane is such that it constitutes a relative impermeability to proteins while fluid and solutes easily pass the membrane. In other words, basic requirements to prevent excessive fluid filtration from the capillaries to the peritoneum are met by the anatomic "construction" of the peritoneal membrane.

The Peritoneal Lymphatic System

The lymphatic system collects fluid, proteins, other macromolecules and cells, to return them to the systemic circulation. The smallest lymphatics consist of one layer of endothelium and drain into lymphatic capillaries. A basement membrane may be present at this level but if so, it is interrupted. The lymphatic capillary net is organized as a plexus along the submesothelial surface and drains to lymph vessels. Lymph vessels have valves and spirally formed smooth muscle cells. They are innervated. Contractions of lymph vessels are generated by myogenic stimuli, and are at least influenced by activation of α-adrenoreceptors, temperature, calcium concentrations, and vasoactive peptides.

A specialised, intriguing anatomic feature of the peritoneal lymphatic system are the so-called stomata. Stomata are open communications between the abdominal cavity and the submesothelial diaphragmatic lymphatics. They are supposed to play a major role in peritoneal lymphatic drainage [1], as most of intraperitoneal fluid is absorbed at this site [6].

The mechanisms involved in lymph formation are still unclear. A hydraulic pressure theory has been proposed [7]. Normally, the interstitial pressure is negative [8], and an increase in intraabdominal pressure will result in increased lymph production. A close correlation between fluid absorption and intra-abdominal pressure has been shown, in line with this theory [9]. Another hypothesis states

that osmotic forces are dominant. This theory postulates a protein concentrating mechanism at the initial lymphatics [10]. Active transendothelial transport of albumin has been shown [11], which could create the necessary osmotic force.

Characteristics of Malignant Ascites - Intraperitoneal Protein Accumulation

Malignant ascites is characterised by positive cytology of malignant cells. Compared to ascites caused by cirrhosis more white blood cells and a higher lactate dehydrogenase (LDH) level are present [12,13]. Interestingly from the viewpoint of capillary hemodynamics is the observation that mean protein levels of ascites are high in patients with PC [13], as are the albumin concentrations [12-14]. The difference between serum- and ascites-albumin concentration is small. Thus, protein and albumin accumulate intraperitoneally in malignant ascites, which will be reviewed further as we discuss Starling's law of capillary hemodynamics.

Impaired Drainage or Increased Production?

Fluid accumulation will occur if lymphatic drainage of the peritoneal cavity is compromised or if net filtration is increased, overwhelming lymphatic capacity. Peritoneal fluid kinetics has been studied extensively in peritoneal dialysis. In dialysis, fluid accumulation is possible only if net filtration exceeds net absorption. Net fluid filtration is the resultant of the osmolality of the dialysate. The higher the osmolality, the higher the force that attracts fluid from the intravascular compartment. Interestingly, the osmolality of the dialysate changes in time. Osmotically active molecules disappear through lymphatic transport and are diluted due to the attracted amount of fluid (water). An important consequence of this mechanism is a reduction in the rate of filtration in time [15]. In contrast, lymphatic drainage proceeds at a fairly constant rate of 40 ml/hr during dialysis. The above mentioned stomata located at the peritoneal membrane lining the diaphragm are the principle site of drainage [15]. Thus, the net effect on intraperitoneal fluid accumulation can be calculated from the combined effects of filtration and lymphatic transport. In malignant ascites, fluid accumulation can likewise be regarded as the resultant of filtration minus drainage.

There is evidence for impaired lymphatic drainage in PC. This was studied in mice in which ascites was induced by injecting tumour cells intraperitoneally [16]. Alterations in diaphragmatic lymphatic absorption were determined radiographically. Diaphragmatic and retrosternal lymph vessels became occluded 5 days after tumour cell injection. Ascites formation was evident 5 to 7 days after injection of tumour cells. Comparable experimental data showing decreased lymphatic drainage have been produced by others [17]. Furthermore, lymphoscintigraphy showed decreased lymphatic drainage in humans [18,19].

Together, there is fair evidence for decreased lymphatic drainage as contributing factor in the pathogenesis of malignant ascites (Fig. 1) [19].

In addition to impaired lymphatic drainage there is evidence for increased fluid production. Using radioactive isotopes, it was shown that the inflow rate of plasma into the peritoneal cavity was increased six- to sixteen-fold [20]. The patho-physiology of increased fluid production is described by Starling's law of capillary hemodynamics.

Starling's law of Capillary Hemodynamics

The exchange of fluid between the plasma and the interstitium is determined by the hydraulic and oncotic pressures in each compartment. The relationship between these parameters can be expressed by Starling's law [21]:

$$\begin{aligned} \text{Net filtration} &= \text{LpS } (\delta \text{ hydraulic pressure} - \delta \text{ oncotic pressure}) \\ &= \text{LpS } [(P_{cap} - P_{if}) - s(\pi_{cap} - \pi_{if})] \end{aligned}$$

In this equation Lp is the unit permeability or porosity of the capillary wall, S is the surface area available for filtration, P_{cap} and P_{if} are the capillary and interstitial fluid hydraulic pressures, π_{cap} and π_{if} are the capillary and interstitial fluid oncotic pressures, and s represents the reflection coefficient of proteins across the capillary wall (with values ranging from 0 if completely permeable to 1 if completely impermeable) [21]. Increased capillary permeability (Lp), increased surface area (S) available for filtration, increased hydraulic pressure difference ($P_{cap} - P_{if}$), a decreased oncotic pressure difference $s(\pi_{cap} - \pi_{if})$ or a combination of these factors could account for an increase of net filtration.

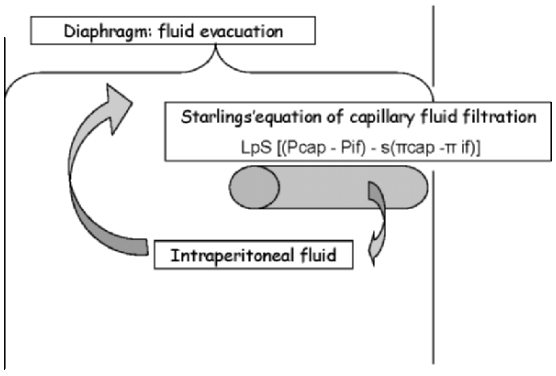


Figure 1. Schematic drawing of the proposed pathogenesis of malignant ascites. Normal physiology of fluid filtration and absorption results in a constant flow of fluid from the vascular compartment to the peritoneal space without significant fluid accumulation

Increased Capillary Permeability

In PC, increased permeability to proteins was observed in mice after intraperitoneal administration of tumour cells [16]. In another study, it was shown that a few days after intraperitoneal injection of Walker 256 carcinoma cells new capillaries were observed and bloody ascites developed. Inhibition of angiogenesis with locally administered protamine prevented new capillaries to develop and also prevented the occurence of ascites [22]. The coincidence of increased permeability and new vessel formation is striking.

It is now well understood that tumour growth is dependent on angiogenesis, the formation of new blood vessels [23]. Angiogenesis starts by stimulation of the endothelium, resulting in hyperpermeability of the endothelial membrane and degradation of the basement membrane and underlying stroma. The next step is the migration and proliferation of endothelial cells, and the formation of new blood vessels and capillaries [23]. Two important factors in angiogenesis are basic fibroblast growth factor (b-FGF) and vascular endothelial growth factor (VEGF) [24]. VEGF was discovered as a factor creating hyperpermeability and was initially named vascular permeability factor (VPF) [25].

In a mouse model, it was shown that small blood vessels lining the peritoneal cavity (mesentery, peritoneal wall, diaphragm) became hyperpermeable several days after intraperitoneal tumour cell injection. The development of hyperpermeable microvessels correlated with ascites VPF concentration [26]. Most tumours express VEGF [27], including ovarian [28,29], gastric and colon carcinomas [30]. A study has been performed confirming the presence of high VEGF concentrations in malignant ascites [31,32]. Furthermore, malignant ascites production, but not tumour growth, was completely inhibited in mice when treated with function-blocking VEGF antibodies. When the treatment was stopped, all mice developed ascites within two weeks [33]. These experimental results have been confirmed by others using anti-VEGF antibodies [34,35], VEGF tyrosine kinase receptor inhibitors [35,36] or exogenous soluble human VEGF receptor [37].

These data strongly suggest that increased capillary permeability due to production of locally active substances such as VEGF is an important factor in the pathophysiology of malignant ascites (Fig. 2). In words of the equation: the value of Lp become larger thus leading to increased net filtration.

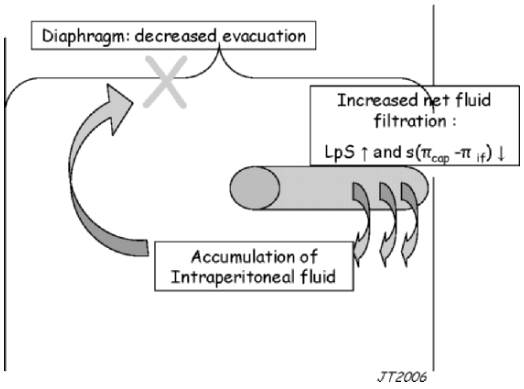


Figure 2. Presence of tumour cells results in the obliteration of lymphatic drainage as depicted with (X). This process seems particularly important at the diaphragm which is an important site of fluid drainage. Furthermore, production of locally active molecules such as VEGF and b-FGF results in changes in Starlings' law of capillary hemodynamics (mostly at the level of LpS and $s(\pi_{cap} - \pi_{if})$, see text). Thus, the balance between fluid production and drainage is disturbed leading to the formation of ascites

Increased Filtration Surface Area

In mice, the size and number of peritoneal lining microvessels and subsequently cross sectional area increased after intraperitoneal tumour cell injection [38]. The site of production of malignant ascites has also been studied in patients using plastic rings with absorbent paper which were placed on peritoneal tumour and tumour-free surface. The rate of production of ascites of tumour-free omentum and small bowel surface was increased. The rate of fluid production from the tumour surface was also higher than the fluid production of peritoneum of control subjects, but less outspoken. The authors concluded that "undoubtedly fluid exuded from the tumour surface but the lion's share comes from the disease free peritoneum" [20]. Thus, in malignant ascites an increased cross sectional area of microvessels lining the peritoneal cavity has been shown in an experimental setting. In addition, it seems that in human subjects the tumour free peritoneal surface is able to produce the surplus of fluid in malignant ascites [20]. These findings are in line with an increased S (experimental) and S or LpS (human) in Starlings' equation.

Increased Hydraulic Pressure Difference

The same paper [20] reported on portal pressure in controls and in patients with ovarian cancer with or without ascites. A minor increase of portal vein pressure was observed in patients with ascites. $(P_{cap} - P_{if})$ will probably not change dramatically.

Decreased Oncotic Pressure Difference

In normal physiology, albumin is known to be an effective osmol which contributes to intravascular oncotic pressure necessary to reabsorb fluid from the interstitial space. If the oncotic pressure difference decreases, reabsorbtion decreases and interstitial fluid accumulation results. In PC, protein accumulates intraperitoneally [12]. These intraperitoneal proteins may be partly degraded to smaller peptides and amino acids, which could contribute to intraabdominal oncotic pressure. This situation is comparable to peritoneal dialysis solutions containing a 5% amino acid concentration, which are very effective in forcing ultrafiltration [15]. As the plasma to peritoneal oncotic pressure difference decreases and even becomes negative reabsorption into the intravascular compartment will diminish and fluid may even be "filtrated" into the peritoneal cavity (Fig. 1).

Thus, regarding Starling's law of capillary hemodynamics we propose that the increased capillary permeability is essential in the pathophysiology of malignant ascites. The resultant decreased or negative oncotic pressure difference attracts fluid into the peritoneal cavity. In the equation: $s(\pi_{cap} - \pi_{if})$ will approximate zero or attain a negative value thus increasing net filtration. Overall the equation will become:

$$\textbf{Net filtration} \uparrow\uparrow = \textbf{LpS} \uparrow [(P_{cap} - P_{if}) - \textbf{s}(\boldsymbol{\pi}_{\textbf{cap}} - \boldsymbol{\pi}_{\textbf{if}}) \downarrow]$$

Conclusion

The pathogenesis of malignant ascites is beginning to be elucidated. Decreased lymphatic absorption and increased fluid production can be identified as contributing features of ascites formation. The increased net capillary fluid production is due to an increase of capillary permeability and surface area, and a subsequent increase of intraperitoneal protein concentration leading to increased intraperitoneal oncotic pressure. This sequence might be the result of biologically active peptides produced by tumour cells such as VEGF and b-FGF. Interference with these mediators may serve as target in future therapeutic strategies.

References

1. Gotloib L, Shostak A (1990) The functional anatomy of the peritoneum as a dialyzing membrane. In: Twardowski ZJ, Nolph KD, Khanna R, editors. Contempory issues in Nephrology vol 22. Peritoneal Dialysis: new concepts and applications. New York: Churchill Livingstone, 1-29

2. Nolph KD, Miller F, Rubin J, Popovich R (1980) New directions in peritoneal dialysis concepts and applications. Kidney Int Suppl 10:S111-S116
3. Renkin EM (1986) Some consequences of capillary permeability to macromolecules: Starling's hypothesis reconsidered. Am J Physiol 250 (5Pt2): H706-H710
4. Grega GJ, Adamski SW, Dobbins DE (1986) Physiological and pharmacological evidence for the regulation of permeability. Fed Proc 45(2):96-100
5. Gotloib L, Digenis GE, Rabinovich S, et al (1983) Ultrastructure of normal rabbit mesentery. Nephron 34(4):248-255
6. Lifshitz S (1982) Ascites, pathophysiology and control measures. Int J Radiat Oncol Biol Phys 8(8):1423-1426
7. Allen L (1931) Volume and pressure changes in terminal lymphatics. Am J Physiol 123:3
8. Guyton AC (1963) A concept of negative interstitial pressure based on pressures in implanted perforated capsules. Circ Res 12:399
9. Zink J, Greenway CV (1977) Control of ascites absorption in anesthetized cats: effects of intraperitoneal pressure, protein, and furosemide diuresis. Gastroenterology 73(5):1119-1124
10. Casley-Smith JR (1978) A fine ultrastructural study of variations in protein concentration in lacteals during compression and relaxation. Lymphology 12:59-65
11. Shasby DM, Shasby SS (1985) Active transendothelial transport of albumin. Interstitium to lumen. Circ Res 57(6):903-908
12. Runyon BA, Hoefs JC, Morgan TR (1988) Ascitic fluid analysis in malignancy-related ascites. Hepatology 8(5):1104-1109
13. Salerno F, Restelli B, Incerti P, et al (1990) Utility of ascitic fluid analysis in patients with malignancy-related ascites. Scand J Gastroenterol 25(3):251-256
14. Jungst D, Xie Y, Gerbes AL (1992) Pathophysiology of elevated ascites fluid cholesterol in malignant ascites. Increased ascites to serum relation of proteins and lipoproteins in patients with peritoneal carcinomatosis as compared to patients with cirrhosis of the liver. J Hepatol 14(2-3):244-248
15. Mactier RA (1990) Kinetics of ultrafiltration with glucose and alternative osmotic agents. In: Twardowski ZJ, Nolph KD, Khanna R, editors. Contemporary issues in nephrology. Peritoneal dialysis, new concepts and applications. New York: Churchill Livingstone, 29-52
16. Fastaia J, Dumont AE (1976) Pathogenesis of ascites in mice with peritoneal carcinomatosis. J Natl Cancer Inst 56(3):547-550
17. Feldman GB, Knapp RC, Order SE, Hellman S (1972) The role of lymphatic obstruction in the formation of ascites in a murine ovarian carcinoma. Cancer Res 32(8):1663-1666
18. Coates G, Bush RS, Aspin N (1973) A study of ascites using lymphoscintigraphy with 99m Tc-sulfur colloid. Radiology 107(3):577-583
19. Bronskill MJ, Bush RS, Ege GN (1977) A quantitative measurement of peritoneal drainage in malignant ascites. Cancer 40(5):2375-2380
20. Hirabayashi K, Graham J (1970) Genesis of ascites in ovarian cancer. Am J Obstet Gynecol 106(4):492-497

21. Rose BD, Post TW (2001) Edematous states. In: Rose BD, Post TW (eds). Clinical physiology of acid-base and electrolyte disorders. New York, NY: McGraw-Hill, 478-534
22. Heuser LS, Taylor SH, Folkman J (1984) Prevention of carcinomatosis and bloody malignant ascites in the rat by an inhibitor of angiogenesis. J Surg Res 36(3):244-250
23. Hanahan D, Folkman J (1996) Patterns and emerging mechanisms of the angiogenic switch during tumourigenesis. Cell 86(3):353-364
24. Neufeld G, Cohen T, Gengrinovitch S, Poltorak Z (1999) Vascular endothelial growth factor (VEGF) and its receptors. FASEB J 13(1):9-22
25. Senger DR, Galli SJ, Dvorak AM, et al (1983) Tumour cells secrete a vascular permeability factor that promotes accumulation of ascites fluid. Science 219(4587):983-985
26. Nagy JA, Masse EM, Herzberg KT, et al (1995) Pathogenesis of ascites tumour growth: vascular permeability factor, vascular hyperpermeability, and ascites fluid accumulation. Cancer Res 55(2):360-368
27. Senger DR, Perruzzi CA, Feder J, Dvorak HF (1986) A highly conserved vascular permeability factor secreted by a variety of human and rodent tumour cell lines. Cancer Res 46(11):5629-5632
28. Yamamoto S, Konishi I, Mandai M, et al (1997) Expression of vascular endothelial growth factor (VEGF) in epithelial ovarian neoplasms: correlation with clinicopathology and patient survival, and analysis of serum VEGF levels. Br J Cancer 76(9):1221-1227
29. Barton DP, Cai A, Wendt K, Young M, et al (1997) Angiogenic protein expression in advanced epithelial ovarian cancer. Clin Cancer Res 3(9):1579-1586
30. Zebrowski BK, Liu W, Ramirez K, et al (1999) Markedly elevated levels of vascular endothelial growth factor in malignant ascites. Ann Surg Oncol 6(4):373-378
31. Kraft A, Weindel K, Ochs A, et al (1999) Vascular endothelial growth factor in the sera and effusions of patients with malignant and nonmalignant disease. Cancer 85(1):178-187
32. Santin AD, Hermonat PL, Ravaggi A, et al (1999) Secretion of vascular endothelial growth factor in ovarian cancer. Eur J Gynaecol Oncol 20(3):177-181
33. Mesiano S, Ferrara N, Jaffe RB (1998) Role of vascular endothelial growth factor in ovarian cancer: inhibition of ascites formation by immunoneutralization. Am J Pathol 153(4):1249-1256
34. Luo JC, Toyoda M, Shibuya M (1998) Differential inhibition of fluid accumulation and tumour growth in two mouse ascites tumours by an antivascular endothelial growth factor/permeability factor neutralizing antibody. Cancer Res 58(12):2594-2600
35. Schlaeppi JM, Wood JM (1999) Targeting vascular endothelial growth factor (VEGF) for anti-tumour therapy, by anti-VEGF neutralizing monoclonal antibodies or by VEGF receptor tyrosine-kinase inhibitors. Cancer Metastasis Rev 18(4):473-481

36. Xu L, Yoneda J, Herrera C, et al (2000) Inhibition of malignant ascites and growth of human ovarian carcinoma by oral administration of a potent inhibitor of the vascular endothelial growth factor receptor tyrosine kinases. Int J Oncol 16(3):445-454
37. Stoelcker B, Echtenacher B, Weich HA, et al (2000) VEGF/Flk-1 interaction, a requirement for malignant ascites recurrence. J Interferon Cytokine Res 20(5):511-517
38. Nagy JA, Morgan ES, Herzberg KT, Manseau EJ, Dvorak AM, Dvorak HF (1995) Pathogenesis of ascites tumour growth: angiogenesis, vascular remodeling, and stroma formation in the peritoneal lining. Cancer Res 55(2):376-385

Natural History of Peritoneal Carcinomatosis from Digestive Origin

JC Lifante, O Glehen, E Cotte, AC Beaujard, FN Gilly

Introduction

Primary peritoneal carcinomatosis (malignant mesothelioma, pseudomyxoma peritonei) are rare while peritoneal carcinomatosis (PC) is a common evolution of digestive cancers. In the past, it has been regarded as a terminal condition only to be palliated. Since the eighties, there has been a renewed interest in PC with new multimodal therapeutic approaches: peritonectomy procedures [1], intraperitoneal chemo hyperthermia [2,3], immediate postoperative intraperitoneal chemotherapy [4], and more. However, the literature available on aetiology, clinical features and natural history of PC from non gynaecological malignancies is not very extensive.

The causes of PC have been listed and reported by Sugarbaker [5]: natural mechanisms (full-thickness penetration of the bowel wall by cancer and then seeding of the peritoneal cavity, cascade phenomenon from the first peritoneal implant that progress into nodules – these nodules then exfoliate malignant cells to exponentially increase the number of cancerous nodules) and iatrogenic mechanisms (narrow margins of resection, leakage of tumour cells from lymphatic channels that have been traumatically divided during the resection, venous blood lost from the tumour at the time of resection, and trauma to the primary malignancy).

The clinical features and natural history of PC were reported in three studies: the first reported was from Chu (1989), including 100 patients [6], the second one was the French multicenter prospective study named EVOCAPE 1 (2000) including 370 patients [7] and the third one is from Jayne [8].

Aetiology of Peritoneal Carcinomatosis

A lot of in vitro and in vivo experimental details have been reported on the progression of a digestive tumour: the very first malignant cells appear in the digestive mucosa, then invade the submucosa, the muscular layers and at last, the serosal surfaces.

During this progression, three ways of dissemination could occur. Malignant cells may invade bowel wall veins and travel via the blood flow up to the portal vein with the risk of developing into liver metastases. A second dissemination way can occur during the progression of malignant cells from the mucosa to the serosal surface: microscopic lymphatic channels within the bowel wall could be invaded and so lymph nodes nearby the primary tumour could be involved by the malignant process and the lymph nodes at distance could be involved as well.

Invasion of the bowel wall up to the serosal surfaces may result in seeding of malignant cells from the primary tumour on adjacent and distant peritoneal surfaces: this represents the third way of dissemination in digestive cancers as well as the main aetiology of PC.

Once malignant cells are free inside the peritoneal cavity, they are entrapped at sites of trauma (abdominal incision, ligatures around blood vessels or lymphatic chains, suture lines and more) where fibrin accumulations and blood clots will secure them and enhance their growth; then, according to Sugarbaker's theory, "*the pace of the disease increases markedly as metastases from metastases develop and implants exfoliate within the peritoneal cavity"* [5].

Preclinical studies have clearly demonstrated a relation between surgical trauma of the peritoneum and tumour implantation both at the site of trauma and at more distant locations [9,10].

These three ways of dissemination are commonly accepted. However, they do not explain the rare occurrence of PC in early stage (pT_1 or pT_2) disease [7]; alternative hypotheses could be formulated in this regard (blood flow dissemination (but peritoneal surface vascularisation is poor), exfoliation from involved lymph nodes (but synchronous peritoneal carcinomatosis also occurs in pN_0 digestive cancers).

Clinical Features of Peritoneal Carcinomatosis from Digestive Origin

Clinical features of PC were reported by Chu [6] on 100 patients (45 colorectal, 20 pancreas, 6 gastric, 4 small bowel, 2 appendix, 2 unknown primaries and 21 miscellaneous) as well as from the French EVOCAPE 1 [7] on 370 patients (125 gastric, 118 colorectal, 58 pancreas, 4 small bowel, 3 liver, 12 pseudomyxoma, 7 mesothelioma and 43 unknown).

Synchronous PC was found in 54.6% of patients (257/470) while ascites (164/470) and bowel obstruction (114/470) were the main clinical symptoms. Resection of the primary tumour was done in 42.0% of patients while only bypass to re-establish gastrointestinal continuity was performed in 34.2% of patients and only exploratory laparotomy and biopsies in 23.8%.

Further details of the four largest subgroups (gastric, colorectal, pancreatic and primary unknown carcinoma) are given below (restricted to the 370 patients included in the french EVOCAPE 1 study). The extent of PC was staged according to the classification detailed in Table 1.

Table 1. Staging of Peritoneal Carcinomatosis

Stage	Peritoneal Carcinomatosis Description
Stage 0	No macroscopic disease
Stage 1	Malignant granulations less than 5mm in diameter Localized in one part of the abdomen
Stage 2	Malignant granulations less than 5 mm in diameter Diffuse to the whole abdomen
Stage 3	Malignant granulations 5mm to 2 cm in diameter
Stage 4	Large malignant deposits (more than 2 cm diameter)

Gastric Cancer

The mean age of these 125 patients (76 males) was 60.5 years (range 21- 96 years). Seventy three patients had synchronous PC (58.4 %) and the most frequent symptom was ascites (35/125) while bowel obstruction was present in 9 patients (0.07%) (Table 2).

Table 2. Clinical features of peritoneal carcinomatosis from non gynecological origin (EVOCAPE-1)

Primary	Clinical features				Surgical procedures			CTh
	Synchr.	Obstruction	Ascites	+ US/CT	Resection	Bypass	Biopsy	
Gastric	73	9	35	13	63	35	27	17
Colorectal	69	23	35	11	75	26	17	46
Pancreas	40	6	25	5	4	30	24	11
Unknown	28	14	21	20		20	23	10
Small bowel	0	3	1	0	1	3	0	1
Liver	2	0	0	0	1	0	2	1
PMP		2	8	4		10	2	6
Mesothelioma		2	2	5		1	6	5
TOTAL	212	59	127	58	144	125	101	97

Synchr, synchronous; US, ultrasound; CTh, chemotherapy

The stage of the disease was advanced as evidenced by the pTNM classification (55 pT_3, 62 pT_4, 117 pN_+); however, 2 patients had a pT_1 and 6 patients a pT_2 gastric cancer (Table 3). Peritoneal carcinomatosis staging [11] showed 72/125 stages 3 and 4, according to the previously described PC staging (Table 4).

Table 3. Pathological stage and differentiation of primary tumours (EVOCAPE-1)

	Differentiation					**pTNM stage**							
	WD	MD	PD	UD	N/A	pT_1	pT_2	pT_3	pT_4	pN_0	pN_+	M_1	**T**
Gastric	22	31	16	9	47	2	6	55	62	8	117	19	125
Colorectal	41	22	14	1	40	0	4	76	38	14	104	27	118
Pancreas	13	15	5	1	24	0	2	30	26	5	53	6	58
Unknown	3	5	10	1	24								43
Small bowel	1	0	1	0	2								4
Liver	0	2	0	0	1								3
PMP													12
Mesothelioma													7
Total	80	75	46	12	138	2	12	161	126	27	274	52	370

T, total; WD, Well differentiated; MD, Moderately differentiated; PD, Poorly differentiated; UD, Undifferentiated; N/A, not available; PMP, pseudomyxoma peritonei

Table 4. Peritoneal carcinomatosis staging (EVOCAPE-1)

	Stage 0	**Stage 1**	**Stage 2**	**Stage 3**	**Stage 4**	**Total**
Gastric	9	22	22	27	45	125
Colorectal	2	11	27	33	45	118
Pancreas	2	11	13	12	20	58
Unknown	0	1	4	7	31	43
Small bowel	0	1	1	0	2	4
Liver	0	1	2	0	0	3
Pseudomyxoma	0	0	2	3	7	12
Mesothelioma	0	0	0	2	5	7
TOTAL	13	47	71	84	155	370

Colorectal Cancer

The mean age of these 118 patients (62 males) was 62.3 years (range 20-92 years). Sixty nine patients had synchronous PC (58.5%) and the most frequent symptom (Table 2) was ascites (35/118) while bowel obstruction occurred for 23 patients (19.5%). The stage of the disease was advanced (Table 3) as evidenced by the pTNM classification (76 pT_3, 38 pT_4, 27 pM_1); however, 4 patients had a pT_2 colorectal cancer. Peritoneal carcinomatosis staging showed 78/118 stages 3 and 4 (Table 4).

Pancreatic Cancer

The mean age of these 58 patients (27 males) was 65.5 years (range 26-89 years). Forty patients had synchronous PC (68.9 %) and the most frequent symptom (Table 2) was ascites (25/58) while bowel obstruction occurred in 6 patients (10.3 %). The stage of the disease was advanced as evidenced by the presence of ascites (43.1 %) and by the PC staging (32/58 stages 3 and 4).

Cancer with Unknown Primary

The mean age of these 43 patients (15 males) was 67.5 years (range 24-83 years). Twenty eight patients had synchronous PC (65.1%) and the most frequent symptom (Table 2) was ascites (21/43) while 14 patients (32.5 %) presented with bowel obstruction. Peritoneal carcinomatosis stages were advanced: 38/43 were stage 3 and 4 (Table 4).

Natural History of Peritoneal Carcinomatosis

While PC is a common evolution of digestive cancers with a poor prognosis, the literature available on the natural history of PC is not very extensive. In 1989, Chu [6] reported a 6 months median survival in PC from colorectal origin, 0.7 months from pancreas origin and 1 month from gastric cancer.

Important additional data were gathered by the EVOCAPE 1 study, which excluded patients who underwent laparoscopy or extensive cytoreductive surgery with intraperitoneal chemotherapy.

Three hundred seventy patients were entered (206 males, 164 females, mean age 67.7, range 20-90 years) with the following tumour types: 125 gastric, 118 colorectal, 58 pancreas, 12 pseudomyxoma peritonei, 7 malignant peritoneal mesothelioma, 4 small bowel, 3 liver and 43 cancers with unknown primary.

pTNM classification and differentiation are underlined in Table 2. In these 370 PC patients, 212 were synchronous and 158 metachronous. All 370 patients underwent surgery. The staging of peritoneal carcinomatosis was as follows: stage 0

(n = 13), stage 1 (n = 47), stage 2 (n = 71), stage 3 (n= 84) and stage 4 (n = 155) as described in Table 4. The procedures performed (Table 2) were resection of primary tumours in 144 patients, bypass to restablish gastrointestinal continuity in 125 and only laparotomy with biopsies in 101 patients. The overall operative mortality and morbidity rates were 21% (77/370) and 16% (60/370) respectively. Ninety seven patients underwent postoperative palliative systemic chemotherapy (1-8 courses, 5-fluorouracil combined with folinate in 64 patients and 5 fluorouracil combined with oxaliplatin in 33 patients). The mean and the median overall survival were 6.0 months (0.1 to 48.0 months) and 3.1 months respectively (Fig. 1).

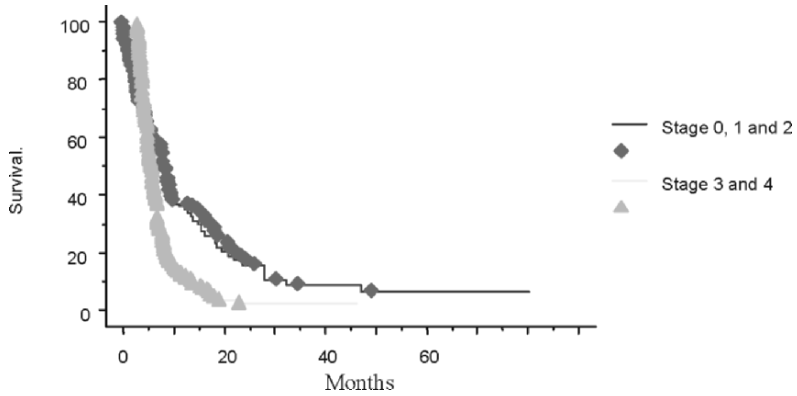


Figure 1. Kaplan Meier survival curves of 370 patients with peritoneal carcinomatosis according to PC stage

Mean and median survival according to the peritoneal carcinomatosis staging are shown in Table 5. For gastric carcinoma patients, overall mean and median survival were 6.5 months (range 0.1-48) and 3.1 months respectively.

Table 5. Survival of peritoneal carcinomatosis patients according to PC stage

	Mean	Range	Median
Stage 0	19.9	0.1 – 24.1	10.0
Stage 1	9.8	1.6 – 48.0	7.0
Stage 2	6.8	0.2 – 40.0	5.0
Stage 3	5.6	0.1 – 24.1	3.9
Stage 4	3.7	0.1 – 36.0	2.0
Overall	6.0	0.1 – 48.0	3.1

Data represent time (months); $p < 0.0001$

Several potential prognostic factors were analyzed for survival differences: synchronous or metachronous peritoneal carcinomatosis, initial pTNM classification, lymph node involvement, peritoneal carcinomatosis staging, presence of ascites and of liver metastases (Table 6).

Table 6. Prognostic factors for survival in gastric cancer with peritoneal metastases

		Median	**Mean**	**p**
Timing of PC	Synchronous	2.8	4.8	0.6
	Not synchronous	3.1	5.1	
Initial pT stage	T1,T2 (n = 8)	9.0	20.4	0.06
	T3 (n = 55)	4.0	9.7	
	T4 (n = 62)	2.5	4.2	
Initial pN stage	N0	8.8	7.0	0.52
	N+	8.5	7.8	
PC stage	Stage 1	7.9	11.2	**0.001**
	Stage 2	6.2	4.5	
	Stage 3	5.8	3.5	
	Stage 4	1.9	2.6	
Differentiation	WD and MD	4.2	5.2	0.4
	PD and UD	2.4	4.7	
Ascites	Yes	1.4	3.6	**0.05**
	No	3.8	5.4	
Hepatic mets	Yes	1.0	2.6	**0.0009**
	No	3.3	5.3	

WD, Well differentiated; MD, Moderately differentiated; PD, Poorly differentiated; UD, Undifferentiated; data represent time (months)

For colorectal cancer patients, overall mean and median survival times were 6.9 months (range 0.6 - 44.9) and 5.2 months respectively. Several potential prognostic factors were analyzed for survival differences as shown in Table 7.

Table 7. Prognostic factors for survival in colorectal cancer with peritoneal metastases

		Median	**Mean**	**p**
Timing of PC	Synchronous	4.1	6.0	0.78
	Not synchronous	5.3	6.2	
Initial pTstage	T_1,T_2 (n=8)	7.3	9.3	0.5
	T_3 (n=55)	5.3	7.2	
	T_4 (n=62)	3.4	4.7	
Initial pN stage	N_0	8.7	10.2	0.13
	N_+	7	6.8	
PC stage	Stage 1	12.5	14.3	**0.001**
	Stage 2	8.3	8.4	
	Stage 3	4.4	6.0	
	Stage 4	2.7	4.4	
Differentiation	WD and MD	3.2	5.3	0.9
	PD and UD	5.5	5.3	
Ascites	Yes	3.7	5.1	0.6
	No	5.1	6.5	
Hepatic mets	Yes	4.4	6.1	0.4
	No	5.9	6.1	

WD, Well differentiated; MD, Moderately differentiated; PD, Poorly differentiated; UD, Undifferentiated; data represent time (months)

For pancreatic carcinoma patients, overall mean and median survival times were 2.9 months (range 0.3 - 13.6) and 2.1 months respectively. Four potential prognostic factors were analyzed for survival differences as shown in Table 8. For primary unknown cancer patients, overall mean and median survival times were 2.9 months (range 0.2 - 12) and 1.5 months respectively.

Table 8. Prognostic factors for survival in pancreatic cancer with peritoneal metastases

		Median	Mean	p
PC Stage	Stage 1 and 2	3.1	3.2	0.45
	Stage 3 and 4	2.3	2.4	
Differentiation	WD and MD	2.1	3.5	0.85
	PD and UD	2.5	3.2	
Ascites	Yes	1.4	3.4	**0.05**
	No	3.8	5.8	
Hepatic mets	Yes	2.8	4.2	0.36
	No	1.8	3.1	

WD, Well differentiated; MD, Moderately differentiated; PD, Poorly differentiated; UD, Undifferentiated; data represent time (months)

Discussion

Peritoneal carcinomatosis was first described as the regional spread in ovarian carcinoma in 1931 [12]. Mechanisms of PC development are still controversial: spreading of free cancer cells due to serosal involvement of the primary tumour [13], implantation of free cancer cells due to the presence of adherence molecules [5], lymphatic and or venous dissemination of malignant cells: it would appear that the mechanisms responsible are multifactorial.

From a clinical point of view, the main clinical indicators of PC are bowel obstruction and ascites [14]: bowel obstruction is mainly reported in colorectal cancer (20% in the present study) while ascites was mainly reported in pancreatic cancer (43%). Since both bowel obstruction and ascites usually reflect advanced disease, sensitive imaging is important to select patients with early stage PC as potential candidates for cytoreductive surgery.

Ultrasonography and CT scan are sensitive for the detection of ascites, but peritoneal implants smaller than 2 cm (ultrasound) or 5 mm (CT scan) are usually missed [15]. The sensitivity of CT in the diagnosis of PC has been reported as 70% for lesions 2 cm in greatest dimension and only 28% for lesions less than 5 mm in greatest dimension [16]. Magnetic resonance imaging is currently under evaluation in our institution with encouraging preliminary results in detecting stage 2 PC; immunoscintigraphy and PET scanning are under evaluation.

Diagnostic laparoscopy with biopsies could be the most effective way to diagnose PC, but the risk of tumour spread has raised concerns [14,17].

The French EVOCAPE 1 study confirms the very poor prognosis in PC patients (overall median survival 3.1 months), although some patients have reached a

survival of more than three years. Survival times differ according to the location of the primary tumour, with upper GI cancer having a worse prognosis (pancreas 2.1 months, stomach 3.1 months) than colorectal cancer (5.2 months). Moreover, survival was greatly influenced by PC stage and the presence of ascites. For PC from gastric origin, the presence of liver metastases also appears to be a negative prognostic factor [18]. The significant difference in survival observed between PC stage 1 and 2 versus stage 3 and 4 is important and highlights the need for stratification of patients according to PC stage (or the Peritoneal Cancer Index) in future clinical trials evaluating cytoreduction and/or systemic chemotherapy [19,20].

Future clinical and genomic studies will have to answer the important question whether PC, in the absence of systemic disease, reflects systemic spread or a locally treatable (and genomically different) entity for which cytoreduction and intraperitoneal chemotherapy could prolong survival.

References

1. Sugarbaker PH (1995) Peritonectomy procedures. Ann Surg 221: 29-42
2. Glehen O, Mithieux F, Osinsky D, et al (2003) Surgery combined with peritonectomy procedures and intraperitoneal chemohyperthermia in abdominal cancers with peritoneal carcinomatosis: A phase II study. J Clin Oncol 21:799-806
3. Fujimoto S, Takahaschi M, Mutou T et al (1997) Improved mortality rate of gastric carcinoma patients with peritoneal carcinomatosis treated with IPCH combined with surgery. Cancer 79: 884-891
4. Elias D, Gachot B, Bonvallot S et al (1997) Carcinoses peritoneales traitées par exerese complete et chimiotherapie intraperitoneale postoperatoire immediate: etude de phase II. Gastroenterol Clin Biol 21:181-187
5. Sugarbaker PH (1996) Peritoneal carcinomatosis: natural history and rational therapeutic using intraperitoneal chemotherapy. In: Sugarbaker PH, Peritoneal carcinomatosis, drugs and diseases. Boston, USA, Kluwer academic publishers, p149-168
6. Chu DZ, Lang NP, Thompson C et al (1989) Peritoneal Carcinomatosis in non gynecologic malignancy. Cancer 63: 364-367
7. Sadeghi B, Arvieux C, Glehen O et al (2000) Peritoneal carcinomatosis from non gynaecologic malignancies: results of EVOCAPE 1 multicentric prospective study. Cancer 88: 358-363
8. Jayne DG, Fook S, Loi C, et al (2002) Peritoneal carcinomatosis from colorectal cancer. Br J Surg 89:1545-1550
9. Van den Tole P, Van Rossen E, Van Eijck C et al (1998) Reduction of peritoneal trauma by using non surgical gauze leads to less implantation metastases of spilled tumour cells. Ann Surg 227, 242-248
10. Zoetmulder F (1996) Cancer cell seeding during abdominal surgery: experimental studies. In: Sugarbaker PH: Peritoneal carcinomatosis: principles of management. Boston, USA. Kluwer Academic publishers, p 155-161

11. Gilly FN, Carry PY, Sayag AC et al (1994) Regional chemotherapy and intraoperative hyperthermia for digestive cancers with peritoneal carcinomatosis. Hepatogastroenterology 41: 124-129
12. Sampson JA (1931) Implantation peritoneal carcinomatosis of ovarian origin. Am J Pathol 7: 423-443
13. Iitsuka H, Kaneshima S, Tenda O et al (1979) Intraperitoneal free cancer cells and their viability in gastric cancer. Cancer 44: 1476-1481
14. Ketcham AS, Hoye RC, Pilch YH et al (1970) Delayed intestinal obstruction following treatment for cancer. Cancer 25: 406-410
15. Weill FS, Costas R, Guetarini S et al (1990) Diagnostic echographiques des métastases péritonéales ches les malades ascitiques. J Radiol 71: 365-368
16. Jacquet P, Jelinek JS, Steves MA et al (1993) Evaluation of computed tomography in patients with peritoneal carcinomatosis. Cancer 72: 1631-1636
17. Targazona EM, Martinez J, Nadal A et al (1998) Cancer dissemination during laparoscopic surgery. World J Surg 22: 55-60
18. Glehen O, Schreiber V, Cotte E et al (2004) Cytoreductive surgery and intraperitoneal chemohyperthermia for peritoneal carcinomatosis arising from gastric cancer. Arch Surg 139: 20 – 26
19. Jacquet P, Sugarbaker PH (1996) Clinical research methodologies in diagnosis and staging of patients with peritoneal carcinomatosis. In: Sugarbaker PH. Peritoneal carcinomatosis. Boston, USA, Kluwer Academic publishers, p 359-374
20. Sugarbaker PH (1999) Successful management of microscopic residual disease in large bowel cancer. Cancer Chemother Pharmacol 43 (suppl) S15-S25

Intraperitoneal Drug Therapy: Physical and and Biological Principles

MF Flessner

Background

Although improvements in the management of gastrointestinal cancer and ovarian adenocarcinoma have been made in recent years, there still are significant problems managing the spread of these cancers throughout the peritoneal cavity, a process called peritoneal carcinomatosis (PC) [1]. Researchers now recognize that peritoneal spread can also occur with endometrial carcinoma [2] and esophageal cancer [3]. Traditional treatments of these diseases include a combination of surgery, radiation therapy, and systemic chemotherapy. Until recently, the prognosis for patients with PC has been dismal. Sugarbaker [4] has reported some success with careful peritonectomy and the use of perioperative intraperitoneal (IP) chemotherapy. There are, however, a number of problems with this technique, including trauma to the peritoneum during surgical removal of tumours that may result in further metastases [5]. Innovative techniques such as perioperative, hyperthermic chemotherapy to treat these residual microscopic lesions after peritonectomy have resulted in five-year survival of 80% [6].

The major challenges for IP therapy consist of: (a) sufficient *residence time* or duration of actual contact time with treatment solution to effect a cure, (b) coverage of the targeted area by the IP treatment solution (*peritoneal contact area*), and (c) the *penetration* or distance that the agent transports into the targeted tumour tissue in sufficient concentration to treat the tumour. The residence time becomes important because the solution can move from place to place in the cavity, and even relatively large volumes in the cavity only cover 30-40% of the anatomic peritoneum [7-9]. Studies in normal mice and rats [9,10] demonstrate that a solution placed in the peritoneal cavity for 24 hours covers all of the surfaces of the peritoneum. This might work well if the drug has absolute killing power on the instant that it reaches the surface of the tumour. However, most medications depend on some duration of exposure to transfer enough drug to effect a change in the tissue. In humans [8], the actual area covered by a large volume such as 2 to 3 liters in a human being is approximately 25 to 30 percent of the anatomic peritoneum.

Depending on adhesions and other abnormalities in the peritoneal cavity, the flow of the treatment fluid may be quite irregular and the residence time of the drug adjacent to the tumour nodule may be quite short or quite long. Once the drug is adjacent to the surface of the tumour, it must penetrate the tumour in order to reach the tumour cells. Since nodules of 0,5 to 1 centimeter in diameter may be missed by the oncologic surgeon in the initial peritonectomy [11-13], it may be anticipated that drugs will have to treat and cure this sized nodule. This chapter will deal with these delivery issues such as residence time, peritoneal contact area, and the penetration of antineoplastic agents administered intraperitoneally.

IP Versus Systemic (IV) Chemotherapy

Because the IP route of therapy is less convenient than IV and fraught with hazard for the inexperienced clinician, the case needs to be made for the advantage of IP therapy. Is there a pharmacokinetic advantage of administering the drug IP vs IV? One major advantage would be the attainment of very high concentrations in the peritoneal cavity relative to concentrations in the systemic circulation. This would minimize side effects and toxicity from systemic administration while increasing the therapeutic advantage in the peritoneal cavity [14].

Pharmacokinetic Advantage

The pharmacokinetic rationale for IP administration of drugs in the treatment of microscopic residual ovarian carcinoma was established in 1978 by Dedrick and colleagues [14]. The quantitative formula for pharmacokinetic advantage (R_d) in its simplest from is [14,15]:

$$R_d = \left(\frac{C_P}{C_B}\right)_{IP} \Big/ \left(\frac{C_P}{C_B}\right)_{IV} \quad (1)$$

where C_P = the concentration in the peritoneal cavity and C_B = concentration in the systemic circulation and the subscripts indicate the route of administration.

Pharmacokinetic studies have established the advantage of the IP vs IV route for treatment of intraabdominal cancer. Goel and colleagues [16] studied the IP administration of cisplatin in combination with etoposide and attempted to protect the kidney against platinum toxicity through the intravenous administration of sodium thiosulfate. The regional pharmacokinetic advantage was 26 compared to patients receiving IV therapy without simultaneous thiosulfate infusion. This is similar to the value of 16 found by Piccart and colleagues [17]. 5-fluorouracil values of R_d have been determined to be as high as 124 as noted by Speyer and colleagues [18] and 298 by Sugarbaker and colleagues [19]. Other drugs have shown similar pharmacokinetic advantages. Antibiotics are often administered IP in

patients with peritonitis. Drugs such as vancomycin have a calculated advantage of 4 to 15 if given intraperitoneally for peritonitis. Intraperitoneal insulin has an advantage of approximately 17 in dogs [20]. For macromolecules such as antibodies, R_d is in the range of 17 to 33 [15,21]. Several clinical trials [12,22,23] have demonstrated the efficacy of the therapy so that now intraperitoneal therapy has been recommended for ovarian carcinoma by the US National Cancer Institute (NCI Clinical Announcement: Intraperitoneal Chemotherapy for Ovarian Cancer, January 5, 2006).

Compartmental Approach to IP Pharmacokinetics

In order to properly plan IP, the R_d should be estimated from Equation (1). The IP and IV concentrations can be predicted from a relatively simple model, which is conceptualized in Fig. 1.

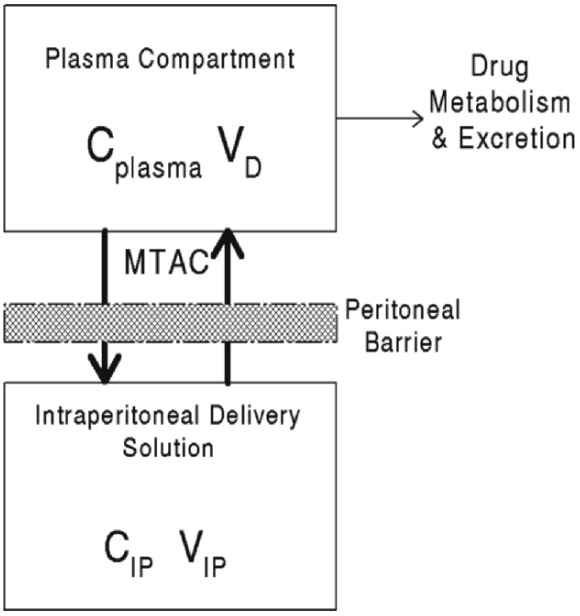


Figure 1. Compartmental Model of Peritoneal Drug Delivery, used to calculate the pharmacokinetic advantage of IP administration versus IV for a particular agent. C_{plasma}, C_{IP} = concentration of antineoplastic agent in plasma and IP solution, respectively; V_{IP} = volume of treatment solution; V_D = volume of distribution for agent; MTAC = mass transfer-area coefficient for transfer of the agent

The body consists of two compartments: (1) the systemic blood circulation that circulates through the drug's volume of distribution (V_D) and the peritoneal cavity

where the therapeutic drug is in solution. The transfer of drug across the so-called "*peritoneal membrane*" modeled as a simple transfer of mass as follows:

$$rate\ of\ mass\ transfer = \frac{d(C_P V_P)}{dt} = MTAC(C_P - C_B) \quad (2)$$

where MTAC = the overall mass transfer-area coefficient for the drug, C_P = the concentration in the peritoneal cavity, V_P = the volume in the peritoneal cavity, and C_B = the concentration in the blood. Fig. 2 provides the typical MTAC for water-soluble drugs in normal dialysis patients and in patients undergoing IP chemotherapy [14,24,25].

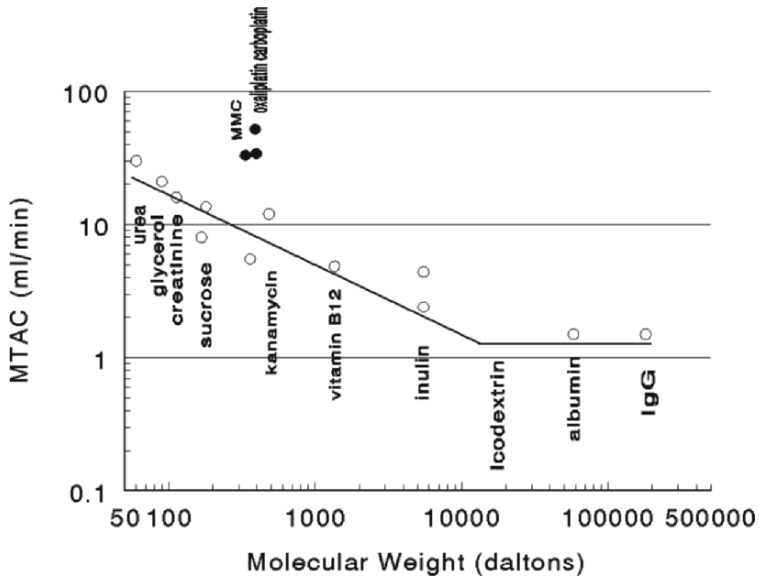


Figure 2. MTAC (mass transfer-area coefficient) vs molecular weight (daltons). Open symbols are derived from the peritoneal dialysis literature [14,24,25]. Closed symbols are from IP studies using heated solutions [26-28]. Data points are labeled with the drug/substance name. The delivery of heated solutions via multiple catheters in the perioperative setting has a distinct advantage in terms of the rate of mass transfer

These may underestimate or overestimate the mass transfer of particular drugs in tumour bearing patients. As can be seen in Fig. 2, the MTAC for heated drugs is considerably higher than the non-heated solutions [26-28]. This likely due to the combination of vasodilation with increased peritoneal blood flow and greater surface contact area with the use of dual catheters and a continuous flow system. The area is not well defined in these perioperative procedures, but the technique can significantly enhance the pharmacokinetic advantage and the efficacy [29].

Drugs which are more *lipid soluble* will have an order of magnitude higher rate of clearance from the peritoneal cavity [30-32].

A more complicated approach is to consider the body compartment to be separated from the peritoneal cavity by groups of tissues including a tumour compartment [15]. This particular model is complicated by the number of parameters required to solve the various differential equations for defining the transfer into each particular tissue bed. For small molecular weight drugs (50-500 daltons), the mass transfer coefficients into normal tissues are approximately the same across the peritoneum [15,33]. On the other hand, the tumour may be present in small mass at multiple sites and not available to the treatment solution during the total treatment time. While the multi-compartment approach is theoretically appealing, it is not practical because the intraperitoneal therapy is designed to treat small peritoneal metastases dispersed throughout the peritoneal cavity. Therefore, the multi-compartmental approach is likely unnecessary in planning intraperitoneal chemotherapy with small solutes (< 6000 Da).

In summary, there are both theoretical reasons for a therapeutic advantage and clinically established guidelines for the intraperitoneal route of chemotherapy. Pharmacokinetic data from the dialysis and oncologic literature can be used to estimate the advantage of IP therapy.

Distributed Model and Challenges of the Peritoneal Barrier in Neoplasms

Optimizing the concentration of the drug at the surface may or may not guarantee penetration of the drug into the tumour to the rapidly dividing tumour cells, which are the real target. The compartmental model concept lumps all of the potential barriers to the solute into one entity and does not differentiate between the variety of tissues, which may have different areas of contact and which may experience different transport forces. While Equation (2) permits calculation of the pharmacokinetic advantage according to Equation (1), the model does not tell us anything about the specific penetration into the tissue or even the area of contact. It merely describes the transfer between the two compartments.

The distributed model concept is illustrated in Fig. 3; mathematical details of this theory are contained in previous publications [34-37]. Because intraperitoneal therapy involves the treatment of normal tissue as well as neoplastic tissue, it is important to differentiate between the properties of both of these. Fig. 3 displays elements of the normal peritoneum with a tumour implant, which has destroyed the peritoneum and is growing within the tissue. The normal peritoneal barrier is made up of peritoneum, interstitial matrix, and the blood capillary wall. Lymphatic vessels are also located between normal tissue planes within smooth muscle or in the diaphragm. The differences between tumour and normal tissue include: lack of a mesothelial layer over the tumour, a very altered interstitium and a

hyper-permeable microcirculation. The following paragraphs will discuss the transport barrier for the normal peritoneum and the abnormalities of neoplastic tissue.

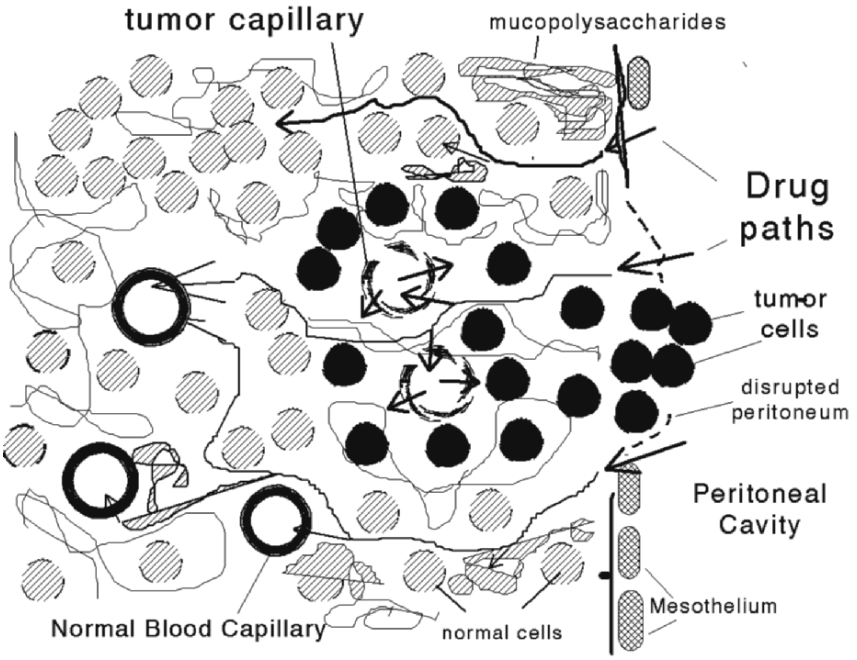


Figure 3. Distributed model concept of metastatic cancer and potential barriers to IP therapy. Solid circles represent the tumour metastasis, which has invaded and destroyed the mesothelium in its vicinity. Tumour capillaries are typically more permeable than the normal microcirculation. The tumour microenvironment (interstitium between cells) is often markedly expanded compared to that of normal tissue. See text for details

Anatomic Peritoneum

While many call the barrier the peritoneal membrane as portrayed in Figure 1, the actual anatomic peritoneum, made up of a layer of mesothelial cells and several layers of connective tissue [38], is not a significant barrier to molecules up to a molecular weight of 160,000 daltons. Studies in rodents and dialysis patients have shown that protein leaves the cavity rates of approximately 10 times the rate at which it appears in blood [39-43]. The only route of transfer of protein in the cavity back to the central circulation is via the lymphatics [44-46]. There must be some other pathway for disappearance of this protein. In experiments with rodents, it has been shown that the protein transports across the peritoneum and into the underlying tissue; there is some adsorption [47] to the peritoneal cells but most of the protein deposition is into the sub-peritoneum.

Further experiments demonstrated that removal of the peritoneum does not eliminate the dialytic properties of the peritoneal barrier [48]. Recent studies in

patients undergoing partial or total peritonectomy for treatment of peritoneal carcinomatosis confirm the findings in rodents; the clearance of mitomycin C from the peritoneal cavity was not significantly affected by an extensive peritoneal resection [1].

Although proteins appear to easily pass the mesothelium into the sub-peritoneum, viral vectors containing gene products are absorbed directly into mesothelial cells with little penetration beyond this single cell layer. Adenoviruses that code for the reporter gene β-galactosidase have been shown to be quantitatively taken up in mesothelium and not to penetrate into underlying tissues unless there is a break in the mesothelium [49,50,50-56].

The *peritoneum at the site of tumour implantation* will likely be destroyed in most cases of neoplastic cellular infiltration of the peritoneum. The loss of the mesothelium promotes adhesions, presents problems to the maintenance of the smoothly gliding peritoneal surface, and decreases the function of the immune system. Without the mesothelium, adhesions form between the visceral and parietal surfaces, and the fluid distribution may become markedly abnormal, which may preclude intracavitary therapy [57]. However, treatment with viral vectors containing anti-sense RNA or other gene products, which might not be capable of passing through the normal mesothelium, have the possibility to penetrate into the tumour from the peritoneal cavity [55].

In summary, the anatomic peritoneum is not a significant barrier to small solutes or to macromolecules, unless there exists a mechanism of uptake by the mesothelial cells, as in the case of viral vectors.

Interstitium and Tumour Microenvironment

Interstitium or the so-called "*micro environment*" is made up of collagen fibers linked through adhesion molecules such as β-1 integrins to fibroblasts, parenchymal cells, and other interstitial cells [58,59]. Hyaluronan molecules, which vary from 50,000 daltons to 40 million, wrap around the collagen fibers and are likely attached to them at some link point. To the hyaluronan are attached large molecules called proteoglycans that also interact with the surrounding cells [60,61]. Hyaluronan molecules are highly negatively charged and imbibe large amounts of water and restrict the passage of negatively charged proteins [62]. Proteins such as immunoglobulins are typically restricted to about 50% of the interstitial space [63,63,64]. Thus, the interstitial space of normal muscle, which is anywhere from 12 to 20 percent of the total tissue volume, restricts proteins to 6 to 10 percent of the tissue. The transport of large solutes such as immunoglobulin G (150 kDa) or adenovirus (900 kDa) will be highly hampered by the microenvironment.

Alterations in the interstitial pressure can change the relative tissue interstitial water space and the proportion of the tissue available to the solute. It has been

shown in animal experiments, that the abdominal wall interstitium will double when the intraperitoneal pressure is increased from 0 to 4 mm Hg [65,66]. This will markedly enhance the transport of both small and large solutes through this space. The hydraulic conductivity or water permeability of the tissue also increases with increasing intraperitoneal pressure and washout of hyaluronan from the tissue interstitium [67]. Since the surface contact area is maximized with increasing peritoneal volumes [8,29], attempts to increase the contact area will increase the pressure as well. Fig. 4 demonstrates the IP pressure vs. the IP volume [68,69] for normal dialysis patients.

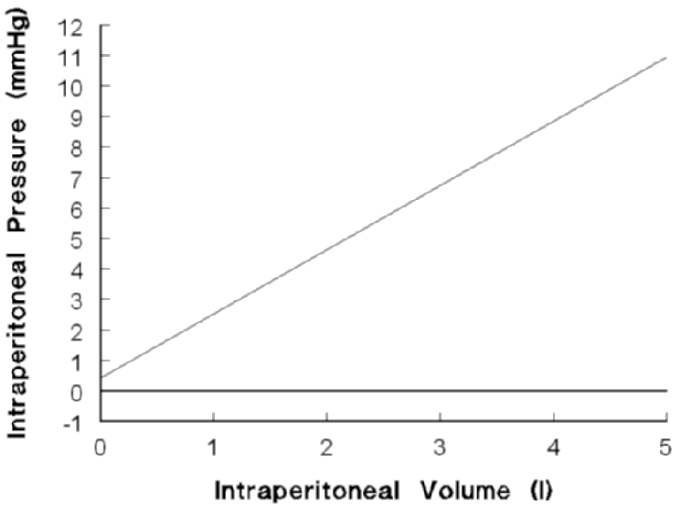


Figure 4. Intraperitoneal pressure versus volume in supine dialysis patients. Data taken from [68,69]

The effect of pressure is greatest in the abdominal wall where a nearly linear pressure gradient from the inside of the peritoneal cavity to the outside has been measured in the rat; these profiles may be quite different than those in tumours or in the human abdominal wall. Patients with adhesions or extensive surgical resection may have restricted volumes and very different pressure-volume characteristics, with increased pressures at lower volumes than those of dialysis patients. In summary, large volumes in the cavity increase the intraperitoneal pressure and expand the interstitial space and, in turn, augment the space within the tissue to which both small and large solutes distribute. Antineoplastic agents will transport at faster rates through the tissue due to increases in both diffusion and convection [36,65,67,70-72].

There exist remarkable differences between the *tumour microenvironment* and that of normal adjacent tissue. Interstitial pressures in normal tissue are in the range of –2 to 0 mm Hg [73,74]. This allows convection due to the hydrostatic pressure gradient from the solution in the cavity (3-10 mm Hg) into the tissue.

Unfortunately, several investigators have observed high interstitial pressures up to 45 mm Hg in neoplastic tissue [75-79]. To deliver macromolecules from the cavity into these tumours, the solution would have to attain a pressure greater than that of the tumour. The tolerance of an ambulatory patient is approximately 8 to 10 mm Hg in the peritoneal cavity [75,80], which may limit the penetration of large solutes that depend on convection or solvent drag. In addition, steady pressures of >15 mm Hg in a closed cavity may suppress the portal circulation [80]. Pressures of >20 mm Hg may prevent the descent of the diaphragm [80] and compromise respiration. Therefore, an unanesthetized ambulatory patient will likely be unable to tolerate therapy which depends on large volumes (> 3-4 liters) to produce high IP pressure. If tumour interstitial pressures are higher than those that can be attained, therapy with a macromolecule may be precluded. Anesthetized patients, who receive mechanical ventilation, may be able to tolerate higher levels of IP pressure, but the mesenteric circulation supplying the gut should be carefully monitored.

Studies of tumour interstitium show that the space between the cells is often markedly expanded in comparison to normal tissue [81]. A recent study in human ovarian carcinoma xenografts demonstrated an interstitial water space of 2-3 times that of normal muscle [76]. Gullino and colleagues have shown similar results in several tumours [81]. Thus the high interstitial pressure results in an expanded interstitium, which would typically result in higher rates of diffusion and convection in normal tissue. However, the high interstitial pressure and intrinsic properties of the tumour interstitium resist any transfer of large molecules into the tumour [77,82-85]. On the other hand, smaller substances (MW < 500 daltons) will diffuse into the tumour parenchyma in a fashion similar to normal tissue [76,86].

Microcirculation

Normal blood capillary endothelia are lined with a glycocalyx, which has been demonstrated to provide the endothelium with its barrier characteristics [87-90]. In portions of the inter-endothelial cleft, it is theorized that the glycocalyx is quite dense and only small molecules up to the size of insulin (~5500 Da) will typically pass through while in other areas a small number of gaps will have a less dense glycocalyx, which will permit protein leakage [72]. This provides the size selective nature of the normal peritoneal barrier. However, inflammation or drugs such as adenosine [91] cause the elimination or degradation of the glycocalyx and an increase the capillary permeability; the vessels of the normal peritoneum are likely affected during inflammation due to invasion by metastatic carcinoma [92].

Capillary permeability is markedly altered in neoplastic tissue, with typically a high permeability but a variable microvascular density [93,94]. Although detailed studies have not been carried out, all indications are that these highly permeable capillaries may be responsible for the rapid clearance of drugs into portions of the

tumour from the systemic circulation [95]. While this can be an advantage in treatment of these tumours, the high pressures in the interstitium may actually result in difficulty in drug penetration [94,96]. The nature of angiogenic vessels is under scrutiny; these may not have the glycocalyx that lines the normal endothelium and provides much of the barrier to solute transfer [93,93,95-97]. Thus many of the characteristics of these *new* vessels may be completely different from those of normal vasculature. In addition, the actual distribution of vessels is very irregular. In small (<1 cm diameter) ovarian xenografts, the vessels are located in the periphery of the tumour, which is expanding into the normal tissue [76]. The central part of the tumour may actually be necrotic and have no vasculature at all. Penetration to non-vascularized portions of the tumour is one of the problems of IV or IP treatment. Targeting the vasculature simultaneously with intraperitoneal therapy may be a method of accessing these portions of the tumour and solving this problem.

Lymph drainage from the cavity is chiefly through the sub-diaphragmatic lymphatics [72]. In normal conditions, the relaxation of the diaphragm will open specialized "*stomata*", which accept proteins, cells, and solution from the peritoneal cavity into collecting lymphatics [98,99]. The subsequent contraction of the diaphragm will close the stomata and propel the material into the parasternal lymphatics and ultimately into the right or left lymph duct. Approximately 70 to 80 percent of peritoneal lymph drainage occur through this route [45]. Lymphatics from the viscera drain to the cisterna chyli at the base of the thoracic duct and ultimately into the left venous system [46].

With peritoneal carcinomatosis, the subdiaphragmatic *lymphatics* and the mesenteric lymphatics may be obstructed [100,101]. This may result in severe ascites because the normal flow of fluid and proteins from the viscera into the peritoneal cavity cannot be cleared properly [101]. In addition, the lymphatics provide a route of metastasis to the remainder of the body; including the periaortic and thoracic nodes [102]; often supra-diaphragmatic nodes are overwhelmed with tumour cells; these same nodes then allow tumour cells to pass into the systemic circulation. However, if these pathways are still functional, intraperitoneal therapy directly targets these routes of metastasis and is a direct route to the systemic circulation for all agents, particularly those with molecular sizes greater than that of albumin.

Summary of Neoplastic versus Normal Peritoneal Barrier

The anatomic peritoneum is not a barrier to most drugs, including immunoglobulins. The mesothelial layer may be absent in a tumour implant on the peritoneum and the vasculature and the microenvironment may be greatly altered. While viral vectors are totally absorbed in the normal mesothelium, its absence at a tumour surface may permit these very large particles (~900 kDa) to pass into the first few

cell layers of the tumour; however viral vectors will still have restricted movement in the tumour interstitium [85].

The interstitium is markedly expanded and theoretically should promote high rates of diffusion and convection [76,85]. However, the high interstitial pressure and the tendency of flow from the center part of the tumour towards the periphery may cause a functional obstruction in the direction of the treatment drug originating from the peritoneum cavity [75,77,79,103]. In addition, there appear to be structural differences in the collagen matrix of the tumour interstitium that prevent significant convection and diffusion of negatively-charged, macromolecular agents [84,85].

The tumour blood capillary and microcirculation are markedly abnormal in distribution and permeability characteristics [93,94,96]. Depending on the location and density of the tumour microvasculature, systemically administered drugs may rapidly distribute to perfused regions of the tumour but not reach poorly vascularized locations altogether. Multi-agent therapies that simultaneously attack the interstitium, vasculature, and the peritoneal side of the tumour will therefore likely be more effective in remitting peritoneal carcinomatosis.

Importance of Contact Area to Intraperitoneal Chemotherapy

Although there are dominant routes of metastatic cellular migration from colorectal or ovarian carcinoma, presumably the entire peritoneum is the target for treatment with IP chemotherapy. Research in animals [9,10] and humans [7,8] has clearly demonstrated that during dialysis only about 30% of the total surface area is covered at any one time. Dialysis solutions containing glucose are gradually absorbed from the cavity and, therefore, there is a receding volume and surface area of contact with time. An alternative to the typical dialysis solution is one containing 4% of Icodextrin (a 20 to 30 kDa starch), which has been shown to maintain the peritoneal volume at a constant for up to 48 hours [104]. Over the next 48 hours the patients lost 50% of the volume. A 7.5% Icodextrin solution has been shown to be effective as a drug carrier for 5-flurouracil [105] for up to 96 hours; this type of solution maintains the volume and therefore, the area of contact relatively constant. However, it does not guarantee that the solution will be in contact with the target areas for any given length of time. The volume of the solution, the size of the patient, and the patient's position all affect the peritoneal contact area. For example, if the patient is ambulatory, even a large volume (3 liters) may pool in the bottom of the peritoneal cavity. Large portions of the peritoneum may not be covered [9], and therefore the residence time of the medication may be a problem for certain regions of the cavity.

Another approach to improve the contact area is to use a surface-active agent. In experiments with animals, diacetyl-sodium sulfosuccinate (DSS) has been shown to increase the surface contact area and to proportionally increase the rate

of mass transfer into the local tissues [9,10,106]. More rapid uptake of the drug would result in a dissipation of the drug concentration from the fluid; this problem could be solved with the use of an automated exchange device such as a peritoneal dialysis machine, programmed to deliver periodic infusions over time of given concentration. Although DSS is used as an oral stool softener (docusate sodium), it unfortunately is quite toxic if administered IP; exposure of fluid containing surfactant to a larger proportion of the peritoneal surface area also accelerated the loss of protein and the dissipation of the drug concentration in the therapeutic solution [10].

In the perioperative setting, drug delivery can be enhanced considerably. Two catheters can be placed in the peritoneal cavity: one catheter for drug input and the other catheter for removal of solution. Solutions warmed to temperatures greater than body temperature (approximately 41° C) may be infused rapidly into the peritoneal cavity and withdrawn in the second catheter. This technique will set up higher concentrations if solution is fed from a large reservoir so that the loss of drug is relatively small. Additionally, heating of the drug causes vasodilation and there is likely an increase in penetration into both normal tissue and neoplastic tissue [26-28,107,108]. This technique may help to solve the problem of residence time as well. If a greater portion of the peritoneal surface area is covered by the solution and the concentration of the drug is maintained constant, then the area under the curve for the surface contact concentration should be maximized. This will be restricted to perioperative patients, and the side effects of these drugs on normal peritoneum have not been studied.

Penetration of Antineoplastic Agents

Tumour penetration of antineoplastic agents in regional therapy depends on the penetration of the agent from the periphery of the tumour nodule into the tumour with ultimate purpose of killing the tumour cells. While either continuous or repeated therapy is necessary for a significant remission of residual tumour, adequate penetration of the agent with each administration in order to kill tumour cells would be advantageous. When drugs are administered systemically, they will generally deposit in regions close to blood vessels. Tumour microvessels are typically quite permeable, but their irregular distribution makes the treatment of tumour nodules challenging. Regions that are not well perfused may receive no drug at all or such minute amounts that there will be no response. In order for intraperitoneal therapy or any regional therapy to work properly, the drug must penetrate from the periphery towards the center of the tumour in significant concentrations. There are typically three regimens which can be utilized to do this: (1) Small molecular weight solutes which are typically in the range of 100 to 5,000 daltons, (2) macromolecular agents which are typically in the range of immunoglobulin G (150,000 daltons), (3) genetic agents carried on viral vectors, which are on the order of 1 million daltons.

Intraperitoneal Chemotherapy with Small Molecular Weight Drugs

Substances of the size of mannitol (180 Da), which are neither bound nor taken up by mammalian cells or the extracellular matrix, are absorbed gradually as they transfer through the interstitium and are removed by blood vessels. Fig. 5 demonstrates the acute penetration of mannitol over three hours into the abdominal wall muscle of a rat and into an SKOV3 xenograft [76,86].

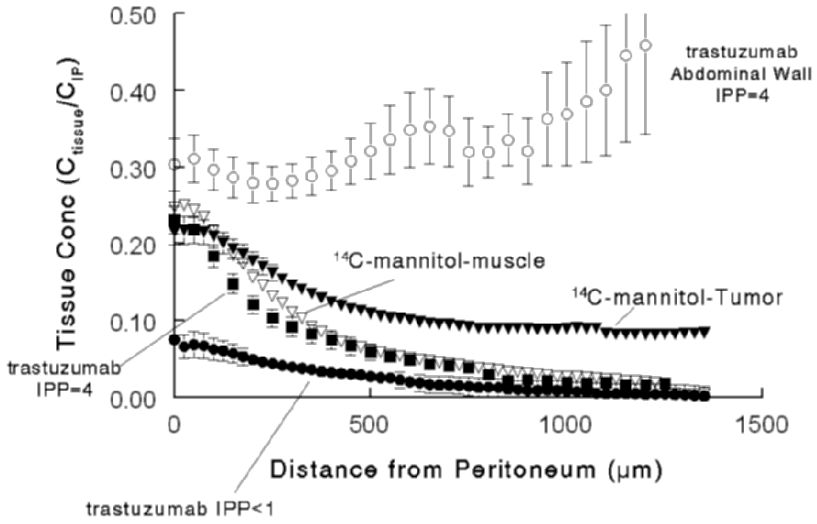


Figure 5. Comparison of penetration of mannitol or trastuzumab (IgG monoclonal, Her2/neu) into normal tissue or IP SKOV3 xenograft of the rat after 3 hours of treatment with a large IP volume. Mean ± SE concentrations vs distance in microns from the peritoneal surface. IPP, intraperitoneal pressure. Replotted from [75,76,111]

The SKOV3 xenografts revealed significant angiogenesis in the periphery of the tumour with very few vessels in the center. Thus small molecular weight drugs entering from the edge of the tumour are rapidly absorbed, and the concentration profile levels off 500 microns from the tumour surface. If one can obtain a significantly higher concentration such as 10 times the toxic dose to the tumour cells, it may be that the first 500 to 600 microns of tissue are easily treated in tumour nodules.

Profiles change when the drug is taken up by tumour cells. Los and colleagues [109] conducted penetration studies of cisplatin on testicular carcinoma on the serosal surfaces of rats. Utilizing proton induced x-ray emission, they showed substantially higher levels in the outer one mm of the tumour after multiple doses of intraperitoneally administered cisplatin when compared to the levels resulting from IV route (Fig. 6).

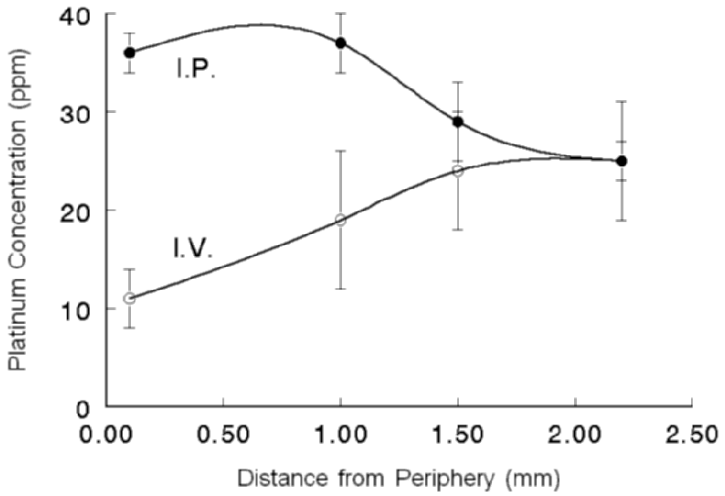


Figure 6. Platinum concentration (mean ± SE, ppm) vs distance (mm) from the periphery of CC531 xenografts in rats after 3 successive doses of cisplatin over 168 hours. Tumours were 3-8 mm in diameter. Replotted from [109]

This demonstrates local penetration into tissue and uptake into the tumour cells. The elevated concentration closer to the edge of the tumour for the IP delivery demonstrates the delivery advantage of IP dosing. Drugs such as Cisplatin penetrated about 0.5 mm but have increased levels very close to the tumour edge and penetrate even further with uptake of platinum in the cells. This has been verified in other studies with Adriamycin [110]. Even with limited penetration of < 1mm, repeated application of the drug should kill layer after layer of cells and ultimately cause the tumour to regress. However, the timing of this should be such that the tumour cannot reestablish itself from cells in the inner part of the tumour that are not treated.

Intraperitoneal Therapy with Macromolecular Agents

Penetration of large molecular weight substances (MW > 60 kDa) into tumours is more challenging. These substances do not diffuse very rapidly and must be carried chiefly by convection (solvent drag, see Fig 5) [76]. Convection requires a positive hydrostatic pressure gradient from outside of the tumour to the interior of the tumour (note in Fig 5 the change in the trastuzumab profiles between IP pressure (IPP) of <1 mm Hg and 4 mm Hg).

Tumour pressures may, however, be high enough to preclude a positive pressure gradient from the outside into the tumour. The limitation of approximately 10-20 mm Hg of pressure in the IP therapeutic solution, may present a major difficulty in obtaining a convective force that can actually carry the antibody beyond the first few cell layers of the tumour nodule [75]. One would also assume that

with an expanded interstitium, there would be open pathways for large antibodies to pass through once a positive gradient was set up. However, even with special maneuvers such as decapsulation of a xenograft with subsequent measurements of near zero pressure in the center of the tumours, penetration was not enhanced [75]. Experiments with hyaluronidase treatment of the tumour to break down hyaluronan resulted in no enhancement of penetration. Use of collagenase, however, resulted in a significant increase in penetration of tumours [85]. Unfortunately collagenase administered into a body cavity would destroy normal tissue as well as tumour tissues. Therefore, some other adjuvant therapy must be utilized to decrease the pressure and interrupt the development of the abnormal interstitium. The interstitium of tumours therefore presents a complex structure that markedly retards the penetration of macromolecules.

Gene therapy that depends on viral vector delivery is fraught with just as many problems as immunotherapy. While uptake of some of the viral particles is observed in the first few cell layers, penetration into the inner part of these tumours via either diffusion or convection will be extremely slow. It may be that such therapy would be more beneficial if given intravenously from the systemic side, with the proviso that the vector was less toxic to normal cells than to the tumour cells.

Acknowledgment

NIH grants CA-085984 and DK-048479 supported this work.

References

1. Vazquez VdL, Stuart OA, Mohamed F, Sugarbaker P (2003) Extent of parietal peritonectomy does not change intraperitoneal chemotherapy pharmacokinetics. Cancer Chemother Rep 52:108-112
2. Yazbeck C, Dhainaut C, Batallan A, Benifila J-L, Thoury A, Madelenat P (2005) Diagnostic hysteroscopy and risk of peritoneal dissemination of tumor cells. Gynecol Obstet Fertil 33:247-252
3. Ludeman L, Shepherd N (2005) Serosal involvement in gastrointestinal cancer: its assessment and significance. Histopathology 47:123-131
4. Colcher D, Esteban J, Carrasquillo JA, Sugarbaker P, Reynolds JC, Bryant G et al (1987) Complementation of intracavitary and intravenous administration of a monoclonal antibody (B72.3) in patients with carcinoma. Cancer Res 47:4218-4224
5. Oosterling S, van der Bij GJ, van Egmond M, van der Sijp J (2005) Surgical trauma and peritoneal recurrence of colorectal carcinoma. Eur J Surg Oncol 31:29-37

6. Lopez-Berlanga J, De Miguel A, Elvira A (2004) Anesthesia and postoperative care of 11 patients undergoing peritonectomy and hypothermic intraperitoneal chemotherapy. Revista Espanola de Anesthesiologia y Reanimacion 51:423-428
7. Chagnac A, Herskovitz P, Weinstein T, Elyashiv S, Hirsh J, Hamel I, Gafter U (1999) The peritoneal membrane in peritoneal dialysis patients: estimation of its functional surface area by applying stereologic methods to computerized tomography scans. J Am Soc Nephrol 10:342-346
8. Chagnac A, Herskovitz P, Ori Y, Weinstein T, Hirsh J, Katz M, Gafter U (2002) Effect of increased dialysate volume on peritoneal surface area among peritoneal dialysis patients. J Am Soc Nephrol 13:2554-2559
9. Flessner MF, Lofthouse J, Zakaria EL (2001) Improving contact area between the peritoneum and intraperitoneal therapeutic solutions. J Am Soc Nephrol 12:807-813
10. Flessner MF, Lofthouse J, Williams A (2001) Increasing peritoneal contact area during dialysis improves mass transfer. J Am Soc Nephrol 12:2139-2145
11. Markman M (1998) Intraperitoneal therapy of ovarian carcinoma. Semin Oncol 25:356-360
12. Fujiwara K, Markman M, Morgan M, Coleman RL (2005) Intraperitoneal carboplatin-based chemotherapy for epithelial ovarian cancer. Gynecol Oncol 97:10-5
13. Gershenson DM, Tortolero-Luna G, Malpica A, Baker VV, Whitaker L, Johnson E, Mitchell MF (1996) Ovarian intraepithelial neoplasia and ovarian cancer. Obstetrics and Gyencol Clinics of North America 23:475-543
14. Dedrick RL, Myers CE, Bungay PM, DeVita VT (1978) Pharmacokinetic rationale for peritoneal drug administration in the treatment of ovarian cancer. Cancer Treat Rep 62:1
15. Flessner MF, Dedrick R (2000) Intraperitoneal chemotherapy. In: Gokal R, Khanna R, Krediet RT, Nolph K, eds. Textbook of Peritoneal Dialysis. Dordrecht, Netherlands: Kluwer Academic Publishers p809-827
16. Goel R, Cleary S, Horton C, Howell S (1989) Effect sodium thiosulfate on the pharmacokinetics and toxicity of cisplatin. J Nat Cancer Inst 81:1552-1560
17. Piccart M, Abrams J, Dodian P, et al (1988) Intraperitoneal chemotherapy with cisplatin and melphalan. J Nat Cancer Inst 80:1118-1124
18. Speyer JL, Sugarbaker PH, Collins JM, Dedrick RL, Klecker RW, Myers CE (1981) Portal levels and hepatic clearance of 5-fluorouracil after intraperitoneal administration in humans. Cancer Res 41:1916
19. Sugarbaker P, Graves T, DeBruijn E, et al (1990) Early postoperative intraperitoneal chemotherapy as an adjuvant therapy to surgery for peritoneal carcinomatosis from gastrointestinal cancer: pharmacological studies. Caner Res 50:5790-5794
20. Selam J-L, Bergman R, Raccah D, Jean-Didier N, Lozano J, Charles M (1990) Determination of portal insulin absorption from peritoneum via novel non-isotopic method. Diabetes 39:1361-1365
21. Dedrick RL, Flessner MF (1989) Pharmacokinetic considerations on monoclonal antibodies. Immunity to Cancer II:429-438

22. Barakat RR, Sabbatini P, Bhaskaran D, Revzin M, Smith A, Venkatraman E et al (2002) Intraperitoneal chemotherapy for ovarian carcinoma: results of long-term follow-up. J Clin Oncol 20:694-698
23. Alberts DS, Liu PY, Hannigan EV, O'Toole R, Williams SD, Young JA et al (1996) Intraperitoneal cisplatin plus intravenous cyclophosphamide versus intravenous cisplatin plus intravenous cyclophosphamide for stage III ovarian cancer. New Engl J Med 335:1950-1955
24. Krediet RT (2000) Physiology of peritoneal solute transport and ultrafiltration. In: Gokal R, Khanna R, Krediet RT, Nolph K, eds. Textbook of Peritoneal Dialysis. Dordrecht: Kluwer Academic Publishers, 135-172
25. Babb AL, Johansen PJ, Strand MJ, Tenckhoff H, Scribner BH (1973) Bidirectional permeability of the human peritoneum to middle molecules. Proc Eur Dial Transplant Assoc 10:247
26. Jacquet P, Averbach A, Stephens A, Stuart O, Chang D, Sugarbaker P (1998) Heated intraoperative intraperitoneal mitomycin C and early postoperative intraperitoneal 5-fluorouracil: pharmacokinetic studies. Oncology 55:130-138
27. Elias D, Bonnay M, Puizillou J, Antoun S, Demirdjian S, ElOtmany A et al (2002) Heated intra-operative intraperitoneal oxaliplatin after complete resection of peritoneal carcinomatosis: pharmacokinetics and tissue distribution. Ann Oncol 13:267-272
28. Steller M, Egorin MJ, Trimble E, Bartlett D, Suhowski E, Alexander H, Dedrick R (1999) A pilot phase I trial of continuous hyperthermic periotoneal perfusion with high-dose carboplatin as primary treatment of patients with small-volume residual ovarian cancer. Cancer Chemother Pharmacol 43:106-114
29. Keshaviah P, Emerson PF, Vonesh EF, Brandes JC (1994) Relationship between body size, fill volume, and mass transfer area coefficient in peritoneal dialysis. J Am Soc Nephrol 4:1820-1826
30. Torres IJ, Litterst CI, Guarino AM (1978) Transport of model compounds across the peritoneal membrane in the rat. Pharmacology 17:161-166
31. Lewis C, Lawson N, Rankin E, et al (1990) Phase I and pharmacokinetic study of intraperitoneal thioTEPA in patients with ovarian cancer. Cancer Chemother Pharmacol 26:283-287
32. Wikes A, Howell S (1985) Pharmacokinetics of hexamethylmelamine administered via the ip route in an oil emulsion vehicle. Cancer Treat Rep 69:657-662
33. Flessner MF (1996) Small-solute transport across specific peritoneal tissue surfaces in the rat. J Am Soc Nephrol 7:225-233
34. Flessner MF, Dedrick RL, Schultz JS (1984) A distributed model of peritoneal-plasma transport: theoretical consideratons. Am J Physiol 246:R597-R607
35. Dedrick RL, Flessner MF, Collins JM, Schultz JS (1982) Is the peritoneum a membrane? Am Soc Artificial Intern Organs J 5:1-5
36. Flessner M.F., Lofthouse J., Zakaria ER (1997) In vivo diffusion of immunoglobulin G in muscle: effects of binding, solute exclusion, and lymphatic removal. Am J Physiol 273:H2783-H2793

37. Flessner M.F (2001) Transport of protein in the abdominal wall during intraperitoneal therapy I. Theoretical approach. Am J Physiol Gastrointest Liver Physiol 281:G424-G437
38. Baron MA (1941) Structure of the intestinal peritoneum in man. Am J Anat 69:439-497
39. Flessner MF (1992) Net ultrafiltration in peritoneal dialysis: Role of direct fluid absorption into peritoneal tissue. Blood Purif 10:136-147
40. Rippe B, Stelin G, Ahlmen J (1986) Frontiers in Peritoneal Dialysis. New York: Field, Rich, p24-30
41. Heimburger O, Waniewski J, Werynski A, Park MS, Lindholm B (1994) Lymphatic absorption in CAPD patients with loss of ultrafiltration capacity. In: Heimburger O, ed. PhD Thesis. Stockholm: Konogl Carolinska Medico Chirurgiska Institute, 1-21
42. Daugirdas JT, Ing TS, Gandhi VC, Hano JE, Chen WT, Yuan L (1980) Kinetics of peritoneal fluid absorption in patients with chronic renal failure. J Lab Clin Med 85:351-361
43. Flessner MF (1991) Peritoneal transport physiology: insights from basic research. J Am Soc Nephrol 2:122-135
44. Granger DN, Parker RE, Quillen EW, Brace RA, Taylor AE (1979) Lymphology. Stuttgart, FRG: Thieme, p61-64
45. Yoffey JM, Courtice FC (1970) Lymphatics, Lymph, and the Lymphomyeloid Complex. London, UK: Academic, p320
46. Courtice FC, Steinbeck AW (1951) Absorption of protein from the peritoneal cavity. J Physiol London 114:336-355
47. Flessner MF, Schwab A (1996) Pressure threshold for fluid loss from the peritoneal cavity. Am J Physiol 270:F377-F390
48. Flessner M.F., Henegar J, Bigler S, Genous L (2003) Is the peritoneum a significant transport barrier in peritoneal dialysis? Perit Dial Int 23:542-549
49. Hekking LHP, Harvey VS, Havenith CEG, van den Born J, Beelen RHJ, Jackman RW, Nagy JA (2003) Mesothelial cell transplantation in models of acute inflammation and chronic peritoneal dialysis. Perit Dial Int 23:323-330
50. Margetts PJ, Kolb M, Galt T, Hoff CM, Shockley TR, Gauldie J (2001) Gene transfer of transforming growth factor-Beta1 to the rat peritoneum: effects on membrane function. J Am Soc Nephrol 12:2029-2039
51. Margetts PJ, Gyorffy S, Kolb M, Yu L, Hoff CM, Holmes CJ, Gaudie J (2002) Antiangiogenic and antifibrotic gene therapy in a chronic infusion model of peritoneal dialysis in rats. J Am Soc Nephrol 13:721-728
52. Jackman RW, Hoff CM, Shockley TR, Nagy JA (1999) Adenovirus-mediated transfer of rat catalase cDNA into rat primary mesothelial cells confers increased resistance to oxidant-induced injury in vitro. J Am Soc Nephrol 10:446A-447A
53. Hoff CM, Piscopo D, Inman K, Shockley TR (2000) Adenovirus-mediated gene transfer to the peritoneal cavity. Perit Dial Int 20:128-136
54. Alvarez RD, Curiel D (1997) A phase I study of recombinant adenovirus ventor-mediated intraperitoneal delivery of herpes simplex virus thymidine

kinase (HSV-TK) gene and intravenous gangciclovir for previously treated ovarian and extraovarian cancer patients. Hum Gene Ther 8:597-613
55. Mujoo K, Maneval D, Anderson S, Gutterman J (1996) Adenoviral-mediated p53 tumor suppressor gene therapy of human ovarian carcinoma. Oncogene 12:1617-1623
56. Tong X-W, Block A, Chen S-H, Contact C, Agoulnik I, Blankenburg K et al (1996) In vivo gene therapy of ovarian cancer by adenovirus-mediated thymidine kinase gene transduction and gangciclovir administration. Gynec Oncol 61:175-179
57. deForni M, Boneu A, Otal P, Martel P, Shubinski R, Bugat R, Lucot H (1993) Anatomic changes in the abdominal cavity during intraperitoneal chemotherapy: prospective study using scintigraphic peritoneography. Bulletin du Cancer 80:345-350
58. Reed RK, Rubin K, Wiig H, Rodt SA (1992) Blockade of β_1-integrins in skin causes edema through lowering of interstitial fluid pressure. Circ Res 71:978-983
59. Rubin K, Sundberq C, Ahlen K, Reed RK (1995) Integrins: transmembrane links between the extracellular matrix and the cell interior. In: Reed RK, Mattale NG, Bert JL, Winlove CP, Laine GA, eds. Interstitium, Connective Tissue, and Lymphatics. London: Portland Press Ltd, 29-40
60. Rubin K, Gullberg D, Tomasini-Johansson B, Reed RK, Ryden C, Borg TK (1996) Molecular recognition of the extracellular matrix by cell surface receptors. In: Comper WD, ed. Extracellular Matrix. Amsterdam: Harwood Academic Publishers, p262-309
61. Laurent TC (1995) Structure of the extracellular matrix and the biology of hyaluronan. In: Reed RK, McHale NG, Bert JL, Winlove CP, Laine GA, eds. Interstitium, Connective Tissue, and Lymphatics. London: Portland Press, p1-12.
62. Fraser JRE, Laurent TC (1996) Hyaluronan. In: Comper WD, ed. Extracellular Matrix. Amsterdam: Harwood Academic Publishers, 141-199
63. Wiig H, DeCarlo M, Sibley L, Renkin EM (1992) Interstitial exclusion of albumin in rat tissues measured by a continous infusion method. Am J Physiol 263:H1222-H1233
64. Wiig H, Kaysen GA, Al-Bander HA, DeCarlo M, Sibley L, Renkin EM (1994) Interstitial exclusion of IgG in rat tissues estimated by continuous infusion. Am J Physiol 266:H212-H219
65. Zakaria ER, Lofthouse J, Flessner MF (1999) In vivo effects of hydrostatic pressure on interstitium of abdominal wall muscle. Am J Physiol 276:H517-H529
66. Zakaria ER, Lofthouse J, and Flessner MF (2000) Effect of intraperitoneal pressures on tissue water of the abdominal muscle. Am J Physiol Renal Physiol 278:F875-F885
67. Zakaria ER, Lofthouse J, Flessner MF (1997) In vivo hydraulic conductivity of muscle: effects of hydrostatic pressure. Am J Physiol 273:H2774-H2782
68. Twardowski ZJ, Prowant BF, Nolph KD (1983) High volume, low frequency continuous ambulatory peritoneal dialysis. Kidney Int 23:64-70

69. Gotloib L, Mines M, Garmizo L, Varka I (1981) Hemodynamic effects of increasing intra-abdominal pressure in peritoneal dialysis. Peritoneal Dial Bull 1:41-43
70. Flessner MF, Dedrick RL, Reynolds JC (1992) Bidirectional peritoneal transport of immunoglobulin in rats: tissue concentration profiles. Am J Physiol 263:F15-F23
71. Flessner M.F (1999) Changes in the peritoneal interstitium and their effect on peritoneal transport. Perit Dial Int 19 Suppl 2:S77-S82
72. Flessner MF (2005) The transport barrier in intraperitoneal therapy. Am J Physiol 288:F433-F442
73. Wiig H, Reed RK, Aukland K (1981) Micropuncture measurement of interstitial fluid pressure in rat subcutis and skeletal muscle: comparison to the wick-in-needle technique. Microvasc Res 21:308-319
74. Wiig H, Reed RK (1985) Interstitial compliance and transcapillary Starling pressures in cat skin and skeletal muscle. Am J Physiol 248:H666-H673
75. Flessner MF, Choi J, Credit K, Deverkadra R, Henderson K (2005) Resistance of tumor interstitial pressure to the penetration of intraperitoneally delivered antibodies into metastatic ovarian tumors. Clin Cancer Res 11:3117-3125
76. Flessner MF, Choi J, He Z, Credit K (2004) Physiological characterization of human ovarian cancer cells in a rat model of intraperitoneal antineoplastic therapy. J Appl Physiol 97:1518-1526
77. Boucher Y, Baxter LT, Jain RK (1990) Interstitial pressure gradients in tissue-isolated and subcutaneous tumors: implications for therapy. Cancer Res 50:4478-4484
78. Boucher Y, Kirkwood JM, Opacic D, Desantis M, Jain RK (1991) Interstitial hypertension in superficial metastatic melanomas in humans. Cancer Res 51:6691-6694
79. Roh HD, Boucher Y, Kalnicki S, Buchsbaum R, Bloomer WD, Jain RK (1991) Interstitial hypertension in carcinoma of uterine cervix in patients: possible correlation with tumor oxygenation and radiation exposure. Cancer Res 51:6695-6698
80. Flessner MF (1981) Transport of Water-Soluble Solutes Between the Peritoneal Cavity and Plasma in the Rat. Ann Arbor, MI: Univ. of Michigan
81. Gullino PM, Grantham FH, Smith SH (1965) The interstitial water space of tumors. Cancer Res 25:727-731
82. Boucher Y, Jain RK (1992) Microvascular pressure is the principal driving force for interstitial hypertension in solid tumors: implications for vascular collapse. Cancer Res 52:5110-5114
83. Jain RK (1987) Transport of molecules in the tumor interstitium: a review. Cancer Res 47:3039-3051
84. Netti PA, Berk DA, Swartz MA, Grodzinsky AJ, Jain RK (2000) Role of extracellular matrix assembly in interstitial transport in solid tumors. Cancer Research 60:2497-2503

85. Choi J, Credit K, Henderson K, Deverkadra R, He Z, Wiig H et al (2006) Intraperitoneal immunotherapy for metastatic ovarian carcinoma: resistance of intratumoral collagen to antibody penetration. Clin Cancer Res 12:1906-1912
86. Flessner MF, Fenstermacher JD, Dedrick RL, Blasberg RG (1985) A distributed model of peritoneal-plasma transport: tissue concentration gradients. Am J Physiol 248:F425-F435
87. Vink H, Duling BR (1996) Identification of distinct luminal domains for macromolecules, erythrocytes, and leucocytes within mammalian capillaries. Circ Res 79:581-589
88. Vink H, Duling BR (2000) Capillary endothelial surface layer selectively reduces plasma solute distribution volume. Am J Physiol 278:H285-H289
89. Fu B, Curry FE, Adamson RH, Weinbaum S (1997) A model for interpreting the tracer labeling of interendothelial clefts. Ann Biomed Engin 25:375-397
90. Fu BM, Curry FE, Weinbaum S (1995) A diffusion wake model for tracer ultrastructure-permeability studies in microvessels. Am J Physiol 269:H2124-H2140
91. Platts SH, Duling BR (2004) Adenosine A3 receptor activation modulates the capillary endothelial glycocalyx. Circ Res 94:77-82
92. Matsuki T, Duling B (2000) TNF-alpha increases entry of macromolecules into luminal endothelial cell glycocalyx. Microcirculation 7:411-418
93. Leunig M, Yuan F, Menger MD, Boucher Y, Goetz AE, Messmer K, Jain RK (1992) Angiogenesis, microvascular architecture, microhemodynamics, and interstitial fluid pressure during early growth of human adenocarcinoma LS174T in SCID mice. Cancer Res 52:6553-6650
94. Nugent LJ, Jain RK (1984) Plasma pharmacokinetics and interstitial diffusion of macromolecules in a capillary bed. Am J Physiol 246:H129-H137
95. Yuan F, Leunig M, Berk DA, Jain RK (1993) Microvascular permeability of albumin, vascular surface area, and vascular volume measured in human adenocarcinoma LS174T using dorsal chamber in SCID mice. Microvasc Res 45:269-289
96. Gerlowski LE, Jain RK (1986) Microvascular permeability of normal and neoplastic tissues. Microvasc Res 31:288-305
97. Henry CBS, Duling BR (2000) TNF-alpha increases entry of macromolecules into luminal endothelial cell glycocalyx. Am J Physiol 279:H2815-H2823
98. Bettendorf U (1978) Lymph flow mechanism of the subperitoneal diaphragmatic lymphatics. Lymphology 11:111-116
99. Bettendorf U (1979) Electronmicroscopic studies on the peritoneal resorption of intraperitioneally injected latex particles via the diaphragmatic lymphatics. Lymphology 12:66-70
100. Courtice FC, Steinbeck AW (1951) The effects of lymphatic obstruction and of posture on absorption of protein from the peritoneal cavity. Aust J Exp Biol Med Sci 29:451-458
101. Dykes PW, Jones JH (1964) Albumin exchange between plasma and ascites fluid. Clin Sci 34:185-197
102. Rusznysk I, et al (1967) Lymphatics and Lymph Circulation. London: Pergamon Press

103. Baxter LT, Jain RK (1989) Transport of fluid and macromolecules in tumor. I. Role of interstitial pressure and convection. Microvasc Res 37:77-104
104. Hosie KB, Gilbert J, Kerr DJ, Brown C, Peers E (2001) Fluid dynamics in man of an intraperitoneal drug delivery solution: 4% icodextrin. Drug Delivery 8:9-12
105. Hosie KB, Kerr DJ, Gilbert JA, Downes M, Lakin G, Pemberton G et al (2003) A pilot study of adjuvant intraperitoneal 5-fluorouracil using 4% icodextrin as a novel carrier solution. Eur J Surg Oncol 29:254-260
106. Penzotti SC, Mattocks AM (1968) Acceleration of peritoneal dialysis by surface active agents. J Pharm Sci 57:1192-1195
107. van Ruth S, Mathot R, Sparidans R, Beijnen J, Verwaal V, Zoetmulder F (2004) Population pharmacokinetics and pharmacodynamics of mitomycin during intraoperative hyperthermic intraperitoneal chemotherapy. Clin Pharmacokin 43:131-143
108. Witkamp A, deBree E, VanGoethem R, Zoetmulder F (2001) Rationale and techniques of intra-operative hyperthermic intraperitoneal chemotherapy. Cancer Treat Rev 27:365-374.
109. Los G, Mutsaers PHA, van der Vijgh WJF, Baldera GS, de Graag PW, McVie JG (1989) Direct diffusion of cis-diaminedichloroplatinum (II) in intraperitoneal rat tumors after intraperitoneal chemotherapy: A comparison with systemic chemotherapy. Cancer Res 49:3380-3384
110. Ozols RF, Locker GY, Doroshow JH (1979) Pharmacokinetics of adriamycin and tissue penetration in murine ovarian cancer. Cancer Res 39:3209-3214
111. Flessner MF, Deverkadra R, Smitherman J, Li X, Credit K (2006) In vivo determination of diffusive transport parameters in a superfused tissue. Am J Physiol Renal Physiol, In press

Current Status of Intraperitoneal Antineoplastic Drug Delivery

M Markman

Intraperitoneal Chemotherapy: Historical Perspective

Following the initial introduction of cytotoxic pharmaceutical agents into the armamentarium of physicians caring for patients with malignant disease, attempts were made to treat cancers involving the abdominal cavity (e.g., advanced cancers of the ovary stomach and colon) by instilling the drugs directly into this body compartment [1]. While these early efforts revealed malignant ascites formation could be reduced by this approach, there was little (if any) evidence for "shrinkage" of tumour masses, and with the drugs employed in these early years considerable local toxicity (abdominal pain) was observed.

With the further observation that systemic drug administration was at least as effective, less cumbersome and resulted in less side effects, the use of intraperitoneal (IP) anti-neoplastic therapy became relegated to those situations where it was hoped malignant fluid accumulation could be controlled to provide meaningful short-term palliation of distressing symptoms [2].

The "Dedrick Model" and Pre-clinical Evaluation of Intraperitoneal Chemotherapy

Then, in 1978, Robert Dedrick and his colleagues at the National Cancer Institute published a landmark paper that provided a compelling, but entirely theoretical, rationale to re-explore the administration of antineoplastic agents as therapy of ovarian cancer [3,4]. Based on existing data regarding the natural history of this malignancy, the pharmacology of cytotoxic agents available at that time, and the known physiology of drug transport into, and out of, the body compartment (e.g., uptake from the peritoneal cavity principally via the portal circulation), the "Dedrick model" suggested it should be possible to expose tumour present within the cavity to substantially higher concentrations (> 100-fold) of specific cytotoxic agents than with systemic delivery.

A number of subsequently conducted pre-clinical evaluations revealed the potential for greater tumour cell kill associated with the concentrations of antineoplastic drugs possibly achievable within the peritoneal cavity following regional drug delivery [5], as well as the fact certain drugs administered by the IP route could result in considerable local toxic effects (e.g. doxorubicin) [6].

Both theoretical considerations, and rather extensive experimental observations, permit the development of a general outline for what might be described as the "ideal antineoplastic drug" for IP administration:

- Active antineoplastic agent against the tumour type being treated
- Clinical, or pre-clinical, data exist for the agent supporting the favorable impact of increasing the dose or duration-of-exposure (AUC) on its cytotoxic potential
- Agent is not a vesicant
- Agent demonstrates slow clearance from the peritoneal cavity and rapid clearance from the systemic circulation
- Agent undergoes extensive metabolism to a non-toxic metabolite during its first passage through the liver
- Agent does not require activation in the liver to become an "active" cytotoxic drug

Penetration of Cytotoxic Antineoplastic Agents into Tumour Tissue

Perhaps the most important observation in these experimental studies, which substantially impacts the potential clinical relevance of IP antineoplastic drug delivery, was the consistent finding that following regional delivery there is *very limited direct penetration* of the agent into tumour tissue [7-10]. Depending on the investigational model employed, the depth of penetration varied from several cell layers to a maximum of a few millimeters from the surface of cancerous or normal tissue.

These data strongly argue that the patient population which potentially may benefit from IP drug delivery, and the group which prospectively should be examined in clinical trials, would be those individuals with very small volume cancer present within this body compartment when the regional treatment strategy is initiated.

Clearly, from the perspective of these experimental considerations, patients with microscopic residual disease (following surgical resection of the primary lesion and metastatic implants) would be the best group to employ regional treatment. However, patients with small volume residual macroscopic cancer may also benefit, particularly as it must be recognized that "standard anti-cancer" therapy includes a number of individual drug administrations (e.g., 4-6 courses), rather than a single therapeutic cycle [11].

Thus, with each delivery cycle, the residual volume will be reduced (assuming, of course, the *cancer is responsive* to the drug regimen), permitting *subsequent treatments* to have a greater impact on this decreased total volume of disease. Of all the intra-abdominal malignancies, this management paradigm is most likely to be relevant in ovarian cancer, where approximately 70-80% of patients will be anticipated to have tumours which are responsive to platinum-based chemotherapy [11,12].

Drug Delivery by Direct Penetration versus Capillary Flow

A final issue which must be considered in the design of an IP chemotherapy program is the question of the adequacy of delivery of the cytotoxic drug to the malignancy by *capillary flow* following regional administration.

This is a critically important point, for if local drug instillation results in a reduction in the total concentration of the antineoplastic agent reaching the tumour through the systemic vascular compartment, patients treated with the regional approach may actually experience an *inferior outcome*, compared to intravenous (systemic) treatment. This could occur despite the fact there may be very high local concentrations of the drug in direct contact with the cancer within the peritoneal cavity.

However, if drugs administered IP subsequently enter the systemic vascular compartment at concentrations comparable to what would be achieved following intravenous delivery, there should be no reason to suspect there will be a reduction in the efficacy of treatment. There are two methods to assure this outcome. First, during the conduct of clinical trials the concentration of active drug can be measured in the systemic compartment and compared to what would be achieved with "standard" intravenous therapy.

Second, if the dose limiting toxicity of a regionally administered agent is found to be identical as what is observed with systemic treatment (e.g., emesis, bone marrow suppression), it is reasonable to assume as much of the active drug is reaching the vascular compartment and tumour by capillary flow as when the agent is given intravenously. Again, this observation can be confirmed by a formal pharmacokinetic analysis.

Phase I studies have revealed that both cisplatin [13-16] and carboplatin [17-19] satisfy the criteria of agents where following IP instillation it is possible to achieve the *same level* of systemic drug exposure as observed with intravenous drug administration. However, in addition, concentrations of the agents *within the peritoneal cavity* are 10 to 20-fold higher than present in the vascular compartment.

In contrast, for a drug whose limiting toxicity is found to be *local effects* (e.g., abdominal pain) [20,21], it is likely there will be less of the active drug found in the vascular compartment than could be safely attained following systemic delivery. While this may be an acceptable situation, particularly if the observed local concentrations are extremely high, an alternative approach would be to treat

patients with this agent by both the IP and systemic routes to optimize drug delivery through local uptake *and* capillary flow.

An example of an agent that fulfills the criteria of this type of drug is paclitaxel, where concentrations within the peritoneal cavity following regional administration are > 1000-fold higher than in the systemic compartment, but this method of treatment results in lower systemic drug levels than attainable with several "standard" intravenous paclitaxel regimens [21,22].

While the impact of this reduction in the systemic exposure to paclitaxel is uncertain, it is interesting to note the reported experience of a phase 2 trial of single agent IP paclitaxel (60 mg/m^2/week x 16 weeks) employed as a second-line treatment regimen in ovarian cancer [23]. Of the 28 patients who initiated this secondline program with *microscopic disease only*, 17 (61%) achieved a surgically-confirmed complete response. In sharp contrast, only 1 of 31 (3%) patients who initiated IP paclitaxel therapy with any *macroscopic tumour nodules* (largest residual mass permitted for entry into this trial was 0.5 cm in maximum diameter) achieved a complete response. These data would suggest that the very high local concentrations achieved with intraperitoneal paclitaxel had an impressive impact in the setting where only direct penetration was necessary (microscopic disease), but these high local drug concentrations alone were insufficient to have much of a biological impact even when a small amount of macroscopic residual cancer was present.

Phase I Trial Experience with Intraperitoneal Antineoplastic Drug Delivery

Over the past two decades a number of antineoplastic agents have been examined to document both their safety and pharmacokinetic properties when administered by the IP route (Table 1) [13-22, 24-27].

Table 1. Pharmacokinetic advantage associated with intraperitoneal delivery of selected antineoplastiv agents

Drug	Cp/Cs	AUC
Carboplatin	-	18
Cisplatin	20	12
Cytarabine	664	474
Doxorubicin	474	-
5-fluorouracil	298	367
FUDR	-	1000
Melphalan	93	65
Methotrexate	92	-
Mitoxantrone	-	1400
Paclitaxel	1000	1000

FUDR, floxuridin; Cp/Cs, ratio of peritoneal cavity to serum peak concentration; AUC, area under the concentration versus time curve

Further, the effects of a variety of combination intraperitoneal regimens have been explored in early phase clinical trials [24]. Much of the effort in the development of combination regimens has been to examine the potential for concentration-dependent synergistic cytotoxic effects observed in pre-clinical model systems.

(For completeness, it should be noted that several biological agents (interferon-gamma, interferon-alpha, tumour necrosis factor, interleukin-2) have also been evaluated for safety and pharmacokinetic profile when delivered IP, but as the focus of this review is on the cytotoxic antineoplastic agents, the experience with biological drugs will not be considered further [28].)

While most of the focus in these clinical trials has been on agents with documented activity in ovarian cancer (e.g., cisplatin, carboplatin, doxorubicin, paclitaxel), studies also explored the potential use of drugs which might subsequently be examined in gastrointestinal malignancies (e.g., 5-fluorouracil) [24-29].

These studies demonstrated the limited local toxic effects of several agents (e.g., cisplatin, carboplatin) and the fact that other drugs produced rather significant abdominal pain even at relatively low concentrations (e.g., doxorubicin, mitoxantrone) [20,24]. For a third category of drugs, while local toxicity was dose limiting (e.g., paclitaxel, 5-fluorouracil), it was possible to find a concentration of the drug where these effects were tolerable in most patients [21,22,29].

Phase II Trials of Intraperitoneal Antineoplastic Drug Delivery

Again, the substantial majority of phase 2 trials examining the biological activity of IP antineoplastic therapy in the management of malignant disease principally confined to this body compartment have focused on ovarian cancer.

Further, due to its established central role in the management of this malignancy, a major emphasis has been placed on cisplatin-based regimens, particularly in the second-line setting [24]. Both single agent and combination cisplatin-based IP programs have been evaluated, as well as an extensive exploration of the use of high dose IP cisplatin (200 mg/m^2) combined with systemically delivered sodium thiosulfate to neutralize the active drug entering the systemic compartment [13,30-32].

Unfortunately, as there have been no phase III randomized trials in the second-line setting in ovarian cancer, it is not possible to determine if any one of the multiple cisplatin-based regimens examined is superior to others in improving either symptom-free or overall survival.

However, examination of this extensive phase II experience does permit several general conclusions regarding the biological activity observed in this clinical setting:

- Surgically-documented responses observed in 20-40% of treated patients
- Responses principally observed in patients with persistent microscopic disease or very small (< 0.5 cm maximum diameter) volume macroscopic cancer when treatment initiated
- Responses observed in patients who had previously demonstrated a degree of sensitivity to platinum-based systemic therapy ("partial response"), and very rarely in individuals whose cancers had actually progressed through initial treatment, even in the presence of only microscopic residual cancer
- Long-term disease free-survival documented in a sub-set of patients treated with second-line IP cisplatin-based chemotherapy.

While meaningful objective response rates were observed [33], and long-term disease free survival was noted in a group of these patients [31,34,35], in the absence of data from randomized phase 3 trials it remains uncertain if the generally good outcome in many of these patients is the direct result of the treatment program, or represents the natural history of the malignancy in the presence of favourable clinical and biological features of the disease process (e.g., small volume residual cancer which has responded to primary chemotherapy).

Other agents have been explored when delivered by the IP route as a second-line treatment approach in ovarian cancer, including carboplatin, mitoxantrone, 5-fluorouracil, and FUDR [36-40].

There has been a limited examination of the use of IP chemotherapy in the treatment of peritoneal mesothelioma [41-48]. Activity associated with a number of approaches, including single agent cisplatin, and a combined modality strategy which included both IP chemotherapy and external beam radiation, have been reported. Finally, there has also been considerable interest in the potential use of IP antineoplastic drug delivery in the management of gastrointestinal malignancies, both in the setting of small volume macroscopic or documented microscopic metastatic disease, as well as an adjuvant strategy, following removal of a primary malignant lesion [49-53].

A particular focus of research in this area has been the combination of aggressive surgical cytoreduction of documented intraperitoneal disease, often (but not always) involving "lower grade" intra-abdominal malignancies (e.g., carcinoma of the appendix and associated pseudomyxoma peritonei), which is quickly followed by the delivery of IP chemotherapy [54-59]. In the reported experiences with this strategy, the antineoplastic regimens have frequently included multiple cytotoxic agents and are most commonly delivered in a hyperthermic environment. Long-term survival has been noted in a number of studies reported involving the combination of this aggressive surgery and hyperthermic IP chemotherapy.

The rationale for this novel, and quite controversial, approach for the management of extensive IP gastrointestinal malignant disease has previously been

described and will not be discussed here [57]. However, it is important to very clearly state that this area of clinical investigation will ultimately require *prospective randomized phase III trials* to define the role of regional antineoplastic drug delivery in these clinical settings.

Phase III Trials of Cisplatin-based Intraperitoneal Chemotherapy as Primary Treatment of Advanced Ovarian Cancer

Over the past decade there have been three large randomized phase III trials conducted in the United States that have examined a specific role for cisplatin-based IP chemotherapy as a primary management approach for small volume residual advanced ovarian cancer following an attempt at optimal surgical cytoreduction (Table 2) [60-62].

Table 2. Summary of results of randomized phase III trials examining primary cisplatin based intraperitoneal therapy of small volume residual advanced ovarian cancer

	Progression-free survival	**Overall survival**
Study 1	-	48 months versus 41 months (p=0.02); HR 0.76
Study 2	28 months versus 22 months (p=0.02) HR 0.78	63 months versus 52 months (p=0.05); HR 0.81
Study 3	24 months versus 18 months (p=0.05) HR 0.79	66 months versus 50 months (p=0.03); HR 0.71

Study 1: Control arm; Cisplatin 100 mg/m^2 IV + Cyclophosphamide 600 mg/m^2 IV every 3 weeks x 6 cycles; Experimental arm: Cisplatin 100 mg/m^2 **IP** + Cyclophosphamide 600 mg/m^2 IV every 3 weeks x 6 cycles; **Study 2:** Control arm: Paclitaxel 135 mg/m^2 IV over 24 hours + Cisplatin 75 mg/m^2 IV (day 2) every 3 weeks x 6 cycles; Experimental arm: Carboplatin (AUC 9) every 28 days x 2 cycles, followed by Paclitaxel 135 mg/m^2 IV over 24 hours + Cisplatin 100 mg/m^2 **IP** (day 2) every 3 weeks x 6 cycles; **Study 3:** Control arm: Paclitaxel 135 mg/m^2 IV over 24 hours + Cisplatin 75 mg/m^2 IV (day 2) every 3 weeks x 6 cycles; Experimental arm: Paclitaxel 135 mg/m^2 over 24 hours + Cisplatin 100 mg/m^2 **IP** (day 2) + Paclitaxel 60 mg/m^2 **IP** (day 8) every 3 weeks x 6 cycles

The results of these trials, all of which demonstrated a statistically significant survival advantage associated with the regional treatment program, have changed the paradigm for the management of women in this clinical setting.

In the first study, conducted by the Southwest Oncology Group and the Gynecologic Oncology Group, patients whose largest residual tumour mass was < 2 cm in maximal diameter were randomized to receive either intravenous or IP cisplatin (both administered at a dose of 100 mg/ m^2) (Table 2) [60]. All patients in the study were also given intravenous cyclophosphamide. Patients receiving the IP regimen experienced a reduced risk of neutropenia, tinnitus and hearing loss (pre-

sumably due to lower peak levels of cisplatin achieved in the systemic compartment following regional delivery), but also a greater incidence of abdominal pain (mild to moderate in severity). There was no difference in treatment-related deaths. However, of greatest importance, treatment with IP cisplatin was associated with a statistically significant improvement in overall survival (49 months versus 41 months; $p < 0.02$) [60].

The second study (conducted by the Gynecologic Oncology Group, the Southwest Oncology Group, and the Eastern Cooperative Oncology Group) was specifically designed to address the question of whether the same benefits associated with IP therapy would be observed if all patients received intravenous paclitaxel [12], rather than cyclophosphamide, as employed in the previous trial (Table 2) [61]. The maximum size of residual tumour nodules permitted for entry into this study was 1 cm (compared to 2 cm in the previously discussed randomized trial). This study added a second novel question in its design, by attempting to determine if the delivery of two cycles of "moderately high dose" intravenous carboplatin (AUC 9), prior to the administration of IP cisplatin, could effectively "chemically debulk" the residual tumour volume and enhance the activity of the regional treatment program [63]. Unfortunately, while potentially an interesting concept, the two cycles of carboplatin resulted in excessive bone marrow suppression (principally thrombocytopenia), such that 19% of the patients randomized to the IP chemotherapy arm received two or fewer courses of the regional treatment, before being required to withdraw from the protocol. Despite this fact, treatment with the experimental regimen was associated with a statistically significant improvement in both progression-free survival (28 months versus 22 months; $p = 0.02$) and overall survival (63 months versus 52 months; $p = 0.05$). It is relevant to note this was the first randomized trial in advanced ovarian cancer to reveal one treatment arm resulted in a median overall survival of greater than 5 years.

The third randomized phase 3 trial, conducted by the Gynecologic Oncology Group, examined both cisplatin and paclitaxel delivered by the IP route (together with intravenous paclitaxel), compared to the "standard intravenous regimen" of cisplatin and paclitaxel (Table 2) [62]. The maximum size of residual tumour masses permitted for entry into this study was 1 cm. This trial again demonstrated the regional strategy resulted in a greater risk of toxicity, including emesis, abdominal pain, and neuropathy. However, the study also included a formal quality-of-life analysis, and while treatment with IP therapy resulted in a poorer overall quality-of-life during treatment, by 12 months following the completion of treatment, there was no difference between the two study arms. This trial again demonstrated that treatment with IP chemotherapy (both cisplatin and paclitaxel) improved progression-free survival (24 months versus 18.3 months, $p = 0.05$) and overall survival (66 months versus 50 months, $p = 0.03$), the third randomized phase III study to reach this conclusion [62].

Options for Use of Primary IP Chemotherapy of Ovarian Cancer

While it would be reasonable to conclude that the regional antineoplastic program utilized in the most recent phase III trials can be employed in routine clinical practice, it is also appropriate to argue that available data would support a modest modification in the specific regimen which may noticeably improve the side effect profile associated with the IP regimen, without compromising efficacy.

As several randomized trials have convincingly demonstrated the *lack of benefit* associated with platinum "dose intensity" approaches in ovarian cancer [64-66], lowering the IP administered dose of cisplatin from 100 mg/m^2 to 75 or 80 mg/m^2 would be predicted to reduce the systemic toxicities of cisplatin, while continuing to achieve very high local concentrations and almost certainly maintaining adequate systemic drug levels.

A second question relates to the appropriateness of substituting IP carboplatin for IP cisplatin [19]. While an intriguing idea, based on the equivalence of the drugs following *systemic delivery* in advanced ovarian cancer, and the clearly superior toxicity profile of carboplatin one must be cautious with such a change, based on the demonstrated *survival advantage* associated with the regional delivery of c*isplatin* [60-62]. It would perhaps be most reasonable to conclude that the first choice for IP treatment in ovarian cancer should be with cisplatin, but for an individual patient who is unable to tolerate the systemic side effects of this agent (e.g., emesis), therapy may be continued with the regional delivery of carboplatin.

Finally, it must be asked if IP paclitaxel is a required component of the treatment program, especially because it is likely that this agent was responsible for much of the abdominal pain documented with this regimen. Further, while the largest absolute survival difference between the control and experimental arms was observed in the third randomized trial which included IP paclitaxel [62], the prior two phase III studies also demonstrated a survival benefit [60,61], and regional paclitaxel was not employed.

Again, while impressive existing data support the routine use of IP paclitaxel, an alternative strategy would be to deliver the first treatment course with only cisplatin delivered by the IP route. Assuming acceptable local side effects (e.g., abdominal pain) with this initial cycle, for subsequent courses IP paclitaxel can be added to the treatment program.

Other Potential Uses of Intraperitoneal Antineoplastic Drug Delivery in the Management of Ovarian Cancer

Based on existing data and knowledge of the natural history of ovarian cancer, it is reasonable to propose other settings where IP drug delivery may be an effective strategy in disease management:

- Primary chemotherapy (cisplatin-based) of small volume residual advanced ovarian cancer (current "Standard-of-Care")
- Consolidation therapy following a surgically documented complete response in a patient with high grade cancer (ultimate risk of relapse > 50%)
- Second-line therapy in a patient achieving an excellent partial response to primary platinum-based systemic chemotherapy, but with persistent microscopic or very small volume macroscopic (< 0.5 cm) disease (includes patients treated with a neoadjuvant approach who have undergone interval cytoreduction)
- Primary chemotherapy of early stage, high risk (e.g., grade 3, stage II) disease

It is important to recognize that phase III randomized trials have yet to be conducted to demonstrate the superiority of this approach, compared to alternative options. Thus, while it may even be reasonable to treat selected patients in this manner outside the setting of a clinical trial, patients must be informed of the absence of definitive data confirming the benefits of this strategy.

Randomized Trial Experience with Intraperitoneal Antineoplastic Drug Delivery in Non-ovarian Cancers

Unfortunately, there have been relatively few randomized phase III trials involving IP antineoplastic drug delivery outside the setting of ovarian cancer [67-74], and the results have often been inconsistent, making it difficult to draw any definitive conclusions regarding the overall clinical utility of this approach in these clinical settings.

A particularly notable relatively recently reported study compared aggressive surgical resection plus the administration of hyperthermic IP chemotherapy as therapy of peritoneal carcinomatosis of colorectal origin, compared to a palliative approach (no surgery or bypass surgery only, standard intravenous chemotherapy), and found a survival advantage associated with the intensive combined modality strategy [74]. Unfortunately, despite the excellent intentions of this group to conduct a randomized trial, they actually asked the *wrong question*.

What needed to be asked was: "Does the hyperthermic chemotherapy add anything to the aggressive surgery?" In the absence of such data, it currently remains unknown if the use of this regional antineoplastic drug delivery approach favourably impacts the natural history of the disease process, following the performance

of extensive surgery. Again, as previously stated, additional appropriately designed randomized trials are urgently needed in this clinical setting.

Of note, there remain a number of areas where IP antineoplastic drug delivery continues to be a potential management option. However, in each of these areas, phase III trials are needed to define an ultimate role for this strategy in standard disease management. Areas deserving future clinical investigation involving the intraperitoneal delivery of antineoplastic cytotoxic agents include:

- Component of an adjuvant chemotherapy strategy for cancers of the stomach and colon
- Management of microscopic or very small volume macroscopic intraperitoneal disease following surgical resection of primary and metastatic cancer
- Treatment of peritoneal mesothelioma following surgical resection of macroscopic cancer
- Aggressive surgical resection of metastatic cancer (gastrointestinal, peritoneal mesothelioma) followed by intensive hyperthermic intraperitoneal cytotoxic chemotherapy

References

1. Suhrland LG, Weisberger AS (1965) Intracavitary 5-fluorouracil in malignant effusions. Arch Intern Med 116:431-433
2. Ostrowski MJ (1986) An assessment of the long-term results of controlling the reaccumulation of malignant effusions using intracavitary bleomycin. Cancer 57:721-727
3. Dedrick RL, Myers CE, Bungay PM and DeVita VT Jr (1978) Pharmacokinetic rationale for peritoneal drug administration in the treatment of ovarian cancer. Cancer Treat Rep 62:1-9
4. Dedrick RL (1985) Theoretical and experimental bases of intraperitoneal chemotherapy. Semin Oncol 12:1-6
5. Alberts DS, Young L, Mason N, Salmon SE (1985) In vitro evaluation of anticancer drugs against ovarian cancer at concentrations achievable by intraperitoneal administration. Semin Oncol 12:38-42
6. Litterst CL, Collins JM, Lowe MC, Arnold ST, Powell DM, Guarino AM (1982) Local and systemic toxicity resulting from large-volume Ip administration of doxorubicin in the rat. Cancer Treat Rep 66:157-161
7. Nederman T, Carlsson J (1984) Penetration and binding of vinblastine and 5-fluorouracil in cellular spheroids. Cancer Chemother Pharmacol 13:131-135.
8. Ozols RF, Locker GY, Doroshow JH, Grotzinger KR, Myers CE and Young RC (1979) Pharmacokinetics of adriamycin and tissue penetration in murine ovarian cancer. Cancer Res 39:3209-3214
9. Los G, Mutsaers PHA, van der Vijgh WJF, Baldew GS, de Graaf PW and McVie JG (1989) Direct diffusion of cis-diamminedichloroplatinum(II) in

intraperitoneal rat tumors after intraperitoneal chemotherapy: A comparison with systemic chemotherapy. Cancer Res 49:3380-3384
10. Los G, Verdegaal EM, Mutsaers PH and McVie JG (1991) Penetration of carboplatin and cisplatin into rat peritoneal tumor nodules after intraperitoneal chemotherapy. Cancer Chemother Pharmacol 28:159-165
11. Cannistra SA (1993) Cancer of the ovary. N Engl J Med 329:1550-1559
12. McGuire WP, Hoskins WJ, Brady MF, Kucera PR, Partridge EE, Look KY, Clarke-Pearson DL and Davidson M (1996) Cyclophosphamide and cisplatin compared with paclitaxel and cisplatin in patients with stage III and stage IV ovarian cancer. N Engl J Med 334:1-6
13. Howell SB, Pfeifle CL, Wung WE, Olshen RA, Lucas WE, Yon JL and Green M (1982) Intraperitoneal cisplatin with systemic thiosulfate protection. Ann Intern Med 97:845-851
14. Casper ES, Kelsen DP, Alcock NW and Lewis JL, Jr (1983) Ip cisplatin in patients with malignant ascites: pharmacokinetic evaluation and comparison with the iv route. Cancer Treat Rep 67:235-238
15. Pretorius RG, Hacker NF, Berek JS, Ford LC, Hoeschele JD, Butler TA and Lagasse LD (1983) Pharmacokinetics of Ip cisplatin in refractory ovarian carcinoma. Cancer Treat Rep 67:1085-1092
16. Lopez JA, Krikorian JG, Reich SD, Smyth RD, Lee FH and Issell BF (1985) Clinical pharmacology of intraperitoneal cisplatin. Gynecol Oncol 20:1-9
17. Degregorio MW, Lum BL, Holleran WM, Wilbur BJ and Sikic BI (1986) Preliminary observations of intraperitoneal carboplatin pharmacokinetics during a phase I study of the Northern California Oncology Group. Cancer Chemother Pharmacol 18:235-238
18. Elferink F, van der Vijgh WJ, Klein I, Bokkel Huinink WW, Dubbelman R and McVie JG (1988) Pharmacokinetics of carboplatin after intraperitoneal administration. Cancer Chemother Pharmacol 21:57-60
19. Fujiwara K, Markman M, Morgan M and Coleman RL (2005) Intraperitoneal carboplatin-based chemotherapy for epithelial ovarian cancer. Gynecologic Oncology 97:10-15
20. Ozols RF, Young RC, Speyer JL, Sugarbaker PH, Greene R, Jenkins J and Myers CE (1982) Phase I and pharmacological studies of adriamycin administered intraperitoneally to patients with ovarian cancer. Cancer Res 42:4265-4269
21. Markman M, Rowinsky E, Hakes T, Reichman B, Jones W, Lewis JL, Jr., Rubin S, Curtin J, Barakat R, Phillips M, et al (1992) Phase I trial of intraperitoneal taxol: a Gynecologic Oncology Group study. J Clin Oncol 10:1485-1491
22. Francis P, Rowinsky E, Schneider J, Hakes T, Hoskins W and Markman M (1995) Phase I feasibility and pharmacologic study of weekly intraperitoneal paclitaxel: a Gynecologic Oncology Group Pilot Study. J Clin Oncol 13:2961-2967
23. Markman M, Brady MF, Spirtos NM, Hanjani P and Rubin SC (1998) Phase II trial of intraperitoneal paclitaxel in carcinoma of the ovary, tube, and peritoneum: a Gynecologic Oncology Group study. J Clin Oncol 16:2620-2624

24. Markman M (1993) Intraperitoneal therapy for treatment of malignant disease principally confined to the peritoneal cavity. Crit Rev Oncol Hematol 14:15-28
25. Markman M, Howell SB, Lucas WE, Pfeifle CE and Green MR (1984) Combination intraperitoneal chemotherapy with cisplatin, cytarabine, and doxorubicin for refractory ovarian carcinoma and other malignancies principally confined to the peritoneal cavity. J Clin Oncol 2:1321-1326
26. Markman M, Hakes T, Reichman B, Hoskins W, Rubin S, Jones W, Almadrones L, Yordan EL, Jr., Eriksson J and Lewis JL, Jr (1991) Intraperitoneal cisplatin and cytarabine in the treatment of refractory or recurrent ovarian carcinoma. J Clin Oncol 9:204-210
27. Muggia FM, Groshen S, Russell C, Jeffers S, Chen SC, Schlaerth J, Curtin J and Morrow CP (1993) Intraperitoneal carboplatin and etoposide for persistent epithelial ovarian cancer: analysis of results by prior sensitivity to platinum-based regimens. Gynecol Oncol 50:232-238
28. Markman M (1987) The intracavitary administration of biological agents. J Biol Response Mod 6:404-411
29. Speyer JL, Collins JM, Dedrick RL, Brennan MF, Buckpitt AR, Londer H, DeVita VT, Jr. and Myers CE (1980) Phase I and pharmacological studies of 5-fluorouracil administered intraperitoneally. Cancer Res 40:567-572.
30. Howell SB, Pfeifle CE, Wung WE and Olshen RA (1983) Intraperitoneal cisdiamminedichloroplatinum with systemic thiosulfate protection. Cancer Res 43:1426-1431
31. Howell SB, Zimm S, Markman M, Abramson IS, Cleary S, Lucas WE and Weiss RJ (1987) Long-term survival of advanced refractory ovarian carcinoma patients with small-volume disease treated with intraperitoneal chemotherapy. J Clin Oncol 5:1607-1612
32. Kirmani S, Lucas WE, Kim S, Goel R, McVey L, Morris J and Howell SB (1991) A phase II trial of intraperitoneal cisplatin and etoposide as salvage treatment for minimal residual ovarian carcinoma. J Clin Oncol 9:649-657
33. Markman M, Reichman B, Hakes T, Jones W, Lewis JL, Jr., Rubin S, Almadrones L and Hoskins W (1991) Responses to second-line cisplatin-based intraperitoneal therapy in ovarian cancer: influence of a prior response to intravenous cisplatin. J Clin Oncol 9:1801-1805
34. Barakat RR, Sabbatini P, Bhaskaran D, Revzin M, Smith A, Venkatraman E, Aghajanian C, Hensley M, Soignet S, Brown C, Soslow R, Markman M, Hoskins WJ and Spriggs D (2002) Intraperitoneal chemotherapy for ovarian carcinoma: results of long-term follow-up. J Clin Oncol 20:694-698
35. Markman M, Reichman B, Hakes T, Lewis JL, Jr., Jones W, Rubin S, Barakat R, Curtin J, Almadrones L and Hoskins W (1992) Impact on survival of surgically defined favorable responses to salvage intraperitoneal chemotherapy in small-volume residual ovarian cancer. J Clin Oncol 10:1479-1484
36. Pfeiffer P, Bennedbaek O and Bertelsen K (1990) Intraperitoneal carboplatin in the treatment of minimal residual ovarian cancer. Gynecol Oncol 36:306-311

37. Speyer JL, Beller U, Colombo N, Sorich J, Wernz JC, Hochster H, Green M, Porges R, Muggia FM, and Canetta R (1990) Intraperitoneal carboplatin: favorable results in women with minimal residual ovarian cancer after cisplatin therapy. J Clin Oncol 8:1335-1341
38. Markman M, George M, Hakes T, Reichman B, Hoskins W, Rubin S, Jones W, Almadrones L and Lewis JL, Jr (1990) Phase II trial of intraperitoneal mitoxantrone in the management of refractory ovarian cancer. J Clin Oncol 8:146-150
39. Ozols RF, Speyer JL, Jenkins J and Myers CE (1984) Phase II trial of 5-FU administered Ip to patients with refractory ovarian cancer. Cancer Treat Rep 68:1229-1232
40. Muggia FM, Liu PY, Alberts DS, Wallace DL, O'Toole RV, Terada KY, Franklin EW, Herrer GW, Goldberg DA and Hannigan EV (1996) Intraperitoneal mitoxantrone or floxuridine: effects on time-to-failure and survival in patients with minimal residual ovarian cancer after second-look laparotomy--a randomized phase II study by the Southwest Oncology Group. Gynecol Oncol 61:395-402
41. Pfeifle CE, Howell SB and Markman M (1985) Intracavitary cisplatin chemotherapy for mesothelioma. Cancer Treat Rep 69:205-207
42. Markman M, Cleary S, Pfeifle C and Howell SB (1986) Cisplatin administered by the intracavitary route as treatment for malignant mesothelioma. Cancer 58:18-21
43. Markman M, Kelsen D (1989) Intraperitoneal cisplatin and mitomycin as treatment for malignant peritoneal mesothelioma. Reg Cancer Treat 2:49-53
44. Langer CJ, Rosenblum N, Hogan M, Nash S, Bagchi P, LaCreta FP, Catalano R, Comis RL and O'Dwyer PJ (1993) Intraperitoneal cisplatin and etoposide in peritoneal mesothelioma: favorable outcome with a multimodality approach. Cancer Chemother Pharmacol 32:204-208
45. Antman KH, Osteen RT, Klegar KL, Amato DA, Pomfret EA, Larson DA and Corson JM (1985) Early peritoneal mesothelioma: a treatable malignancy. Lancet 2:977-981
46. Lederman GS, Recht A, Herman T, Osteen R, Corson J and Antman KH (1987) Long-term survival in peritoneal mesothelioma: The role of radiotherapy and combined modality treatment. Cancer 59:1882-1886
47. Vlasveld LTh, Gallee MPW, Rodenhuis S and Taal BG (1991) Intraperitoneal chemotherapy for malignant peritoneal mesothelioma. Eur J Cancer 27:732-734
48. Feldman AL, Libutti SK, Pingpank JF, Bartlett DL, Beresnev TH, Mavroukakis SM, Steinberg SM, Liewehr DJ, Kleiner DE and Alexander HR (2003) Analysis of factors associated with outcome in patients with malignant peritoneal mesothelioma undergoing surgical debulking and intraperitoneal chemotherapy. J Clin Oncol 21:4560-4567
49. Arbuck SG, Trave F, Douglass HO, Jr., Nava H, Zakrzewski S and Rustum YM (1986) Phase I and pharmacologic studies of intraperitoneal leucovorin and 5-fluorouracil in patients with advanced cancer. J Clin Oncol 4:1510-1517

50. Sugarbaker PH, Cunliffe W, Belliveau JF, DeBruijn EA, Graves T, Mullins R, Schlag P and Gianola F (1988) Rationale for perioperative intraperitoneal chemotherapy as a surgical adjuvant for gastrointestinal malignancy. Reg Cancer Treat 1:66-79
51. Atiq OT, Kelsen DP, Shiu MH, Saltz L, Tong W, Niedzwiecki D, Trochanowski B, Lin S, Toomasi F and Brennan M (1993) Phase II trial of postoperative adjuvant intraperitoneal cisplatin and fluorouracil and systemic fluorouracil chemotherapy in patients with resected gastric cancer. J Clin Oncol 11:425-433
52. Leichman L, Silberman H, Leichman CG, Spears CP, Ray M, Muggia FM, Kiyabu M, Radin R, Laine L, and Stain S (1992) Preoperative systemic chemotherapy followed by adjuvant postoperative intraperitoneal therapy for gastric cancer: a University of Southern California pilot program. J Clin Oncol 10:1933-1942
53. Kelsen D, Karpeh M, Schwartz G, Gerdes H, Lightdale C, Botet J, Lauers G, Klimstra D, Huang Y, Saltz L, Quan V and Brennan M (1996) Neoadjuvant therapy of high-risk gastric cancer: a phase II trial of preoperative FAMTX and postoperative intraperitoneal fluorouracil-cisplatin plus intravenous fluorouracil. J Clin Oncol 14:1818-1828
54. Beaujard AC, Glehen O, Caillot JL, Francois Y, Bienvenu J, Panteix G, Garbit F, Grandclement E, Vignal J and Gilly FN (2000) Intraperitoneal chemohyperthermia with mitomycin C for digestive tract cancer patients with peritoneal carcinomatosis. Cancer 88:2512-2519
55. Sugarbaker PH, Zhu BW, Sese GB and Shmookler B (1993) Peritoneal carcinomatosis from appendiceal cancer: results in 69 patients treated by cytoreductive surgery and intraperitoneal chemotherapy. Dis Colon Rectum 36: 323-329
56. Glehen O, Mithieux F, Osinsky D, Beaujard AC, Freyer G, Guertsch P, Francois Y, Peyrat P, Panteix G, Vignal J and Gilly FN (2003) Surgery combined with peritonectomy procedures and intraperitoneal chemohyperthermia in abdominal cancers with peritoneal carcinomatosis: a phase II study. J Clin Oncol 21:799-806
57. Glehen O, Mohamed F and Gilly FN (2004) Peritoneal carcinomatosis from digestive tract cancer: new management by cytoreductive surgery and intraperitoneal chemohyperthermia. Lancet Oncol 5:219-228
58. Elias D, Blot F, El Otmany A, Antoun S, Lasser P, Boige V, Rougier P and Ducreux M (2001) Curative treatment of peritoneal carcinomatosis arising from colorectal cancer by complete resection and intraperitoneal chemotherapy. Cancer 92:71-76
59. Rossi CR, Foletto M, Mocellin S, Pilati P, De SM, Deraco M, Cavaliere F, Palatini P, Guasti F, Scalerta R and Lise M (2002) Hyperthermic intraoperative intraperitoneal chemotherapy with cisplatin and doxorubicin in patients who undergo cytoreductive surgery for peritoneal carcinomatosis and sarcomatosis: phase I study. Cancer 94:492-499
60. Alberts DS, Green S, Hannigan EV, O'Toole R, Stock-Novack D, Anderson P, Surwit EA, Malvlya VK, Nahhas WA and Jolles CJ (1992) Improved

therapeutic index of carboplatin plus cyclophosphamide versus cisplatin plus cyclophosphamide: final report by the Southwest Oncology Group of a phase III randomized trial in stages III and IV ovarian cancer. J Clin Oncol 10:706-717

61. Markman M, Bundy BN, Alberts DS, Fowler JM, Clark-Pearson DL, Carson LF, Wadler S and Sickel J (2001) Phase III trial of standard-dose intravenous cisplatin plus paclitaxel versus moderately high-dose carboplatin followed by intravenous paclitaxel and intraperitoneal cisplatin in small-volume stage III ovarian carcinoma: an intergroup study of the Gynecologic Oncology Group, Southwestern Oncology Group, and Eastern Cooperative Oncology Group. J Clin Oncol 19:1001-1007
62. Armstrong DK, Bundy B, Wenzel L, Huang HQ, Baergen R, Shashikant L, Copeland LJ, Walker JL and Burger RA (2006) Phase III randomized trial of intravenous cisplatin and paclitaxel versus an intensive regimen of intravenous paclitaxel, intraperitoneal cisplatin and intraperitoneal paclitaxel in stage III ovarian cancer: A Gynecologic Oncology Group Study. N Engl J Med 354:34-43
63. Shapiro F, Schneider J, Markman M, Reichman BS, Venkatraman E, Barakat R, Almadrones L and Spriggs D (1997) High-intensity intravenous cyclophosphamide and cisplatin, interim surgical debulking, and intraperitoneal cisplatin in advanced ovarian carcinoma: a pilot trial with ten-year follow-up. Gynecol Oncol 67:39-45
64. Gore M, Mainwaring P, A'Hern R, MacFarlane V, Slevin M, Harper P, Osborne R, Mansi J, Blake P, Wiltshaw E and Shepherd J (1998) Randomized trial of dose-intensity with single-agent carboplatin in patients with epithelial ovarian cancer. London Gynaecological Oncology Group. J Clin Oncol 16:2426-2434
65. McGuire WP, Hoskins WJ, Brady MF, Homesley HD, Creasman WT, Berman ML, Ball H, Berek JS and Woodward J (1995) Assessment of dose-intensive therapy in suboptimally debulked ovarian cancer: a Gynecologic Oncology Group study. J Clin Oncol 13:1589-1599
66. Conte PF, Bruzzone M, Carnino F, Gadducci A, Algeri R, Bellini A, Boccardo F, Brunetti I, Catsafados E, Chiara S, Foglia G, Gallo L, Iskra L, Mammoliti S, Parodi G, Ragni N, Rosso R, Rugiati S and Rubagotti A (1996) High-dose versus low-dose cisplatin in combination with cyclophosphamide and epidoxorubicin in suboptimal ovarian cancer: a randomized study of the Gruppo Oncologico Nord-Ovest. J Clin Oncol 14:351-356
67. Sugarbaker PH, Gianola FJ, Speyer JC, Wesley R, Barofsky I and Meyers CE (1985) Prospective, randomized trial of intravenous versus intraperitoneal 5-fluorouracil in patients with advanced primary colon or rectal cancer. Surgery 98:414-422
68. Yu W, Whang I, Suh I, Averbach A, Chang D and Sugarbaker PH (1998) Prospective randomized trial of early postoperative intraperitoneal chemotherapy as an adjuvant to resectable gastric cancer. Ann Surg 228:347-354
69. Vaillant JC, Nordlinger B, Deuffic S, Arnaud JP, Pelissier E, Favre JP, Jaeck D, Fourtanier G, Grandjean JP, Marre P and Letoublon C (2000) Adjuvant in-

traperitoneal 5-fluorouracil in high-risk colon cancer: A multicenter phase III trial. Ann Surg 231:449-456

70. Fujimoto S, Takahashi M, Mutou T, Kobayashi K and Toyosawa T (1999) Successful intraperitoneal hyperthermic chemoperfusion for the prevention of postoperative peritoneal recurrence in patients with advanced gastric carcinoma. Cancer 85:529-534
71. Rosen HR, Jatzko G, Repse S, Potrc S, Neudorfer H, Sandbichler P, Zacherl J, Rabl H, Holzberger P, Lisborg P and Czeijka M (1998) Adjuvant intraperitoneal chemotherapy with carbon-adsorbed mitomycin in patients with gastric cancer: results of a randomized multicenter trial of the Austrian Working Group for Surgical Oncology. J Clin Oncol 16:2733-2738
72. Nordlinger B, Rougier P, Arnaud JP, Debois M, Wils J, Ollier JC, Grobost O, Lasser P, Wals J, Lacourt J, Seitz JF, Guimares dos SJ, Bleiberg H, Mackiewickz R, Conroy T, Bouche O, Morin T, Baila L, van Cutsem E and Bedenne L (2005) Adjuvant regional chemotherapy and systemic chemotherapy versus systemic chemotherapy alone in patients with stage II-III colorectal cancer: a multicentre randomised controlled phase III trial. Lancet Oncol 6:459-468
73. Hagiwara A, Takahashi T, Kojima O, Sawai K, Yamaguchi T, Yamane T, Taniguchi H, Kitamura K, Noguchi A, and Seiki K (1992) Prophylaxis with carbon-adsorbed mitomycin against peritoneal recurrence of gastric cancer. Lancet 339:629-631
74. Verwaal VJ, van Ruth S, de Bree E, van Sloothen GW, van Tinteren H, Boot H and Zoetmulder FA (2003) Randomized trial of cytoreduction and hyperthermic intraperitoneal chemotherapy versus systemic chemotherapy and palliative surgery in patients with peritoneal carcinomatosis of colorectal cancer. J Clin Oncol 21:3737-3743

The Biologic Rationale of Hyperthermia

B Hildebrandt, P Wust

Abstract

The clinical efficacy of various of hyperthermia approaches has been demonstrated in the scope of various randomised trials. In addition, a large body of preclinical data on alterations of cellular and molecular pathways under the circumstance of elevated temperatures is available. However, there is still uncertainty about the mechanisms which are acutally responsible for the beneficial effect of clinical hyperthermia when applied as an adjunt to radiotherapy, chemotherapy, or radiochemotherapy. This chapter gives a clinically orientated overview on the cytotoxic effect of heat alone and in conjunction with radiation and drugs, heat-induced alterations of the tumour microenvironment, and the immunological targets of hyperthermia.

Introduction

The term “hyperthermia” describes various approaches to increase the temperature of a tumour-loaded body region to 39°C - 43°C by using an external energy source. The different hyperthermia techniques are best categorized by their target volume (local vs. regional vs. whole-body hyperthermia), and the physical mode of power deposition (radiant vs. capacitive vs. convective). For practical purposes, radiant local, interstitial, and regional hyperthermia are distinguished from capacitive hyperthermia, radiant whole-body hyperthermia, and convective techniques.

The latter can be further subdivided into those in which the patients´ blood is warmed by an external device before it is retransfused to the target volume (e.g. isolated limb perfusion and convective whole-body hyperthermia), or those in which contact heating is employed (hyperthermic peritoneal and vesical perfusion) [1-5] (Table 1).

Table 1. Synopsis of different hyperthermia techniques

Target volume	Mode of application		Indication	RT
Local	Radiative		Superficial lymph node metastases	1
			breast cancer (chest wall recurrence)	1
			malignant melanoma	1
	Interstitial		Superficial lymph node metastases	2
			Glioblastoma	2
	Capacitive		Head and neck cancer	1
Regional	Radiative		Rectal Cancer	1
			Cervix cancer	1
			Bladder Cancer	1
	Capacitive		Rectal Cancer	1
			Cervix Cancer	1
			Esophagus Cancer	1,4
	Convective	HILP	Malignant melanoma	1
			Soft-tissue sarcoma (adult)	0
		HIPEC	Gastric cancer	1
			Colon cancer	1
Systemic	Radiative		Metastatic carcinoma / sarcoma	0
	Convective		obsolete	0

RT: Randomised trial available (at least one): 0 = not available, 1 = comparing radiotherapy vs. hyperthermic radiotherapy, 2 = comparing brachytherapy vs. hyperthermic brachytherapy, 2 = comparing radiotherapy vs. hyperthermic chemotherapy, 4 = comparing radiochemotherapy vs. hyperthermic radiochemotherapy; HILP: hyperthermic isolated limb perfusion; HIPEC: hyperthermic intraperitoneal chemoperfusion. For detailed references see [5]

All hyperthermia modalities have in common that they are not effective enough to replace any of the established oncological treatment modalities. However, some of them have been demonstrated to improve the results of radio- and chemotherapy in the scope of randomised trials. Thus the administration of hyperthermia aims to optimise the results of the classic treatment strategies within the framework of multimodal treatment concepts.

Most randomised hyperthermia trials have been performed on radiotherapy combined with radiant or capacitive hyperthermia [4,5]. Clinical improvements have been particularly observed in patients with superficial lymph node metastases of various primaries, and in patients with locally advanced malignancies of the pelvis. In addition, the postoperative application of hyperthermic limb perfusion and hyperthermic peritoneal perfusion in patients with malignant melanoma and gastric cancer, respectively, colon cancer has been demonstrated to improve local recurrence and/or survival when compared with no adjuvant treatment [1,3-6].

Discussing the different approaches in a general context, one has to consider that their therapeutical potentials, expenditure of treatment, technical problems and evidence of efficacy are diverse. Whereas local and regional radiofrequency hyperthermia can be regarded as well-established, non-toxic treatment which is carried out according to standardised protocols worldwide [7], the corresponding

capacative techniques are lacking detailed technical evaluations and - from a physical point of view - major drawbacks with regard to efficacy (as discussed in [8-10]). Radiant whole-body hyperthermia as an adjunct to chemotherapy has been evaluated in a number of phase II trials, but no phase III trial has been yet completed [2]. Finally, hyperthermic peritoneal and isolated limb perfusion have a demonstrated efficacy for certain indications in randomized trials, but are associated with a relatively high technical expenditure and occurrence of potentially severe side-effects [1,11]. However, locoregional radiofrequency hyperthermia, hyperthermic isolated limb perfusion and hyperthermic intraperitoneal chemoperfusion can be regarded as well-established and effective treatment options today.

The major argument for the clinical efficacy of hyperthermia is its proven benefit in the scope of randomised clinical trials. In addition, a clear dose-response relationship has been established from analyses of thermometric data obtained from clinical studies on radiative locoregional hyperthermia (see below). Regarding hyperthermic chemoperfusion, only one clinical study has demonstrated a possible dependency of thermal dose and clinical outcome so far [12]. Therefore, it is largely unclear to which extent this principle can be applied for the method. The third argument for the efficacy of hyperthermia is the availability of detailed preclinical data on the cellular and molecular effectors of hyperthermia:

- **Cytoskeleton**
  - changes in stability and fluidity of cell membrane
  - alterations of cell shape
  - impaired transmembranal transport
  - alterations of membrane potential
  - modulation of efflux pumps
  - induction of apoptosis
- **Intracellular proteins**
  - impairment of protein synthesis
  - denaturation of proteins
  - aggregation of proteins at nuclear matrix
  - induction of HSP-synthesis
- **Nucleic acids**
  - decrease of RNA-/DNA synthesis
  - inhibition of DNA-repair enzymes
  - altered DNA-conformation
- **Other alterations of cell function**
  - intracellular metabolism of other substrates
  - gene expression, signal transduction

Again, most of these data refer to models employing radiative heat application, a fact that surely curtails the transferability of preclinical results to the specific situation in peritoneal chemoperfusion. However, engagement with the principles and history of preclinical hyperthermia research from light microscopy to molecular biology may be helpful for researchers involved in hyperthermic chemoperfusion with regard to the planning of their future projects on this very exciting and promising method.

Basic Principles of Hyperthermic Cell Death

Early experiments on the cytotoxic effect of *in vitro* hyperthermia on cultured cells revealed a time- and dose-dependant relationship in the temperature range between 41° C and 47° C. The slope of the corresponding survival curves show a typical shoulder, indicating a potential of cells to recover from a thermal insult on the one, and a transition into an exponential phase of cell death beyond a certain thermal dose on the other hand. It was also found that the capability of heat to induce cell death at temperatures < 42° C- 43° C is markedly lower than above 43° C, e.g. above a certain "threshold" (Fig. 1).

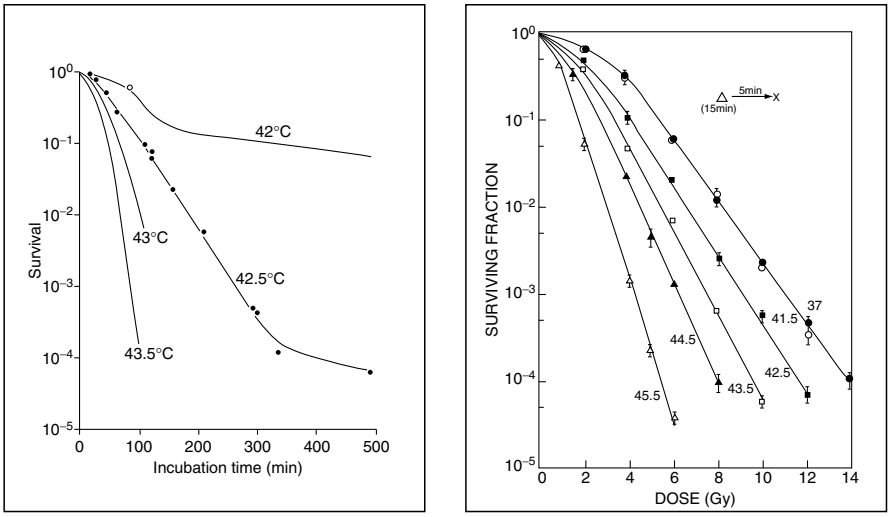


Figure 1. Dose-response relationship of thermal cell killing and thermal radiosenzitation. Left: survival fraction of asynchronous CHO cells heated at different temperatures, emphazising the typical "shoulder" (reprinted with permission from [61]); right: survival fractions of V79 cells heated for 15 min. for various temperatures 10 min. before exposure to different dosages of radiation. Reprinted with permission from [62]

Therefore, the most common definition of the thermal dose (D) is derived from exposure time (t) and given temperature (T), according to the formula

$$D = t\, R^{T-43}$$

where R is a constant with R = 2 for temperatures ≥ 43° C, and R = 4 for temperatures < 43° C, or - in the case of varying T - a summation of thermal doses with respect to temperature T and its duration Δt (reviewed in [13-15]). In clinical hyperthermia, the calculation of the "thermal isoeffect dose" (TID) according the the formula has evolved into a practical tool to compare different hyperthermia exposures with each other when local or regional hyperthermia is applied as an adjunt to radiotherapy, chemotherapy, or radiochemotherapy. In addition, a number of studies have established a clear-cut relationship between thermal dose and clinical response for local and regional radiofrequency hyperthermia [16-19]. However, it is still unclear to which extent the thermal dose concept can also be applied for whole-body hyperthermia and hyperthermic chemoperfusion.

When cultured cells are exposed to temperatures < 43° C, or are cooled down to 37° C between two heat shock treatments > 43° C, a decrease of heat-induced cell killing can be observed which results in a flattened inactivation curve ("thermotolerance"). Thermotolerance is of multifactorial origin and reversible in principle. It is not inherited in cell cultures, is at least partially based on the induction of heat shock proteins and other posttranslational adaption processes (e.g. cell cycle arrest in the G2-phase, changes in cell metabolism), and may occur in association with some forms of aquired or inherited drug resistance. The ability of a cell to become thermotolerant is influenced by various environmental conditions such as (suddenly) lowered intracellular pH [20-22]. As the temperatures achievable in clinical hyperthermia are usually below 43° C, it has long been proposed that the occurrence of thermotolerance may counteract the efficacy of heat application. Indeed, this "43° C-dogma" has largely hampered hyperthermia research although a clinical dose-relationship had long been established for the temperature range of 39° C - 42° C for clinical applications [5, 23, 24].

Another groundbreaking observation from early *in vitro* studies was that hyperthermia not only acts in a cytotoxic way by itself, but also sensitizes tumour cells to radiotherapy and various cytostatic drugs at markedly lower temperatures than 43° C ("thermal radiosensitization" and "thermal chemosensitization") (reviewed in [20, 25, 26]). The extent of thermal sensitization in a given model can be quantified by the quotient of survival fraction of cells treated with radiation or chemotherapy alone and those treated with radiation or chemotherapy at the same dose plus heat ("thermal enhancement ratio", TER). Both thermal radio- and chemosensitization are reproducible *in vivo,* and sufficiently explain the beneficial clinical effect of radiative locoregional hyperthermia which can be already observed at temperatures below 43° C [5, 20, 27].

Molecular and Cellular Effectors of Hyperthermia

The thermal doses required to induce hyperthermic cell death varies with a factor 10 between different cell types, whereby the thermal energy dose required to induce exponetial cell death is similar to that required for cellular protein denaturation in vitro and in experimental tumours (ca. 140 kcal/mol). This supports the hypothesis that the cytotoxic effect of hyperthermia is mainly based on denaturation of cytoplasmatic and membrane proteins [21]. Regarding the role of the cell membrane, heat application has been demonstrated to affect fluidity and stability of cellular membranes, and to impede the function of transmembranal transport proteins and cell surface receptors in vitro [28, 29]. In addition, several studies indicate that hyperthermia-induced changes of cytoskeletal organisation (cell shape, mitotic apparatus, intracytoplasmatic membranes such as endoplasmatic reticulum and lysosomes) is correlated with the extent of hyperthermic cell death [28]).

In the 1960s, heat application was suggested to act similarly to radiation by directly damaging nuclear DNA, thereby inducing double-strand breaks. Later, it has been demonstrated that heat does not primarily cause DNA-damage by itself, but rather impedes the repair of radiation-induced cell damage thus boosting radiation-induced DNA-fragmentation. Recent data suggest that inhibition of the "base damage repair" system may be the crucial pathogenetic step in hyperthermic radiosensitization (reviewed in [30]).

In the 1980s, Borelli and coworkers firstly described the occurrence of "membrane blebbing" in cultured cells exposed to heat [31], which is a typical feature of programmed cell death (apoptosis). Further studies revealed that heat sensitivity is highest during the mitotic phase, where hyperthermia induces microscopically detectable damage of the mitotic apparatus leading to inefficient mitosis and consecutive polyploidy. In contrast, G1-cells exposed to hyperthermia do not exhibit corresponding alterations, but may instead undergo a "rapid mode of cell death" immediately after heat exposure. The varying behaviour of cells in the different cell cycle phases indicate the diversity of molecular mechanisms of cell death following hyperthermia , which include apoptosis, necrosis, and cell cylce arrest (reviewed in [13,14,20,21]).

But even if a number of in vitro studies have subjected the various modes of cell death during hyperthermia, detailed data obtained from living organisms are not available. Animal studies on "moderate" whole-body hyperthermia at 39° C found that this application is suitable to induce significant tumour growth delay in a xenotransplanted colon carcinoma. Analyses of the host tissues revealed a high rate of apoptotic cell death particularly in various lymphatic tissues, a moderately increased apoptosis within the small intestine, but not in any of the remaining organs [32,33]. Regarding the different mechanisms of apoptosis induction, data from concomitant clinical research suggest that response of patients to hyperthermia in conjunction to radio - or radiochemotherapy largely depends on the intratumoural status of bax-proteins, e.g. high bax-expression is associated with better, and loss of bax with a poor response [34,35].

In a non-oncological context, heat-induced apoptosis is suggested to be one of the major pathogenetic mechanism mediating heat-induced developmental defects in foetuses by inducing an irreversible damage to neurogenic cells. A similar mechanism has also been proposed for heat damage of the central nervous system in adults (reviewed in [36,37]).

Alterations of the Tumour Microenvironment

The main features of the tumour microenvironment are hypoxia, acidosis and energy deprivation, which are consequences of a altered blood vessel architecture, density, and blood flow. In early preclinical studies, hyperthermia > 42° C has been demonstrated to further impair the unfavourable environment by inducing a reduction of tumour blood supply. The thermal dose required has been demonstrated to vary between different tumours, and to largely depend on the percentage of responsive vessels that have maintained their ability of thermal regulation. However, the inhomogenicity of blood supply within the same tumour regularly persisted after application of temperatures > 42° C. Heat-induced alterations mainly consisted of endothel swelling, shift of plasma fluid into the interstitium, microthrombosis due to hemostasis activation, and changes of viscosity.

All of those factors promote a further reduction of oxygen and nutrient supply, as well as intratumoural lactate acidosis, and may be further enhanced by reactive hyperaemia of surrounding (healthy) tissues that have maintained their ability of thermoregulation in the sense of a "steal-effect " [38-40].

Contrary to those earlier studies, the application of hyperthermia at temperatures < 42° C has later been demonstrated to improve tumour blood flow and oxygen content [41]. On the background of the proven efficacy of locoregional hyperthermia approaches – where intratumoural temperatures rarely exceed 42° C - such alterations would favourably explain the benefical effect of heat treatment, as they would increase both the efficacy of radiotherapy (which is more effective in tumours with higher pH and oxygen content) and chemotherapy (which is more effective at higher intratumoural drug concentrations). However, the actual effect of clinical hyperthermia on the tumour environment, as well as its extent in the various temperature ranges (>/< 43°C), still has to be determined.

Recently, the introduction of novel technologies has enabled non-invasive investigations of tumour perfusion during hyperthermia. The first clinical data employing positron-emmision tomography with ^{15}O-labelled water before and immediately after treatment revealed that regional pelvic hyperthermia enhances the arterial input of the tumour in patients with recurrent rectal cancer [42]. More detailed information on intratumoural perfusion changes during hyperthermia are to be expected from analyses of datasets provided during MR-guided online thermometry, where first results have just been published [43]. Fig. 2 gives an example of the MR-temperature in comparison to the MR-based imaging of the perfusion before and during regional hyperthermia in an adult patient with a

locally advanced soft tissue sarcoma. It appears that the perfusional changes under regional hyperthermia are complex, and may differ between the marginal and central parts of the tumour, and the surroundung tissue.

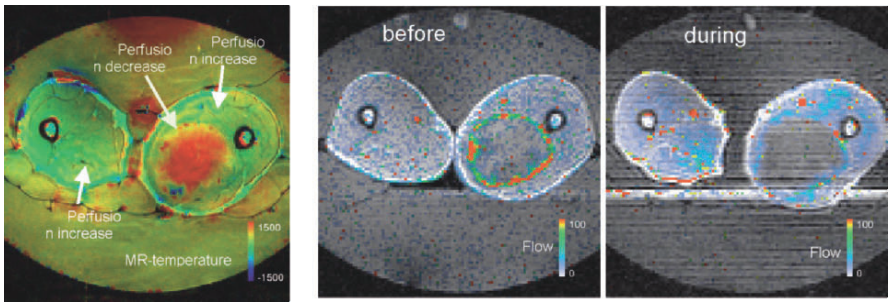


Figure 2. MR-temperature distribution (MR-deg) and perfusion before and during regional hyperthermia. Note that the perfusion increases in some parts of the muscle, which also increases the MR-temperature. In the tumour margin the perfusion decreases, also lowering the MR-temperature. In the tumour centre the perfusion remains constant (near zero). Here, the MR-temperature is identical with the temperature in ° Celsius

Hyperthermia and the Immune System

Cellular Immune Response

The stimulation of the cellular immune system by hyperthermia is firstly mediated by direct effects of heat on the behaviour of immunocompetent cells. There is increasing evidence that heat stress may affect signalling pathways responsible for the extravasation of lymphocytes from the vasculature into the tumour environment across intratumoural microvessels [44,45].

In addition, an activation of the cellular immune response by systemic heat application in the context of therapeutic purposes has been assumed. However, clinical data on this topic have long been limited to studies on healthy volunteers or patients suffering from heat stroke. During the past decade, a novel generation of applicators for whole-body hyperthermia (WBH) has been introduced, which enables the application of systemic heat up to 42° C with acceptable side-effects [2]. Results of a number of recent clinical phase II trials on this approach suggest a rather limited benefit achievable with WBH as an adjunct to systemic chemotherapy, but concomitant research has highlighted different aspects of heat-induced alterations of the cellular immune system.

Several groups have demonstrated that the application of WBH at 42° C to cancer patients induces the secretion of pro-inflammatory cytokines such as Interleukin-1 (IL-1), Interleukin-8 (IL-8), and tumour necrosis factor alpha (TNF-α),

as well as Interleukin-6 (IL-6) through monocytes and macrophages [46-48]. Similar findings have been obtained in patients suffering from heat stroke [49], or healthy volunteers undergoing moderate WBH [50].

Regarding the course of lymphocyte subpopulations during WBH, a reversible increase of blood natural killer cells (NK-cells) as well as natural killer T-cells (NKT-cells) and γδ-T-cells have been reported. Analyses of all alterations of T-lymphocyte subpopulations suggest a short period of impaired T-cell proliferation and reduced T-cell activity which was followed by a prolonged T-cell activation, whereby corresponding changes did not occur in a control group of patients treated with chemotherapy alone [46,51,52].

To sum it up, it appears that WBH induces an activation of the monocyte/ macrophage system, as well as a reversible anti-inflammatory stress response which is followed by prolonged T-cell activation, although it cannot be ruled out that those effects are partly caused by the induction of stress hormones during systemic heat application.

Heat Shock Proteins

Hyperthermia and various other stress conditions induce the synthesis of "heat shock proteins" (HSP) which are mediated by activation of nuclear "heat shock factors" (HSF) within minutes. HSPs are also expressed constitutively, and consist of at least 5 subgroups with different molecular mass and varying biological function. Those are usually divided into small HSPs (molecular mass < 40 kDa), and the HSP 60, HSP 70, HSP 90 and HSP 100 protein families. All HSPs have the property to unselectively bind to hydrophobic protein sequences liberated by denaturation, and thereby prevent irreversible interaction of neighboured proteins ("chaperoning function"). In particular the proteins of the HSP 27 and 70 families are able to defend cells against a variety of potentially lethal stimuli, e.g. by increased resistance to apoptosis. They are thus regarded as "general survival proteins" [53-55].

Besides their chaperoning function, HSPs are involved in antigen presentation, cross-presentation, and tumour immunity. In addition, HSPs isolated from cancer tissues have been demonstrated to form complexes with tumour specific peptides that are internalised into antigen presenting cells by specific receptors and then presented together with MHC (major histocompatibility complex) class I molecules, thereby inducing a cytotoxic T-cell-activation. It has been shown that HSPs interact with antigen presenting cells through the CD 91 receptor, inducing the re-presentation of chaperoned peptides by MHC-molecules and activation of NF-κB [56]. Multhoff and coworkers characterised solid tumour cell lines expressing a stress-inducible form of HSP 70 which mediates MHC-independent lysis. They demonstrated that a cell-surface presentation of HSP 70 may occur constitutively or heat-induced. Thereby, membrane expression of HSP70 epitopes may represent a target for NK-cells, but also protect tumour cells from radiation damage [57,58]. Because of their unique immunologic features, HSPs are believed to procure more or less specific immunogenic effects induced by hyperthermia

and other exogenous stimuli and have attracted particular interest for tumour vaccination strategies [59,60].

Summary

A large body of research data dealing with the molecular and cellular targets of hyperthermia is available. Early cell culture experiments demonstrated a cell killing effect of hyperthermia which is markedly enhanced at temperatures above 43° C, as well as in combination with radiation and various cytostatic drugs ("thermal radiosensitization", "thermal chemosensitization"). More recent research has focused on the effect of hyperthermia on distinct cellular signalling pathways, particularly of those involved in "heat shock response", cell cycle regulation, and apoptosis.

The clinical application of hyperthermia to cancer patients is very complex, due to alterations of the tumour microenvironment, immunological pathways, and other interactions that cannot be simulated in preclinical models. In addition, hyperthermia is usually applied in the scope of multimodal treatment concepts, a fact that makes it difficult to extract reliable information on the effects of heat alone in the scope of concomitant research. Another point to consider, investigating the molecular effects of hyperthermia in a clinical context requires repetitive extraction of tumour samples from the patient which is problematic for practical and ethical reasons.

As a conclusion, there is still little certainty on the biological mechanisms contributing to the clinical effect of hyperthermia, although the efficacy of many hyperthermia techniques has already been proven in a number of prospective randomised trials.

References

1. Eggermont AM, Brunstein F, Grunhagen D, ten Hagen TL (2004) Regional treatment of metastasis: role of regional perfusion. State of the art isolated limb perfusion for limb salvage. Ann Oncol 15 Suppl 4:iv107-12
2. Hildebrandt B, Hegewisch-Becker S, Kerner T, Nierhaus A, Bakhshandeh-Bath A, Janni W, et al (2005) Current status of radiant whole-body hyperthermia at temperatures > 41.5°C and practical guidelines for the treatment of adults. The German "Interdisciplinary Working Group on Hyperthermia". Int J Hyperthermia 21(2):169-183
3. Stewart JHt, Shen P, Levine EA (2005) Intraperitoneal hyperthermic chemotherapy for peritoneal surface malignancy: current status and future directions. Ann Surg Oncol 12(10):765-777
4. van Der Zee J (2002) Heating the patient: a promising approach? Ann Oncol 13(8):1173-1184

5. Wust P, Hildebrandt B, Sreenivasa G, Rau B, Gellermann J, Riess H, et al (2002) Hyperthermia in combined treatment of cancer. Lancet Oncol 3(8):487-497
6. Sugarbaker PH (2006) New standard of care for appendiceal epithelial neoplasms and pseudomyxoma peritonei syndrome? Lancet Oncol 7(1):69-76
7. Lagendijk JJ, Van Rhoon GC, Hornsleth SN, Wust P, De Leeuw AC, Schneider CJ, et al (1998) ESHO quality assurance guidelines for regional hyperthermia. Int J Hyperthermia 14(2):125-133
8. Jones EL, Prosnitz LR, Dewhirst MW, Vujaskovic Z, Samulski TV, Oleson JR, et al (2005) In regard to Vasanathan et al. (Int J Radiat Oncol Biol Phys 2005;61:145-153). Int J Radiat Oncol Biol Phys 63(2):644
9. van der Zee J, van Rhoon GC, Wust P (2005) In regard to Dr. Vasanthan et al. (Int J Radiat Oncol Biol Phys 2005;61:145-153). Int J Radiat Oncol Biol Phys 1;62(3):940-941
10. Vasanthan A, Mitsumori M, Park JH, Zhi-Fan Z, Yu-Bin Z, Oliynychenko P, et al (2005) Regional hyperthermia combined with radiotherapy for uterine cervical cancers: a multi-institutional prospective randomized trial of the international atomic energy agency. Int J Radiat Oncol Biol Phys 1;61(1):145-153
11. Verwaal VJ, van Ruth S, de Bree E, van Sloothen GW, van Tinteren H, Boot H, et al (2003) Randomized trial of cytoreduction and hyperthermic intraperitoneal chemotherapy versus systemic chemotherapy and palliative surgery in patients with peritoneal carcinomatosis of colorectal cancer. J Clin Oncol 15;21(20):3737-3743
12. Yonemura Y, de Aretxabala X, Fujimura T, Fushida S, Katayama K, Bandou E, et al (2001) Intraoperative chemohyperthermic peritoneal perfusion as an adjuvant to gastric cancer: final results of a randomized controlled study. Hepatogastroenterology 48(42):1776-1782
13. Dewhirst MW, Viglianti BL, Lora-Michiels M, Hanson M, Hoopes PJ (2003) Basic principles of thermal dosimetry and thermal thresholds for tissue damage from hyperthermia. Int J Hyperthermia 19(3):267-294
14. Dewey WC (1994) Arrhenius relationships from the molecule and cell to the clinic. Int J Hyperthermia 10(4):457-483
15. Hildebrandt B, Rau B, Gellermann J, Wust P, Riess H (2004) Hyperthermic intraperitoneal chemotherapy in patients with peritoneal carcinosis. J Clin Oncol 15;22(8):1527-1529
16. Issels R, Prenninger SW, Nagele A, Boehm E, Sauer H, Jauch K-W, et al (1990) Ifosfamide plus etoposide combined with regional hyperthermia in patients with locally advanced sarcomas. J Clin Oncol 11:1818-1829
17. Jones EL, Oleson JR, Prosnitz LR, Samulski TV, Vujaskovic Z, Yu D, et al (2005) Randomized trial of hyperthermia and radiation for superficial tumours. J Clin Oncol 1;23(13):3079-3085
18. Sapareto SA, Dewey WC (1984) Thermal dose determination in cancer therapy. Int J Radiat Oncol Biol Phys 10(6):787-800

19. Tilly W, Wust P, Rau B, Harder C, Gellermann J, Schlag P, et al (2001) Temperature data and specific absorption rates in pelvic tumours: predicitve factors and correlations. Int J Hyperthermia 17(2):172-188
20. Hildebrandt B, Wust P, Ahlers O, Dieing A, Sreenivasa G, Kerner T, et al (2002) The cellular and molecular basis of hyperthermia. Crit Rev Oncol Hematol 43(1):33-56
21. Lepock JR (2005) How do cells respond to their thermal environment? Int J Hyperthermia 21(8):681-687
22. Li GC, Mivechi NF, Weitzel G (1995) Heat shock proteins, thermotolerance, and their relevance for clinical hyperthermia. Int J Hyperthermia 11(4):459-488
23. Corry PM, Armour EP (2005) The heat shock response: role in radiation biology and cancer therapy. Int J Hyperthermia 21(8):769-778
24. Dewhirst MW, Vujaskovic Z, Jones E, Thrall D (2005) Re-setting the biologic rationale for thermal therapy. Int J Hyperthermia 21(8):779-790
25. Dewey WC (1989) Mechanism of thermal radiosenzitation. In: Urano M, Douple E, editors. Biology of thermal potentiation of radiotherapy. Utrecht, Tokyo: VSP; p1-16
26. Vujaskovic Z, Song CW (2004) Physiological mechanisms underlying heat-induced radiosensitization. Int J Hyperthermia 20(2):163-174
27. Myerson RJ, Roti Roti JL, Moros EG, Straube WL, Xu M (2004) Modelling heat-induced radiosensitization: clinical implications. Int J Hyperthermia 20(2):201-212
28. Lepock JR (2003) Cellular effects of hyperthermia: relevance to the minimum dose for thermal damage. Int J Hyperthermia 19(3):252-266
29. Coss RA, Linnemanns WAM (1996) The effects of hyperthermia on the cytoskeleton: a review. Int J Hyperthermia 12(2):173-196
30. Kampinga HH, Dynlacht JR, Dikomey E (2004) Mechanism of radiosensitization by hyperthermia (> or = 43 degrees C) as derived from studies with DNA repair defective mutant cell lines. Int J Hyperthermia 20(2):131-139
31. Borrelli MJ, Garlini WG, Ransom BR, Dewey WC (1986) A direct correlation between hyperthermia induced membrane blebbing and survival in synchronous G1 CHO cells. J Cell Physiol 126:181-190
32. Yonezawa M, Otsuka T, Matsui N, Tsuji H, et al (1996) Hyperthermia induces apoptosis in malignant fibrous histiocytoma cells in vitro. Int J Cancer 66(3):347-351
33. Sakaguchi Y, Stephens LC, Makino M, Kaneko T, et al (1995) Apoptosis in tumours and normal tissues induced by whole body hyperthermia in rats. Cancer Res 55(22): 5459-5464
34. Harima Y, Nagata K, Harima K, Oka A, Ostapenko VV, Shikata N, et al (2000) Bax and Bcl-2 protein expression following radiation therapy versus radiation plus thermoradiotherapy in stage IIIB cervical carcinoma. Cancer 1;88(1):132-138

35. Sturm I, Rau B, Schlag PM, Wust P, Hildebrandt B, Riess H, et al (2006) Genetic dissection of apoptosis and cell cycle control in response of colorectal cancer treated with preoperative radiochemotherapy. BMC Cancer 10;6(1):124
36. Sharma HS, Hoopes PJ (2003) Hyperthermia induced pathophysiology of the central nervous system. Int J Hyperthermia 19(3):325-354
37. Edwards MJ, Saunders RD, Shiota K (2003) Effects of heat on embryos and foetuses. Int J Hyperthermia 19(3):295-324
38. Folkman J (1990) What is evidence that tumors are angiogenesis dependent ? J Natl Cancer Inst 82:4-6
39. Vaupel P, Kallinowski F, Okunieff P (1989) Blood flow, oxygen and nutrient supply, and metabolic microenvironment of human tumors: a review. Cancer Res 49(23):6449-6465
40. von Ardenne M, Reitnauer PG (1982) Manipulated selective inhibition of microcirculation in cancer tissues, Article in German. J Cancer Res Clin Oncol 103(3):269-279
41. Song CW, Park HJ, Lee CK, Griffin R (2005) Implications of increased tumour blood flow and oxygenation caused by mild temperature hyperthermia in tumour treatment. Int J Hyperthermia 21(8):761-767
42. Hildebrandt B, Wust P, Drager J, Ludemann L, Sreenivasa G, Tullius SG, et al (2004) Regional pelvic hyperthermia as an adjunct to chemotherapy (oxaliplatin, folinic acid, 5-fluorouracil) in pre-irradiated patients with locally recurrent rectal cancer: a pilot study. Int J Hyperthermia 20(4):359-369
43. Ollek JT, Wlodarczyk W, Ludemann L, Gellerman J, Hildebrandt B, Wust P (2006) Evaluation of perfusion changes induced by hyperthermia using DCE-MRI. 23rd Annual Meeting of the European Society for Hyperthermic Oncology; May 24-27th, 2006; Berlin; p. 80
44. Calderwood SK, Theriault JR, Gong J (2005) How is the immune response affected by hyperthermia and heat shock proteins? Int J Hyperthermia 21(8):713-716
45. Chen Q, Evans SS (2005) Thermal regulation of lymphocyte trafficking: hot spots of the immune response. Int J Hyperthermia 21(8):723-729
46. Atanackovic D, Nierhaus A, Neumeier M, Hossfeld DK, Hegewisch-Becker S (2002) 41.8 degrees C whole body hyperthermia as an adjunct to chemotherapy induces prolonged T cell activation in patients with various malignant diseases. Cancer Immunol Immunother 51(11-12):603-613
47. Katschinski D, Wiedemann GJ, Longo W, d'Oleire FR, Spriggs D, Robins HI (1999) Whole body hyperthermia cytokine induction: a review, and unifying hypothesis for myeloprotection in the setting of cytotoxic therapy. Cytokine Growth Factor Rev 10(2):93-97
48. Robins HI, Kutz M, Wiedemann GJ, Katschinski DM, Paul D, Grosen E, et al (1995) Cytokine induction by 41.8 degrees C whole body hyperthermia. Cancer Lett 97:195-201
49. Bouchama A, Knochel JP (2002) Heat stroke. N Engl J Med 20;346(25):1978-1988

50. Zellner M, Hergovics N, Roth E, Jilma B, Spittler A, Oehler R (2002) Human monocyte stimulation by experimental whole body hyperthermia. Wien Klin Wochenschr 15;114(3):102-107
51. Ahlers O, Hildebrandt B, Dieing A, Deja M, Bohnke T, Wust P, et al (2005) Stress induced changes in lymphocyte subpopulations and associated cytokines during whole body hyperthermia of 41.8-42.2 degrees C. Eur J Appl Physiol 95(4):298-306
52. Dieing A, Ahlers O, Kerner T, Wust P, Felix R, Loffel J, et al (2003) Whole body hyperthermia induces apoptosis in subpopulations of blood lymphocytes. Immunobiology 207(4):265-273
53. Kregel KC (2002) Heat Shock proteins: modifying factors in physiological stress response and aquired thermotolerance. J Appl Physiol 92:2177-2186
54. Jolly C, Morimoto RI (2000) Role of the heat shock response and molecular chaperones in oncogenesis and cell death. J Natl Cancer Inst 92(19):1564-1572
55. Jaattelä M (1999) Heat shock proteins as cellular lifeguards. Ann Med 31:261-271
56. Srivastava P (2002) Roles of heat-shock proteins in innate and adaptive immunity. Nat Rev Immunol 2(3):185-194
57. Multhoff G (2002) Activation of natural killer cells by heat shock protein 70. Int J Hyperthermia 18(6):576-585
58. Gehrmann M, Marienhagen J, Eichholtz-Wirth H, Fritz E, Ellwart J, Jaattela M, et al (2005) Dual function of membrane-bound heat shock protein 70 (Hsp70), Bag-4, and Hsp40: protection against radiation-induced effects and target structure for natural killer cells. Cell Death Differ 12(1):38-51
59. Krause SW, Gastpar R, Andreesen R, Gross C, Ullrich H, Thonigs G, et al (2004) Treatment of colon and lung cancer patients with ex vivo heat shock protein 70-peptide-activated, autologous natural killer cells: a clinical phase i trial. Clin Cancer Res 10(11):3699-3707
60. Li CY, Dewhirst MW (2002) Hyperthermia-regulated immunogene therapy. Int J Hyperthermia 18(6):586-596
61. Streffer C (1990) Biological basis of thermotherapy (with special reference to oncology). In: Gautherie M, editor. Biological basis of oncologic thermotherapy. Berlin, Heidelberg, New York: Springer; p1-72
62. Raaphorst GP (1989) Thermal radiosenzitation in vitro. In: Urano M, Douple E, editors. Biology of thermal potentiation of radiotherapy. Utrecht, Tokyo: VSP; p17-52

Interactions between Hyperthermia and Cytotoxic Drugs

B Hildebrandt, P Wust

Abstract

Pioneering studies of the 1970s and 1980s demonstrated that heat application induces cell death at temperatures of more than 43° C in cell cultures and animal experiments, and sensitises tumour cells to radiation and certain cytotoxic drugs in the temperature range of 39° C - 43° C. Further research revealed further details of the complex interactions of heat and drugs in the living organism. In this chapter, we discuss the fundamentals of heat-drug interactions, their variations between certain classes of cytotoxic agents, induction and reversal of drug resistance under hyperthermic conditions, and pharmacological aspects of drug administration during hyperthermia.

Introduction

The terms “thermal radiosensitization” and “thermal chemosensitization” describe the capacity of hyperthermia to enhance the cytotoxicity of radiation and antineoplastic drugs. In experimental models, the extent of an agents’ thermal sensitization can be calculated as “thermal enhancement ratio” (TER; see chapter 3.3.), e.g. the quotient of the survival fractions of cells treated with radiation or chemotherapy at basal temperatures, and at the same dose plus heat. The TER mainly depends on the rate of cell survival at normal temperature, drug concentration, and duration of heat exposure. In the living organism, the susceptibility of a malignant tissue to hyperthermic radio- or chemotherapy additionally depends on environmental factors such as tumour blood flow, tumour oxygenation, and pH [1-5].

Today, it is widely accepted that hyperthermia in the temperature range < 43° C can increase tumour perfusion, vascular permeability, and thereby oxygenation, whereas higher temperature typically induces vascular damage and hypoxia. In hyperthermic radiotherapy, improvement of tumour oxygenation may co-determine the favourable effect of adjunctive heat application, in particular as the

energy absorption (radiation dose) is not relevantly influenced by elevated temperatures. In hyperthermic chemotherapy, the situation is much more complex, as the extent of thermal enhancement varies among different drugs and temperatures. It is determined by a variety of factors including pharmacodynamic interactions (such as acceleration of primary mode of action and cellular drug uptake), modulation of side effects, as well as pharmacokinetic alterations.

Pharmacodynamic Interactions

- Primary mode of action
  - alkylating reaction, DNA-strand breaks
  - enzyme induction
  - protein damage
  - receptor density
- Intracellular drug concentration
  - drug uptake
  - membrane alterations
  - pH changes
  - drug resistance (MDR)
- Modulation of side effects

Pharmacokinetic Interactions

- Drug uptake
  - decreased gastrointestinal absorption
  - altered transdermal absorption
- Drug distribution
  - perfusional changes
  - fluid sequestration
  - pH changes
- Metabolism and excretion
  - changes in hepatic/renal blood flow
  - enzyme induction

Other interactions to be considered are chemical instability at higher temperatures, impairment of drug action by contact with heated glass or plastic, or independent heat interactions of solvents or additives [1-6].

In this chapter, we summarise the recent knowledge on the different modes of heat-drug interaction, the interactions of heat and drug-resistance, and drug pharmacology under hyperthermic conditions.

Principles of heat-drug interaction

The cell-killing potential of various cytotoxic agents is increased at elevated temperatures. A number of studies analysed heat-drug interactions *in vitro* and in animal models, describing different modes of heat-drug interactions (Table 1).

Table 1. Thermal enhancement ratios (TER) of selected drugs. Derived from animal models only; as calculated by Urano et al. 1999 [9]

Compound	Range of TER at 40° C-42° C	Range of TER at 42.5° C-45° C
BCNU	1.5–2.96	2.71
Bleomycin	1.24	1.65–2.90
Cisplatin	1.48–3.9	1.39–4.96
Cyclophosphamide	1.52–2.28	1.27–2.74
Doxorucibin	1.0	1.0
5-Flourouracil	1.0	1.0
Melphalan	1.5–3.9	n.a.
Mitomycin C	1.0	2.8

In this context, the terms “additive” or “supraadditive” describe an increase in TER with increasing temperature. In particular platinum compounds show a linear enhancement of efficacy in the temperature range between 37-41° C, as well as most alkylating agents. Other cytostatics, such as doxorubicin and mitomycin, rather exhibit a “threshold behaviour”, e.g. there is little evidence of an increased cytotoxicity at lower temperatures, but a marked synergism above a distinct threshold temperature. For a third group of agents (antimetabolites, pyrimidine analogues, vinca alkaloids) no relevant thermal chemosensitization was found in most studies, and their mode of heat interaction was classified as “independent”. Another class of drugs, the so-called “thermosensitisers”, only act in a cytotoxic way under hyperthermic conditions but do not have antineoplastic properties at normal temperatures, such as the local anesthetic lidocaine and the antimycotic amphotericin B [2,5,7-9].

In the opinion of the authors, the classification of a drug-heat interaction as “additive”, “threshold” or “independent” on the basis of experimental research was a very useful tool to identify the most suitable drugs for hyperthermic chemotherapy in the 1970s and 1980s. However, application of a wide range of temperatures (including those > 43° C), the restriction of analyses to synchronous application of hyperthermia and drugs, as well as, the lack of combination schedules and detailed pharmacological analyses largely curtail the transferability of these findings into clinical practice. In addition, different heating schedules - some of them may have promoted thermoresistance - have been applied to xenotransplanted tumours which generally show a great variability with regard to their susceptibility to a particular cytostatic drug at room temperature.

Results contradicting synergisms with heat have especially been reported for non-alkylating cytotoxic drugs (antimetabolites, purine- and pyrimidine analogues, vinca alkaloids, etoposide, and taxanes). In contrast to alkylating agents

and platinum compounds, those drugs do not immediately interact with the cellular DNA. Therefore, it may be reasonable to administer them to hyperthermia in a sequential (e.g. 24 or 48 hours before heat treatment) rather than in a synchronous way [10,11]. As another point to consider, some drugs like 5-flourouracil or taxanes are potent radiosensitisers so that their thermal enhancement properties may vary between different application schedules (hyperthermic chemotherapy vs. hyperthermic radiotherapy/radiochemotherapy) [12]. On the contrary, alkylating drugs like cyclophosphamide have been demonstrated to exhibit favourable TERs in animal models, but undergo an extensive hepatic metabolism in man which cannot be simulated in animal experiments.

As a conclusion, preclinical data on thermal chemosensitization have identified alkylating agents and platinum compounds as suitable drugs for hyperthermic chemotherapy. However, negative data on a drug's chemosensitizing potential should be interpreted with caution, because preclinical experiments may not cover all aspects of conceivable heat-drug interactions.

Hyperthermia and drug resistance

Drug resistance is the major cause of failure of cytostatic treatment of human malignancies. It can be induced by different mechanisms, including the pleiotropic "multidrug resistance (MDR)" which is mainly mediated by the transmembranal glycoprotein p170 and the multidrug resistance protein 1 efflux pumps. Experimental data suggest that hyperthermia is a good candidate to overcome various modes of drug resistance, whereby the most persuading data are available for the reversal of drug resistance to cisplatin (DDP). Mechanisms of DDP-resistance include changes in transmembrane conductivity, activity of sodium/potassium-ATPase, glutathion metabolism, and DNA repair [13,14]. Other experimental studies suggest that hyperthermia is also suitable to reverse multidrug resistance under certain conditions, although this phenomenon was not reproduced in studies performed concomitant to clinical trials so far [15-17]. On the contrary, moderate heat exposure has been described to induce thermotolerance (see chapter 10) by induction of heat shock protein-synthesis, a condition that promotes certain forms of drug resistance [3,18,19].

As a summary, data on the modulation of the different forms of drug resistance by heat application are still too incomplete to draw final conclusions. Clinical experience mostly suggests a favourable effect of various hyperthermia approaches on drug sensitivity. Indeed, a number of phase I- and II-trials reported successful treatments of patients with chemorefractory tumours by adding hyperthermia to antineoplastic chemotherapy [20,21]. Regarding the results in peritoneal chemoperfusion (PC), at least one randomised study suggested that the efficacy of adjuvant PC in patients with gastric cancer is actually enhanced by the application of perfusates with elevated temperatures [22]. However, one should consider the possibility to induce thermotolerance and drug resistance by heat treatment, especially if the heat is applied in the run-up to chemotherapy in the sense of "pre-heating".

Pharmacological studies

Only few studies have subjected pharmacological alterations of drugs applied synchronously to hyperthermia so far. Mechanisms of external heating to affect the pharmacology of a drug include changes of the primary mode of drug action and side-effects, alterations of drug uptake and distribution (via gastral hyperchlorhydria, gastrointestinal fluid sequestration, changes of tumour blood supply, tumour-pH, fluid and electrolyte balance etc.), as well as drug metabolism and excretion [2,6]. In a clinical phase I study on whole-body hyperthermia (WBH) and chemotherapy, the authors detected a slight decrease in the renal elimination of carboplatin (CBCDA) which was probably the reason for the increased rates of nephrotoxicity observed [23]. In another trial, occurrence of excess nephrotoxicity with CBCDA was mainly due to the use of a haemodialysis-system to induce WBH, a method which is thought to produce a relevant rate of nephrotoxicity by itself and which is regarded as obsolete today [24]. In regional hyperthermia of the pelvis, a trend towards a higher peritoneal clearance after intraperitoneal carboplatin application was detected in patients with ovarian cancer, whereas another trial did not report on the correlation between the pharmacokinetics of liposomal doxorubicin and thermal parameters [25,26].

As a conclusion, data suggest that longer lasting systemic heat exposure or regional hyperthermia may influence the pharmacokinetics of cytotoxic drugs, mainly due to changes in the organ circulation, to temperature-dependent metabolism rates, or to fluid shifts. However, further research concomitant to clinical trials is required to better understand drug pharmacology under hyperthermic conditions.

Since the beginning of the new millenium, most pharmacological studies performed in the context of hyperthermia refer to the specific situation in hyperthermic chemoperfusion, an issue that is discussed in separate chapters of this book.

Heat interactions of novel compounds

Therapeutic monoclonal antibodies (tMAbs) represent a class of drugs which has revolutionised the pharmacotherapy of malignant lymphoma and distinct solid malignancies, as well as, of certain chronic inflammatory diseases. Data available so far on the application of tMAbs in parallel to hyperthermia suggest a synergism that goes far beyond sole thermal radio- and chemosensitization. Indeed, interactions between heat and native as well as radiolabelled tMAbs include a disproportionally high increase in drug-target-interactions, interferences with the immunological target (e.g. receptor density), and a more pronounced and uniform intratumoural drug distribution [27-30]. Therefore, the combination of hyperthermia with tMAbs appears to be a promising principle, although clinical experience is still very limited.

Some other, exciting innovations in hyperthermia research do not represent drugs in the narrower sense, but are to be mentioned here for the sake of completeness:

- *Hyperthermia-induced gene therapy* is based on the principle that heat application is suitable to induce the expression of some highly-conserved, ubiquitary genes which may be linked to a heat-inducible promotor with a corresponding effector gene such as TNF-α or IL-12. The first clinical studies with adenoviral vectors under control of the promotor of the heat-shock protein 70b (hsp-70b) are immanent [31-33].
- A new generation of *thermosensitive liposomes* has been developed which reliably enables the liberation of drugs into a heated tissue at a predefined temperature. Recent research suggests that those liposomes may largely improve the thermal control of hyperthermia-guided drug-targeting [34,35].
- In *magnetic fluid hyperthermia* (MFH), magnetic nanoparticles are directed into the tumour and heated within an alternating magnetic field (e.g. 100 kHz). The probably most exciting aspect of MFH is that it represents the only technology by which heat can be either applied at a "hyperthermic" or "ablational" target temperature [36,37].

Summary

Hyperthermia enhances the cytotoxicity of various antineoplastic agents and radiation effects. Based on calculations of the "thermal enhancement ratio" (TER) in experimental systems, different modes of heat-drug interactions have been described. However, the calculation of a drug's TER mainly describes pharmacodynamic features, and largely neglects pharmacokinetic aspects. In addition, the TER is usually estimated during synchronous applications of heat and drug, and thus favours compounds with intermediate onset of action, such as platinum derivatives and alkylating agents. Therefore, some drugs with complex or delayed mechanims of thermal enhancement may have been missed by using this concept.

Hyperthermia has been demonstrated to both induce and reverse certain forms of drug resistance, although the clinical relevance of these interactions is still poorly understood. From a clinical point of view, the chance to reverse drug resistance by hyperthermia application clearly outweighs the hazard to induce thermotolerance, a circumstance that might be accompanied with drug resistance.

Only few studies on clinical pharmacology during hyperthermia have been performed so far. Results available suggest that heat exposure can relevantly affect the pharmacokinetics of synchronously administered cytotoxic drugs. Further studies are required to more clearly define the consequences of the different hyperthermia approaches on drug uptake, distribition, and excretion, as well as the modulation of side effects by heat application.

The combination of heat treatment in conjunction with the administration of monoclonal antibody administration appears to be one of the most promising future applications of hyperthermia. In addition, the clinical introduction of heat-induced gene-therapy and novel interstitial techniques such as thermolabile liposomes and magnetic fluid hyperthermia are immanent.

References

1. Dewhirst MW, Vujaskovic Z, Jones E, Thrall D (2005) Re-setting the biologic rationale for thermal therapy. Int J Hyperthermia 21(8):779-790
2. Hildebrandt B, Wust P, Ahlers O, Dieing A, Sreenivasa G, Kerner T, et al (2002) The cellular and molecular basis of hyperthermia. Crit Rev Oncol Hematol 43(1):33-56
3. Lepock JR (2005) How do cells respond to their thermal environment? Int J Hyperthermia 21(8):681-687
4. Song CW, Park HJ, Lee CK, Griffin R (2005) Implications of increased tumor blood flow and oxygenation caused by mild temperature hyperthermia in tumor treatment. Int J Hyperthermia 21(8):761-767
5. Streffer C (1990) Biological basis of thermotherapy (with special reference to oncology). In: Gautherie M, editor. Biological basis of oncologic thermotherapy. Berlin, Heidelberg, New York: Springer; p1-72
6. Vanakoski J, Seppälä T (1998) Heat exposure and drugs. Clin Pharmacokinet 34(4):311-322
7. Kampinga HH (2006) Cell biological effects of hyperthermia alone or combined with radiation or drugs: a short introduction to newcomers in the field. Int J Hyperthermia 22(3):191-196
8. Takemoto M, Kuroda M, Urano M, Nishimura Y, Kawasaki S, Kato H, et al (2003) The effect of various chemotherapeutic agents given with mild hyperthermia on different types of tumours. Int J Hyperthermia 19(2):193-203
9. Urano M, Kuroda M, Nishimura Y (1999) For the clinical application of thermochemotherapy given at mild temperatures. Int J Hyperthermia. 15(2):79-107
10. Katschinski DM, Jacobson EL, Wiedemann GJ, Robins HI (2001) Modulation of VP-16 cytotoxicity by carboplatin and 41.8 degrees C hyperthermia. J Cancer Res Clin Oncol 127(7):425-432
11. van Bree C, Beumer C, Rodermond HM, Haveman J, Bakker PJ (1999) Effectiveness of 2',2'difluorodeoxycytidine (Gemcitabine) combined with hyperthermia in rat R-1 rhabdomyosarcoma in vitro and in vivo. Int J Hyperthermia 15(6):549-556
12. Cividalli A, Cruciani G, Livdi E, Pasqualetti P, Tirindelli Danesi D (1999) Hyperthermia enhances the response of paclitaxel and radiation in a mouse adenocarcinoma. Int J Radiat Oncol Biol Phys 1;44(2):407-412

13. Beck WT, Dalton WS (1997) Mechanisms of drug resistance. In: DeVita VTj, Hellmann S, Rosenberg SA, editors. Cancer-Principles and practice of Oncology. Philadelphia, New York: Lippincott-Raven publishers, p498-512
14. Hettinga JV, Konings AW, Kampinga HH (1997) Reduction of cellular cisplatin resistance by hyperthermia--a review. Int J Hyperthermia 13(5):439-457
15. Souslova T, Averill-Bates DA (2004) Multidrug-resistant hela cells overexpressing MRP1 exhibit sensitivity to cell killing by hyperthermia: interactions with etoposide. Int J Radiat Oncol Biol Phys 1;60(5):1538-1551
16. Stein U, Rau B, Wust P, Walther W, Schlag PM (1999) Hyperthermia for treatment of rectal cancer: evaluation for induction of multidrug resistance gene (mdr1) expression. Int J Cancer 80(1):5-12
17. Stein U, Jurchott K, Schlafke M, Hohenberger P (2002) Expression of multidrug resistance genes MVP, MDR1, and MRP1 determined sequentially before, during, and after hyperthermic isolated limb perfusion of soft tissue sarcoma and melanoma patients. J Clin Oncol 1;20(15):3282-3292
18. Kregel KC (2002) Heat Shock proteins: modifying factors in physiological stress response and aquired thermotolerance. J Appl Physiol 92:2177-2186
19. Li GC, Mivechi NF, Weitzel G (1995) Heat shock proteins, thermotolerance, and their relevance for clinical hyperthermia. Int J Hyperthermia 11(4):459-488
20. Hildebrandt B, Hegewisch-Becker S, Kerner T, Nierhaus A, Bakhshandeh-Bath A, Janni W, et al (2005) Current status of radiant whole-body hyperthermia at temperatures > 41.5°C and practical guidelines for the treatment of adults. The German "Interdisciplinary Working Group on Hyperthermia". Int J Hyperthermia 21(2):169-183
21. Wust P, Hildebrandt B, Sreenivasa G, Rau B, Gellermann J, Riess H, et al (2002) Hyperthermia in combined treatment of cancer. Lancet Oncol 3(8):487-497
22. Yonemura Y, de Aretxabala X, Fujimura T, Fushida S, Katayama K, Bandou E, et al (2001) Intraoperative chemohyperthermic peritoneal perfusion as an adjuvant to gastric cancer: final results of a randomised controlled study. Hepatogastroenterology 48(42):1776-1782
23. Robins Hl, Cohen JD, Schmitt CL, Tutsch KD, Feierabend C, Arzoomanian RZ, et al (1993) Phase I Clinical Trial of Carboplatin and 41.8°C Whole-Body Hyperthermia in Cancer Patients. J Clin Oncol 9:1787-1794
24. Wiedemann G, d'Oleire F, Knop E, Eleftheriadis S, Bucsky P, Feddersen S, et al (1994) Ifosfamide and Carboplatin combined with 41.8°C whole-body hyperthermia in patients with refractory sarcoma and malignant teratoma. Cancer Res 54:5346-5350
25. Formenti SC, Shrivastava PN, Sazozink M, et al (1996) Abdominopelvic hyperthermia and intraperitoneal carboplatin in epithelial ovarian cancer: feasibility, tolerance, and pharmacology. Int J Radiat Oncol Biol Phys 35(5):993-1001

26. Jones E, Alvarez Secord A, Prosnitz LR, Samulski TV, Oleson JR, Berchuck A, et al (2006) Intra-peritoneal cisplatin and whole abdomen hyperthermia for relapsed ovarian carcinoma. Int J Hyperthermia 22(2):161-172
27. Hauck ML, Zalutsky MR (2005) Enhanced tumour uptake of radiolabelled antibodies by hyperthermia: Part I: Timing of injection relative to hyperthermia. Int J Hyperthermia 21(1):1-11
28. Hauck ML, Zalutsky MR (2005) Enhanced tumour uptake of radiolabelled antibodies by hyperthermia. Part II: Application of the thermal equivalency equation. Int J Hyperthermia 21(1):13-27
29. Kinuya S, Yokoyama K, Hiramatsu T, Konishi S, Watanabe N, Shukei N, et al (2000) Optimal timing of hyperthermia in combined radioimmunotherapy. Cancer Biother Radiopharm 15(4):373-379
30. Kinuya S, Yokoyama K, Michigishi T, Tonami N. Optimization of radioimmunotherapy interactions with hyperthermia. Int J Hyperthermia. 2004 Mar;20(2):190-200
31. Brade AM, Ngo D, Szmitko P, Li PX, Liu FF, Klamut HJ (2000) Heat-directed gene targeting of adenoviral vectors to tumor cells. Cancer Gene Ther 7(12):1566-1574
32. Huang Q, Hu JK, Lohr F, Zhang L, Braun R, Lanzen J, et al (2000) Heat-induced gene expression as a novel targeted cancer gene therapy strategy. Cancer Res 1;60(13):3435-3439
33. Lee YJ, Lee H, Borrelli MJ (2002) Gene transfer into human prostate adenocarcinoma cells with an adenoviral vector: Hyperthermia enhances a double suicide gene expression, cytotoxicity and radiotoxicity. Cancer Gene Ther 9(3):267-274
34. Kong G, Dewhirst MW (1999) Hyperthermia and liposomes. Int J Hyperthermia 15(5):345-370
35. Lindner LH, Eichhorn ME, Eibl H, Teichert N, Schmitt-Sody M, Issels RD, et al (2004) Novel temperature-sensitive liposomes with prolonged circulation time. Clin Cancer Res 15;10(6):2168-2178
36. Johannsen M, Thiesen B, Gneveckow U, Taymoorian K, Waldofner N, Scholz R, et al (2006) Thermotherapy using magnetic nanoparticles combined with external radiation in an orthotopic rat model of prostate cancer. Prostate 1;66(1):97-104
37. Moroz P, Jones SK, Gray BN (2002) Magnetically mediated hyperthermia: current status and future directions. Int J Hyperthermia. 18(4):267-284

Pharmacodynamic Aspects of Intraperitoneal Cytotoxic Therapy

WP Ceelen, L Påhlman, H Mahteme

Introduction

The pharmacokinetic (PK) properties of cytotoxic drugs, described by parameters such as plasma half live and distribution volume, are generally well studied and have implications for toxicity and development of dosage regimens. The PK rationale for intraperitoneal (ip) cytotoxic drug therapy is discussed in chapter 8.

In order to exert their anticancer effects, drugs have to gain access to tumour cells by penetrating into tissue. The available data on tumour tissue distribution of cytotoxic drugs and their relation with antitumour efficacy are limited, and mainly stem from in vitro multicellular models such as tumour spheroids (spherical tumour aggregates; diameter approximately 1 mm) and multilayered cell cultures [1]. Tissue penetration in these models is studied following incubation in a medium containing anticancer drugs, and generally the results show a very limited cytotoxic drug penetration. Since abstraction is made of vascular drug supply and the geometry of drug penetration is from the periphery towards the centre, the results of these models apply even more to ip chemotherapy than to intravenous administration. On the other hand, the renewed interested in ip chemotherapy in the management of peritoneal surface malignancy generated data relating specifically to tissue penetration of ip administered cytotoxic drugs, combined or not with locoregional hyperthermia.

This chapter provides a summary of the available data concerning the pharmacodynamics of cytotoxic drug administration with an emphasis on drugs used clinically during hyperthermic intraperitoneal chemoperfusion (HIPEC).

General Pharmacodynamic Aspects of Intraoperative Intraperitoneal Chemotherapy

Results from experiments with multicellular models have shown that direct tissue penetration of most cytotoxic agents is very limited in space (usually less than 1

mm) [2]. Intraperitoneal chemotherapy effectiveness will therefore be limited to tumour nodules of a very small dimension or to loose cancer cells. The presence of small tumour nodules will result in an additional advantage related to the population kinetics of tumour growth. Indeed, human cancers are known to obey Gompertzian growth kinetics, implying that instead of a continuous exponential growth, a plateau is reached when nutrient and oxygen supply no longer meet demands resulting in a decline in growth when the tumour size increases. Small residual tumour will have the largest growth fraction and therefore the fractional kill by chemotherapy will be much higher than later in the course of the disease.

The penetration of cytotoxic drugs into peritoneal tumour nodules is a complex, multi-step process summarized in Fig. 1.

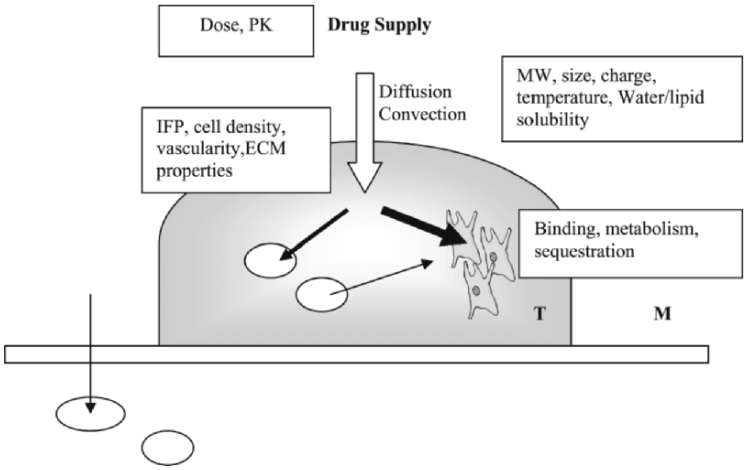


Figure 1. Schematic representation of drug penetration into peritoneal metastatic tumours. Drug supply is a function of pharmacokinetic parameters such as dose, concentration, and exposure time. The periphery of the tumour is entered by diffusion and convection. The extent of penetration will depend on drug properties and properties related to the tumour. Once intracellular, drug will accumulate into tumour cells by binding to target structures, non-specific binding, and sequestration in cellular organelles. A fraction of the drug will be altered by metabolic pathways. Tumour in the immediate vicinity of blood vessels (ovals) will also be reached by absorbed drug present in the microcirculation. Systemic drug absorption occurs both in submesothelial tissue and in tumour tissue. T, tumour nodule; M, mesothelium; PK, pharmacokinetics; IFP, interstitial tissue pressure; ECM, extracellular matrix; MW, molecular weight

Pharmacodynamics of Cytotoxic Drugs used with HIPEC

A rational choice of a cytotoxic agent for HIPEC therapy should consider the following elements:

- Activity against the disease process
- Cell cycle specificity
- Relation between dose/exposure time and response
- Thermal enhancement ratio (when used in combination with hyperthermia)
- Pharmacokinetic advantage (peritoneal/plasma AUC ratio)
- Pharmacodynamic properties
- Local and systemic toxicity

Table 1. Pharmacokinetic and pharmacodynamic properties of cytotoxic agents used during intraoperative or early postoperative intraperitoneal chemotherapy. Data taken in part from [3]

Drug	MW (Da)	Ip dose (mg/m^2)	AUC ratio*	Drug penetration distance	TE
Alkylating agents					
Mitomycin C	334.3	35	10-23.5	2 mm	+
Platinum compounds					
Cisplatin	300.1	90-120	13-21	1-3 mm	+
Carboplatin	371.3	350-800	1.9-5.3	0.5 mm	+
Oxaliplatin	397.3	460	3.5	1-2 mm	+
Antimicrotubule agents					
Paclitaxel	853.9	20-175	NA	> 80 cell layers	?
Docetaxel	861.9	40-156	207	NA	+
Topoisomerase Interactive Agents					
Topotecan	457.9		NA	NA	?
Irinotecan	677.2		NA	NA	±
Mitoxantrone	517.4	28	15.2	5-6 cell layers	±
Doxorubicin	543.5	60-75	162	4-6 cell layers	+
Antimetabolites					
5- Fluorouracil	130.1	650	NA	0.2 mm	-

MW, molecular weight; ip, intraperitoneal; TE, thermal enhancement; NA, not available; AUC, area under the concentration-time curve; *only data referring to clinical studies with hyperthermic chemoperfusion

Alkylating Drugs

Mitomycin C

Mitomycin C (MMC) is an antibiotic extracted from a Streptomyces species and exhibits activity in breast and gastrointestinal cancer.

In vitro data suggest thermal enhancement of MMC cytotoxicity [4]. Little clinical data are available concerning the pharmacodynamic properties of ip mitomycin with or without heat. Fujimoto et al. studied tissue penetration and histological effects of HIPEC with MMC in a group of gastric cancer patients [5]. They found that one in three patients showed signs of apoptosis in all cancer cells, but submesothelial penetration of heat and drug was limited. Others confirmed the limited penetration of hyperthermia during clinical HIPEC procedures [6].

The clinical pharmacokinetics of ip MMC (10 mg/l of perfusate) with HIPEC were reported by Jacquet et al., who found a mean peritoneal/plasma AUC ratio of 23.5 [7]. Following extensive peritonectomy (as compared to limited peritonectomy), the AUC ratio was significantly lower due to increased plasma concentrations.

In the Netherlands Cancer Institute, a dose finding study was performed suggesting that a dose of 35 mg/m^2 resulted in the highest peritoneal/plasma AUC ratio (mean value of 13) with acceptable toxicity [8]. In order to maintain perfusate MMC concentration, the dose was divided in three fractions: 50% at the start, 25% after 30 min., and 25% after 60 min. of perfusion (total perfusion time 90 min.). The same group successfully fitted pharmacokinetic and pharmacodynamic data to a population model consisting of a single peritoneal compartment with first order elimination and a two-compartment plasma model with first order absorption and elimination (Fig. 2) [9]. Important observations included: 1. the central distribution volume increases with duration of surgery (probably related to dilution by infused blood and fluids); 2. the perfusate/plasma AUC ratio was 10.1 ± 4.6; 3. a sigmoidal 'maximum effect' model best explained the pharmacodynamic relation between plasma AUC and degree in leucopenia; the estimated ip dose to result in a grade 3/4 percentage of 10% was 25 mg/m^2.

Since the ip MMC concentration determines both antitumour efficacy and systemic toxicity, the volume of perfusate is an important therapy variable. Sugarbaker et al. studied the PK effects of HIPEC (10 mg/m^2 or 15 mg/m^2) administered in various volumes of perfusate and found, as expected, significantly lower ip and iv concentrations with increasing perfusate volume [10]. They suggested therefore to base calculations of perfusate volume on the body surface area.

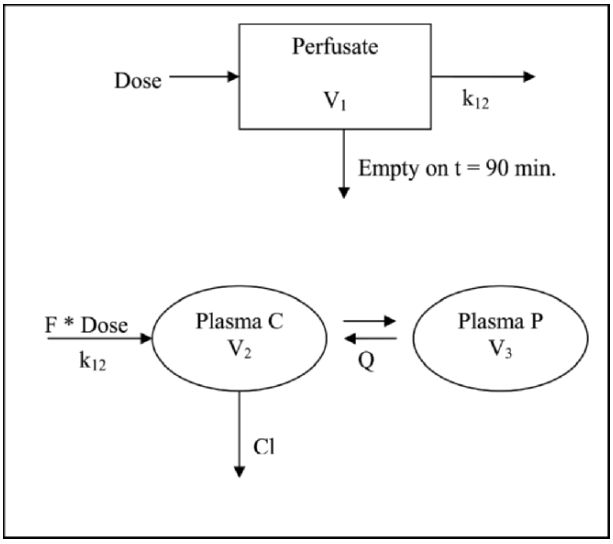


Figure 2. Pharmacokinetic model of hyperthermic chemoperfusion with mitomycin C (35 mg/m^2 in three doses) consisting of three compartments: perfusate (distribution volume V_1), central plasma compartment (distribution volume V_2), and peripheral plasma compartment (distribution volume V_3). k_{12}, rate constant from perfusate to plasma; F, bioavailability; Q, intercompartmental clearance; Cl, clearance. Redrawn from [9]

Platinum Compounds

The platinum compounds represent a unique class of anticancer agents. Cisplatin, carboplatin and oxaliplatin are among the best studies agents for ip therapy and cause apoptotic cell death by the formation of DNA adducts.

Cisplatin

Cisplatin ([cis-diamminedichloroplatinum II], CDDP) has been well studied in the setting of adjuvant ip therapy in small volume residual disease ovarian cancer, where three randomized trials have shown a significant survival advantage associated with regimens containing ip CDDP 100 mg/m^2 alone or in combination with ip paclitaxel [11]. Combined with hyperthermia, CDDP has been used for intracavitary therapy of ovarian cancer, gastric cancer, and peritoneal mesothelioma.

Rossi et al. studied the pharmacokinetics of HIPEC with doxorubicin and cisplatin in a phase I study; the peritoneal/plasma AUC ratio of cisplatin was 20,6 ± 6.0 [12]. Similarly, Cho et al. found an AUC ratio of 13 in a clinical study involving 56 patients treated with HIPEC (90 min. at 42.5° C) using cisplatin in a dose ranging from 100 mg/m^2 to 400 mg/m^2 [13]. These authors fitted several PK models

to the obtained data and found the best fit with a two compartment model supplemented with two extra compartments representing the peritoneal cavity and a drug reservoir (Fig. 3).

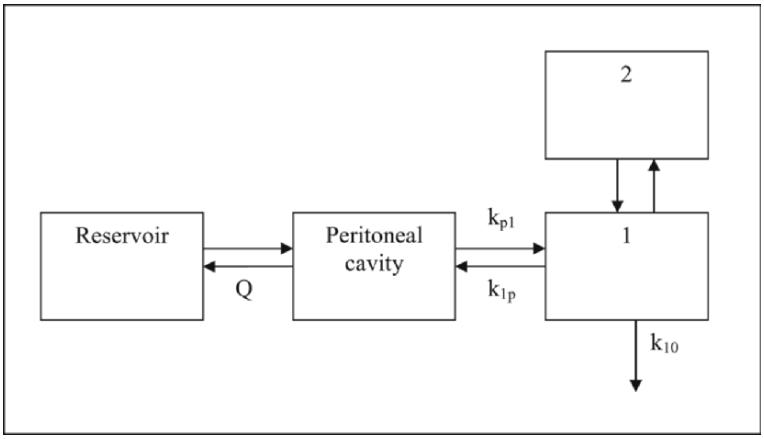


Figure 3. Pharmacokinetic model of HIPEC with cisplatin (100-400 mg/m^2) consisting of a standard two compartment model with an additional peritoneal compartment and reservoir. Q, flow rate; k; rate constants. Redrawn from [13]

Leopold et al. combined ip CDDP (80-120 mg/m^2) with regional hyperthermia in a phase I ovarian cancer study and found the AUC ratio to be between 30 and 35 [14]. In patients with mesothelioma, Ma et al. found an AUC ratio of 21 using a similar HIPEC protocol; CDDP was, however, dosed per ml perfusate (120 mg/l) [15].

Zeamari et al. compared normothermic (37° C) with mild hyperthermic (40° C) ip CDDP chemoperfusion in a rat model and did not observe any difference in tumour drug uptake [16]. Los et al., however, found that the addition of hyperthermia to ip CDDP significantly increased intracellular platinum uptake in vitro and tumour platinum concentration in vivo (four-fold increase) [17]. The same group later reported that compared with ip chemotherapy alone, combined hyperthermia (60 min. at 41.5° C) with CDDP or carboplatin significantly enhanced DNA adduct formation and antitumour efficacy in a rat colorectal cancer model [18]. In an important clinical study, van de Vaart and coworkers found a significantly higher DNA-adduct formation in exposed tumour nodules excised after the perfusion compared to buccal cells from ovarian cancer patients treated with HIPEC (90 min. at 42° C) using cisplatin (50-70 mg/m^2) [19]. They also demonstrated that the penetration depth into tumour nodules as judged from a nuclear staining intensity assay is at least 3-5 mm.

Carboplatin

Due to its higher hydrophilicity and higher molecular weight, carboplatin [diammine (1,1-cyclobutanedicarboxylato) platinum(II)] is cleared more slowly from the peritoneal cavity than cisplatin (6 vs. 15 ml/min).

In clinical studies without hyperthermia, the peritoneal/plasma AUC ratio was found to be around 6-17 [20,21]. Despite the pharmacokinetic advantage of carboplatin (slower clearance from the peritoneal cavity), Los et al. found that, in a rat peritoneal tumour model, tissue penetration of carboplatin was far less than that of CDDP [22].

In a similar model, Los et al. found that ip carboplatin resulted in significantly enhanced tumour platinum concentrations when combined with mild hyperthermia [23]. Interestingly, however, they found that the effect of hyperthermia resulted from an increased *systemic* platinum exposure due to a slower elimination from the blood. Similarly, a three fold increase in therapeutic ratio was noted when carboplatin was combined with whole body hyperthermia in a rat tumour model [24].

Steller et al. reported a phase I trial of HIPEC in 6 advanced ovarian cancer patients using ip carboplatin. The dose administered was 800-1200 mg/m^2 with a regional advantage of 1.9-5.3 [25]. Formenti et al. combined ip carboplatin (200-400 mg/m^2 every 4 weeks) with regional RF hyperthermia in a feasibility study and found the mean peritoneal/plasma AUC ratio to be lower in patients who received hyperthermia (5.2 versus 6.1) due to an increased drug clearance by hyperthermia [26].

Oxaliplatin

Oxaliplatin [oxalato-1,2-diaminocyclohexane platinum(II)], is a third generation platinum complex. This agent has become the standard of care in first line iv chemotherapy of metastatic colorectal cancer.

In vitro, hyperthermia enhanced the cytotoxicity and DNA adduct formation of oxaliplatin, although less so than that of cisplatin [27]. Of note, compared to exposure to 41° C, hyperthermia at 43° C did not further increase cytotoxicity of oxaliplatin.

In a rat model, ip administration of oxaliplatin resulted in a peritoneal/plasma AUC ratio of 16-17; hyperthermia increased normal tissue concentrations although not significantly [28]. Interestingly, the highest concentrations were found in colonic tissue.

Elias et al. studied kinetics and tissue drug concentrations (both in normal and in tumour tissue) in peritoneal carcinomatosis patients treated with HIPEC (30 min. at 43° C) using oxaliplatin in increasing doses [29]. Half of the dose was absorbed after the perfusion, and at the highest dose level (460 mg/m^2) the maximal drug concentration was 25 times higher in the perfusate than in plasma. Interestingly, oxaliplatin concentrations were significantly higher in tumour tissue and peritoneum (339 ng/mg and 392 ng/mg respectively) compared to unbathed normal muscle (19 ng/mg) although plasma AUC values were higher than those commonly obtained during iv administration of oxaliplatin. The peritoneal/plasma

AUC ratio as estimated from the published data is limited (approximately 3.5). Of note, Elias et al. chose to combine oxaliplatin with 5-fluorouracil (400 mg/m^2) and folate which were administered intravenously immediately before HIPEC as both agents cannot be mixed in the perfusate.

Since oxaliplatin can only be administered ip in a dextrose 5% solution, severe hyperglycaemia and hyponatriemia develops during chemoperfusion and continuous high dose insulin infusion has to be provided (WP Ceelen, unpublished observations). In a small retrospective comparison of HIPEC using MMC versus oxaliplatin using open perfusion, morbidity was higher in the oxaliplatin group, although the difference did not reach statistical significance (62% versus 15%, p = 0.2, Fisher exact test) [30].

Topoisomerase Interactive Agents

The camptothecin analogues (topotecan and irinotecan) interact with the topoisomerase I – DNA complex and prevent resealing of single strand DNA breaks. The resulting DNA damage ultimately leads to apoptotic cell death. The anthracyclin analogue doxorubicin (produced by a Streptomyces species) inhibits both topoisomerase I and II enzymes while mitoxantrone inhibits religation of DNA cleaved by topoisomerase II and induces protein-linked breaks in the DNA.

Topotecan

In a phase I study in advanced ovarian cancer patients, the peritoneal/plasma AUC ratio was 46 ± 30 and the maximal tolerated dose (MTD) determined to be 20 mg/m^2 [31]. A similar phase I study found an AUC ratio of 31.2 and recommended 3 mg/m^2 as the dose for phase II studies [32]. In vitro, hyperthermia enhanced topotecan sensitivity of human glioma cells, but not that of murine mammary carcinoma cells [33,34]. There are no in vivo or clinical data on thermal enhancement by this agent.

Irinotecan (CPT-11)

The PK advantage of ip irinotecan administration was demonstrated by Guichard et al. in a mouse colon carcinoma model [35]. The cytotoxicity of irinotecan was noted to be enhanced by whole body hyperthermia in a rat mammary adenocarcinoma model [36]. Mohamed et al. studied thermal enhancement of irinotecan with moderate hyperthermia (41.5° C) in a murine fibrosarcoma model and found a mean tumour growth delay of 4.03 ± 0.58 days with chemotherapy alone and 6.69 ± 1.12 days with thermochemotherapy (p = 0.001) [37].

Elias et al. reported a phase I study of HIPEC with combined ip oxaliplatin and escalating doses of irinotecan [38]. They found that tumour tissue irinotecan concentration was 18 times higher than that measured in unbathed tissue, although hematological toxicity was worrisome (58% grade 3-4).

Mitoxantrone

Intraperitoneal mitoxantrone has been studied clinically as a second line therapy of platinum insensitive ovarian cancer patients [39]. Thermal enhancement of the cytotoxicity of mitoxantrone has been established both in vitro and in vivo [40].

Nicoletto et al. reported a phase I trial investigating HIPEC (42° C - 43° C during 90 minutes) using mitoxantrone in advanced ovarian cancer patients, and found that with a dose of 28 mg/m^2 the peritoneal/plasma AUC ratio was 15.2 [41].

Doxorubicin

The cytotoxicity of doxorubicin is moderately enhanced by hyperthermia [34]. Jacquet et al. studied ip doxorubicin with and without hyperthermic perfusion (60 min. at 43° C) in a rat model [42]. They observed a peritoneal/plasma AUC ratio of 87.9 without and 82.9 with hyperthermia; the addition of hyperthermia significantly increased drug concentrations in small bowel, omentum, and spleen. In an interesting experiment, Pilati et al. performed ex vivo vascular perfusion of resected colon cancer specimens and studied PK and pharmacodynamic aspects of iv doxorubicin [43]. The results suggested that hyperthermia increases drug uptake and sensitizes tumour cells (but not normal mucosal cells) to the effects of doxorubicin.

The above mentioned phase I trial by Rossi et al., who used HIPEC with cisplatin and doxorubicin, found that the peritoneal/plasma AUC ratio of doxorubicin was 162 ± 113 with higher drug levels in the peritoneum than in tumour or normal tissue samples [12]. Several other authors have reported small phase II trials using HIPEC with a combination of cisplatin and doxorubicin with acceptable (although sometimes considerable) local toxicity results [44-46].

Antimetabolites

5-Fluorouracil (5-FU)

This agent is metabolized intracellularly in two steps to its active form, 5-fluoro-2'-deoxyuridine monophosphate (FdUMP), which in the presence of reduced folate inhibits thymidylate synthase (TS) and results in impaired DNA synthesis. The action of 5-FU is therefore cell cycle specific. The thermal enhancement of 5-FU was absent or very limited in preclinical models [47,48]. Given these limitations, clinical ip administration has been largely confined to early postoperative intraperitoneal therapy (EPIC). In the setting of resected high risk stage II and III colorectal cancer this approach was shown to reduce the recurrence rate of resected stage II colon cancer; in a recent large randomized trial, however, no survival benefit was reaped [49,50].

Although a small molecule, 5-FU is characterized by a pronounced ip PK advantage explained by rapid metabolization by dihydropyrimidine dehydrogenase (DPD), an enzyme present not only in the liver but also in other tissues including gastrointestinal mucosae and peripheral lymphocytes. The pharmcokinetics of EPIC with 5-FU were studied clinically by Jacquet et al., who reported a very high peritoneal/plasma AUC ratio (> 400) that was not influenced by a previous HIPEC procedure [7]. Mahteme et al. studied the uptake of radioactively labelled 5-FU following ip or iv administration in a rat tumour model [51]. They found that both cytoreduction and ip administration were associated with an increased drug uptake in tumour tissue.

Antimicrotubule Agents

Among the microtubule targeting agents, the taxanes paclitaxel and docetaxel represent one of the most important new classes of anticancer agents due to their unique chemical structure, mechanism of action, and activity against a broad range of tumour types. The taxanes stabilize the microtubule against depolymerization, thereby disrupting normal microtubule dynamics.

Paclitaxel

Paclitaxel is well studied in the context of ip therapy of advanced ovarian cancer. In this setting, the large MW of the agent resulted in prolonged exposure of the peritoneal cavity and a very high peritoneal/plasma AUC ratio (1350 ± 500 in a study by Hofstra et al. [52] and 996 ± 93 in a study by Markman et al. [53]). A study in 3D histiocultures showed that paclitaxel penetration is limited to the tumour periphery in the first 24 hours; later, however, increasing apoptosis resulted in more extensive penetration of the xenografts (at least 1 mm or 80 cell layers) [54].

Paclitaxel is heat stable, but thermal enhancement of its cytotoxicity is not clearly defined and likely depending on the cell type under scrutiny. Since hyperthermia itself disorganizes microtubules, a synergism with the cytotoxicity of the taxanes theoretically could exist [55]. In cervical cancer cells in vitro, moderate hyperthermia did not enhance paclitaxel cytotoxicity [56]. Similarly, Rietbroek et al. could not demonstrate thermal enhancement of cytotoxicity for the taxanes in R1- and SW 1573-cells exposed to 41.8° C or 43° C [57]. Paclitaxel cytotoxicity was even found to be *inhibited* by 43° C hyperthermia in human breast cancer cells [58]. However, moderate hyperthermia (39.5° C) enhanced paclitaxel cytotoxicity in lung, melanoma, and fibrosarcoma cell lines significantly [59]. Similarly, hyperthermia at 43° C exhibited synergism with paclitaxel in murine breast cancer both in cell lines and in an in vivo model [60,61]. To further complicate matters, however, moderate hyperthermia (41.5° C) did not enhance paclitaxel cytotoxicity in a mouse fibrosarcoma model grown in the foot [37]. Interestingly, the cytotoxicity of docetaxel did increase with hyperthermia in this model.

Clinically, paclitaxel was used with local hyperthermia in unresectable breast cancer recurrences by Zoul et al.; a promising response rate was observed [62]. Orlando et al. used HIPEC (60 min. at 41° C – 42° C) with paclitaxel in 7 patients with advanced ovarian cancer; local toxicity in this small study was limited [63].

Docetaxel

Intraperitoneal docetaxel (combined with carboplatin) has been used in the neoadjuvant management of gastric cancer with peritoneal metastases [64]. In a clinical phase I study in advanced cancer patients, normothermic ip administration of docetaxel resulted in a mean peritoneal/plasma AUC ratio of 181 [65]. De Bree et al. studied HIPEC with docetaxel (75 mg/m^2) at 41° C – 43° C and found a similar mean AUC ratio of 207 [66].

Approaches to Increase Tumour Drug Distribution

Increasing Drug Supply

Pharmacokinetic approaches to increase drug delivery include maximizing the peritoneal/plasma AUC ratio and drug exposure time. Recent modelling data suggest that when exposure times are short (typically 30-90 min. with HIPEC), increasing the concentration gradient will not always result in an equivalent tissue penetration. The dose response curves and their dependency on exposure time have been theoretically modelled by Gardner [67]. Assuming a constant drug concentration throughout the exposure period, the survival fraction is described by

$$S = e^{-q*(1-e^{-ay})}$$

with S = survival fraction, y = drug concentration, a = level of drug resistance, and q = exposure time in hours for non cell cycle specific drugs. From the resulting log dose response curves, it becomes clear that a plateau in cell kill will be reached depending on the exposure time (Fig. 4), as confirmed in various in vitro studies. This suggests that increasing the drug dose will not always compensate for a shorter exposure time, and adds a theoretical argument for adding early postoperative intraperitoneal chemotherapy courses when macroscopic tumour is left after cytoreduction.

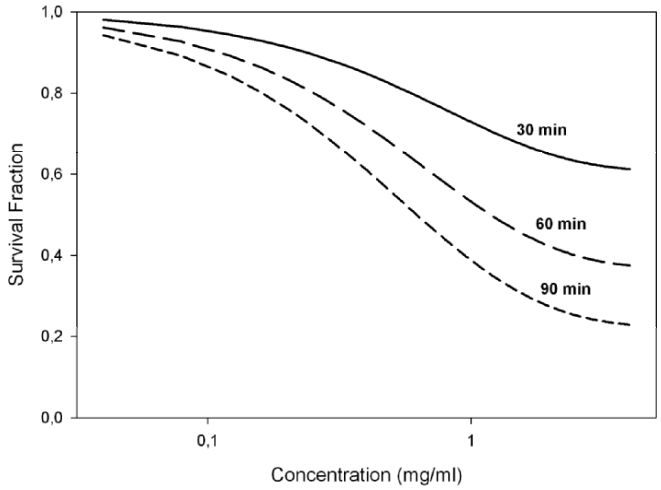


Figure 4. Theoretical calculation of the tumour survival fraction as a function of concentration of a non cell cycle specific cytotoxic drug and exposure time, based on the exponential kill model by Gardner [67]. Note that the plateau in cell kill depends on the exposure time

Alternatively, drug penetration can be enhanced by new pharmacological formulations such as pegylated liposomes, thermosensitive liposomes, or nanoparticles [68-70]. The PK advantage could be further enhanced by reducing systemic drug uptake. The proof of principle in a pig model was described by Lindner et al., who noted a significant increase in AUC ratio of ip carboplatin following reduction of splanchnic circulation with a vasopressin analogue [71].

Enhancing Drug Penetration

Combining cytotoxic drug administration with hyperthermia results in increased uptake, probably related to membrane alterations (chapter 4). Another approach is to reduce the physicochemical resistance to diffusion represented by the increased interstial fluid pressure (IFP) commonly observed in tumours. This can be achieved by targeting the vascular architecture; monoclonal therapy against VEGF has been shown to 'normalize' tumour microvessels resulting in a reduced IFP [72].

Other therapies that have shown to reduce tumour IFP include hyperthermia, radiotherapy, photodynamic therapy, TNF alpha, and steroids [73]. Degradation of the extracellular matrix by hyaluronidase also resulted in increased drug penetration in multicellular models [74]. Since drugs are known to diffuse less in tumours with a high cellular density [54], 'priming' of the tumour by apoptosis-inducing pre-treatment could enhance penetration of subsequently added drug by decreasing cell density. This concept was successfully tested using paclitaxel in a xenograft model [75].

Preclinical ip chemotherapy models have shown that use of a hypotonic carrier solution increases cytotoxic drug uptake, presumably mediated by increased convective drug transport [76]. Clinical studies, however, did not substantiate the presumed advantage of hypotonic carrier solutions; moreover troublesome toxicity was observed [77].

Esquis et al. demonstrated in a rat tumour model that increasing the intraperitoneal pressure resulted in a significantly higher cisplatin penetration in tumour tissue [78]. Similarly, Jacquet et al. found a significant enhancement of doxorubicin uptake in the abdominal wall and diaphragm of rats when the intraperitoneal pressure was increased to 20-30 mm Hg [79]. The clinical applicability of this approach seems, however, rather limited.

Summary and Conclusion

The rationale for ip administration as an adjunct to surgery is firmly based on theoretical and pharmacokinetic grounds. The superiority of combined ip and intravenous chemotherapy over intravenous chemotherapy alone has been established in randomized trials in stage IIIc ovarian cancer patients.

Intraoperative ip cytotoxic therapy results in a definite pharmacological advantage, since high peritoneal concentrations are achieved with limited systemic absorption. At present, however, it is not clearly established to what extent this PK advantage will result in enhanced anticancer activity and, ultimately, in a survival benefit. Preclinical models show that direct penetration into tumour tissue is limited to a few millimeters. Furthermore, the limited exposure time of intraoperative chemoperfusion could limit cytotoxic activity despite high local concentrations. Among the cytotoxic agents currently used, the pharmacodynamic aspects of the platinum compounds are the best studied both with and without associated hyperthermia. Newer agents such as the taxanes and the camptothecins appear promising for ip chemoperfusion during or immediately after surgery.

Pharmacodynamic aspects of HIPEC needing further preclinical study-including mathematical modeling - are the establishment of tumour tissue penetration of the newer agents and its relation to hyperthermia, the definition of the relative contribution of direct penetration versus vascular supply by absorbed drug, and the efficacy of combined ip and intravenous regimens. Ultimately, however, randomised trials of ip chemotherapy with surgery will have to provide the evidence base to further build upon.

References

1. Minchinton AI, Tannock IF. (2006) Drug penetration in solid tumours. Nat Rev Cancer 6(8):583-592

2. Tannock IF, Lee CM, Tunggal JK, Cowan DSM, Egorin MJ. (2002) Limited penetration of anticancer drugs through tumor tissue: A potential cause of resistance of solid tumors to chemotherapy. Clin Cancer Res 8(3):878-884
3. Sugarbaker PH, Mora JT, Carmignani P, Stuart OA, Yoo D. (2005) Update on chemotherapeutic agents utilized for perioperative intraperitoneal chemotherapy. Oncologist 10(2):112-122
4. Barlogie B, Corry PM, Drewinko B. (1980) Invitro Thermochemotherapy of Human-Colon Cancer-Cells with Cis-Dichlorodiammineplatinum(Ii) and Mitomycin-C. Cancer Res 40(4):1165-1168
5. Fujimoto S, Takahashi M, Kobayashi K, Nagano K, Kure M, Mutoh T, Ohkubo H. (1992) Cytohistologic Assessment of Antitumor Effects of Intraperitoneal Hyperthermic Perfusion with Mitomycin-C for Patients with Gastric-Cancer with Peritoneal Metastasis. Cancer 70(12):2754-2760
6. Van Ruth S, Verwaal VJ, Hart AAM, Van Slooten GW, Zoetmulder FAN. (2003) Heat penetration in locally applied hyperthermia in the abdomen during intra-operative hyperthermic intraperitoneal chemotherapy. Anticancer Res 23(2B):1501-1508
7. Jacquet P, Averbach A, Stephens AD, Stuart OA, Chang D, Sugarbaker PH. (1998) Heated intraoperative intraperitoneal mitomycin C and early postoperative intraperitoneal 5-fluorouracil: Pharmacokinetic studies. Oncology 55(2):130-138
8. van Ruth S, Verwaal VJ, Zoetmulder F. (2003) Pharmacokinetics of intraperitoneal mitomycin C. Surg Oncol Clin N Am 12:771-780
9. van Ruth S, Mathot RAA, Sparidans RW, Beijnen JH, Verwaal VJ, Zoetmulder FAN. (2004) Population pharmacokinetics and pharmacodynamics of mitomycin during intraoperative hyperthermic intraperitoneal chemotherapy. Clinical Pharmacokinetics 43(2):131-143
10. Sugarbaker PH, Stuart OA, Carmignani CP. (2006) Pharmacokinetic changes induced by the volume of chemotherapy solution in patients treated with hyperthermic intraperitoneal mitomycin C. Cancer Chemother Pharmacol 57(5):703-708
11. Markman M, Walker JL. (2006) Intraperitoneal chemotherapy of ovarian cancer: A review, with a focus on practical aspects of treatment. J Clin Oncol 24(6):988-994
12. Rossi CR, Foletto M, Mocellin S, Pilati P, De Simone M, Deraco M, Cavaliere F, Palatini P, Guasti F, Scalerta R, Lise M. (2002) Hyperthermic Intraoperative intraperitoneal chemotherapy with cisplatin and doxorubicin in patients who undergo cytoreductive surgery for peritoneal carcinomatosis and sarcomatosis - Phase I study. Cancer 94(2):492-499
13. Cho HK, Lush RM, Bartlett DL, Alexander HR, Wu PC, Libutti SK, Lee KB, Venzon DJ, Bauer KS, Reed E, Figg WD. (1999) Pharmacokinetics of cisplatin administered by continuous hyperthermic peritoneal perfusion (CHPP) to patients with peritoneal carcinomatosis. J Clin Pharmacol 39(4):394-401
14. Leopold KA, Oleson JR, Clarkepearson D, Soper J, Berchuck A, Samulski TV, Page RL, Blivin J, Tomberlin JK, Dewhirst MW. (1993) Intraperitoneal

Cisplatin and Regional Hyperthermia for Ovarian-Carcinoma. Int J Radiat Oncol Biol Physics 27(5):1245-1251
15. Ma GY, Bartlett DL, Reed E, Figg WD, Lush RM, Lee KB, Libutti SK, Alexander HR. (1997) Continuous hyperthermic peritoneal perfusion with cisplatin for the treatment of peritoneal mesothelioma. Cancer J Sci Am 3(3):174-179
16. Zeamari S, Floot B, Van der Vange N, Stewart FA. (2003) Pharmacokinetics and pharmacodynamics of cisplatin after Intraoperative Hyperthermic Intraperitoneal Chemoperfusion (HIPEC). Anticancer Res 23(2B):1643-1648
17. Los G, Sminia P, Wondergem J, Mutsaers PHA, Havemen J, Huinink DT, Smals O, Gonzalezgonzalez D, McVie JG. (1991) Optimization of Intraperitoneal Cisplatin Therapy with Regional Hyperthermia in Rats. Eur J Cancer 27(4):472-477
18. Los G, Vanvugt MJH, Pinedo HM. (1994) Response of Peritoneal Solid Tumors after Intraperitoneal Chemohyperthermia Treatment with Cisplatin or Carboplatin. Br J Cancer 69(2):235-241
19. van de Vaart PJM, van der Vange N, Zoetmulder FAN, van Goethem AR, van Tellingen O, Huinink WWT, Beijnen JH, Bartelink H, Begg AC. (1998) Intraperitoneal cisplatin with regional hyperthermia in advanced ovarian cancer: Pharmacokinetics and cisplatin-DNA adduct formation in patients and ovarian cancer cell lines. Eur J Cancer 34(1):148-154
20. Czejka M, Jager W, Schuller J, Teherani D. (1991) Pharmacokinetics of Carboplatinum in Serum and Intraperitoneal-Fluid after Intraperitoneal Administration. Arch Pharm (Weinheim) 324(3):183-184
21. Miyagi Y, Fujiwara K, Kigawa J, Itamochi H, Nagao S, Aotani E, Terakawa N, Kohno I. (2005) Intraperitoneal carboplatin infusion may be a pharmacologically more reasonable route than intravenous administration as a systemic chemotherapy. A comparative pharmacokinetic analysis of platinum using a new mathematical model after intraperitoneal vs. intravenous infusion of carboplatin - A Sankai Gynecology Study Group (SGSG) study. Gynecol Oncol 99(3):591-596
22. Los G, Verdegaal EME, Mutsaers PHA, McVie JG. (1991) Penetration of Carboplatin and Cisplatin into Rat Peritoneal Tumor Nodules after Intraperitoneal Chemotherapy. Cancer Chemother Pharmacol 28(3):159-165
23. Los G, Smals OAG, Vanvugt MJH, Vandervlist M, Denengelse L, McVie JG, Pinedo HM. (1992) A Rationale for Carboplatin Treatment and Abdominal Hyperthermia in Cancers Restricted to the Peritoneal-Cavity. Cancer Res 52(5):1252-1258
24. Ohno SJ, Siddik ZH, Baba H, Stephens LC, Strebel FR, Wondergem J, Khokhar AR, Bull JMC. (1991) Effect of Carboplatin Combined with Whole-Body Hyperthermia on Normal Tissue and Tumor in Rats. Cancer Res 51(11):2994-3000
25. Steller MA, Egorin MJ, Trimble EL, Bartlett DL, Zuhowski EG, Alexander HR, Dedrick RL. (1999) A pilot phase I trial of continuous hyperthermic peritoneal perfusion with high-dose carboplatin as primary treatment of patients

with small-volume residual ovarian cancer. Cancer Chemother Pharmacol 43(2):106-114
26. Formenti SC, Shrivastava PN, Sapozink M, Jozsef G, Chan KK, Jeffers S, Morrow PC, Muggia FM. (1996) Abdomino-pelvic hyperthermia and intraperitoneal carboplatin in epithelial ovarian cancer: Feasibility, tolerance and pharmacology. Int J Radiat Oncol Biol Phys 35(5):993-1001
27. Rietbroek RC, vandeVaart PJM, Haveman J, Blommaert FA, Geerdink A, Bakker PJM, Veenhof CHN. (1997) Hyperthermia enhances the cytotoxicity and platinum-DNA adduct formation of lobaplatin and oxaliplatin in cultured SW 1573 cells. J Cancer Res Clin Oncol 123(1):6-12
28. Pestieau SR, Belliveau JF, Griffin H, Stuart OA, Sugarbaker PH. (2001) Pharmacokinetics of intraperitoneal oxaliplatin: Experimental studies. J Surg Oncol 76(2):106-114
29. Elias D, Bonnay A, Puizillou JM, Antoun S, Demirdjian S, El Otmany A, Pignon JP, Drouard-Troalen L, Ouellet JF, Ducreux M. (2002) Heated intraoperative intraperitoneal oxaliplatin after complete resection of peritoneal carcinomatosis: pharmacokinetics and tissue distribution. Ann Oncol 13(2):267-272
30. Rouers A, Laurent S, Detroz B, Meurisse M. (2006) Cytoreductive surgery and hyperthermic intraperitoneal chemotherapy for colorectal peritoneal carcinomatosis: Higher complication rate for oxaliplatin compared to mitomycin C. Acta Chir Belg 106(3):302-306
31. Bos AME, De Vos F, de Vries EGE, Beijnen JH, Rosing H, Mourits MJE, van der Zee AGJ, Gietema JA, Willemse PHB. (2005) A phase I study of intraperitoneal topotecan in combination with intravenous carboplatin and paclitaxel in advanced ovarian cancer. Eur J Cancer 41(4):539-548
32. Plaxe SC, Christen RD, O'Quigley J, Braly PS, Freddo JL, McClay E, Heath D, Howell SB. (1998) Phase I and pharmacokinetic study of intraperitoneal topotecan. Invest New Drugs 16(2):147-153
33. Teicher BA, Holden SA, Khandakar V, Herman TS. (1993) Addition of a Topoisomerase-I Inhibitor to Trimodality Therapy [Cis-Diamminedichloroplatinum(Ii) Heat Radiation] in a Murine Tumor. J Cancer Res Clin Oncol 119(11):645-651
34. Hermisson M, Weller M. (2000) Hyperthermia enhanced chemosensitivity of human malignant glioma cells. Anticancer Res 20(3A):1819-1823
35. Guichard S, Chatelut E, Lochon I, Bugat R, Mahjoubi M, Canal P. (1998) Comparison of the pharmacokinetics and efficacy of irinotecan after administration by the intravenous versus intraperitoneal route in mice. Cancer Chemother Pharmacol 42(2):165-170
36. Sumiyoshi K, Strebel FR, Rowe RW, Bull JMC. (2003) The effect of whole-body hyperthermia combined with 'metronomic' chemotherapy on rat mammary adenocarcinoma metastases. Int J Hyperthermia 19(2):103-118
37. Mohamed F, Marchettini P, Stuart OA, Urano M, Sugarbaker PH. (2003) Thermal enhancement of new chemotherapeutic agents at moderate hyperthermia. Ann Surg Oncol 10(4):463-468

38. Elias D, Matsuhisa T, Sideris L, Liberale G, Drouard-Troalen L, Raynard B, Pocard M, Puizillou JM, Billard V, Bourget P, Ducreux M. (2004) Heated intra-operative intraperitoneal oxaliplatin plus irinotecan after complete resection of peritoneal carcinomatosis: pharmacokinetics, tissue distribution and tolerance. Ann Oncol 15(10):1558-1565
39. Markman M. (1993) Salvage Intraperitoneal Chemotherapy for Ovarian-Cancer - a Possible Role for Mitoxantrone. Eur J Cancer 29A(9):1225-1226
40. Wiedemann G, Mella O, Roszinski S, Weiss C, Wagner T. (1992) Hyperthermia Enhances Mitoxantrone Cytotoxicity on Human Breast-Carcinoma and Sarcoma Xenografts in Nude-Mice. Int J Radiat Oncol Biol Physics 24(4):669-673
41. Nicoletto MO, Padrini R, Galeotti F, Ferrazzi E, Cartei G, Riddi F, Palumbo M, De Paoli M, Corsini A. (2000) Pharmacokinetics of intraperitoneal hyperthermic perfusion with mitoxantrone in ovarian cancer. Cancer Chemother Pharmacol 45(6):457-462
42. Jacquet P, Averbach A, Stuart OA, Chang D, Sugarbaker PH. (1998) Hyperthermic intraperitoneal doxorubicin: pharmacokinetics, metabolism, and tissue distribution in a rat model. Cancer Chemother Pharmacol 41(2):147-154
43. Pilati P, Mocellin S, Rossi CR, Scalerta R, Alaggio R, Giacomelli L, Geroni C, Nitti D, Lise M. (2003) Doxorubicin activity is enhanced by hyperthermia in a model of ex vivo vascular perfusion of human colon carcinoma. World J Surg 27(6):640-646
44. Deraco M, De Simone M, Rossi CR, Cavaliere F, Difilippo F, Scuderi S, Pilatti P, Kusamura S. (2003) An Italian multicentric phase 11 study on peritonectomy and intra peritoneal hyperthermic perfusion (IPHP) to treat patients with peritoneal mesothelioma. J Exp Clin Cancer Res 22(4):41-45
45. Kusamura S, Deraco M, Baratti D, Inglese MG, Costanzo P, Favaro M, Manzi R, Gavazzi C. (2003) Cytoreductive surgery followed by intra peritoneal hyperthermic perfusion in the treatment of peritoneal surface malignancies: Morbidity and mortality with closed abdomen technique. J Exp Clin Cancer Res 22(4):207-212
46. Rossi CR, Deraco M, De Simone M, Mocellin S, Pilati P, Foletto M, Cavaliere F, Kusamura S, Gronchi A, Lise M. (2004) Hyperthermic intraperitoneal intraoperative chemotherapy after cytoreductive surgery for the treatment of abdominal sarcomatosis - Clinical outcome and prognostic factors in 60 consecutive patients. Cancer 100(9):1943-1950
47. Takemoto M, Kuroda M, Urano M, Nishimura Y, Kawasaki S, Kato H, Okumura Y, Akaki S, Kanazawa S, Asaumi J, Joja I, Hiraki Y. (2003) The effect of various chemotherapeutic agents given with mild hyperthermia on different types of tumours. Int J Hyperthermia 19(2):193-203
48. Harada S, Ping L, Obara T, Oikawa H, Miyata M, Matsuo M, Takahashi T, Yanagisawa T. (1995) The Antitumor Effect of Hyperthermia Combined with Fluorouracil and Its Analogs. Radiat Res 142(2):232-241
49. Vaillant JC, Nordlinger B, Deuffic S, Arnaud JP, Pelissier E, Favre JP, Jaeck D, Fourtanier G, Grandjean JP, Marre P, Letoublon C. (2000) Adjuvant

intraperitoneal 5-fluorouracil in high-risk colon cancer - A multicenter phase III trial. Ann Surg 231(4):449-456
50. Nordlinger B, Rougier P, Arnaud JP, Debois M, Wils J, Ollier JC, Grobost O, Lasser P, Wals J, Lacourt J, Seitz JF, dos Santos JG, Bleiberg H, Mackiewickz R, Conroy T, Bouche O, Morin T, Baila L, van Cutsem E, Bedenne L. (2005) Adjuvant regional chemotherapy and systemic chemotherapy versus systemic chemotherapy alone in patients with stage II-III colorectal cancer: a multicentre randomised controlled phase III trial. Lancet Oncol 6(7):459-468
51. Mahteme H, Larsson B, Sundin A, Khamis H, Graf W. (2004) Uptake of 5-fluorouracil (5-FU) in peritoneal metastases in relation to the route of drug administration and tumour debulking surgery: an autoradiographic study in the rat. Eur J Cancer 40(1):142-147
52. Hofstra LS, Bos AME, de Vries EGE, van der Zee AGJ, Willemsen ATM, Rosing H, Beijnen JH, Mulder NH, Aalders JG, Willemse PHB. (2002) Kinetic modeling and efficacy of intraperitoneal paclitaxel combined with intravenous cyclophosphamide and carboplatin as first-line treatment in ovarian cancer. Gynecol Oncol 85(3):517-523
53. Markman M, Reichman B, Hakes T, Lewis JL, Jones W, Rubin S, Barakat R, Curtin J, Almadrones L, Hoskins W. (1992) Impact on Survival of Surgically Defined Favorable Responses to Salvage Intraperitoneal Chemotherapy in Small-Volume Residual Ovarian-Cancer. J Clin Oncol 10(9):1479-1484
54. Kuh HJ, Jang SH, Wientjes MG, Weaver JR, Au JLS. (1999) Determinants of paclitaxel penetration and accumulation in human solid tumor. J Pharmacol Exp Ther 290(2):871-880
55. Knox JD, Mitchel REJ, Brown DL. (1991) Effects of Hyperthermia on Microtubule Organization and Cytolytic Activity of Murine Cytotoxic Lymphocytes-T. Exp Cell Res 194(2):275-283
56. Michalakis J, Georgatos SD, Romanos J, Koutala H, Georgoulias V, Tsiftsis D, Theodoropoulos PA. (2005) Micromolar taxol, with or without hyperthermia, induces mitotic catastrophe and cell necrosis in HeLa cells. Cancer Chemother Pharmacol 56(6):615-622
57. Rietbroek RC, Katschinski DM, Reijers MHE, Robins HI, Geerdink A, Tutsch K, dOleire F, Haveman J. (1997) Lack of thermal enhancement for taxanes in vitro. Int J Hyperthermia 13(5):525-533
58. Leal BZ, Meltz ML, Mohan N, Kuhn J, Prihoda TJ, Herman TS. (1999) Interaction of hyperthermia with Taxol in human MCF-7 breast adenocarcinoma cells. Int J Hyperthermia 15(3):225-236
59. Schrump DS, Zhai SP, Nguyen DM, Weiser TS, Fisher BA, Terrill RE, Flynn BM, Duray PH, Figg WD. (2002) Pharmacokinetics of paclitaxel administered by hyperthermic retrograde isolated lung perfusion techniques. J Thorac Cardiovasc Surg 123(4):686-694
60. Othman T, Goto S, Lee JB, Taimura A, Matsumoto T, Kosaka M. (2001) Hyperthermic enhancement of the apoptotic and antiproliferative activities of paclitaxel. Pharmacology 62(4):208-212

61. Cividalli A, Cruciani G, Livdi E, Pasqualetti P, Danesi DT. (1999) Hyperthermia enhances the response of paclitaxel and radiation in a mouse adenocarcinoma. Int J Radiat Oncol Biol Phys 44(2):407-412
62. Zoul Z, Filip S, Melichar B, Dvorak J, Odrazka K, Petera J. (2004) Weekly paclitaxel combined with local hyperthermia in the therapy of breast cancer locally recurrent after mastectomy - a pilot experience. Onkologie 27(4):385-388
63. Orlando M, Huertas E, Salum G, Loza J, Colo F, Vilanova M, Chacon M, Medina L, Chacon RD. (1998) Intraperitoneal Hyperthermic chemotherapy (IPHC) as consolidation treatment for ovarian cancer in pathological complete response. Proc Am Soc Clin Concol 17:abstract 1432
64. Yonemura Y, Bandou E, Sawa T, Yoshimitsu Y, Endou Y, Sasaki T, Sugarbaker PH. (2006) Neoadjuvant treatment of gastric cancer with peritoneal dissemination. Eur J Surg Oncol 32(6):661-665
65. Morgan RJ, Doroshow JH, Synold T, Lim D, Shibata S, Margolin K, Schwarz R, Leong L, Somlo G, Twardowski P, Yen Y, Chow W, Lin P, Paz B, Chu D, Frankel P, Stalter S. (2003) Phase I trial of intraperitoneal docetaxel in the treatment of advanced malignancies primarily confined to the peritoneal cavity: Dose-limiting toxicity and pharmacokinetics. Clin Cancer Res 9(16):5896-5901
66. de Bree E, Rosing H, Beijnen JH, Romanos J, Michalakis J, Georgoulias V, Tsiftsis DD. (2003) Pharmacokinetic study of docetaxel in intraoperative hyperthermic i.p. chemotherapy for ovarian cancer. Anticancer Drugs 14(2):103-110
67. Gardner SN. (2000) A mechanistic, predictive model of dose-response curves for cell cycle phase-specific and -nonspecific drugs. Cancer Res 60(5):1417-1425
68. Vail DM, Amantea MA, Colbern GT, Martin FJ, Hilger RA, Working PK. (2004) Pegylated liposomal doxorubicin: Proof of principle using preclinical animal models and pharmacokinetic studies. Semin Oncol 31(6):16-35
69. Aoki H, Kakinuma K, Morita K, Kato M, Uzuka T, Igor G, Takahashi H, Tanaka R. (2004) Therapeutic efficacy of targeting chemotherapy using local hyperthermia and thermosensitive liposome: evaluation of drug distribution in a rat glioma model. Int J Hyperthermia 20(6):595-605
70. Nyman DW, Campbell KJ, Hersh E, Richardson K, Patrick K, Trieu V, Desai N, Von Hoff DD. (2004) A phase I trial of ABI-007, nanoparticle paclitaxel, administered to patients with advanced non-hematologic malignancies. J Clin Oncol 22(14):133s-133s
71. Lindner P, Heath D, Howell S, Naredi P, Hafstrom L. (1996) Vasopressin modulation of peritoneal, lymphatic, and plasma drug exposure following intraperitoneal administration. Clin Cancer Res 2(2):311-317
72. Jain RK. (2001) Normalizing tumor vasculature with anti-angiogenic therapy: A new paradigm for combination therapy. Nat Med 7(9):987-989
73. Jain RK. (1989) Delivery of Novel Therapeutic Agents in Tumors - Physiological Barriers and Strategies. J Natl Cancer Inst 81(8):570-576

74. St Croix B, Man S, Kerbel RS. (1998) Reversal of intrinsic and acquired forms of drug resistance by hyaluronidase treatment of solid tumors. Cancer Letters 131(1):35-44
75. Jang SH, Wientjes MG, Au JLS. (2001) Enhancement of paclitaxel delivery to solid tumors by apoptosis-inducing pretreatment: Effect of treatment schedule. J Pharmacol Exp Ther 296(3):1035-1042
76. Kondo A, Maeta M, Oka A, Tsujitani S, Ikeguchi M, Kaibara N. (1996) Hypotonic intraperitoneal cisplatin chemotherapy for peritoneal carcinomatosis in mice. Br J Cancer 73(10):1166-1170
77. Elias D, El Otmany A, Bonnay M, Paci A, Ducreux M, Antoun S, Lasser P, Laurent S, Bourget P. (2002) Human pharmacokinetic study of heated intraperitoneal oxaliplatin in increasingly hypotonic solutions after complete resection of peritoneal carcinomatosis. Oncology 63(4):346-352
78. Esquis P, Consolo D, Magnin G, Pointaire P, Moretto P, Ynsa MD, Beltramo JL, Drogoul C, Simonet M, Benoit L, Rat P, Chauffert B. (2006) High intra-abdominal pressure enhances the penetration and antitumor effect of intraperitoneal cisplatin on experimental peritoneal carcinomatosis. Ann Surg 244(1):106-112
79. Jacquet P, Stuart OA, Chang D, Sugarbaker PH. (1996) Effects of intra-abdominal pressure on pharmacokinetics and tissue distribution of doxorubicin after intraperitoneal administration. Anticancer Drugs 7(5):596-603

Patient Selection for Cytoreduction and Hyperthermic Intraperitoneal Chemoperfusion

JH Stewart, P Shen, EA Levine

Introduction

In his Presidential Address to the Society of Surgical Oncology, Blake Cady elegantly stated that tumour biology is King, patient selection is Queen and technical procedures are the Princes of the kingdom. Only rarely can the Prince usurp the kingdom [1]. This statement has never more applicable than cytoreductive surgery and intraperitoneal cytoreductive surgery combined with intraperitoneal hyperthermic chemotherapy (HIPEC). Natural history studies have shown that peritoneal carcinomatosis is uniformly fatal with median survival in the range of approximately 6 months. For more than a decade, a handful of centers have pursued aggressive HIPEC as an alternative approach to this disease.

Patient selection is one of the most important aspects of the HIPEC treatment paradigm. Due to the extent of surgery necessary to obtain optimal cytoreduction, the morbidity and mortality of HIPEC are significant [2-4]. Current morbidity rates experienced by centers performing HIPEC range between 27% and 56%. The most common complications of HIPEC include abscess, fistula, prolonged ileus, pneumonia, and hematologic toxicity. The national mortality rate for HIPEC has been reported to be between 0% and 11% [2]. Given the significant risks associated with this procedure, it is necessary to select patients who will derive the maximal benefit with lower risks of postoperative morbidity and mortality.

Our group and others utilize rather strict criteria in selecting patients for HIPEC. The candidate must present with a tumour histology that responds favorably to cytoreduction and chemoperfusion. Furthermore, preoperative imaging must demonstrate that the patient's tumour burden is amendable to this treatment modality. The presence of extra-abdominal disease, liver metastasis, bulk retroperitoneal disease, or tumour that cannot be completely resected obviates the utility of HIPEC as these patients typically do not derive significant benefit from this procedure. Finally, the patient must be medically fit to undergo the rigors of this aggressive treatment modality similar to other major surgical procedures. The present work establishes a conceptual framework for the role of patient selection

and diagnostic procedures in the treatment of peritoneal surface malignancy with HIPEC.

Indications for Cytoreduction and HIPEC

The most important aspect of selecting patients for HIPEC is understanding which tumour histologies respond most favourably to this treatment modality. The common indications for HIPEC are listed in Table 1 [5-15].

Table 1. Common indications for intraperitoneal hyperthemic chemoperfusion

Primary Tumour	US Incidence (cases/year)	Percent with Peritoneal Disease at Exploration	Median Survival (months)
Colorectal Cancer	130,000	10-15 [5,6]	5.2 [13]
Gastric Cancer	22,000	50 [8]	3.1 [13]
Ovarian Cancer	27,000	75 [7]	36 [14]
Peritoneal Mesothelioma	1500	100	12 [10,12,15]
Appendiceal Cancer	2500	31 [11]	N/A

N/A, Not Available. Numbers between brackets refer to references.

Pseudomyxoma Peritonei

Pseudomyxoma peritonei (PMP) has been considered the classic indication for HIPEC. Pseudomyxoma peritonei is a rare disease with a median survival of six years in surgically treated patients, and may be simply considered mucinous ascites. The vast majority of PMP arise from tumours of the appendix. Ronnett et al evaluated 109 cases of PMP and categorized them into disseminated peritoneal adenomucinosis (DPAM) (58%), peritoneal mucinous carcinomatosis (PMCA) (28%) and intermediate histologies (14%). DPAM is marked by its abundant extracellular mucin, scant, focally proliferative mucinous epithelium and lack of cytological atypia or mitotic activity. PMCA has histologic characteristics that include abundant mucinous epithelium with architectural and cytologic features of carcinoma. The remainder of patients with PMP will have intermediate/hybrid histology. The outcome with DPAM and intermediate histology is significantly better than that of PMCA [16]. Whether PMP is from the benign DPAM or malignant PCMA etiology, it is important to keep in mind that these lesions are uniformly fatal if untreated.

Four series have evaluated HIPEC for PMP. Five year survival rates have ranged between 66% and 97% [17-20]. However, aggressive cytoreduction and HIPEC resulted in morbidity rates between 27% and 44% and mortality rates

ranging between 2.7% and 13% [17-20]. The largest series to date evaluated a total of 385 patients of PMP secondary to appendiceal primary cancers. Of these, 205 underwent HIPEC with MMC followed by postoperative intraperitoneal 5-FU, while 180 patients underwent exploratory laparotomy, cytoreduction and perioperative intraperitoneal chemotherapy. This work suggested that patients with DPAM have a better prognosis than patients with mucinous adenocarcinoma or intermediate histology after exploratory laparotomy and cytoreduction. However, it is unclear if this survival difference persists after HIPEC [17]. A subsequent study revealed a borderline statistically significant difference in 5-year survival between DPAM (64%) and hybrid/PMCA (54%) with HIPEC ($p = 0.05$). There were, however, very few PMCAs (3/36) in the follow-up study [19].

We recently completed a study of 110 patients treated with HIPEC for PMP. A total of 116 HIPECs were performed on 110 patients for appendiceal PD between 1993 and 2004. The 1-, 3-, and 5-year survival rates for all cases were 79.9 ± 4.1%, 59.0 ± 5.7%, and 53.4 ± 6.5%, respectively (Fig. 1). When stratified by histology, low-grade disseminated peritoneal adenomucinosis and intermediate tumours had better 3-year survival rates (77 ± 7% and 81 ± 10%) than PMCA and high-grade nonmucinous lesions (35 ± 10% and 15 ± 14%; $p = 0.0032$ for test of differences between groups) [21]. Patients with PMP should undergo cytoreduction and HIPEC as primary therapy if they are acceptable surgical candidates.

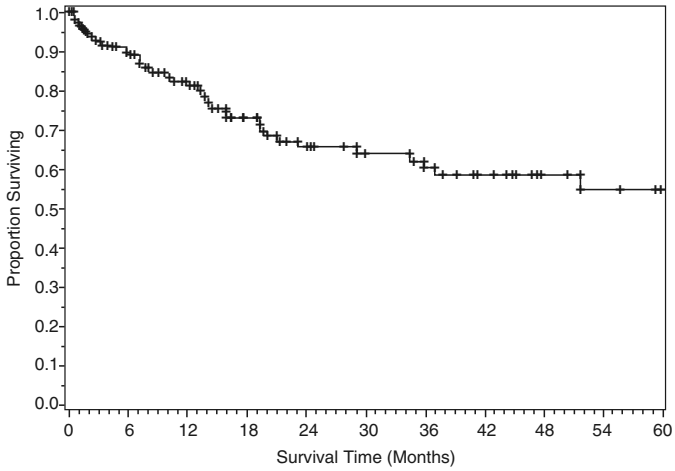


Figure 1. Overall survival of patients undergoing HIPEC for pseudomyxoma peritonei. Reprinted with permission from Ann Surg Oncol 2006;13:624-634

Colorectal Cancer

Several trials have investigated the utility of cytoreductive surgery and HIPEC for carcinomatosis from colorectal carcinoma. These studies, which report on a relatively small numbers of patients, showed a three year survival rate ranging between 25% and 39%, which is clearly superior to that achieved by systemic chemotherapy alone [2,18,22-24]. Single institution results of a phase III randomized study of HIPEC with MMC has been reported by the Netherlands Cancer Institute. Patients with colorectal carcinomatosis were randomized to undergo systemic 5-fluorouracil/leucovorin ± palliative surgery or cytoreduction, HIPEC and systemic chemotherapy. A median survival time of 12.6 months was seen in the palliative chemotherapy arm, while the median survival of the experimental arm was 22.3 months ($p = 0.032$). The trial was stopped prematurely due to the large survival difference in favor of HIPEC [25].

Recently, Glehen et al reported data from an international registry of 506 patients undergoing HIPEC for PC from colorectal cancer at 28 institutions. The overall median survival was 19.2 months after HIPEC. Moreover, the three and five year survival rates were 39% and 19% respectively [26]. The 5 year survival in this setting is indeed remarkable, as such survivors without HIPEC are extremely rare.

Gastric Cancer

Multiple non-randomized trials have evaluated HIPEC in the setting of gastric carcinomatosis. These trials demonstrate that even with complete cytoreduction and HIPEC the prognosis for patients with gastric carcinomatosis is worse than that from colorectal cancer [22,27-29]. In the largest series to date, Yonemura and his colleagues at the Second Department of Surgery performed cytoreduction and HIPEC with MMC, etoposide, and cisplatin in 83 patients with gastric carcinomatosis. The one and five year survival rates in this group of patients were 43% and 11% respectively [29].

A recent study from the Centre Hospitalier Lyon-Sud reported one and five year survival rates of 48% and 16% with a median survival of 10.3 months [22].

We currently consider patients with gastric cancer only if an R0/1 resection can be anticipated or a T_4M_0 lesion is encountered. Current data from our institution suggest that only resection status is significantly correlated with improved survival. Patients undergoing R0/1 resections had a median survival of 11.2 months while those undergoing R2 resections had a significantly lower median survival of 4.6 months ($p = 0.0068$) (Fig. 2) [27].

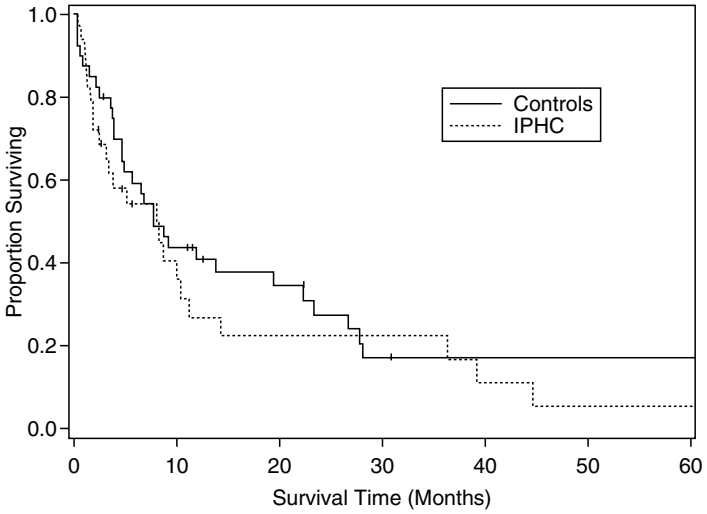


Figure 2. Kaplan-Meier survival curve, comparing standard treatment of advanced gastric cancer to HIPEC. Reprinted with permission from J Gastrointest Surg 2004;8:454-463

Ovarian Cancer

The conventional therapy for FIGO stage III/IV ovarian cancer consists of debulking surgery followed by systemic cisplatin and paclitaxel [14]. However, many patients will recur within five years. At present, there is no consensus on the treatment of women with persistent or recurrent ovarian cancer after front line therapy of cytoreduction and chemotherapy. As a result, HIPEC for ovarian cancer has been investigated in phase I/II studies.

Investigators from the National Cancer Institute of Milan performed a phase II trial with cisplatin and MMC perfusate in 27 patients with recurrent ovarian cancer. Two-year overall survival was 55% while the median time to local progression was 21.8 months [30]. However, unlike gastrointestinal sources of peritoneal carcinomatosis, ovarian cancer is much more responsive to systemic therapy suggesting HIPEC may be most useful as a secondary procedure after initial platinum-based therapy has failed.

Peritoneal Mesothelioma

To date, five non-controlled trials have evaluated HIPEC for peritoneal mesothelioma [31-35]. These studies demonstrate median survival times of 34 months to 67 months which is a significant improvement over the previously reported

median survival times of 12 months to 17 months [9,10,12,15]. Furthermore, palliation of ascites is an essential consideration in the treatment of peritoneal mesothelioma. The current studies show an 86% to 99% relief from ascites after HIPEC for malignant mesothelioma. The National Cancer Institute group recently delineated the factors associated with outcome in individuals undergoing HIPEC with cisplatin for peritoneal mesotheliomas. This analysis demonstrated that a history of previous debulking surgery, absence of deep tissue invasion, and maximum cytoreduction at an age younger than 60 years, were associated with improved survival [36]. Together, these data suggest that patients with mesothelioma are excellent candidates for HIPEC.

Peritoneal Sarcomatosis

Peritoneal sarcomatosis most commonly results from gastrointestinal stromal tumours (GIST) and retroperitoneal sarcomas. Although the best chance of curing these patients is complete resection during the initial operation, recurrence rates range between 25% and 85% [37-39]. Moreover, there is no evidence that adjuvant therapy affects the prognosis of these patients [40].

A recent phase I study from Italy evaluated the utility of HIPEC in the setting of peritoneal sarcomatosis. In the 60 patients followed in this study, the median time to local progression was 22 months and the median overall survival was 34 months [41]. Histologic grading and completeness of cytoreduction were key prognostic indicators. The treatment of GIST with imatinib mesylate (Gleevec™) has demonstrated impressive response rates in visceral disease and therefore HIPEC should probably be reserved for Gleevec™ failures. Patients with peritoneal sarcomatosis from non-GIST sources, although unusual, may also be candidates for HIPEC.

Patient Selection

In considering if patients are medically fit to undergo cytoreductive surgery and HIPEC, one must evaluate the individual's performance status and assess whether or not the patient has a bowel obstruction or malignant ascites.

Data from our institution demonstrate that patients with Eastern Cooperative Oncology Group (ECOG) performance scores of 2 to 3 have a significantly poorer overall survival (median survival of 9.5 months) than patients with ECOG scores of 0 or 1 (median survival of 21.7 months) ($p = 0.02$) (Fig. 3) [42].

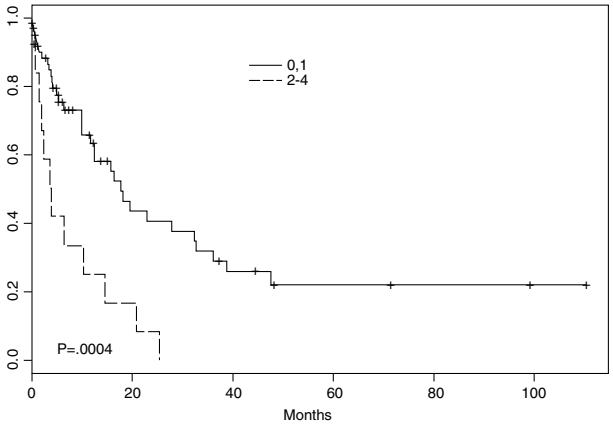


Figure 3. Kaplan-Meier survival curve, comparing overall survival as related to performance status. Reprinted with permission from Ann Surg Oncol 2004;11:178-186

Patients with bowel obstruction and subsequent malnutrition, have a poorer overall survival than those without these co-morbidities, as evidenced by median survivals of 6.3 and 23.0 months respectively (p = 0.03) (Fig. 4) [42].

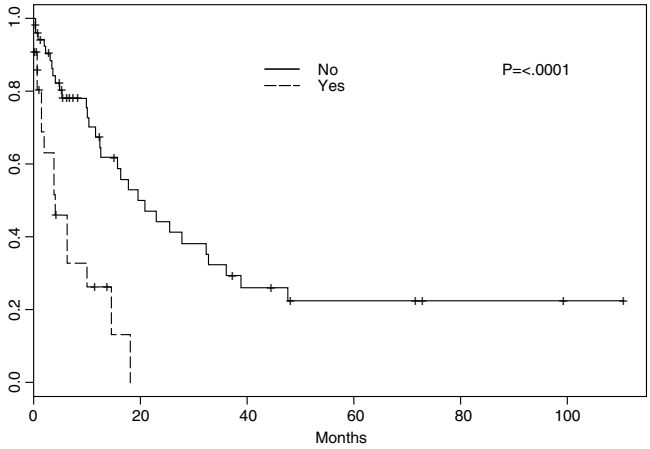


Figure 4. Kaplan-Meier survival curve, comparing overall survival as related to bowel obstruction. Reprinted with permission from Ann Surg Oncol 2004;11:178-186

Although malignant ascites has been shown to predict a poor clinical outcome, HIPEC is an effective means by which to provide palliation. In a phase I/II study of patients with PC and malignant ascites conducted at our institution, HIPEC

prevented recurrence of malignant ascites in 75% of patients, most of whom were chemotherapy failures. Furthermore, HIPEC prevented the development of ascites in all patients with positive intraperitoneal cytology [43]. We continue to offer HIPEC to selected patients with malignant ascites.

Preoperative Imaging

Accurate preoperative imaging of peritoneal surface malignancy not only assists in planning cytoreduction, but also evaluates the presence of extra-abdominal, retroperitoneal and hepatic disease. Hence, optimal preoperative imaging prevents unwarranted laparotomy in patients who have unresectable disease.

The modern armamentarium of preoperative imaging includes computed tomography (CT) scans, magnetic resonance imaging (MRI), and positron emission tomography (PET). Patients found to have extraperitoneal disease by any modality are excluded from HIPEC. CT scans of the solid organs and retroperitoneum have demonstrated great accuracy in detecting primary or recurrent lesions. However, its sensitivity in evaluating disease within the peritoneal cavity and pelvis is limited. In a recent study from the Netherlands Cancer Institute, helical spiral CT scans in 25 consecutive patients with carcinomatosis were compared with intraoperative findings. The overall sensitivity of CT scans in this study was between 25% and 37% with a negative predictive value that ranged between 47% and 51% [44].

These data highlight the limitations of CT scans in detecting and determining the size and location of peritoneal implants. Conversely, MRI with dilute oral barium and intravenous gadolinium has been shown to be superior to CT scans in detecting peritoneal metastasis with a sensitivity of 84% to 100% [45,46]. We utilize either CT or MRI but do not routinely obtain both. Although PET imaging is very sensitive for high volume disease, it has been shown to have decreased sensitivity (10%) in patients with low volume peritoneal carcinomatosis [47]. Moreover, PET is of very limited value for low grade or predominantly mucinous lesions such as PMP. Hence, we do not routinely obtain PET imaging for PMP or mesothelioma. Further studies are indicated to improve preoperative imaging of peritoneal carcinomatosis.

Assessing the patient for resectability is no trivial matter. All trials of HIPEC have demonstrated a correlation between the completeness of cytoreduction and survival. Presently two classification systems are used to describe the extent of cytoreduction. The resection classification system used at Wake Forest includes complete (R0 - no gross disease with negative microscopic margins, R1- no gross disease with positive microscopic margins) versus incomplete (R2a-c) cytoreduction. A resection classification of R2a indicates residual tumour of up to 5mm, R2b designates 6-20 mm of gross disease and R2c identifies more than 20 mm gross residual disease. Data from our institution, and others, demonstrate a significant survival advantage for patients undergoing R0/R1 resection compared to those with R2 resections [2,48,49].

When it is not possible to perform a significant cytoreduction, HIPEC is rarely indicated; the one year survival in these individuals is exceedingly low. A recent study of 56 patients with peritoneal carcinomatosis demonstrated a 79% two year survival rate in patients undergoing complete cytoreduction and HIPEC, while those undergoing incomplete cytoreduction had a two year survival of only 44.7% [50]. Similarly, Yonemura et al demonstrated a 40% three year survival in patients with gastric carcinomatosis treated with complete cytoreduction and HIPEC. This is a dramatic improvement over the three year survival rate of 10% seen in a similar group of patients treated with HIPEC only [51].

In our experience, patients undergoing R0 resection followed by HIPEC experienced three year survival rates of 72.4%, while those undergoing R1, R2a, R2b, or R2c resections experienced five year survival rates of 50%, 44%, 22.2%, and 9.3% respectively (Fig. 5) [2].

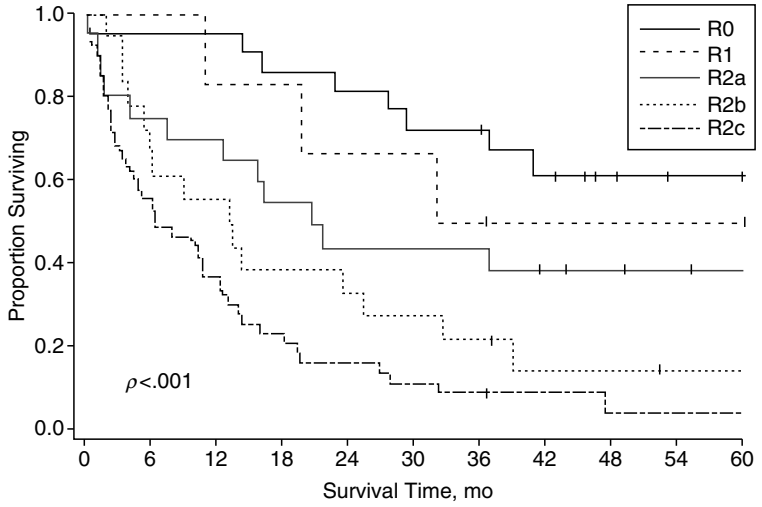


Figure 5. Kaplan-Meier survival curve, comparing overall survival as related to resection status. Reprinted with permission from Arch Surg 2003;138:26-33

Preoperative imaging also allows for the detection of liver metastasis as well as extra-abdominal disease. When hematogenous spread of tumour leads to hepatic parenchymal metastasis, patient outcome is uniformly poor. This represents spread beyond the peritoneal cavity, which indicates systemic disease. Based on data from our group, we generally do not perform cytoreductive surgery and HIPEC in this subset of patients (Fig. 6) [2].

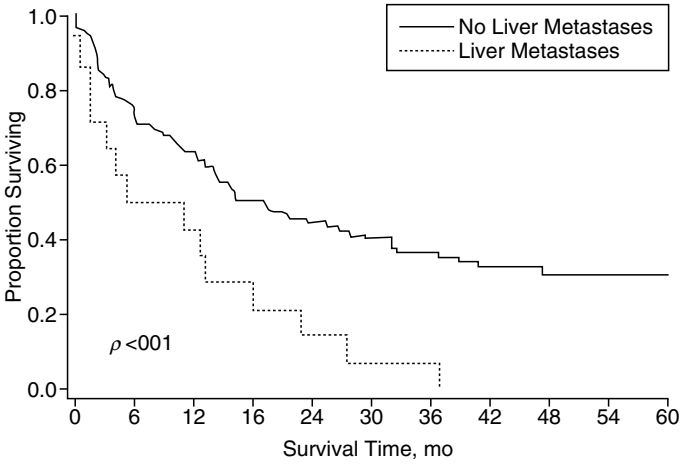


Figure 6. Kaplan-Meier survival curve, comparing overall survival as related to the presence of liver metastases. Reprinted with permission from Arch Surg 2003;138:26-33

Repeat Operations

While reported results from "perfusion centers" represent a substantial improvement in duration and likely quality of life [11,52,53] the majority of patients undergoing these procedures will experience tumour recurrence.

Evaluating patients for second cytoreduction and additional chemoperfusion will become an ever more common problem as these procedures move into the mainstream. The majority of failures after HIPEC occur exclusively at intraabdominal sites. This certainly supports the contention that there is a subset of patients who will manifest intraabdominal disease without manifesting hematogenous metastases. We, and others, assume that in selected patients, a second cytoreductive procedure and chemoperfusion may be of value.

In evaluating patients for a second cytoreduction, the same criteria which are used to select patients for the first remain important. Specifically, the patients must remain medically fit to tolerate a major operative procedure, be free of extra abdominal or hepatic parenchymal metastases, and have disease that seems amenable to complete cytoreduction. Additionally, the time to recurrence after initial cytoreduction and the completeness of the initial cytoreduction should be considered in deciding to proceed with another procedure. Patients with bulk residual disease after an initial cytoreduction for colorectal carcinoma should not be considered candidates for second cytoreductive procedures [54,55].

References

1. Cady B (1990) The Society of Surgical Oncology at a crossroads: thoughts for the future. Presidential address. Arch Surg 125(2):153-157
2. Shen P, Levine EA, Hall J, Case D, Russell G, Fleming R et al (2003) Factors predicting survival after intraperitoneal hyperthermic chemotherapy with mitomycin C after cytoreductive surgery for patients with peritoneal carcinomatosis. Arch Surg 138(1):26-33
3. Stephens AD, Alderman R, Chang D, Edwards GD, Esquivel J, Sebbag G et al (1999) Morbidity and mortality analysis of 200 treatments with cytoreductive surgery and hyperthermic intraoperative intraperitoneal chemotherapy using the coliseum technique. Ann Surg Oncol 6(8):790-796
4. Ahmad SA, Kim J, Sussman JJ, Soldano DA, Pennington LJ, James LE et al (2004) Reduced morbidity following cytoreductive surgery and intraperitoneal hyperthermic chemoperfusion. Ann Surg Oncol 11(4):387-392
5. Dawson LE, Russell AH, Tong D, Wisbeck WM (1983) Adenocarcinoma of the sigmoid colon: sites of initial dissemination and clinical patterns of recurrence following surgery alone. J Surg Oncol 22(2):95-99
6. Russell AH, Tong D, Dawson LE, Wisbeck WM, Griffin TW, Laramore GE et al (1983) Adenocarcinoma of the retroperitoneal ascending and descending colon: sites of initial dissemination and clinical patterns of recurrence following surgery alone. Int J Radiat Oncol Biol Phys 9(3):361-365
7. Deraco M, Raspagliesi F, Kusamura S (2003) Management of peritoneal surface component of ovarian cancer. Surg Oncol Clin N Am 12(3):561-583
8. Sugarbaker PH, Yonemura Y (2000) Clinical pathway for the management of resectable gastric cancer with peritoneal seeding: best palliation with a ray of hope for cure. Oncology 58(2):96-107
9. Moertel CG (1972) Peritoneal mesothelioma. Gastroenterology 63(2):346-350
10. Chan PS, Balfour TW, Bourke JB, Smith PG (1975) Peritoneal nesothelioma. Br J Surg 62(7):576-580
11. McQuellon RP, Loggie BW, Fleming RA, Russell GB, Lehman AB, Rambo TD (2001) Quality of life after intraperitoneal hyperthermic chemotherapy (HIPEC) for peritoneal carcinomatosis. Eur J Surg Oncol 27(1):65-73
12. Brenner J, Sordillo PP, Magill GB, Golbey RB (1981) Malignant peritoneal mesothelioma: review of 25 patients. Am J Gastroenterol 75(4):311-313
13. Sadeghi B, Arvieux C, Glehen O, Beaujard AC, Rivoire M, Baulieux J et al (2000) Peritoneal carcinomatosis from non-gynecologic malignancies: results of the EVOCAPE 1 multicentric prospective study. Cancer 88(2):358-363
14. McGuire WP, Hoskins WJ, Brady MF, Kucera PR, Partridge EE, Look KY et al (1996) Cyclophosphamide and cisplatin compared with paclitaxel and cisplatin in patients with stage III and stage IV ovarian cancer. N Engl J Med 334(1):1-6
15. Antman KH, Osteen RT, Klegar KL, Amato DA, Pomfret EA, Larson DA et al (1985) Early peritoneal mesothelioma: a treatable malignancy. Lancet 2(8462):977-981

16. Ronnett BM, Zahn CM, Kurman RJ, Kass ME, Sugarbaker PH, Shmookler BM (1995) Disseminated peritoneal adenomucinosis and peritoneal mucinous carcinomatosis. A clinicopathologic analysis of 109 cases with emphasis on distinguishing pathologic features, site of origin, prognosis, and relationship to "pseudomyxoma peritonei". Am J Surg Pathol 19(12):1390-1408
17. Sugarbaker PH, Chang D (1999) Results of treatment of 385 patients with peritoneal surface spread of appendiceal malignancy. Ann Surg Oncol 6(8):727-731
18. Witkamp AJ, de Bree E, Kaag MM, van Slooten GW, van Coevorden F, Zoetmulder FA (2001) Extensive surgical cytoreduction and intraoperative hyperthermic intraperitoneal chemotherapy in patients with pseudomyxoma peritonei. Br J Surg 88(3):458-463
19. Elias D, Laurent S, Antoun S, Duvillard P, Ducreux M, Pocard M et al (2003) [Pseudomyxoma peritonei treated with complete resection and immediate intraperitoneal chemotherapy]. Gastroenterol Clin Biol 27(4):407-412
20. Deraco M, Baratti D, Inglese MG, Allaria B, Andreola S, Gavazzi C et al (2004) Peritonectomy and intraperitoneal hyperthermic perfusion (IPHP): a strategy that has confirmed its efficacy in patients with pseudomyxoma peritonei. Ann Surg Oncol 11(4):393-398
21. Stewart JH, Shen P, Russell GB, Bradley RF, Hundley JC, Loggie BL et al (2006) Appendiceal neoplasms with peritoneal dissemination: outcomes after cytoreductive surgery and intraperitoneal hyperthermic chemotherapy. Ann Surg Oncol 13(5):624-634
22. Glehen O, Schreiber V, Cotte E, Sayag-Beaujard AC, Osinsky D, Freyer G et al (2004) Cytoreductive surgery and intraperitoneal chemohyperthermia for peritoneal carcinomatosis arising from gastric cancer. Arch Surg 139(1):20-26
23. Pestieau SR, Sugarbaker PH (2000) Treatment of primary colon cancer with peritoneal carcinomatosis: comparison of concomitant vs. delayed management. Dis Colon Rectum 43(10):1341-1346
24. Glehen O, Cotte E, Schreiber V, Sayag-Beaujard AC, Vignal J, Gilly FN (2004) Intraperitoneal chemohyperthermia and attempted cytoreductive surgery in patients with peritoneal carcinomatosis of colorectal origin. Br J Surg 91(6):747-754
25. Verwaal VJ, van Ruth S, de Bree E, van Sloothen GW, van Tinteren H, Boot H et al (2003) Randomized trial of cytoreduction and hyperthermic intraperitoneal chemotherapy versus systemic chemotherapy and palliative surgery in patients with peritoneal carcinomatosis of colorectal cancer. J Clin Oncol 21(20):3737-3743
26. Glehen O, Kwiatkowski F, Sugarbaker PH, Elias D, Levine EA, De Simone M et al (2004) Cytoreductive surgery combined with perioperative intraperitoneal chemotherapy for the management of peritoneal carcinomatosis from colorectal cancer: a multi-institutional study. J Clin Oncol 22(16):3284-3292
27. Hall JJ, Loggie BW, Shen P, Beamer S, Douglas CL, McQuellon R et al (2004) Cytoreductive surgery with intraperitoneal hyperthermic chemotherapy for advanced gastric cancer. J Gastrointest Surg 8(4):454-463

28. Yonemura Y, de Aretxabala X, Fujimura T, Fushida S, Katayama K, Bandou E et al (2001) Intraoperative chemohyperthermic peritoneal perfusion as an adjuvant to gastric cancer: final results of a randomized controlled study. Hepatogastroenterology 48(42):1776-1782
29. Yonemura Y, Fujimura T, Nishimura G, FallaR, Sawa T, Katayama K et al (1996) Effects of intraoperative chemohyperthermia in patients with gastric cancer with peritoneal dissemination. Surgery 119(4):437-444
30. Deraco M, Rossi CR, Pennacchioli E, Guadagni S, Somers DC, Santoro N et al (2001) Cytoreductive surgery followed by intraperitoneal hyperthermic perfusion in the treatment of recurrent epithelial ovarian cancer: a phase II clinical study. Tumori 87(3):120-126
31. Loggie BW, Fleming RA, McQuellon RP, Russell GB, Geisinger KR, Levine EA (2001) Prospective trial for the treatment of malignant peritoneal mesothelioma. Am Surg 67(10):999-1003
32. Costamagna D, Scuderi S, Vaira M, Barone R, De Simone M (2003) [Treatment of peritoneal mesothelioma using cytoreduction and intraperitoneal hyperthermic chemotherapy]. Tumori 89(4 Suppl):40-42
33. Sugarbaker PH, Welch LS, Mohamed F, Glehen O (2003) A review of peritoneal mesothelioma at the Washington Cancer Institute. Surg Oncol Clin N Am 12(3):605-21, xi
34. Park BJ, Alexander HR, Libutti SK, Wu P, Royalty D, Kranda KC et al (1999) Treatment of primary peritoneal mesothelioma by continuous hyperthermic peritoneal perfusion (CHPP). Ann Surg Oncol 6(6):582-590
35. Deraco M, Casali P, Inglese MG, Baratti D, Pennacchioli E, Bertulli R et al (2003) Peritoneal mesothelioma treated by induction chemotherapy, cytoreductive surgery, and intraperitoneal hyperthermic perfusion. J Surg Oncol 83(3):147-153
36. Feldman AL, Libutti SK, Pingpank JF, Bartlett DL, Beresnev TH, Mavroukakis SM et al (2003) Analysis of factors associated with outcome in patients with malignant peritoneal mesothelioma undergoing surgical debulking and intraperitoneal chemotherapy. J Clin Oncol 21(24):4560-4567
37. Berthet B, Sugarbaker TA, Chang D, Sugarbaker PH (1999) Quantitative methodologies for selection of patients with recurrent abdominopelvic sarcoma for treatment. Eur J Cancer 35(3):413-419
38. Kinsella TJ, Sindelar WF, Lack E, Glatstein E, Rosenberg SA (1988) Preliminary results of a randomized study of adjuvant radiation therapy in resectable adult retroperitoneal soft tissue sarcomas. J Clin Oncol 6(1):18-25
39. Karakousis CP, Gerstenbluth R, Kontzoglou K, Driscoll DL (1995) Retroperitoneal sarcomas and their management. Arch Surg 130(10):1104-1109
40. Pirayesh A, Chee Y, Helliwell TR, Hershman MJ, Leinster SJ, Fordham MV et al (2001) The management of retroperitoneal soft tissue sarcoma: a single institution experience with a review of the literature. Eur J Surg Oncol 27(5):491-497
41. Rossi CR, Deraco M, De Simone M, Mocellin S, Pilati P, Foletto M et al (2004) Hyperthermic intraperitoneal intraoperative chemotherapy after

cytoreductive surgery for the treatment of abdominal sarcomatosis: clinical outcome and prognostic factors in 60 consecutive patients. Cancer 100(9):1943-1950
42. Shen P, Hawksworth J, Lovato J, Loggie BW, Geisinger KR, Fleming RA et al (2004) Cytoreductive surgery and intraperitoneal hyperthermic chemotherapy with mitomycin C for peritoneal carcinomatosis from nonappendiceal colorectal carcinoma. Ann Surg Oncol 11(2):178-186
43. Loggie BW, Perini M, Fleming RA, Russell GB, Geisinger K (1997) Treatment and prevention of malignant ascites associated with disseminated intraperitoneal malignancies by aggressive combined-modality therapy. Am Surg 63(2):137-143
44. de Bree E, Koops W, Kroger R, van Ruth S, Witkamp AJ, Zoetmulder FA (2004) Peritoneal carcinomatosis from colorectal or appendiceal origin: correlation of preoperative CT with intraoperative findings and evaluation of interobserver agreement. J Surg Oncol 86(2):64-73
45. Kubik-Huch RA, Dorffler W, von Schulthess GK, Marincek B, Kochli OR, Seifert B et al (2000) Value of (18F)-FDG positron emission tomography, computed tomography, and magnetic resonance imaging in diagnosing primary and recurrent ovarian carcinoma. Eur Radiol 10(5):761-767
46. Low RN, Barone RM, Lacey C, Sigeti JS, Alzate GD, Sebrechts CP (1997) Peritoneal tumor: MR imaging with dilute oral barium and intravenous gadolinium-containing contrast agents compared with unenhanced MR imaging and CT. Radiology 204(2):513-520
47. Rose PG, Faulhaber P, Miraldi F, Abdul-Karim FW (2001) Positive emission tomography for evaluating a complete clinical response in patients with ovarian or peritoneal carcinoma: correlation with second-look laparotomy. Gynecol Oncol 82(1):17-21
48. Culliford AT, Brooks AD, Sharma S, Saltz LB, Schwartz GK, O 'Reilly EM et al (2001) Surgical debulking and intraperitoneal chemotherapy for esta blished peritoneal metastases from colon and appendix cancer. Ann Surg Oncol 8(10):787-795
49. Marcus EA, Weber TK, Rodriguez-Bigas MA, Driscoll D, Meropol NJ, Petrelli NJ (1999) Prognostic factors affecting survival in patients with colorectal carcinomatosis. Cancer Invest 17(4):249-252
50. Glehen O, Mithieux F, Osinsky D, Beaujard AC, Freyer G, Guertsch P et al (2003) Surgery combined with peritonectomy procedures and intraperitoneal chemohyperthermia in abdominal cancers with peritoneal carcinomatosis: a phase II study. J Clin Oncol 21(5):799-806
51. Yonemura Y, Fujimura T, Fushida S, Fujita H, Bando E, Nishimura G et al (1999) A new surgical approach (peritonectomy) for the treatment of peritoneal dissemination. Hepatogastroenterology 46(25):601-609
52. McQuellon RP, Loggie BW, Lehman AB, Russell GB, Fleming RA, Shen P et al (2003) Long-term survivorship and quality of life after cytoreductive surgery plus intraperitoneal hyperthermic chemotherapy for peritoneal carcinomatosis. Ann Surg Oncol 10(2):155-162

53. Alexander HR, Mavroukakis SM, Libutti SK, Pingpank JF, Beresnev TH, Marden S et al (2004) Impact of Tumor Resection and Intraperitoneal Chemotherapy on Health Related Quality of Life in Patients with Peritoneal Surface Malignancies. Proceedings of the 57th Annual Society of Surgical Oncology (SSO) Cancer Symposium
54. Portilla AG, Sugarbaker PH, Chang D (1999) Second-look surgery after cytoreduction and intraperitoneal chemotherapy for peritoneal carcinomatosis from colorectal cancer: analysis of prognostic features. World J Surg 23(1):23-29
55. Levine EA (2004) Problems of success and problems of failure: recurrent disease after cytoreductive surgery and intraperitoneal chemoperfusion. Ann Surg Oncol 11(4):351-353

Staging and Scoring of Peritoneal Carcinomatosis

S Kübler, J Jähne

Introduction

For several decades, the only therapeutical approach for carcinomatosis of the peritoneal surface arising from gastrointestinal cancer was short-term palliation, using either systemic chemotherapy or limited surgery for the relief of complications such as intestinal obstruction. Therefore, classifications apart from carcinoma tosis yes or no were not needed as they did not have any therapeutical consequences.

Beginning in the 1980s, evidence slowly evolved that peritoneal carcinomatosis (PC) in certain circumstances might be treatable with a combination of aggressive cytoreductive surgery and intraperitoneal hyperthermic chemotherapy (HIPEC) in a curative intent. It was only with this evidence that the need for classification systems arose [9,26,33,39,40]. Several different classification and scoring systems have been proposed and evaluated by the specialised centres worldwide engaged in the treatment of PC. These are on one side descriptive systems classifying the extent of disease and a patient's status before such a therapeutical approach. These systems do not only serve as selection criteria to identify patients who will most probably benefit from this treatment, but are also of prognostic relevance. The other group of classification systems describes and assesses the success in achieving a complete cytoreduction or the extent of surgery done. These classifications again serve as prognostic indicator or factor and, in particular regarding the extent of surgery, as indicators for morbidity and mortality of this treatment regime which are again strongly related to prognosis. An overview of the common classification systems is given in Table 1.

Table 1. Overview of staging systems in peritoneal carcinomatosis diagnosis and therapy

Parameter	Score	Abbrev.	S	P	C
Surgical History	Prior Surgical Score [27]	PSS	+	+	-
Extent of disease	Japanese Research Society for Gastric Cancer Classification [25]	P score	+	+	-
	Peritoneal Cancer Index [27]	PCI	+	+	-
	Simplified Peritoneal Cancer Index [32]	SPCI	+	+	-
	Region Count [30]	N score	+	+	-
	Gilly Score [26]		+	+	-
Cytoreduction	Completeness of cytoreduction score [27]	CC or CCS	-	+	-
	Residual tumour classification [15,19,32]	R stage	-	+	-
Extent of Surgery	Extent of surgery score [18]	ESS	-	+	+
	Extent of cytoreduction score [23]		-	+	+

Abbrev., abbreviation; S, selection; P, prognosis; C, complications

Extent of Prior Surgery

It is an accepted fact in cancer that the initial treatment has the lowest morbidity and mortality and the highest success rate in regard to cure and preservation of function [28]. Therefore, the extent of prior surgery has a negative impact on prognosis and survival.

This is well understandable if one takes into account that wound surfaces induced by surgical trauma have been shown to promote cell implantation at wound sites and adhesions following surgery disturb the recirculation phenomenon and lead to entrapment of tumour cells at different locations. A tool to assess this extent is the prior surgery score.

Prior Surgery Score (PSS)

The extent of prior surgery can assessed by the prior surgical score (PSS), which was first established by Jaquet et al. in 1996 [27]. It quantifies the number and extent of surgical procedures already done before an attempt at maximal cytoreductive surgery including HIPEC is made. It is based on the number of regions involved or the number of procedures performed or a mixture of both. The region count is based on the regions defined in the Peritoneal Cancer Index (PCI), the number of procedures is determined according to the surgical steps listed in Sugarbaker's principles of peritonectomy. The definition of the PSS is given in Table 2.

Table 2. Prior surgery score

PSS	Text	Definition	Number of regions/ procedures
0	None	Diagnosis by laparoscopy or biopsy only	
1	Minimal	exploratory laparatomy only	1-2
2	Moderate	exploratory laparotomy with some resections	2-5
3	Heavy	Extensive previous cytoreduction	> 5

The PSS has been mostly used in patients with low grade malignancies such as pseudomyxoma peritonei and peritoneal mesothelioma [1,17,35,36,40]. These are, due to their histology, a distinct entity especially with DMAP or low grade appendiceal carcinoma. In this setting, multiple operations and reoperations may be possible and be of profit for the patient. In analyses in appendiceal malignancy by Glehen et al. [17], Loungnarath et al [35] and Sugarbaker and Chan [1], it was shown that a high PSS of 3 made a complete cytoreduction by surgical means unlikely. Complete cytoreduction in turn is the most important prognostic factor, and a lower PSS achieves a better prognosis. The same applies for peritoneal mesothelioma [40].

A different entity is carcinomatosis of the peritoneal surface caused by intestinal tumours with regard to biological behaviour, especially invasiveness on a histopathological level. Most of these patients will undergo one or two surgical attempts only which mostly are the removal of the primary tumour and eventually secondary treatment of metastases, e.g. liver resection or possibly cytoreductive surgery. Operations for recurrence of PC are rare. An analysis of the effects of prior surgery in PC of colorectal origin has been made by Portilla et al. [39]. They did not use the PSS explicitly but formed two groups with one prior surgery or more than one operative procedure beforehand. They could not show any difference in survival between both groups. This can be well understood because of the above mentioned biological characteristics.

Even as the amount of prior surgery can be precisely described and classified by the PSS, its clinical relevance especially in PC of intestinal origin as a tool in the selection of patients for treatment is limited. This is even more true as a “cut off point” to exclude patients from further surgery, not even for low grade malignancies, not to speak of any other distinctive cancer origin, has been established yet. Accordingly, in selecting patients to undergo an attempt at maximal cytoreductive surgery in PC the number and type of former surgery should be taken into account, but the PSS score in itself is of limited value only.

Extent and Distribution of Disease

Naturally, the exact description and quantisation of tumour extent and distribution in form of size and location is of central interest in the treatment. It forms the basis of selecting patients for treatment which is the most important feature in the clinical management because the extent and distribution of peritoneal seeds is in most

cases directly related to the probability to achieve a complete cytoreduction. Complete cytoreduction in turn is the most important prognostic factor. Several classification and scoring systems have been described and used [25-27,30].

Carcinomatosis Staging by the Japanese Research Society for Gastric Cancer (P-Score)

This staging system has been developed and extensively used in Japan in the assessment and treatment of PC in gastric cancer patients. The general rules were established by the Japanese Research Society in Gastric Cancer in 1981 [25] as listed in the Table 3. The P-Score is simple to apply and has been validated mostly for gastric cancer, where it was shown to be a good prognostic indicator. An adapted version of this classification for other tumour entities with the main reference point being the site of the primary tumour in general has also been used by Younan et al [23].

Table 3. Japanese Research Society for Gastric Cancer P-Score

Score	Findings
P0	No disseminating metastases to the gastric serosa, greater or lesser omentum, mesenterium, visceral and parietal peritoneum and retroperitoneum
P1	Disseminating metastasis to the adjacent peritoneum (above the transverse colon and including the greater omentum) without metastasis to the distant peritoneum, i.e. the peritoneum below the transverse colon and the abdominal surface of the diaphragm
P2	A few to several scattered metastases to the distant peritoneum, e.g. only ovarian metastases
P3	Numerous metastases to the distant peritoneum

As can be seen in the original definition, the main reference points are the stomach as the primary tumour site and the omentum majus. This system has two main deficits:

1. The location of the carcinomatosis is inaccurate and not very precise;
2. Only the number but not the size of the cancerous implants is taken into account.

Whether these inaccuracies have any impact on either prognosis or resectability in a clinical setting is still unclear. Because of these deficiencies, however, the P-score is of limited value as a descriptive system for peritoneal cancer in general.

Gilly Staging for Peritoneal Carcinomatosis

The Gilly classification was first published by Gilly et al. [26] in 1994. Its definitions are given in the table (Table 4) below.

Table 4. Gilly staging for peritoneal carcinomatosis

Stage	Description
0	No peritoneal carcinomatosis
1	Regional microgranulations (nodules < 5 mm around the primary tumour)
2	Diffuse microgranulations (nodules < 5 mm on the whole peritoneum)
3	Malignant nodules < 2 cm (diffuse throughout the peritoneum)
4	Malignant nodules ≥ 2 cm (large and diffuse malignant deposits)

This system combines localization and tumour size and is simple and reproducible. It has been used for the prediction of survival and has been validated in patients receiving combined treatment for PC of different origins [3,10,26,35].

Beaujard et al. [3] showed a direct relation between survival and clinical stage for gastric and colorectal carcinoma when comparing stage 1 and 2 against stage 3 and 4. Glehen et al. [10] could show the same relation again in colorectal carcinoma.

In a multi-institutional analysis of the treatment effects of extensive cytoreductive surgery and HIPEC in PC of colorectal origin [11], the Gilly score was combined with the Peritoneal Cancer Index (PCI) (Table 5). Both the Gilly score and the PCI discriminated two prognostic categories according to extent of PC.

Table 5. Grouping in a multi-institutional analysis [11]

Group	**Gilly**	**PCI**	**Survival (%)**		
			1-year	3-year	5-year
1 (limited disease)	1 or 2	<13	92	50	33
2 (extensive disease)	3 or 4	≥13	62	22	11

PCI, peritoneal cancer index

The Gilly classification has one main weakness in its definition of stage 4. Stage 4 comprises a combination of tumour size and localisation, which in this combination groups together different patients. Clearly, patients with diffuse and extensive PC belong to this stage. With their chance of having a complete cytoreduction being low, they certainly will have the worst prognosis. On the other side, also patients who have only one tumour nodule bigger than 2 cm are automatically grouped into stage 4, even if this one sole tumour node is easily removable and therefore their prognosis should be much better.

Therefore, the Gilly score has only a limited use in selection of patients for surgery.

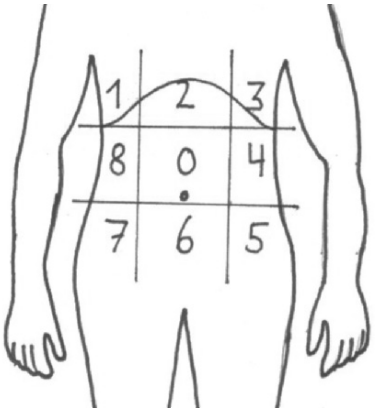
Peritoneal Cancer Index (PCI)

The Peritoneal Cancer Index (PCI) was first described by Jaquet and Sugarbaker in 1996 [27]. It represents a clinical integration of tumour distribution and size with the most detailed definition of location on one side and a clear definition of the size on the other. It has been widely used [2,4,5,9,11,16,22,31,34,38-41] in

describing the extent of disease in appendiceal carcinoma, colonic carcinoma, sarcoma and mesothelioma. The rules to calculate the PCI are the following.

Localisation as defined in PCI

To achieve a detailed location, 13 abdominopelvic regions are assessed. Nine of these regions are defined by the intersection of two transverse and two sagittal planes that divide the abdomen into nine equal sized abdominopelvic regions (AR-0 to AR-8). The two transversal planes are the lowest aspect of the costal margin and the line that connects the two spinae iliacae anteriores. The two sagittal planes are defined as the mid-clavicular lines bilaterally. With this grid, nine equal sized regions are accurately described. The central region is numbered 0, the other regions are numbered clockwise beginning in the upper right sector (Fig. 1).



Region	Definition
0	Central region
1	Right upper quadrant
2	Epigastrium
3	Left upper quadrant
4	Left flank
5	Left lower abdomen
6	Pelvis
7	Right lower abdomen
8	Right flank

Figure 1. Anatomical regions defined within the Peritoneal Cancer Index

The small bowel is assessed using four additional abdominopelvic regions, designated abdominal regions 9 to 12, beginning on the upper jejunum (left upper abdomen) and continuing to the lower ileum (lower right abdomen).

Table 6. Scoring of small bowel involvement within the Peritoneal Cancer Index

Designation	Anatomical definition			Site
9	Upper	Jejunum	left	upper quadrant
10	Lower			lower quadrant
11	Upper	Ileum	right	upper quadrant
12	Lower			lower quadrant

Lesion size defined in PCI

The lesion size is registered in each of the above mentioned areas following the below listed rules. For the same gross findings, several different names have been used, being the lesion size score (LS-score) or the V-classification (volume)

(Table 7). The size is determined during intraoperative evaluation after complete lysis of adhesions.

Table 7. Lesion size score

Lesion Size	Disease status	Score	
		V	LS
No tumour	No detectable disease	0	0
< 0,5 cm	Minimal	1	1
0,5 cm- < 5 cm	Moderate	2	2
> 5 cm or confluent cancer layer	Gross	3	3

The Peritoneal cancer index (PCI) is calculated by adding the score given to the 13 different regions according to regional tumour size. The extent of disease within all the regions of the abdomen and pelvis is thus indicated by a numerical score ranging from 0 to 39 which represents a clinical integration of tumour distribution and size.

Its value as a prognostic indicator has been demonstrated by Sebbag et al. [40] and Pestieau et al. [41] for peritoneal mesothelioma and sarcoma, among others by Mahteme et al. [36] for appendiceal carcinoma and by Tentes [29] for ovarian cancer with different cut off points defined and used in statistical analysis but with a clear correlation between PCI and survival. The same is true for colorectal cancer. Elias et al. [2] found a recurrence rate at 24 months after cytoreductive surgery of 17 % with a PCI < 24 and of 63 % when the PCI was > 24. Three year survival rates were different when a PCI of 15 was used as cut off value (60.3% vs. 32.5%) [9]. Sugarbaker and Chang established survival in colon cancer patients using the PCI with a five-year survival of 50% with a PCI less than 10, 20% for 11–20 and 0% in those with a PCI score greater than 20 [43].

Its value as a selection tool is still disputed. For PC from colonic cancer, currently a PCI of greater than 20 is regarded as a relative contraindication to an elective intervention by some centers [28] because it is associated with approximately the same low median survival as without surgical measures. For other tumour entities, true cut off points have not been clarified and remain to be defined.

The PCI has two flaws that impair its direct and sole utilisation in selecting patients to undergo treatment. First, a direct relation between score and resectability can not be formulated. Indeed, even a low PCI can well mean irresectability because of the location of peritoneal implants on vital structures or extensive involvement of the small bowel. Secondly, the PCI does obviously not take into account the biology of the cancer, which remains one of the most important prognostic determinants.

Simplified Peritoneal Cancer Index

The Simplified Peritoneal Cancer Index (SPCI) was established at the Netherlands Cancer Institute [32]. The Index is calculated similar to Sugarbaker's Peritoneal cancer index by adding up scores related to tumour size in defined regions (Table 8). In contrast to the PCI, only seven regions based on anatomical findings are defined and used. The lesion size is classified into three groups as listed in Table 8. Other features of this score are very similar to the PCI, the maximum score being 21.

Table 8. Regions and lesion size specified in the simplified peritoneal cancer index (SPCI)

Regions according to SPCI	**Lesion Size according to SPCI**	
Small pelvis	Definition	Score
Ileocaecal region	No cancer	0
Omentum/transverse colon	< 1cm	1
Small intestine/mesentery	1 - 5 cm	2
Subhepatic area/stomach	> 5 cm	3

This scoring system has been employed by Verwaal et al. [30, 32] and De Bree et al. [8] in colorectal and appendiceal carcinoma, where higher scores were linearly associated with worsening prognosis. De Bree et al. [8] could show for the same entity that with a SPCI of more than 6, the probability to achieve only an incomplete cytoreduction was 83%.

Apart from that, the SPCI has up to now not been evaluated as a selection tool, but as it is very similar to the PCI the same flaws as mentioned above should be mentioned.

Involved Regions (N-score)

Based on the definitions of regions used in the SPCI, this score has also been published and evaluated at the Netherlands Cancer Institute. The direct number of regions involved is decided upon radiological findings or at intraoperative exploration.

Verwaal et al. [30] showed that the prognosis differed significantly between patients with 1-5 regions involved vs. patients with 6 or more. In an analysis by de Bree et al [8], the probability to achieve a complete cytoreduction decreased to 10% with an N-score of more than 4.

In evaluating the classification systems currently in use to describe the extent of disease and to serve as a prognostic tool as well as selection criteria, the main features and aspects of these classification systems are summarized in Table 9. Most of these classifications already have proven their applicability. All of them seem to be easy to apply.

Table 9. Comparison of classification systems

Classification system	# Regions	Size groups	CT	surgeon assessment	Selection criterion	Prognostic factor
P-Score	2	-	-	+	-	+
Peritoneal Cancer Index (PCI)	13	3	+	+	+	+
Simplified peritoneal Cancer Index (SPCI)	7	3	+	+	+	+
Gilly Score	2	3	-	+	-	+
Region count (N-Score)	7	-	+	+	+	+

The P-score, N-score and Gilly score seem to offer a less precise description of the extent of PC; whether this translates into a meaningful clinical or scientific difference is at present unclear. Indeed, even the PCI is based on a quite subjective assessment and neither its reproducibility nor its superiority over the less extensive staging systems has been verified.

The main advantage of using the above mentioned staging and scoring systems is the demonstrated relation with clinical outcome. Moreover, they allow stratifying patients according to disease burden in the context of clinical trials. They do not, however, allow to accurately predict resectability in individual patients.

Assessment of Completeness or Extent of Surgery

Prognosis in PC is mainly depending on the completeness of surgical removal of all visible tumour nodes [4,5,8,10,11,14,15,17-20,22,35-40,42]. Two different systems, the completeness of cytoreduction score and the R-classification with several definitions regarding the size of tumour nodules left behind have been used. The classification of patients is determined by the largest tumour mass left behind at the completion of the resection.

The rationale behind different sizes in the definition of complete cytoreduction might derive from the fact that a different sensitivity towards different chemotherapeutical agents and for different tumour entities is assumed. As surgical cytoreduction normally is combined with intraperitoneal chemotherapy, the combination of surgery up to a certain size in combination with chemotherapy could well lead to the explicit goal of leaving the patient free of tumour.

Completeness of Cytoreduction Score

The completeness of cytoreduction score (CC or CCS) has been evaluated in several different tumour entities by numerous work groups [4,5,8,10,11,14,17,35-40]. Its definitions are given in Table 10.

Table 10: Completeness of cytoreduction score (CC or CCS)

Score	Definition	Description
0	Complete	no visible tumour remains
1		tumour implants < 0,25 cm
2	Incomplete	tumour implants 0,25 - 2,5 cm in greatest dimension
3		tumour nodules > 2,5 cm in greatest dimension or a layering of disease

Residual Disease (R) - Score

Since the presence or absence of residual tumour determines the likelihood of cure following surgery, the residual tumour (R) stage following surgery is of paramount importance.

The R-stage has been defined in the internationally accepted TNM staging system. In the context of cytoreductive surgery for PC, several authors have proposed a modified or extended version of the R stage that is not always consistent with the TNM stages [15,19,20,32,42,44] (Table 11).

It should be stressed, however, that a correct definition of a 'complete resection' is very important not only in determining the chance of long term survival but also in the communication with pathologists and oncologists. Therefore, it is advisable to reserve the term 'complete resection' for a true TNM R0 resection, i.e. without microscopic tumour left behind. After cytoreduction for PC including fulguration of the liver or bowel surface, intraperitoneal chemotherapy and other chemical or physical measures it is impossible to ascertain a true R0 resection and the term *'macroscopically complete'* or *'clinically complete'* resection should be used in these cases where the surgeon did not leave visible tumour behind.

Table 11: R-Classification as used by Shen [19], Verwaal [32] and Ahmad [15]

R-Score	TNM 6th	Shen [19]	Verwaal [32]	Ahmad [15]
0	Complete resection with microscopically free margins	Complete removal of all visible tumour and negative cytology or negative microscopic margins	Not defined	Complete eradication of all visible tumour
1	Macroscopically complete resection with microscopic residual disease	complete removal of all visible tumour and positive cytology or microscopic margins	no residual tumour	< 4 mm
2a	Macroscopically incomplete resection	minimal residual tumour, nodule(s) ≥ 0,5 cm	residual tumour ≥ 2,5 mm	R 2 > 4 mm
2b		gross residual tumour, nodule > 0,5 cm but ≥ 2 cm	residual tumour > 2,5 mm	Not defined
2c		extensive disease remaining nodules > 2 cm.	Not defined	Not defined

The most elaborate system is the system by Shen [19], which also includes cytology and microscopic margins. In the definition of Verwaal [32], as a complete cytoreduction defined as R0 by the TNM-system is not likely in the context of extensive cytoreduction for PC, this group does not exist. The rationale behind this is the same as in the definition of the CC.

Another definition has been used by Miner et al. [18] in pseudomyxoma according to the extent of cytoreduction (Table 12):

Table 12: Cytoreduction according to Miner [18]

designation	cytoreduction
no gross residual disease	complete
minimal residual disease	90%-99%
gross residual disease	< 90%

This system depends on the amount of tumour implants before surgery takes place. It is therefore not very well defined or precise and is not easily comparable and seems less suited for PC of different origins.

Extent of Surgery

The extent of surgery is related to postoperative morbidity and mortality and therefore has a direct impact on early survival. Two systems have been described for assessing it.

Extent of Surgery Score

The Extent of Surgery Score (ESS) as an adaption of the Sugarbaker PSS-score was described by Miner et al. [18] in assessing the extent of surgery used in the treatment of pseudomyxoma peritonei (Table 13).

Table 13: Extent of surgery score (ESS)

ESS	Definition	Regions involved and dissected
0	biopsy only or laparoscopy plus biopsy	
1	exploratory laparotomy with cytoreduction	1-2
2	exploratory laparotomy with some resections	3-5
3	Extensive cytoreduction	≥6

Level of Cytoreduction

A similar system to the above listed was devised by Younan et al. [23] defining a level of cytoreduction by the number of procedures according to Sugarbakers principles of peritonectomy at different tumour locations (Table 14).

Table 14: Level of cytoreduction

Level	Definition
I	one or two procedures
II	three or four procedures
III	five or more procedures

Both systems were used in assessing the probability of postoperative morbidity and mortality. However, the clinical implications to be drawn out of these scores are at present not clear.

Conclusion

Peritoneal carcinomatosis represents a very heterogeneous disease, and staging or scoring systems are needed to stratify patients according to disease burden and to predict postoperative outcome and long term survival. Classification will therefore facilitate comparison of different reported outcomes or comparison of therapeutic approaches.

The presently available staging and scoring systems differ mainly in the detail of the description of disease burden either before or after cytoreduction. The advantage associated with elaborate scoring systems is offset by their lack of easy clinical applicability, and none of the reported scoring systems have been formally scrutinized in terms of reproducibility. Care has to be taken in the definition of

complete resection or R0 resection, as the reported use in the context of PC therapy is not always consistent with the original TNM classification.

Clearly, a consensus is needed to formulate a uniform staging and scoring system to describe the extent of disease, the completeness of resection, and the presence of visible or microscopic residual tumour.

References

1. Sugarbaker PH, Chang D (1999) Results of Treatment of 385 Patients with Peritoneal Surface Spread of Appendiceal Malignancy. Ann Surg Oncol 6:727–731
2. Elias D, Sideris L, Pocard M, Edè C, Ben Hassouna D, Ducreux M, Boige V, Côté JF, Lasser P (2004) Efficacy of intraperitoneal chemohyperthermia with oxaliplatin in colorectal peritoneal carcinomatosis. Preliminary results in 24 patients. Ann Oncol 15:781–785
3. Beaujard AC, Glehen O, Caillot JL, Francois Y, Bienvenu J, Panteix G, Garbit F, Grandclement E, Vignal J, Gilly FN (2000) Intraperitoneal Chemohyperthermia with Mitomycin C for Digestive Tract Cancer Patients with Peritoneal Carcinomatosis. Cancer 88: 2512-2519
4. Carmignani, CP, Sugarbaker PH (2004) Synchronous extraperitoneal and intraperitoneal dissemination of appendix cancer. Eur J Surg Oncol 30: 864–868
5. Cavaliere F, Perri P, Di Filippo F, et al (2000) Treatment of peritoneal carcinomatosis with intent to cure. J Surg Oncol 74:41-44
6. Ceelen WP, Hesse U, de Hemptinne B, Pattyn P (2000) Hyperthermic intraperitoneal chemoperfusion in the treatment of locally advanced intraabdominal cancer. Br J Surg 87:1006-1015
7. De Bree E, Koops W, Kröger R, Van Ruth S, Witkamp AJ, Zoetmulder FAN (2004) Peritoneal Carcinomatosis From Colorectal or Appendiceal Origin: Correlation of Preoperative CT With Intraoperative Findings and Evaluation of Interobserver Agreement. J Surg Oncol 86:64–73
8. De Bree E, Koops W, Kröger R, Van Ruth S,Verwaal VJ, Zoetmulder FAN (2006) Preoperative computed tomography and selection of patients with colorectal peritoneal carcinomatosis for cytoreductive surgery and hyperthermic intraperitoneal chemotherapy. Eur J Surg Oncol 32:65–71
9. Elias D, Blot F, El Otmany A, Antoun S, Lasser P, Boige V, Rougier P, Ducreux M (2001) Curative Treatment of Peritoneal Carcinomatosis Arising from Colorectal Cancer by Complete Resection and Intraperitoneal Chemotherapy. Cancer 92:71-76
10. Glehen O, Cotte E, Schreiber V, Sayag-Beaujard AC, Vignal J, Gilly FN (2004) Intraperitoneal chemohyperthermia and attempted cytoreductive surgery in patients with peritoneal carcinomatosis of colorectal origin. Br J Surg 91: 747–754

11. Glehen O, Kwiatkowski F, Sugarbaker PH, et al (2004) Cytoreductive surgery combined with perioperative intraperitoneal chemotherapy for the management of peritoneal carcinomatosis from colorectal cancer: a multi-institutional study. J Clin Oncol 22:3284–3292
12. Glehen O, Mohamed F, Gilly FN (2004) Peritoneal carcinomatosis from digestive tract cancer: new management by cytoreductive surgery and intraperitoneal chemohyperthermia. Lancet Oncol 5:219-228
13. Jacquet P, Sugarbaker PH (1996) Clinical research methodologies in diagnosis and staging of patients with peritoneal carcinomatosis. In: Sugarbaker PH, ed. Peritoneal Carcinomatosis: Principles of Management. Boston, MA: Kluwer Academic Publishers; p359–374
14. Deraco M, Baratti D, Inglese MG, MD, Allaria B, Andreola S, Gavazzi C, and Kusamura S (2004) Peritonectomy and Intraperitoneal Hyperthermic Perfusion (IPHP): A Strategy That Has Confirmed its Efficacy in Patients with Pseudomyxoma Peritonei. Ann Surg Oncol 11:393–398
15. Ahmad SA, Kim J, Sussman JJ, Soldano DA, Pennington LJ, James LE, and Lowy AM (2004) Reduced Morbidity Following Cytoreductive Surgery and Intraperitoneal Hyperthermic Chemoperfusion. Ann Surg Oncol 11:387–392
16. Elias D, Matsuhisa T, Sideris L, Liberale G, Drouard-Troalen L, Raynard B, Pocard M, Puizillou JM, Billard V, Bourget P and Ducreux M (2004) Heated intra-operative intraperitoneal oxaliplatin plus irinotecan after complete resection of peritoneal carcinomatosis: pharmacokinetics, tissue distribution and tolerance. Ann Oncol 15:1558–1565
17. Glehen O, Mohamed F, Sugarbaker PH (2004) Incomplete Cytoreduction in 174 Patients With Peritoneal Carcinomatosis From Appendiceal Malignancy. Ann Surg 240: 278–285
18. Miner TJ, Shia J, Jaques DP, Klimstra DS, Brennan MF, and Coit DG (2005) Long-term Survival Following Treatment of Pseudomyxoma Peritonei. Ann Surg 241: 300–308
19. Shen P, Hawksworth J, Lovato J, Loggie BW, Geisinger KR, Fleming RA and Levine EA (2004) Cytoreductive Surgery and Intraperitoneal Hyperthermic Chemotherapy With Mitomycin C for Peritoneal Carcinomatosis from Nonappendiceal Colorectal Carcinoma. Ann Surg Oncol 11:178–186
20. Verwaal VJ, Boot H, Aleman BMP, van Tinteren H and Zoetmulder FAN (2004) Recurrences after Peritoneal Carcinomatosis of Colorectal Origin Treated by Cytoreduction and Hyperthermic Intraperitoneal Chemotherapy: Location, Treatment, and Outcome. Ann Surg Oncol 11:375–379
21. Yan TD, Haveric N, Carmignani CP, Chang D, Sugarbaker PH (2005) Abdominal Computed Tomography Scans in the Selection of Patients with Malignant Peritoneal Mesothelioma for Comprehensive Treatment with Cytoreductive Surgery and Perioperative Intraperitoneal Chemotherapy. Cancer 103:839-849
22. Pestieau SR, Sugarbaker PH (2000) Treatment of primary colon cancer with peritoneal carcinomatosis: comparison of concomitant versus delayed management. Dis Colon Rectum 43: 1341–1346

23. Younan R, Kusamura S, Baratti D, Oliva GD, Costanzo P, Favaro M, Gavazzi C and Deraco M (2005) Bowel Complications in 203 Cases of Peritoneal Surface Malignancies Treated With Peritonectomy and Closed-Technique Intraperitoneal Hyperthermic Perfusion. Ann Surg Oncol 12: 910-918
24. Glehen O, Gilly FN (2003) Quantitative prognostic indicators of peritoneal surface malignancy: carcinomatosis, sarcomatosis, and peritoneal mesothelioma. Surg Oncol Clin N Am 12:649-671
25. Japanese research society for gastric cancer (1981) The general rules for gastric cancer study in surgery and pathology. Jpn J Surg 11:127–139
26. Gilly FN, Carry PY, Sayag AC (1994) Regional chemotherapy with mitomycin C and intraoperative hyperthermia for digestive cancers with peritoneal carcinomatosis. Hepato-gastroenterology 41:124–129
27. Jacquet P and Sugarbaker PH (1996) Current methodologies for clinical assessment of patients with peritoneal carcinomatosis. J Exp Clin Cancer Res 15:49 –58
28. Harmon RL and Sugarbaker PH (2005) Prognostic indicators in peritoneal carcinomatosis from gastrointestinal cancer. International Seminars in Surgical Oncology 2:3
29. Tentes AAK, Tripsiannis G, Markakidis SK, Karanikiotis CN, Tzegas G, Georgiadis G and Avgidou K (2003) Peritoneal cancer index: a prognostic indicator of survival in advanced ovarian cancer. Eur J Surg Oncol 29: 69-73
30. Verwaal VJ, van Tinteren H, van Ruth S and Zoetmulder FAN (2004) Predicting the survival of patients with peritoneal carcinomatosis of colorectal origin treated by aggressive cytoreduction and hyperthermic intraperitoneal chemotherapy. Br J Surg 91: 739–746
31. Yan TD, Haveric N, Carmignani CP, Bromley CM, Sugarbaker PH (2005) Computed tomographic characterization of malignant peritoneal mesothelioma. Tumori 91:394-400
32. Verwaal VJ, van Ruth S, de Bree E, van Slooten GW, van Tinteren H, Boot H and Zoetmulder FAN (2003) Randomized Trial of Cytoreduction and Hyperthermic Intraperitoneal Chemotherapy Versus Systemic Chemotherapy and Palliative Surgery in Patients With Peritoneal Carcinomatosis of Colorectal Cancer. J Clin Oncol 21:3737-3743
33. Fujimoto S, Takahashi M, Mutou T, Kobayashi K, Toyosawa T, Isawa E, Sumida M and Ohkubo H (1997) Improved Mortality Rate of Gastric Carcinoma Patients with Peritoneal Carcinomatosis Treated with Intraperitoneal Hyperthermic Chemoperfusion Combined with Surgery. Cancer 79:884–891
34. Kusamura S, Younan R, Dario Baratti D, Costanzo P, Favaro M, Gavazzi C, Deraco M (2006)Cytoreductive Surgery Followed by Intraperitoneal Hyperthermic Perfusion. Cancer 106:1144–1153
35. Loungnarath R, Causeret S, Bossard N, Faheez M, Sayag-Beaujard A-C, Brigand C, Gilly F and Glehen O (2005) Cytoreductive Surgery With Intraperitoneal Chemohyperthermia for the Treatment of Pseudomyxoma Peritonei: A Prospective Study. Dis Colon Rectum 48: 1372–1379

36. Mahteme H and. Sugarbaker PH (2004) Treatment of peritoneal carcinomatosis from adenocarcinoid of appendiceal origin. Br J Surg 91: 1168–1173
37. Nonaka D, Kusamura S, Baratti D, Casali P, Cabras AD, Younan R, Rosai J and Deraco M (2005) Diffuse Malignant Mesothelioma of the Peritoneum. Cancer 104:2181–2188
38. Pestieau SR, MD, Jelinek JS, Chang D, Jacquet P and Sugarbaker PH (2000) CT in the Selection of Patients with Abdominal or Pelvic Sarcoma for Reoperative Surgery. J Am Coll Surg 190:700-710
39. Portilla AG, Sugarbaker PH and Chang D (1999) Second-look Surgery after Cytoreduction and Intraperitoneal Chemotherapy for Peritoneal Carcinomatosis from Colorectal Cancer: Analysis of Prognostic Features. World J Surg 23:23–29
40. Sebbag G, Yan H, Shmookler M, Chang D, Sugarbaker PH (2000) Results of treatment of 33 patients with peritoneal mesothelioma. Br J Surg 87:1587-1593
41. Sugarbaker PH (2002) Intraperitoneal Chemotherapy for Treatment and Prevention of Peritoneal Carcinomatosis and Sarcomatosis Semin Oncol 29:51-61
42. Shen P, Levine EA, Hall J, et al (2003) Factors predicting survival after intraperitoneal hyperthermic chemotherapy with mitomycin C after cytoreductive surgery for patients with peritoneal carcinomatosis. Arch Surg 138:26-33
43. Sugarbaker PH (1999) Successful management of microscopic residual disease in large bowel cancer. Cancer Chemother Pharmacol 43(Suppl):S15-S25
44. Loggie BW, Fleming RA, McQuellon RP, Russell GB, Geisinger KR (2000) Cytoreductive surgery with intraperitoneal hyperthermic chemotherapy for disseminated peritoneal cancer of gastrointestinal origin. Am Surg 66:561-568

Peritonectomy Procedures

PH Sugarbaker

Introduction

Treatment of peritoneal surface malignancy requires a combined approach that utilizes peritonectomy procedures and perioperative intraperitoneal chemotherapy. In addition, knowledgeable patient selection is mandatory. The visceral and parietal peritonectomy procedures that one must utilize in an attempt to resect all visible evidence of disease is illustrated below.

Complete cytoreduction is essential for treatment of peritoneal surface malignancy to result in long-term survival. All procedures are required in a single surgical event that may require 8-12 hours [1]. The distribution and extent of the malignancy disseminated within the peritoneal space determines the selection of the peritonectomy procedures [2].

Resections are used in the areas of visible cancer progression in an attempt to leave the patient with only microscopic residual disease. In addition to stripping of peritoneal surfaces small tumour nodules on bowel surfaces are removed using electroevaporation. Involvement of visceral peritoneum frequently requires resection of a portion of the stomach, small intestine, or colorectum.

Electroevaporative surgery

In order to adequately perform cytoreductive surgery, the surgeon must use high voltage electrosurgery. Peritonectomies and visceral resections using the traditional scissor and knife dissection will result in a large volume of small vessel bleeding. Also, peritonectomized surfaces devoid of cancer cells are not likely to occur with sharp dissection. Electroevaporative surgery leaves a margin of heat necrosis that is devoid of viable malignant cells [3]. Electroevaporation of tumour at the margins of resection minimizes the likelihood of persistent disease and also minimizes blood loss.

The standard tool used to dissect tumour on peritoneal surfaces from the normal tissues is a 3-mm ball-tipped electrosurgical handpiece (Valleylab, Boulder, CO, USA). The ball-tipped instrument is placed at the interface of tumour and normal tissues with the focal point for further dissection placed on strong traction. The electrosurgical generator is used on pure cut at high voltage. Dissection proceeds cautiously with frequent saline irrigation for tumour removal on tubular structures, especially the ureters, small bowel, and colon.

Using ball-tipped electrosurgery on pure cut creates a large volume of plume because of the electroevaporation (carbonization) of tissue. To maintain visualization of the operative field and to preserve a smoke-free atmosphere, a smoke filtration unit is used (Stackhouse Inc., El Segunda, CA, USA). The vacuum tip is maintained 2 to 3 inches (5 to 7.5 cm) from the field of dissection.

Patient Positioning

The patient is supine with the gluteal fold advanced to the end of the operating table to allow full access to the perineum during the surgical procedure (Fig. 1).

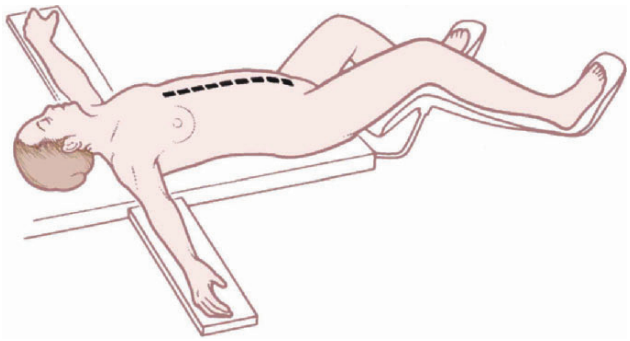


Figure 1. Modified lithotomy position. Reprinted with permission from [1]

This lithotomy position is achieved with the legs extended in St. Mark's leg holders (AMSCO, Erie, PA, USA). The weight of the legs must be directed to the soles of the feet by positioning the foot rest so that minimal weight is on the calf muscle. Myonecrosis within the gastrocnemius muscle may occur unless the legs are protected by foam padding. The legs are surrounded by alternating-pressure boots (SCB Compression Boots, Kendall Co., Boston, MA). These should be operative before the start of anesthesia for maximal protection against venothrombosis. A heating apparatus is placed over the chest and arms of the patient (Bair Hugger Upper Body Cover, Augustine Medical, Eden Prairie, MN, USA) and also beneath the torso (Cincinnati Sub-Zero, Cincinnati, OH, USA).

The Foley catheter is placed in position. A Silastic 18-gauge nasogastric sump tube is placed within the stomach (Argyle Salem Sump Tube, Sherwood Medical, St. Louis, MO) and confirmed at a later time to be positioned along the greater curvature of the stomach.

Construction of the Surgical Field to provide Simultaneous Exposure of the Abdomen and Pelvis

A self-retaining retractor (Thompson Surgical Instruments, Traverse City, MI, USA) is positioned so that continuous retraction of all parts of the abdominal incision occurs (Fig. 2). The retraction system must be securely anchored to the operating table in order to provide for continuous unencumbered visualization of the large operative field.

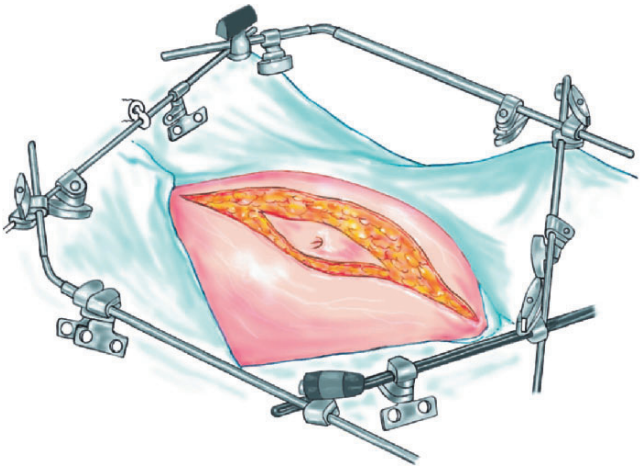


Figure 2. Self-retaining retractor and elliptical incision. Reprinted with permission from [1]

An incision starting above the xiphi-sternal junction to pubis through the midline is constructed. An ellipse is created around the prior abdominal incision and the umbilicus to allow for the peritoneal plane to be clearly exposed throughout the extent of the abdominal incision. Retaining the umbilicus leads to a high incidence of recurrence at this site. The fascia is divided through the linea alba from xiphoid bone to pubic bone. Routinely, the xiphoid is completely resected at the xiphi-sternal junction as part of the specimen [4]. With the fascia divided the parietal peritoneum remains intact.

Parietal Peritoneal Stripping from the Anterior Abdominal Wall

A single entry into the peritoneal cavity in the upper portion of the incision allows the surgeon to assess the requirement for a complete parietal peritonectomy (Fig. 3). If cancer nodules are palpated on the parietal peritoneum a decision for a complete anterior parietal peritonectomy is made [5]. Except for the small defect in the peritoneum required for this peritoneal exploration the remainder of the peritoneum is kept intact. Adair clamps are placed on the skin edge in order to provide broad traction along the complete line for tissue transection. The dissecting tool is the ball-tip and smoke evacuation is used continuously.

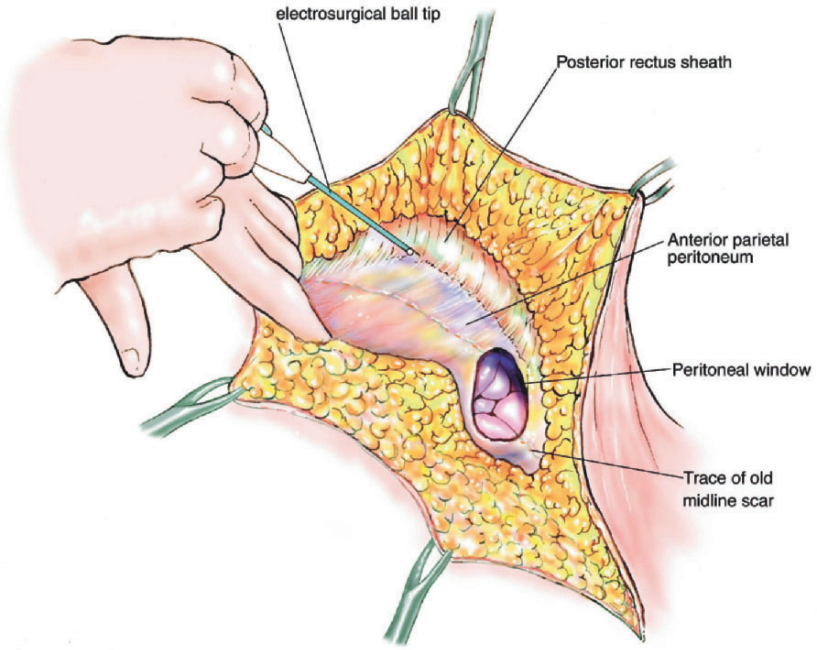


Figure 3. Dissection of the parietal peritoneum from the inferior surface of the anterior abdominal wall. Reprinted with permission from [1]

Stripping the Visceral Peritoneum from the Surface of the Bladder

After dissecting generously the peritoneum on the right and left sides of the bladder, the apex of the bladder is localized and placed on strong traction using Babcock clamps (Fig. 4). The peritoneum with the underlying fatty tissues are stripped away from the surface of the bladder. Broad traction on the entire anterior parietal peritoneal surface and frequent saline irrigation reveals the point for tissue transection that is precisely located between the bladder musculature and its adherent

fatty tissue. This dissection is continued inferiorly down to the cervix in the female or to the seminal vesicles in the male.

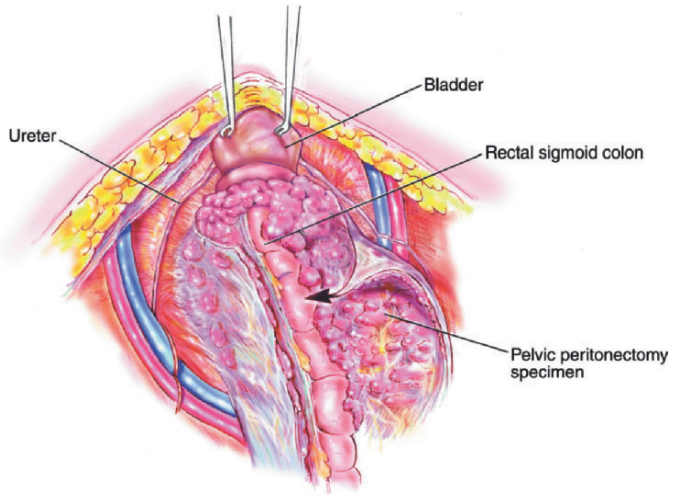


Figure 4. Stripping the visceral peritoneum from the surface of the bladder. Reprinted with permission from [1]

Parietal Peritoneal Dissection to the Paracolic Sulcus and Beyond

The self-retaining retraction system is steadily advanced more deeply into the abdominal cavity (Fig. 5).

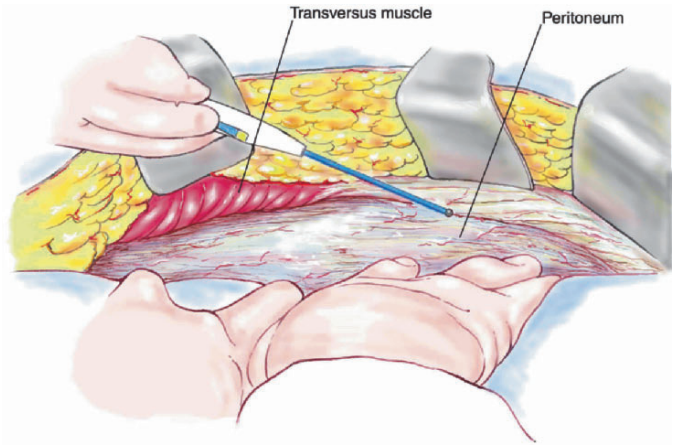


Figure 5. Parietal peritoneal dissection to the paracolic sulcus and beyond. Reprinted with permission from [1]

This optimizes the broad traction at the point of dissection of the peritoneum and its underlying tissues.

The peritoneum strips readily from the undersurface of the hemidiaphragm. It is most adherent directly overlying the transversus muscle. In some instances blunt dissection from inferior to superior aspects of the abdominal wall facilitates clearing in this area. The dissection joins the right and left subphrenic peritonectomy superiorly and the complete pelvic peritonectomy inferiorly. As the dissection proceeds beyond the peritoneum overlying the paracolic sulcus (line of Toldt) the dissection becomes more rapid because of the loose connections of the peritoneum to the underlying fatty tissue at this anatomic site.

Peritoneal Stripping from Beneath the Left Hemidiaphragm

To begin peritonectomy of the left upper quadrant, the peritoneum is progressively stripped off the posterior rectus sheath (Fig. 6). Broad traction must be exerted on the tumour specimen throughout the left upper quadrant. Strong traction combined with ball tip electrosurgical dissection allows separation of surface tumour from all normal tissue in the left upper quadrant including the diaphragmatic muscle, the left adrenal gland, and the perirenal fat. The splenic flexure of the colon is released from the peritoneum of the left abdominal gutter and moved medially.

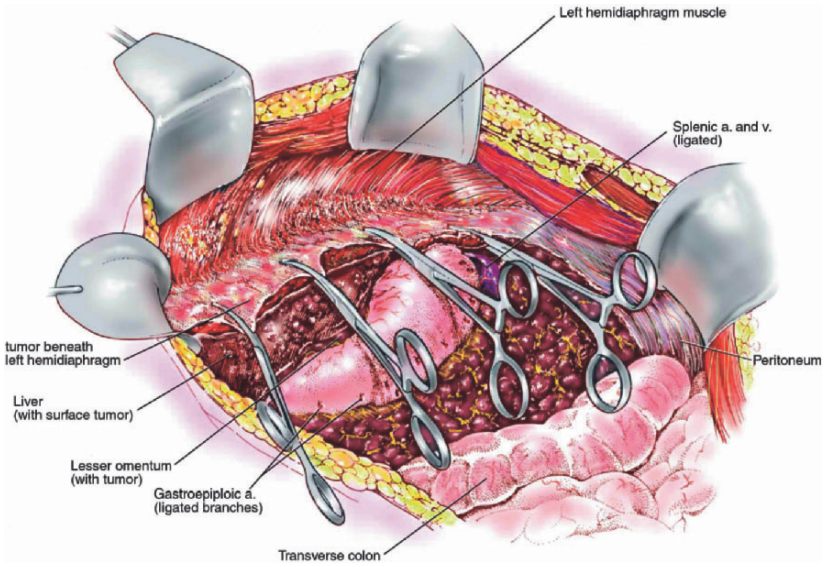


Figure 6. Left subphrenic peritonectomy. Reprinted with permission from [1]

The dissection between diaphragm muscle and its peritoneal covering must be performed with electroevaporative surgery, not by blunt dissection. Numerous blood vessels between the diaphragm muscle and its peritoneal surface must be electrocoagulated before their transection or unnecessary bleeding will occur as the divided blood vessel retracts into the muscle of the diaphragm. Tissues are dissected using ball-tipped electrosurgery on pure cut; all blood vessels are electrocoagulated before their division.

Greater Omentectomy and Splenectomy with Completion of the Left Subphrenic Peritonectomy

The greater omentum is elevated and then separated from the transverse colon using electrosurgery (Fig. 7). This dissection continues beneath the peritoneum that covers the transverse mesocolon so as to expose the pancreas. The gastroepiploic vessels on the greater curvature of the stomach are ligated and divided. Also, the short gastric vessels are transected.

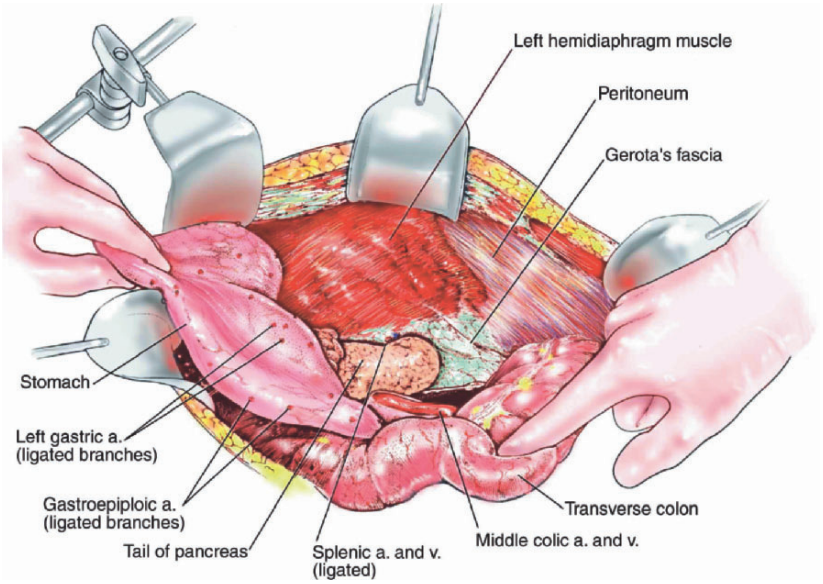


Figure 7. Greater omentectomy and splenectomy with completion of the left subphrenic peritonectomy. Reprinted with permission from [1]

The mound of tumour that covers the spleen is identified. The splenic artery and vein at the tail of the pancreas are ligated in continuity and proximally suture-ligated. This allows the greater curvature of the stomach to be reflected to the right from the pylorus to the gastroesophageal junction.

If clear access to the splenic vessels cannot be achieved by an anterior approach, they can be visualized from posterior after completion of the left subphrenic stripping. Generous dissection posterior to the body of the pancreas will allow its elevation without a crack in the pancreas capsule.

When the left upper quadrant peritonectomy is completed, the stomach may be reflected medially. Numerous branches of the gastroepiploic arteries that have been ligated are evident. The left adrenal gland, pancreas, and left perirenal fatty tissue are visualized completely, as is the anterior peritoneal surface of the transverse mesocolon. The surgeon must avoid the right and left gastric arteries and vein to preserve the vascular supply to the stomach.

Peritoneal Stripping from Beneath the Right Hemidiaphragm

The peritoneum is stripped away from the right posterior rectus sheath to begin the peritonectomy in the right upper quadrant of the abdomen (Fig. 8). Strong traction on the specimen is used to elevate the hemidiaphragm into the operative field. Again, ball-tipped electrosurgery on pure cut is used to dissect at the interface of tumour and normal tissue. Coagulation current is used to divide the blood vessels as they are encountered and before they bleed and retract into the muscle.

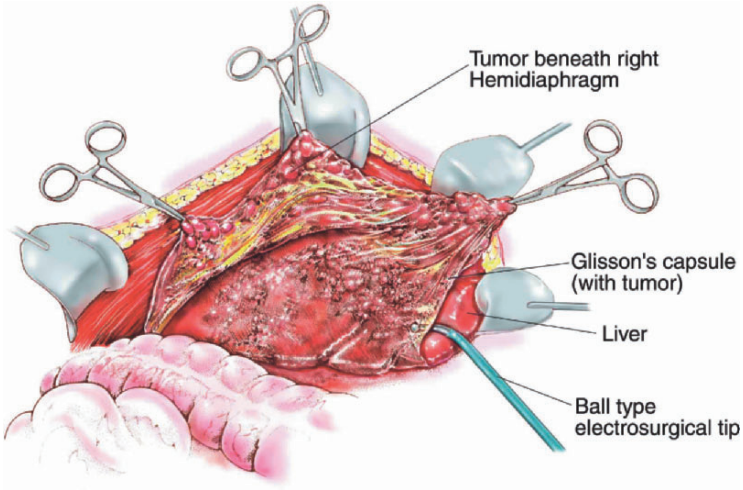


Figure 8. Peritoneal stripping from beneath the right hemidiaphragm and electroevaporation of tumour from the surface of the liver. Reprinted with permission from [1]

The stripping of the tumour from the undersurface of the diaphragm continues until the bare area of the liver is encountered. At that point, tumour on the superior surface of the liver is electroevaporated until the liver surface is cleared. With ball-tipped electrosurgical dissection, a thick layer of tumour may be lifted off the dome of the liver by removing Glisson's capsule. Isolated patches of tumour on

the liver surface are electroevaporated with the distal 2 cm of the ball tip bent and stripped of insulation ("hockey stick" configuration). Ball-tipped electrosurgery is also used to extirpate tumour from attachments of the falciform ligament and round ligament.

Removal of an Envelope of Tumour from Beneath the Right Hemidiaphragm, from the Right Subhepatic Space, and from the Surface of the Liver

Tumour from beneath the right hemidiaphragm, from the right subhepatic space, and from the surface of the liver forms an envelope as it is removed en bloc (Fig. 9). The dissection is greatly facilitated if the tumour specimen can be maintained intact. The dissection continues laterally on the right to encounter the perirenal fat covering the right kidney. Also, the right adrenal gland is visualized and carefully avoided as tumour is stripped from the right subhepatic space. Care is taken not to traumatize the vena cava or to disrupt the caudate lobe veins that pass between the vena cava and segment 1 of the liver.

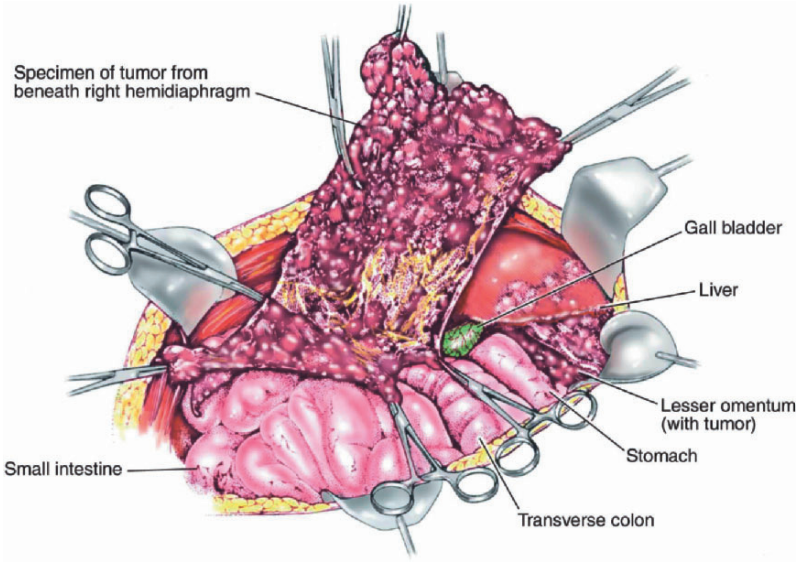


Figure 9. Removal of an envelope of tumour from beneath the right hemidiaphragm from the right subhepatic space and from the surface of the liver. Reprinted with permission from [1]

Completed Right Subphrenic Peritonectomy

With strong upward traction on the right costal margin by the self-retaining retractor and medial displacement of the right liver, one can visualize the completed right subphrenic peritonectomy (Fig. 10). The anterior branches of the phrenic artery and vein on the hemidiaphragm are seen and have been preserved. The right hepatic vein and the vena cava below have been exposed. The right subhepatic space, including the right adrenal gland and perirenal fat covering the right kidney, constitutes the base of the dissection.

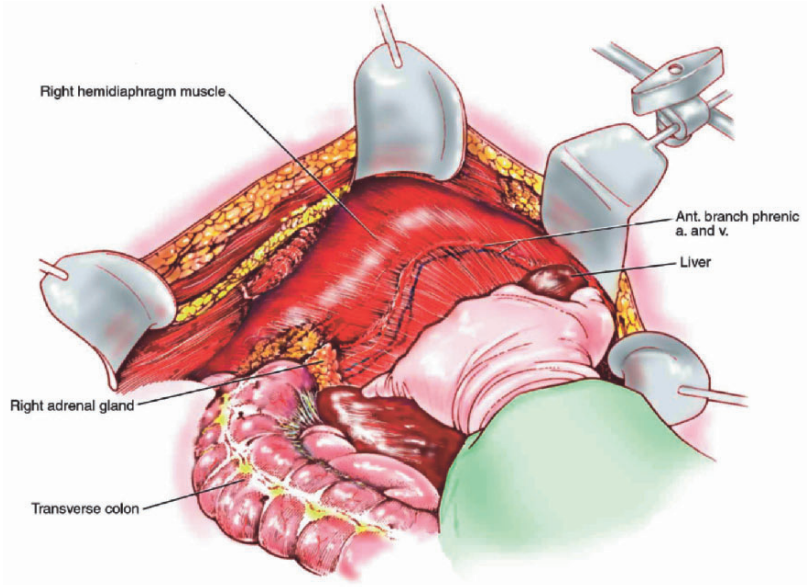


Figure 10. Completed right subphrenic peritonectomy. Reprinted with permission from [1]

Frequently, tumour has invaded the tendinous central portion of the left or right hemidiaphragm. If this occurs, the tissue infiltrated by tumour must be resected. This usually requires an elliptical excision of a central portion of the hemidiaphragm. It may be necessary on the right or the left but is more frequently needed on the right. The defect in the diaphragm is closed with interrupted sutures after the intraoperative chemotherapy is completed.

Cholecystectomy with Resection of the Hepatoduodenal Ligament

The gallbladder is resected in a routine fashion from its fundus toward the cystic artery and cystic duct (Fig. 11). Blunt dissection of the base of the gallbladder away from the common duct and right hepatic artery distinguishes these structures from the surrounding tumour and fatty tissue. These structures are ligated and divided.

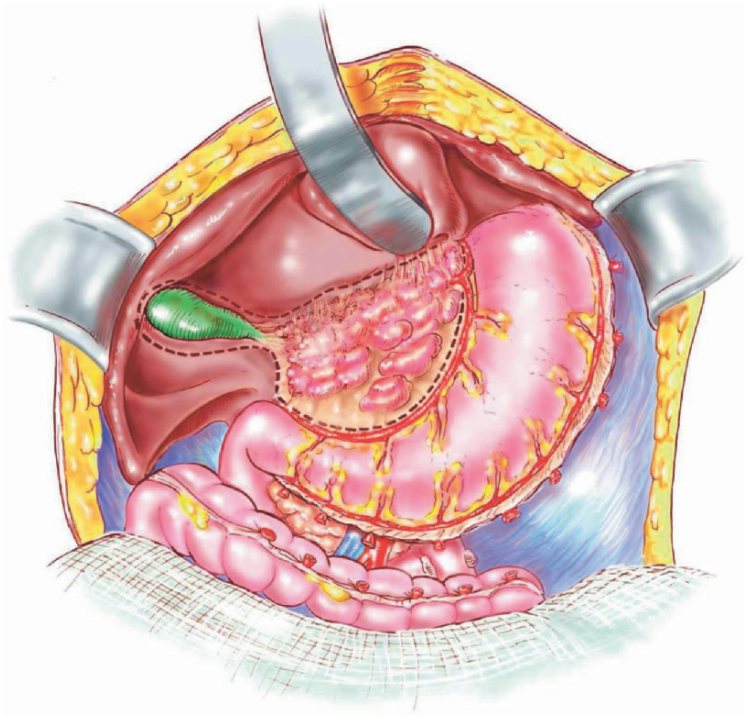


Figure 11. Cholecystectomy with resection of the hepatoduodenal ligament. Reprinted with permission from [1]

To remove the peritoneum from the anterior aspect of the hepatoduodenal ligament, its peritoneal reflection to the liver surface is released. Special care is taken not to injure the left hepatic artery which is usually the most superficial of the portal structures. The peritoneum is firmly grasped using a Russian forceps and peeled away from the common bile duct and hepatic artery. Peritoneal stripping of the undersurface of the porta hepatis is frequently necessary.

Circumferential Resection of the Hepatogastric Ligament and Lesser Omentum by Digital Dissection

The triangular ligament of the left lobe of the liver was resected in performing the left subphrenic peritonectomy. This completed, the left lateral segment of the liver is retracted left to right to expose the hepatogastric ligament in its entirety (Fig. 12). A circumferential release of this ligament from the hepatogastric fissure and from the arcade of right gastric artery to left gastric artery along the lesser curvature of the stomach is required. After electrosurgically dividing the peritoneum on

the lesser curvature of the stomach, digital dissection with extreme pressure from the surgeon's thumb and index finger separates lesser omental fat and tumour from the vascular arcade. As much of the anterior vagus nerve is spared as is possible. The tumour and fatty tissue surrounding the right and left gastric arteries are morcelated away from the vascular arcade. In this manner the specimen is centralized over the major branches of the left gastric artery. With strong traction on the specimen, the lesser omentum is released from the left gastric artery and vein.

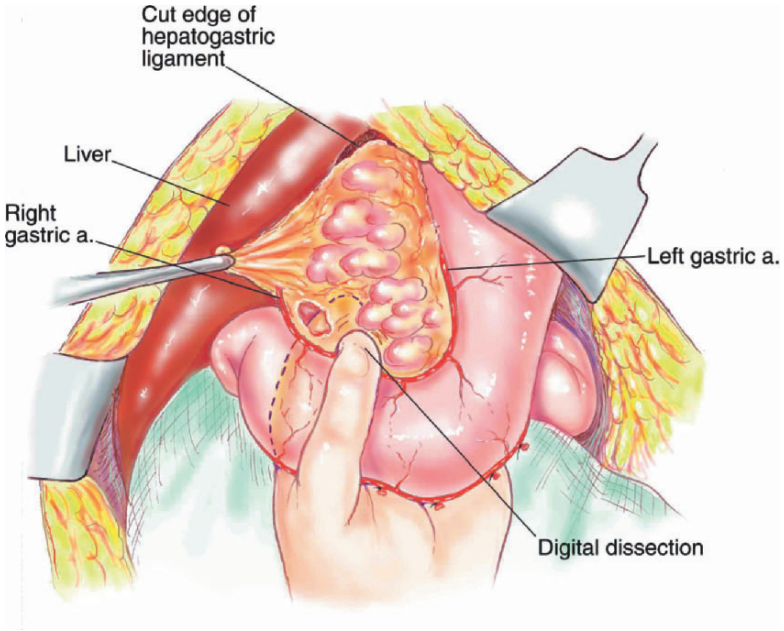


Figure 12. Circumferential resection of the hepatogastric ligament and lesser omentum by digital dissection. Reprinted with permission from [1]

If an accessory left hepatic artery off the left gastric artery is present it is resected with the lesser omentum specimen. This must occur in preparation for resection of the peritoneal surfaces below this structure.

Limits of the Lesser Omentectomy with Stripping of the Floor of the Omental Bursa

A Dever retractor or the assistant's fingertips beneath the left caudate lobe are positioned to expose the entire floor of the omental bursa (Fig. 13). Electroevaporation of tumour from the caudate process of the left caudate lobe of the liver may be necessary to achieve this exposure. Ball-tip electrosurgery is used to cautiously divide the peritoneal reflection of liver onto the left side of the subhepatic vena

cava. After the peritoneum is divided, a Russian forceps assists in a blunt stripping of the peritoneum from the superior recess of the omental bursa, from the crus of the right hemidiaphragm, and from beneath the portal vein.

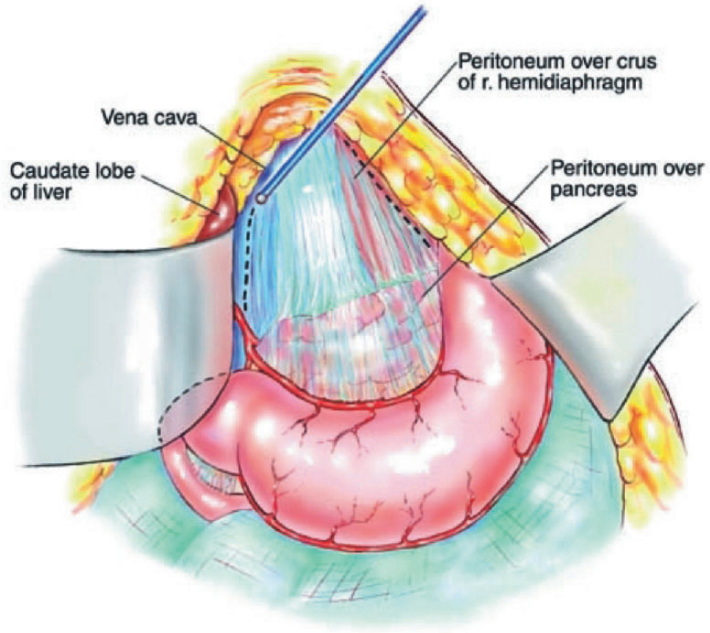


Figure 13. Stripping of the floor of the omental bursa. Reprinted with permission from [1]

Electroevaporation of tumour from the shelf of liver parenchyma beneath the portal vein and joining right and left aspects of the caudate lobe may be required (Fig. 14). Care is taken while stripping the floor of the omental bursa to stay superficial to the right phrenic artery.

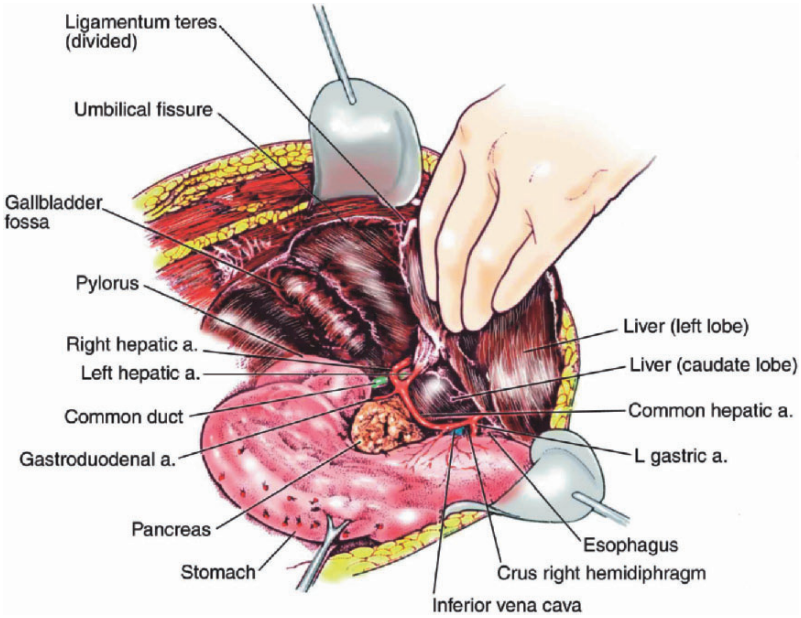


Figure 14. Lesser omentectomy and omental bursectomy completed. Reprinted with permission from [1]

Limits of the Complete Pelvic Peritonectomy

The peritoneal incision around the pelvis is completed (Fig. 15). The right and left ureters are identified and preserved. In women, the right and left ovarian veins are ligated at the level of the lower pole of the kidney and divided. In the male special care is taken to avoid the testicular vessels.

Resection of Rectosigmoid Colon, Uterus, and Cul-de-sac of Douglas

To begin the rectosigmoid colon resection a linear stapler is used to divide the sigmoid colon just above the limits of the pelvic tumour; this is usually at the junction of sigmoid and descending colon. The vascular supply of the distal portion of the bowel is traced back to its origin on the aorta. The inferior mesenteric artery and vein are ligated, suture-ligated and divided. This allows one to pack all the viscera, including the proximal descending colon into the upper abdomen.

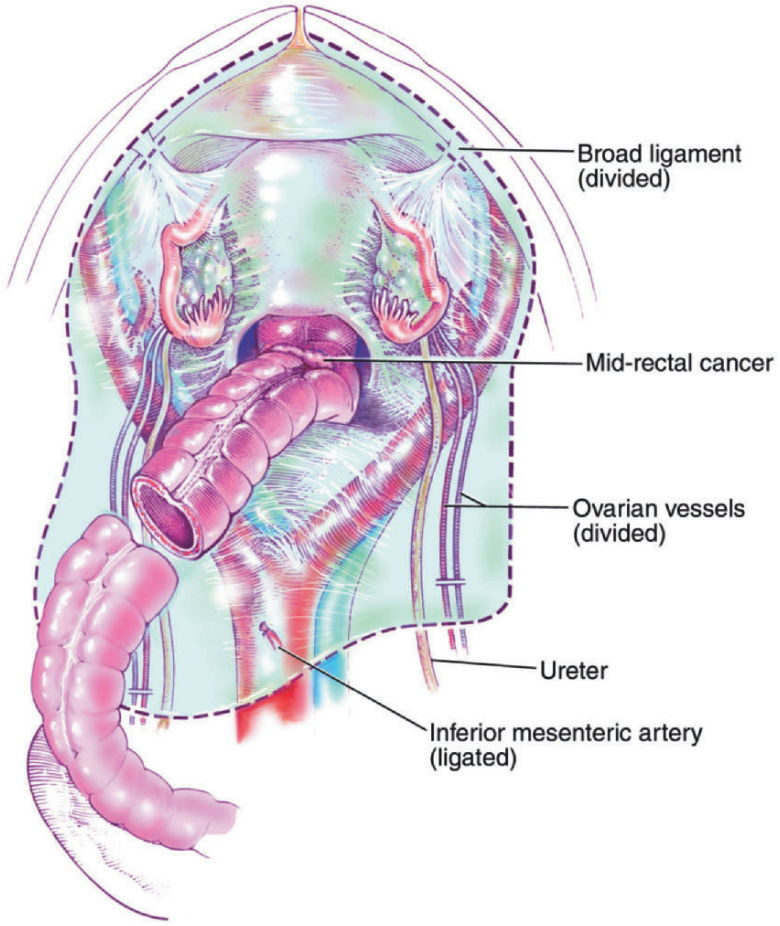


Figure 15. Limits of the complete pelvic peritonectomy. Reprinted with permission from [1]

Ball-tipped electrosurgery is used to dissect at the limits of the mesorectum. The surgeon works in a centripetal fashion. Extraperitoneal ligation of the uterine arteries is performed just above the ureter and close to the base of the bladder. In women, the bladder is moved gently off the cervix and the vagina is entered. The vaginal cuff anterior and posterior to the cervix is transected using ball-tipped electrosurgery, and the rectovaginal septum is entered. Ball-tipped electrosurgery is used to divide the perirectal fat beneath the peritoneal reflection. This ensures that tumour occupying the cul-de-sac is removed intact with the specimen. The rectal musculature is skeletonized using ball-tipped electrosurgery. Preservation of the lower half of the rectum will allow for a larger stool reservoir and diminish

frequent bowel movements. A roticulator stapler (Autosuture, Norwalk, CT, USA) is used to close off the rectal stump and the rectum is sharply divided above the stapler (Fig. 16).

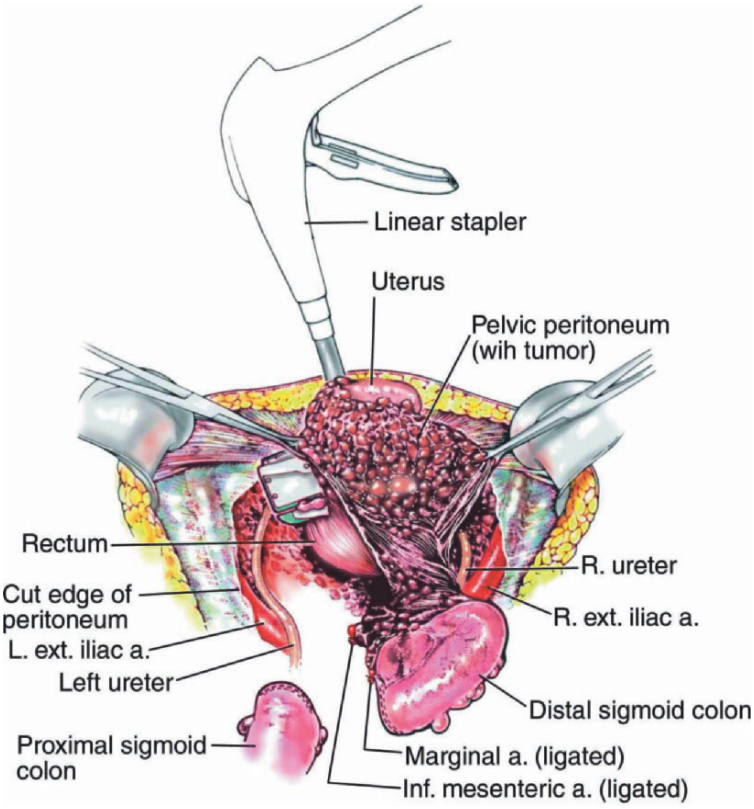


Figure 16. Resection of the rectosigmoid colon, uterus, and cul-de-sac of Douglas. Reprinted with permission from [1]

Preparation for Perioperative Intraperitoneal Chemotherapy

In females with a transection of the vagina, a loose suture repair must be performed prior to the intraperitoneal chemotherapy or large volume leakage may occur. This is the only suture repair performed prior to the intraperitoneal chemotherapy treatments. Four closed-suction drains are placed through the abdominal wall to lie in the right upper quadrant, left upper quadrant, right side of the pelvis and left side of the pelvis. A Tenckhoff catheter is positioned within the mid-abdomen. All tubes and drains are secured at the skin with a purse string stitch to prevent leakage of chemotherapy solution.

If hyperthermic intraoperative intraperitoneal chemotherapy is used, temperature probes are secured to the inflow catheter (Tenckhoff catheter) and to a remote site. The inflow catheter (maximal hyperthermia) may be placed at a site at high risk for cancer recurrence to maximize cytotoxicity at this site. All bowel anastomoses and repair of seromuscular tears are performed after the hyperthermic intraoperative intraperitoneal chemotherapy has been completed.

Discussion

An important concept in the modern treatment of malignancy is dose intensity. In the combined treatment, the maximal effects of surgical cytoreduction plus maximal effects of chemotherapy cytoreduction occur at the same time and at the same anatomic location. This results in maximal dose intensity and treatment success in selected patients with peritoneal surface spread of cancer.

Surgical attempts to cure peritoneal carcinomatosis have never been successful in the past. Palliative attempts to remove even limited quantities of peritoneal carcinomatosis always have resulted in a rapidly recurring confluence of tumour within the abdominal cavity. Also, intraperitoneal chemotherapy alone has been singularly unsuccessful in treating large volumes of intraabdominal cancer. Only when the combined treatments are used have treatment successes been reported.

Pelvic peritonectomy may be the most frequently performed procedure. It may be used in the treatment of primary ovarian malignancy with peritoneal spread. Also, advanced rectal and rectosigmoid colon cancers with full-thickness penetration of the bowel wall and peritoneal seeding in the pelvis should have a pelvic peritonectomy. If a large volume of grade 1 cancer is present within the abdomen, the pelvis often has the largest volume of disease.

The right and left upper quadrant peritonectomy also is frequently required in appendiceal, colon, and ovarian cancer patients. Lymphatic stomata (large peritoneal pores) exist on the undersurface of the diaphragms. These open lymphatic channels draw tumour cells to the superficial layer of the diaphragm's undersurface. These tumour cells then grow as a sheet of cancer adherent to the undersurface of the hemidiaphragm. As tumour beneath the diaphragm progresses, this malignancy may involve the dome of right or left lobes of the liver. Complete removal of this tumour requires stripping of the undersurface of the diaphragm and a dissection of Glisson's capsule away from liver parenchyma.

Perhaps the most difficult peritonectomy is the lesser omentectomy with stripping of the omental bursa. Vital structures here are of great density and mistakes in dissection can lead to life-endangering hemorrhage or severe damage to the liver. The left hepatic artery is the most commonly traumatized vessel. Also, loss of the left gastric artery may result in the need for total gastrectomy. Ligation of the left gastric vein may cause gastric portal hypertension when all other venous

drainage of the stomach is removed by dissection around this organ. The left hepatic vein or left inferior phrenic vein are thin-walled and may be damaged inadvertently by sudden and unpredictable diaphragmatic contractions stimulated by electrosurgical dissection near the crus of the right hemidiaphragm.

Changes in the use of chemotherapy in patients with peritoneal surface malignancy are occurring and show favourable results of treatment. A change in *route* of drug administration has occurred. Chemotherapy is given intraperitoneally. In this new strategy, intravenous chemotherapy alone is rarely indicated. Also, a change in *timing* has occurred in that chemotherapy begins in the operating room and may be continued for the first five postoperative days. Third, a change in *selection* criteria for treatment of cancer has occurred, with the nonaggressive peritoneal surface malignancies most likely to benefit from this approach. The lesion size of residual peritoneal implants following cytoreduction is of crucial importance. In patients with invasive cancer only small intraperitoneal tumour nodules that have a limited distribution within the abdomen and pelvis are likely to be eradicated. Meticulous cytoreductive surgery is necessary prior to the intraperitoneal chemotherapy instillation. Aggressive treatment strategies for an invasive intraperitoneal malignancy with large volume residual disease after debulking will not produce long-term benefits, and are often the cause of excessive morbidity or mortality. The initiation of treatments for peritoneal surface malignancy must occur as early as is possible in the natural history of these diseases in order to achieve the greatest benefits. A great change that now needs to occur with peritoneal surface malignancy is a change in oncologists' attitudes toward these diseases. They may be cured if treated with early application of combined treatments.

References

1. Sugarbaker PH (2003) Peritonectomy procedures. Surg Oncol Clin N Am 12:703-727
2. Carmignani CP, Sugarbaker TA, Bromley CM, Sugarbaker PH (2003) Intraperitoneal cancer dissemination: Mechanisms of the patterns of spread. Cancer Metastasis Rev 22:465-472
3. Sugarbaker PH (1996) Laser-mode electrosurgery. In: Sugarbaker PH, editor. Peritoneal Carcinomatosis: Principles of Management. Boston: Kluwer; p. 375-385
4. De Lima Vazquez V, Sugarbaker PH (2003) Xiphoidectomy. Gastric Cancer 6:127-129
5. De Lima Vazquez V, Sugarbaker PH (2003) Total anterior parietal peritonectomy. J Surg Oncol 83:261-263

Continuous Peritoneal Perfusion: Techniques, Methods and Applications

MH Dahlke, HJ Schlitt, P Piso

Introduction

There is no standardized methodology for hyperthermic intraperitoneal chemotherapy. Technical parameters, such as open or closed abdomen, drugs used, temperature, perfusate volume, carrier solution, flow or duration of perfusion differ from institution to institution. However, there is some consensus on what requirements are necessary: e.g. that a complete cytoreduction should be achieved prior to chemohyperthermia, that target intraperitoneal temperature should be 39° to 42° Celsius and that the duration of chemoperfusion should be 60 min to 120 min (Peritoneal Surface Oncology Group Consensus, Ann Surg Oncol 2006 - in press).

Whether an open or closed method for the hyperthermic intraoperative chemotherapy is used, is still based on the surgeon's personal preference. However, techniques with open abdomen have the advantage of a homogenous intraabdominal temperature and diffusion of the perfusate to the areas at risk, those with closed abdomen that of low contamination risk with safe environment.

Methods of Hyperthermic Intraperitoneal Chemotherapy

Major advantages compared to normothermic early postoperative chemotherapy make hyperthermic intraperitoneal chemotherapy (HIPEC) a highly promising approach. First, active concentrations of the chemotherapeutic drug in the region of interest are significantly higher than in normothermic chemoinstillation without the use of a continously working pump. Compared to systemic chemotherapy the benefit is even higher with a reduced severity of side effects at equal local drug concentrations. Secondly, hyperthermia increases the depth of tissue penetration of the drug from 1-2 mm to 3-4 mm. In addtion, hyperthermia has an intrinsic cytotoxic effect, which works synergistically with the cytotoxicity of the chemotherapeutic. This synergistic effect has been shown to increase the drug effect beyond the limit of tumours formerly classified as chemoresistant (as shown for

cisplatin). The homogenous distribution of the HIPEC-solution under direct control of the surgeon is another major benefit.

In the following the technical aspects of HIPEC are discussed in respect to the most important features of the procedure: basic method, cytotoxic agent, temperature, fluid volume, carrier solution, duration, and flow.

Open Perfusion Methods

The HIPEC procedure is carried out before closing the abdominal cavity. A complete macroscopical cytoreduction must have been achieved by parietal and visceral peritonectomy prior to HIPEC. The most frequently performed *open procedure* was established by Paul Sugarbaker at the Washington Cancer Institute in the 1980s. He introduced the so-called *Coliseum technique* [10], which uses a plastic foil that is attached to the abdominal fascia by sutures (Fig. 1).

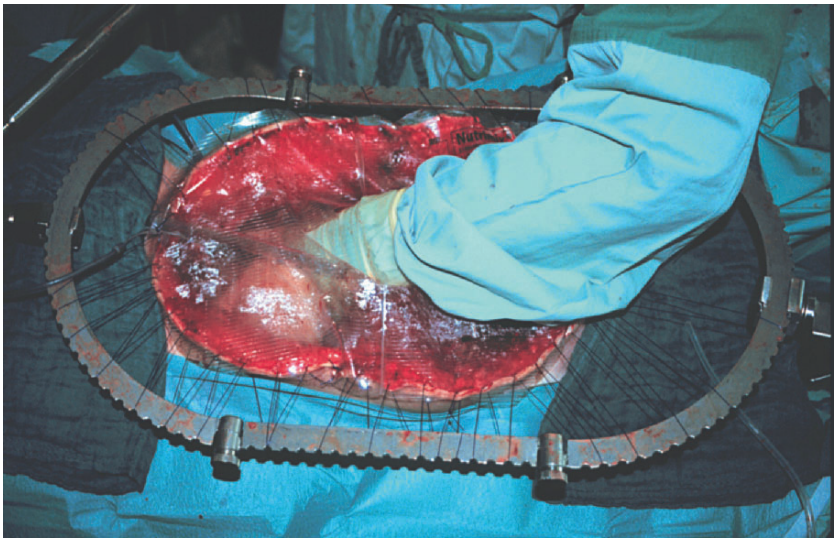


Figure 1. Open HIPEC technique with manual distribution of the perfusate

The foil is opened in the midline to allow the surgeon's hand to be introduced into the abdominal cavity while the chemotherapeutic is circulated continously by a roller pump into the abdominal cavity. Tubes and drains needed for the chemotherapy are positioned into the peritoneal space (one or two inflow catheters, which are placed by the surgeons hand in all quadrants and three out-flow catheters, usually placed into the right and left subphrenium and the small pelvis). A major advantage of the open technique is a homogeneous distribution of the drug under direct visual control of the surgeon. The technique combines a mechanical effect of removing cells from wound surfaces with a chemical effect of the

cytostatic agent that is used. Cancer cells from surfaces that would otherwise not be accessible are therefore mechanically released. This is especially important for areas at risk of recurrence, such as the small intestine, the mesentery or fascial margins. In the original Coliseum technique, the stumps of the intestine are spilled by the perfusate and the anastomoses are performed at the end of the procedure. However, many European surgeons perform the anastomoses prior to HIPEC. At the end of the procedure, all abdominal areas are inspected and than the abdominal wall closed. Drainage tubes remain in the abdomen for additional postoperative normothermic intraperitoneal chemotherapy.

A variation of the last year is a new containment instrument for the delivery of heated chemotherapy which has been developed by Sugarbaker [11]. It is a modification of the "Coliseum Technique". The instrument is placed few cm above the skin of the anterior abdominal wall. The skin is sutured to the instrument and transforms the abdomen into a reservoir for chemotherapy solution. A steel lid permits an access to the abdominal cavity by the surgeons hand and allows a manual distribution under direct visual control in all areas. The components of this instrument are covered by a disposable drape. The instrument has for that reason an extremely low risk of leakage with contamination.

Another open technique, which is comparable to the coliseum technique, uses a *Peritoneal cavity expander* [6]. This is an acrylic cylinder with a spindle-shaped cross section, attached to the abdominal wound. This waterproof device is sutured to the fascia and the peritoneal cavity is perfused with heated chemotherapeutic solution via two drains placed inside the peritoneal cavity expander. The perfusion is performed with the roller pump and the temperature is achieved with a heat exchanger, similar to the open Coliseum technique described above. The extended abdominal cavity can be filled up with higher volumes of chemotherapy solution in this manner allowing the entire small intestine to be surrounded by fluid. This technique has been used in the 1980s in particular in Japan, less in Europe, and has been more and more replaced over the last decade by the Coliseum technique.

Partially Closed and Closed Perfusion Methods

Safety environmental aspects are an argument for the use of *closed HIPEC techniques* that start chemoperfusion after the closure of the abdominal cavity. Neither contamination of the operating room nor a direct exposure to the personnel involved occurs when certain safety precautions are taken. Moreover, the closed technique does not require the surgeon to continuously distribute the chemosolution within the abdomen.

There are two variations of the "non-open" methods. In the first one, only the skin is sutured. The fascia remains open and is in contact with the chemotherapy solution. The anastomoses of the intestinum are not performed yet. This is the so-called *"partially closed"* technique. After HIPEC, the abdomen is reopened, anastomoses performed and finally the abdominal wall is closed.

The second variation is the *"closed"* technique. In this technique intestinal anastomoses are performed prior to HIPEC, the abdominal wall closed (skin and fascia) and the abdomen is not reopened at the end of the procedure.

For both techniques one or two inflow-catheters are placed below the epigastrium between small bowel loops and three outflow catheters are positioned in the Douglas pouch, subhepatically, and right and left subphrenically. A variation consists in having two inflow catheters into the right and left subphrenium and two outflows catheters along the right and left paracolic rim into the small pelvis (used in particular by US centers). Temperature probes are placed next to the inflow-catheter and in those regions, which are thought to be especially 'cold' during hyperthermic perfusion (the subphrenic left and paracolic right space). Up to 5 liters of fluid carrier solution are used to fill the abdominal cavity before the circulation of this fluid is started. A roller-pump and a heater device allow for continous distribution at 42° C within the abdominal space.

The chemotherapeutic agent is added to the circulating solution when optimal flow and heat parameters are established. After adding the chemotherapeutic the heated circulation is maintained for at least one additional hour. During the time of perfusion the operating table is moved in all possible directions to guarantee optimal distribution of the heated agent within the abdomen. Discontinuous clamping of the outflow-catheters can prevent the establishment of single "flow-routes". Moreover, intermittent strong and repeated manual pressure of the anterior abdominal wall can support the distribution of the intraabdominal fluid. Closed perfusion may have a disadvantage with regard to the risk of enteric fistulas. In a study of 200 patients treated by the open technique, the rate of fistulas was 4.5%. In 216 patients treated by the closed technique, 6.5% had fistulas [3,8], which is higher. However, the difference is small and the patient collectives were not tested on comparability.

Advantages of open abdomen and manual distribution of heated chemotherapy are the possibility of avoiding excess hyperthermia in some regions, improved distribution into all abdominal areas, reduced incidence of small bowel fistula and anastomotic leak. The pleural space can also be perfused if the diaphragm has been incidentally opened during surgery.

Although no clear data are availabe, the closed technique may bear the risk of suboptimal distribution of the drug in the abdominal cavity. However, since the handling of the chemotherapeutics is much easier, the risk of contamination is lower for the operating staff and the man-power needed for the procedure is lower, closed HIPEC procedures are used by an increasing number of centers. A prospective randomized trial to answer this question would be very difficult to perform, as other issues have to be investigated by priority, e.g. new cytostatic drugs.

Elias et al. investigated different techniques of chemohyperthermia after complete cytoreduction in prospective fashion [1,2]. Seven procedures were tested in 32 patients, mainly based of four variations: closed abdominal fascia, closed abdominal skin with non-sutured fascia, peritoneal cavity expander technique, and coliseum technique. During the procedure thermal homogeneity was monitored by six probes, and methylene blue was added to the perfusate to assess spatial diffusion

in the peritoneal cavity. Among all techniques, the coliseum one was the best, allowing optimal temperatures and diffusion of chemotherapy to all areas. The closed procedures did not allow adequate treatment of all surfaces at risk and it was impossible to obtain thermal homogeneity if the abdominal wall has been closed. However, the distribution improved if only the skin was closed. The peritoneal cavity expander did not permit treatment of the wound margins.

Safety Considerations for HIPEC

Stuart et al (2002) investigated the open technique with respect to safety for the personnel in the operating room. Large volumes of air were suctioned from 5 to 35 cm above the skin edge. Urine from surgeon and perfusionist were assayed. The permeability of the gloves used for the manipulation of the viscera was assayed.

The analysis of all samples of operating room air and urine from perfusionist and surgeon showed no detectable levels of mitomycin C. The least permeable gloves leaked a mean of 3.8 parts per million over 90 minutes. The authors concluded that no detectable safety hazard to the surgeon or other operating room personnel could be demonstrated. However, droplets of chemotherapy solution may escape into the operating room environment and complete elimination all risks may not be possible.

Spanish authors reviewed the literature with regard to HIPEC [4]. They recommend guidelines for safe administration of the chemotherapy: restriction of personnel inside the operating room, air conditioning with efficient HEPA filters, smoke evacuators, high power filtration mask and eye protection for the operating staff, absorbent towels, protective barrier garments, protective disposable impervious gowns and Latex gloves that are changed every 30 min.

The safest method is clearly the closed technique. However, at least theoretically, it may not use the full benefit of hyperthermic intraperitoneal chemotherapy.

The HIPEC devices that are necessary to perform a continuous hyperthermic intraperitoneal chemotherapy are usually ‘homemade’ from different cardiovascular devices modified in order to enable the irrigation of the peritoneal cavity [15]. Many of the cytostatic drugs that are used for the hyperthermic intraperitoneal chemotherapy at present are not approved specifically for intraperitoneal application for the treatment of peritoneal carcinomatosis. The European community has recently approved several commercial heater circulators for hyperthermic intraperitoneal administration. Some of these devices can also be used for the chemohyperthermic perfusion of limbs or intraabdominal organs.

Technical Parameters of Hyperthermic Chemoperfusion

Cytostatic Agents Suitable for HIPEC

Several agents have been used for intraperitoneal chemotherapy. Most often substances with high molecular weights have been used to increase the degree of intraperitoneal persistence of the fluid and favourable pharmacokinetic behaviour of the drug. Most of these agents can also be used together with hyperthermia. The most commonly used substances are mitomycin C, cisplatin, doxorubicin, oxaliplatin, and irinotecan. The use of mitoxantron, gemcitabine, carboplatin, docetaxel, etoposid, ciclophosphamid and melphalan is uncommon for HIPEC. Possible substances for normothermic intraabdominal chemotherapy include 5-FU and paclitaxel.

The most frequent used drug for HIPEC is mitomycin C. There are two strategies described: either use of a moderate dose e.g. 20 mg/m^2 or perform of early postoperative intraperitoneal chemotherapy with 5-FU; or use of 35 mg/m^2 without postoperative chemotherapy. Mitomycin C is used in particular for colorectal cancer and for pseudomyxoma peritonei.

Although used by some groups for gastrointestinal malignancy, cisplatinum is part of HIPEC mainly for ovarian cancer, gastric cancer or malignant mesothelioma. It has many pharmacological advantages for the intraperitoneal route and a good heat synergism. The described dosages vary from 50 to 250 mg/m^2. Cisplatinum can be combined with doxrubicin, a very attractive drug for the intraperitoneal route as it accumulates into the tumour nodules during HIPEC. However, the total amount of docxorubicin should not exceed 15 mg/m^2 due to an extensive peritoneal fibrosis that may occur [12].

Over the last year, in particular supported by French data, oxaliplatin and irinotecan seem to be suitable for HIPEC after complete cytoreduction in patients with peritoneal carcinomatosis from colorectal cancer. Elias reported the simultaneous administration of high dose oxaliplatin 460 mg/m^2 and irinotecan 400 mg/m^2 intraperitoneal in combination with 5-FU 400 mg/m^2 i.v. [2].

The HIPEC procedure is shortened to 30 min. and long term results described until now are encouraging. However, the whole procedure has a high Grade III and IV bone marrow toxicity with 58% of the patients presenting leucopenia.

Intraperitoneal Temperature

Hyperthermia increases cytotoxicity of chemotherapeutic agents and drug penetration into the tissue [7]. It also acts cytotoxic in itself and inhibits mechanisms of repair. Clinical and experimental data indicate that malignant cells are selectively destroyed by hyperthermia in the range of 41° C - 43° C. With every degree centrigrade less, efficacy is reduced. However, more than 44° C can produce

coagulation necrosis and bowel leakage. This is why a continuous temperature monitoring during HIPEC is necessary.

It is recommendable that an intraperitoneal temperature of 42° C is reached before the cytostatic agent is added to the carrier solution. Whatever technique is used, temperature probes have to be placed at the inflow catheters and at least in the small pelvis and upper abdomen. Intraperitoneal temperature has to be continuously monitored in order to maintain an optimum of heat distribution. However, the central temperature should be also monitored, e.g. with an esophageal temperature probe, as an increase of 1.5° C - 2.5° C is to be expected.

Carrier solution

Different carrier solutions have been investigated, e.g. isotonic solutions, dextrose 1.5% or NaCl 0.9%. The main disadvantage is the rapid resorption from the abdominal cavity. This can be diminished if a dialysis solution is used.

Of note, oxaliplatin must not be combined with chlorine and therefore the HIPEC carrier solution is limited to dextrose 5%. Both the surgical and anesthesiological teams should anticipate severe hyperglycemia and associated hyponatriemia in these patients by continuous infusion of insulin during the chemoperfusion.

Hypotonic solutions may lead to diffuse bleeding and should be avoided. Recent data show the superiority of hetastarch 6% solution [5]. The authors added paclitaxel to the carrier solutions and could demonstrate that hetastarch increased the exposure of peritoneal surfaces to paclitaxel by increasing the volume of solution with no decrease in drug concentration. However, these data refer to early postoperative intraperitoneal chemotherapy.

Volume

In a study from Sugarbaker et al. a marked variation in total amount of drug reaching the plasma compartment was noted when different volumes of chemotherapy were used [14]. They found that the systemic effect of chemotherapy is less marked for 2 L compared to 6 L carrier solution. As the volume changes the toxicity profile it is recommendable to base the volume of chemotherapy solution on the patient's body surface area. It seems that a volume of 1.5 L/m^2 is appropriate.

Duration

HIPEC is usually performed for 60 to 90 min. However, centers using very high doses of chemotherapy (oxaliplatin) combined with i.v. route perform HIPEC for only 30 min. On the other hand, some Asian centres reported a duration of 120 min. The majority of the cytostatic agents (up to 90%) would be expected to be

resorbed systemically after this time interval, so that longer procedures do not seem to be justified.

Flow rate

An optimal flow of the cytostatic solution should enable extensive contact with all areas at risk. In the literature, flow rates from 500 to 3,000 ml/min have been described. It depends on the device that is used, if an optimum of heat can be reached intraperitoneally by increasing or reducing the flow. This is different for open procedures as compared to close one.

Conclusions

Hyperthermic intraperitoneal chemotherapy is part of a multimodality treatment for peritoneal surface malignancies. Complete surgical cytoreduction has to be achieved prior HIPEC. Several Phase II trials and one Phase III trial demonstrated superiority of the whole treatment concept as compared to systemic chemotherapy. No less than for colon cancer with peritoneal carcinosis there are data suggesting that this should be the new standard of care in selected patients. At present, a wide spectrum of variations regarding the technique and drugs used is observed in different institutions. However, the “open” technique seems to have the most theoretical advantages while the “closed” technique shows more safety for the environment in the operating room. Published data indicate that the optimal intraperitoneal temperature is 41° C - 42° C. New drugs, e.g. oxaliplatin and irinotecan are used for HIPEC and bidirectional treatment concepts (i.v. and i.p. administration) have to be considered for the future. Main purpose for the future should be to standardize HIPEC techniques worldwide and answer still open questions like optimal duration, volume, fluid, drugs, or carrier solution.

References

1. Elias D, Antoun S, Goharin A et al. (2000) Research on the best chemohyperthermia technique of treatment of peritoneal carcinomatosis after complete resection. Int J Surg Investig 1:431-439
2. Elias D, Matsuhisa T, Sideris L, G. Liberale, L. Drouard-Troalen, B. Raynard, et al. (2004) Heated intra-operative intraperitoneal oxaliplatin plus irinotecan after complete resection of peritoneal carcinomatosis: pharmacokinetics, tissue distribution and tolerance. Ann Oncol 15:1558-1565
3. Glehen O, Osinksy D, Cotte E et al. (2003) Intraperitoneal chemothyperthermia using a closed abdominal procedure for the treatment of peritoneal carcinomatosis: Morbidity and mortality analysis of 216 consecutive procedures. Ann Surg Oncol 10:853-869

4. Gonzalez Bayon L, Gonzalez Moreno S, Ortega Perez G. (2006) Safety considerations for operating room personnel during hyperthermic intraperitoneal chemotherapy perfusion. Eur J Surg Oncol 32(6):619-624
5. Mohamed F, Marchettini P, Stuart OA, Yoo D, Sugarbaker PH. (2003) A comparison of hetastarch and peritoneal dialysis solution for intraperitoneal delivery. Eur J Surg Oncol 29:261-265
6. Shido A, Ohmura S, Yamamoto K et al. (2000) Does hyperthermia induce peritoneal damage in continous hyperthermic peritoneal perfusion? World J Surg 24:507-511
7. Sticca RP, Dach BW. (2003) Rationale for hyperthermia with intraoperative intraperitoneal chemotherapy agents. Surg Oncol Clin N Am 12:689-701
8. Stephens AD, Alderman R, Chang D et al. (1999) Morbidity and mortality of 200 treatments with cytoreductive surgery and hyperthermic intraoperative intraperitoneal chemotherapy using the Coliseum technique. Ann Surg Oncol 6:790-796
9. Stuart OA, Stephens AD, Welch L, Sugarbaker PH. (2002) Safety monitoring of the Coliseum technique for heated intraoperative intraperitoneal chemotherapy with Mitomycin C. Ann Surg Oncol 9:186-191
10. Sugarbaker PH, Averbach AM, Jaquet P et al. (1996) A simplified approach of hyperthermic intraoperative intraperitoneal chemotherapy using a self-sis: retaining retractor. In: Sugarbaker PH, editor, "Peritoneal carcinomato-Principles of management" Boston: Kluwer, p414-421
11. Sugarbaker PH. (2005) An instrument to provide containment of intraoperative intraperitoneal chemotherapy with optimized distribution. J Surg Oncol 2005,92:142-146
12. Sugarbaker PH, Mora JT, Carmignani P, Stuart OA, Yoo D. (2005) Update on Chemotherapeutic Agents Utilized for Perioperative Intraperitoneal Chemotherapy. Oncologist 10:112-122
13. Sugarbaker PH, Stuart OA, Yoo D. (2005) Strategies for management of the peritoneal surface component of cancer: cytoreductive surgery plus perioperative intraperitoneal chemotherapy. J Oncol Pharm Practice 11:111-119
14. Sugarbaker PH, Stuart OA, Carmignani PC. (2006) Pharmakokinetic changes induced by the volume of chemotherapy solution in patients treated with hyperthermic intraperitoneal mytomycin C. Cancer Chemother Pharmacol 57:703-708
15. Sugarbaker PH, Clarke L. (2006) The approval process for hyperthermic intraoperative intraperitoneal chemotherapy. Eur J Surg Oncol 32(6):637-643

Handling of Chemotherapeutic Drugs in the OR: Hazards and Safety Considerations

T Kiffmeyer, C Hadtstein

Introduction

Chemotherapeutic drugs (CD) are hazardous agents and effective precautions have to be taken in order to minimize workers exposure. This applies to all personnel who handle hazardous drugs as well as employees who work in the respective areas. According to European Guidelines (Corrigendum to Directive 2004/37/EG), any use of carcinogenic, mutagenic or teratogenic substances, including the application in health care settings, are assigned to the highest risk level.

The situation during intraoperative chemotherapy (IC) is a special case since the exposure risk is rather high compared to other work places and because many protective measures, particularly most of the technical equipment, cannot be applied. Contamination of the workplace and uptake of the active compounds by exposed individuals often remains unnoticed since most CDs are color-, odor- and tasteless. Most antineoplastic agents are not caustic, some are irritant or allergenic but the majority of acute toxic effects, such as skin irritation, nausea or vomiting only occur after high dosage uptake. However, serious damages such as mutagenic, teratogenic and carcinogenic effects remain concealed for up to decades. These effects are not dose dependant and no threshold values can be defined. Thus best effort must be made to prevent constant occupational exposure even to very little concentrations.

Within this chapter we will discuss sources and routes of contamination and analyze the special situation during handling of chemotherapeutic drugs in the OR. Based on well proven concepts from other areas of application, recommendations for precaution measures will be given, especially considering the *feasibility* in the OR. Although the different national regulations will not be discussed here, it has to be pointed out that the persons in charge have to ensure that all national regulations in force are observed.

Exposure and Effect Studies

There are numerous studies on biological and especially ambient monitoring of chemotherapeutic drugs in pharmacies and on hospital wards, showing that despite high safety standards, contamination frequently occurs [1-17]. For a good summary see [17,18]. Traces of the pharmaceutical compounds can be found particularly in the direct working area (safety cabinet, work tops, floor), on frequently touched equipment (switcher, handles, telephone receivers, computer keyboards), on drug vials and ready prepared applications as well as in areas where drugs and contaminated waste is stored. In some studies spread of active compounds into adjacent rooms was also detected. Work clothes and gloves have been found to be contaminated as well as bed linen and patient clothing.

In recent investigations, CDs have been detected on unprotected skin (forehead) and beneath the safety clothing of oncology nurses [19-21]. Determination of the active drug or metabolites in urine (biological monitoring) of exposed individuals has been carried out less frequently. The results show inner exposure in part of the staff, sometimes even without obvious contact to the respective compound [13,14].

Many studies have used biological effect monitoring in order to measure and assess early biological effects caused by absorption of CDs. Several biological endpoints established in occupational medicine have been employed e.g. urinary mutagenicity, chromosomal aberrations, sister chromatid exchanges, micronuclei induction, DNA damage, HPRT mutations, and thioether excretion [3,4,22-27]. However, the correlation between these parameters, occupational exposure and analytical detectable uptake of the compounds seem to be limited and superposed by confounders.

In contrast, only a limited number of epidemiological and few case reports address the occupational cancer risk related to CDs. An increased risk of leukemia among oncology nurses and physicians who handled antineoplastic agents has been reported [28,29]. An elevated risk was also found especially for long-term pharmacy personnel [30]. Sessink et al. have calculated the risk of excess cancer in workers exposed to cyclophosphamide based on data from ambient and biological monitoring [31].

There are incidences for teratogenic and reproductive toxic effects in exposed personnel. In a recent review of 14 studies 9 showed some positive association [32]. The major reproductive effects found were increased fetal loss, congenital malformations depending on the length of exposure, low birth weight, congenital abnormalities, and infertility. In another review, an association was identified between exposure to chemotherapy and spontaneous abortions, but not to congenital malformations and stillbirths [33].

However, very little is known about the situation in the OR, although the problem has been identified and addressed in recent publications [34-37]. In two studies [38,39] no detectable amounts of mitomycin were found in air of the OR and in urine of workers exposed during HT intraoperative treatment with this compound. However, these findings may be the result of some limitations in the sampling

procedures applied and the fact that only small part of incorporated mitomycin is excreted with the urine [40].

Routes and mechanisms of exposure

Workers can be exposed to CD by way of inhalation, ingestion, skin and eye contact and also by direct injection (e.g. needle stick injuries). Monitoring studies as well as laboratory experiments and modeling suggest that direct skin contact is the main route of absorption [41]. However, other pathways may significantly contribute to overall uptake of hazardous compounds.

Inhalation of Airborne Compounds

Airborne contamination possesses a significant risk because once released the compound is distributed rapidly and normally undetected. Hazardous drugs may be inhaled as small solid or liquid particles as well as in gaseous form. Formation and incorporation of airborne particles have been considered as one major exposure factor [18]. We experimentally determined the vapor pressure of five frequently applied antineoplastic substances according to standard procedures [42]. Carmustine, cisplatin, cyclophosphamide, etoposide, and fluorouracil were examined at 20° C and 40° C. From this, the equilibrium concentrations of the respective compounds in air have been calculated, which is the maximum value that can be reached under optimum conditions for evaporation.

Table 1. Vapour pressure and equilibrium concentration [10]

Substance	**Measured Vapour pressure [Pa]**		**Calculated equilibrium concentration [mg/m^3]**	
	T=20° C	T=40° C	T=20° C	T=40° C
Carmustine	0.019	0.530	1.7	44
Cisplatin	0.0018	0.0031	0.22	0.36
Cyclophosphamide	0.0033	0.0090*	0.36	0.90
Etoposide	0.0026	0.0038	0.63	0.86
5-Fluorouracil	0.0014	0.0039	0.08	0.20

*Extrapolated

Measurements in pharmacies have shown gaseous cyclophosphamide concentrations in the ng/m^3 range in the exhaust air of the safety cabinet and close to the working aperture [10]. Conner et al. investigated the ability of selected antineoplastic agents to vaporize at 23° C and 37° C, determining the airborne mutagenicity by a bacterial assay. Carmustine, cyclophosphamide, ifosfamide, thiotepa and mustard demonstrated vaporization at 37° C. Doxorubicin, cisplatin, etoposide,

5-fluorouracil and mitomycin were not detected as producing a mutagenic gas phase in this assay [43].

The special conditions of HIPEC with solutions being heated up to 40° C - 45° C increases the vapour pressure and allows certain substances, such as mitomycin, to evolve their characteristic odor. It is important to state, that for the actual formation of a gas phase the rate of evaporation is even more important then the vapor pressure. The evaporation time is strongly dependent on the surface to mass ratio, i.e. the particle size.

Table 2. Evaporation time for particles of selected chemotherapeutic drugs [10]

Substance	**Calculated evaporation time [s]**	
	Particle diameter = 1 µm	Particle diameter = 100 µm
Carmustine	12	1.2×10^5
Cisplatin	110	11×10^5
Cyclophosphamide	44	4.4×10^5
Etoposid	51	5.1×10^5
Fluorouracil	210	21×10^5

Consequently, the formation of large numbers of small particles, especially droplets, e.g. during withdrawing or de-aeration of syringes is the main risk factor for drug evaporation. During intraoperative chemotherapy, leaks in over pressure systems may result in sudden release of drug containing aerosols. Spread of the active substance over large surfaces, e.g. caused by improper cleaning procedures also accelerates the process. Although comparatively large volumes of drug containing solutions are handled, if formation of aerosols is avoided, HIPEC is not expected to generate very high airborne concentrations. However, especially when open perfusion methods are used, there still is a significant lack of knowledge on airborne drug concentrations in the operating room and further investigations are required to finally assess the risk related to this route of exposure.

Ingestion and Eye Contact

Oral incorporation usually does not result from direct uptake, but from unnoticed hand to mouth transfer. In a recent conceptual analysis, hand-to-mouth and object-to-mouth events have been identified as the primary exposure processes [44]. Direct ingestion is possible if splashes reach the mouth. This represents a less likely but yet more effective way of uptake compared to contact with normal skin. Gastrointestinal uptake is a minor problem compared to buccal resorption. Here again possible DNA damage is of greater importance than direct intoxications.

Eye contact with hazardous drugs is not very likely but yet more dangerous. It can lead to local irritations as well as systematic resorption. Working with pumps and other over pressure systems increases the possibility of sudden splashes of hazardous solutions. Therefore the use of safety goggles is recommended. Absorption through eyes also can result from contact with contaminated hands or objects.

Skin Contact

Especially during intraoperative chemotherapy, direct and indirect contact of skin with the compounds is considered to be the most relevant route of exposure. Direct splashes may reach unprotected skin. But penetration through gloves and clothing is also possible under certain circumstances [19,20,45,46]. The most problematic substance in this context is carmustine. Lately the results of the Ansell Cytostatic Permeation Program have been published [47]. Different gloves of this manufacturer were investigated considering the barrier function against numerous cytostatic agents. Even after 60 min the tested gloves did not show alarming penetration. The least permeable gloves leaked a mean of 3.8 parts per million over 90 minutes.

Contact of unprotected skin with contaminated working materials, countertops, and other surfaces as well as any containers delivered from the pharmacy or the drug supplier is an often underestimated risk. Bare skin may be contaminated as well by involuntarily touching of the face with contaminated gloves and objects. Absorption of CDs through normal skin has hardly been investigated so far. However, initial studies revealed that skin penetration has to be considered [41].

Injection through Accidental Injury with Sharp Tools

Analogous to hygienical precautions to be taken when handling sharp objects such as needles and surgical blades, accidential injections must be avoided. This applies for patient treatment as well as for drug preperation; it is strongly recommended to perform drug preperation using technical devices such as safety cabinets or isolators. The use of safe-needle devices, needleless systems, dispensing pins, and closed-system devices reduces the likelihood of exposure by injection.

Recommendations for Safety Precautions

During the last two decades awareness of health risks and safety standards for handling of CDs have been improved considerably throughout the whole life-cycle of these products. This has resulted in a measurable decrease of environmental and personal contamination.

For effective protection an integrated safety concept comprising all potentially hazardous handling procedures is required. Since the work flow differs from institution to institution, the details of the program should be tailor made to the respective conditions. For optimum results all concerned groups and authorities e.g. medical and non-medical staff, cleaning personnel, head of the pharmacy, company doctor etc. should be involved.

The main elements of such a safety concept are:

- comprehensive risk assessment
- organizational measures
- use of technical and personal protective equipment
- spill management
- cleaning and decontamination protocols
- proper waste handling and disposal
- adequate information and training
- medical and analytical surveillance
- monitoring and reviewing the effectiveness of the measures

Such a risk assessment results in a prioritized list combining relative likeliness and possible consequences. All measures should aim at reducing the area and the person subgroup with potential exposure to CDs and to minimize exposure. In many cases, standards and procedures from other departments of the hospital can be considered as a starting point and can be adjusted to the specific situation in the OR. Since patients excreta and body fluids may contain significant amounts of CDs up to several days after treatment it has to be assured that precaution measures are continued during post operative treatment.

Organizational measures

Safe standard procedures for all work steps have to be elaborated as well as for critical incidents such as spillage or other accidental release. Areas of different contamination levels should be defined and outlined. With consequent changes of gloves and shoes at defined barriers, contamination will be limited to restricted areas.

When using hazardous drugs in the OR, areas can be outlined along hygienic directives, keeping in mind that in this case, the way out is more curtail than the way in. The access to the working areas has to be restricted and warning notices have to be affixed. Special care has to be taken for juveniles, pregnant and breast-feeding women, including nurses and technicians as well as housekeeping staff. If possible, these employees should be posted to a different workplace.

According to European Guidelines, any handling of CMR (Carcinogenic, mutagenic, toxic to reproduction) agents has to be documented, stating the personnel involved, the type of compounds, the working period and the exact working conditions. All documents have to be updated regularly, e.g. annually or after changes in the work routines. Hazardous properties of each handled substance should be outlined and used for staff instructions. As these data is not part of drug information, it has to be extracted from material safety data sheets (MSDSs) provided by the supplier on request or from other sources. Table 3 gives the relevant data for some pharmaceutical compounds frequently used in HIPEC today.

Table 3. Hazardous Properties of important chemotherapeutic drugs

	Risk Phrase	C	M	R	Effect of Direct Contact
Cisplatin	R45 May cause cancer R46 May cause heritable genetic damage R60 May impair fertility R61 May cause harm to the unborn child	1	1	1	Low irritant
5-Fluorouracil	R45 May cause cancer R46 May cause heritable genetic damage R60 May impair fertility R61 May cause harm to the unborn child	1	1	1	low irritant
Mitomycin C	R45 May cause cancer R46 May cause heritable genetic damage R63 Possible risk of harm to unborn child	1	1	3	necrotic
Oxaliplatin	R45 May cause cancer R46 May cause heritable genetic damage R60 May impair fertility R61 May cause harm to the unborn child	1	1	1	low irritant

CMR, Carcinogenic, mutagenic, toxic to reproduction

Use of Technical and Personal Protective Equipment

Prevention of contamination has mainly to be assured through the use of personal protective equipment. Implementation of containment devices such as biological safety cabinets or isolators used for pharmaceutical preparations cannot be realized in the OR. However, installation of a (mobile) exhausting device above the application area or the operation of the smoke evacuation system can help to reduce airborne contamination. Drug containing solutions should be handled in closed systems where possible. Before the drug containing solution is circulated, the integrity of the system can be checked with a non-toxic test-solution. No recommendation can be given based on the available data of whether covering or closing of patient's abdomen during chemotherapy provides better protection.

Adequate and well fitted personal protective equipment (PPE) should be worn consequently. Covering of bare skin is the most important measure in minimizing exposure and reducing the risk. Especially hands and arms should be covered with suitable gloves with long gauntlets (elbow-length). In addition, gowns with closed fronts, long sleeves, and elastic closed cuffs made of watertight or at least water-repellant material should be worn by all staff possibly getting in contact with the drug. Even if only small areas of clothing are drenched or damaged, clothes should be changed immediately, because soaked fabric on skin exhibits excellent absorption conditions. Considering that an immediate change of clothing is rather difficult during surgery, watertight fabric is favored. After skin contact, areas should be rinsed generously with water. Water temperature should be moderately

cold. Hot water increases the local blood flow, favoring penetration and absorption. Soap may only be used after the area has been rinsed with an excess of water, no oily skin care should be applied. Disposable gowns are favored by the Centers for Disease Control and Prevention (CDC), USA. However, treatment procedures applied to infectious laundry have shown to be successful in decontamination of contaminated clothes [49].

Further more, double gloving should be used. The outer glove should extend the cuff of the gown. Gloves made for handling of antineoplastic drugs provide good protection and should be favoured. In general the double gloving confers a high protection, using an air layer as an effective barrier. Double gloves made of different colored gloves can be recommended as the color contrast allows immediate spotting of perforations and cuts. Gloves must be changed immediately when torn, punctured, or contaminated. Considering the influence of mechanical and chemical stress to the material, which is not simulated in the respective permeability tests, it is recommended to change the gloves regularly every 30 minutes. Goggles should be worn to protect eyes from unexpected splashes. A safety shield is even more effective, protecting the whole face from direct contact with the hazardous compounds.

Surgical masks do not protect the person wearing it from airborne CDs, since they are not designed to hold back particles or vapors while breathing in. Thus they can serve only as splash protection. If the surgeon or other OR personnel seek a protection from possible inhalation of drug containing aerosols, respirator face masks must be fit tested to ensure effective filtration of air. Filtering half masks (Filtering Face Piece = FFP) class 3 provide the best protection against solid and liquid particles according to European Norms (EN 149). As mentioned above, the usage of overshoes or special shoes used exclusively in the direct working area is recommended to prevent wide spreading. In compliance with hygienic standards, shoes should be closed and easy to clean.

Spill Management, Cleaning and Decontamination Protocols

During intraoperative chemotherapy, spillage of small amounts of drug containing solutions is not completely avoidable. Therefore, effective protocols and procedures for both immediate removal of larger spillage and regular cleaning are of utmost importance. Cleaning procedures normally applied in hospitals are predominantly designed to meet hygienic requirements, but often fail to remove pharmaceutical compounds from contaminated surfaces [14].

Since most antineoplastic drugs are colourless, contamination can only be detected by analytical methods. To prevent widespread and unnoticed contact with antineoplastic agents, any noticed spill should be removed immediately. Adsorbent pads and sheets as well as removable work trays should be used where possible, but have to be replaced as soon as they are wet. It is recommended that one experienced person is consigned solely to this task during the whole chemotherapeutic treatment, so that the rest of the team can focus on their work. For small amounts of drug solution, absorbent tissues, which should be at hand in sufficient

quantities, may be used to remove the liquid. Afterwards the surface should be cleaned with first an alkaline solution (1M NaOH) and afterwards with an alcohol based solvent e.g. 80% isopropanol. The wiping solution should not be applied – especially not sprayed! - onto the contaminated surface but put on the tissue in order to avoid aerosolization of the drug. When high concentrated solutions have to be removed the procedure has to be repeated several times.

For larger spillages it is recommended to hold one or more emergency kits ready. Such kits are commercially available in many countries but can easily be assembled individually. It should contain personal safety equipment for one or two persons (water-resistant long-sleeved coat, two pairs of gloves, goggles, and over-shoes), gripper, tissues, a dustpan, disposal bags and tape to mark the area. In such cases, first the personal safety equipment has to be put on, and then the area of the accident should be generously outlined to prevent wide spreading by accidental contact. The liquid should be covered with tissue to absorb the spillage. Spillage of solid substances should be swabbed with wet tissue to prevent dispersion of fine particles into air. Soaked tissue and wet outer gloves are disposed into a bag which will be closed thoroughly. The gripper should be used to pick up sharp objects, without the risk of cuts and glove damage. After this first decontamination, the area should be well cleaned (s. a.).

Since spill kits probably are not used very frequently, their correct handling should be part of regular training. Because small droplets or splashes often remain unnoticed, the daily cleaning procedures have to ensure that these drug residues are removed from floors and work tops but also from furniture, door handles, light switchers etc. In cooperation with the department or company responsible for housekeeping, effective and economic procedures have to be developed to ensure that no cumulative contamination of the whole area results.

Proper Waste Handling and Disposal

A clear policy for the management of CDs containing waste should be enforced. Collection, labeling, storage, transport and disposal of contaminated waste have to follow the valid regulations in the respective country. The definition of hazardous waste is dependent upon the content of CMR substances. In most countries, all objects which have only little contact with CD are treated as domestic waste and require no specific disposal e.g. high temperature incineration. Hazardous waste is defined as items with a content of more than 3% by weight of the respective compounds in the USA and more than 20 ml in Germany.

During risk assessment, all types and amounts of waste possibly accumulating during IC (including accidents) should be listed and assigned to one of these categories. Sufficient bags and containers have to be provided for safe storage of even unexpected large volumes of contaminated material before starting IC. Double gloving should be used throughout while cleaning and discharging of hazardous waste. We recommend to seal- or zip-lock waste containing CDs in plastic bags prior to collection in disposal bins and the usage of containers with closed lids. All manipulations (congesting, stuffing, shaking etc.) which may result in release of

active compounds to surfaces or into the air have to be avoided and bags and containers should only be reopened if necessary. Finally all contaminated waste has to be sealed in approved containers which have to be labelled and handled according the respective national guidelines. Reusable equipment, e.g. pumps, should be safely stored in closed, labelled boxes or containers for transport and subsequent decontamination.

Information and Training

Adequate information and training is the most important tool in protecting workers from health risks. This is of special relevance in IR since this still is a comparatively uncommon technique and the OR personnel involved normally is not as well certified and experienced as staff from pharmacies or oncological wards. All important information and safety measures have to be communicated and discussed with those who have to implement them in their daily work. All staff working within the area where antineoplastic agents are handled have to be involved (doctors, nurses, technicians, housekeeping personnel etc.).

Important parts of regular courses are:

- properties and effects of the hazardous compounds
- legislative requirements, guidelines and regulations
- protective equipment and clothing
- instructions for waste handling, cleaning, decontamination and spill response
- safe handling procedures and sources of errors

Contents should be adjusted to the special institution and the tasks and exposure situation of the respective participants. Additional practical training, e.g. for management of spills or other hazardous situations, is very valuable. Coloured ore fluorescent solutions (e.g. quinine) can be used during simulated handling to make contamination visible. Any incidents should be discussed within the whole team as a chance for further improvement of the safety standards.

External courses for safe handling of hazardous drugs are offered by various institutions in most countries and should be available as additional source of information. Although mainly preparation and administration of CDs are addressed, many solutions and recommendations are transferable to the OR. Information and training should be provided regularly and has to be compulsory for all exposed individuals. In addition, any new staff members should receive a comprehensive briefing and training.

Medical Surveillance and Monitoring

In order to control and minimize the exposure of workers to CDs, regular measurements of ambient concentrations and medical checks of exposed employees are useful. According to revised European Guidelines [49] regular ambient monitoring must be accomplished by the employer at workplaces where CMR substances of the category 1 or 2 are handled. However, no threshold values for workplace concentration are defined.

Medical Health Surveillance

Beside very rare severe accidents, the amounts possibly incorporated at the workplace are several orders of magnitude below the therapeutic doses. Therefore direct health effects as known from cancer patients are not likely. However, the company medical officer or occupational physician should be informed about the potential exposure and adjust his examination procedures accordingly.

Again, juvenile employees, pregnant and breastfeeding women need special attention. Additional examination should be carried out after any larger exposition, in particular after accidents. In such cases collection and storage of urine (deep freezing in single batches) for subsequent chemical analysis is reasonable. Any workplace exposure record created in connection with CD handling shall be kept, transferred, and made available for at least 30 years and medical records shall be kept for the duration of employment plus 30 years.

Monitoring Programs

An ideal procedure for contamination control would need to meet several requirements - it should be sensitive, specific, quantitative, rapid, reproducible and inexpensive. In general one can distinguish biological effect monitoring on one hand, and biological and ambient monitoring of the active compounds on the other hand. As mentioned before, currently available methods of biological effect monitoring lack significance to detect uptake and health damage of CDs. As any biological monitoring cannot give information on the route of exposure, these tests are not recommended for routine use in contamination and health surveillance.

During the last ten years the analytical determination of the substances themselves has become the main tool in research and contamination control. Validated and field-tested methods for sampling and determination of most of the currently applied substances at trace levels have been developed in many countries [17,50]. These procedures can be applied in biological and ambient monitoring to determine the concentration of the respective compound on hard surfaces (worktops, floors, shelves etc.), on textiles (working clothes, gowns, gloves etc.) as well as in exposed individuals (urine, blood etc.). Because biological monitoring does not provide information about the route of exposure it should be combined with an ambient monitoring. However, it is an important tool in control of possible uptake after accidents.

While for determination of CD concentrations in air, clothing etc. comparatively complex procedures are required, wipe sampling of hard surfaces can be performed with special designed kits by the user himself. This has made wipe sampling an affordable and wide spread used monitoring instrument [51].

Determination of selected compounds at the work place by wipe sampling not only gives information on the actual exposure level but also provides information on critical steps and possible improvements as well as on the efficiency of safety measures and cleaning procedures. Regular repetitions are recommended and allow control of temporal developments. If standardized methods (same compounds, surfaces and procedures) are applied, a comparison with other drug handling institutions is possible, which provides important information in order to assess the potential occupational risk.

Altogether, many years of experiences in other drug handling institutions have shown that a serious, open discussion of the risks and the implementation of practicable protective measures not only reduces contamination, but results in a significantly improved long term workplace satisfaction.

References

1. Acampora A, et al (2005) A case study: surface contamination of cyclophosphamide due to working practices and cleaning procedures in two Italian hospitals. Ann Occup Hyg 49:611-618
2. Baker ES and Connor TH (1996) Monitoring occupational exposure to cancer chemotherapy drugs. Am J Health Syst Pharm 53:2713-2723
3. Ursini CL, Cavallo D, Colombi A, Giglio M, Marinaccio A, Iavicoli S (2006) Evaluation of early DNA damage in healthcare workers handling antineoplastic drugs. Int Arch Occup Environ Health (in press)
4. Cavallo D, Ursini CL, Perniconi B, Francesco AD, Giglio M, Rubino FM, Marinaccio A, Iavicoli S (2005) Evaluation of genotoxic effects induced by exposure to antineoplastic drugs in lymphocytes and exfoliated buccal cells of oncology nurses and pharmacy employees. Mutat Res 587:45-51
5. Connor TH, et al (1999) Surface contamination with antineoplastic agents in six cancer centers in Canada and the United States. Am J Health Syst Pharm 56:1427-1432
6. Crauste-Manciet S, Sessink PJ, Ferrari S, Jomier JY, and Brossard D (2005) Environmental contamination with cytotoxic drugs in healthcare using positive air pressure isolators. Ann Occup Hyg 49:619-628
7. Ensslin AS, Pethran A, Schierl R, and Fruhmann G (1994) Urinary platinum in hospital personnel occupationally exposed to platinum-containing antineoplastic drugs. Int Arch Occup Environ Health 65:339-342
8. Ensslin AS, Huber R, Pethran A, Rommelt H, Schierl R, Kulka U, and Fruhmann G (1997) Biological monitoring of hospital pharmacy personnel occupationally exposed to cytostatic drugs: urinary excretion and cytogenetic studies. Int Arch Occup Environ Health 70:205-208

9. Fransman W, Vermeulen R, and Kromhout H (2004) Occupational dermal exposure to cyclophosphamide in Dutch hospitals: a pilot study. Ann Occup Hyg 48:237-244
10. Kiffmeyer TK, Kube C, Opiolka S, Schmidt KG, Schöppe G, and Sessink PJM (2002) Vapour pressures, evaporation behaviour and airborne concentrations of hazardous drugs – Implications for occupational safety. The Pharmaceutical Journal 268:331-337
11. Larson RR, Khazaeli MB, and Dillon HK (2002) Monitoring method for surface contamination caused by selected antineoplastic agents. Am J Health Syst Pharm 59:270-277
12. Mason HJ, Blair S, Sams C, Jones K, Garfitt SJ, Cuschieri MJ, and Baxter PJ (2005) Exposure to Antineoplastic Drugs in Two UK Hospital Pharmacy Units. Ann Occup Hyg 49:603-610
13. Pethran A, Schierl R, Hauff K, Grimm CH, Boos KS, and Nowak D (2003) Uptake of antineoplastic agents in pharmacy and hospital personnel. Part I: monitoring of urinary concentrations. Int Arch Occup Environ Health 76:5-10
14. Schreiber C, Radon K, Pethran A, Schierl R, Hauff K, Grimm CH, Boos KS, and Nowak D (2003) Uptake of antineoplastic agents in pharmacy personnel. Part II: study of work-related risk factors. Int Arch Occup Environ Health 76:11-6
15. Sessink PJ, and Bos RP (1999) Drugs hazardous to healthcare workers. Drug Saf 20:347-359
16. Turci R, Sottani C, Ronchi A, and Minoia C (2002) Biological monitoring of hospital personnel occupationally exposed to antineoplastic agents. Toxicol Lett 134:57-64
17. Turci R, Sottani C, Spagnoli G, Minoia C (2003) Biological and environmental monitoring of hospital personnel exposed to antineoplastic agents: a review of analytical methods. J Chromatogr B 789:169-209
18. NIOSH (2004) NIOSH Alert: Preventing occupational exposure to antineoplastic and other hazardous drugs in healthcare settings. U.S. Department of Health and Human Services, Centers for Disease Control and Prevention, National Institute for Occupational Safety and Health. Prepublication copy [www.cdc.gov/niosh/docs/2004-HazDrugAlert].
19. Minoia C, Turci R, Sottani C, Schiavi A, Perbellini L, Angeleri S, Draicchio F, and Apostoli P (1998) Application of high performance liquid chromatography/tandem mass spectrometry in the environmental and biological monitoring of health care personnel occupationally exposed to cyclophosphamide and ifosfamide. Rapid Commun Mass Spectrom 12:1485-1493
20. Fransman W, Vermeulen R, and Kromhout H (2005) Dermal exposure to cyclophosphamide in hospitals during preparation, nursing and cleaning activities. Int Arch Occup Environ Health 78:403-412
21. Fransman W, Vermeulen R, and Kromhout H (2004) Occupational dermal exposure to cyclophosphamide in Dutch hospitals: a pilot study. Ann Occup Hyg 48:237-244
22. Burgaz S, Karahalil B, Canhi Z, Terzioglu F, Ancel G, Anzion RB, Bos RP, and Huttner E (2002) Assessment of genotoxic damage in nurses occupationally

exposed to antineoplastics by the analysis of chromosomal aberrations. Hum Exp Toxicol 21:129-135
23. Hessel H, Radon K, Pethran A, Maisch B, Grobmair S, Sautter I, and Fruhmann G (2001) The genotoxic risk of hospital, pharmacy and medical personnel occupationally exposed to cytostatic drugs - evaluation by the micronucleus assay. Mutat Res 18;497:101-109
24. Kevekordes S, Gebel TW, Hellwig M, Dames W, and Dunkelberg H (1998) Human effect monitoring in cases of occupational exposure to antineoplastic drugs: a method comparison. Occup Environ Med 55:145-149
25. Laffon B, Teixeira JP, Silva S, Loureiro J, Torres J, Pasaro E, Mendez J, and Mayan O (2005) Genotoxic effects in a population of nurses handling antineoplastic drugs, and relationship with genetic polymorphisms in DNA repair enzymes. Am J Ind Med 48:128-136
26. Maluf SW, and Erdtmann B (2000) Follow-up study of the genetic damage in lymphocytes of pharmacists and nurses handling antineoplastic drugs evaluated by cytokinesis-block micronuclei analysis and single cell gel electrophoresis assay. Mutat Res 20;471:21-27
27. Pilger A, Kohler I, Stettner H, Mader RM, Rizovski B, Terkola R, Diem E, Franz-Hainzl E, Konnaris C, Valic E, and Rudiger HW (2000) Long-term monitoring of sister chromatid exchanges and micronucleus frequencies in pharmacy personnel occupationally exposed to cytostatic drugs. Int Arch Occup Environ Health 73:442-448
28. Skov T, Maarup B, Olsen J, Rørth M, Winthereik H, and Lynge E. Leukaemia and reproductive outcome among nurses handling antineoplastic drugs. Br J Ind Med 1992;49:855-61.
29. Skov T, Lynge E, Maarup B, Olsen J, Rørth M, Winthereik H (1990) Risk for physicians handling antineoplastic drugs. Lancet 336:1446
30. Hansen, J and Olsen JH (1994) Cancer morbidity among Danish female pharmacy technicians. Scand J Work Environ Health 20:22-26
31. Sessink PJM, Kroese ED, van Kranen HJ and Bos RP (1993) Cancer risk assessment for health care workers occupationally exposed to cyclophosphamide. Inter Arch Occup Environ Health 67:317-323
32. Harrison BR (2001) Risks of handling cytotoxic drugs. In M.C. Perry (Ed.), The chemotherapy source book (3rd Ed), Lippincott, Williams, & Wilkins, Philadelphia, p566-582
33. Dranitsaris G, Johnston M, Poirier S, Schueller T, Milliken D, Green E, and Zanke B (2005) Are health care providers who work with cancer drugs at an increased risk for toxic events? A systematic review and meta-analysis of the literature. J Oncol Pharm Practice 1:69-78
34. Foltz P, Wavrin C, and Sticca R (2004) Heated intraoperative intraperitoneal chemotherapy-the challenges of bringing chemotherapy into surgery. AORN J 80:1055-1063
35. Gonzalez-Bayon L, Gonzalez-Moreno S, amd Ortega-Perez G (2006) Safety considerations for operating room personnel during hyperthermic intraoperative intraperitoneal chemotherapy perfusion. Eur J Surg Oncol 32(6):619-624

36. Jahne J, Piso P, Schmoll E, Haulitschek-Hauss R, Sterzenbach H, Paul H, and Pichlmayr R (1997) [Intraoperative (hyperthermic) intraperitoneal chemotherapy-considerations and aspects of safe intra- and postoperative treatment with cytostatic drugs]. Langenbecks Arch Chir 382:8-14
37. White SK, Stephens AD, and Sugarbaker PH (1996) Hyperthermic intraoperative intraperitoneal chemotherapy safety considerations. AORN J 63:716-724
38. Stuart OA, Stephens AD, Welch L, and Sugarbaker PH (2002) Safety monitoring of the coliseum technique for heated intraoperative intraperitoneal chemotherapy with mitomycin C. Ann Surg Oncol 9:186-191
39. Schmid K, Boettcher MI, Pelz JO, Meyer T, Korinth G, Angerer J and Drexler H (2006) Investigations on safety of hyperthermic intraoperative intraperitoneal chemotherapy (HIPEC) with Mitomycin C. Eur J Surg Oncol (in press)
40. Connor TH, van Balen P, and Sessink PJM (2003) Monitoring for Hazardous Drugs in the Operating Room. Ann Surg Oncol 10:821-822; reply 822-823
41. Kromhout H, Hoek F, Uitterhoeve R, Huijbers R, Overmars RF, Anzion R, Vermeulen R (2000) Postulating a dermal pathway for exposure to antineoplastic drugs among hospital workers. Applying a conceptual model to the results of three workplace surveys. Ann Occup Hyg 44:551-560.
42. Organisation for economic development and cooperation, OECD (1995) Guidelines for the testing of chemicals. 104: Vapour Pressure Curve – vapour pressure balance, Paris
43. Connor TH, Shults M, and Fraser MP (2000) Determination of the vaporization of solutions of mutagenic antineoplastic agents at 23 and 37 degrees C using a desiccator technique. Mutat Res 470:85-92
44. Cherrie JW, Semple S, Christopher Y, Saleem A, Hughson GW, and Philips A (2006) How Important is Inadvertent Ingestion of Hazardous Substances at Work? Ann Occup Hyg (in press)
45. Connor TH (1999) Permeability of nitrile rubber, latex, polyurethane, and neoprene gloves to 18 antineoplastic drugs. Am J Health Syst Pharm 56:2450–2453
46. Klein M, Lambov N, Samev N, and Carstens G (2003) Permeation of cytotoxic formulations through swatches from selected medical gloves. Am J Health Syst Pharm 60:1006-1011
47. Wallemacq PE, Capron A, Vanbinst R, Boeckmans E, Gillard J, and Favier B (2006) Permeability of 13 different gloves to 13 cytotoxic agents under controlled dynamic conditions. Am J Health Syst Pharm 63:547-556
48. Türk J, Stellwag N, Reinders M, Kiffmeyer TK, Schöppe G, and Schmidt KG (2002) Kontamination von Krankenhauswäsche mit hochwirksamen Arzneimitteln. Wie effektiv sind die derzeitigen Reinigungsverfahren? WRP 11:24-27
49. Council Directive 98/24/EC of April 7, 1998 on the Protection of the Health and Safety of Workers from the Risks Related to Chemical Agents at Work fourteenth individual Directive within the Meaning of Article 16(1) of Directive 89/391/EEC). Official Journal L 131, 05/05/1998, p11–23

50. Bos RP, and Sessink PJ (1997) Biomonitoring of occupational exposures to cytostatic anticancer drugs. Rev Environ Health 12:43-58
51. Schulz H, Bigelow S, Dobish R, and Chambers CR (2005) Antineoplastic agent workplace contamination study: the Alberta Cancer Board Pharmacy perspective. J Oncol Pharm Pract 11:101-109

Results of Cytoreduction followed by HIPEC in Carcinomatosis of Colorectal Origin

VJ Verwaal

Introduction

Stage IV colorectal cancer is defined as colon or rectal cancer with metastasis, disregarding the site of the metastasis. It traditionally includes liver metastasis, lung metastasis, bone metastasis as well as metastasis on the peritoneum. Historically, patients suffering from stage IV colorectal cancer have a 5-year survival of 10% and a median survival just beyond one year [1].

Recent studies in this field show a more promising survival with a median survival of up to 20 months reported [2]. Peritoneal carcinomatosis (PC) is a special form of stage IV colorectal cancer. The existence of PC limits the survival in stage IV colorectal carcinoma patient even further to only half a year [3].

There are many studies concerning the chemotherapeutic treatment of metastatic colorectal cancer. These studies include patients with metastases of colorectal cancer of any location. However, to enable proper response evaluation most studies include only those patients who have measurable disease. For practical reason measurements are most often made on liver metastasis or lymph nodes. Thus, the data of stage IV colorectal cancer trials are dominated by the results found in the treatment of liver and lung metastases. For patients with metastases elsewhere (including PC), the data are limited limited in these studies.

Peritoneal carcinomatosis forms an all-covering thin layer of metastasis on the peritoneum. Imaging this layer is difficult. It lacks a volume density, which means that there is only a limited amount of tumour per cubical unit. It is like a paper sheet, if one looks at it, it can be seen quite easily. But if the paper is in a box it does not fill any significant part of the volume of the box. The consequence of this is that CT scan, MRI scan and even PET scan, which detect volume-density differences, leave PC undetected until the total amount of tumour is important [4]. In fact, most cases of PC stay undetected until there is secondary evidence of its presence, e.g. bowel or urinary tract obstruction.

Peritoneal carcinomatosis is often associated with bowel dysfunction resulting from tumour deposits that grow on the visceral peritoneum causing a so-called “hosepipe” phenomenon, indicating that peristaltic movements are blocked the

encasing tumour shelf. As a consequence, many PC patients suffer from malnutrition. This malnutrition is one of the elements explaining the poor tolerance of systemic chemotherapy in PC patients, as opposed to patients with metastatic disease confined to the liver or lungs.

As detailed in chapter 7, data from retrospective studies have highlighted that the natural history of PC is characterized by a median survival of approximately half a year [5-7]. Treatment in these studies was not standardized and varied from no treatment at all to the most modern chemotherapy at that time.

The only prospective study in this field stems from an update of a randomised trial [8]. In this study 5-fluorouracil (5FU) with leucovorin was given according to the Laufmann scheme, representing the most effective therapy in stage IV colorectal cancer at the time the study was done [9]. This study resulted in a median survival of 12 months.

Surgery for Peritoneal Carcinomatosis of Colorectal Origin

In the last decennia a number of phase two studies reported the outcome cytoreduction for PC. The first results were reported by Sugarbaker et al., who described a three-year survival of 61% [10]. In the next years, results of different studies from all over the world became available showing a median survival of approximately two years (Table 1).

Table 1. Phase II studies of cytoreduction followed by HIPEC for peritoneal carcinomatosis of colorectal origin

Author	Year	Number of patients	Median survival (months)
Elias [22]	2001	64	36
Pilati [23]	2003	46	18
Shen [24]	2004	77	16
Glehen [25]	2004	53	13
Verwaal [12]	2005	117	22

There are a number of open questions in these studies. Since most studies did not state clearly which patients were entered in the analysis, the most important one is the question of patient selection. Indeed, it could be assumed that patients who underwent an incomplete cytoreduction or were not perfused were not included in the survival analysis which would indicate a selection bias.

The question of patient selection was answered in the randomised controlled trial comparing cytoreduction followed by HIPEC and adjuvant systemic chemotherapy to systemic chemotherapy [11]. In this trial mitomycin C (MMC) was used for HIPEC and 5FU - leucovorin was used for systemic chemotherapy.

5FU-leucovorin was standard therapy for systemic therapy of stage IV colorectal cancer at the time of writing of the study protocol.

In this study the patients in both treatment arms are treated with systemic chemotherapy. In the standard arm patients were treated with systemic chemotherapy in combination with palliative surgery; in the experimental arm patients were treated with cytoreduction followed by HIPEC and followed by the same systemic chemotherapy, but now in the adjuvant setting. Thus the question of the study can be rephrased to: "Does cytoreduction followed by HIPEC ad survival time to systemic chemotherapy", which was answered positively by this trial.

The inclusion for the trial was histologically proven PC of colorectal origin without evidence of systemic metastasis. There was no limitating extent of the PC burden. Survival analysis was based on the intention to treat principle. The survival results showed a median survival in the systemic chemotherapy arm of 12.6 months and of 22.4 months in the so-called "HIPEC" arm. This difference was statistically significant ($p < 0.05$) (Fig. 1).

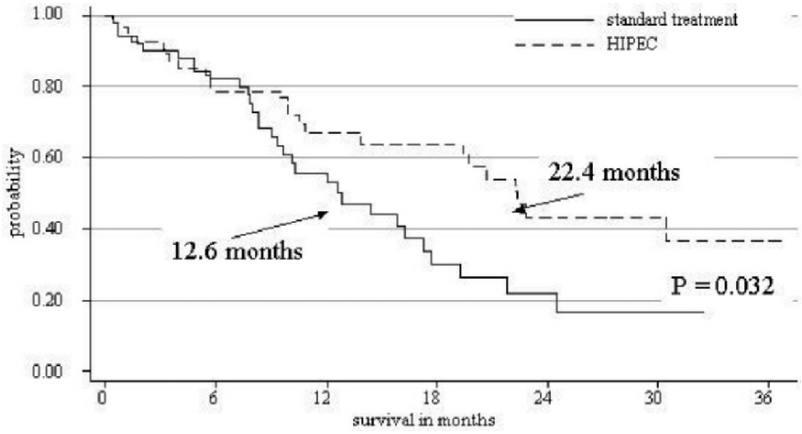


Figure 1. Overall survival in the Netherlands Cancer Institute randomized trial comparing cytoreduction followed by systemic chemotherapy (5-fluorouracil-LV) with systemic chemotherapy alone in patients with peritoneal carcinomatosis of colorectal origin

Long-term results of cytoreduction and HIPEC are now showing even better results. Five-year survival above 40% is seen in patients who underwent a 'complete' cytoreduction [12] (Fig. 2).

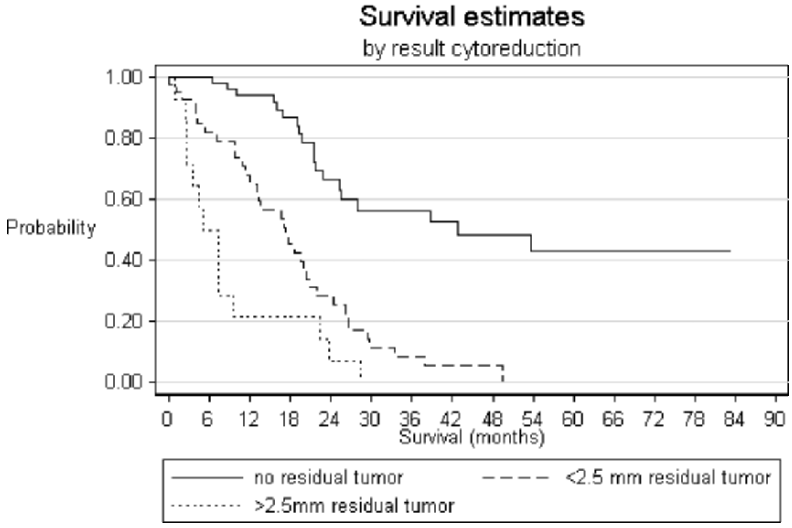


Figure 2. Survival according to residual tumour after cytoreduction with HIPEC. Reprinted with permission from [12]

Prognostic Factors

Completeness of Cytoreduction

There are many known prognostic factors predicting the outcome of cytoreduction followed by HIPEC. The completeness of cytoreduction is usually seen as the most important factor predicting outcome. The completeness of cytoreduction can either be scored in the CC score [13] or by the Dutch modification of the IUCC / AJCC R classification [14]. The CC score varies from CC-0 to CC-3. Score CC-0 indicates that there is no *macroscopic* tumour left behind, CC-1 indicates nodules up to 2.5 mm in diameter left behind while CC-2 designates residual tumour between 2.5 mm and 25 mm. CC-3 represents gross residual disease. The Dutch modification of the AJCC R classification details an R1 group without macroscopic disease (but possible microscopic disease), an R2a group with a maximum of 2.5 mm diameter residual disease and an R2b group with more extensive residual disease. Survival results following CC-2 or R2b cytoreduction are generally very poor [15,16], and these patients should not be treated with HIPEC.

Extent of Disease before Surgery

The extent of the peritoneal carcinomatosis can be measured and scored in various ways (chapter 14). None of the scoring system is superior over the other. The four systems of scoring the abdomen can be divided into two classes. The first are more descriptive. This group consist of the Gilly [17] system and the Japanese system [18]. Roughly they divided patients into locally limited carcinomatosis and diffuse carcinomatosis. The other group consists of the more anatomically oriented systems. The most widely used system is the peritoneal cancer index [19]. The maximum score of this system is 39. Involvement of the small bowel is heavily weighted in this system. The competing system is the Dutch Simplified Cancer Index [14]. This system divides the abdomen into 7 regions and for each region the maximum tumour size is measured, scored form 0 (none) to 3 (> 5 cm). Both the PCI and simplified PCI show similar ROC curves and are therefore interchangeable. All staging systems show that, when the entire or nearly the entire abdomen is affected by PC a complete cytoreduction is not likely to be achieved.

Other Prognostic Factors

Other, less strong, predictors are location of the primary tumour (appendix vs. colon vs. rectum), presentation (carcinomatosis at recurrence vs. at first presentation of the tumour) and differentiation grade (good / moderate differentiation vs. poor differentiation). Fig. 3 shows the multilevel confidence bars of prognostic factors [14].

The most complex scoring system includes all factors after exploring the abdomen. Although this system describes the prognosis best it is highly unpractical. The formula for this system Prognostic score (PS) is:

$$PS = 0.592*C + 1.875*R + 0.448*D + 0.478*H + 0.343*Re$$

with C: colon (yes =1, no = 0); R: rectum (yes =1, no = 0); D: tumour differentiation grade (good or moderate = 1, poor = 2); H: histology (signet cell = 2; other = 1) and Re: number of regions effected. Although this system probably provides the most accurate prognostic prediction it is far from practical and therefore will not be used in many institutes.

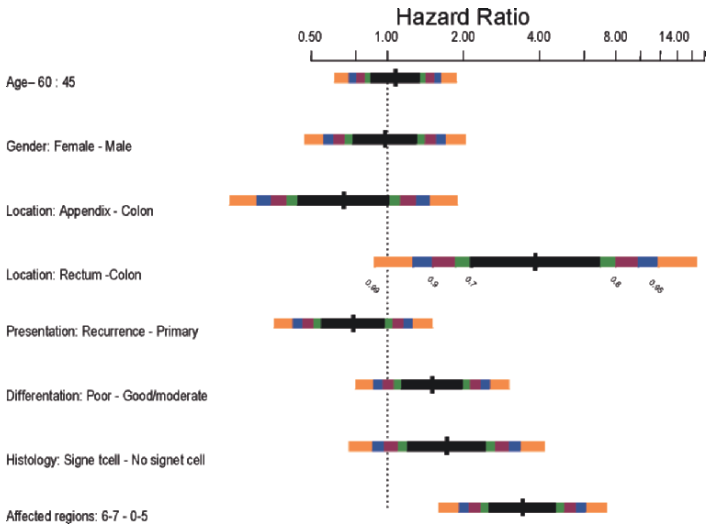


Figure 3. Hazard ratios of factors predicting survival in patients who underwent cytoreduction and HIPEC for peritoneal carcinomatosis of colorectal origin. Reprinted with permission from [14]

Complications of Surgery

The promising results of cytoreduction followed by HIPEC come with a trade-off for complications. The most eyecatching complications are infectious, often related to bowel leakage. The risk of bowel leakage arises both from bowel anastomoses and from adhesiolysis in patients who had previous surgery [20].

Another main cause of toxicity is related to the local and systemic side effects of the chemotherapy used during the chemoperfusion. Table 2 lists the toxicity in 102 patients according to the NCI common toxicity criteria. Treatment toxicity is related to both patient and treatment related factors. Table 3 summarizes a univariate analysis of the most important variables determining treatment toxicity. The risk of toxicity was significantly higher in patients who presented with metachronous disease, a finding probably related to the risk of extensive adhesiolysis. Not surprisingly, the risk of toxicity was also higher in patients with a high tumour burden as reflected by the simplified peritoneal cancer index.

Table 2. toxicity of 102 patients treated with cytoreduction followed by HIPEC

	Grade 3	Grade 4	Grade 5
Bone marrow	17	2	1
Cardiac	2	5	1
Skin	4	-	-
Gastrointestinal	7	16	1
Hemorrhage	1	1	1
Hepatic	1	-	-
Infections	16	1	4
Neurological	3	-	-
Pulmonary	8	2	-
Renal	3	2	-
All	62	29	8

Of note, postoperative toxicity was higher in patients who underwent an incomplete resection. Unsuccessful attempts at cytoreduction therefore carry a definite risk without any survival benefit for the patient and should therefore be avoided by an optimal preoperative patient selection [16].

Cytoreductive surgery followed by HIPEC is a demanding therapeutic approach both for the patients and for the medical team. Postoperative complications can be severe, and the treating staff should be experienced in intensive care management of general postoperative morbidity as well as in the specific risks associated with intraperitoneal chemotherapy.

The Learning Curve of Cytoreduction with HIPEC

Over the last years, the postoperative morbidity and mortality associated with cytoreduction and HIPEC have substantially decreased. One of the main reasons for this improvement is better selection and earlier patient referral. Indeed, in the eighties and nineties this treatment was generally regarded as experimental. Most patients presented with a history of previous laparotomies and multiple courses of ultimately failing systemic chemotherapy. From the presently available data it is clear that this category of patients to whom cytoreduction and HIPEC is offered as a desperate measure in very advanced and therapy resistant disease does not reap any survival benefit. Patients are nowadays referred earlier and selected according to strict criteria, implying that patients with 6 or 7 of the 7 abdominal regions

Table 3. Univariate analysis of factors determining postoperative complications in 102 patients suffering from PC of colorectal origin treated with cytoreduction and HIPEC

Factor		N	%	p
Gender	Male	57	40	0.229
	Female	45	28	
Timing of PC	Synchronous	60	25	**0.009**
	Metachronous	42	50	0.346
Location*	Appendix	15	40	
	Colon	78	32	
	Rectum	5	40	
	NS	4	75	
Differentiation*	Good / Moderate	67	33	0.468
	Poor	27	41	
Histology*	Signet cell	15	27	0.449
	Non-signet cell	87	37	
Obstruction present	Yes	42	38	0.620
	No	60	33	
PC extent	Locally	52	40	0.594
	Ovary	14	14	
	Extensive	36	36	
# Affected regions	0- 5	77	30	**0.044**
	6 - 7	25	52	
SPCS	< 13	25	30	**0.012**
	• 13	11	61	
Completeness of cytoreduction	R-1	50	28	**0.035**
	R-2a	37	35	
	R-2b	15	60	
Blood loss (liter)	• 6	68	28	**0.028**
	> 6	34	50	
Operating time (hours)	• 10	71	30	0.067
	> 10	31	48	
# Suture lines	• 2	69	28	**0.018**
	> 2	33	35	

N, number of patients; %, percentage of patients with a complication; *refers to the characteristics of the primary cancer; PC, peritoneal carcinomatosis; SPCS, simplified peritoneal cancer score

involved will not be offered cytoreduction and HIPEC. This approach has not only improved the percentage of R1 (or CC1) resections, it also has decreased the postoperative risks.

From the technical point of view the procedure has become nearly standardized in the past decades. Standard protocols are made available pertaining to the work-up before therapy to the cytoreduction, the chemoperfusion and the postoperative ICU and ward care. Similarly, the standardized approach of complications has significantly reduced their impact on the patient's postoperative course, and very

prolonged ICU or hospital stays are nowadays rarely necessary. The overall effect of this learning curve is illustrated in Fig. 4 [21].

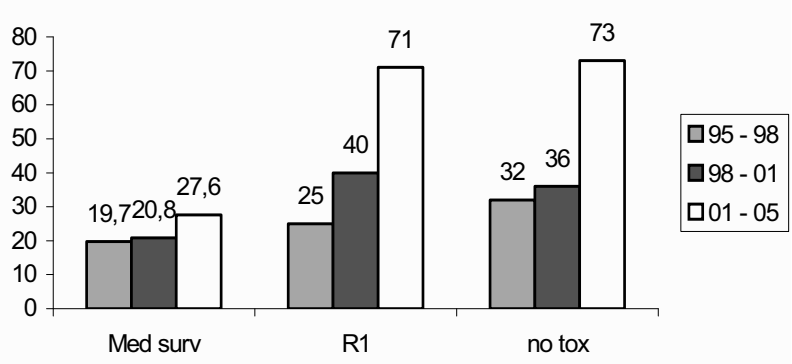


Figure 4. Learning curve effect on median survival (months), percentage R1 resections and percentage of patients without serious toxicity in patients treated with cytoreduction and HIPEC

Conclusion

Before the establishment of intensive locoregional therapy, stage IV colorectal cancer with PC was associated with a dismal prognosis. Since the introduction of cytoreduction and HIPEC in selected patients, a 5-year survival up to 40% can be reached provided an R1 resection is feasible. These results are similar to the survival observed after resection of isolated liver metastasis.

The trade-off for this survival benefit is a 27% probability of treatment related grade III or higher toxicity. The extent of disease in the abdomen is the main prognostic factor for survival and toxicity. Early patient referral and careful selection therefore are the cornerstones of this form of multimodal therapy.

References

1. Greene FL, Balch CM, Flemming ID (2002) AJCC cancer staging handbook
2. Tournigand C, Andre T, Achille E et al (2004) FOLFIRI followed by FOLFOX6 or the reverse sequence in advanced colorectal cancer: a randomized GERCOR study. J Clin Oncol 22:229-237
3. Rosen SA, Buell JF, Yoshida A et al (2000) Initial presentation with stage IV colorectal cancer: how aggressive should we be? Arch Surg 135:530-534

4. de Bree E, Koops W, Kroger R et al (2006) Preoperative computed tomography and selection of patients with colorectal peritoneal carcinomatosis for cytoreductive surgery and hyperthermic intraperitoneal chemotherapy. Eur J Surg Oncol 32:65-71
5. Chu DZ, Lang NP, Thompson C et al (1989) Peritoneal carcinomatosis in nongynecologic malignancy. A prospective study of prognostic factors. Cancer 63:364-367
6. Jayne DG, Fook S, Loi C, Seow-Choen F (2002) Peritoneal carcinomatosis from colorectal cancer. Br J Surg 89:1545-1550
7. Sadeghi B, Arvieux C, Glehen O et al (2000) Peritoneal carcinomatosis from non-gynecologic malignancies: results of the EVOCAPE 1 multicentric prospective study. Cancer 88:358-363
8. Bloemendaal AL, Verwaal VJ, van Ruth S et al (2005) Conventional surgery and systemic chemotherapy for peritoneal carcinomatosis of colorectal origin: a prospective study. Eur J Surg Oncol 31:1145-1151
9. Laufman LR, Krzeczowski KA, Roach R, Segal M (1987) Leucovorin plus 5-fluorouracil: an effective treatment for metastatic colon cancer. J Clin Oncol 5:1394-1400
10. Sugarbaker PH, Gianola FJ, Speyer JC et al (1985) Prospective, randomized trial of intravenous versus intraperitoneal 5-fluorouracil in patients with advanced primary colon or rectal cancer. Surgery 98:414-422
11. Verwaal VJ, van Ruth S, de Bree E et al (2003) Randomized trial of cytoreduction and hyperthermic intraperitoneal chemotherapy versus systemic chemotherapy and palliative surgery in patients with peritoneal carcinomatosis of colorectal cancer. J Clin Oncol 21:3737-3743
12. Verwaal VJ, Van Ruth S, Witkamp A et al (2005) Long-term survival of peritoneal carcinomatosis of colorectal origin. Ann Surg Oncol 12:65-71
13. Jacquet P, Sugarbaker PH (1996) Clinical reseach methodologies in diagnosis and staging of patients with peritoneal carcinomatosis. In Sugarbaker PH (ed.): Kluwer Avademic publishers, p359-374.
14. Verwaal VJ, van Tinteren H, van Ruth S, Zoetmulder FA (2004) Predicting the survival of patients with peritoneal carcinomatosis of colorectal origin treated by aggressive cytoreduction and hyperthermic intraperitoneal chemotherapy. Br J Surg 91:739-746
15. Beaujard AC, Glehen O, Caillot JL et al (2000) Intraperitoneal chemohyperthermia with mitomycin C for digestive tract cancer patients with peritoneal carcinomatosis. Cancer 88:2512-2519
16. Verwaal VJ, van Tinteren H, Ruth SV, Zoetmulder FA (2004) Toxicity of cytoreductive surgery and hyperthermic intra-peritoneal chemotherapy. J Surg Oncol 85:61-67
17. Gilly FN, Carry PY, Sayag AC et al (1994) Regional chemotherapy (with mitomycin C) and intra-operative hyperthermia for digestive cancers with peritoneal carcinomatosis. Hepatogastroenterology 41:124-129
18. Japanese research society for Gastric Cancer (1981) Jpn J Surg 11:127-139

19. Sugarbaker PH, Jablonski KA (1995) Prognostic features of 51 colorectal and 130 appendiceal cancer patients with peritoneal carcinomatosis treated by cytoreductive surgery and intraperitoneal chemotherapy. Ann Surg 221:124-132
20. Jacquet P, Stephens AD, Averbach AM et al (1996) Analysis of morbidity and mortality in 60 patients with peritoneal carcinomatosis treated by cytoreductive surgery and heated intraoperative intraperitoneal chemotherapy. Cancer 77:2622-2629
21. Verwaal VJ, Smeenk R, Zoetmulder FAN et al (2006) Learning curve of cytoreduction and hyperthermic intra peritoneal chemotherapy for peritoneal carcinomatosis of colorectal origin and pseudomyxoma peritonei. Ann Surg Oncol 13:46
22. Elias D, Blot F, El Otmany A et al (2001) Curative treatment of peritoneal carcinomatosis arising from colorectal cancer by complete resection and intraperitoneal chemotherapy. Cancer 92:71-76
23. Pilati P, Mocellin S, Rossi CR et al (2003) Cytoreductive surgery combined with hyperthermic intraperitoneal intraoperative chemotherapy for peritoneal carcinomatosis arising from colon adenocarcinoma. Ann Surg Oncol 10: 508-513
24. Shen P, Hawksworth J, Lovato J et al (2004) Cytoreductive surgery and intraperitoneal hyperthermic chemotherapy with mitomycin C for peritoneal carcinomatosis from nonappendiceal colorectal carcinoma. Ann Surg Oncol 11:178-186
25. Glehen O, Cotte E, Schreiber V et al (2004) Intraperitoneal chemohyperthermia and attempted cytoreductive surgery in patients with peritoneal carcinomatosis of colorectal origin. Br J Surg 91:747-754

HIPEC with Oxaliplatin in the Treatment of Peritoneal Carcinomatosis of Colorectal Origin

D Elias, M Pocard, D Goere

Introduction

Cisplatin is one of the most frequently used antineoplastic agents in association with hyperthermic intraperitoneal chemoperfusion (HIPEC) [1-4]. The rationale for its use is its potentiation at high temperatures and its ability to act at any stage of malignant cell replication [1]. However, while this drug has a proven activity in the treatment of intraperitoneal malignancies such as gastric and ovarian carcinoma [5-7], no such efficacy has been demonstrated in colorectal or appendiceal adenocarcinoma.

Oxaliplatin (LOHP) is a third generation platinum complex with a diaminocyclohexane carrier group, and an oxalate leaving ligand. It induces no renal or hepatic toxicity, but may cause cumulative sensory neuropathy, thus limiting the total dose to be delivered. It yields high response rates and improves survival in patients with metastatic colorectal cancer. The objective response rate was 24% when administered as a single agent in second-line intravenous chemotherapy, and around 55% when given upfront combined with 5-FU and leucovorin at a dose intensity of 40-50 mg/m²/week [8-10].

Recently, cytoreduction followed by HIPEC has been introduced in the management of peritoneal carcinomatosis (PC) [5,11-13]. Cytoreduction should be as complete as possible since experimental studies have shown that drug penetration is limited to a few cell layers under the tumour surface [14]. Intraperitoneal chemotherapy must be immediate, thus avoiding the trapping of residual tumour cells in postoperative fibrin adhesions [15,16]. HIPEC ensures a high local concentration of antineoplastic agents [1], and their cytotoxicity is improved by hyperthermia [1] while that of LOHP is increased by 180% [17].

LOHP should be a more interesting agent for HIPEC in colorectal carcinomatosis than the drugs currently used: mainly mitomycin [1,2,4-6], which has a limited efficacy against such tumours, and 5-FU, a long-acting drug which is not potentiated by hyperthermia (18).

In this chapter, we report the results of consecutive prospective trials of HIPEC with LOHP following complete cytoreductive surgery for colorectal PC.

Patient Eligibility and Surgical Procedures

Patients in good general condition with preoperatively identified PC were included in these consecutive trials that were reviewed and approved both by our Institutional Review Board and by an independent Ethics Committee. All patients gave written informed consent for participation in the studies. Patients with extraperitoneal metastases could be enrolled in these trials provided these were completely resectable.

At laparotomy, the diagnosis of PC was confirmed by frozen section histology and the disease extent scored using Sugarbaker's peritoneal cancer index [2]. Macroscopically detectable disease had to be completely resected before including the patient in the trial. Resection of PC obeyed principles described in detail in chapter 15 and in [19].

Hyperthermic Intraperitoneal Chemotherapy (HIPEC)

We performed HIPEC with a continuous closed circuit using four 36-French drains (two inlets and two outlets) connected to two pumps. We used one heating unit and two heat exchangers to eliminate a Y connector that could reduce flow rates and heat homogeneity [4]. HIPEC was performed with the open abdomen 'coliseum' technique following a study demonstrating that this technique was the only one allowing temperature homogeneity and complete spatial diffusion of the peritoneal instillation throughout the peritoneal cavity [4]. The flow rate was 1 L/min for each pump. Temperature data from four thermal probes inside the peritoneal cavity were continuously monitored and stored as an electronic computer file. The intra-abdominal temperature was maintained between 42° C and 44° C during HIPEC.

The duration of the perfusion was exactly 30 minutes from the time when the optimal temperature (> 42° C) was reached. The reasons for this short duration were: 1. the high cost of every minute in a functioning operating room; 2. the marked increase in tumour oxygenation occurring mainly during the first 30 minutes at 42.5° C [20], and 3. the choice of increasing the drug concentration in order to decrease the duration of HIPEC. Usually, 5 to 10 minutes were necessary to reach a high homogeneous temperature, leading to a total peritoneal infusion duration of close to 40 minutes. Afterwards, the infusion was completely evacuated.

The total LOHP dose was administered as a bolus mixed with a 5% dextrose solution at the beginning of the procedure. The total amount of peritoneal liquid used was based, as for LOHP, on the body surface area: 2 L/m² (the dimension of the abdominal cavity varying with size and weight), in order to obtain the same drug concentration in all patients. One hour before HIPEC, we delivered systemic intravenous leucovorin 20 mg/m² and 5-FU 400 mg /m², because 5-FU potentiates LOHP activity [9]. However, as 5-FU cannot be mixed with LOHP in the peritoneal cavity due to pH incompatibility, it was administered intravenously.

Results of a Phase I Study of HIPEC with Oxaliplatin

Methods

Twenty consecutive patients with PC underwent complete cytoreductive surgery followed by intra-operative HIPEC with increasing doses of LOHP. We treated at least 3 patients at each of the six intraperitoneal LOHP dose levels (from 260 to 460 mg/m²) before progressing to the next. We analyzed intraperitoneal, plasma and tissue samples with atomic absorption spectrophotometry [21].

Results

Pharmacokinetics

The mean duration of the entire procedure was 8.4 ±2.7 hours. Half of the LOHP dose was absorbed in 30 min at all dose levels. The area under the curve (AUC) and maximal plasma concentration (Cmax) increased with dose. At the highest dose level (460 mg/m²), peritoneal LOHP concentration was 25-fold that in plasma (Table 1).

Table 1. Tissue oxaliplatin concentrations after HIPEC

Dose level (mg/m^2)	Tumour	Peritoneum	Muscle*
260	228	230	29
310	248	273	31
360	327	296	20
410	323	287	21
460	339	392	19

All concentrations are in ng /mg of dry tissue. *not in contact with the chemoperfusion solution

AUCs following intraperitoneal (ip) administration were consistently below historical control AUCs after intravenous LOHP (130 mg/m²). Intratumour LOHP penetration was high, similar to absorption at the peritoneal surface and 17.8-fold higher than that in unbathed tissues. Increasing the instillate volume to 2.5 L/m² instead of 2 L/m² dramatically decreased LOHP concentration and absorption.

Tolerance

There were no deaths, nor severe hematological, renal or neurological toxicity, but two fistulas and 3 deep abscesses were observed.

Conclusion

HIPEC yielded high peritoneal and tumour LOHP concentrations with limited systemic absorption. An LOHP dose of 460 mg/m² in 2 L/m² of 5% dextrose is recommended for HIPEC at a temperature of 42° C - 44° C over 30 min.

Results of a Phase I Study with Oxaliplatin using Hypotonic Solutions

Introduction

Experimentally, ip hypotonic solutions increase platinum accumulation in tumour cells and enhance its cytotoxicity in vitro [23,24]. Experiments in rats showed that the amount of platinum taken up by solid tumours from an ip hypotonic solution of 103 mosm/l was about twice that taken up from isotonic solutions [25]. We therefore conducted a phase I clinical study of HIPEC with LOHP administered with increasingly hypotonic solutions, following complete cytoreductive surgery for PC [22]. Our main aim was to test the possibility that hypotonic ip LOHP might be preferable to and cheaper than ip LOHP dose escalation.

Methods

Patients underwent complete cytoreductive surgery followed by HIPEC with successive dextrose solutions of 300, 200, 150, and 100 mosm/l. LOHP (460 mg/m²) was administered in 2 liters of solution per m², at an ip temperature of 42° C to 44° C for 30 min. Sixteen consecutive patients with PC either of gastrointestinal or peritoneal origin were treated. The safety of the procedure was studied.

Results

Pharmacokinetics

The mean duration of the entire procedure was 7.7 ± 2.6 hours. Half of the LOHP dose was absorbed within 30 min at all dose levels. Absorption was not higher with hypotonic solutions than with isotonic solutions. The area under the curve (AUC) of LOHP in plasma did not increase with decreasing osmolarity of the ip solutions. Intratumour LOHP penetration was high; it was similar to that at the peritoneal surface, and about 18-fold higher than that in unbathed tissues. LOHP penetration was not significantly increased by using hypotonic solutions.

Safety

There was a very high incidence of unexplained postoperative peritoneal bleeding (50%) and unusually severe thrombocytopenia, in the 150 and 100 mosm/l groups.

Conclusion

Contrary to the findings of animal studies, this clinical study showed no increase in tumour or systemic penetration of LOHP with ip hypotonic solutions (200, 150 or 100 mosm/l) during HIPEC. A high incidence of ip hemorrhage and thrombocytopenia was observed.

Results of a Phase II Study of HIPEC with Oxaliplatin

Patients and methods

From June 1998 to December 2003, thirty patients with macroscopic colorectal PC underwent complete resection of PC followed by HIPEC with LOHP performed in an open abdominal [26]. The LOHP dose was 460 mg/m² in 2 L/m² of iso-osmotic 5% dextrose, over 30 min at 43° C and at a flow rate of 2 L/min. During the hour preceding HIPEC, patients received 5-fluorouracil (400 mg/m²) and leucovorin (20 mg/m²) intravenously. All patients received neoadjuvant and adjuvant systemic chemotherapy.

Results

Mean peritoneal tumour extension (Sugarbaker's PCI) was 14.3 ± 3.8, median operative duration, 450 min, and median blood loss, 940 ml. Eleven (37%) patients had associated extra-peritoneal lesions which were resected during the same procedure. There were no postoperative deaths (0%), and grade 2-3 morbidity (requiring specific treatment) was 40% (Table 2).

Table 2. Postoperative morbidity following cytoreduction and HIPEC with oxaliplatin

	N** (%)	Type*	Therapy
Abdominal complications	7 (23)	Digestive fistula: 4	Repeat surgery: 3
		Pancreatic fistula: 1	Percutaneous drain
		Urinary leak: 1	Ureteral catheter
		Deep abscess: 1	Percutaneous drain
Extraabdominal complications	8 (27,5)	Pneumonia: 2	
		Aplasia grade 2 or 3: 2	
		Catheter infection: 2	
		Transient renal failure: 1	
		Popliteal nerve damage: 1	
Total	12 (40)		

HIPEC, hyperthermic intraperitoneal chemoperfusion. Only grade 2 and grade 3 complications according to Feldman et al. [31] were collected. Grade 1 complications (i.e. minor complications which resolve if left untreated or which require simple bedside procedures without drugs excepted analgesics, antipyretics, antidiarrheals or oral antibiotics) were not counted. *Different types of complications could be associated in the same patient.**Number of patients; both intra- and extraabdominal complications were seen in three patients

Median follow-up was 55 months (range: 31-84). Twenty-two patients (73%) relapsed after a median interval of 14 months, but 7 of them (32%) were amenable to curative repeat surgery. At 3 and 5 years, overall survival rates (95% confidence interval) were 53% (9-72), and 48.5% (31-66) respectively (Fig. 1). At 3 and 5 years, disease-free survival rates were 41.5% (27-59), and 34% (19-52) respectively. Median survival was 60.1 months.

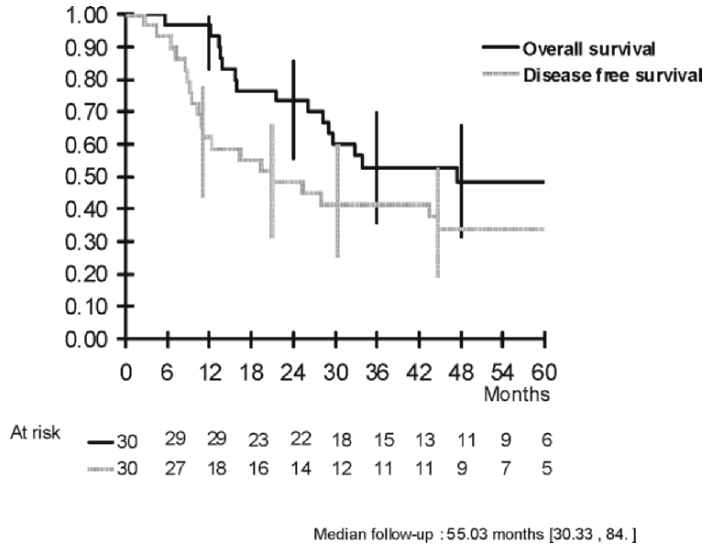


Figure 1. Overall and disease-free survival rates of 30 patients with colorectal carcinomatosis treated with maximal cytoreductive surgery and hyperthermic intraperitoneal chemotherapy with oxaliplatin

Discussion

With a 5-year overall survival rate of 48.5%, a 5-year disease-free survival rate of 34%, median survival attaining 60.1 months, this short series provides two new and important findings.

First, some patients with colorectal PC can be offered long term survival with complete cytoreductive surgery combined with IPCH, and the impact of this combined treatment on survival is far greater than previously thought. Recently, Verwaal et al. reported a 5-year survival rate of 43% (median survival: 42.9 months) in 59 patients who underwent macroscopically complete cytoreduction plus HIPEC with mitomycin C at 40° C to 41° C for 90 min [27]. In contrast, the 5-year survival rate was 0% when gross macroscopic tumour was left behind, despite HIPEC [27]. Piso et al. also reported a 75% 4-year survival rate [28]. These concordant results reported by different teams indicate that in selected patients suffering from PC of colorectal origin long term survival is within reach with combined surgery and HIPEC.

Second, only selected patients are likely to benefit from this treatment. Our patients were selected according to the following criteria: a good general health status, the absence of extra-abdominal lesions on preoperative imaging and completeness of cytoreductive surgery which can only be appraised at laparotomy. In the literature, median survival is 7 months for unselected patients with colorectal PC without surgical treatment [29]. This median survival increases to 12.6 months

for selected patients (good general status and no extra-abdominal lesions) treated with chemotherapy alone (5-FU and leucovorin and second-line CPT-11) [30]. Two other parameters may also explain our survival results. First, there was no postoperative mortality in this series which is unusual. Usually, our mortality rate approximates 5%, and therefore the absence of fatalities was likely due to chance in this study. Unfortunately, postoperative deaths have been observed after HIPEC since closure of the trial. Second, 7 (32%) of the 22 patients who relapsed underwent successful repeat surgery, underlining the role of an "aggressive" approach in these selected patients.

Conclusion

When feasible, cytoreduction followed by HIPEC with oxaliplatin in patients with PC of colorectal origin yields a 5-year survival rate of 48.5%, with a median survival of 60.1 months.

Comparison of HIPEC with Oxaliplatin versus Standard Systemic Chemotherapy

We performed a comparative study of similar patients with PC of colorectal origin treated with or without complete cytoreductive surgery plus HIPEC with LOHP (data not yet published).

HIPEC group

All patients with gross PC from colorectal adenocarcinoma who underwent cytoreductive surgery plus HIPEC at the Gustave Roussy Institute from June 1st 1998 to December 31st 2003 were studied. The preoperative selection of these patients was according to the following criteria: 1. no very extensive and symptomatic PC; 2. no extra-abdominal lesions; 3. a good general status and age less than 66 years; and 4. no progression after 2-3 months of neoadjuvant chemotherapy. Cytoreductive surgery was always complete with no remaining peritoneal disease exceeding 1 mm in diameter. HIPEC was performed with ip LOHP (460 mg/m^2), and iv 5-FU plus leucovorin, as previously described. These patients also received neoadjuvant (n = 48) and adjuvant (n = 37) systemic chemotherapy when an objective response was obtained preoperatively based on imaging or blood markers.

Standard Group

We asked the digestive oncologists of five French Cancer Centers situated in Lyons, Nantes, Toulouse, Nancy, and Caen, to very carefully select retrospectively 8

to 10 patients in their own center, as similar as possible to those who were treated with HIPEC. Thus, 48 patients were selected during the same period as the HIPEC group, and with the same selection criteria. However, although we have strong arguments to consider that their PC was potentially completely resectable, we cannot prove it. However, we had the operative reports for 36/48 patients who underwent a laparotomy confirming PC, with a precise description of peritoneal seeding which seemed to be resectable. In 12 patients, no laparotomy was performed and the diagnosis was based on a transcutaneous biopsy in 4 patients, and on a modification of the rectal examination (appearance of a tumour nodule in Douglas' pouch) and/or imaging in 8 patients, associated with an increasing blood CEA level. Retrospectively, we consider that these 48 patients could have been treated with HIPEC, but they received standard systemic chemotherapy. All of them received first-line chemotherapy, 33 second line, 17 third line and 12 fourth line chemotherapy, resulting in a mean of 2.3 lines of chemotherapy per patient including LOHP and irinotecan.

Overall survival rates

Overall survival curves are reported in Fig. 2. The survival rate of the HIPEC group was significantly higher than that of the standard group (p = 0.0001). At 3 years, it was 72% (95% CI: 57-83) and 32% (95% CI: 20-48) respectively. Median survival was 24.8 months in the standard group versus 60.1 months in the HIPEC group.

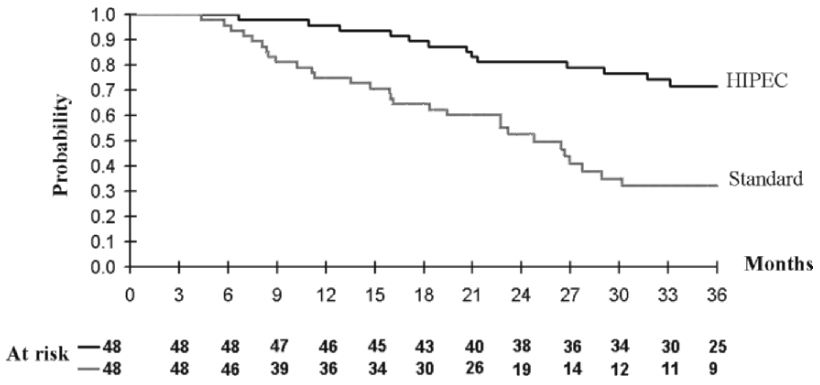


Figure 2. Survival curves of similar patients with colorectal PC treated with cytoreduction and HIPEC or with standard systemic chemotherapy (retrospective study); p = 0.0001

This study has the drawback of not being a randomized study. However, the retrospective comparison of almost similar patients shows a therapeutic benefit for patients with gross colorectal PC treated with complete cytoreductive surgery plus

HIPEC compared with patients treated with different lines of systemic chemotherapy alone.

Discussion

The usual main reservation regarding HIPEC is the stringent selection of patients. It is commonly thought that the survival rate with standard chemotherapy is probably very close than that obtained with HIPEC in these highly selected patients. In this study, 8 to 10 patients who did not undergo HIPEC but were treated with standard treatment were carefully chosen with highly selective criteria in each cancer center. The two groups were similar except in terms of age and the fact that we had no absolute proof that PC was completely resectable in the standard group, even if we had strong arguments to believe so, mainly based on a precise description of the extent of PC during laparotomy in 36/48 patients in the standard group.

Another point indicates that the patients in this group were highly selected: their median survival was 24.8 months with systemic chemotherapy, far better than the 7 months of the 349 patients with colorectal PC studied prospectively by Jayne et al [29], who were not selected and mainly treated with 5-fluorouracil and leucovorin. In addition, it is higher than the median survival of 12.8 months reported in patients who were selected as eligible for the randomized study of HIPEC conducted by the Amsterdam group [30]. These 50 patients were randomized to the control group and were treated with intravenous first-line 5-fluorouracil and leucovorin and second-line irinotecan. It could be argued that the chemotherapy regimen was not optimal in the control arm. We know, however, that the addition of the most recent agents increased the median survival of these patients from 4 to only 8 months. We could therefore expect a maximum median survival of 20 months for the selected patients in this control group. This is why the unusually high (24.8 months) median survival observed in our standard group is indirect proof of stringent selection. We can speculate that a small percentage of the patients in the standard group ultimately could not undergo a complete resection of PC, and that their exclusion would result in a higher median survival rate, approximating 30 months. Such a high median survival rate has never been reported in the literature for patients with colorectal PC treated with systemic chemotherapy. It is the highest median survival rate currently attainable with systemic treatment. However, these 30 months are only half of the 60 months of median survival that we obtained using complete cytoreductive surgery plus HIPEC.

Phase I study of HIPEC combining Oxaliplatin and Irinotecan

In 2002-2003, we studied the pharmacokinetics (PK), tissue distribution and tolerance of HIPEC combining irinotecan with LOHP after complete resection of PC [32].

Methods

Thirty-nine consecutive patients with PC of either gastrointestinal or peritoneal origin underwent complete cytoreductive surgery followed by HIPEC with a stable dose of oxaliplatin (460 mg/m²), and one of seven incremental doses of irinotecan (from 300 to 700 mg/m²). Patients received iv leucovorin (20 mg/m²) and 5-fluorouracil (400 mg/m²) just before HIPEC to maximize the effect of LOHP and irinotecan.

Results

Plasma irinotecan peaked at 30 minutes whereas it decreased exponentially from the peritoneal perfusion. The active metabolite of irinotecan, SN-38, was present in the peritoneal instillate immediately after the beginning of HIPEC. Irinotecan concentration in tumor tissue increased up to 400 mg/m² and then remained stable despite dose increments. It was 16 to 23 - fold higher than in unbathed tissues. Incremental doses of intraperitoneal irinotecan did not modify the PK of intraperitoneal oxaliplatin, and the drug concentration was 17.8 - fold higher in tumor tissue (bathed) than in unbathed tissues. Half of the oxaliplatin and irinotecan was absorbed during the procedure. The hospital mortality rate was 2.5% and the non hematological complication rate was 25%. However, grade 3-4 hematological toxicity attained 58%.

Conclusion

HIPEC with LOHP (460 mg/m²) combined with irinotecan (400 mg/m²), along with an i.v. 5-FU-leucovorin, had an advantageous PK profile and was tolerated by patients despite high hematological toxicity. We used only 360 mg/m² for the two drugs in further studies to in order to decrease mortality and morbidity.

Relation between the Extent of Cytoreductive surgery and the Rate of Postoperative Hematological Toxicity

We therefore studied the impact of the extent and duration of surgery on postoperative hematological toxicity after HIPEC [33].

Background

Peritoneal carcinomatosis is a major disease, currently treated using complete cytoreductive surgery and HIPEC. Morbidity, usually related to the extent of surgery, is a significant limitation of this procedure, and hematological toxicity which is solely dependent on the chemotherapy dose. The aim of our study was to investigate whether surgery alone had an impact on hematological toxicity associated with the standard drug protocol routinely prescribed.

Methods

Data were prospectively recorded from 83 consecutive patients who underwent complete cytoreductive surgery followed by HIPEC with LOHP (360 mg/m²) and irinotecan (360 mg/m²), in 2 L/m² of dextrose over 30 min at 42-45 °C. Patients also received an intravenous infusion of leucovorin (20 mg/m²) and 5-FU (400 mg/m²). Severe aplasia was defined as a leukocyte count of < 500/ml, platelets < 50 000/ml, and reticulocytes < 6.5gr Hb/l.

Results

Postoperatively, severe aplasia was seen in 40/83 patients (48%). There was no difference in the characteristics of patients with and without aplasia, other than the extent of surgery. The incidence of severe aplasia was only related to the duration of surgery (537 min in the aplasia group versus 444 min in the no-aplasia group) ($p = 0.002$) (Fig. 3), and to the extent of peritoneal disease (peritoneal cancer index of 19.5 in the aplasia group, versus 15.3 in the no-aplasia group) ($p = 0.02$).

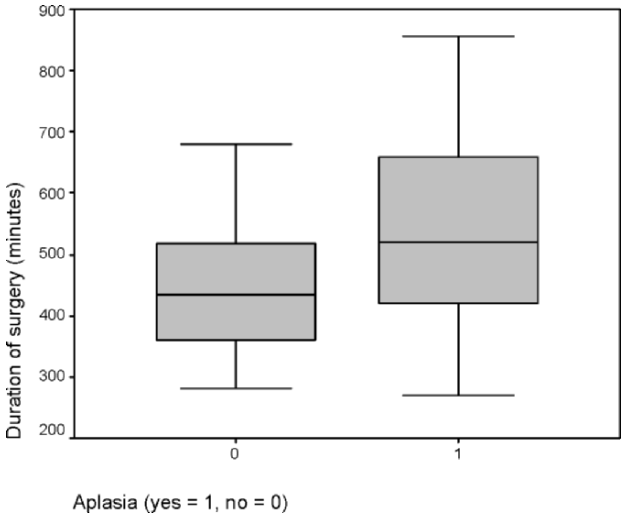


Figure 3. Box plots showing the incidence of severe aplasia as a function of the duration of cytoreductive surgery in 83 patients; $p = 0.002$

Conclusion

We report for the first time that the duration of surgery may increase the incidence of hematological toxicity following HIPEC. We also hypothesized that transient intra- and postoperative biochemical disorders, such as hypoalbuminemia, hemodilution, liver and renal insufficiency and stress can be involved in this process. These hypotheses may allow improved postoperative care.

Future Directions and Conclusion

The rationale for using LOHP rather that mitomycin C during HIPEC for colorectal PC is strongly supported. Local ip LOHP is theoretically potentiated by iv infusion of 5-FU and leucovorin (which cannot be added to the peritoneal instillate). We demonstrated that this regimen, after complete cytoreductive surgery resulted in a 5-year survival rate of 48.5%, a figure that has never been reported thus far. In addition, there is strong evidence that combination chemotherapy is better than a single agent in this setting. This is why we tested the feasibility of adding irinotecan to LOHP inside the abdominal cavity during HIPEC. We completed in January 2006 a phase II study of 100 consecutive patients treated with this new combination (triple therapy containing LOHP, irinotecan and 5-FU), but the results of this study are awaited.

Aknowledgement

The authors thank Lorna Saint Ange for editing the manuscript.

References

1. Elias D, Detroz B, Debaene B, Damia E, Leclercq B, Rougier P, et al (1994) Treatment of peritoneal carcinomatosis by intraperitoneal chemohyperthermia: reliable and unreliable concepts. Hepatogastroenterol 41: 207-213
2. Sugarbaker PH. Intraperitoneal chemotherapy and (1998) cytoreductive surgery for the prevention and treatment of peritoneal carcinomatosis and sarcomatosis. Semin Surg Oncol 14: 254-261
3. Yonemura Y, Fujimura T, Nishimura G, Falla R, Sawa T, Katayama K, et al (1996) Effects of intraoperative chemohyperthermia in patients with gastric cancer with peritoneal dissemination. Surgery 119: 437-444
4. Elias D, Antoun A, Goharin A, El Otmany A., Puizillout JM, Lasser P (2000) Research on the best chemohyperthermia technique for treatment of peritoneal carcinomatosis after complete resection. Int J Surg Invest 1: 431-439
5. Fujimoto S, Shresta RD, Kokubun M, Kobayashi K, Kiushu S, Konna C et al (1990) Positive results of combined therapy of surgery and intraperitoneal hyperthermic perfusion for far advanced gastric cancer. Ann Surg 212: 592-596
6. Hamazoe R, Maeta M, Keibara N (1994) Intraperitoneal thermochemotherapy for prevention of peritoneal recurrence of gastric cancer. Cancer 73: 2048-2052
7. Van der Vaart PJ, van der Vange N, Zoetmulder FA, van Goethtel AR, van Tellingen O, ten Bokkel WW, et al (1998) Intraperitoneal cisplatin with regional hyperthermia in advanced ovarian cancer: pharmacokinetics and cisplatin-DNA adducts formation in patients and ovarian cancer cell lines. Eur J Cancer 34 : 148-154
8. Becouarn Y, Ychou M, Ducreux M, Borel C, Bertheault-Cvitkovic F, Seitz JF, et al (1998) Phase II trial of oxaliplatin as first line chemotherapy in metastatic colorectal cancer. J Clin Oncol 16 : 2739-2744
9. Giachetti S, Perpoint B, Zidani R, Le Bail R, Faggiuolo R, Focan C, et al (2000) Phase III multicenter randomized trial of oxaliplatin added to chronomoduled fluorouracil-leucovorin as first line treatment of metastatic colorectal cancer. J Clin Oncol 18 : 136-147
10. De Gramont A, Figer A, Seymour M, Homerin M, Hmissi A, Cassidy J, et al (2000) Leucovorin and fluorouracil with or without oxaliplatin as first line treatment in advanced colorectal cancer. J Clin Oncol 18: 2938-2947
11. Sugarbaker PH, Cuniffe W, Belliveau JF, de Bruin E, Graves T (1988) Rationale for perioperative intraperitoneal chemotherapy as a surgical adjuvant for gastrointestinal malignancy. Reg Cancer Treat 1: 66-79

12. Sugarbaker PH, Jablonski KH (1995) Prognostic features of 51 colorectal and 130 appendiceal cancer patients with peritoneal carcinomatosis treated by cytoreductive surgery and intraperitoneal chemotherapy. Ann Surg 221: 124-132
13. Elias D, Gachot B, Bonvalot S, Blot F, Sabourin JC, Ducreux M, et al (1997) Carcinoses péritonéales traitées par exérèse complète et chimiothérapie intrapéritonéale postopératoire immédiate (CIPPI). Etude de phase II portant sur 54 malades. Gastroenterol Clin Biol 21: 181-187
14. Didkhoff T, van der Heider J, Dubbelman R, ten Bokkel Huinink W (1985) Tissue concentration of platinum after intraperitoneal cisplatin administration in patients. Proc AACR 26: 162
15. Zoetmulder FA (1996) Cancer cell seeding during abdominal surgery: experimental studies. In: Peritoneal carcinomatosis: principles of management, P.H. Sugarbaker (ed.), Kluwer Acad Pubisher, Boston; p155-162
16. Jacquet P, Elias D, Sugarbaker P (1996) L'implantation tumorale dans les sites de cicatrisation après chirurgie des cancers digestifs. J Chir (Paris) 133: 175-182
17. Rietbroeck RC, van de Vaart PJ, Haveman J, Blommaert FA, Geerdink A, Bakker PJ, et al (1997) Hyperthermia enhances the cytotoxicity and platinum-DNA adducts formation of lobaplatin and oxaliplatin in cultured SW 1573 cells. J Cancer Res Clin Oncol 123: 6-12
18. Hahn GM (1979) Potential for therapy of drugs and hyperthermia. Cancer Res 39: 2264-2268
19. Sugarbaker PH (1995) Peritonectomy procedures. Ann Surg 221: 29-42
20. Song CW, Shakil A, Osborn JL, Iwata K (1996) Tumour oxygenation is increased by hyperthermia at mild temperatures. In J Hyperthermia 12: 367-373
21. Elias D, Bonnay M, Puizillou JM, Antoun S, Demirdjian S, El Otmany A, et al (2002) Heated intraoperative intraperitoneal oxaliplatin after complete resection of peritoneal carcinomatosis: pharmacokinetic and tissue distribution. Ann Oncology 13: 267-272
22. Elias D, El Otmany A, Bonnay M, Paci A, Ducreux M, Antoun S, et al (2002) Human pharmacokinetic study of heated intraperitoneal oxaliplatin in increasingly hypotonic solutions, after complete resection of peritoneal carcinomatosis. Oncology 63: 346-352
23. Groose E, Walker L, Masters JR (1986) The influence of osmolarity on drug cytotoxicity in vitro. Br J Cancer 54: 181-182
24. Smith E, Brock AP (1989) The effect of reduced osmolarity on platinum drug cytotoxicity. Br J Cancer 59: 873-875
25. Tsujitani S, Oka A, Kondo A, Katano K, Oka S, Saito H, et al (1999) Administration in a hypotonic solution is preferable to dose escalation in intraperitoneal cisplatin chemotherapy for peritoneal carcinomatosis in rats. Oncology 57: 77-82
26. Elias D, Raynard B, Farkhondeh F, Goéré D, Rouquie D, Ciuchende R, et al (2006) Peritoneal carcinomatosis of colorectal origin: long-term results of

intraperitoneal chemohyperthermia with oxaliplatin following complete cytoreductive surgery. Gastroenterol Clin Biol (in press).
27. Verwaal VJ, van Ruth S, Witkamp A, Boot H, van Slooten G, Zoetmulder F (2005) Long-term survival of peritoneal carcinomatosis of colorectal origin. Ann Surg Oncol 12: 65-71
28. Piso P, Bektas H, Werner U, Schiltt HJ, Kubicka S, Bornscheuer A, et al (2001) Improved prognosis following perinectomy procedures and hyperthermic intraperitoneal chemotherapy for peritoneal carcinomatosis from appendiceal carcinoma. Eur J Surg Oncol 27: 286-290
29. Jayne DG, Fook S, Loi C, Seow-Choen F (2002) Peritoneal carcinomatosis from colorectal cancer. Br J Surg 89; 1545-1550
30. Verwaal VC, van Ruth S, de Bree E, van Sloten GW, van Tinteren H, Boot H, et al (2003) Randomized trial of cytoreduction and hyperthermic intraperitoneal chemotherapy versus systemic chemotherapy and palliative surgery in patients with peritoneal carcinomatosis from colorectal cancer. J Clin Oncol 21: 3737-3743
31. Feldman L, Barkun J, Barkun A, Sampalis J, Rosenberg L (1997) Measuring postoperative complications in general surgery patients using an outcome-based strategy: comparison with complications presented at morbidity and mortality rounds. Surgery 122: 711-720
32. Elias D, Matsuhisa T, Sideris L, Liberale G, Drouard-Troalen L, Raynard B, Pocard M, Puizillou JM, Billard V, Bourget P, Ducreux M (2004) Heated intra-operative intraperitoneal oxaliplatin plus irinotecan after complete resection of peritoneal carcinomatosis: pharmacokinetics, tissue distribution and tolerance. Ann Oncol 15: 1558-1565
33. Elias D, Raynard B, Boige V, Laplanche A, Estphan G, Malka D, Pocard M (2005) Impact of the extent and duration of cytoreductive surgery on postoperative haematological toxicity after intraperitoneal chemohyperthermia for peritoneal carcinomatosis. J Surg Oncol 90: 220-225

Clinical Results of Cytoreduction and HIPEC in Pseudomyxoma Peritonei

NJ Lutton, BJ Moran

Introduction

Pseudomyxoma peritonei (PMP) is a rare condition with a reported incidence of approximately one per million per year and classically presents at laparotomy with "jelly belly" [1]. It is characterized by copious production of mucinous ascites that, over time, fills the peritoneal cavity. The majority of cases originate from a perforated appendiceal epithelial neoplasm [1]. Appendiceal neoplasms are uncommon, accounting for 0.4-1% of all gastro-intestinal tract malignancies [2,3]. The majority of appendix neoplasms are carcinoid tumours, with the next most common being epithelial neoplasms [4]. Frequently epithelial appendiceal neoplasms present insidiously, after an occult rupture, with the features of PMP. The earliest description of the condition was by Rokitansky in 1842 in a patient with a benign mucocele of the appendix [5]. Werth first introduced the term "PMP" 42 years later in 1884 when he described a patient with a ruptured pseudomucinous ovarian cyst with implantation of the cystic contents on the peritoneal surfaces [6]. In 1901, Frankel was the first to describe PMP from a ruptured appendiceal cyst [7].

PMP has generally been considered a benign condition but its behaviour over time suggests that it should be considered, at best, a "borderline malignant" condition with inevitable disease persistence and progression.

Site of Origin of PMP

Since the publications by Werth and Frankel there has been ongoing controversy as to the site of origin of PMP, particularly in women. Synchronous disease is found in the ovary and appendix in most females with PMP, and the disease is reported to be more prevalent in females. Recent morphological, immunohistochemical, molecular and genetic evidence support the theory that the majority of

cases of classical PMP originate from a perforated mucinous tumour of the appendix [1,8-14]. In women, the ovarian mucinous tumours, occasionally massive in size, represent secondary spread from the appendix. Mukherjee et al [15] reported a similar male to female distribution in an analysis of their reported series of patients with PMP. It therefore seems unlikely that the ovary is the usual site of origin in females unless the male and female appendix behave in a completely different manner.

Undoubtedly, a small proportion of cases arises from other intra-abdominal organs and it is possible that an ovarian primary mucinous tumour may be the commonest in this diverse group which also includes the stomach, colon, pancreas, gallbladder and urachus [1,16].

Pathological Classification of Appendiceal Neoplasms

There has also been considerable confusion in the published literature about the pathological classification of epithelial appendiceal neoplasms and their relationship to PMP [1,2,9,17-19]. However, it is now generally agreed that classical PMP arises from a perforated mucinous neoplasm of the appendix. High grade colonic mucinous neoplasms, adenocarcinomas of the appendix and mucinous adenocarcinomas originating from any other intra-abdominal organ, particularly the colon, can simulate the clinical, radiological, and pathological features of PMP [8]. Additionally, there appears to be a spectrum of disease from low to high-grade mucinous appendiceal neoplasms, though the pathological appearances do not correlate with the clinical behaviour of the tumour in a significant number of cases [1].

These difficulties in pathological classification of the clinical entity of PMP have led to diverse reports in the literature and ongoing confusion as to the outcomes following intervention. Thus, many series include all cases of PMP, of whatever origin, and include patients with mucinous adenocarcinoma of the appendix whereas others have reported only on classical pseudomyxoma from appendiceal cystadenomas. Ronnett and colleagues, in a retrospective review of a series of patients who had undergone complete cytoreduction by Sugarbaker's group, have reported a pathological system commonly quoted in the literature [9]. They classified low-grade tumours as disseminated peritoneal adenomucinosis (DPAM) and high-grade tumours as peritoneal mucinous carcinomatosis (PMCA), with an intermediate group (IG) demonstrating a mixture of DPAM and PMCA [9]. Survival was significantly higher in the low-grade (DPAM) group as compared with the high-grade tumours (IG and PMCA) with actuarial 5-year survivals of 84%, 37.6% and 6.7% respectively. They were unable to show a statistically significant difference between the IG and PMCA groups and in subsequent articles have grouped these together [8,20]. Dichotomous categorisations of mucinous tumours of the appendix have been adopted elsewhere and what is emerging is that the optimal outcomes result from the management of PMP originating from low-grade mucinous tumours of the appendix as shown in Table 1.

The reported 5-year survival of low-grade tumours (DPAM) varies from 74-100%. Loungnarath et al's [22] excellent actuarial results of 100% survival were in a group of only 8 patients with a median follow-up of 23 months. High-grade tumours have a predictably lower 5-year survival ranging from 28-65%. Moran et al [23] and Elias et al [24] figures include only patients with complete cytoreduction with similar survival outcomes for low-grade tumours as in other studies but a marked difference in the high-grade group.

Table 1. Studies describing pathological grade and 5 year survival for pseudomyxoma peritonei

Study	Year	Patients	Pathological Grades	5-year survival	Ref.
Ronnett	1995	107	DPAM	84	9
			Hybrid	38	
			PMAC	7	
Sugarbaker	1999	385	DPAM	80	20
			Hybrid+ adenocarcinoma	28	
Elias[a]	2003	36	Low Grade	74	24
			High Grade	54	
Deraco	2004	33	Low Grade	96	25
			High Grade	NS	
Loungnarath	2005	27	Low Grade	100	22
			High Grade	32	
Miner[b]	2005	88	Low Grade	87	21
			High Grade	40	
Moran[a]	2006	65	Low Grade	80	23
			High Grade	65	

[a] Reported on complete cytoreduction patients only; [b] Four patients only received hyperthemic intraperitoneal chemotherapy; DPAM, disseminated peritoneal adenomucinosis; PMCA, peritoneal mucinous carcinomatosis; NS, not significant

Clinical Trials of Cytoreduction and Intraperitoneal Chemotherapy

Traditionally patients with PMP have been treated with repeated interval debulking procedures for relief of symptoms, but with limited expectation of long-term survival, and no prospect of cure [18,21,26].

The aim of surgery in PMP is complete cytoreduction as described by Sugarbaker [27]. This involves up to six different peritonectomy procedures in combination with visceral resections as required, to remove all visible tumour, or if this was not possible, to leave tumour deposits less than 3 mm (3 mm being the maximum direct penetration of locally applied chemotherapy). In brief peritonectomy includes a greater omentectomy and splenectomy, left upper quadrant peritonectomy, right upper quadrant peritonectomy, lesser omentectomy and cholecystectomy, appendicectomy or right hemicolectomy, total colectomy, partial or total

gastrectomy, and pelvic peritonectomy with anterior resection of the recto-sigmoid colon. Females require hysterectomy and bilateral salpingo-oophorectomy, if not already performed.

This structured approach to achieve complete cytoreduction has been shown in multiple studies to be an independent variable for survival and these studies are shown in Table 2.

Table 2. Recent case series of cytoreduction and intraperitoneal chemoperfusion

Study	Year	N	CC (%)	Mortality (%)	Major Morbidity (%)	5 YS CC	5 YS IC	Ref
Sugarbaker	1999	385	65	2.7	27	80	20	20
Witkamp	2001	46	87	9	39	NR[a]	NR	38
Elias	2003	36	71	13.8	44	66	NR	24
Deraco	2004	33	91	3	33	96	NR	25
Guner	2004	28	40	7	36	80	17	39
Loungnarath	2005	27	41	0	22	NR	NR	22
Miner	2005	97	55	4	16	89	41	21
Moran	2006	100	65	8	43	75	NR	23
Stewart	2006	110	64	6	38	70[b]	39[b]	40

N, number of patients; CC, complete cytoreduction; IC, incomplete cytoreduction; 5 YS, 5 year survival; NR, Not reported; [a] 3-year actuarial survival figures of 81% for all patients with no subset analysis; [b] Interpreted from graph and incomplete survival is for patients with gross residual disease nodule > 0.5 cm and < 2 cm. Patients with extensive residual disease nodules (> 2 cm) had a 5-year survival of 16%

The rate of complete cytoreduction varies from 40-91% with the 2 largest studies, one European and the other Northern American, both having complete macroscopic tumour removal in 65% of cases [20,23]. This supports the benefits of centralising this aggressive surgical treatment at institutions with an interest in PMP.

Most patients are referred from other surgical or gynaecological units and have usually had variable degrees of surgery prior to formal attempts at complete cytoreduction. Extensive previous attempts to reduce tumour load were shown by one large study to have a negative impact on survival [20]. It is the authors' preference that the referring team performs the minimal amount of surgery required to establish a histological diagnosis prior to referral to a specialist centre.

The types of chemotherapy agents utilized, the dosage, the temperature or duration of intraperitoneal chemotherapy have not been subjected to randomised trials but have been chosen on knowledge of the agents' intraperitoneal pharmacokinetics. The commonly used intraoperative agents are mitomycin C alone, cisplatin alone, 5-fluorouracil alone or a combination of these and are usually administered during 30-120 minutes. For postoperative intraperitoneal chemotherapy mitomycin C, 5-fluorouracil, and cyclophosphamide are most frequently used for up to 6 days.

Despite multiple recent publications on intraperitoneal chemotherapy there has never been a randomised trial evaluating its effectiveness compared to optimal surgery alone in patients with PMP. Retrospective reviews and phase II trials are used to support the arguments but are conflicting [26,28-30]. On analysis of the literature for high level evidence in other peritoneal neoplastic diseases one randomised controlled trial looking into the effectiveness of heated intraperitoneal chemotherapy in carcinomatosis from colonic malignancies [31] and four as adjuvant treatment of gastric cancer [32-35] are available. The colonic and three of the four gastric cancer trials showed a significant survival benefit with the use of hyperthermic intraperitoneal chemotherapy (HIPEC) as adjuvant treatment but it is impossible to translate these results directly into the management of PMP, a disease with completely different tumour biology.

The move from early postoperative intraperitoneal chemotherapy to intraoperative chemotherapy was mainly due to work by Sugarbaker [36] who showed inconsistent tumoricidal effects throughout the abdomen from postoperative intraperitoneal chemotherapy. This was thought to be due to the uneven delivery of the treatment. This theoretical problem has been overcome by manually encouraging intraperitoneal chemotherapy to all areas of the abdomen intraoperatively in the "open" technique but a randomised controlled trial of this compared to a "closed" technique, or compared to post-operative intraperitoneal chemotherapy has not been performed.

In 1993 Sugarbaker and colleagues [29] published their experience with 69 cases of appendiceal cancer using cytoreduction and early postoperative intraperitoneal chemotherapy and reported a 3 year survival of 91.6% in those with complete cytoreduction and 47.8% for those with moderate residual disease. Pathological classification of the appendiceal tumours was 55% PMP, 36% cystadenocarcinoma and 9% adenocarcinoma. These patients were also treated with delayed intraperitoneal chemotherapy and postoperative systemic chemotherapy. No patient received HIPEC.

This combined treatment approach of cytoreduction and early postoperative intraperitoneal chemotherapy was further supported by the early Mayo Clinic experience, which comprised of 56 patients with heterogeneous primary tumours treated over 26 years. Various adjuvant treatment modalities were used, including radiotherapy in 28%, and although not statistically significant a trend was seen towards improved disease free survival in the 13% of patients that received intraperitoneal chemotherapy [21].

Sugarbaker published a large series of 385 patients in 1999, which included patients from his 1993 data, and of these 205 received HIPEC [20]. He showed survival advantages in those who had complete vs. incomplete cytoreductions (80% vs. 20%) and in those with low-grade tumours (80% vs. 28% with high-grade tumours) but did not comment on whether the introduction of HIPEC made any difference to survival. Glehen analysed the data from the same institution over a 30-year period and found a survival benefit for those receiving HIPEC in addition to early postoperative intraperitoneal chemotherapy (27.2% vs. 7.3% 5-year survival) but he was specifically looking at patients with incomplete cytoreductions [37]. No study has reported on the effect of hyperthermic compared to normothermic

intraperitoneal chemotherapy in patients in whom a complete cytoreduction was performed.

There have been a number of recent case reports in the literature addressing the morbidity, mortality and outcome in patients with PMP treated by cytoreduction and intraperitoneal chemotherapy (Table 2). All have generally combined both treatment modalities as routine treatment with the exception of Miner et al [21] who reported the Memorial Sloan Kettering cumulative experience with 97 patients with PMP over a 22 year period. Minimal intraperitoneal chemotherapy was used with only four patients receiving HIPEC and 20 others having 5FU based early postoperative chemotherapy with the remainder having surgery alone. In 55% this was considered complete cytoreduction and repeat surgery was performed in a number of cases with diminishing effectiveness as would be expected. Nevertheless, overall survival at 5 years was 89% for low-grade tumours and 41% for high-grade tumours outlining the effectiveness of maximal safe surgery.

All series, including Miner et al [21] reported significant morbidity and procedure related mortality ranging from 0-13% (median 5.5%). These figures undoubtedly include institutional and individual learning curves, but also show consistently good 5 year survival figures particularly in patients who had a complete cytoreduction. One confounding feature, particularly applicable to survival, of the published reports is that some report all cases, from all causes, treated with combination therapy whilst others only include favourable cases of appendiceal origin that had complete cytoreduction combined with intraperitoneal chemotherapy.

One aspect that has not been fully addressed is a strategy for the many patients whose tumours were preoperatively considered unlikely to be completely removable, either due to tumour extent and distribution, or as a result of serious co-morbidity or age. There is increasing evidence that many of these, in addition to patients where complete tumour removal is impossible at laparotomy, benefit from a major palliative resection with reasonable intermediate-term survival of 43% at 2 years and 15% at 5 years and improved quality of life [37,40]. In these situations our approach generally involves an extended right hemicolectomy, greater omentectomy and splenectomy with an ileocolic anastomosis or on occasions a total colectomy and end ileostomy [41]. Glehen et al [37] recommended combination of comprehensive surgical debulking with HIPEC except for patients with signet ring histology or lymph node involvement in their experience of 174 patients with incomplete cytoreduction.

Conclusion

Recent evidence suggests that optimal surgical resection (complete cytoreduction if possible) combined with heated intraoperative, intraperitoneal chemotherapy as popularised by Sugarbaker is the most fundamentally based strategy for PMP. A combined approach makes both common and scientific sense, in that surgery attempts to remove all macroscopic disease whilst intraperitoneal chemotherapy

addresses residual microscopic disease. This treatment strategy is a complex procedure, associated with significant morbidity and mortality with a substantial institutional and individual "learning curve" phenomenon [42].

The clinical results for cytoreduction and HIPEC in PMP show good survival for those patients with low-grade histology amenable to complete cytoreduction. Poorly defined and often confusing terms in the literature have resulted in most series being a heterogeneous population. This, combined with the relatively low incidence has limited the quality of the evidence with a lack of randomised controlled trials [4]. This deficiency is not unique to PMP surgery as few, if any, major surgical techniques are amenable to randomisation. Recent publications in the last 15 years with increasing numbers of medium to large case series reports in the literature reflect an improved awareness and understanding of the disease. This trend should continue with the development of centralised treatment centres throughout the world such that the quality of care and information available for patients with PMP should ultimately improve.

An emerging network of specialized centres may facilitate multicentre studies on aspects of chemotherapy type, duration and temperature to help allay the criticisms of many surgical, and in particular, medical oncologists on the lack of hard scientific evidence in PMP management.

Meanwhile good evidence is rapidly accumulating and surgical nihilism is no longer acceptable in this inexorably progressive, universally fatal, but eminently treatable disease.

References

1. Moran BJ, Cecil TD (2003) The etiology, clinical presentation, and management of Pseudomyxoma Peritonei. Surg Oncol Clin N Am 12: 585-603
2. Deans GT, Spence RA (1995) Neoplastic lesions of the appendix. Br J Surg 82: 299-306
3. Esmer-Sanches DD, Martinez-Ordaz JL, Roman-Zepeda P, Sanchez-Fernandez P, Medina-Gonzalez E (2004) Appendiceal tumors. Clinicopathologic review of 5,307 appendectomies. Cir Cir 72: 375-378
4. Connor SJ, Hanna GB, Frizelle FA (1998) Appendiceal tumors: retrospective clinicopathologic analysis of appendiceal tumors from 7,970 appendectomies. Dis Colon Rectum 41: 75-80
5. Weaver CH (1937) Mucocele of the appendix with pseudomucinous degeneration. Am J Surg 36: 523-526
6. Werth R (1884) "Klinische und Anastomische Untersuchungen Zur Lehre von der Bauchgeschwullsten und der laparotomie". Arch Gynecol Obstet 84: 100-118
7. Frankel E (1901) Ueber das sogenannte Pseudomyxoma Peritonei. Med Wochenschr 48: 965-970
8. Sugarbaker PH, Ronnett BM, Archer A et al (1996) Pseudomyxoma Peritonei syndrome. Adv Surg 30: 233-280

9. Ronnett BM, Zahn CM, Kurman RJ, Kass ME, Sugarbaker PH, Shmookler BJ (1995) Disseminated peritoneal adenomucinosis and peritoneal mucinous carcinomatosis. A clinicopathologic analysis of 109 cases with emphasis on distinguishing pathologic features, site of origin, prognosis, and relationship to "Pseudomyxoma Peritonei". Am J Surg Pathol 19: 1390-1408
10. Ronnett BM, Schmooker BM, Diener-West M, Sugarbaker PH, Kurman RJ (1997) Immunohistochemical evidence supporting the appendiceal origin of Pseudomyxoma Peritonei in women Int J Gynecol Pathol 16: 1-19
11. Prayson RA, Hart WR, Petras RE (1994) Pseudomyxoma Peritonei: a clinicopathological study of 19 cases with emphasis on site of origin and nature of associated ovarian tumours. Am J Surg Pathol 18: 591-603
12. Young RH, Gilks CB, Scully RE (1993) Pseudomyxoma peritonei. Am J Surg Pathol 17: 1068-1071
13. Chuaqui RF, Zhuang Z Emmert-Buck MR, et al (1996) Genetic analysis of synchronous mucinous tumours of the ovary and appendix. Hum Pathol 27: 165-171
14. Szych C, Steabler A Connolly DC, et al (1999) Molecular genetic evidence supporting the clonality and appendiceal origin or Pseudomyxoma Peritonei in women. Am J Pathol 154: 1849-1855
15. Mukherjee A, Parvaiz A, Cecil TD, Moran BJ (2004) Pseudomyxoma Peritonei usually originates from the appendix: a review of the evidence. Eur J Gynaecol Oncol 25: 411-414
16. Moran BJ, Mukherjee A, Sexton R (2006) Operability and early outcome in 100 consecutive laparotomies for peritoneal malignancy. Br J Surg 93: 100-104
17. Carr NJ, Sobin LH (1995) Epithelial noncarcinoid tumors and tumor-like lesions of the appendix. Cancer 76: 2383-2384
18. Misdraji J, Yantiss RK, Graeme-Cook FM, Balis UJ, Young RH (2003) Appendiceal mucinous neoplasms: a clinicopathologic analysis of 107 cases. Am J Surg Pathol 27: 1089-1103
19. Misdraji J, Young RH (2004) Primary epithelial neoplasms and other epithelial lesions of the appendix (excluding carcinoid tumours). Semin Diagn Pathol 21: 120-133
20. Sugarbaker PH, Chang D (1999) Results of treatment of 385 patients with peritoneal surface spread of appendiceal malignancy. Ann Surg Oncol 6: 727-731
21. Miner TJ, Shia J, Jaques DP, Klimstra DS, Brennan MF, Coit DG (2005) Long-term survival following treatment of Pseudomyxoma Peritonei: an analysis of surgical therapy. Ann Surg 241: 300-308
22. Loungnarath R, Causeret S, Bossard N et al (2005) Cytoreductive surgery with intraperitoneal chemohyperthermia for the treatment of Pseudomyxoma Peritonei: a prospective study. Dis Colon Rectum 48: 1372-1379
23. Moran BJ, Mukherjee A, Sexton R (2006) Operability and early outcome in 100 consecutive laparotomies for peritoneal malignancy. Br J Surg 93: 100-104

24. Elias D, Laurent S, Antoun S, Duvillard P, Ducreux M, Pocard M, Lasser P (2003) [Pseudomyxoma Peritonei treated with complete resection and immediate intraperitoneal chemotherapy]. Gastroenterologie Clinique et Biologique 27: 407-412
25. Deraco M, Baratti D, Inglese MG, Allaria B, Andreola S, Gavazzi C, Kusamura S (2004) Peritonectomy and intraperitoneal hyperthermic perfusion (IPHP): a strategy that has confirmed its efficacy in patients with Pseudomyxoma Peritonei. Ann Surg Oncol 11: 393-398
26. Gough DB, Donohue JH, Schutt AJ et al (1994) Pseudomyxoma Peritonei. Long-term patient survival with an aggressive regional approach. Am Surg 219: 112-119
27. Sugarbaker PH, Kern K, Lack E (1987) Malignant Pseudomyxoma Peritonei of colonic origin. Natural history and presentation of a curative approach to treatment. Dis Colon Rectum 30: 772-779
28. Smith JW, Kemeny N, Caldwell C, Banner P Sigurdson E, Huvos A (1992) Pseudomyxoma of appendiceal origin. The Memorial Sloan-Kettering Cancer Centre Experience. Cancer 70: 396-401
29. Sugarbaker PH, Zhu BW, Sese GB, Shmookler B (1993) Peritoneal carcinomatosis from appendiceal cancer: results in 69 patients treated by cytoreductive surgery and intraperitoneal chemotherapy. Dis Colon Rectum 36: 323-329
30. Mann WJ, Wagner J, Chumas J, Chalas E (1990) The Management of Pseudomyxoma Peritonei. Cancer 66: 1636-1640
31. Verwaal VJ, van Ruth S, de Bree E, et al (2003) Randomised trial of cytoreduction and hyperthermic intraperitoneal chemotherapy versus systemic chemotherapy and palliative surgery in patients with peritoneal carcinomatosis of colorectal cancer. J Clin Oncol 21: 3737-3743
32. Yeomura Y, de Aretxabala X, Fujimura T, et al (2001) Intraoperative chemohyperthermic peritoneal perfusion as an adjuvant to gastric cncer: final results of a randomised controlled study. Hepatogastroenterology 48: 1776-1782
33. Fujimoto S, Takahashi M, Mutou T, et al (1999) Successful intraperitoneal hyperthermic chemoperfusion for the prevention of postoperative peritoneal recurrence in patients with advanced gastric carcinoma. Cancer 85: 529-534
34. Huang Y, Hagiwara A, Wang W, et al (2001) Local injection M-CH combined with i.p. hyperthermic intraperitoneal chemotherapy in patients with Pseudomyxoma Peritonei. Br J Surg 88: 458-463
35. Hamazoe R, Maeta M, Kaibara N (1994) Intraperitoneal thermochemotherapy for prevention of peritoneal recurrence of gastric cancer. Final results of a randomised controlled study. Cancer 73: 2048-2052
36. Sugarbaker PH, Landy D. Jaffe G, Pascal R (1990) Histologic changes induced by intraperitoneal chemotherapy with 5-fluorouracil and mitomycin C in patients with peritoneal carcinomatosis and cystadenocarcinoma of the colon or appendix. Cancer 65: 1495-1501
37. Glehen O, Mohamed F, Sugarbaker PH (2004) Incomplete cytoreduction in 174 patients with peritoneal carcinomatosis from appendiceal malignancy. Ann Surg 240: 278-285

38. Witkamp AJ, de Bree E, Kaag MM et al (2001) Extensive cytoreductive surgery followed by intra-operative hyperthermic intraperitoneal chemotherapy with mitomycin-C in patients with peritoneal carcinomatosis of colorectal origin. Eur J Cancer 37: 979-984
39. Guner Z, Schmidt U, Dahlke MH, Schlitt HJ, Klempnauer J, Piso P (2005) Cytoreductive surgery and intraperitoneal chemotherapy for Pseudomyxoma Peritonei. Int J Colorectal Dis 20: 155-160
40. Stewart JH, Shen P, Russell GB et al (2006) Appendiceal Neoplasms With Peritoneal Dissemination: Outcomes After Cytoreductive Surgery and Intraperitoneal Hyperthermic Chemotherapy. Ann Surg Oncol 13: 624-634
41. Farquharson SM, Murphy E, Sexton R, Cecil TD, Moran BJ (2005) What happens to patients following surgery for peritoneal malignancy with incomplete tumour removal? Analysis of 100 consecutive laparotomies. J Roy Coll Surg Ed & Ireland 92, Supp, p7.
42. Moran BJ (2006) Establishment of a peritoneal malignancy treatment centre in the United Kingdom. Eur J Surg Oncol 32(6): 614-618
43. Bryant J, Clegg AJ, Sidhu MK, Brodin H, Royle P, Davidson P (2005) Systematic review of the Sugarbaker procedure for Pseudomyxoma Peritonei. Br J Surg 92: 153-158

The Impact of Therapy in the Treatment of Pseudomyxoma Peritonei

TJ Miner

Introduction

Epithelial neoplasms of the appendix are rare tumours that make up approximately 1% (about 1500 cases per year) of colorectal cancer cases in the USA [1]. These tumours have biologic features that make them unique among gastrointestinal malignancies. Most patients with appendiceal neoplasms present with disseminated peritoneal disease. Tumour usually does not show evidence of histological aggressiveness (10%), lymphatic spread (2%) or hematogenous (2%) metastases. This contrasts to colorectal cancer that usually does not present with carcinomatosis (15%) and is associated more commonly with aggressive pathology (95%), lymph node metastasis (50%), and liver metastasis (20%) at initial diagnosis [2].

Appendiceal neoplasms display a range of both biologic and clinical aggressiveness. Although many are non-invasive and grow slowly, allowing long-term survival even in patients without specialized treatment, some tumours progress rapidly and can lead to death shortly after diagnosis. Pseudomyxoma peritonei syndrome (PMP) constitutes a large proportion of the cases of appendiceal neoplasm and is characterized by the progressive accumulation of peritoneal implants and mucinous ascites.

Inconsistent or imprecise classification of this entity by surgeons, pathologists and oncologists has caused confusion in the understanding of its natural history. It is now generally thought that PMP arises as the result of neoplastic mucin secreting cells with low-grade cytologic features disseminating within the peritoneal cavity. These cells are derived from a ruptured appendiceal neoplasm in almost all cases. In order of aggressiveness (least to most) these tumours have been classified histologically as diffuse peritoneal adenomucinosis (DPAM), hybrid or intermediated discordant features and peritoneal mucinous carcinoma (PMCA) [2-4]. Although this morphologic designation has greatly helped in the understanding of PMP, some patients with DPAM demonstrate a rapidly progressive disease process. It has been proposed that further investigation of PMP at a molecular and genetic level may help to develop an even more precise and comprehensive classification in the future [5].

As PMP progresses, the peritoneal cavity fills in a characteristic pattern with mucinous neoplasm and ascites, presumptively at a rate correlated to its biologic aggressiveness. The classic pattern of PMP dissemination within the peritoneal cavity was best defined by Sugarbaker as a redistribution phenomenon, demonstrating a complete and sequential invasion of the peritoneal cavity with large tumour volume localized at predictable anatomic sites and minimal invasion at other sites. Grossly, the greater omentum is usually thickened and infiltrated with tumour. Parts of the abdomen that can entrap malignant cells also commonly contain tumour, including the undersurface of the right and left hemidiagphragms, the right subhepatic space, the splenic hilus, the right and left abdominal gutters, the pelvis and the cul-de-sac [6]. Carmingnani et al. has reported that the almost constant peristaltic activity of the small bowel prevents tumour cells from adhering to its surface or the small-bowel mesentery, perhaps explaining the observation that PMP tumours usually spare the mobile portions of the small bowel [7]. Although patients can present with appendicitis, a clinical manifestation of a ruptured appendiceal mucocele with local inflammation, the sings and symptoms of PMP generally progress as peritoneal implants and mucous accumulates. The most common presenting symptom in PMP is increased abdominal girth. Women can also present with an ovarian mass caused by tumour and men can present with a new hernia caused by the accumulation disease in a hernia sac [3,8]. In patients with increasing abdominal girth because of presumed malignant ascites, diagnosis is usually established with paracentesis or laparoscopy and biopsy.

In PMP, the observation of localized disease within the peritoneum without distant spread makes an aggressive local-regional approach theoretically appealing. Although some authors have argued that surgical debulking of PMP should be performed on a selective basis, most agree that patients with PMP are best treated, at least initially, with aggressive local therapy [9]. Innovative improvements in surgical techniques have been developed to enable the eradication of cancer implants widely distributed around the abdomen and pelvis. Peritonectomy procedures combined with visceral resections have greatly facilitated the optimal cytoreduction of PMP [2]. Since it is unlikely that these techniques can remove all microscopic tumour, additional modalities have been proposed. Systemic chemotherapy for peritoneal surface spread is largely ineffective because of its limited entry into the peritoneum and the fact that some tumour cells are resistant to chemotherapy. Additional modalities such as external beam radiation, photodynamic therapy, normothermic intraperiteonal chemotherapy coupled with maximal tumour debulking have not significantly improved the outcomes of patients with PMP [10]. Sugarbaker, who has published extensively on PMP, advocates aggressive cytoreduction with the use of radical peritonectomy procedures followed by hyperthermic intraperitoneal chemotherapy (HIPEC) [2,8,11]. Although reports of this therapy have demonstrated prolonged survival, several concerns, in combination with the associated morbidity and mortality, have caused others to apply this modality cautiously and selectively [3].

The optimal treatment of patients with PMP remains open to debate. Recommendations from the literature are contradictory and skewed by observations of dissimilar patient groups. Series frequently have different inclusion criteria that

bias their populations towards more or less aggressive extremes of the disease. Studies are further limited by small numbers of patients collected over long periods of time during which treatment paradigms change, by inadequate length and quality of follow-up data, patient selection bias, and by conclusions based on inappropriate endpoints [3].

Much of the literature debating the appropriate treatment of PMP is based on overall survival. Survival is traditionally the most important outcome in cancer treatment [12]. Overall survival signifies death from any cause and represents a discrete, reproducible, and generally recognized measurement. To properly evaluate outcome different endpoints may be more appropriate; disease-free survival is important in the adjuvant setting, progression-free survival in patients with metastatic disease, symptom-free survival in the palliative setting, and event-free survival in the long-term assessment of potentially curative treatments [13]. Evaluation of more appropriate endpoints to effectively determine the value of various therapies in PMP, however, is problematic. The determination of disease-free interval is hazardous and imprecise in PMP. A comprehensive evaluation of quality of life is difficult to accurately assess over the long time intervals required to properly evaluate this endpoint in PMP. The value of overall survival as a study endpoint when considering patients with PMP is limited, as it fails to characterize the impact of disease recurrence, ongoing treatment, and treatment related toxicity on the quality of life of patients with this insidious, slowly progressive disease. Without knowing the natural history of a disease process (i.e. expected survival without specific treatment), it is difficult if not impossible to properly design a clinical trial to assess how patients might benefit from treatment [14]. Because of our limited understanding of the natural history of patients with PMP, conclusions based mainly on overall survival should be interpreted with caution [3].

Memorial Sloan-Kettering Cancer Center Experience

At the Memorial Sloan-Kettering Cancer Center (MSKCC) the approach to PMP has been to focus primarily on optimal symptom management with surgical therapy [3]. Function preserving debulking is performed when possible. Complete cytoreduction is attempted, especially at the first operation, but not at the expense of patients' quality of life. Major organ resection, including gastrectomy or proctectomy, is performed rarely. Intra-peritoneal chemotherapy is used selectively in patients who are able to undergo complete, or near-complete, cytoreduction. The timing of subsequent procedures is driven largely by symptoms. The purpose of this study was to review the institutional experience with PMP, to define its natural history, and to examine the clinical and pathologic features that might aid in clinical decision making. A critical analysis of our results utilizing previously published standards allowed comparisons to other therapeutic philosophies.

Methods

Patients treated at MSKCC between 1980 and 2002 with a diagnosis of PMP were identified and analyzed retrospectively. Patient data were obtained from clinical records, surgical reports, pathology reports, and pathology specimens were reviewed. All patients in the study underwent at least one abdominal operation at the MSKCC during the course of their disease. The sequence, indications, and time interval between operations was noted for each patient. The extent of each abdominal operation was graded using an Extent of Surgery Score (ESS) based on a modification of Sugarbaker's Previous Surgery Score [15]. The completeness of cytoreduction was determined as no gross residual disease, minimal residual disease (90-99% cytoreduction), or gross residual disease (< 90% cytoreduction). The occurrence of a death or a major operative complication (resulting in reoperation, ICU admission, chronic disability, or death) within 30 days was noted.

Patients were designated into histopathologic groups based on the work of Ronnett et al [4]. Lesions were classified as mucinous adenocarcinoma with a low or high-grade modifier to reflect the histological grade of the neoplastic epithelial cells. By definition, the designation of mucinous adenocarcinoma low grade is synonymous to the term *disseminated peritoneal adenomucinosis (DPAM)* as defined in their reports. Although Sugarbaker et al. has stated that the term PMP should be applied only to benign cases of the disease, others suggest that it should be applied to low-grade malignant conditions as well [16,17]. Patients with low-grade primary lesions and well-differentiated intraperitoneal material with low cellularity (0-10%) were defined as having mucinous adenocarcinoma, low grade. Patients with high-grade primary and mucinous material with high cellularity (>50%) were classified as having mucinous adenocarcinoma, high grade. The three intermediate pathologic features included moderately differentiated primary, moderately differentiated mucinous material and cellularity between 11-50%. Patients having one of these intermediate features were categorized as mucinous adenocarcinoma, low grade while those with two or more were placed in the mucinous adenocarcinoma, high-grade group.

From 1980 to 2002, 97 patients were treated for PMP at MSKCC. All patients had a history of gross mucinous ascites and had evidence of visible, localized or generalized accumulation of mucin in the peritoneal cavity either attached to the peritoneal surfaces or incorporated within dense fibrous tissue. No patients were eliminated from data analysis. The mean age at diagnosis was 53 ± 1.5 years (range 19-84). There was a slight preponderance of women (55%). Long-term follow-up was achieved in 92% of the subjects in this study. The mean follow-up was 70 ± 5.5 months (median 57.5, range 3- 220). Two foreign patients were lost to follow-up after 36 months. One additional patient was lost after 7 years.

Clinicopathologic Features

The appendix was grossly determined to be the site of the primary tumour in 97% (94/97) at the time of surgery. Adequate archival pathologic specimens for further pathologic classification were available from 91% (88/97) of the patients. Patients were designated into groups as mucinous adenocarcinoma, low grade in 52% (46/88) and high grade in 48% (42/88). Thirty-one percent (13/42) of the high grade cases were classified as mucinous adenocarcinoma, high grade, based on two or more intermediate features. The grade of intraperitoneal cells was closely associated with the grade of primary tumour ($p < 0.001$). When comparing low to high-grade groups, there was no significant difference in age ($p = 0.94$), gender ($p = 0.09$), or ovarian involvement in women ($p = 0.62$). Although none of the low-grade patients presented with evidence of nodal or distant metastasis, there was no observed difference in the development of metastasis over the course of their disease ($p = 0.32$).

Operative Results

A total of 202 operations were performed in the 97 patients. Patients received an average of 2.2 ± 0.1 operations (range 1- 6). Thirty percent (29/97) had one, 39% (38/97) had two, 21% had three (20/97), 7% had four (7/97), and 3% (3/97) had five or more operations. Sufficient information for detailed analysis of each individual operation was available in 98% (197/202) of the procedures performed.

Symptoms were reported before operation in 76% (149/197) of the evaluable procedures. In those patients who were asymptomatic, indications for the procedure often were based on identification of disease on physical exam or radiographic studies. Symptoms were reported less frequently at the time of the second operation ($p = 0.006$) than the first operation. Operations to explicitly manage symptoms were performed explicitly with palliative intent in 15% (29/197) of the cases and were most commonly encountered after the third operation ($p = 0.004$). The durability of symptom control tended to decrease after each operation and was significantly shorter after the third ($p < 0.001$) and fourth operations ($p = 0.02$).

Complete resection of all PMP associated tumour was achieved during at least one operation in 55% (53/97) of patients. Complete cytoreduction was more commonly associated with the first and second operations (operations 1 and 2 (35% (53/153) versus operation ≥ 3 (7% (3/44), $p < 0.001$). At the initial operations, biopsy alone was performed in 18% (17/97) of patients and others had an unsuccessful attempt at complete cytoreduction before specialty center referral. The ability to achieve complete cytoreduction was not associated with pathologic subtypes (mucinous adenocarcinoma, low grade (65% (30/46)) versus mucinous adenocarcinoma, high grade (55% (23/42)), $p = 0.32$). Of the 53 patients who underwent complete cytoreduction, 91% (48/53) recurred at a median of 24 months (range 2-103). The disease-free interval was not associated with pathologic subtype ($p= 0.30$), the extent of surgery ($p = 0.92$), or the operation number ($p = 0.83$). Sixty

percent (29/48) of the patients who recurred after complete cytoreduction underwent further operations, with 17% (5/29) of the operable patients obtaining complete cytoreduction a second time.

The extent of surgery score (ESS) was 0 in 10% (20/197), 1 in 20% (39/197), 2 in 49% (96/197), and 3 in 21% (42/197) of the procedures. At some time in their clinical history, 39% (38/97) of patients underwent an ESS-3 operation. There was a significant increase in the proportion of ESS-3 procedures performed at the second operation (27% (17/65), $p = 0.04$) compared to the first. Complete cytoreduction was associated with an ESS-3 procedure in 33% (14/42), an ESS-2 procedure in 34% (33/96), and an ESS-1 procedure in 21% (8/39). There was no difference in the frequency of complete cytoreduction comparing ESS-1, ESS-2 and ESS-3 operations ($p = 0.17$) respectively.

Fifty-eight percent (117/202) of all and 43% (42/97) of the initial operations were performed at the MSKCC. An ESS-3 procedure was performed in 25% (29/117) of the patients. An adjacent organ resection was performed in 33% (36/117) with patients receiving a small bowel resection in 15% (17/117), a splenectomy in 9% (10/117), a colectomy in 8% (9/117), a subtotal gastrectomy in 3% (3/117), a hysterectomy in 2% (2/117), and a segmental liver resection in 2% (2/117). A colectomy associated with the formation of a colostomy or ileostomy was required in 4% (4/117). A total gastrectomy was performed in a single patient (1% (1/117)).

Fifty-nine percent (59/97) of the patients received systemic chemotherapy at some time during their clinical course. 5-FU was the most commonly used agent (78% (46/59)). Thirty one percent (30/97) of the patients had a catheter placed for intraperitoneal chemotherapy. A 5-FU based agent was used for intraperitoneal chemotherapy in 67% (20/30) of these patients. Four patients received hyperthermic intraperitoneal chemotherapy. A therapeutic response to any type of chemotherapy was not documented in any subject.

The 30-day operative mortality rate was 4% (4/97). Uncontrolled intraabdominal sepsis after ESS-1 operations caused the deaths of two patients. Two additional deaths following ESS-3 operations resulted from a pulmonary embolism and a gastric perforation. A major complication resulting in reoperation, ICU admission, or chronic disability was identified after 16% (32/197) of the operations. ESS-3 operations had the highest major complication rate (38%) compared to ESS-1 (18% (7/39), $p = 0.19$) and ESS-2 procedures (9% (9/96), $p = 0.003$). A variety of complications was associated with each type of procedure, but a perforated viscus (gastric or colonic) was most commonly associated with an ESS-3 operation ($p = 0.01$). Even though 86% (24/28) of the patients who survived a major complication later had symptoms associated with PMP, an additional operation was performed in only 11% (3/28).

Long term overall survival

The median survival of the whole patient population (n= 97) was 9.8 years (range, 0.3 to 18.3 years) from the date of the initial operation. In patients with complete pathologic data, median survival was significantly longer in patients with mucinous adenocarcinoma low grade (12.8 years) versus high grade (4.0 years, $p < 0.001$), as seen in Fig. 1.

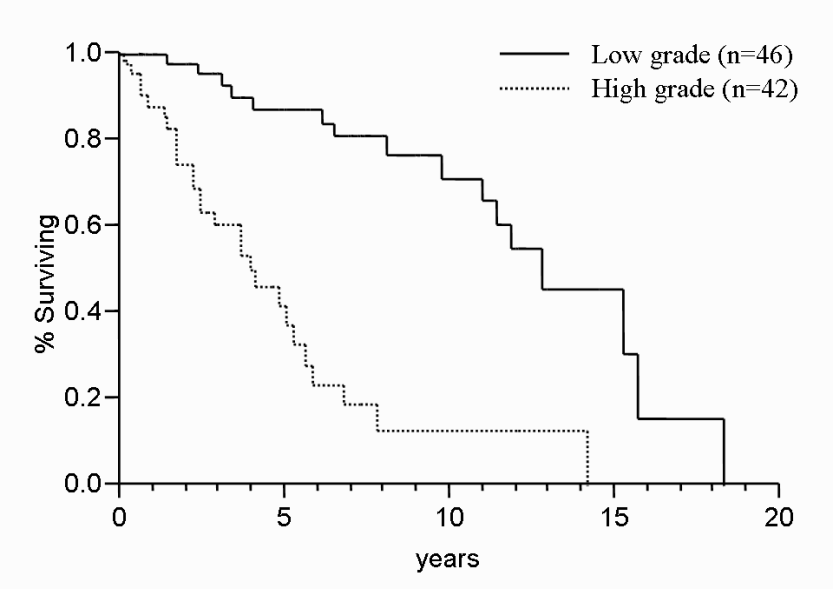


Figure 1. Long-term survival associated with pathologic designation ($p< 0.001$). Reprinted with permission from [3]

To identify factors important in determining survival, clinical and pathological factors were analyzed using univariate and multivariate analysis (Table 1). Univariate analysis showed that improved survival was associated with a clinical history of complete cytoreduction (median 12.8 years versus 4.2 years, $p < 0.001$), female gender (median 11.6 years versus 5.1 years, $p < 0.016$), a previous ESS-3 surgery (median 11.9 years versus 6.6 years, $p = 0.032$), and a prolonged disease free interval ($\geq$ 24 months) following complete cytoreduction (median 12.8 years versus 8.2 years, $p = 0.048$). On multivariate analysis, however, only the designation of low-grade mucinous adenocarcinoma and the history of a complete cytoreduction were independently associated with prolonged survival.

Table 1. Uni- and multivariate analysis of variables predicting survival in pseudomyxoma peritonei

Variable	N	Univariate	Multivariate	
All patients	88	p	Hazard ratio (CI)	p
Low grade mucinous adenocarcinoma	48	<0.001	5.1 (2.6-10)	**<0.001**
History of complete cytoreduction	53	<0.001	2.7 (1.4-5.3)	**0.003**
History of radical ESS-3 surgery	38	0.032	1.7 (0.8-3.1)	0.21
Female gender	45	0.016	1.1 (0.8-1.6)	0.38
Disease free interval ≥ 24 months	23	0.048	1.1 (0.4-2.1)	0.88
Number of operations	88	0.24	-	-
Age > 55 years	41	0.35	-	-
Systemic chemotherapy	56	0.39	-	-
Intraperitoneal chemotherapy	29	0.41	-	-

Patients experienced 10-year survival in 21% (43% low grade versus 2% high grade). Among all patients with PMP, 10-year survival was most likely to be associated with female gender (75% (15/20), $p = 0.04$) and a pathologic classification of low-grade mucinous adenocarcinoma (90% (18/20), $p < 0.001$). Although 10-year survivors more frequently had a complete cytoreduction at some time in their clinical course (75% (15/20), $p = 0.04$), they did not have radical ESS-3 operations at higher rates (50% (10/20), $p = 0.27$). Due to the advanced age and chronicity of their disease, the precise cause of death in some patients could not be determined adequately in this analysis. At the time of death or the completion of follow-up, however, 23% (6/20) of the 10-year survivors were disease-free. Only twelve percent (12/97) of the patients in this study were alive with no evidence of disease at the time of last follow-up [3].

Evaluating the Impact of Therapy for PMP

The MSKCC study suggests that PMP remains a disease that follows "an unremitting but prolonged clinical course" [15]. Despite a much-improved understanding of the biology of this condition, the impact of therapy is still incompletely understood. Even though complete cytoreduction is associated with prolonged overall survival, recurrence of disease is common and multiple operations are frequently required. Patients may enjoy sustained periods of remission, free of symptoms, but a long-term disease-free survival is uncommon [3].

The most important common observation between the MSKCC and other studies is the dominant impact that biology plays on long-term outcome. Pathology data were analyzed in the MSKCC series using a schema comparable to that proposed by Sugarbaker's group [4]. The overall median survival was 9.8 years. Patients with mucinous adenocarcinoma, low grade, had an improved overall sur-

vival of 12.8 years compared to those with the high grade variant, where the median survival was 4 years. This report confirms observations made by others that biologic characteristics associated with low-grade forms of PMP are independently associated with improved survival [4,18].

Support for more aggressive therapy of PMP is based often on comparisons of overall survival in patient groups with diverse or poorly specified pathologic subtypes. Patients with PMP selected for aggressive therapeutic paradigms, such as those advocated by Sugarbaker's group, by definition, only have benign or low-grade lesions [6,8,11]. The demonstration of improved survival in a favourable group following maximal therapy does not allow one to properly conclude whether superior results were caused by the biology of the disease process, by good patient selection, or by the specific treatment. As shown in Fig. 2, the overall survival using a treatment strategy based on the selective application of extensive debulking procedures and priority given to function preservation and symptom management was equivalent to the 5-year and 10-year survival rates of 75% and 68% reported by Sugarbaker et al [8,18]. This observation does not support the conclusions of authors who propose that radical cytoreduction and HIPEC are responsible for improved survival in patients with PMP. It suggests that the biology of the disease, rather than the aggressiveness of treatment, ultimately defines outcome.

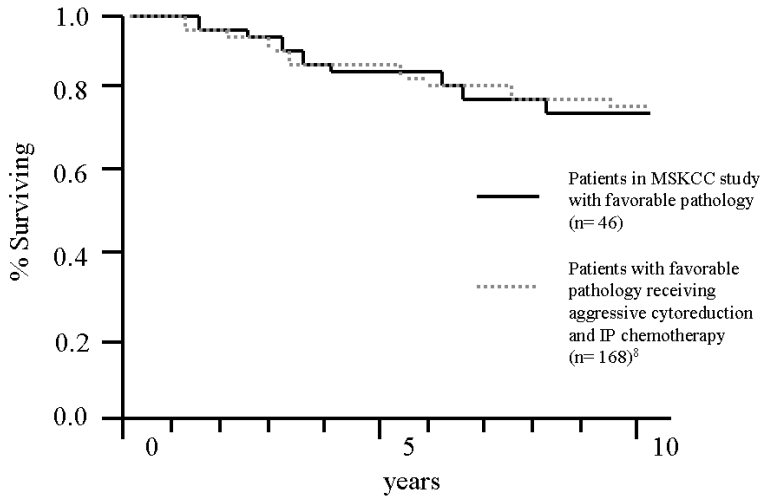


Figure 2. Comparison of long-term survival in patients with PMP from studies representing different treatment philosophies. Survival curves were adjusted to reflect similar ten-year time intervals (years on the x axis). The dotted line represents the overall survival of patients receiving aggressive surgical cytoreduction and intraperitoneal chemotherapy [8]. In order to be selected for this therapy, patients, by definition, had favorable pathologic characteristics. The black line represents patients from the MSKCC study that had comparable favorable pathologic features. Reprinted with permission from [3]

In the MSKCC study, improved survival was associated with complete cytoreduction. Patients able to undergo complete cytoreduction at some point in their therapy had a median survival of 12.8 years. This finding is consistent with other reports that suggest that improved survival is associated with complete cytoreduction [19-22] and refutes claims of authors who state that long term survival has never been identified in patients undergoing cytoreduction alone [23,24]. The cause-effect role of surgical cytoreduction, however, is not clear. In the MSKCC study, more extensive ESS-3 operations were not clearly associated with either improved survival or a greater likelihood of complete cytoreduction. This suggests that the extent of surgery, as demonstrated by the ESS score, may reflect a surgeon-dependent phenomenon that is not independently associated with improved survival. The completeness of cytoreduction may reflect a disease-phenomenon (extent of disease) and emerges as more predictive of outcome. Stewart et al. have recently shown that age at time of perfusion, performance status, the completeness of debulking and the duration of IPHC are independently associated with overall survival in patients with PMP [10]. Although it is impossible to determine the relative contributions of each of these factors on overall survival because of patient selection factors, these data suggest that technical aspects of therapy for PMP under the control of the surgeon may have an impact on patient outcome.

Analysis of recurrence data from the MSKCC series underscores the limitations of using overall survival as the principal endpoint in evaluating patients with PMP. Following complete cytoreduction, 91% of the patients experienced disease recurrence, with a median disease-free interval of only 24 months. The disease-free interval was not associated with pathologic subgroup, the extent of surgery, or operation number. Even in patients who experienced the best outcomes, disease recurrence was common. Ninety percent of the 10-year survivors required multiple operations for PMP recurrence and 77% had evidence of disease either at death or at the completion of follow-up. Other authors have noted that recurrence is common following an operation for PMP. In the Mayo series, 67% of patients ultimately developed recurrence and 50% of recurrence occurred within 2.5 years [19]. Although the short-term recurrence of PMP following aggressive treatment protocols has been stated to be in the 35%-40% range, recurrence data from the literature on long-term survivors is unclear thereby limiting the ability to make useful comparisons to the MSKCC report [9,22]. This observation suggests, however, that a disease-free state is not an absolute requirement for long-term survival in PMP [3].

Although a demonstrable long-term survival makes it tempting to claim that surgery for PMP is potentially curative, the high likelihood of recurrent disease suggests that such claims are imprecise. Although cure, defined as long-term recurrence- free survival, is rare, careful application of surgical interventions may benefit carefully selected patients. Unfortunately, surgical therapy is viewed often in an overly simplistic manner based on either “curative” or “noncurative” designations [25]. The use of terminology such as “cure” when discussing a disease such as PMP often leads to arguments that fail to appropriately characterize the complexity of a disease in which recurrence is commonly observed during a prolonged natural history. Use of the term *remission*, frequently used to describe stable

disease in patients with hematologic malignancies, would be a more accurate way to describe the course of patients with PMP in a disease-free or symptom-free state [3].

The significance of such terminology is not simply a matter of semantics. During the curative phase of therapy, consequences of treatment such as severe acute toxicity, patient discomfort, and even mortality may be viewed as acceptable risks in order to achieve prolongation of life [26]. Although recent reports suggest improved morbidity and mortality with more aggressive therapy for PMP, the functional and quality of life issues sometimes encountered after aggressive surgical procedures for PMP cannot be understated [27]. By predicating decisions with expectations of cure, the surgeon may be encouraging the patient to accept risks that he or she might not otherwise find acceptable. In addition, the presentation of survival data out of context to the natural history of PMP or without relevant recurrence data, can potentially "frame" or bias patient decisions in an inappropriate manner. Despite an individual patient's enthusiasm regarding such therapy, in such circumstances, the surgeon must avoid minimizing the risks of morbidity and reduced quality of life that might result. By understating the known uncertainties regarding PMP, surgeons not only weaken the informed consent process but also endanger the foundations of a strong and enduring therapeutic alliance that will surely be required for the optimal care of the patient during the long-term survival associated with this disease [28]. Claims that suggest that more aggressive forms of therapy for PMP are "standard of care" are similarly problematic and potentially misleading [2,28]. A basic definition of standard of care is the degree of care a reasonable surgeon would take to prevent harm to similar patients in similar situations [29]. This legal term should not be used to convey encouraging results, physician enthusiasm and convictions, or international support. The very nature of this debate on appropriate therapy for PMP clearly shows that reasonable surgeons do not agree on the interpretation of available data. Clearly, more work is needed before any therapeutic approach for PMP can be accurately or appropriately called "standard of care."

Like most other reports on PMP, patient selection plays a major role in any therapy that is utilized. In the MSKCC series, the surgical intent of each subsequent operation evolved through the course of the patient's disease. During the initial procedures, operations tended to be more aggressive and more often resulted in complete cytoreduction. Although patients usually had symptoms, asymptomatic patients were brought to operation more frequently for disease only appreciated on radiographic studies or physical exam. This report demonstrates several factors that may explain, in part, surgeons' changing approach to patients with PMP. Following an ESS-3 procedure, subsequent attempts at complete cytoreduction were rarely successful. Perhaps surgeons chose not to offer patients further radical surgery having already failed at an earlier attempt. Following an earlier operation associated with a major complication, furthermore, patients infer-quently received an additional operation, suggesting that surgeons choose not to select patients for additional procedures following serious morbidity. After initial attempts at more aggressive therapy, operations became progressively more palliative in nature. It is impossible for the MSKCC report to determine which factors

were used by surgeons to select PMP patients for palliative operations. It appears that symptom severity, physical and functional status, expected durability of the procedure, and expected survival of the patient play significant roles in this decision making process. In attempts to create theoretically attractive and uniform treatment protocols, the critical role of patient selection should not be minimized, but rather, explored to understand key factors involved in good clinical decision-making [3].

Over the past two decades great improvement in the care of patients with PMP has resulted from the efforts of those dedicated to overcoming therapeutic nihilism from physicians in order to serve those patients suffering with PMP better. Studies to date have revealed important and interesting associations, but they have not been able to fully define the impact of therapy on patient outcome. Ultimately, the superiority of various treatment protocols will need to be determined by randomized prospective trials carefully designed to look at recurrence-free survival and quality of life. However, a phase III trial in PMP will be very difficult to conduct in this rare disease due to potentially insurmountable issues of equipoise between different treatment arms and appropriate designation of patients in a disease that displays a wide spectrum of biologic and clinical aggressiveness often over what is often a decades-long natural history. Until such trials occur, those entrusted with the care of patients with PMP will be challenged as they help their patients cope with many challenging decisions regarding risk and benefit, extensive surgical procedures and symptomatic debulking, curative or palliative strategies and the appropriate timing of selected interventions [30]. The thoughtfulness required to address these problems will continue to benefit our patients as we strive to achieve the ultimate goal of the surgical oncologist: helping our patients live as well as they can for as long as they can.

References

1. Fann JI, Vierra M, Fisher D, et al (1993) Pseudomyxoma peritonei. Surg Gynecol Obstet 177:441-447
2. Sugarbaker PH (2006) New standard of care for appendiceal epithelial neoplasm and pseudomyxoma peritonei syndrome? Lancet Oncol 7:69-76
3. Miner TJ, Shia J, Jaques DP, et al (2005) Long-term survival following treatment of pseudomyxoma peritonei: an analysis of surgical therapy. Ann Surg 241:300-308
4. Ronnett BM, Zahn CM, Kurman RJ, Kass ME, Sugarbaker PH, Shmookler BM (1995) Disseminated peritoneal adenomucinosis and peritoneal mucinous carcinomatosis. A clinicopathologic analysis of 109 cases with emphasis on distinguishing pathologic features, site of origin, prognosis, and relationship to “pseudomyxoma peritonei”. Am J Surg Path 19:1390-1408
5. Mohamed F, Gething S, Haiba M, Brun EA and Surgarbaker PH (2004) Clinically aggressive pseudomyxoma peritonei: a variant of a histologically indolent process. J Surg Oncol 86:10-5

6. Sugarbaker PH (1994) Pseudomyxoma peritonei: a cancer whose biology is characterized by a redistribution phenomenon. Ann Surg 219:109-111
7. Carmignani P, Sugarbaker TA, Bromley CM, Sugarbaker PH (2003) Intraperitoneal cancer dissemination: mechanisms of the patterns of spread. Cancer Metastasis Rev 22:465-472
8. Esquivel J, Sugarbaker PH (2000) Clinical presentation of the Pseudomyxoma peritonei syndrome. Brit J Surg 87:1414-1418
9. Hinson FL, Ambrose NS (1998) Pseudomyxoma peritonei. Brit J Surg 85:1332-1339
10. Stewart JH, Shem P, Russell GB, et al (2006) Appendiceal neoplasm with peritoneal dissemination: outcomes after cytoreductive surgery and intraperitoneal hyperthermic chemotherapy. Ann Surg Oncol 13:624-634
11. Sugarbaker PH (2001) Cytoreductive surgery and peri-operative intraperitoneal chemotherapy as a curative approach to pseudomyxoma peritonei syndrome. Eur J Surg Oncol 27:239-243
12. American Society of Clinical Oncology: Outcomes of Cancer Treatment for Technology Assessment and Cancer Treatment Guidelines (1996) J Clin Oncol 14:671-679
13. Tamburini M, Casali PG, Miccinesi G (2000) Outcome Assessment in Cancer Management. Surg Clin N Am 80:1-14
14. Spertus J (2001) Selecting end points in clinical trials: what evidence do we really need to evaluate a new treatment? Am Heart J 142:1-4
15. Portilla AG, Sugarbaker PH, Chang D (1999) Second-look surgery after cytoreduction and intraperitoneal chemotherapy for peritoneal carcinomatosis from colorectal cancer: analysis of prognostic features. World J Surg 23: 13-29
16. Wirtzfeld DA, Rodriguez-Bigas M, Weber T, Petrelli NJ (1999) Disseminated peritoneal adenomucinosis: a critical review. Ann Surg Oncol 6:797-801
17. Prayson RA, Hart WR, Petras RE (1994) Pseudomyxoma peritonei. A clinicopathologic study of 19 cases with emphasis on site of origin and nature of associated ovarian tumours. Am J Surg Path 18:591-603
18. Ronnett BM, Yan H, Kurman RJ, Shmookler BM, Wu L, Sugarbaker PH (2001) Patients with pseudomyxoma peritonei associated with disseminated peritoneal adenomucinosis have a significantly more favorable prognosis than patients with peritoneal mucinous carcinomatosis. Cancer 92:85-91
19. Gough DB, Donohue JH, Schutt AJ, et al (1994) Pseudomyxoma peritonei. Long-term patient survival with an aggressive regional approach. Ann Surg 219:112-119
20. Smith JW, Kemeny N, Caldwell C, Banner P, Sigurdson E, Huvos A (1992) Pseudomyxoma peritonei of appendiceal origin. The Memorial Sloan-Kettering Cancer Center experience. Cancer 70:396-401
21. Wertheim I, Fleischhacker D, McLachlin CM, Rice LW, Berkowitz RS, Goff BA (1994) Pseudomyxoma peritonei: a review of 23 cases. Obstet Gyn 84:17-19
22. Sugarbaker PH, Ronnett BM, Archer A, Averback AM, Bland R, Chang D, et al (1996) Pseudomyxoma peritonei syndrome. Adv Surg 30:233-280

23. Glehen O, Mohamed F, Sugarbaker PH (2004) Incomplete cytoreduction in 174 patients with peritoneal carcinomatosis from appendiceal malignancy. Ann Surg 240;278-285
24. Sugarbaker PH, Alderman R, Edwards G, et al (2006) Prospective morbidity and mortality assessment of cytoreductive surgery plus perioperative intraperitoneal chemotherapy to treat peritoneal dissemination of appendiceal mucinous malignancy. Ann Surg Oncol 13:635-644
25. Miner TJ, Jaques DP, Tavaf-Motamen H, Shriver CD (1999) Decision making on surgical palliation based on patient outcome data. Am J Surg 177:150-154
26. Lustig A, Scardino P (1998) Elective patients. Surgical Ethics, Oxford University Press, NY, p133-151
27. Sugarbaker PH (2003) Clinical research to standard of care: when does the transition occur? Ann Surg Oncol 10;825-826
28. McCullough LB, Jones JW, Brody BA (1998) Informed consent: autonomous decision making of the surgical patient. Surgical Ethics, Oxford University Press, NY, p15-37
29. Strasberg SM (2005) Biliary injury in laparoscopic surgery: processes used in determination of standard of care in misidentification injuries. J Am Coll Surg 201;598-603
30. Miner TJ, Jaques DP, Brennan MF, Coit DG (2005) Are there curative options for pseudomyxoma? Ann Surg 242(5):750-751

Clinical Results of Cytoreduction and HIPEC for Malignant Peritoneal Mesothelioma

HR Alexander, N Hanna, JF Pingpank

Introduction

Malignant Peritoneal Mesothelioma (MPM) is rare primary neoplasm that arises from the serosal membranes of the abdominal cavity. The Surveillance, Epidemiology, and End Results (SEER) program from the U.S. National Cancer Institute maintains data from 11 cancer registries representing approximately 27% of the U.S. population; analysis of this database indicate that about 400 new cases of MPM arise annually in the U.S. [1]. This represents about 17 and 7 percent of all mesothelioma cases diagnosed in females and males, respectively.

Histologically, MPM are generally characterized as low-grade which includes adenomatoid and tubulopapillary tumours and high-grade which includes epitheliod, sarcomatoid, or biphasic tumours; biphasic mesothelioma is defined as a tumour that has both epithelial and sarcomatoid components. High-grade tumours make up between 62% - 76% of all MPM [2,3]. Nonaka and colleagues have evaluated histopathological features of MPM from patients undergoing cytoreduction and heated intraoperative intraperitoneal chemotherapy (HIPEC) and found that most tumours express calretinin and EGFR [4]. In an initial series they reported that patients with tumours that had a high nuclear grade or those with ≥ 5 mitoses per 50 high powered field (HPF) had a significantly shorter survival than patients without those tumour features; however, nuclear grade was not a significant prognostic factor in a follow-up analysis of a larger cohort [5].

Grossly, tumours are diffusely disseminated throughout the peritoneal cavity and depending on the extent of progression can range from a few millimeters to large nodular masses which, in the late stages, will coalesce to form large nodular masses that can replace the greater and less omentum and encase viscera (Fig. 1). Ascites is a common consequence of MPM. The disease remains confined to the abdominal cavity until very late stages in the course when it can spread, usually by direct extension, through the diaphragm into the thorax; hematogenous metastases generally are rare.

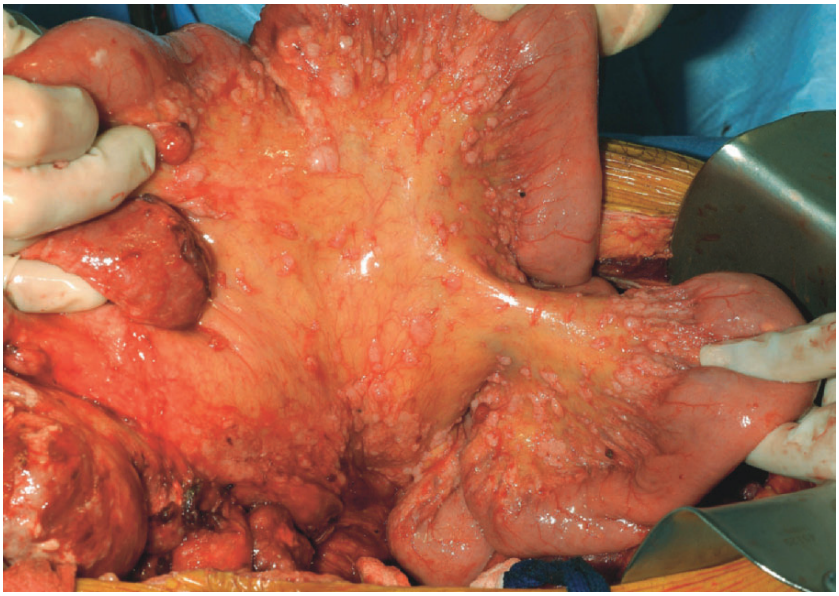


Figure 1. Intra-operative photograph of diffuse MPM on the small bowel mesentery

MPM presents with non-specific signs and symptoms; patients present with complaints related to ascites or large tumour burden. Occasionally, patients are diagnosed incidentally. The median age of presentation is between 40 and 65 [6]. Unfortunately, due to the indolent progression of nonspecific symptoms, many patients present with advanced disease. Abdominal distension is the most frequent initial symptom and is associated with early satiety, loss of lean body mass and overall inanition. Increased abdominal girth is the presenting symptom in 56-82% of patients [7,8]. Pain is the second most common symptom, found in 27-58% of presenting patients [7-10]. Up to a third of patients present with a palpable abdominal mass [9,11].

The vast majority of patients die from complications of intraperitoneal tumour progression and based on this consistent natural history and the lack of effective systemic therapies, regional therapies designed to control disease progression with the peritoneal cavity have been actively developed over the past 20 years [12]. Reports of patients with MPM treated primarily with chemotherapy or biological agents have resulted in median survivals of less than one year [13,14]. Antman and colleagues were the first to note over 20 years ago that surgical resection (or cytoreduction) combined with intraperitoneal chemotherapy was associated with prolonged survival in some patients with MPM [15]. A number of medical centers throughout the world are now reporting long-term overall survival and median survival between 30 and 90 months for patients with MPM following cytoreduction and HIPEC; this approach has now emerged as the standard of care for selected patients with MPM (Table 1).

Table 1. Summary of results of clinical series of cytoreduction and HIPEC for patients with malignant peritoneal mesothelioma

Center	N	Optimal Cytoreduction	HIPEC Agents	Median Survival (months)	Prognostic Factors
Centre Hospitalier Lyon Sud, France	15	11	Cisplatin MMC	36	Optimal resection, Stage at Rx
National Cancer Institute, Milan	49	43	Cisplatin and MMC or doxorubicin	57%*	Optimal resection, Mitotic count
Columbia-Presbyterian, NY	27	-	Cisplatin MMC	68	
Wake Forest, NC	12	5	MMC	34	
Washington Hospital Center	68	41	Cisplatin and Doxorubicin	67	Optimal resection, Female gender
NCI, Bethesda	49	43	Cisplatin	92	Optimal resection, Age <60, No deep invasion

HIPEC, hyperthermic intraperitoneal chemotherapy; *5 year survival

Results of Cytoreduction and HIPEC

The selection criteria and treatment parameters used at various medical centers that treat patients with cytoreduction surgery and HIPEC vary to one degree or another but, in principal, all share the same goals of complete resection of all gross disease combined with a 60 to 120 minute perfusion using either an open or closed technique with one of several chemotherapy agents, the most commonly used being mitomycin C or cisplatin [16].

Optimal selection criteria have been better defined in great part because of the contribution of Sugarbaker and colleagues at the Washington Hospital Center, Washington, D.C. He and his colleagues systematically scored findings on preoperative CT scans from 30 individuals with MPM treated with cytoreduction surgery and HIPEC and identified features associated with adequacy of cytoreduction [17]. Scoring was done by a reader who was blinded as to the operative findings. They identified the presence of a > 5 cm mass in the epigastric region and loss of normal architecture of the small bowel and its mesentery as two radiographic features strongly associated with suboptimal cytoreduction (Fig. 2). In patients who had neither of these 2 radiographic findings there was a 94% probability of adequate cytoreduction defined as all residual tumour nodules < 2.5 cm in diameter. Kebapci and colleagues also reported features of CT findings in 11 patients with MPM and concluded that they are frequently non-specific and not sufficiently characteristic to pinpoint a specific diagnosis [8].

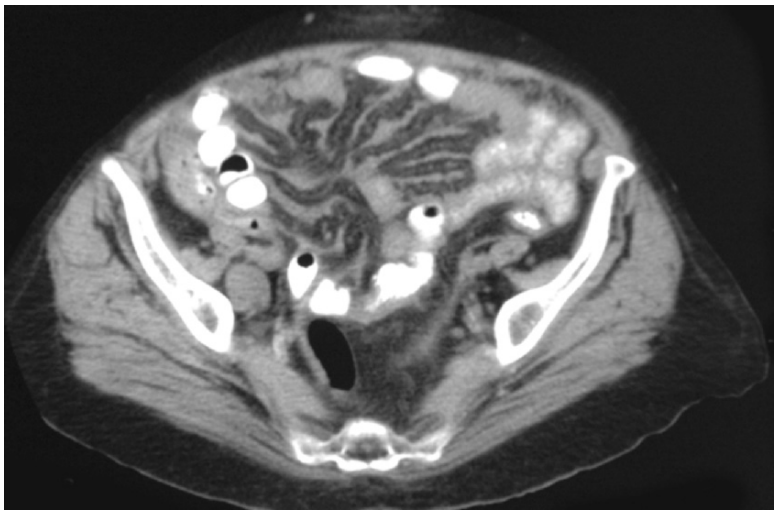


Figure 2. Contrast enhanced CT scan demonstrating diffuse serosal thickening and nodularity in a woman with unresectable MPM

Serum chemistries and markers have no value in establishing a diagnosis. Hyaluronan, CA-125, alpha fetoprotein, carcinoembryonic antigen, and tissue polypeptide antigen have been evaluated and although some patients have elevated levels concordant with disease progression, the specificity of serum tumour markers remains low. Many patients have an elevated CA-125 and it is a useful marker to verify response to therapy or for surveillance. Recently, Hassan and colleagues have shown that mesothelin, a cell surface glycoprotein highly expressed in mesothelioma, is shed into the circulation and can be quantified in the serum in a high percentage of individuals with mesothelioma [18]. They developed a mesothelin specific ELISA and noted elevated levels in 40 of 56 (71%) of patients with mesothelioma. Moreover, serum mesothelin levels were measured in 4 of 6 patients with MPM pre-operatively; the mesothelin levels decreased in the 3 of 4 patients who successfully underwent cytoreduction and HIPEC and were undetectable by day 7. These data suggest that serum mesothelin may be a useful and sensitive marker for response and recurrence in patients with MPM.

Based on a growing number of clinical reports, the outcome for selected patients with MPM undergoing cytoreduction and HIPEC is very good (Table 1). Durable progression-free survival, overall survival, palliation of symptoms secondary to ascites, and improved health-related quality-of-life (HRQOL) endpoints have been described by centers in the United States and Europe.

Glehen and colleagues at the Centre Hospitalier Lyon Sud reported outcomes in 15 MPM patients treated over a 15 year period with cytoreduction and HIPEC [19]. Eleven of 15 patients were scored as having an optimal resection defined as gross residual disease < 2.5 mm. Treatment consisted of a 90 minute perfusion with mitomycin C, 0.5 mg/kg, and cisplatin, 0.7 mg/kg and target intraperitoneal temperature of between 42.0 and 42.5 degrees C (Table 2).

Table 2. Typical HIPEC parameters used at various centers

Duration (min)	90-120
Target temperature (°C)	40-42.5
Perfusate volume (liter)	4-6
Flow rate (liter/min)	1-1.5
Agents (alone or in combination)	Mitomycin C, doxorubicin, cisplatin

The median overall survival was 35.6 months and the 5-year actuarial survival rate was 29%. Factors associated with poor outcome (shortened survival) were advanced stage of disease at presentation and suboptimal resection. In patients who had suboptimal resection the median survival was only 6.5 months. Deraco and colleagues from the National Cancer Institute, Milan, Italy have published 2 papers detailing their institutional experience with cytoreduction and HIPEC in this clinical setting [5,20]. In the most recent series, a detailed analysis of 49 patients with MPM was presented. The mean operative time was almost 10 hours (529 minutes, range: 250-720 min) and 43 patients (89%) had optimal cytoreduction, defined as before as all residual disease $\leq$ 2.5 mm. The treatment regimen consisted of cisplatin, 25 mg/m^2/L, with mitomycin C, 3.3 mg/m^2/L, or doxorubicin, 15.25 mg/m^2/L administered under hyperthermic conditions for 60 to 90 minutes. They evaluated a number of clinical and pathological factors for association with survival and found, as did others, that completeness of cytoreduction correlated with overall survival. In addition, a high mitotic count (• 5/50 HPF) was correlated with significantly shortened survival.

A number of medical centers in the United States have also published institutional experiences with cytoreduction surgery with HIPEC or some type of intraperitoneal, intravenous, or multimodal chemotherapy. Investigators at Roswell Park reported outcomes in 15 women with MPM treated over a 32 year interval [21]. The median overall survival was 12.5 months and was significantly longer in the cohort selected for cytoreduction surgery versus those treated without surgery. Optimal cytoreduction was associated with the best outcome but based on the very small number of patients no statistically significant differences in outcome based on the completeness of cytoreduction could be identified. Interestingly, survival was also significantly longer in the cohort selected for chemotherapy after surgery. One limitation of this report is the long and retrospective nature of the study. Under these circumstances, outcomes associated with surgical resection or chemotherapy may have been principally due to a selection bias to offer more aggressive therapy to women who had better performance status or a more limited tumour burden.

Investigators at Columbia-Presbyterian Medical Center have employed an ambitious multimodal two staged approach for patients with MPM [12,22]. In stage 1, patients undergo surgical debulking via laparotomy, placement of an intraperitoneal catheter, and receive intraperitoneal cisplatin, doxorubicin, and gamma interferon for four months. Stage 2 commences with a second laparotomy, complete cytoreduction of residual disease, and HIPEC using cisplatin and mitomycin C

followed by whole abdominal radiotherapy. The median overall survival of the 27 patients treated in this study was 68 months.

Loggie, Levine, and colleagues from Wake Forest University reported outcomes in 12 patients with MPM who underwent cytoreduction and HIPEC using mitomycin C, given in divided doses of 30 mg at time 0 and 10 mg at time 60 minutes, of a planned 90 minute perfusion [23]. The median overall survival was 34 months; of note, in 7 patients who had symptomatic ascites, 6 had permanent control of their ascites. This observation has been made by others and is widely acknowledged as one of the major palliative benefits associated with treatment for patients with MPM. In an early report of 18 patients with MPM treated at the National Cancer Institute, Bethesda, Maryland, Bartlett, Alexander and colleagues noted resolution of symptomatic ascites in 9 of 10 patients (Fig. 3) [24].

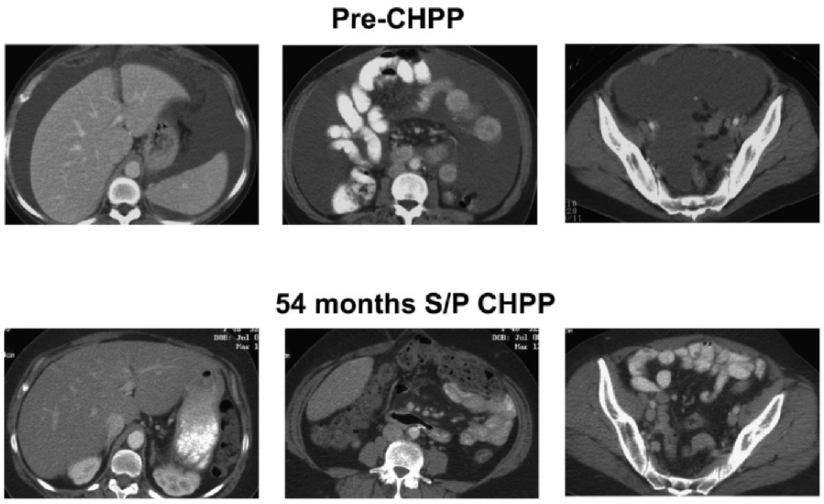


Figure 3. Axial abdominal computed tomography scans showing complete resolution of ascites in a patient with malignant mesothelioma after tumor resection and HIPEC

Three developed recurrent ascites at 10, 22, and 27 months after therapy. It is noteworthy that resolution of ascites after HIPEC occurred in patients who had minimal cytoreduction or bulky residual disease suggesting a direct role for HIPEC in this setting.

Alexander and colleagues from the NCI, Bethesda, Maryland, have published an updated report of 49 patients with MPM treated with cytoreduction and HIPEC using cisplatin, 250 mg/m^2 administered under hyperthermic conditions for 90 minutes. This dose of cisplatin is administered with systemic sodium thiosulfate as a nephroprotective agent. Thirty-five patients also received a single intraperitoneal dwell of 5-fluorouracil and paclitaxel in 2 L of saline between post-operative days 7 to 10. Approximately 50% of patients were debulked to residual disease < 5 mm and 88% to residual disease < 10 mm (Table 3).

Table 3. Clinical and pathology characteristics in patients with malignant peritoneal mesothelioma treated at the NCI, Bethesda, Md, USA

		N	%
Total number		49	
Gender	Female	21	
	Male	28	
Age (years)	Range	16-76	
	Mean	49	
	Median	47	
Prior chemotherapy	None	41	84
	Paclitaxel/cisplatin	8	16
Prior surgery	Exploration/adhesiolysis	18	37
	Cytoreduction/organ resection	7	14
	None	24	49
Histological type*	High grade	30	64
	Epitheloid	26	
	Sarcomatoid	4	
	Low grade	17	36
	Tubulopapillary	16	
	Adenomatoid	1	

*available in 47 patients

The median overall survival was 92 months (Fig. 4).

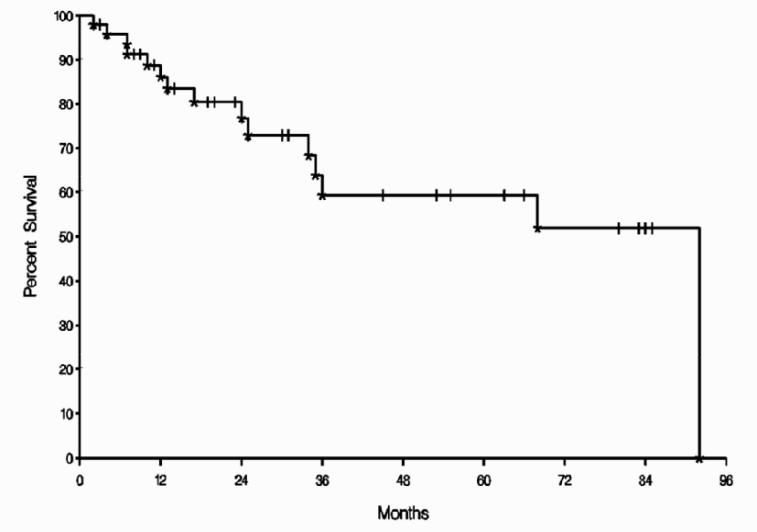


Figure 4. Actuarial overall survival in 49 patients with malignant peritoneal mesothelioma after cytoreduction and HIPEC

A multivariate analysis of factors associated with progression-free (PFS) and overall survival (OS) identified several factors that were independently correlated with outcome (Table 4).

Table 4. Prognostic significance of clinicopathologic variables based on Cox proportional hazards model analysis

End Point	Variable (in terms of poor prognosis)	Param. Est. (SE)	p	Hazard Ratio (95% CI)
PFS	No previous debulking (vs. debulking)	1.55 (0.43)	0.0003	4.70 (2.02-10.9)
	Deep invasion (v.no deep invasion)	1.28 (0.44)	0.003	3.60 (1.53-8.48)
OS	Age >60 (v. age $\leq$ 60)	1.29 (0.61)	0.034	3.65 (1.10-12.1)
	No previous debulking (vs. debulking)	1.67 (0.80)	0.036	5.33 (1.12-25.4)
	Deep invasion (vs. no deep invasion)	1.44 (0.71)	0.041	4.24 (1.06-16.9)
	Residual disease > 1 cm (vs. none, <1 cm)	1.75 (0.82)	0.032	5.76 (1.16-28.5)

Param. Est, parameter estimate; SE, standard error; CI, confidence interval; PFS, progression free survival; OS, overall survival

A history of previous cytoreductive surgery was a factor associated with improved PFS and OS and most likely represents a surrogate marker for indolent tumour biology. As with other reports, the completeness of cytoreduction was also an important predictor of OS. Actuarial survival curves showing the effects of age, residual disease status, and history of previous cytoreduction are shown in Fig. 5.

Sugarbaker and colleagues from the Washington Hospital Center have made many important contributions to the field of management of patients with peritoneal dissemination from cancer; this is amply exemplified by their reports on the management of MPM patients. They have published a detailed report of 33 patients with MPM treated with cytoreduction, HIPEC, and early post-operative intraperitoneal chemotherapy [25]. The HIPEC regimen consisted of cisplatin, 50 mg/m^2 and doxorubicin, 15 mg/m^2 delivered under hyperthermic conditions for 90 minutes. Nine patients who had massive ascites at presentation underwent induction chemotherapy with intraperitoneal cisplatin and doxorubicin administered for 5 consecutive days monthly and patients with moderate or greater residual disease received the same regimen. Seven other patients received systemic chemotherapy before surgery. Seventeen of 33 patients had residual disease less than 2.5 cm; median overall survival in this series was 30 months. As in other reports, the completeness of cytoreduction was associated with improved survival. In an updated report of 68 patients with MPM the median overall survival had improved to 67 months; female gender remained a favourable prognostic parameter [7].

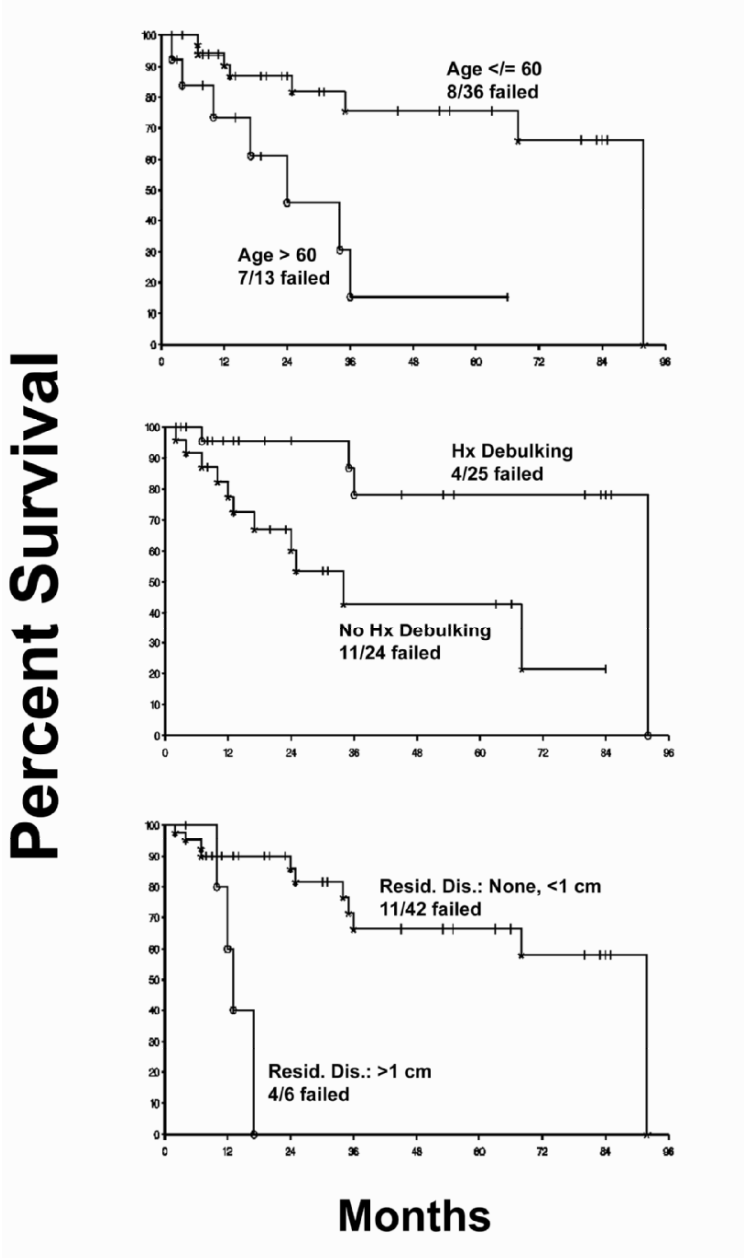


Figure 5. Effects of age (A), history (Hx) of previous debulking (B), and status of residual disease after debulking (C) on overall survival after treatment

Morbidity and mortality from treatment in these series is in line with those observed using this technique for peritoneal dissemination from other types of cancer. The mortality in the published series of more than 20 patients ranges from 0% to 7%. Morbidity appears to be related to the extent of surgery and the intensity of perioperative chemotherapy; on average the complication rate is 25%. Complications related to laparotomy and cytoreduction include fistula, bleeding, wound infection, and sepsis. Complications related to chemotherapy are almost invariably related to myelosupression.

Summary

Taken together, these reports provide very provocative and encouraging data that have prompted some to conclude that cytoreduction and HIPEC represents a "new standard of care" for patients with MPM [26]. Certainly, for selected patients who have good performance status (low operative risk) and in whom complete or near complete cytoreduction can be achieved, this form of therapy is associated with a very notable overall survival ranging from 67 to 92 months in 2 larger series.

Patient selection remains the central criteria for successful outcome. Patients should be carefully evaluated for co-morbid illnesses that would make them an unacceptable operative risk. Subsequently, CT scan and possibly laparoscopy should be performed to assess resectability with the appreciation that patients with suboptimal resection do very poorly. Pre-operative assessment of disease resectability is difficult to ascertain but some useful information can be obtained from a careful review of the CT scan; some investigators have advocated routine laparoscopy. Technically, details of HIPEC vary from center to center to some degree with respect to type of chemotherapy, dose of chemotherapy, duration of HIPEC, degree of hyperthermia, and method of recirculating the chemotherapy using either the open or closed technique. The use of the HIPEC technique, however, is considered the optimal method of ensuring complete distribution of therapeutic agents to the peritoneal cavity. Hyperthermia is routinely used for its synergistic actions with chemotherapy and its direct tumoricidal activity in experimental models. However, the therapeutic contribution of HIPEC above the effects of successful cytoreduction cannot be determined with available data although palliation of ascites is observed with HIPEC even without cytoreduction. There are no data indicating that one intra-operative chemotherapy regimen is superior to any other. The centers that report use of prolonged induction or post-operative intraperitoneal chemotherapy do not appear to have superior outcomes to those centers that use a more simple treatment regimen. Finally, although the intensity of therapy is considerable, once recovered, the patients appear to enjoy a good HRQOL. Although not specific for patients with MPM, 2 reports have convincingly demonstrated that HRQOL is significantly improved after HIPEC [27,28].

References

1. Hassan R, Alexander R (2005) Nonpleural mesotheliomas: mesothelioma of the peritoneum, tunica vaginalis, and pericardium. Hematol Oncol Clin North Am 19(6):1067-1087
2. Suzuki Y (1992) Diagnostic criteria for human diffuse malignant mesothelioma. Acta Pathol Jpn 42(11):767-786
3. Kannerstein M, Churg J (1977) Peritoneal mesothelioma. Hum Pathol 8:83-94
4. Nonaka D, Kusamura S, Baratti D, Casali P, Cabras AD, Younan R, Rosai J, Deraco M (2005) Diffuse malignant mesothelioma of the peritoneum: a clinicopathological study of 35 patients treated locoregionally at a single institution. Cancer 104(10):2181-2188
5. Deraco M, Nonaka D, Baratti D, Casali P, Rosai J, Younan R, Salvatore A, Cabras Ad AD, Kusamura S (2006) Prognostic analysis of clinicopathologic factors in 49 patients with diffuse malignant peritoneal mesothelioma treated with cytoreductive surgery and intraperitoneal hyperthermic perfusion. Ann Surg Oncol 13(2):229-237
6. Averbach AM, Sugarbaker PH (1996) Peritoneal mesothelioma: treatment approach based on natural history. Cancer Treat Res 81:193-211:193-211
7. Sugarbaker PH, Welch LS, Mohamed F, Glehen O (2003) A review of peritoneal mesothelioma at the Washington Cancer Institute. Surg Oncol Clin N Am 12(3):605-621
8. Kebapci M, Vardareli E, Adapinar B, Acikalin M (2003) CT findings and serum ca 125 levels in malignant peritoneal mesothelioma: report of 11 new cases and review of the literature. Eur Radiol 13(12):2620-2626
9. Antman KH (1981) Clinical presentation and natural history of benign and malignant mesothelioma. Semin Oncol 8(3):313-320
10. van Gelder T, Hoogsteden HC, Versnel MA, de Beer P, Vandenbroucke JP, Planteydt HT (1989) Malignant peritoneal mesothelioma: A series of 19 cases. Digestion 43:222-227
11. Antman KH, Blum RH, Greenberger JS, Flowerdew G, Skarin AT, Canellos GP (1980) Multimodality therapy for malignant mesothelioma based on a study of natural history. Am J Med 68:356-362
12. Hassan R, Alexander R, Antman K, Boffetta P, Churg A, Coit D, et al (2006) Current treatment options and biology of peritoneal mesothelioma: meeting summary of the first NIH peritoneal mesothelioma conference. Ann Oncol, in press
13. Markman M, Kelsen D (1992) Efficacy of cisplatin-based intraperitoneal chemotherapy as treatment of malignant peritoneal mesothelioma. J Cancer Res Clin Oncol 118(7):547-550
14. Langer CJ, Rosenblum N, Hogan M, Nash S, Bagchi P, LaCreta FP, Catalano R, Comis RL, O'Dwyer PJ (1993) Intraperitoneal cisplatin and etoposide in peritoneal mesothelioma: favorable outcome with a multimodality approach. Cancer Chemother Pharmacol 32:204-208

15. Antman K, Osteen R, Klegar K, Amato DA, Pomfret EA, Larson DA, Corson JM (1985) Early peritoneal mesothelioma: a treatable malignancy. Lancet 2(8462):977-981
16. Alexander H.R., Kavanagh MA, Libutti SK, Pingpank J.F (2004) Regional Therapy of Cancer Using Continuous Hyperthermic Peritoneal Perfusion or Vascular Isolation and Perfusion Techniques. In: Figg W, McLeod HL, editors. Handbook of Anticancer Pharmacokinetics and Pharmacodynamics. Totowa: Humana Press Inc., p327-348
17. Yan TD, Haveric N, Carmignani CP, Chang D, Sugarbaker PH (2005) Abdominal computed tomography scans in the selection of patients with malignant peritoneal mesothelioma for comprehensive treatment with cytoreductive surgery and perioperative intraperitoneal chemotherapy. Cancer 103(4): 839-849
18. Hassan R, Remaley AT, Sampson ML, Zhang J, Cox DD, Pingpank J, Alexander R, Willingham M, Pastan I, Onda M (2006) Detection and quantitation of serum mesothelin, a tumor marker for patients with mesothelioma and ovarian cancer. Clin Cancer Res 12(2):447-453
19. Brigand C, Monneuse O, Mohamed F, Sayag-Beaujard AC, Isaac S, Gilly FN, Glehen O (2006) Peritoneal mesothelioma treated by cytoreductive surgery and intraperitoneal hyperthermic chemotherapy: results of a prospective study. Ann Surg Oncol 13(3):405-412
20. Deraco M, Casali P, Inglese MG, Baratti D, Pennacchioli E, Bertulli R, Kusamura S (2003) Peritoneal mesothelioma treated by induction chemotherapy, cytoreductive surgery, and intraperitoneal hyperthermic perfusion. J Surg Oncol 83(3):147-153
21. Eltabbakh GH, Piver MS, Hempling RE, Recio FO, Intengen ME (1999) Clinical picture, response to therapy, and survival of women with diffuse malignant peritoneal mesothelioma. J Surg Oncol 70(1):6-12
22. Mongero LB, Beck JR, Kroslowitz RM, Argenziano M, Chabot JA (1999) Treatment of primary peritoneal mesothelioma by hyperthemic intraperitoneal chemotherapy. Perfusion 14(2):141-145
23. Loggie BW, Fleming RA, McQuellon RP, Russell GB, Geisinger KR, Levine EA (2001) Prospective trial for the treatment of malignant peritoneal mesothelioma. Am Surg 67(10):999-1003
24. Park BJ, Alexander HR, Libutti SK, Wu P, Royalty D, Kranda KC, Bartlett DL (1999) Treatment of primary peritoneal mesothelioma by continuous hyperthermic peritoneal perfusion (CHPP). Ann Surg Oncol 6(6):582-590
25. Sebbag G, Yan H, Shmookler BM, Chang D, Sugarbaker PH (2000) Results of treatment of 33 patients with peritoneal mesothelioma. Br J Surg 87(11):1587-1593
26. Sugarbaker PH, Yan TD, Stuart OA, Yoo D (2006) Comprehensive management of diffuse malignant peritoneal mesothelioma. Eur J Surg Oncol 32(6):686-691
27. McQuellon RP, Loggie BW, Lehman AB, Russell GB, Fleming RA, Shen P, Levine EA (2003) Long-term survivorship and quality of life after cytoreduc-

tive surgery plus intraperitoneal hyperthermic chemotherapy for peritoneal carcinomatosis. Ann Surg Oncol 10(2):155-162

28. Alexander H.R., Mavroukakis SM, Libutti SK, Pingpank J.F., Beresnev T, Marden S, Steinberg SM, Liewehr DJ (2004) Impact of tumor resection (Rxn) and intraperitoneal (IP) chemotherapy (CHRx) on health related quality of life (HRQL) in patients (Pts) with peritoneal surface malignancies (PSM). Society of Surgical Oncology 57th Annual Cancer Symposium. Ann Surg Oncol S109

Cytoreduction and Intraperitoneal Chemotherapy for Carcinomatosis from Gastric Cancer

Y Yonemura, E Bando, T Kawamura, H Ito, Y Endo, M Miura, K Kiyosaki, and T Sasaki

Introduction

Peritoneal carcinomatosis (PC) is the most common cause of metastasis from gastric cancer, and is detected in 30% of all gastric cancer patients [1]. However, the survival after surgery alone in patients with PC remains very poor [2,3]. PC is more frequently found in T_3/T_4 tumours and diffuse infiltrating type (Borrmann type 3/4) than T_1/T_2 tumours and localized type (Borrmann type 1/2). Peritoneal recurrence is found in about 60% after curative resection of patients with T_3/T_4 tumours or diffuse infiltrating type.

The poor results after treatment of PC are due to 1. low preoperative diagnostic accuracy of PC by the conventional diagnostic tools; 2. unavailability of effective systemic chemotherapeutic agents; 3. limited effect of surgical cytoreduction on survival; and 4. limited knowledge of the molecular mechanisms of peritoneal dissemination and possible targeted therapy.

In this review, recent advances in the diagnosis and multimodal therapy for PC from gastric cancer are described.

Diagnosis of Peritoneal Carcinomatosis

The diagnosis of PC is made by computed tomography (CT), fluorine-18 fluorodeoxyglucose positron emission tomography scan (PET-CT), laparoscopy, and peritoneal wash cytology/ immunocytology/ molecular diagnosis using real time polymerase chain reaction (RT-PCR).

The presence of PC is often overlooked by conventional CT [4]. In our experience, use of high speed spiral CT (HSSCT) in the diagnosis of PC resulted in a diagnostic accuracy of 77.2% (88/114), sensitivity of 38.2% (13/34), specificity of 93.8% (75/80), positive predicting value (PPV) of 72.2% (13/18), and negative predicting value (NPV) of 78.1% (75/96).

Recently, PET-CT was shown to be helpful in the preoperative staging of gastric cancer [4]. For the primary tumour of a gastric adenocarcinoma, PET demonstrated an increased uptake in 64 of 68 patients (sensitivity 94%) [4]. A comparison of FDG uptake and clinicopathological features showed a significant association between FDG uptake and lymph node metastasis and TNM stage. Furthermore, PET had a higher specificity than CT (92% vs. 62%) in assessing local lymph node status [4]. Moreover, PET had additional diagnostic value in 10 (15%) of 68 patients by upstaging 4 (6%) and downstaging 6 (9%) patients. PET combined with CT was more accurate for preoperative staging than either modality alone (66% vs. 51%, 66% vs. 47%, respectively).

There are, however, no reports describing the use of PET-CT in the diagnosis of PC from gastric cancer. In our experience, PET-CT had an accuracy of 87.9% (124/141), sensitivity of 74.4% (29/39), specificity of 93.1% (95/102), PPV of 80.6% (29/36) and NPV of 90.5% (95/105), significantly better than CT (Fig. 1). Accordingly, PET-CT can be used as a useful adjunct to HSSCT in predicting PC, since FDG-PET improves the preoperative TNM staging of gastric adenocarcinoma.

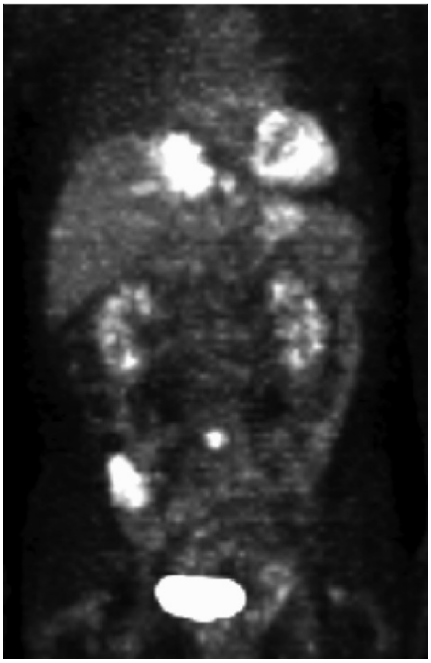


Figure 1. PET CT scan in a patient with peritoneal carcinomatosis from gastric cancer

Recently, preoperative laparoscopy has been described to diagnose PC and the existence of the peritoneal free cancer cells (PFCCs). Laparoscopy was performed under general anesthesia with CO_2 pneumoperitoneum under general anesthesia. A

trocar and two manipulating forceps were inserted, and the surfaces of the peritoneum, omentum, stomach, spleen, pancreas, liver, and diaphragm were examined. In addition, the existence of PFCCs can be examined by peritoneal washing during laparoscopy. Shiraishi et al reported a mean time for laparoscopy of only 20 minutes and a high sensitivity and specificity related to the procedure. In addition, a significant positive correlation between the extent of PC and the prevalence of positive cytology was noted [6]. Laparoscopy is useful for the evaluation of peritoneal spread of advanced gastric cancer, and can avoid unnecessary laparotomy. It appears to be a safe and effective staging modality, avoiding unnecessary exploration and is important for the choice of therapy in patients with T_3/T_4 tumours or diffusely infiltrating gastric cancer.

In the Japanese general rules of gastric cancer treatment, peritoneal lavage cytological examination is recommended to be done just after laparotomy to confirm the existence of PFCCs [6]. Positive cytology is recorded as Cy1, and P0 means no macroscopic peritoneal dissemination. The PFCCs are exfoliated from the serosal surface of primary tumour. Because almost all patients with P0/Cy1 status exclusively die of peritoneal recurrence even after curative gastrectomy, these patients are classified as Stage 4 in the Japanese staging system. Since peritoneal cytology is usually not performed in other countries, a Cy1 status is not included in the current UICC/AJCC TNM stage [8]. However, since more than 30% of potentially curable patients with advanced gastric cancer show positive lavage cytology routine examination for PFCCs is very important [9].

Bando et al. reported that a tumour size larger than 6 cm, a diameter of serosal invasion greater than 2.5 cm, stage T_3/T_4 tumours and infiltrating growth pattern are independent predictors of peritoneal recurrence [1]. Although the sensitivity of these clinicopathological parameters is low to predict peritoneal recurrence, the specificity of the peritoneal lavage cytology is very high in predicting peritoneal recurrence. However, the sensitivity of peritoneal wash cytology for peritoneal recurrence is only 56%, and a significant number of patients with negative cytology will develop peritoneal recurrence.

Recently, more sensitive methods and combination assays using several markers have been proposed to detect peritoneal dissemination. Immunocytological detection using monoclonal antibodies against tumour-associated antigens (CEA, CA19-9, Ber-EP4), and no unwarranted reactions were found in the control samples. With immunocytochemical detection of peritoneal micrometastasis in gastric cancer, it was possible to identify PFCCs in 35% of the patients, with a 14% improvement over routine cytopathology results [10,11]. Furthermore, combination analysis with conventional methods and immunocytologic studies offer more sensitive results than the conventional staining alone [12].

Several authors have used CEA protein levels in peritoneal washing fluid as a sensitive predictor of peritoneal recurrence [13,14]. CEA levels in peritoneal washings were statistically independent of those in sera and, in comparison to standard cytology, more reliably predicted the presence of peritoneal dissemination [13,14].

Others have used RT-PCR to detect markers such as CEA, matrix metalloproteinase 7, and DOPA decarboxylase in peritoneal washing samples to detect or predict peritoneal recurrence [15-18]. Although these assays are highly sensitive, their results should be interpreted cautiously due to possible expression by normal or inflammatory cells.

Results of Systemic Chemotherapy in the Treatment of PC of Gastric Origin

The prognosis of patients with peritoneal dissemination from gastrointestinal cancer is very poor with a median survival time of about 3 months [19]. No standard systemic chemotherapy for PC has yet been reported. At present, intravenous 5-fluorouracil (5-FU) has been used either alone or as part of a combination therapy such as FAM (5-FU, doxorubicin, and mitomycin C) or FAMTX (5-FU, methotrexate, and adriamycin) in advanced gastric cancer [20,21]. However, systemic chemotherapy does not improve the survival of patients with PC [22], possibly due to the blood-peritoneal barrier which inhibits the movement of drugs, oxygen and nutrients from the submesothelial capillary to the peritoneal cavity [23].

Recent phase II chemotherapy studies in gastric cancer patients have shown promising results using capecitabine, docetaxel, or oxaliplatin [24-29].

TS-1 is a novel oral fluoropyrimidine anticancer drug based on a biochemical modulation of 5-FU and is composed of tegafur, gimestat (CDHP) and otastat potassium at a molar ratio of 1: 0.4: 1. In Japan, it is currently the first line anticancer drug as the standard therapy for gastric cancer. 5FU is metabolized from tegafur by P-450 and glutathione in the liver, and is delivered to the blood stream and peritoneal cavity. In an experimental peritoneal dissemination model, the 5-FU concentration in ascites after oral administration of TS-1 is maintained at a high level for 1-6 hours, while the 5-FU level in ascites after oral administration of 5-FU alone was very low [30-32]. Dihydropyridine dehydrogenase (DPD) is the degradation enzyme for 5FU and exists at a high concentration in the peritoneal mononuclear cells. CDHP inhibits the activity of DPD, resulting in the inhibition of 5FU degradation. CDHP was detectable in ascites at a high level from 30 min after oral administration of TS-1, and maintained the level of 5-FU in the ascites [31].

Yonemura et al. reported the effect of postoperative TS-1 in potentially curable gastric cancer patients with PFCCs (P0/Cy1) [34]. After gastrectomy and lymphadenectomy, patients were treated with oral TS-1 (80 mg/m^2) for 28 consecutive days and 14 day rest, and the schedule was repeated every 6 weeks (TS-1 group).

Patients treated with TS-1 group survived significantly longer than the control group (Fig. 2 and Table 1).

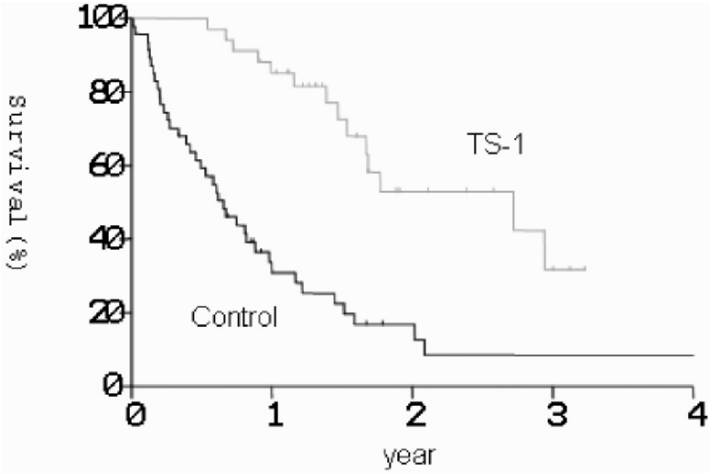


Figure 2. Survival of patients with P0/Cy1 status, treated with oral TS-1 administration. After a gastrectomy and lymphadenectomy, patients were treated with oral TS-1 (80 mg/m^2) for 28 consecutive days and 14 day rest, and the schedule was repeated every 6 weeks (TS-1 group). The patients treated with TS-1 group survived significantly longer than the control group ($p < 0.0001$, log rank test)

Table 1. Survival data in patients without established peritoneal carcinomatosis (P0/Cy1)

Treatment	N	1 year	2 years	3 years	median
TS-1	34	85%	53%	32%	21.1 m
Control	47	30%	11%	7%	5.9 m

In patients with established PC (P1), treatment with adjuvant TS-1 similarly resulted in a significant survival advantage (Fig. 3 and Table 2). Major adverse reactions included myelosuppression; gastrointestinal toxicities were generally mild and there were no treatment-related deaths after TS-1 administration. Accordingly, oral TS-1 treatment is considered to be safe and effective adjuvant therapy as for patients with a P0/Cy1 and P1 status.

Currently, the therapeutic efficacy of the taxanes (paclitaxel and docetaxel) and irinotecan as a single agent and in combination are being evaluated in clinical studies in advanced gastric cancer [35,36,58,59].

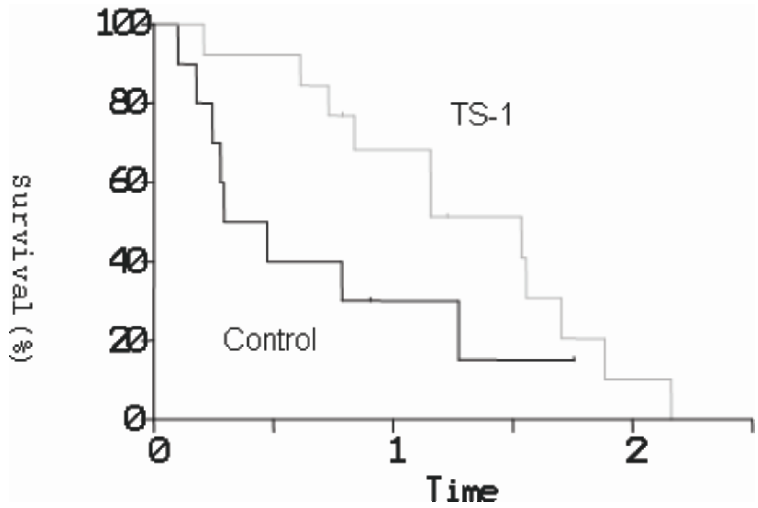


Figure 3. Survival of patients with established peritoneal dissemination (P1), treated with oral TS-1. Patients treated with TS-1 group survived significantly longer than the control group ($p < 0.005$, log rank test)

Table 2. Survival data in patients with established peritoneal carcinomatosis (P1)

Treatment	N	1 year	2 years	median
TS-1	13	68%	10%	14.7 m
Control	10	30%	0%	3.5 m

Treatment of PC of Gastric Origin with Intraperitoneal Chemotherapy

Intraperitoneal chemotherapy (IP) offers potential therapeutic advantages over systemic chemotherapy by generating high local drug concentrations [37,38]. Pharmacokinetic studies have shown significantly higher AUC (area under the curve) values in the peritoneal cavity after IP chemotherapy compared to systemic administration [39,40]. Recently, taxanes are considered as candidates for IP chemotherapy, because of their high molecular weight and longer retention time after intraperitoneal injection. In a mouse model, IP injection of docetaxel resulted in significantly higher drug concentrations in the peritoneal cavity, peritoneal solid cancer tissue, and free cancer cells compared to IV injection (Fig. 4) [41,42].

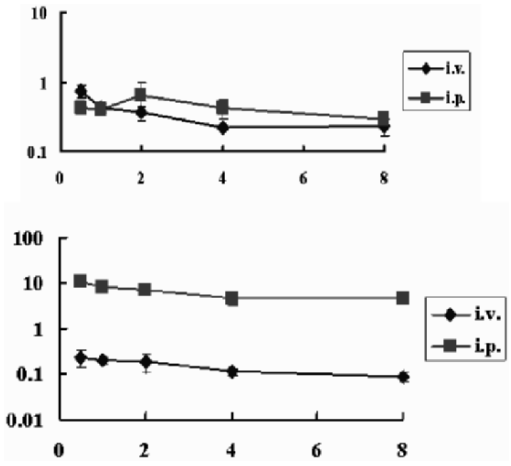


Figure 4. Time course (hours) of docetaxel concentration (μg/ml) in plasma (above) and ascites (below) after i.v. or i.p. injection of 8 mg/kg docetaxel into tumor-bearing mice

In this experimental peritoneal dissemination model of gastric cancer, the survival time of mice treated with IP administration of docetaxel was markedly prolonged in comparison to that in the control group [42]. Similarly, in an animal model of PC of ovarian cancer origin, IP docetaxel resulted in a significantly longer survival compared to systemic administration using an identical dose [43]. These results indicate that IP administration of docetaxel could represent an effective treatment method for PC without causing any increase in systemic toxicity.

Perioperative Intraperitoneal Chemotherapy

Perioperative IP chemotherapy can be administerd preoperatively, intraopertively, or early postoperatively. Preoperative (neoadjuvant) IP chemotherapy aims to reduce the tumour burden and increase the probability of achieving a complete cytoreduction. Moreover, preoperative chemotherapy can eradicate PFCCs and can provide a measure of the cancer's chemosensitivity.

Neoadjuvant Intraperitoneal and Systemic Chemotherapy

Neoadjuvant intraperitoneal and systemic chemotherapy (NIPS) was developed to increase the rate of a complete cytoreduction [44]. According to Cunliffe [45], intraabdominal metastases derive their nutritional supply both from the peritoneal surface and from the blood supply. The penetration depth of IP chemotherapy is limited to approximately 2 mm for CDDP/ carboplatin and less than one mm for

several other drugs; as a consequence deeper tumour regions will not be adequately treated by IP chemotherapy alone [46]. By combining systemic and IP chemotherapy, NIPS allows treating both superficial and deeper tumour regions simultaneously.

During NIPS, docetaxel (30 mg/m^2) and carboplatin (100 mg/m^2) are administered IP in 1 liter of saline using an implanted port system. At the same time, methotrexate (100 mg /m^2) and 5-FU (600 mg/m^2) are administered intravenously. This regimen is repeated weekly.

Generally, systemic or IP chemotherapy alone show response rates of less than 30%. In contrast, NIPS showed a fairly good response rate of 65% [44]. Accordingly, the two-route chemotherapy may be the best route for the preoperative chemotherapy. After several cycles of NIPS, complete cytoreduction is attempted.

One of the aims of NIPS is to eradicate PFCCs before operation. The PFCCs are viable and may be trapped on the peritoneal wound injured by the surgical procedure. After NIPS, positive cytology changed to a negative cytology in two thirds of patients treated with NIPS. NIPS, therefore, establishes the containment of PFCCs prior to cytoreduction.

Seventy-one patients with PC from gastric cancer were treated by NIPS, and 37 (51%) patients underwent cytoreduction including total gastrectomy, resection of involved organs, and peritonectomy. Seventeen (46%) patients received complete cytoreduction by peritonectomy. The median survival of patients who received cytoreduction after NIPS was 16.8 months, while that of patients who did not receive operation was 9.7 months (Fig. 5). Patients who received a complete cytoresection (CC-0) had a significantly better prognosis than those who received an incomplete cytoreduction (Fig. 6).

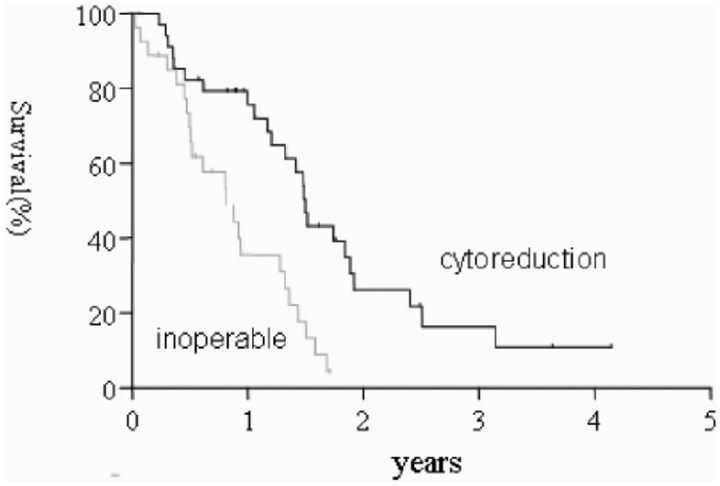


Figure 5. Survival after neoadjuvant intraperitoneal and systemic chemotherapy (NIPS) in gastric cancer patients. Survival of patients who underwent cytoreduction was significantly better than that of inoperable patients ($p < 0.01$, log rank test)

Table 3. Survival data in gastric cancer patients who received NIPS

Treatment	N	1 year	2 years	3 years	median
Cytoreduction	37	76%	22%	11%	16.8 m
Inoperable	34	36%	4%	0%	9.7 m

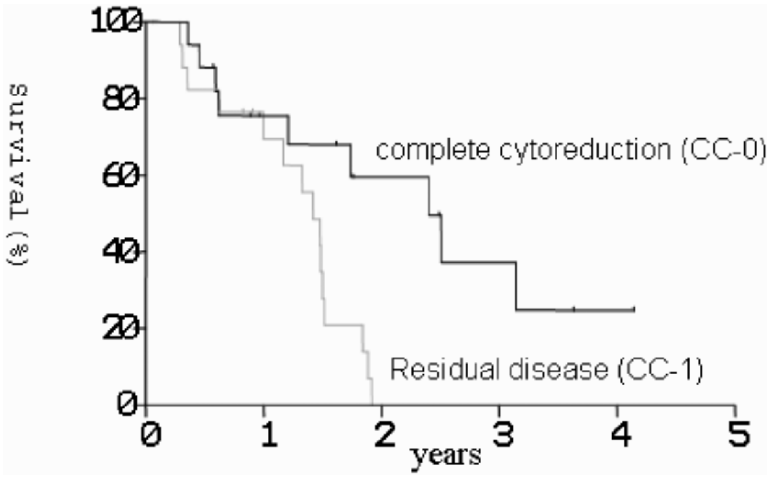


Figure 6. Survival after neoadjuvant intraperitoneal and systemic chemotherapy (NIPS) followed by surgery in gastric cancer patients. Survival was significantly better after complete cytoreduction ($p < 0.01$, log rank test)

Table 4. Survival data in gastric cancer patients who received NIPS followed by surgery according to completeness of cytoreduction (CC)

Treatment	N	1 year	2 years	3 years	median
CC-0	18	76%	49%	25%	20.4 m
CC-1	19	70%	0%	0%	15.6 m

Hyperthermic Intraperitoneal Chemoperfusion

Hyperthermic intraperitoneal chemoperfusion (HIPEC) is a novel method to treat the whole peritoneal cavity by circulating heated anti-cancer drugs in a high dose. Hyperthermia has synergism with certain anticancer drugs, and results in a higher anticancer drug concentration in experimental peritoneal tumours than chemotherapy alone [47]. Fujimoto et al. reported that the PFCCs vanished after HIPEC [48]. Kiyosaki et al. reported a significant survival benefit in patients with P0/Cy1 status by HIPEC using MMC and CDDP [49]. Fifteen patients with P0/Cy1 status were treated by HIPEC after D2 gastrectomy, and the 5-year survival rate was

42% (Fig. 7) while survival in the 39 control patients only 12%. Furthermore, HIPEC significantly decreased the rate of peritoneal recurrence, as compared with surgery alone (Tables 5 and 6).

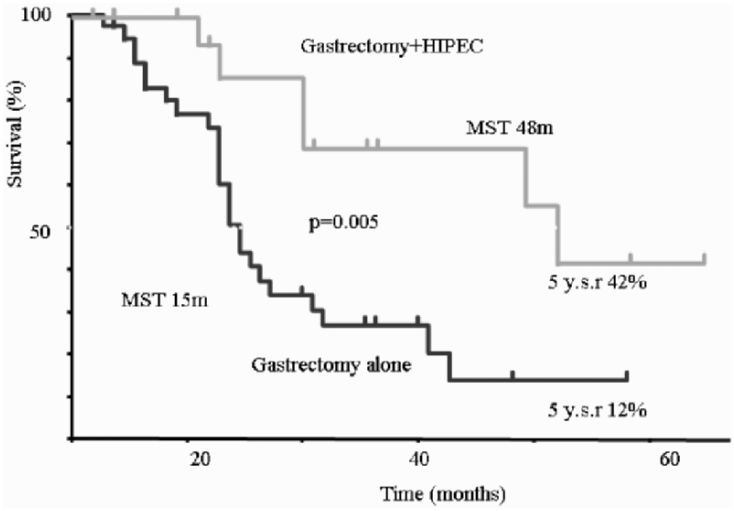


Figure 7. Survival in gastric cancer patients with P0/Cy1 status following gastrectomy alone or gastrectomy with HIPEC. MST, median survival time

Table 5. Clinical and pathological variables in gastric cancer patients who received surgery with HIPEC or surgery alone

Variable		Surgery + HIPEC	Surgery alone	p
N		15	39	
Gender	Male	12	12	
	Female	3	27	
Age (y) mean ± SD		57.1 ± 9.2	66.6 ± 9.6	
T stage	T1	1	1	NS
	T2	2	5	
	T3	11	31	
	T4	1	2	
N stage	N0	4	5	NS
	N1	3	13	
	N2	8	15	
	N3	1	6	

Table 6. Peritoneal recurrence in gastric cancer patients who received surgery with HIPEC or surgery alone

	Peritoneal recurrence		Total
	Present	Absent	
Surgery + HIPEC	7 (46.7%)	8	15
Surgery alone	25 (64.1%)	14	29

Yonemura et al. reported the results of cytoreduction with HIPEC in patients with peritoneal dissemination [50]. After resection of the primary tumour, lymph nodes, and peritoneal metastases, HIPEC was performed during 60 minutes using mitomycin C (30 mg), etoposide (150 mg), and cisplatin (300 mg) at a temperature of 42° C - 43° C.

Among 55 evaluable patients with residual macroscopic peritoneal seeding, complete and partial response was noted in five (9%) and 17 (30%) patients respectively. The overall 1- and 5-year survival rates were 53% and 7%, and median survival time was 12.9 months (Fig. 8 and Table 7). Patients with a complete response had a significantly better prognosis than those with a partial response and nonresponders. One-year survival rates in patients with complete response, partial response or nonresponders were 80%, 44% and 33%, respectively.

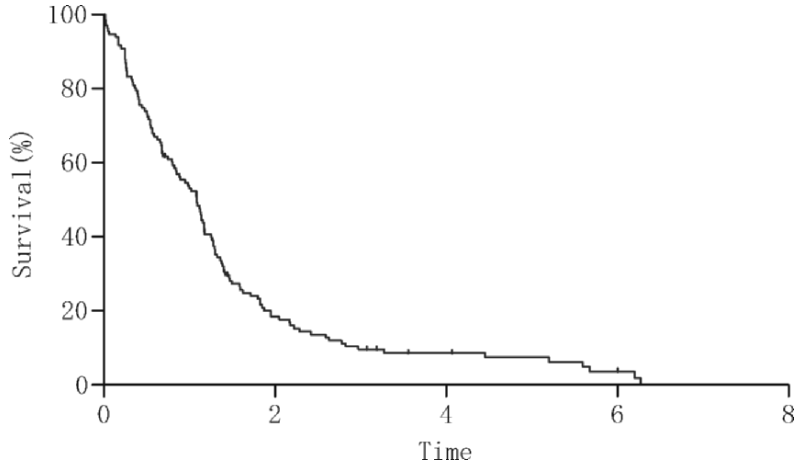


Figure 8. Overall survival in 133 gastric cancer patients with peritoneal dissemination treated with cytoreduction and HIPEC

Table 7. Survival data

N	1 year	2 years	3 years	5 years	median
133	53%	18%	10%	7%	12.9 m

Early Postoperative Intraperitoneal Chemotherapy

Early Postoperative Intraperitoneal Chemotherapy (EPIC) is instilled with 500 ml of saline with anti-cancer agents from postoperative day 1 for several postoperative days. The aim of EPIC is to kill the residual intraperitoneal cancer cells by IP chemotherapy before they become trapped by adhesions or fibrin formation. Jeung [51] and Yu et al. [52] reported the feasibility of EPIC for the treatment and for the prophylaxis of PC. Jeung et al. reported that EPIC started on the day of operation with 5-FU/cisplatin (days 1-3) over a 4-week interval or MMC/5FU. There was no grade 3 or grade 4 adverse effects and the overall survival was 12 months. EPIC is thus considered to be a simple and useful method for the treatment of peritoneal dissemination.

Peritonectomy Procedures

Recent literatures indicates that the complete removal of peritoneal dissemination is an independent prognostic factor gastric and colorectal cancer [53,54]. The aim of a peritonectomy is to remove PC by dissection between the mesothelial layer and underlying parietal or visceral tissue. The survival of patients treated with peritonectomy is shown in Fig. 9 and Table 8.

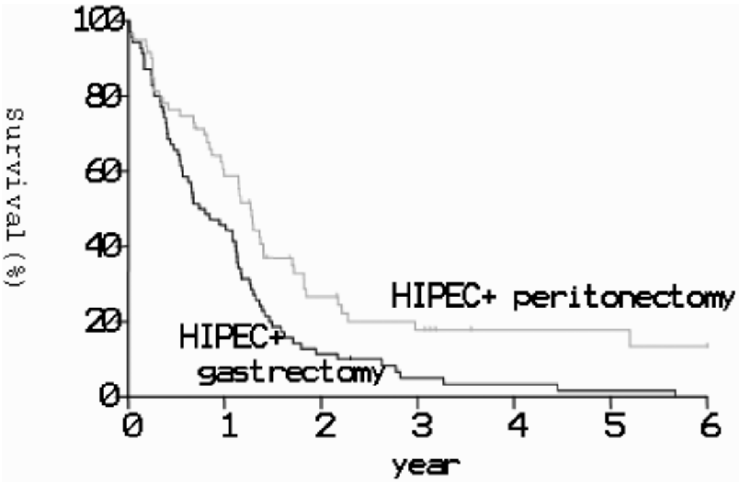


Figure 9. Survival of P1 gastric cancer with or without additional peritonectomy; $p < 0.05$

Table 8. Survival data of P1 gastric cancer treated with gastrectomy and HIPEC with or without additional peritonectomy

Treatment	N	1 year	2 years	3 years	median
HIPEC	71	46%	11%	3%	8.8 m
HIPEC + peritonectomy	62	59%	27%	18%	15 m

Conclusion

Neoadjuvant intraperitoneal-systemic chemotherapy (NIPS), hyperthermic intraperitoneal perfusion chemotherapy (HIPEC), and early postoperative intraperitoneal chemotherapy (EPIC) are newly developed perioperative chemotherapies. Moreover, peritonectomy is a novel surgical procedure to perform a complete cytoreduction for peritoneal dissemination. The combined use of these techniques will likely improve the survival of patients, and these approaches may be a main treatment modality for peritoneal dissemination in the near future in combination with novel chemotherapeutic and biological agents [59,61-63].

References

1. Bando E, Yonemura Y, Takeshita Y, Taniguchi K, Yasui T, Yoshimitsu Y, Fushida S, Fujimura T, Nishimura G, Miwa K (1999) Intraoperativve lavage for cytological examination in 1, 297 patients with gastric carcinoma. Am J Surg 78:256-262
2. Benevolo M, Mottolese M, Coamelli M, Tedesco M, Giannarelli D, Vasselli S, Carlini M, Garofalo A, Natali PG (1998) Diagnostic and prognostic value of peritoneal immunocytology in gastric cancer. J Clin Oncol 16:3406-3411
3. Chu ZD, Lang NP, Thompson C, Osteen PK, Westbrook KC (1989) Peritoneal carcinomatosis in nongynecological malignancy. A prospective study of prognostic factors. Cancer 63:364-367
4. Chen J, Cheong JH, Yun MJ, Kim J, Lim JS, Hyung WJ, Noh SH (2005) Improvement in preoperative staging of gastric adenocarcinoma with positron emission tomography. Cancer 103:2383-2390
5. Shiraishi N, Morimoto A, Sato K, Bandoh T, Adachi Y, Kitano S (1999) Laparoscopy in the management of scirrhous gastric cancer. Gastric Cancer 2:109-114
6. Conlon KC (2001) Staging laparoscopy for gastric cancer. Ann Ital Chir 72:33-37
7. Japanese Research Society for Gastric Cancer (1995) The general rules for gastric cancer study in surgery and pathology. First English Edition, Tokyo, Kanehara Shuppan
8. UICC International Union Against Cancer. TNM Classification of Malignant Tumours, Sixth Edition (2002) LH Sobin, Ch Wittekind, Eds. John Wiley & Sons
9. Wu CC, Chen JT, Chang MC, Ho WL, Chen CY, Yeh DH, Liu TJ, P'eng FK (1997) Optimal surgical strategy for potentially curable serosa-involved gastric carcinoma with intraperitoneal free cancer cells. J Am Coll Surg 184: 611-617

10. Benevolo M, Mottolese M, Cosimelli M, Tedesco M, Giannarelli D, Vasselli S, Carlini M, Garofalo A, Natali PG (1998) Diagnostic and prognostic value of peritoneal immunocytology in gastric cancer. J Clin Oncol 16:3406-3411
11. Rosenberg R, Nekarda H, Bauer P, Schenck U, Hoefler H, Siewert JR (2006) Free peritoneal tumour cells are an independent prognostic factor in curatively resected stage IB gastric carcinoma. Brit J Surg 93:325-332
12. Juhl H, Stritzel M, Wroblewski A, Henne-Bruns D, Kremer B, Schmiegel W, Neumaier M, Wagener C, Schreiber HW, Kalthoff H (1994) Immunocytological detection of micrometastatic cells: comparative evaluation of findings in the peritoneal cavity and the bone marrow of gastric, colorectal and pancreatic cancer patients. Int J Cancer 57:330-335
13. Asao T, Fukuda T, Yazawa S, Nagamachi Y (1991) Carcinoembryonic antigen levels in peritoneal washings can predict peritoneal recurrence after curative resection of gastric cancer. Cancer 68:44-47
14. Nishiyama M, Takashima I, Tanaka T, Yoshida K, Toge T, Nagata N, Iwamori S, Tamura Y (1995) Carcinoembryonic antigen levels in the peritoneal cavity: useful guide to peritoneal recurrence and prognosis for gastric cancer. World J Surg 19:133-137
15. Yonemura Y, Endou Y, Fujimura T, Fushida S, Bandou E, Kinoshita K, Sugiyama K, Sawa T, Kim BS, Sasaki T (2001) Diagnostic value of preoperative RT-PCR-based screening method to detect carcinoembryonic antigen-expressing free cancer cells in the peritoneal cavity from patients with gastric cancer. ANZ J Surg 71:521-528
16. Kodera Y, Nakanishi H, Ito S, Yamamura Y, Kanemitsu Y, Shimizu Y, Hirai T, Yasui K, Kato T, Tatematsu M (2002) Quantitative detection of disseminated free cancer cells in peritoneal washes with real-time reverse transcriptase-polymerase chain reaction: a sensitive predictor of outcome for patients with gastric carcinoma. Ann Surg 235:499-506
17. Yonemura Y, Fujimura T, Ninomiya I, Kim BS, Bandou E, Sawa T, Kinoshita K, Endo Y, Sugiyama K, Sasaki T (2001) Prediction of peritoneal micrometastasis by peritoneal lavaged cytology and reverse transcriptase-polymerase chain reaction for matrix metalloproteinase-7 mRNA. Clin Cancer Res 7:1647-1653
18. Nakanishi H, Kodera Y, Yamamura Y, Ito S, Kato T, Ezaki T, Tatematsu M (2000) Rapid quantitative detection of carcinoembryonic antigen-expressing free tumour cells in the peritoneal cavity of gastric-cancer patients with real-time RT-PCR on the lightcycler. Int J Cancer 89:411-417
19. Chu DZ, Lang NP, Thompson C, Osteen PK and Westbrook KC (1989) Peritoneal carcinomatosis in nongynecologic malignancy. A prospective study of prognostic factors. Cancer 63:364-367
20. MacDonald JS, Schein PS, Woolley PV, et al (1980) 5-Fluorouracil, doxorubicin, and mitomycin (FAM) combination chemotherapy for advanced gastric cancer. Ann Intern Med 93:533-536
21. Wils JA, Klein HO, Wagener DJ, et al (1991) Sequential high-dose methotrexate and fluorouracil combined with doxorubicin-a step ahead in the

treatment of advanced gastric cancer: a trial of the European Organization for Research and Treatment of Cancer Gastrointestinal Tract Cooperative Group. J Clin Oncol 9:827-831
22. Ajani JA, Ota DM, Jessup JM, et al (1991) Resectable gastric carcinoma. An evaluation of preoperative and postoperative chemotherapy. Cancer 68: 1501-1506
23. P. Jacquet, P.H. Sugarbaker (1996) Peritoneal-plasma barrier. In P.H. Sugarbaker (ed). Peritoneal Carcinomatosis: Principles of Management, Kluwar Academic Pubisher, Boston p53-63
24. Sastre J, Garcia-Saenz JA and Diaz-Rubio E (2006) Chemotherapy for gastric cancer. World J Gastroenterol 12:204-213
25. Ajani JA (2005) Evolving chemotherapy for advanced gastric cancer. Oncologist 10 Suppl 3:49-58
26. Park YH, Kim BS, Ryoo BY and Yang SH (2006) A phase II study of capecitabine plus 3-weekly oxaliplatin as first-line therapy for patients with advanced gastric cancer. Br J Cancer 94:959-963
27. Kim JG, Sohn SK, Kim DH, et al (2005) Phase II study of docetaxel and capecitabine in patients with metastatic or recurrent gastric cancer. Oncology 68:190-195
28. Sakamoto J, Chin K, Kondo K, et al (2006) Phase II study of a 4-week capecitabine regimen in advanced or recurrent gastric cancer. Anticancer Drugs 17:231-236
29. Park YH, Kim BS, Ryoo BY and Yang SH (2006) A phase II study of capecitabine plus 3-weekly oxaliplatin as first-line therapy for patients with advanced gastric cancer. Br J Cancer 94:959-963
30. Mori T, Fujiwara Y, Yano M, et al (2003) Experimental study to evaluate the usefulness of S-1 in a model of peritoneal dissemination of gastric cancer. Gastric Cancer 6 Suppl 1:13-18
31. Yonemura Y, Endou Y, Tochiori S, et al (2005) [Effect of intraperitoneal chemotherapy on experimental peritoneal dissemination of gastric cancer]. Gan To Kagaku Ryoho 32:1635-1639
32. Yamagata S, Nakata B and Hirakawa K (2004) Dihydropyrimidine dehydrogenase inhibitory fluoropyrimidine S-1 may be effective against peritoneal dissemination in gastric cancer. Oncol Rep 12:973-978
33. Japanese Research Society for Gastric Cancer (1993) The general rules for the gastric cancer study in surgery and pathology. 12th ed. Tokyo, Kanehara Shuppan
34. Yonemura Y (2006) The usefulness of oral TS-1 treatment for potentially curable gastric cancer patients with intraperitoneal free cancer cells. Cancer Treat 4:135-142
35. Yoshida K, Ninomiya M, Takakura N, Hirabayashi N, Takiyama W, Sato Y, Todo S, Terashima M, Gotoh M, Sakamoto J, Nishiyama M (2006) Phase II Study of Docetaxel and S-1 Combination Therapy for Advanced or Recurrent Gastric Cancer. Clin Cancer Res 12:3402-3407

36. Kii T, Takiuchi H, Gotoh M, Kawabe S, Ohta S, Tanaka T, Kuwakado S, Nishitani H, Katsu K (2006) Weekly administration regimen of paclitaxel (PTX) in patient with inoperable or recurrent gastric cancer. Gan To Kagaku Ryoho 33:621-624
37. Markman M (1991) Intraperitoneal chemotherapy. Semin Oncol 18:248-254
38. Armstrong DK, Bundy B, Wenzel L, et al (2006) Intraperitoneal cisplatin and paclitaxel in ovarian cancer. N Engl J Med 354:34-43
39. Goel R, Cleary SM, Horton C, et al (1989) Effect of sodium thiosulfate on the pharmacokinetics and toxicity of cisplatin. J Natl Cancer Inst 81:1552-1560
40. O'Dwyer PJ, LaCreta F, Hogan M, Rosenblum N, O'Dwyer JL and Comis RL (1991) Pharmacologic study of etoposide and cisplatin by the intraperitoneal route. J Clin Pharmacol 31:253-258
41. Yonemura Y, Endou Y, Bando E, et al (2004) Effect of intraperitoneal administration of docetaxel on peritoneal dissemination of gastric cancer. Cancer Lett 210:189-196
42. Shimada T, Nomura M, Yokogawa K, et al (2005) Pharmacokinetic advantage of intraperitoneal injection of docetaxel in the treatment for peritoneal dissemination of cancer in mice. J Pharm Pharmacol 57:177-181
43. Dykes DJ, Bissery MC, Harrison SD, Jr. and Waud WR (1995) Response of human tumour xenografts in athymic nude mice to docetaxel (RP 56976, Taxotere). Invest New Drugs 13:1-11
44. Yonemura Y, Bandou E, Kinoshita K, et al (2003) Effective therapy for peritoneal dissemination in gastric cancer. Surg Oncol Clin N Am 12:635-648
45. Cunliffe WJ (1991) The rationale for early postoperative intraperitoneal chemotherapy for gastric cancer. P Sugarbaker (ed) Management of gastric cancer. Kluwer Academic Publication, Boston, p143-157
46. Yonemura Y (1998) Principles of the treatment of peritoneal dissemination. Peritoneal Dissemination. Yonemura Y Ed., Maeda Shoten, Kanazawa, Japan p175-190
47. Los G, van Vugt MJ and Pinedo HM (1994) Response of peritoneal solid tumours after intraperitoneal chemohyperthermia treatment with cisplatin or carboplatin. Br J Cancer 69:235-241
48. Fujimoto S, Takahashi M, Kobayashi K, et al (1993) Relation between clinical and histologic outcome of intraperitoneal hyperthermic perfusion for patients with gastric cancer and peritoneal metastasis. Oncology 50:338-343
49. Kiyosaki K (2004) Efficacy of prophylactic continuous hyperthermic peritoneal perfusion (CHPP) for gastric cancer with intraperitoneal cytological positivity for malignancy. 12th International postgraduate course. New frontiers in the diagnosis and management of GI diseases. Abstract 18
50. Yonemura Y, Fujimura T, Nishimura G, FallaR, Sawa T, Katayama K, Tsugawa K, Fushida S, Miyazaki I, Tanaka M, Endou Y, Sasaki T (1996) Effects of intraoperative chemohyperthermia in patients with gastric cancer with peritoneal dissemination. Surgery 119:437-444

51. Jeung HC, Rha SY, Jang WI, Noh SH and Chung HC (2002) Treatment of advanced gastric cancer by palliative gastrectomy, cytoreductive therapy and postoperative intraperitoneal chemotherapy. Br J Surg 89:460-466
52. Yu WS, Sugarbaker PH (1991) Early postoperative intraperitoneal chemotherapy for gastric cancer. Cancer Treat Res 55:265-275
53. Culliford AT, Brooks AD, Sharma S, et al (2001) Surgical debulking and intraperitoneal chemotherapy for established peritoneal metastases from colon and appendix cancer. Ann Surg Oncol 8:787-795
54. Glehen O, Kwiatkowski F, Sugarbaker PH, Elias D, Levine EA, De Simone M, Barone, Yonemura Y, Cavaliere F, Quenet F, Gutman M, Tentes AAK, Lorimier G, Bernard JL, Bereder JM, Porcheron J, Gomez-Portilla A, Shen P, Deraco M, Rat P (2004) Cytoreductive surgery combined with perioperative intraperitoneal chemotherapy for the management of peritoneal carcinomatosis from colorectal cancer. A multi-institutional study of 506 patients. J Clin Oncol 15:3284-3292
55. Yonemura Y, Kawamura T, Bandou E, Takahashi S, Sawa T and Matsuki N (2005) Treatment of peritoneal dissemination from gastric cancer by peritonectomy and chemohyperthermic peritoneal perfusion. Br J Surg 92:370-375
56. Yonemura Y, Bandou E, Sawa T, et al (2006) Neoadjuvant treatment of gastric cancer with peritoneal dissemination. Eur J Surg Oncol; in press.
57. Rhee J Hoff PM (2005) Angiogenesis inhibitors in the treatment of cancer. Expert Opin Pharmacother 6:1701-1711
58. Inokuchi M, Yamashita T, Yamada H, Kojima K, Ichikawa W, Nihei Z, Kawano T, Sugihara K (2006) Phase I/II study of S-1 combined with irinotecan for metastatic advanced gastric cancer. Br J Cancer 94(8):1130-1135
59. Park SR, Chun JH, Yu MS, Lee JH, Ryu KW, Choi IJ, Kim CG, Lee JS, Kim YW, Bae JM, Kim HK (2006) Phase II study of docetaxel and irinotecan combination chemotherapy in metastatic gastric carcinoma. Br J Cancer. 94:1402-1406
60. Miura S, Yoshimura Y, Endo M, Satoh H, Machida H and Sasaki T (1999) Comparison of 1-(2-deoxy-2-fluoro-4-thio-ß-D-arabinofuranosyl)cytosine with gemcitabine in its antitumour activity. Cancer Lett 144:177-182
61. Matsuda A and Sasaki T (2004) Antitumour activity of sugar-modified cytosine nucleosides. Cancer Sci 95:105-111
62. Kazuno H, Sakamoto K, Fujioka A, Fukushima M, Matsuda A and Sasaki T (2005) Possible antitumour activity of 1-(3-C-ethynyl-ß-D-ribo-pentofuranosyl) cytosine (ECyd, TAS-106) against an established gemcitabine (dFdCyd)-resistant human pancreatic cancer cell line. Cancer Sci 96:295-302
63. Miura S, Endo Y, Yoshimura Y, Endo M, Yonemura Y and Sasaki T (2002) Potent antitumour effect of 1-(2-deoxy-2-fluoro-4-thio-ß-D-arabinofuranosyl) cytosine on peritoneal dissemination models of gastrointestinal cancers. Oncol Rep 9:1319-1322

Cytoreduction and Intraperitoneal Chemotherapy for Peritoneal Carcinomatosis of Ovarian Cancer

JM Lee

Introduction

Ovarian cancer is the second most frequent malignant tumour of the female genital tract and the fifth cause of death caused by cancer among women. It affects aproximately 25,000 women per year, and more than 14,000 women die of the disease in the United States [1]. Although ovarian cancer is one of the most chemosensitive solid tumours, it has the highest fatality rate among gynecologic cancers. The median age at diagnosis is 63 years and peaks age ranged 70 to 74 years with the incidence of 59.4 per 100,000 women [2,3].

Epithelial cancer is the most common ovarian malignancy, and patients with advanced disease (Stage III or IV) at diagnosis make up almost 75% of cases because they are usually asymptomatic. Ovarian cancer represents a major surgical challenge, and requires intensive and complex therapies. It is extremely demanding of the patient's psychological and physical energy [4].

The 5-year survival rate for all patients with ovarian cancer is approximately 40%. Although the cure rate is more than 90% in a small proportion of patients with disease confined to the ovary, the 5-year disease-free survival rate is only 12% in patients with stage III disease. These tendencies have not changed for the past three decades [5].

Bristow et al. found that there was a statistically significant positive correlation between survival and maximal cytoreduction. Their study also showed that each 10% increase in maximal cytoreduction was associated with a 5.5% increase in median survival time [6]. A number of studies have demonstrate that residual disease at the end of primary debulking surgery correlates with survival, and aggressive cytoreduction is the basis of initial therapy.

Cytoreductive Surgery

Primary surgery in ovarian cancer is unique because radical resections for maximal surgical debulking are undertaken even in patients in whom the likelihood of complete macroscopic removal of the tumour is small. The concept of maximal surgical debulking was introduced in the 1970s. Griffiths established the concept of optimal cytoreduction in 1975 when he published a retrospective study demonstrating improved survival in women with advanced stage disease who had no residual disease after primary surgery [7,8]. Since then, the ideal goal of cytoreduction is to leave no residual disease, but more commonly, the goal is to reduce the residual tumour to less than 1 or 2 cm. Gynecologic Oncology Group (GOG) studies comprising more than 500 patients demonstrated improved pathologic response, survival, disease-free interval, and complete clinical response in patients with no residual nodules greater than 1 cm [9,10].

The benefits of cytoreductive surgery are clear in the diagnosis and initial treatment of ovarian cancer. There are no prospective randomized trials comparing patients with optimal versus suboptimal cytoreduction, but accumulated retrospective data shows a strong correlation between the volume of the residual tumour and survival (Table 1) [11].

Table 1. Survival after primary cytoreductive surgery in advanced ovarian carcinoma according to residual tumour size

Author	Year of Study	No. of Patients	Median Overall Survival (Months)	
			< 2 cm	> 2 cm
Hoskins et al. [10]	1992	31	32	20
DelCampo et al. [12]	1994	91	31	22
Griffith et al.[13]	2002	74	38.4	14
Ryu et al.[14]	2004	57	40.6	13.2
Sharma et al. [5]	2005	140	52	26

Hacker and Berek [15,16] showed that patients whose largest residual lesions were ≤ 5 mm had superior survival. This finding was substantiated by Hoskins et al., who presented the GOG data, and Tingulstad et al. showed a survival benefit in patients with minimal residual disease after primary cytoreduction (Fig. 1) [17, 18].

Heinz et al. reported that a group with no macroscopic disease had a slightly better survival than a group with no microscopic disease in their study [3]. In fact, patients with no residual disease had the best survival. In addition, within the group with macroscopic residual disease, there was a striking difference in survival between those with residual disease of < 2 cm and those with residual tumours > 2 cm. Both results are arguments for improving the outcome of primary and interval debulking operations (Fig. 2).

Multiple studies have confirmed this observation [19-25]. Surgery remains the cornerstone of diagnosis and initial therapy for patients with advanced ovarian

carcinoma. Some authors have suggested that intrinsic tumour biology, rather than surgical intervention, determines not only prognosis but also the feasibility of surgical debulking [6,26].

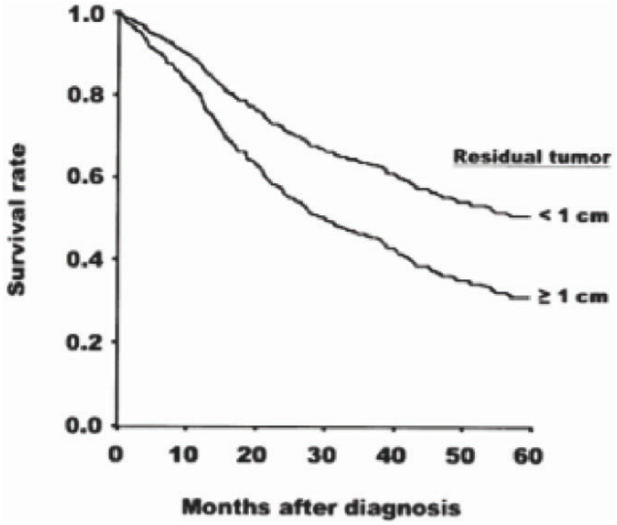


Figure 1. Estimated 5-year survival by residual tumour after primary cytoreduction in ovarian cancer. Reprinted with permission from [18]

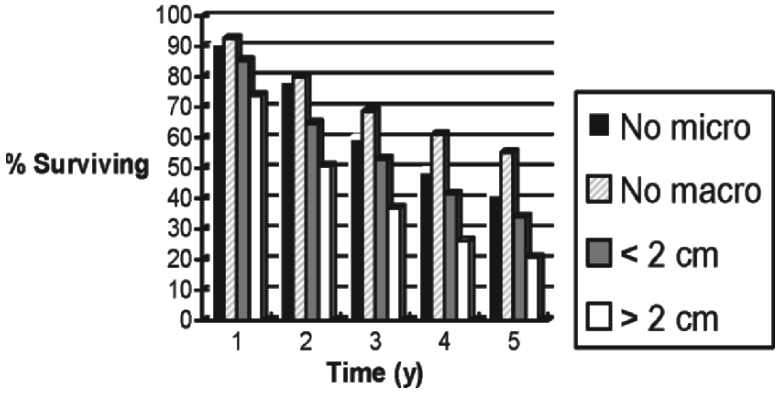


Figure 2. Survival of patients with stage IIIc epithelial ovarian cancer based on the maximum size of the residual tumour after primary cytoreduction

Hyperthermic Intraperitoneal Chemotherapy

Intraperitoneal spread is the most common and recognized characteristic of ovarian cancer. Carcinomatosis of advanced ovarian cancer is confined to the intraperitoneal cavity without distant metastasis. Several clinical trials have targeted itraperitoneal cancer cells in advanced ovarian cancer. These include immunotherapy such as intraperitoneal instillation of monoclonal antibodies, intraperitoneal radiotherapy, and direct administration of chemotherapeutic agents into the peritoneal cavity.

Intraperitoneal Chemotherapy

This concept of intraperitoneal (IP) chemotherapy was developed in the 1970s by Jones et al [27]. The rationale for IP chemotherapy is to eradicate residual disease by concentrating on the cytotoxic effect, introducing high drug concentrations directly into the peritoneal cavity, and reducing systemic toxic effects associated with standard intravenous administration [28,29].

There have been several randomized phase III trials comparing intravenous (IV) chemotherapy to a combination of IV and IP chemotherapy in optimally debulked stage III ovarian cancer [30,31]. The median duration of follow-up was 48.2 months in the IV therapy group and 52.6 months in the IP therapy group, with 5 and 11 patients, respectively, lost to follow-up. The median progression-free survival was 18.3 months in the IV therapy group and 23.8 months in the IP therapy group. The median overall survival was 49.7 and 65.6 months, respectively [31].

Intraperitoneal Hyperthermic Chemotherapy

This therapy combines cytoreductive surgery with direct instillation of chemotherapeutic agents into the peritoneal cavity intraoperatively with regional hyperthermia (at 42° C - 44° C) [32]. Because dissemination or recurrence of ovarian cancer occurs mostly within the peritoneal cavity, hyperthermic intraperitoneal chemotherapy (HIPEC) is an attractive modality to treat these patients. When combined with adequate cytoreductive surgery, HIPEC can markedly improve survival in patients with disseminated intraperitoneal malignancies [33,34].

Effects of HIPEC

On the basis of the results of several clinical trials, IP and HIPEC showed comparable survival benefits compared with standard IV chemotherapy in the therapeutic management of ovarian cancer, especially with small-volume, residual disease. Armstrong et al. showed that IP treatment was associated with an improvement in both progression-free and overall survival [31].

In the largest, retrospective, controlled study for HIPEC treatment in ovarian cancer [14], there was a significant improvement in the HIPEC group in both progression-free (median, 48.7 months vs. 19.8 months, respectively; p = 0.0021) and 5-year overall survival (63.4% vs. 52.8%, respectively; p = 0.0078) (Fig. 3). These findings agree with those of Gori et al., who showed prolongation of both disease-free (median, 57.1 vs. 46.4 months, respectively; p = 0.227) and overall survival (median, 64.4 vs. 60.1 months, respectively; p = 0.598) [39] (Table 2).

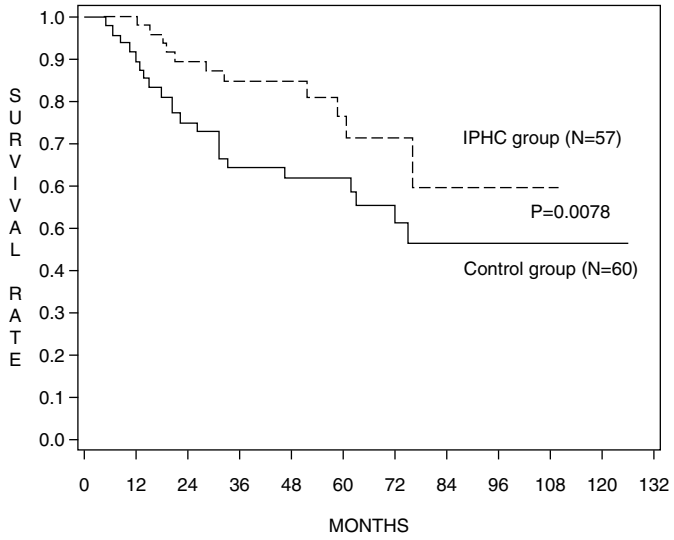


Figure 3. Overall 5-year survival. There was a significant difference between the intraperitoneal hyperthermic chemotherapy (HIPEC) and control groups (p = 0.0078). HIPEC group, dotted line; control group, solid line (Kaplan-Meier method). Reprinted with permission from [14]

Table 2. Results of Intraperitoneal Chemotherapy for Ovarian Cancer

Authors	year	N	Drugs	Type	MS	PFS	FU	SR
Markman et al. [36]	2001	235	Cisplatin*	IP	63.2	27.9		
Hager et al. [37]	2001	36	Cisplatin or Carboplatin	IPHC	49		5	35
Piso et al. [38]	2004	19	Cisplatin + Mitoxantrone	IPHC	33		5	15
Ryu et al. [14]	2004	57	Carboplatin	IPHC	NR	48.7	5	63.4
Gori et al. [39]	2005	29	Cisplatin	IPHC	64.4	57.1		
Raspagliesi et al. [40]	2006	40	Cisplatin + MMC or Cisplatin + Doxorubicin	IPHC	41.4	23.9	5	15
Armstrong et al . [31]	2006	205	Cisplatin + Paclitaxel**	IP	65.6	23.8	5	37.6

MS, median survival (m); PFS : progression free survival (m); NR, not reached; * Carboplatin AUC 9 × 2 cycles IV, then Cisplatin 100 mg/ m^2 IP + Paclitaxel 135 mg/ m^2 IV q 21 days × 6 cycles; ** Paclitaxel 135 mg/ m^2 IV (day 1) + Cisplatin 100mg/m^2 IP (day 2) + Paclitaxel 60 mg/ m^2 IP (day 8) q 21 days × 6 cycles

In the study by Ryu et al [14], of the 53 patients with optimally cytoreducted (< 1 cm), stage III ovarian cancer, 26 patients in the HIPEC group showed a significantly increased median disease-free survival compared to 27 patients in the control group (40.6 vs. 13.2 months, respectively; p = 0.0027). In addition, the 5-year survival rate was also significantly higher at 65.6% in the HIPEC vs. 40.7% in the control group (p = 0.0046) (Fig. 4).

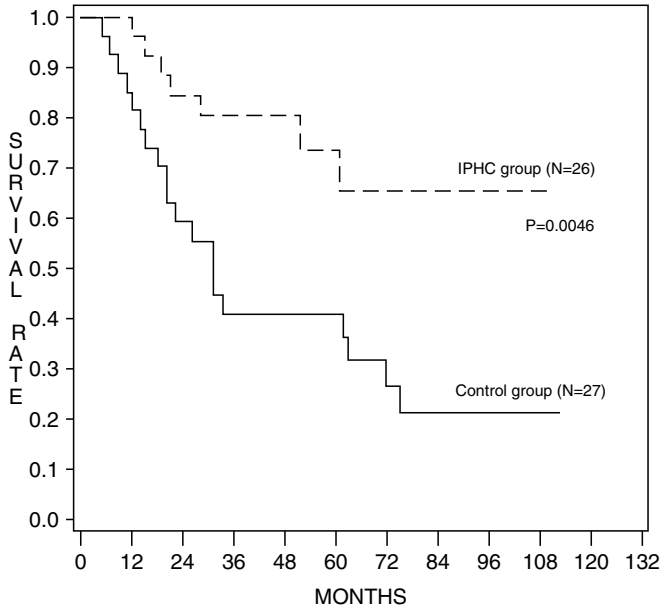


Figure 4. Overall survival of stage III ovarian cancer patients with a residual mass less than 1 cm after the second surgery. There was a significant difference between the intraperitoneal hyperthermic chemotherapy (HIPEC) and control groups (p = 0.0046). HIPEC group, dotted line; control group, solid line (Kaplan–Meier method). Reprinted with permission from [14]

The authors recommend that candidates for successful HIPEC are [35]:

- medically fit to undergo aggressive cytoreductive surgery and HIPEC;
- without extra-abdominal disease;
- patients whose peritoneal disease is potentially completely respectable or could be significantly reduced;
- without parenchymal hepatic metastases; and
- without bulk retroperitoneal disease.

Refractory Ovarian Cancer

Many patients present persistent or recurrent disease after first-line chemotherapy with complete clinical response. Several drugs can be used as a second-line therapy, and response rates range from 14% to 34% [41]. There is no standardized second-line treatment for recurrent or refractory ovarian cancer. The combination of secondary cytoreductive surgery and HIPEC represents a feasible, potential locoregional therapeutic option for this subset of patients [40].

In addition, direct cytotoxic hyperthermia effects and synergistic antineoplastic effects with anticancer agents are clinically and experimentally observed. The penetration of the chemotherapeutic agent into cancer tissues is improved by thermal effects, and drug resistance can be reduced by heat. Immunologic modulation to the cells and heat-induced secretion of cytokines may also contribute to the synergistic anticancer effect. Therefore, increased response rates and survival are expected with HIPEC. Higher response rates are obtained in patients with recurrent, chemotherapy-resistant/refractory, peritoneal-disseminated ovarian cancer. Reversal of drug resistance is obviously possible in combination with heat at temperatures above 42° C [37].

Recently, it has been proven that the synergistic antineoplastic effect of chemotherapeutic drugs with hyperthermia results from increased penetration of cancer tissues by the drug, the promotion of cytotoxicity by the formation of a carboplatin–DNA adduct form, and the induction of apoptosis [42].

Prognostic Factors Affecting Patient Survival

Using a multivariate analysis, Ryu et al. analyzed the prognostic factors affecting the survival of ovarian cancer patients, including patient age, cancer stage (Ic + II vs. III), tumour size after the second surgery, and the use of HIPEC [14]. These four factors were each independent prognostic factors of disease-free and overall survival (Table 3). In stage III, the hazard ratio was 12.4 and the risk of death increased by 8.8-fold as compared to stages Ic and II. When the remnant tumours after the second surgery were smaller than 1 cm, the recurrence rate decreased by 0.36 and the risk of death decreased by 0.38 compared with larger tumours. In the HIPEC group, the recurrence rate decreased by 0.55 and the risk of death decreased by 0.5. The relative risks of recurrence and death increased by 1.031 and 1.026 with a 1-year increase in age, respectively.

Table 3. Multivariate analysis of the effects on disease-free and overall survival [14]

	Disease-free interval		Survival	
	p	Hazard ratio	P value	Hazard ratio
Stage III	<0.0001	12.396	0.0032	8.759
< 1 cm*	0.0005	0.355	0.003	0.382
HIPEC	0.0181	0.554	0.0176	0.496
Age	0.0035	1.031	0.0278	1.026

* Residual mass size at second surgery

Conclusion

In advanced ovarian cancer, extended cycles of consolidation chemotherapy after optimal primary cytoreduction have been performed to prevent recurrence and prolong overall survival. Besides systemic chemotherapy, regional anticancer therapy is essential to control intraperitoneal disease progression. Sustained intraperitoneal temperature and even distribution of heat and anticancer drug are essential for successful HIPEC; thus, development of a new delivery system is necessary. HIPEC can be an effective and feasible therapeutic modality for locoregional consolidation therapy in advanced ovarian cancer.

The optimal duration, temperature, and chemotherapeutic agents remain unknown. Additionally, the integration of HIPEC with systemic therapy needs to be evaluated. The advancement of centers of excellence and the initiation of cooperative group trials will help to define the optimal treatment approach for intraperitoneal chemotherapy. The future of HIPEC lies in multicenter and randomized trials that investigate not only response and survival but also standardization of technique.

References

1. Landis SH, Murray T, Bolden S, Wingo PA (1999) Cancer statistics, CA Cancer J Clin 49:8-31
2. Rock JA, Thompson JD (1997) Te Linde's operative gynecology (ed 8). Philadelphia, PA, Lippincott-Raven Publisher, p1557-1558.
3. Sharma S, Driscoll D, Odunsi K, Venkatadri A, Lele S (2005) Safety and efficacy of cytoreductive surgery for epithelial ovarian cancer in elderly and high-risk surgical patients. Am J Obstet Gynecol 193:2077-2082
4. Berek JS (2005) Epithelial ovarian cancer, in Berek JS, Hacker NF: Practical gynecologic oncology (ed 4). Philadelphia, PA, Lippincott Williams and Wilkins, p 443-509

5. Heintz AP, Odicino F, Maisonneuve P, Beller U, Benedet JL, Creasman WT, et al (2003) Carcinoma of the ovary. Int J Gynaecol Obstet 83(Suppl 1):135-166
6. Bristow RE, Tomacruz RS, Armstrong DK, Trimble EL, Montz FJ (2002) Survival effect of maximal cytoreductive surgery for advanced ovarian carcinoma during the platinum era: a meta-analysis. J Clin Oncol 20:1248-1259
7. Griffiths CT (1975) Surgical resection of tumor bulk in the primary treatment of ovarian carcinoma. Natl Cancer Inst Monogr 42:101-104
8. Griffiths CT, Parker LM, Fuller AF Jr (1979) Role of cytoreductive surgical treatment in the management of advanced ovarian cancer. Cancer Treat Rep 63:235-240
9. Omura GA, Bundy BN, Berek JS, Curry S, Delgado G. Mortel R (1989) Randomized trial of cyclophosphamide plus cisplatin with or without doxorubicin in ovarian carcinoma: a Gynecologic Oncology Group Study. J Clin Oncol. 7:457-465.
10. Hoskins WJ, Bundy BN, Thigpen JT, Omura GA (1992) The influence of cytoreductive surgery on recurrence-free interval and survival in small-volume stage III epithelial ovarian cancer: a Gynecologic Oncology Group study. Gynecol Oncol 47:159-166
11. Mutch DG (2002) Surgical management of ovarian cancer. Sem Oncol 29(Suppl 1):3-8
12. Del Campo JM, Felip E, Rubio D, Vidal R, Bermejo B, Colomer R, et al (1994) Long-term survival in advanced ovarian cancer after cytoreduction and chemotherapy treatment. Gynecol Oncol 53:27-32
13. Griffiths CT, Parker LM, Lee S, Finkler NJ (2002) The effect of residual mass size on response to chemotherapy after surgical cytoreduction for advanced ovarian cancer: Long-term results. Int J Gynecol Cancer 12:323-331
14. Ryu KS, Kim JH, Ko HS, Kim JW, Ahn WS, Park YG, Kim SJ, Lee JM (2004) Effects of intraperitoneal hyperthermic chemotherapy in ovarian cancer. Gynecol Oncol 94:325-332
15. Hacker NF, Berek JS (1986) Cytoreductive surgery in ovarian cancer. In: Albert PS. Surwit EA. Eds. Ovarian cancer: Boston: Martinus Nijhoff, p53-67
16. Berek JS (1996) Complete debulking of advanced ovarian cancer. Cancer J Sci Am 2:134-135
17. Hoskins WJ, McGuire WP, Brady MF, Homesley HD, Creasman WT, Berman M, Ball H, Berek JS (1994) The effect of diameter of largest residual disease on survival after primary cytoreductive surgery in patients with suboptimal residual epithelial ovarian carcinoma. Am J Obstet Gynecol 170:974-979
18. Tingulstad S, Skjeldestad FE, Halvorsen TB, Hagen B (2003) Survival and prognostic factors in patients with ovarian cancer. Obstet Gynecol 101:885-891
19. Eisenkop SM, Friedman RL, Wang HJ (1998) Complete cytoreductive surgery is feasible and maximizes survival in patients with advanced epithelial ovarian cancer: a prospective study. Gynecol Oncol 69:103-108

20. Heintz AP, Hacker NF, Berek JS, Rose TP, Munoz AK, Lagasse LD (1986) Cytoreductive surgery in ovarian carcinoma: feasibility and morbidity. Obstet Gynecol 67:783-788
21. Piver MS, Baker T (1986) The potential for optimal (≥2 cm) cytoreductive surgery in advanced ovarian carcinoma at a tertiary medical center: a prospective study. Gynecol Oncol 24:1-8
22. Piver MS, Lele SB, Marchetti DL, Baker TR, Tsukada Y, Emrich LJ (1988) The impact of aggressive debulking surgery and cisplatin-based chemotherapy on progression-free survival in stage III and IV ovarian carcinoma. J Clin Oncol 6:983-989
23. Bertelsen K (1990) Tumor reduction surgery and long-term survival in advanced ovarian cancer: a DACOVA study. Gynecol Oncol 38:203-209
24. Hacker NF, Berek JS, Lagasse LD, Nieberg RK, Elashoff RM (1983) Primary cytoreductive surgery for epithelial ovarian cancer. Obstet Gynecol 61:413-420
25. Guidozzi F, Ball JH (1994) Extensive primary cytoreductive surgery for advanced epithelial ovarian cancer. Gynecol Oncol 53:326-330
26. Covens AL (2000) A critique of surgical cytoreduction in advanced ovarian cancer. Gynecol Oncol 78:269-274
27. Jones RB, Myers CE, Guarino AM, Dedrick RL, Hubbard SM, DeVita VT (1978) High volume intraperitoneal chemotherapy ("belly bath") for ovarian cancer. Pharmacologic basis and early result. Cancer Chemother Pharmacol 1:161-166
28. Jaaback K, Johnson N (2006) Intraperitoneal chemotherapy for the initial management of primary epithelial ovarian cancer. Cochrane Database Syst Rev 1:CD005340
29. Sugarbaker PH, Mora JT, Carmignani P, Stuart OA, Yoo D (2005) Update on chemotherapeutic agents utilized for perioperative intraperitoneal chemotherapy. Oncologist 10:112-122
30. Walker JL, Armstrong DK, Huang HQ, Fowler J, Webster K, Burger RA, et al (2006) Intraperitoneal catheter outcomes in a phase III trial of intravenous versus intraperitoneal chemotherapy in optimal stage III ovarian and primary peritoneal cancer: a Gynecologic Oncology Group Study. Gynecol Oncol 100:27-32
31. Armstrong DK, Bundy B, Wenzel L, Huang HQ, Baergen R, Lele S, et al (2006) Intraperitoneal cisplatin and paclitaxel in ovarian cancer. New Engl J Med 354:34-43
32. Sugarbaker PH (1999) Management of peritoneal-surface malignancy: the surgeon's role. Langenbecks Arch Surg 384:576-587
33. Verwaal VJ, van Ruth S, de Bree E, van Sloothen GW, van Tinteren H, Boot H, Zoetmulder FA (2003) Randomized trial of cytoreduction and hyperthermic intraperitoneal chemotherapy versus systemic chemotherapy and palliative surgery in patients with peritoneal carcinomatosis of colorectal cancer. J Clin Oncol 21:3737-3743

34. Ceelen WP, Hesse U, de Hemptinne B, Pattyn P (2000) Hyperthermic intraperitoneal chemoperfusion in the treatment of locally advanced intra-abdominal cancer. Br J Surg 87:1006-1015
35. Deraco M, Rossi CR, Pennacchioli E, Guadagni S, Somers DC, Santoro N, et al (2001) Cytoreductive surgery followed by intraperitoneal hyperthermic perfusion in the treatment of recurrent epithelial ovarian cancer: a phase II clinical study. Tumori 87:120-126
36. Markman M, Bundy BN, Alberts DS, Fowler JM, Clark-Pearson DL, Carson LF, et al (2001) Phase III trial of standard-dose intravenous cisplatin plus paclitaxel versus moderately high-dose carboplatin followed by intravenous paclitaxel and intraperitoneal cisplatin in small-volume stage III ovarian carcinoma: an intergroup study of the Gynecologic Oncology Group, Southwestern Oncology Group, and Eastern Cooperative Oncology Group. J Clin Oncol 19:1001-1007
37. Hager ED, Dziambor H, Hohmann D, Muhe N, Strama H (2001) Intraperitoneal hyperthermic perfusion chemotherapy of patients with chemotherapy-resistant peritoneal disseminated ovarian cancer. Int J Gynecol Cancer 11:57-63
38. Piso P, Dahlke MH, Loss M, Schlitt HJ (2004) Cytoreductive surgery and hyperthermic intraperitoneal chemotherapy in peritoneal carcinomatosis from ovarian cancer. World J Surg Oncol 2:21
39. Gori J, Castano R, Toziano M, Habich D, Staringer J, De Quiros DG, Felci N (2005) Intraperitoneal hyperthermic chemotherapy in ovarian cancer. Int J Gynecol Cancer 15:233-239
40. Raspagliesi F, Kusamura S, Campos Torres JC, de Souza GA, Ditto A, Zanaboni F, Younan R, Baratti D, Mariani L, Laterza B, Deraco M (2006) Cytoreduction combined with intraperitoneal hyperthermic perfusion chemotherapy in advanced/recurrent ovarian cancer patients: The experience of National Cancer Institute of Milan. Eur J Surg Oncol 32(6):671-675
41. Conte PF, Gadducci A, Cianci C (2001) Second-line treatment and consolidation therapies in advanced ovarian cancer. Int J Gynecol Cancer 11:52-56
42. Kong G, Braun RD, Dewhirst MW (2001) Characterization of the effect of hyperthermia on nanoparticle extravasation from tumor vasculature. Cancer Res 61:3027-3032

The role of Neoadjuvant Chemotherapy versus Primary Surgery in the Management of Stage III Ovarian Cancer

T Van Gorp, F Amant, P Neven, P Berteloot, K Leunen, I Vergote

Introduction

Primary cytoreductive (or cytoreduction) surgery is an operation to remove as much of the tumour, and its metastases, as possible before subsequent therapy is instituted. This is in contrast to *interval* cytoreductive (or cytoreduction) surgery, which is an operation performed in patients after a short course of induction chemotherapy, usually two, three or four cycles of chemotherapy, to remove as much primary and metastatic disease as possible in order to facilitate response to subsequent chemotherapy and to improve survival [1].

The importance of primary cytoreductive surgery in the treatment of advanced ovarian cancer (International Federation of Gynaecology and Obstetrics (FIGO) Stage III and IV) was already suggested as early as 1934 by Meigs [2] and later by Hudson [3], but, it wasn't until the 1970s, that Aure et al. [4] and Griffiths et al. [5] showed that the amount of residual tumour following primary surgery was an important prognostic factor in advanced ovarian carcinoma. Unfortunately, no prospective randomized controlled trials concerning the role of primary cytoreductive surgery in advanced ovarian carcinoma have been performed. Despite this lack of randomized controlled trials, primary cytoreductive surgery is considered as the standard of care in advanced ovarian cancer [6]. In the 1980s, the European Organisation for Research and Treatment of Cancer - Gynaecological Cancer Group (EORTC-GCG) launched a randomized study to investigate the role of interval cytoreduction surgery in women who did not or could not have a successful primary cytoreduction operation (reduction of disease to < 1 cm). During the same time period, several institutions started with neoadjuvant chemotherapy in patients with advanced ovarian cancer (without primary attempt at cytoreduction) followed by an interval cytoreduction surgery. The aim of this chapter is to clarify the role of primary cytoreductive surgery versus neoadjuvant chemotherapy followed by interval cytoreductive surgery in patients with locally advanced ovarian cancer.

Primary Cytoreductive Surgery

Biological basis of Cytoreductive Surgery

There is also a biological explanation why the act of cytoreductive surgery can be successful in improving the prognosis of ovarian cancer patients [7,8]. Large tumours have a relatively small percentage of blood vessels compared to their volume and have more often hypoxic or necrotic areas. Small tumours have more central blood perfusion and seem to have a higher percentage of dividing or proliferating cells. In the latter group, the increased blood flow will favour the transport and diffusion of the cytotoxic drugs to the tumour cells and since rapidly dividing cells are more sensitive to the action of cytotoxic drugs, the impact of these drugs on the cells will be greater. Cytoreductive surgery will therefore increase the susceptibility of smaller lesions to chemotherapy. Skipper [7] also coined the 'fractional cell kill' hypothesis that postulates that the proportion of tumour cells killed with each treatment is constant. Since the number of tumour cells is reduced by cytoreductive surgery, these smaller lesions will also require fewer chemotherapy cycles. The faster a tumour can be eradicated, the lower the chance it will develop drug-resistance during the course of therapy.

Optimal versus Suboptimal Cytoreductive Surgery

It has become clear that primary cytoreduction surgery is only advantageous to the patient if the primary cytoreductive surgery results in a minimal residual tumour load. In studies conducted in the 1970s, and even in more recent multicentric trials investigating different chemotherapeutic regimens, the rate of optimal primary cytoreductive surgery was only 20% tot 30% [4,9-13]. A meta-analysis of surgery in advanced ovarian cancer by Hunter et al. [13] suggested that cytoreductive surgery only had a small improvement in median survival, but this meta-analysis has been criticized because of two reasons. First, there was only a low rate of optimally debulked patients, and second, a large number of patients were not treated with optimal platinum-containing chemotherapy. More recently, Bristow et al. [14] performed a new meta-analysis on the survival effect of maximal cytoreduction surgery for advanced ovarian carcinoma, and this during the platinum era. In this meta-analysis 81 cohorts of patients with stage III or IV ovarian carcinoma were included (6,885 patients in total). The analysis showed a statistically significant positive correlation between percent maximal cytoreduction and median survival time, even after controlling for all other variables. In addition, each 10% increase in maximal cytoreduction was associated with a 5.5% increase in median survival time. They concluded that maximal cytoreduction surgery is one of the most powerful determinants of cohort survival among patients with advanced ovarian cancer.

The definition of 'optimal' or 'maximal' cytoreduction surgery remains controversial. The definition of an optimal cytoreduction has changed many times in the past 20 years, from a largest residual tumour mass of 2 cm to no residual tumour and even recent trials differ often in the definition they use for optimal cytoreduction. Griffiths et al. [5] originally proposed that the residual tumour mass for an optimal cytoreduction should be less than 1.5 cm. Later, many studies showed that patients without residual tumour had a better survival than those with less than 0.5 cm as the largest residual tumour mass, and the latter group had a better prognosis than patients with 0.5 tot 1.5 cm residual tumour [15-21]. Vergote et al. [22] suggested that optimal cytoreductive surgery should be defined as no macroscopic residual tumour, and this proposal was endorsed by the meta-analysis of Bristow et al. [14]. Despite this clear evolution towards a more radical approach during primary cytoreduction surgery, a questionnaire among US gynaecologic oncologists showed that only 12% of the responders regarded optimal cytoreduction surgery as no residual tumour, 13.7% as ≤ 0,5 cm, 60.8% as ≤ 1 cm, and 12.3% as ≤ 1.5 or 2 cm [23].

Variables influencing Cytoreductive Surgery

Cytoreductive surgery has an important role in the treatment of advanced ovarian cancer, in contrast to its limited use in other abdominal malignancies. This is mainly due to the relatively good resectability of ovarian cancer. Metastatic disease is usually confined to the abdominal cavity, and despite an extraordinarily large tumour burden, resection of metastatic tumour masses may be feasible as well as efficacious in these circumstances. Whether the observed survival benefits for optimally cytoreduced patients are a function of tumour biology or of surgical skill remains a fiercely debated issue. Indirect evidence is available that inherent tumour biology relates to resectability. For example, Heintz et al. [15] observed that cytoreduction was easier to achieve in patients who had low-grade tumours, small metastases, and no ascites. Burghardt et al. [24] showed that women in whom optimal cytoreduction was impossible had a higher number of positive pelvic and para-aortic lymph node metastases. In addition, Friedlander et al. [25] reported that the size of the largest residual tumour mass was not an independent factor when newer prognostic variables such as DNA ploidy were included in the multivariate analyses. Recently, microarrays were used to screen for differences in gene expression profiles between optimally and suboptimally debulked ovarian cancers [26]. Microarrays were able to differentiate between optimal and suboptimal cytoreduction with 72.7% accuracy. The authors concluded that the achievement of optimal cytoreduction is, at least in part, linked to tumour biology.

However, apart from this, the expertise of the surgeon cannot be misunderstood. Comparison of overall survival of patients treated by surgeons with or without a subspecialist training in gynaecologic oncology showed a survival benefit for those patients that were treated by a surgeon trained in performing this type of

operations [27]. This may also not be solely attributed to the surgical skills of the surgeon but also to the qualifications of the whole team in which the surgeon is working. The interdisciplinary approach and the continuous education and self-evaluation in such 'expert' centres are of a great importance. The suggested requirement for an 'expert' centre is an optimal resection rate of at least 75% [15]. Even in patients with advanced ovarian carcinoma initially thought to be unresectable by less experienced surgeons, an optimal cytoreductive cytoreduction can be achieved in 71% - 76% by gynaecologic oncologists, who are an integral part of such centres [15,19].

Patient variables also influence the resectability of widespread disease. Indeed, patients with good prognostic variables may be easier to debulk than patients with poor prognosis. For example, Heintz et al. [15] observed that cytoreduction was easier to achieve in young patients. Age is an important prognostic factor for patients with ovarian cancer. Older patients often have multiple medical comorbidities, and often only those patients with good performance statuses are selected for exploration. Moreover, these patients are very often treated less aggressively than younger patients. However, patients should not be offered less aggressive treatment based upon age alone. Carefully selected elderly patients tolerate surgical cytoreduction remarkably well with a complication and recovery rate similar to that of younger women [28].

Outcome of Cytoreductive Surgery

Not only the residual tumour load but also the initial metastatic tumour load is of prognostic significance. In an analysis of the data from the University of California, Los Angeles, Hacker et al. [19] first observed that patients with extensive metastatic disease prior to cytoreduction (> 10 cm in diameter) or with clinical ascites, had a poor prognosis even if the disease was cytoreduced to an optimal status. A later study from the same centre showed that the only prognostic factors influencing resectability to optimal status were metastatic disease larger than 5 cm and the presence of more than 1 litre of ascites [15]. Furthermore, in a study from The Netherlands, Heintz et al. [20] observed that the prognosis was influenced by the diameter of the largest metastasis before cytoreduction, and the presence of ascites or peritoneal carcinomatosis. In a study by the Gynecologic Oncology Group (GOG) of 349 patients with optimally resected (≤ 1 cm) disease, the multivariate analyses revealed that the presence of 20 or more residual lesions was an independent unfavourable prognostic variable [21]. Potter et al. [16] analyzed 302 patients with ovarian carcinoma and concluded that the role of bowel resection should be questioned when residual disease remained at the completion of the operative procedure. Vergote et al. [22] reported that patients with more than 1,000 g of total metastatic tumour load treated with primary cytoreduction surgery, had a poor survival despite optimal surgery. In addition, also Farias-Eisner et al. [29] found that the extent of peritoneal carcinomatosis before surgery was the most important prognostic factor in patients with less than 0.5 cm residual tumour. On the

other hand, some studies suggested that a complete excision of all peritoneal implants is feasible, even in the presence of an initial extensive peritoneal carcinomatosis, and that this excision will still improve survival [30,31].

FIGO Stage IV Disease

The role of cytoreductive surgery in FIGO stage IV remains controversial. Some studies have reported an improved survival in optimally debulked stage IV ovarian cancer patients, even when liver or lung metastases were present [32,33]. In contrast, Vergote et al. [22] reported that patients with stage IV disease, treated with primary cytoreduction surgery, had a poor survival despite optimal surgery. During the consensus meeting in 1998 it was agreed that patients with only a pleural effusion, or a supraclavicular node or a single cutaneous metastasis can be treated as stage III disease. Extensive primary cytoreduction in patients with liver or lung metastases was regarded as most likely of no benefit [1]. This was confirmed by a retrospective study of Zang et al. [34]. In this study, 71 patients with stage IV ovarian cancer were reviewed. Optimal cytoreductive surgery was an important prognostic factor of survival, but mainly in those with malignant pleural effusion or positive supraclavicular lymph node pathology.

From the above mentioned evidence we can conclude that primary cytoreduction surgery is currently one of the cornerstones in the standard care of patients with advanced stage ovarian cancer. This operation should be performed in expert centres by surgeons or gynaecologist with a certain degree of subspecialisation in the field of gynaecologic oncology in order to obtain a high percentage of optimal cytoreduction (approximately 75% or higher). The goal of cytoreductive surgery should be no residual disease, or, when this is impossible, minimal residual disease. FIGO stage IV ovarian cancer can be treated similar to FIGO stage III disease in case of a malignant pleural effusion or a supraclavicular lymph node metastasis. In our experience, only a few categories of patients are not considered suitable for primary cytoreduction surgery:

- poor general medical condition (e.g. age > 80 years)
- intrahepatic multiple metastases larger than 2 cm
- extra-abdominal metastatic disease (>2 cm in diameter), except for supraclavicular and inguinal lymph node metastases
- intra-abdominal metastatic disease, > 2 cm in diameter, at the level of the porta hepatis or at the level of the superior mesenteric artery
- extensive serosal invasion (plaques) of the small bowel necessitating resection of > 1.5 m of bowel

Neoadjuvant Chemotherapy and Interval Cytoreduction

Neoadjuvant Chemotherapy followed by Interval Cytoreduction after Suboptimal Primary Cytoreduction

When an optimal cytoreduction cannot be achieved, either an immediate re-operation by an experienced surgeon can be performed, or the operation can be completed after several cycles of chemotherapy. Possible advantages of neoadjuvant chemotherapy include an increased rate of optimal residual disease, less extensive surgery, reduced blood loss, lower morbidity, shortened hospital stay, improved quality of life, and the possibility to select out patients with platinum resistance. Basically, it will enable us to select out patients who are platinum resistant and probably benefit less from aggressive surgery.

Interval cytoreduction surgery after suboptimal primary cytoreduction followed by 3 courses of platinum-based chemotherapy has been investigated in 2 prospective randomized trials [35,36]. The first study was performed by the EORTC-GCG and the second by the GOG. In the EORTC-GCG study, 278 patients with epithelial ovarian carcinoma FIGO stage IIb-IV, that were suboptimally debulked, were included. A suboptimally cytoreduction was defined as residual disease of more than 1 cm. After having received three cycles of cisplatin plus cyclophosphamide (CP) chemotherapy, the patients that were not progressive during these three first cycles, were randomized either to continue with another three cycles of CP or undergo interval cytoreduction surgery followed by three additional cycles of CP. The 2-year and progression-free survival rates were significantly higher in the group of interval cytoreduction surgery (56% vs. 46%, and 38% vs. 26%, respectively), as was median survival (26 months vs. 20 months), while a 49% death reduction rate was seen in the first group At the time of an update of the study in 2001, after a median follow-up of 6.3 years, the survival remained improved up to 9 years after randomization ($p = 0.0032$). It is important to know that in this study the overall survival of patients with less than 1 cm tumour at the time of opening the abdomen for interval cytoreduction surgery is exactly the same as for those patients who were debulked to less than 1 cm during this procedure (19.4 months vs. 20 months). Furthermore, no subgroups of patients could be identified (stage, age, grading, or peritoneal carcinomatosis, number of lesions, tumour size at the time of interval cytoreduction surgery) that did not show an improved survival.

The outline of the GOG 152 can be summarized as follows. Patient accrual criteria were silimar to the EORTC trial: patients with FIGO stage III-IV ovarian cancer who underwent a suboptimal cytoreduction (residual tumour load > 1 cm). However, one of the important differences with the EORTC trial is that in the GOG-trial, one of the eligibility criteria was appropriate ovarian cancer surgery, defined as a laparotomy with an adequate excision to explore the entire abdominal cavity with a *maximal effort* to resect uterus, tubes, ovaries, omentum and all gross disease at the time of primary surgery. In total, 425 patients who had been subop-

timally cytoreduced and had subsequently received three courses of cisplatin and paclitaxel chemotherapy were randomized to either continuation of chemotherapy for another three courses or interval cytoreduction plus three additional chemotherapy courses. The median survival and progression-free survival were similar for the two groups (32 months vs. 33 months and 10.5 months vs. 10.8 months, respectively). The authors concluded that interval cytoreduction surgery did not improve the overall and progression-free survival in patients with stage III or IV ovarian cancer who had previously undergone a maximal but suboptimal primary cytoreduction.

The main differences between the two trials are summarized in Table 1. Basically, in the EORTC study there were more patients with Stage IV disease, poor WHO performance status and a higher residual tumour load after primary surgery. From both studies can be concluded that, based on the EORTC trial, interval cytoreduction surgery by an experienced gynaecological oncologist improves survival in some patients who have not been optimally operated primarily (poor medical condition, inexperienced surgeon). On the other hand, based on the GOG 152 trial, interval cytoreduction surgery does not seem to be indicated in patients who underwent primarily a *maximal surgical effort* by a gynaecological oncologist.

Table 1. Patient characteristics of two randomized trials of interval debulking surgery after 3 courses of first-line chemotherapy in ovarian cancer: GOG 152 [36] and EORTC [35]

	GOG 152	EORTC IDS
Chemotherapy	TP	CP
FIGO Stage IV	6%	21%
WHO Performance Status 2	7%	17%
Residual tumour after primary surgery		
1 – 2 cm	12%	6 %
2 – 5 cm	56%	30%
5 – 10 cm	23%	38%
> 10 cm	9%	26%

IDS, interval debulking surgery; TP, paclitaxel 135 mg/m^2/24 h + cisplatin 75 mg/m^2 q. day 21; CP, cyclophosphamide 750mg/m^2 + cisplatin 75 mg/m^2 q. day 21

Neoadjuvant Chemotherapy followed by Interval Cytoreduction as an Alternative to Primary Cytoreduction

Alternatively to primary cytoreduction surgery, neoadjuvant chemotherapy can be administered before attempting cytoreductive surgery. This approach has been advocated by some authors, especially for the treatment of stage IV ovarian cancer or for patients with a very high metastatic tumour load (e.g. more than 1 kg) or for patients with a poor general condition.

From several retrospective phase II studies it appears that the outcome of these women, treated with neoadjuvant chemotherapy followed by interval cytoreduction surgery, is essentially the same as for patients treated with primary cytoreduction surgery followed by chemotherapy. These studies are summarized in Table 2 [22,37-52,52-60]. In most of these studies, interval cytoreduction surgery was performed after 3 or 4 courses of neoadjuvant chemotherapy. The arguments for the timing of the interval surgery are firstly, chemotherapy induced fibrosis will be less extensive after 3 when compared to 6 courses, secondly, more patients might have developed chemoresistance after 6 courses than after 3 courses, and lastly, earlier studies investigated the role of cytoreduction surgery at the time of second-look surgery after 6 courses of chemotherapy. In this study no survival benefit was found when cytoreduction surgery was performed at this time point. Summarizing the published data, the survival results for 1102 patients with FIGO stage III and/or stage IV ovarian cancer treated with neoadjuvant chemotherapy (usually followed by interval cytoreduction surgery) are similar or better than those treated with primary cytoreduction surgery, but unfortunately no firm conclusions can be drawn because all these studies were retrospective. To illustrate this, we [22] observed a better overall survival when selecting 45% of our patients for neoadjuvant chemotherapy and 55% for primary cytoreduction surgery compared with a historical series with very aggressive cytoreduction (82% < 0.5 cm residual tumour). However, in the historical series only 76% of the patients were treated with platinum and none with paclitaxel, while in the group treated with neoadjuvant chemotherapy 94% was treated with platinum (and 30% also platinum and paclitaxel).

Bristow et al. [61] recently performed a meta-analysis on 22 cohorts of patients with stage III-IV disease. The median overall survival time of the 835 patients included in this analysis was 24,5 months. In contrast to the conclusions that were drawn in the individual studies, the authors of the meta-analysis concluded that neoadjuvant chemotherapy is associated with a poor prognosis when compared to primary cytoreduction surgery. The authors base their conclusion on the fact that the median survival rate is equivalent to that of patients with advanced stage disease and suboptimal residual disease (>1cm) following primary surgery. However, we must take into consideration that the majority of the patients within these cohorts were primarily patients with poor prognostic factors and extensive disease, leading to a poor prognosis to start with.

Table 2. Retrospective studies of ovarian cancer patients treated with neoadjuvant chemotherapy followed by interval cytoreduction

Authors	n	Main conclusion	Ref.
Donadio et al.	24	NAC increase the chances of optimal debulking	[37]
Lawton et al.	36	78 % IDS of which 89% < 2 cm	[38]
Tummarello et al.	24	NAC could be a valid alternative to surgery	[39]
Jacob et al.	22	Same survival as 18 matched controls	[40]
Lim et al.	30	NAC can make patients operable	[41]
Shimizu et al.	74	46 % IDS to < 2 cm	[42]
Onnis et al.	88	42 % IDS to < 2 cm	[43]
Surwit et al.	29	Median survival = 22 months (= primary debulking)	[44]
Vergote et al.	75	Crude survival higher when selecting about ½ of the patients for NAC	[22]
Schwartz et al.	59	Similar survival compared with those treated during the same time period with primary debulking	[45]
Ansquer et al.	54	Better survival for patients treated with NAC compared with non-debulked tumours	[46]
Kuhn et al.	37	Better median survival in the group treated with NAC compared with primary debulked group	[47]
Recchia et al.	34	Only stage IV, median survival 28 months	[48]
Kayikcioglu et al.	45	NAC followed by IDS does not appear to worsen prognosis	[49]
Ushijima et al.	65	Similar survival in NAC group compared with primary debulking group	[50]
Fanfani et al.	73	NAC followed by successful IDS can achieve good results in terms of survival outcomes	[51]
Shibata et al.	29	The long-term outcome was not statistically different in patients treated with NAC,	[52]
Morice et al.	34	IDS in patients with advanced stage ovarian cancer offers the same survival as PDS, but it is better tolerated	[53]
Morice et al.	57	Survival rates were similar in patients with advanced stage ovarian cancer who underwent IDS or PDS.	[54]
Mazzeo et al.	45	NAC followed by optimal IDS may be a safe treatment alternative in patients with primarily unresectable advanced ovarian cancer	[55]
Vrscaj et al.	20	NAC does not have an unfavourable effect on prognosis.	[52]
Chan et al.	17	Overall quality of life improves after NAC	[56]
Le et al.	61	NAC with IDS appears to be safe and feasible	[57]
Hegazy et al.	27	NAC followed by IDS does not appear to worsen the prognosis, but it permits a less aggressive surgery to be performed.	[58]
Loizzi et al.	25	No difference in overall disease-specific survival and disease-free survival between NAC and PDS	[59]
Lee et al.	18	NAC provides an equivalent survival with less invasive surgery and reduced morbidity compared with PDS	[60]
TOTAL	1102		

IDS, Interval Debulking Surgery; NAC, Neoadjuvant Chemotherapy; PDS, Primary Debulking Surgery

Although there is evidence from retrospective studies that neoadjuvant chemotherapy followed by interval cytoreduction surgery is a valid alternative in a selected group of patients with stage III or IV ovarian carcinoma, this needs to be confirmed in a prospective randomized trial. Therefore, the EORTC Gynaecological Cancer Group, in cooperation with the NCI-Canada, launched a prospective randomized trial (EORTC 55971) to compare primary cytoreduction surgery with neoadjuvant chemotherapy followed by surgery. To be eligible the patients should have biopsy proven stage IIIc or IV epithelial ovarian cancer or peritoneal or fallopian tube carcinoma. Patients are being randomized between upfront cytoreduction surgery, followed by at least 6 cycles of platinum-based chemotherapy, or 3 cycles of neoadjuvant platinum-based chemotherapy, followed by interval cytoreduction surgery and at least 3 more cycles of platinum-based chemotherapy. Patients that are progressive during or after the three first cycles of neo-adjuvant chemotherapy are taken out of the protocol. The study is expected to close in the summer of 2006 with a target accrual of 704 patients.

Laparoscopy to select Patients for Neoadjuvant Chemotherapy

Nelson et al. [62] proposed computerized tomographic (CT) scan criteria to predict operability in patients with suspect ovarian masses. Tumour localization on the spleen or tumours larger than 2 cm on the diaphragm, liver surface, mesentery, gallbladder on CT scan were regarded as inoperable. However, 6 out of 18 patients (33%) judged to be inoperable based on these criteria were optimally debulked. Therefore, we do not believe that operability can be judged based on CT scan findings. Others proposed newer CT scan criteria, CA 125 or microarray analyses to predict operability [63-65]. We [66] concluded that CT scan with peritoneography was superior to standard CT scan but still less sensitive than laparoscopy to evaluate operability.

The technique of an open laparoscopy decreases the risk of a 'blind' insertion of a Veres needle or trocar. During open laparoscopy, a small incision in or underneath the umbilicus is made. Consecutively, the different layers of the abdominal wall are opened (ie, a mini-laparotomy), and a blunt trocar is introduced under direct vision. Between 1995 and 2002, we performed an open laparoscopy in 173 patients to establish the diagnosis of stage III or IV ovarian carcinoma and found that open laparoscopy was the best technique to evaluate the operability. This procedure also provides the opportunity to perform biopsies and to exclude other primary tumours metastatic to the pelvis (eg, intestinal tumours or pancreatic tumours) [67].

The possible development of port site metastases might hold back some surgeons to perform laparoscopy. We explored this issue further and completely excised all port sites at the time of primary cytoreductive surgery or interval cytoreductive surgery in the last 71 cases. Twenty-two of these contained malignant cells. The total number of port site metastases in the whole series of 173 patients

was 30 (17%) [67]. It should be noted that in this series all port site metastases disappeared during the neoadjuvant chemotherapy or were excised at the time of surgery. None of the patients recurred later during the follow-up in the port sites, and none of the patients had a port site metastasis at the time of death. Therefore, we believe that port site metastases in advanced ovarian cancer are frequent but not of prognostic significance. We can conclude that open laparoscopy is an important tool in the evaluation of the operability of patients with ovarian cancer. Until present, this technique does not have any proven adverse effects on the prognosis of these patients.

References

1. Berek JS, Bertelsen K, du Bois A, Brady MF, Carmichael J, Eisenhauer EA, Gore M, Grenman S, Hamilton TC, Hansen SW, Harper PG, Horvath G, Kaye SB, Luck HJ, Lund B, McGuire WP, Neijt JP, Ozols RF, Parmar MKB, Piccart-Gebhart MJ, van Rijswijk R, Rosenberg P, Rustin GJS, Sessa C, Thigpen JT, Trope C, Tuxen MK, Vergote I, Vermorken JB, Willemse PHB (1999) Advanced epithelial ovarian cancer: 1998 consensus statements. Ann Oncol 10: 87-92
2. Meigs JV (1934) Tumors of the pelvic organs. Macmillan: New York, NY
3. Hudson CN (1968) A radical operation for fixed ovarian tumours. J Obstet Gynaecol Br Commonw 75: 1155-1160
4. Aure JC, Hoeg K, Kolstad P (1971) Clinical and histologic studies of ovarian carcinoma. Long-term follow-up of 990 cases. Obstet Gynecol 37: 1-9
5. Griffiths CT, Fuller AF (1978) Intensive Surgical and Chemotherapeutic Management of Advanced Ovarian Cancer. Surg Clin North Am 58: 131-142
6. Berek JS, Trope C, Vergote I (1999) Surgery during chemotherapy and at relapse of ovarian cancer. Ann Oncol 10: 3-7
7. Skipper HE (1974) Thoughts on Cancer Chemotherapy and Combination Modality Therapy (1974). Jama-Journal of the American Medical Association 230: 1033-1035
8. Goldie JH, Coldman AJ (1979) Mathematic Model for Relating the Drug Sensitivity of Tumors to Their Spontaneous Mutation-Rate. Cancer Treat Rep 63: 1727-1733
9. Smith JP, Day TG (1979) Review of Ovarian-Cancer at the University-Of-Texas-Systems-Cancer-Center, Md-Anderson-Hospital-And-Tumor-Institute. Am J Obstet Gynecol 135: 984-993
10. Bertelsen K, Jakobsen A, Andersen JE, Ahrons S, Pedersen PH, Kiaer H, Arffmann E, Bichel P, Boestofte E, Christophersen IS, Gregersen E, Hansen MK, Holund B, Jacobsen M, Jensen HK, Jepsen FL, Larsen G, Nielsen ES, Nyland M, Olsen J, Panduro J, Rank F, Sell A, Sogaard H (1987) A Randomized Study of Cyclophosphamide and Cisplatinum with Or Without Doxorubicin in Advanced Ovarian-Carcinoma. Gynecol Oncol 28: 161-169

11. Marsoni S (1987) Randomized Comparison of Cisplatin with Cyclophosphamide Cisplatin and with Cyclophosphamide Doxorubicin Cisplatin in Advanced Ovarian-Cancer. Lancet 2: 353-359
12. Voest EE, Vanhouwelingen JC, Neijt JP (1989) A Meta-Analysis of Prognostic Factors in Advanced Ovarian-Cancer with Median Survival and Overall Survival (Measured with the Log (Relative Risk)) As Main Objectives. Eur J Cancer Clin Oncol 25: 711-720
13. Hunter RW, Alexander NDE, Soutter WP (1992) Metaanalysis of Surgery in Advanced Ovarian-Carcinoma - Is Maximum Cytoreductive Surgery An Independent Determinant of Prognosis. Am J Obstet Gynecol 166: 504-511
14. Bristow RE, Tomacruz RS, Armstrong DK, Trimble EL, Montz FJ (2002) Survival effect of maximal cytoreductive surgery for advanced ovarian carcinoma during the platinum era: A meta-analysis. J Clin Oncol 20: 1248-1259
15. Heintz APM, Hacker NF, Berek JS, Rose TP, Munoz AK, Lagasse LD (1986) Cytoreductive Surgery in Ovarian-Carcinoma - Feasibility and Morbidity. Obstet Gynecol 67: 783-788
16. Potter ME, Partridge EE, Hatch KD, Soong SJ, Austin JM, Shingleton HM (1991) Primary Surgical Therapy of Ovarian-Cancer - How Much and When. Gynecol Oncol 40: 195-200
17. Redman JR, Petroni GR, Saigo PE, Geller NL, Hakes TB (1986) Prognostic Factors in Advanced Ovarian-Carcinoma. J Clin Oncol 4: 515-523
18. Neijt JP, Tenbokkelhuinink WW, Vanderburg MEL, Vanoosterom AT, Willemse PHB, Heintz APM, Vanlent M, Trimbos JB, Bouma J, Vermorken JB, Vanhouwelingen JC (1987) Randomized Trial Comparing 2 Combination Chemotherapy Regimens (CHAP-5 v CP) in Advanced Ovarian-Carcinoma. J Clin Oncol 5: 1157-1168
19. Hacker NF, Berek JS, Lagasse LD, Nieberg RK, Elashoff RM (1983) Primary Cytoreductive Surgery for Epithelial Ovarian-Cancer. Obstet Gynecol 61: 413-420
20. Heintz APM, Vanoosterom AT, Baptist J, Trimbos MC, Schaberg A, Vandervelde EA, Nooy M (1988) The Treatment of Advanced Ovarian-Carcinoma. 1. Clinical-Variables Associated with Prognosis. Gynecol Oncol 30: 347-358
21. Hoskins WJ, Bundy BN, Thigpen JT, Omura GA (1992) The Influence of Cytoreductive Surgery on Recurrence-Free Interval and Survival in Small-Volume Stage-III Epithelial Ovarian-Cancer - A Gynecologic Oncology Group-Study. Gynecol Oncol 47: 159-166
22. Vergote I, De Wever I, Tjalma W, Van Gramberen M, Decloedt J, van Dam P (1998) Neoadjuvant chemotherapy or primary debulking surgery in advanced ovarian carcinoma: A retrospective analysis of 285 patients. Gynecol Oncol 71: 431-436
23. Eisenkop SM, Spirtos NM (2001) What are the current surgical objectives, strategies, and technical capabilities of gynecologic oncologists treating advanced epithelial ovarian cancer? Gynecol Oncol 82: 489-497

24. Burghardt E, Girardi F, Lahousen M, Tamussino K, Stettner H (1991) Patterns of Pelvic and Paraaortic Lymph-Node Involvement in Ovarian-Cancer. Gynecol Oncol 40: 103-106
25. Friedlander ML, Hedley DW, Swanson C, Russell P (1988) Prediction of Long-Term Survival by Flow Cytometric Analysis of Cellular Dna Content in Patients with Advanced Ovarian-Cancer. J Clin Oncol 6: 282-290
26. Berchuck A, Iversen ES, Lancaster JM, Dressman HK, West M, Nevins JR, Marks JR (2004) Prediction of optimal versus suboptimal cytoreduction of advanced-stage serous ovarian cancer with the use of microarrays. Am J Obstet Gynecol 190: 910-922
27. Kehoe S, Powell J, Wilson S, Woodman C (1994) The Influence of the Operating Surgeons Specialization on Patient Survival in Ovarian-Carcinoma. Br J Cancer 70: 1014-1017
28. Wright JD, Herzog TJ, Powell MA (2004) Morbidity of cytoreductive surgery in the elderly. Am J Obstet Gynecol 190: 1398-1400
29. Farias-Eisner R, Teng F, Oliveira M, Leuchter R, Karlan B, Lagasse LD, Berek JS (1994) The influence of tumor grade, distribution, and extent of carcinomatosis in minimal residual stage III epithelial ovarian cancer after optimal primary cytoreductive surgery. Gynecol Oncol 55: 108-110
30. Eisenkop SM, Nalick RH, Wang HJ, Teng NNH (1993) Peritoneal Implant Elimination During Cytoreductive Surgery for Ovarian-Cancer - Impact on Survival. Gynecol Oncol 51: 224-229
31. vanDam PA, Tjalma W, Weyler J, Vanoosterom AT, Buytaert P (1996) Ultraradical debulking of epithelial ovarian cancer with the ultrasonic surgical aspirator: A prospective randomized trial. Am J Obstet Gynecol 174: 943-950
32. Naik R, Nordin A, Cross PA, Hemming D, Lopes AD, Monaghan JM (2000) Optimal cytoreductive surgery is an independent prognostic indicator in stage IV epithelial ovarian cancer with hepatic metastases. Gynecol Oncol 78: 171-175
33. Akahira J-I, Yoshikawa H, Shimizu Y, Tsunematsu R, Hirakawa T, Kuramoto H, Shiromizu K, Kuzuya K, Kamura T, Kikuchi Y (2001) Prognostic Factors of Stage IV Epithelial Ovarian Cancer: A Multicenter Retrospective Study. Gynecol Oncol 81: 398-403
34. Zang RY, Zhang ZY, Cai SM, Li ZT, Chen J, Tang MQ, Liu Q (1999) Cytoreductive surgery for stage IV epithelial ovarian cancer. J Exp Clin Cancer Res 18: 449-454
35. van der Burg ME, van Lent M, Buyse M, Kobierska A, Colombo N, Favalli G, Lacave AJ, Nardi M, Renard J, Pecorelli S (1995) The effect of debulking surgery after induction chemotherapy on the prognosis in advanced epithelial ovarian cancer. Gynecological Cancer Cooperative Group of the European Organization for Research and Treatment of Cancer. N Engl J Med 332: 629-634
36. Rose PG, Nerenstone S, Brady MF, et al (2002) A phase III randomised study of interval secondary cytoreducion in patients with advanced stage ovarian

carcinoma with suboptimal residual disease: a Gynecologic Oncology Group study. Proceedings of ASCO 21: 201a
37. Donadio M, Bonardi G, Iberti V, Bertetto O, Carnino F, Iskra L, Mossetti C, Calciati A (1989) The role of induction chemotherapy in inoperable ovarian cancer. Tumori 75: 609-614
38. Lawton FG, Redman CWE, Luesley DM, Chan KK, Blackledge G (1989) Neoadjuvant (Cytoreductive) Chemotherapy Combined with Intervention Debulking Surgery in Advanced, Unresected Epithelial Ovarian-Cancer. Obstet Gynecol 73: 61-65
39. Tummarello D, Menichetti ET, Miseria S, Torresi U, Guidi F, Gramazio A, Vissani L, Cellerino R (1990) Advanced epithelial ovarian cancer: no difference in survival rate between exploratory laparotomy and inadequate debulking surgery as treatment approach before chemotherapy. J Chemother 2: 260-263
40. Jacob JH, Gershenson DM, Morris M, Copeland LJ, Burke TW, Wharton JT (1991) Neoadjuvant Chemotherapy and Interval Debulking for Advanced Epithelial Ovarian-Cancer. Gynecol Oncol 42: 146-150
41. Lim JT, Green JA (1993) Neoadjuvant carboplatin and ifosfamide chemotherapy for inoperable FIGO stage III and IV ovarian carcinoma. Clin Oncol (R Coll Radiol) 5: 198-202
42. Shimizu Y, Hasumi K (1993) [Treatment of stage III and IV ovarian cancer-is neoadjuvant chemotherapy effective?]. Nippon Sanka Fujinka Gakkai Zasshi 45: 1007-1014
43. Onnis A, Marchetti M, Padovan P, Castellan L (1996) Neoadjuvant chemotherapy in advanced ovarian cancer. Eur J Gynaecol Oncol 17: 393-396
44. Surwit E, Childers J, Atlas I, Nour M, Hatch K, Hallum A, Alberts D (1996) Neoadjuvant chemotherapy for advanced ovarian cancer. Int J Gynecol Cancer 6: 356-361
45. Schwartz PE, Rutherford TJ, Chambers JT, Kohorn EI, Thiel RP (1999) Neoadjuvant chemotherapy for advanced ovarian cancer: Long-term survival. Gynecol Oncol 72: 93-99
46. Ansquer Y, Leblanc E, Clough K, Morice P, Dauplat J, Mathevet P, Lhomme C, Scherer C, Tigaud JD, Benchaib M, Fourme E, Castaigne D, Querleu D, Dargent D (2001) Neoadjuvant chemotherapy for unresectable ovarian carcinoma - A French multicenter study. Cancer 91: 2329-2334
47. Kuhn W, Rutke S, Spathe K, Schmalfeldt B, Florack G, von Hundelshausen B, Pachyn D, Ulm K, Graeff H (2001) Neoadjuvant chemotherapy followed by tumor debulking prolongs survival for patients with poor prognosis in International Federation of Gynecology and Obstetrics Stage IIIC ovarian carcinoma. Cancer 92: 2585-2591
48. Recchia F, De Filippis S, Rosselli M, Saggio G, Carta G, Rea S (2001) Primary chemotherapy in stage IV ovarian cancer. A prospective phase II study. Eur J Gynaecol Oncol 22: 287-291
49. Kayikcioglu F, Kose MF, Boran N, Caliskan E, Tulunay G (2001) Neoadjuvant chemotherapy or primary surgery in advanced epithelial ovarian carcinoma. Int J Gynecol Cancer 11: 466-470

50. Ushijima K, Ota S, Komai K, Matsuo G, Motoshima S, Honda S, Tomonari R, Sugiyama T, Kamura T (2002) Clinical assessment of neoadjuvant chemotherapy and interval cytoreductive surgery for unresectable advanced ovarian cancer. Int Surg 87: 185-190
51. Fanfani F, Ferrandina G, Corrado G, Fagotti A, Zakut HV, Mancuso S, Scambia G (2003) Impact of interval debulking surgery on clinical outcome in primary unresectable FIGO stage IIIc ovarian cancer patients. Oncology 65: 316-322
52. Shibata K, Kikkawa F, Mika M, Suzuki Y, Kajiyama H, Ino K, Mizutani S (2003) Neoadjuvant chemotherapy for FIGO stage III or IV ovarian cancer: Survival benefit and prognostic factors. Int J Gynecol Cancer 13: 587-592
53. Morice P, Brehier-Ollive D, Rey A, Atallah D, Lhomme C, Pautier P, Pomel C, Camatte S, Duvillard P, Castaigne D (2003) Results of interval debulking surgery in advanced stage ovarian cancer: an exposed-non-exposed study. Ann Oncol 14: 74-77
54. Morice P, Dubernard G, Rey A, Atallah D, Pautier P, Pomel C, Lhomme C, Duvillard P, Castaigne D (2003) Results of interval debulking surgery compared with primary debulking surgery in advanced stage ovarian cancer. J Am Coll Surg 197: 955-963
55. Mazzeo F, Berliere M, Kerger J, Squifflet J, Duck L, D'Hondt V, Humblet Y, Donnez J, Machiels JP (2003) Neoadjuvant chemotherapy followed by surgery and adjuvant chemotherapy in patients with primarily unresectable, advanced-stage ovarian cancer. Gynecol Oncol 90: 163-169
56. Chan YM, Ng TY, Ngan HY, Wong LC (2003) Quality of life in women treated with neoadjuvant chemotherapy for advanced ovarian cancer: a prospective longitudinal study. Gynecol Oncol 88: 9-16
57. Le T, Faught W, Hopkins L, Fung Kee FM (2005) Primary chemotherapy and adjuvant tumor debulking in the management of advanced-stage epithelial ovarian cancer. Int J Gynecol Cancer 15: 770-775
58. Hegazy MA, Hegazi RA, Elshafei MA, Setit AE, Elshamy MR, Eltatoongy M, Halim AA (2005) Neoadjuvant chemotherapy versus primary surgery in advanced ovarian carcinoma. World J Surg Oncol 3: 57
59. Loizzi V, Cormio G, Resta L, Rossi CA, DI Gilio AR, Cuccovillo A, Selvaggi L (2005) Neoadjuvant chemotherapy in advanced ovarian cancer: a case-control study. Int J Gynecol Cancer 15: 217-223
60. Lee SJ, Kim BG, Lee JW, Park CS, Lee JH, Bae DS (2006) Preliminary results of neoadjuvant chemotherapy with paclitaxel and cisplatin in patients with advanced epithelial ovarian cancer who are inadequate for optimum primary surgery. J Obstet Gynaecol Res 32: 99-106
61. Bristow RE, Chi DS (2006) Platinum-based neoadjuvant chemotherapy and interval surgical cytoreduction for advanced ovarian cancer: A meta-analysis. Gynecol Oncol
62. Nelson BE, Rosenfield AT, Schwartz PE (1993) Preoperative Abdominopelvic Computed Tomographic Prediction of Optimal Cytoreduction in Epithelial Ovarian-Carcinoma. J Clin Oncol 11: 166-172

63. Bristow RE, Duska LR, Lambrou NC, Fishman EK, O'Neill MJ, Trimble EL, Montz FJ (2000) A model for predicting surgical outcome in patients with advanced ovarian carcinoma using computed tomography. Cancer 89: 1532-1540
64. Tate S, Hirai Y, Takeshima N, Hasumi K (2005) CA125 regression during neoadjuvant chemotherapy as an independent prognostic factor for survival in patients with advanced ovarian serous adenocarcinoma. Gynecol Oncol 96: 143-149
65. Berchuck A, Iversen ES, Lancaster JM, Dressman HK, West M, Nevins JR, Marks JR (2004) Prediction of optimal versus suboptimal cytoreduction of advanced-stage serous ovarian cancer with the use of microarrays. Am J Obstet Gynecol 190: 910-925
66. Gryspeerdt S, Clabout L, Van Hoe L, Berteloot P, Vergote IB (1998) Intraperitoneal contrast material combined with CT for detection of peritoneal metastases of ovarian cancer. Eur J Gynaecol Oncol 19: 434-437
67. Vergote I, Marquette S, Amant F, Berteloot P, Neven P (2005) Port-site metastases after open laparoscopy: a study in 173 patients with advanced ovarian carcinoma. Int J Gynecol Cancer 15: 776-779

Morbidity and Quality of Life following Cytoreduction and HIPEC

M Deraco, D Baratti, S Kusamura

Introduction

The evolution of locoregional therapy in the last 2 decades has changed the perpective in the clinical management of patients affected by peritoneal surface malignancies from mere palliation to possible cure [1]. Results from Phase II studies testing the combination of cytoreductive surgery (CRS) and hyperthermic intraperitoneal chemoperfusion (HIPEC) in the treatment of peritoneal carcinomatosis (PC) of various origins have been encouraging [2]. In previous papers, we reported 5-year overall survival rates of 97% and 57%, respectively, for patients with pseudomyxoma peritonei [3] and malignant peritoneal mesothelioma [4-5] treated with CRS and HIPEC. Other authors have reported 5-year overall survival rates of 63.4% and 19%, respectively, in ovarian [6] and colorectal cancer [7]. Moreover, results of a phase III trial have confirmed the superiority of CRS and HIPEC in the treatment of patients with PC from colorectal cancer over other standard surgical and/or systemic chemotherapy (CT) modalities [8].

Morbidity and Mortality following Cytoreduction and HIPEC

The institution of a program in peritoneal malignancy requires not only highly specialized human resources but also complex technological facilities to perform CRS and HIPEC safely, minimizing treatment-related morbidity and mortality, and maximizing results in terms of survival and quality of life [9]. Morbidity and mortality are relevant issues in this surgical procedure which combines unusually lengthy operative times, multiple and complex visceral resections, peritoneal stripping and heated intraperitoneal (ip) chemotherapy. Morbidity of cytoreductive surgery with HIPEC can be categorized into surgical complications and chemotherapy-related toxicity. Major morbidity rates after CRS and HIPEC varied from 14% to 56%. Mortality ranged from 0% to 19% in the literature and from 0% to

8% in the main series, which may be considered acceptable in a major surgical procedure [2].

The main papers focusing on the toxicity and complications related to CRS and HIPEC are summarized in Table 1. However, CRS and HIPEC are characterized by a broad variability with regard to therapeutic indication, patient selection and surgical technique which renders the clinical results difficult to compare. Moreover, timing, modality, duration, degree of hyperthermia, type and dose of drugs for loco-regional CT are not consistent from one center to another.

Table 1. Overview of operative complications associated with cytoreductive surgery and intraperitoneal chemotherapy

Ref	N	Histology	PM	OT	Mb	Mt	Complications	Risk factors
[10]	45	Appendix Colon	O	NA	37.7	0	Bowel, bleeding	Induction ip chemotherapy
[11]	60	Appendix Colon	C	10.9[a]	35	5	Bowel, bleeding	Gender, intraabdominal temperature, duration
[12]	102	Colon	O	7.5[b]	35	8	Bowel, intraabdominal abscess	Recurrent form, carcinomatosis extent, CC, blood loss, # anastomoses
[13]	200	PMP colon	O	NA	27	1.5	Peripancreatitis, bowel, bleeding	# peritonectomy procedures
[14]	216	Colon, PMP ovarian	C	6.1[a]	24.5	3.2	Digestive fistula; hematologic toxicity	Carcinomatosis extension, duration, # peritonectomy procedures
[21]	77	Colon	C	9[b]	30	12	Bowel infection, respiratory failure, sepsis	Bowel anastomoses (with sepsis)

PM, perfusion method; OT, operating time; Mb, morbidity (%); Mt, mortality (%); NA, not available; ip, intraperitoneal; HIPEC, hyperthermic intraperitoneal chemoperfusion; PMP, pseudomyxoma peritonei; CC, completeness of cytoreduction; [a] Mean; [b] Median

Esquivel [10] reported on the complications observed in 44 patients with PC of appendiceal, colonic, small bowel, or fallopian tube origin treated with CRS and early postoperative ip chemotherapy (EPIC) using mitomycin C (MMC) and 5-fluorouracil (5-FU). Twenty-two patients had been treated with induction ip chemotherapy prior to CRS and EPIC. The median duration of postoperative ileus was 21 days and related to age and to the extent of the surgical cytoreduction. Postoperative haemorrhage requiring re-intervention was recorded in 4 patients, pneumonia and respiratory failure requiring orotracheal intubation in 2. Enteric complications, including small bowel fistula, anastomotic disruption, bile leak, and pancreatitis occurred in 7 patients, of whom 6 had been treated with induction ip chemotherapy. The authors concluded that, since induction ip chemotherapy

carries an increased risk of postoperative enteric complications, this treatment modality should be reserved for patients with small volume disease.

Jacquet [11] conducted a study on 60 patients with PC from adenocarcinoma of the colon or appendix treated with CRS and HIPEC with MMC followed by 1 cycle of EPIC using 5-FU. Major morbidity developed in 35% of patients. Anastomotic leakage or bowel perforations were the most frequent complications. After a multivariate analysis including 11 clinical variables, gender, intrabdominal temperature and operating time were identified as the best predictors of major morbidity. Three patients (5%) died as a consequence of treatment-related complications.

Verwaal et al [12] reported the complications and the toxicity of CRS and HIPEC with MMC in a series of 102 patients with PC of colorectal or appendiceal origin. Patients were treated according to the same protocol in consecutive prospective phase I, II, or III trials. Grade III, IV, or V (according to the Common Toxicity Criteria [CTC] by the National Cancer Institute [NCI]) toxicity rate was 65%. Eight patients died of treatment-related causes, 6 of whom due to abdominal sepsis. The surgical complication (defined as any postoperative event that needed re-intervention) rate was 35%. Fistulae were observed in 18 patients, abdominal sepsis in 16. The risk of complications was higher in the following situations:

- metachronous PC ($p = 0.009$)
- more than 5 regions affected ($p = 0.044$)
- Simplified Peritoneal Cancer Index $\geq$ 13 ($p = 0.012$)
- incomplete initial cytoreduction ($p = 0.035$)
- blood loss exceeding 6 L ($p = 0.028$)
- three or more anastomoses ($p = 0.018$).

Stephens [13] reported on 183 patients with PC of gastrointestinal origin who underwent 200 procedures of CRS followed by HIPEC using the coliseum technique. Twenty morbidity categories, rated according to the NCI's CTC, were analyzed for association with 25 preoperative, surgical and HIPEC-related variables. Overall grade III/IV morbidity was 27%. Peripancreatitis was the most frequent complication observed in 6% of patients, followed by fistulization (4.5%), hemorrhage (4.5%), and haematological toxicity (4%). Three patients (1.5%) died of treatment-related causes, two of whom due to severe haematological toxicity. Duration of surgery ($p < 0.0001$), the number of peritonectomy procedures and resections ($p < 0.0001$) and the number of anastomoses ($p = 0.0078$) were significantly associated with the occurrence of grade III, IV or V morbidity at univariate analysis. After multivariate analysis, the number of peritonectomies and visceral resections was the only variable significantly associated with major morbidity ($p = 0.0002$).

Glehen et al. [14] analyzed 216 consecutive treatments performed on 201 patients with PC. Patients were treated with closed abdomen HIPEC associated to CRS when needed. Most patients suffered from ovarian, colorectal, or gastric cancer. Grade III/IV toxicity rate was 23.6%. Bowel fistulization (6.5%) and haematological toxicity (4.5%) were the most frequent complications. Seven patients

(3.4%) died of treatment-related complications. After univariate analysis, morbidity was proven to be linked with the carcinomatosis stage ($p = 0.016$), duration of surgery ($p = 0.005$), and the number of resections and peritonectomy procedures ($p = 0.042$).

Milano National Cancer Institute Experience

A prospective phase-II study was conducted at the National Cancer Institute of Milan (Italy) to analyze morbidity and mortality of cytoreductive surgery and HIPEC in the treatment of peritoneal surface malignancies [15]. Two hundred and five patients undergoing 209 procedures for peritoneal mesothelioma (50 patients), pseudomyxoma peritonei (49), ovarian cancer (41), abdominal sarcomatosis (32), colorectal cancer (13), gastric cancer (12) and other-type carcinomatoses (8) were enrolled into the study.

The technique of cytoreductive surgery has been described elsewhere [16-17]. CRS was aimed at reducing peritoneal disease to residual nodules < 2,5 mm in diameter. HIPEC was performed according to the closed abdomen techniques. Intraperitoneal chemotherapy regimens were the following: cisplatin (CDDP, 25 mg/m^2/L) and MMC (3,3 mg/m^2/L) for pseudomyxoma peritonei, colorectal, and gastric carcinomatosis; CDDP (43 mg/L of perfusate) and doxorubicin (Dx, 15,25 mg/L of perfusate) for mesothelioma, ovarian carcinomatosis, and abdominal sarcomatosis. The HIPEC was carried out using an extracorporeal perfusion device (Performer LRT; RAND, Medolla (MO), Italy) at a temperature of 42° C - 43° C for 60-90 minutes, depending on the drug schedule.

The mean number of peritonectomy procedures was 4.9 per patient and the mean operative time was 532 minutes (range 240-1320). The mean doses of drugs administered were 218 mg (range: 100-300 mg) for CDDP, 31 mg (15-50 mg) for MMC, and 63 mg (25-90 mg) for Dx. Postoperative complications were scored according to the scale adopted by the surgical department of the National Cancer Institute of Milan, which was coined by Bozzetti to grade surgical morbidity. It classifies complications as follows: G1 = no complications; G2 = minor self-limiting complications; G3 = major complications requiring re-operation, intensive care unit admission or interventional radiology; G4 = in-hospital mortality [18].

Major morbidity occurred in 25 cases (12%). The most common morbidities were 17 anastomotic leaks. The other postoperative complications, their anatomic location, description, and management are outlined in Table 2.

Table 2. Anatomic location, description, and management of main complications

Type of complication	No. of complications	Surgical treatment or ICU recovery	Conservative management
Anastomotic leak	17	12	5
Digestive tract perforation	6	6	0
Biliary fistula	1	1	0
Pancreatic fistula	2	0	2
Ileus/gastric stasis	4	0	4
Pneumonia	9	0	9
Pleural effusion	4	0	4
Pulmonary embolism	1	1	0
Respiratory failure	1	1	0
Abdominal abscess	3	1	2
Sepsis	4	0	4
Fever[a]	6	0	6
Abdominal bleeding	14	4	0
Other complications	10	0	10

ICU, intensive care unit; [a] Unrelated to infectious problems

One patient presented an acute hypotensive episode, clinically diagnosed as cardiac arrest, on the 8th day after the procedure; he was urgently resuscitated without any short- or long-term sequelae. Ten (4.8%) patients developed grade III or IV toxicity, scored according to World Health Organization (WHO) criteria. There were 3 cases of grade III hematologic toxicity, one patient with grade III gastrointestinal toxicity, 2 cases of grade III nephrotoxicity, 2 cases of grade IV nephrotoxicity, one case of grade IV pulmonary toxicity, and one case of grade III alopecia. The 2 cases of nephrotoxicity were peritoneal mesothelioma patients who required haemodialysis in the postoperative period and developed chronic renal failure.

A multivariate analysis was performed using logistic regression to determine the correlation between clinical variables and major morbidity (Table 3).

Table 3. Univariate and multivariate analysis of clinical risk factors for major morbidity

Independent variables	Univariate[a]		Multivariate[b]
	OR	p	OR (95% CI) p
Tumour histology of GI origin	1.54	.213	
Male gender	2.76	.016	
Performance status (ECOG) ≥1	0.43	.050	
Age ≥52 yrs	1.02	.571	
BMI ≥ 25 kg/m^2	0.76	.343	
No previous chemotherapy	3.69	.004	2.7 (98-7.51) .054
Previous radiotherapy	1.49	.539	
Carcinomatosis extension (PCI ≥ 20)	2.88	.027	
No. of anastomoses ≥ 2	2.65	.028	
Procedure duration ≥ 530 minutes	3.35	.014	
Extent of cytoreduction: Levels I/II vs. III[c]	2.68	.019	2.9 (1.29-6.4) **.010**
Completeness of cytoreduction: 0/1 vs. 2/3	1.27	.439	
CDDP HIPEC dose ≥ 240 mg	2.70	.020	3.1 (1.24-7.9) **.016**

OR, odds ratio; CI, confidence interval; GI, gastrointestinal; BMI, body mass index; CDDP, cisplatin; HIPEC, hyperthermic intraperitoneal chemoperfusion; [a] Chi square test or Fisher's exact test, [b] logistic regression model with backward elimination method; [c] Cytoreduction was classified into 3 levels according to the number of procedures performed: Level I: 1-2 procedures; Level II: 3 or 4 procedures; Level III: more than 5 procedures; ECOG: Eastern Cooperative Oncology Group

After the backward elimination method, no previous systemic chemotherapy, extent of cytoreduction and dose of CDDP for HIPEC •240 mg remained in the model. However, no previous systemic chemotherapy presented a borderline significance (p = 0.054), and therefore only the extent of cytoreduction and the dose of CDDP were considered the best predictors of major morbidity after CRS and HIPEC.

The most significant complication in our series was digestive fistula due to anastomotic leak and/or digestive perforation. This complication constituted about 70% of all cases with major morbidity. The rate of fistula in the whole series was 11%. Such a figure is somewhat higher than the 5% rate reported for common elective surgeries with bowel anastomoses [19-20]. Our results seem to be consistent with those reported in other trials evaluating CRS and HIPEC, ranging from 3.9% to 34% (Table 4) [10-14,21-28]. However, such a comparison should be made cautiously for several reasons. First, throughout the published literature, we found great variability in the definition of bowel-related complications. Some authors report digestive fistulas [12,14,21-22], others differentiate fistulas from anastomotic leaks [13] and still others define bowel leaks as the presence of either an anastomotic leak or a bowel perforation [27]. Second, the series are heterogeneous in terms of the distributions of potential risk factors for bowel complications.

Third, intuitively, the more anastomoses performed, the higher the risk of complications. In the overview of published series (Table 4), the mean number of anastomoses per patient varies widely from 0.4 to 2.8 anastomoses per patient. This is a several fold difference and could account for a major statistical difference. Some authors promote performing a proximal diverting stoma for low rectal anastomoses [12,21,28], while others do not [14,22]. Another difference relates to the fact that many authors advocate completing the anastomoses before HIPEC, [22] whereas most do so after the perfusion [8,13].

Table 4. Overview of bowel complications associated with CRS and HIPEC

Ref	N	Male (%)	#	Protective ostomy (%)	Timing	BC/A (%)	BCR (%)	Risk factors for bowel complications
[11]	60	62	1.8	None	After	9.3	17	Duration, # of peritonectomy procedures[a]
[13]	200	53	NA	NA	After	NA	7.5	Intraoperative blood loss[a]
[23]	46	41	2	39	After	17.4	34	
[22]	64	42	2.6	NA	Before and after	8	18.8	
[24]	36	39	2.8	NA	NA	7.2	22.2	
[25]	43	NA	NA	NA	NA	NA	5	
[14]	56	37	.6	NA	Before	NA	10.7	Carcinomatosis extent, duration, # anastomoses[a]
[26]	73	37		NA	Before	NA	10.7	
	56	48	.4	NA	Before	17.3	8	
[12]	102	56	>2	42	After	NA	17.6	
[29]	77	58	NA	13	After	NA	NA	
[33]	203	33	1	0.5	Before	11.3	10.8	Duration, Male sex, no previous CT[b] Cytoreduction extent, # anastomoses[a]

N, number of procedures; #, number of anastomoses per patient; BC/A, bowel complications/anastomoses ratio; BCR, bowel complication rate; NA, not available; [a] on univariate analysis; [b] on multivariate analysis.

Following the gastrointestinal tract, the respiratory tract was the second most affected system by postoperative complications. Pulmonary morbidity was found in 15 cases: most of them of Grade I/II, with the exception of one case of pulmonary embolism and one case of respiratory failure. This finding is in line with reports in the literature [29]. Several factors can account for respiratory morbidity. The stripping of the diaphragmatic peritoneum elicits a mechanical and thermal injury on the diaphragm, with the formation of clinically nonevident communications between the abdominal and pleural cavities that allow passage of perfusate inside the thorax during HIPEC. Moreover, inflammatory reaction secondary to tissue injury could be responsible for continuous production of exudates by the pleura during the postoperative period.

The possibly impaired contractive function of the diaphragmatic muscle due to surgical trauma, formation of pleural effusion, along with general causes related to any major surgery (prolonged anaesthesia time, inappropriate postoperative analgesia) should all be considered as potential factors for the emergence of pulmonary morbidity. The prevention and management of such complications includes careful inspection of the integrity of diaphragmatic muscle after the stripping of its peritoneum and the prompt repair of eventual macroscopic defects, the prophylactic insertion of a chest drain after the cytoreduction, [30] careful control of postoperative pain, judicious management of respiratory rehabilitation and administration of antibiotics.

In our study the dose of CDDP used for HIPEC was an independent risk factor for major procedure-related morbidity. Two drug schedules were used for HIPEC according to the tumour type, namely, CDDP + MMC and CDDP + Dx. The second combination was established formally by a Phase I dose-finding study [31]. The dose of CDDP for HIPEC in each of the combinations was calculated in different ways and, in our study, ranged from 100 to 300 mg. We chose 240 mg as the cut-off value, as it represents the theoretical maximal tolerable dose. It is the approximate result of the product of 43 mg by 6 liters (maximal volume of perfusate used in our series). Patients receiving CDDP $\geq$ 240 mg presented a significantly higher rate of combined major morbidity, with a 3 fold higher risk of developing grade III/IV postoperative complications compared with those who were treated with a lower CDDP dose, when adjusted for the other variables. This finding should not be surprising as it can be supported by an experimental study, which demonstrated the negative influence of CDDP on the healing of bowel anastomosis after HIPEC [32].

Bowel Complications following Cytoreduction and HIPEC

A study to assess the bowel complication rate and the risk factors for their occurrence in loco-regional procedures performed with closed-technique HIPEC was conducted at the National Cancer Institute of Milan [33]. One hundred ninety-eight consecutive patients undergoing 203 cytoreductive and HIPEC procedures from 1995 to 2004 constituted the study population. The mean number of organ resections was 2.4 per patient; overall, 480 visceral resections were performed, excluding peritonectomy procedures. A total of 194 anastomoses were performed, with a mean of 0.96 anastomoses per patient (range, 0–4). Ninety-four patients (46%) had none performed. Conversely, 53 patients (26%) had a single anastomotic site, 34 (17%) had two anastomoses, 15 (8%) received 3, and 7 (3%) received 4 anastomoses.

During the entire study period all anastomoses were carried before HIPEC. When a partial or total gastrectomy was performed, Roux-en-Y reconstruction was always used. End-to-side esophago-jejunal anastomoses were performed with a circular stapler, usually with a 25 mm diameter. During a subtotal gastrectomy, the end-to-side gastrojejunal anastomosis was hand-sewn with a single layer of

continuous extra-mucosal Maxon 4-0 stitches (United States Surgical Corporation, Norwalk, CT). The distal end-to-side jejuno-jejunal anastomosis was also hand-sewn in the same fashion. Small-bowel and colic anastomoses were always hand-sewn in an end-to-end fashion. In case of low anterior resection, the lower margin of bowel transection was usually below the level of the peritoneal reflection and low colorectal anastomoses were performed with an intraluminal stapler of 29- to 33-mm diameter. Anastomotic integrity was tested using air insufflation from below.

No protecting stoma was performed after a low anterior rectal resection. During the study period, a terminal ileostomy was performed in only one patient (0.5%). This was a female patient who underwent a total colectomy with small-bowel resection and in whom the ileorectal anastomosis could not be fashioned because of undue tension. Bowel complications were defined as bowel perforation or anastomotic leak. A bowel perforation occurs at a site away from an anastomosis. An anastomotic leak is a breach and/or complete dehiscence at the suture line.

After 203 consecutive procedures, 22 patients (10.8%) developed bowel complications occurring at a mean of 11.5 days after the operation (range 3–28 days). Overall, two patients (1%) died. In 17 patients the complication occurred at an anastomotic site or suture line. Six complications occurred away from anastomoses or suture lines. The ileocolic anastomosis was the most common site of bowel complications. Five patients with ileocolic anastomotic leaks underwent reoperation and bowel resection with reanastomosis. Two patients of this group had a protective ileostomy. The remaining two patients were conservatively treated with total parenteral nutrition and had spontaneous resolution. One small-bowel anastomotic leak was surgically treated, and another was conservatively managed. Three small-bowel perforations occurred at random sites not related to a suture line and were all surgically treated. One patient with a colorectal anastomotic dehiscence was surgically treated and given a colostomy, whereas the other was conservatively treated with drainage and parenteral nutrition. Overall, six patients (27%) received a stoma as part of their final management, including one performed to gain access to a major presacral bleed. Anatomical location, description, and management of bowel complications are shown in Table 5.

Table 5. Anatomical location, description, and management of bowel complications.

Anatomical location	N	Bowel perforations	Anastomo-Tic leaks	Surgical treatment	Conservative management
Ileocolic anastomosis	7	0	7	5	2
Small bowel	5	3	2	4	1
Colon	4	1	3	-	-
Colorectal anastomosis	2	0	2	1	1
Duodenum	2	1	1	2	0
Stomach	1	1	0	1	0
Not available	2	0	2	-	-
Total[a]	23				

[a] One patient had two simultaneous bowel complications

We designated a bowel complications/anastomoses (BC/A) ratio, which is the total number of bowel complications divided by the total number of anastomoses performed. We found a BC/A ratio of 11.3%. After univariate analysis, we found a statistically significant association between bowel complications and the following variables: gender, no previous systemic chemotherapy, number of anastomoses (fewer than two vs. two or more), duration of the procedure (< 8.7 vs. ≥ 8.7 hours), and extent of cytoreduction (level III vs. levels I-II). After multivariate analysis, the following variables remained in the model and were considered independent risk factors for the occurrence of bowel complications: male gender, no previous systemic chemotherapy, and duration of the procedure ≥ 8.7 hours (Table 6).

Table 6. Univariate and multivariate analysis of clinical risk factors for bowel complications

Independent variables	OR (crude)	p	OR (95%CI)[a]	p
Tumour histology GI	1.6	NS		
Male gender	4.1	.002	4.2 (1.5–12.1)	**.01**
Performance status 0	.6	NS		
Age ≥ 52 y	.9	NS		
BMI > 25 kg/m^2	.5	.1		
No previous systemic chemotherapy	3.9	.005	3.5 (1.1–11.6)	**.04**
Previous radiotherapy	1.7	NS		
Carcinomatosis extent 3	2.4	.07		
Number of anastomoses ≥ 2	4.3	.002		
Procedure duration ≥ 8.7 h	6.4	.0003	6.3 (1.7–23.2)	**.01**
Extent of cytoreduction level III	3.1	.01		
Completeness of cytoreduction score 2/3	.8	NS		
HIPEC drug schedule CDDP+MMC	1.6	NS		
CDDP HIPEC dose ≥ 240 mg (no)	1.7	NS		

OR, odds ratio; CI, confidence interval; GI, gastrointestinal tumours; BMI, body mass index; HIPEC; hyperthermic intraperitoneal chemoperfusion; CDDP, cisplatin; MMC, mitomycin-C; NS, not significant; [a] logistic regression model with backward elimination method

Glehen [14] found on univariate analysis that carcinomatosis extent, duration of surgery, and number of anastomoses were significantly associated with the occurrence of digestive fistulas. Jacquet et al. [11] found on univariate analysis that bowel leakage was related to the duration of surgery and number of peritonectomy procedures. Stephens found that intraoperative blood loss was the only associated risk factor for anastomotic leaks [13] (Table 4).

Our data confirmed that the longer the procedure, the higher the risk of intestinal complications. Such findings are in line with those previously reported by other authors. We estimated by multivariate analysis that patients undergoing a procedure longer than 8.7 hours had almost a 7-fold increase in bowel complication risk. This implies that the duration of operation reflects the extent of surgical

procedures, the carcinomatosis extent, the total number of anastomoses, or all of these variables. The number of anastomoses was not shown in our series to be an independent risk factor. Theoretically, more anastomoses should increase bowel complications. In our opinion, this unexpected finding can be explained only by the small number of events (a total of 22). However, we found that male sex and no previous systemic chemotherapy were unfavourable risk factors. Such findings may be explained by the differences between cohorts concerning the distribution of several potential risk factors (e.g., gender, duration of the operation, number of anastomoses, and HIPEC techniques), thus rendering comparison of the results somewhat problematic. Moreover, not all the studies used multivariate statistical analysis. In addition, the analysis performed in our study included only clinical and surgical variables related to the preoperative and intraoperative phases of the procedure. One can raise the hypothesis that by including preoperative, intraoperative, and immediate postoperative parameters, reflecting, for example, the nutritional, hemodynamic, and/or respiratory status, new independent risk factors for bowel complications could emerge.

The first independent risk factor on multivariate analysis was male gender, which had an OR of 4.2 favouring complications. It is well known that the male pelvis is more difficult to dissect for anatomical reasons. Nevertheless, this cannot totally account for our results for two reasons: first, we encountered only two colorectal anastomotic fistulas in our series, and, second, one of these was in a man and the other in a woman.

Previous systemic chemotherapy was a protective factor in our series. Patients who did not receive chemotherapy had an OR of 3.8 for developing complications. This could be explained by the fact that patients eligible to receive chemotherapy have a better performance status and a more favourable prognosis. A selection bias could have occurred in that setting.

Digestive tract perforations occurred in 6 cases in our series. They all occurred away from suture lines. Possible explanations could be partial-thickness mechanical and/or thermal damage to intestinal surfaces. This, in turn, could have been aggravated by subsequently heated chemotherapy. Other possible explanations for digestive perforation are 1. focal heat injury at the tip of the inflow catheter; 2. mechanical trauma elicited by the suctioning effect of the outflow catheter; 3. postoperative shrinking of infiltrating metastatic nodules on the intestinal wall because of the antiblastic effect of heated chemotherapy. The risk for such complications should be minimized by careful lysis of adhesions and dissection, with a judicious use of the ball-tip electrocautery on the serosal surfaces of the intestine in case the cytoreduction requires an extensive fulguration of metastatic disseminated implants. Another important surgical step is the final inspection of the abdominal cavity after the drainage of perfusate at the end of HIPEC. This phase should be performed as accurately as possible to identify and treat all the risky damaged areas on the organs and intestinal tract.

The higher incidence of anastomotic leak or of intrabdominal abscess in locoregional treatment of PC with respect to common elective surgical procedures has guided several surgeons to perform protective proximal ostomies more liberally. Indications are not uniform, suffering a range of variation. Verwaal recommend

colostomy for all rectal resections [12]. Moran et al. and Sugarbaker advocate a proximal diverting stoma in cases of low anterior resections in which the preservation of the rectum is not possible [16,20]. Conversely, Shen, despite having found an unacceptably high rate of sepsis correlated with bowel anastomoses, adopted a more flexible policy that suggested the surgical performance of a protective stoma is an alternative [26]. During the entire study period, we performed only 1 diverting ostomy, despite a total of 58 low colorectal anastomoses fashioned. We found that only two patients had an anastomotic leak at this site. Therefore, in our opinion, to primarily complete unprotected colorectal anastomoses seems to be a viable alternative. The decision to perform a diverting stoma should not be guided by the type of operation, but rather should be based on the established risk factors for a leak in the surgical practice. An interesting finding in this study was that most patients had bowel complications at or distal to the ileocolic anastomoses (13 patients; 60%). On the basis of these data, it is now our policy in case of the need for a protecting stoma to perform a temporary ileostomy rather than a colostomy.

Another technical variation of CRS and HIPEC is the optimal timing for bowel anastomoses. They can be performed either after or just before the completion of the HIPEC. Proponents of the first alternative argue that delaying the anastomosis permits a better distribution of heat and drugs inside the peritoneal cavity. In addition, they state that the risk of postoperative bowel complications can be diminished as a result of the avoidance of the potential adverse effects of heat and chemotherapy on the suture line healing. However, others have proposed the second alternative, which is supported by experimental and clinical evidence. In fact, the influence of chemotherapy on suture healing depends on the type of drug. In animal studies, anastomotic healing can be impaired by intra-peritoneal MMC, but not by 5-FU at a normal temperature [34] or by paclitaxel [35]. Local hyperthermia alone has no adverse effect on rat anastomotic healing [36]. Moreover, there seems to be no increased morbidity due to postoperative bowel fistula or anastomotic leak when anastomoses are constructed before HIPEC [37].

Investigators have not achieved agreement on open or closed abdomen techniques of HIPEC. Proponents of the coliseum technique (open abdomen) claim better drug and heat distribution by continuous manipulation of the abdominal organs [13]. Deficiencies were noted in the distribution of methylene blue dye with the closed abdomen technique, which, in turn, was blamed for a higher rate of complications [37]. Conversely, the closed technique permits an increase in the intra-abdominal pressure that may lead to increased convection-driven drug penetration of macromolecular agents such as TNF-α inside the tumour [38]. Moreover, Jacquet et al. reported that, in animal models, an intraabdominal pressure of 20 mm Hg and 30 mm Hg increased tissue uptake of doxorubicin in bladder, diaphragm, and abdominal wall during the first 10 minutes of intra-peritoneal administration [39]. Furthermore, a recent study carried out by Glehen reported morbidity and mortality results on 216 procedures of ip chemohyperthermia using the closed abdomen technique [14]. They observed a postoperative mortality rate of 3.2% and morbidity of 24.5%, comparable to other reports. Since up to now, no prospective controlled clinical trial has been conducted that specifically addresses

the superiority of 1 technique over the other, the issue remains unclear with no striking differences between the 2 techniques in terms of operative morbidity.

Quality of Life following Cytoreduction and HIPEC

Only 2 studies have investigated the quality of life (QOL) after CRS followed by HIPEC. McQuellon [40] assessed QOL in 64 patients with various non-gynecologic peritoneal surface malignancies, 16 of whom of colonic origin, during the first year after therapy. QOL was assessed by means of the Functional Assessment of Cancer Therapy - Colon scale, analysis of various activities of daily living, the Brief Pain Inventory, the Center for Epidemiologic Studies - Depression scale, and the Eastern Cooperative Oncology Group performance status rating scale. Before surgery, patients with ascites had a significantly lower QOL in comparison to those without ascites. Patients with ascites reported an improved overall QOL immediately after surgery. Patients without ascites reported decreased QOL during the first 3 months after CRS and HIPEC. From 3 months postoperatively onwards, most patients returned to baseline or better levels of functioning. One year after surgery, 58% of patients reported a normal performance status, whereas 14% had to spend extra time in bed during the day due to either disease- or treatment-related symptoms. The mean scores at activities of daily living, however, were still lower than the general population, even after successful treatment and symptom reduction.

In a second publication, McQuellon [41] reported the quality of life of 17 patients who had survived more than 3 years after CRS and HIPEC. Sixteen patients reported no limitations on moderate activities, whereas 10 patients described their health as very good or excellent. In a similar study, the National Cancer Institute (Bethesda) reported improved QOL scores at 3, 6 and 9 months following cytoreductive surgery and HIPEC [42].

References

1. Sugarbaker PH (2006) New standard of care for appendiceal epithelial neoplasms and pseudomyxoma peritonei syndrome? Lancet Oncol 7: 69-76
2. Stewart JH, Shen P, Levine EA (2005) Intraperitoneal hyperthermic chemotherapy for peritoneal surface malignancy: current status and future directions. Ann Surg Oncol 12: 765-777
3. Deraco M, Baratti D, Inglese MG et al (2004) Peritonectomy and intraperitoneal hyperthermic perfusion (IPHP): a strategy that has confirmed its efficacy in patients with pseudomyxoma peritonei. Ann Surg Oncol 11: 393-398
4. Deraco M, Nonaka D, Baratti D et al (2006) Prognostic Analysis of Clinicopathologic Factors in 49 Patients With Diffuse Malignant Peritoneal

Mesothelioma Treated With Cytoreductive Surgery and Intraperitoneal Hyperthermic Perfusion. Ann Surg Oncol 13: 229-237
5. Nonaka D, Kusamura S, Baratti D et al (2005) Diffuse malignant mesothelioma of the peritoneum: a clinicopathological study of 35 patients treated locoregionally at a single institution. Cancer 104: 2181-2188
6. Ryu KS, Kim JH, Ko HS et al (2004) Effects of intraperitoneal hyperthermic chemotherapy in ovarian cancer. Gynecol Oncol 94: 325-332
7. Glehen O, Kwiatkowski F, Sugarbaker PH et al (2004) Cytoreductive surgery combined with perioperative intraperitoneal chemotherapy for the management of peritoneal carcinomatosis from colorectal cancer: a multi-institutional study. J Clin Oncol 22: 3284-3292
8. Verwaal VJ, van Ruth S, de Bree E et al (2003) Randomized trial of cytoreduction and hyperthermic intraperitoneal chemotherapy versus systemic chemotherapy and palliative surgery in patients with peritoneal carcinomatosis of colorectal cancer. J Clin Oncol 21: 3737-3743
9. Bayo'n LG, Sugarbaker PH, Moreno SG et al (2003) Initiation of a program in peritoneal surface malignancy. Surg Oncol Clin North Am 12: 741-753
10. Esquivel J, Vidal-Jove J, Steves MA et al (1993) Morbidity and mortality of cytoreductive surgery and intraperitoneal chemotherapy. Surgery 113: 631-636
11. Jacquet P, Stephens AD, Averbach AM et al (1996) Analysis of morbidity and mortality in 60 patients with peritoneal carcinomatosis treated by cytoreductive surgery and heated intraoperative intraperitoneal chemotherapy. Cancer 77: 2622-2629
12. Verwaal VJ, van Tinteren H, Ruth SV et al (2004) Toxicity of cytoreductive surgery and hyperthermic intra-peritoneal chemotherapy. J Surg Oncol. 85:61-67
13. Stephens AD, Alderman R, Chang D, et al (1999) Morbidity and mortality analysis of 200 treatments with cytoreductive surgery and hyperthermic intraoperative intraperitoneal chemotherapy using the coliseum technique. Ann Surg Oncol 6: 790-796
14. Glehen O, Osinsky D, Cotte E et al (2003) Intraperitoneal chemohyperthermia using a closed abdominal procedure and cytoreductive surgery for the treatment of peritoneal carcinomatosis: morbidity and mortality analysis of 216 consecutive procedures. Ann Surg Oncol 10: 863-869
15. Kusamura S, Younan R, Baratti D et al (2006) Cytoreductive surgery followed by intraperitoneal hyperthermic perfusion: analysis of morbidity and mortality in 209 peritoneal surface malignancies treated with closed abdomen technique. Cancer 106: 1144-1153
16. Sugarbaker PH (1995) Peritonectomy procedures. Ann Surg 221: 29-42
17. Deraco M, Casali P, Inglese MG et al (2003) Peritoneal mesothelioma treated by induction chemotherapy, cytoreductive surgery, and intraperitoneal hyperthermic perfusion. J Surg Oncol 83: 147-153
18. Bozzetti F, Braga M, Gianotti L et al (2001) Postoperative enteral versus parenteral nutrition in malnourished patients with gastrointestinal cancer: a randomised multicentre trial. Lancet 358: 1487-1492

19) Abete M, Ronchetti V, Casano A (2003) [Anastomotic leakage after traditional surgery of the colon and rectum]. Minerva Chir 58: 167-174
19. Montesani C, De Milito R, Chiappalone S et al (1992) Critical evaluation of the anastomoses in large bowel surgery: experience gained in 533 cases. Hepatogastroenterology 39: 304-308
20. Moran BJ, Cecil TD (2003) The etiology, clinical presentation, and management of pseudomyxoma peritonei. Surg Oncol Clin North Am 12: 585–603
21. Elias D, Blot F, El Otmany A et al (2001) Curative treatment of peritoneal carcinomatosis arising from colorectal cancer by complete resection and intraperitoneal chemotherapy. Cancer 92: 71–76
22. Witkamp AJ, de Bree E, Kaag MM et al (2001) Extensive surgical cytoreduction and intraoperative hyperthermic intraperitoneal chemotherapy in patients with pseudomyxoma peritonei. Br J Surg 88: 458–463
23. Elias D, Laurent S, Antoun S et al (2003) Pseudomyxoma peritonei treated with complete resection and immediate intraperitoneal chemotherapy. Gastroenterol Clin Biol 27: 407–412
24. Parvaiz A, Amin AI, Howell R et al (2002) First one hundred referrals, predominantly of pseudomyxoma peritonei, to a peritoneal surface malignancy unit: operability and early outcome. Br J Surg 89(Suppl 1):13
25. Glehen O, Mithieux F, Osinsky D, et al (2003) Surgery combined with peritonectomy procedures and intraperitoneal chemohyperthermia in abdominal cancers with peritoneal carcinomatosis: a phase II study. J Clin Oncol 21:799–806
26. Shen P, Hawksworth J, Lovato J et al (2004) Cytoreductive surgery and intraperitoneal hyperthermic chemotherapy with mitomycin C for peritoneal carcinomatosis from nonappendiceal colorectal carcinoma. Ann Surg Oncol 11: 178–186
27. Murio JE, Sugarbaker PH (1993) Gastrointestinal fistula following cytoreductive procedures for peritoneal carcinomatosis: incidence and outcome. J Exp Clin Cancer Res 12: 3.
28. Chen MY, Chiles C, Loggie BW et al (1997) Thoracic complications in patients undergoing intraperitoneal heated chemotherapy with mitomycin following cytoreductive surgery. J Surg Oncol 66: 19-23
29. Ahmad SA, Kim J, Sussman JJ et al (2004) Reduced morbidity following cytoreductive surgery and intraperitoneal hyperthermic chemoperfusion. Ann Surg Oncol 11: 387-392
30. Rossi CR, Foletto M, Mocellin S et al (2002) Hyperthermic intraoperative intraperitoneal chemotherapy with cisplatin and doxorubicin in patients who undergo cytoreductive surgery for peritoneal carcinomatosis and sarcomatosis: phase I study. Cancer 94: 492-499
31. Makrin V, Lev-Chelouche D, Even Sapir E et al (2005) Intraperitoneal heated chemotherapy affects healing of experimental colonic anastomosis: an animal study. J Surg Oncol 89: 18-22
32. Younan R, Kusamura S, Baratti D et al (2005) Bowel complications in 203 cases of peritoneal surface malignancies treated with peritonectomy and

closed-technique intraperitoneal hyperthermic perfusion. Ann Surg Oncol 12: 910-918
33. Kuzu M, Koksoy C, Kale T, et al (1998) Experimental study of the effect of preoperative 5-fluorouracil on the integrity of colon anastomosis. Br J Surg 85: 236–239
34. Arikan AY, Gunal O, Pehlivan M et al (2000) The effect of intraperitoneal paclitaxel administration on colonic anastomosis. Hepatogastroenterology 47: 1273–1276
35. Shimizu T, Maeta M, Koga S (1991) Influence of local hyperthermia on the healing of small intestinal anastomoses in the rat. Br J Surg 78:57–59
36. Sugarbaker PH, Jacquet P, Stephens AD et al (1996) Comparison of covered versus closed technique for heated intraoperative chemotherapy for peritoneal carcinomatosis from gastrointestinal cancer. In: Abe O, Inokuchi K , Takasaki K, editors. XXX World Congress of the International College of Surgeons. Bologna, Italy: Monduzzi Editore, p389-393
37. Milosevic MF, Fyles AW, Hill RP (1999) The relationship between elevated interstitial fluid pressure and blood flow in tumours: a bioengineering analysis. Int J Radiat Oncol Biol Phys 43: 1111-1123
38. Jacquet P, Stuart OA, Chang D et al (1996) Effects of intra-abdominal pressure on pharmacokinetics and tissue distribution of doxorubicin after intraperitoneal administration. Anticancer Drugs 7: 596-603
39. McQuellon RP, Loggie BW, Fleming RA et al (2001) Quality of life after intraperitoneal hyperthermic chemotherapy (IPHC) for peritoneal carcinomatosis. Eur J Surg Oncol 27: 65–73
40. McQuellon RP, Loggie BW, Lehman AB et al (2003) Long-term survivorship and quality of life after cytoreductive surgery plus intraperitoneal hyperthermic chemotherapy for peritoneal carcinomatosis. Ann Surg Oncol 10: 155–162
41. Alexander HR, Mavroukakis SM, Libutti SK et al (2004) Impact of tumour resection and intraperitoneal chemotherapy on health related quality of life in patients with peritoneal surface malignancies. In: Proceedings of the 57th Annual Society of Surgical Oncology Cancer Symposium. Philadelphia: Lippincott, Williams and Wilkins

Detection and Treatment of Recurrent Disease after Cytoreduction and HIPEC

VJ Verwaal

Follow-up after Cytoreduction

Despite major achievements in the treatment of peritoneal malignancies, recurrences still occur after cytoreduction followed by HIPEC in approximate 50% in the first 5 years. These recurrences often cause symptoms which have a major impact on the patient's well being.

At the outset, the major aim of follow-up is to assess the initial results of therapy and to deal with treatment related problems. The result of therapy is mainly determined by the completeness of surgery as measured by either the completeness of cytoreduction (CC) [1] score or the AJCC residual tumour (R) classification with Dutch modification [2]. Other ways of measurement are a comparison of CT scan before and after the procedure and tumour marker levels [3].

Treatment related medical problems are related to bowel function and malnutrition. Special care should be taken to detect deficits of vitamins such as B12 and folic acid. Furthermore, ostomy related problems can occur in patients with a short or completely removed large bowel. In general, however, the quality of life is good and the medical problems are manageable [4].

Subsequently, follow up focuses on early detection of recurrent disease. The rationale for regular follow up is early detection of recurrent disease to provide a renewed chance of long-term survival in selected patients. Based on this rationale, often costly investigations are performed. The effectiveness of these investigations is difficult to assess, and most follow up schedules are empirical and not based on published evidence [5]. Some authors prefer not to install follow up and advise patients to return only if they develop symptoms [6].

The effectiveness of follow-up depends mainly on the possibilities to treat recurrent disease. Only selected patients are expected to benefit from treatment of a recurrence of carcinomatosis of colorectal origin [7]. After an incomplete cytoreduction, treatment of a recurrence is unlikely to be successful. Follow-up of these patients will never be effective in terms of renewed chances of survival. However, following these patients during the final stage of their disease will be useful to provide palliative care and moral support. As in primary colorectal cancer, follow-up should be tailored to the stage of the disease, to the results of earlier treatment and to the potential of second-line treatment [8].

The original study protocols required visits at the outpatient clinic every three months for two years and at six-monthly intervals thereafter. The follow-up protocol was as follows: history, physical examination and serum CEA and CA 19.9 at each visit, and a CT-scan of the abdomen six- monthly. If symptoms arose, a CT-scan or endoscopy was performed as required to determine their cause. A PET scan was obtained in case of a tumour marker rise and inconclusive CT-scan findings [9].

Cytoreduction followed by HIPEC is a relatively novel treatment and therefore initially most patients were followed according to fixed protocols. Since cytoreduction is nowadays an established clinical procedure, follow up schedules should be based on efficacy and cost effectiveness.

Only a small numbers of studies reported on the follow-up after cytoreduction and HIPEC. Portilla et al. studied an intensive follow up approach including standard second-look procedures [10]. Verwaal et al. studied the results of the above mentioned follow-up schedule based on CT-scan and CEA levels (Table 1) [3].

Table 1. Results of investigations detecting 63 recurrences in 92 patients who had an effective treatment of peritoneal carcinomatosis of colorectal origin by cytoreduction and HIPEC

	Test result	All	Intra-abdominal	Hepatic	Chest	Intraabdominal and hepatic	Unknown
Results of history and physical examination	None	24	11	11	-	-	2
	Obstruction	23	21	-	-	2	-
	Pain	6	2	1	-	3	-
	Mass	4	2	-	1	1	-
	Blood loss	5	4	-	-	1	-
	Unknown	1	-	-	-	-	1
CT-scanning results	None	21	16	2	-	1	2
	Mass	35	18	10	1	6	-
	Obstruction	2	2	-	-	-	-
	Not done	5	4	-	-	-	1
CEA and CA 19.9 testing	No change	18	12	3	1	2	-
	Rise	39	24	9	-	4	2
	Not done	6	4	-	-	1	1

The latter study demonstrated that most recurrences can be found after relatively simple and inexpensive initial diagnostic tests. Of all CT-scans made in the follow-up only one showed a recurrence which had not been detected in another way. As a result of this a reasonable follow-up schedule could be: baseline CT-scan with CEA and CA 19.9 testing followed by standard physical examination and tumour marker testing at regular intervals. A CT scan can be repeated after one and a half years for the screening of liver metastasis. Abnormal physical examination or elevated tumour markers are the only indications for more invasive and costly tests. Table 2 shows the proposed follow-up scheme [3]. This scheme encompasses both a postoperative screening for complications and the follow-up for detecting recurrence. The scan starts with a new base-line CT-scan to compare any future investigation with.

Table 2. Proposed follow-up schedule in patients who underwent cytoreduction for peritoneal carcinomatosis

	Preop	6w	3m	6 m	9m	1y	1½ y	2 y	2½ y	3 y	3½ y	4 y	4½ y	5y	6 y
Physical exam	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*
CT Chest/Abd.	*		*				*								
CEA, CA19.9	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*
Hb, WBC	*	*	*	*											
BUN, Kreat, Bilir, Tot prot.	*	*	*												

The time to recurrence is, as is obvious, dictated by the completeness of the cytoreduction. Table 3 shows the median time to recurrence related to the cytoreduction status. The location recurrence in peritoneal malignancies is different from the usual distribution seen in recurrent colorectal carcinoma. Whereas recurrence in colorectal cancer usually involves the liver, in patients treated for isolated peritoneal carcinomatosis recurrence predominantly take place in the abdominal cavity [8,10].

Table 3. Time to recurrence in 69 patients with recurrence after cytoreduction and HIPEC by initial cytoreduction result

Residual tumour	Number of patients at risk	Number of recurrences	Median time to recurrence (months)	S.E.
R-1	54	25	13.7	1.0
R-2a	37	33	10.8	1.7
R-2b	15	11	4.8	0.4

R-1, no residual macroscopic tumour; R-2a, residual tumour ≤ 2.5 mm; R2b, residual tumour > 2.5 mm; SE, standard error

Table 4 shows the locations of recurrence after a complete or near complete cytoreduction.

Table 4. Location of recurrences after treatment by cytoreduction and HIPEC

Location	Initial cytoreduction		Disease free interval (months) (S.E.)
	R-1 (N= 54)	R-2a (N=37)	
Intra-abdominal	13	26	12.7 (1.6)
Liver	8	2	9.0 (0.6)
Lung	-	1	14.7
Intra-abdominal and systemic	3	4	13.7 (1.1)
Unknown location	1	-	3.9
All	25	33	12.3 (2.1)

R-1, no residual macroscopic tumour; R-2a, residual tumour ≤ 2.5 mm; R2b, residual tumour > 2.5 mm; SE, standard error

Recurrence of pseudomyxoma peritonei is usually found by a tumour marker rise, CT scan, or during laparotomy. Recurrence on surgical wound surfaces was seen in 11 patients and was confined to the stomach/bowel, abdominal wall/scar, colostomy, and vaginal stump. Extraperitoneal recurrence was observed in the pleural cavity and retroperitoneal in the ureter [11-13].

Pathology of the recurrence in the study by Smeenk et al. [12] was categorized as DPAM or PMCA-I in 38% patients and 47% patients, respectively. This distribution was different for the original categorization. This demonstrated that dedifferentiation can occur (change of pathology form DPAM to PMCA-I or from PMCA-I to PMCA) in recurrent PMP.

Treatment of Recurrent Disease

Recurrent disease after cytoreduction and HIPEC develops in approximately 50% of the patients within the first 5 years. A typical recurrence of peritoneal carcinomatosis occurs between halve or two-third of the life span after the initial treatment of peritoneal carcinomatosis. The recurrences can occur predominantly within the abdomen but can occur at distant sites as well.

The median survival after the recurrence of peritoneal carcinomatosis of colorectal cancer is once again influenced by the successfulness of the first cytoreduction. Patients who had undergone R-1 and R-2a resections had a median survival of 11.1 months (S.E. 0.9) and 5.9 months (S.E. 0.8) after recurrence, respectively. When there was gross residual tumor (R-2b resection) at the initial cytoreduction, median survival was a mere 3.7 months (S.E. 0.3) [8].

Furthermore, the length of the interval between the initial treatment of carcinomatosis and recurrence influences the survival after the recurrence. A short interval was related to a shorter survival after recurrence [14]. Also the presence of signet cell carcinoma and older age were significant risk factors for a shorter survival. The factors gender, location of the primary tumour, synchronous or metachronous carcinomatosis and malignancy grade had no prognostic value.

Recurrences are treated according to general guidelines. A local recurrence is likely to cause bowel obstruction, and should be treated surgically. Radiotherapy may be indicated if the local recurrence cannot be removed surgically with free margins. Systemic chemotherapy is the treatment of choice in patients who have distant metastases. Systemic therapy is also given in case of multiple intra-abdominal recurrences [8,12-14].

In the study by Verwaal et al. [8] twelve patients did not receive any treatment for their recurrence, mostly due to a poor performance state. They survived for a median period of 1.9 months. Fifty-eight patients who had an effective initial cytoreduction (R-1 and R-2a) were further analyzed. Seven of them received no treatment at all, while five patients underwent an explorative laparotomy only. In six patients, who had bypass surgery for the recurrence, the median survival was 4.5 months. The median survival of the 15 patients who underwent a second surgical debulking was 10.3 months. The 16 patients who received systemic chemotherapy for recurrence survived a median of 8.5 months. Most of those patients were treated with irinotecan, three patients received 5-fluorouracil/leucovorin and three patients were treated in phase I and II trials. Radiotherapy was given in eight patients who had a recurrence in the pelvis that was unsuitable for surgery and one patient received radiotherapy for a metastasis in the abdominal wound. Five patients received radiotherapy with long-term palliative intent and their survival was 11.2 months. Survival was 8.7 months in the four patients in whom short-term palliative radiotherapy was administered.

Taken together, the data above indicate that in selected peritoneal carcinomatosis patients who underwent cytoreduction and HIPEC treatment of a recurrence can prolong survival. Usually, patients who agreed to undergo the risks associated with cytoreduction and HIPEC tend to take any possible chance to increase their life expectancy when a recurrence arises, despite the morbidity or mortality risk involved.

References

1. Jacquet P, Sugarbaker PH (1996) Clinical reseach methodologies in diagnosis and staging of patients with peritoneal carcinomatosis. In Sugarbaker PH (ed): Kluwer Academic publishers, p 359-374
2. Verwaal VJ, van Tinteren H, van Ruth S, Zoetmulder FA (2004) Predicting the survival of patients with peritoneal carcinomatosis of colorectal origin treated by aggressive cytoreduction and hyperthermic intraperitoneal chemotherapy. Br J Surg 91:739-746

3. Verwaal VJ, Zoetmulder FA (2004) Follow-up of patients treated by cytoreduction and chemotherapy for peritoneal carcinomatosis of colorectal origin. Eur J Surg Oncol 30:280-285
4. McQuellon RP, Loggie BW, Fleming RA et al (2001) Quality of life after intraperitoneal hyperthermic chemotherapy (IPHC) for peritoneal carcinomatosis. Eur J Surg Oncol 27:65-73
5. Kjeldsen BJ, Kronborg O, Fenger C, Jorgensen OD (1997) A prospective randomized study of follow-up after radical surgery for colorectal cancer. Br J Surg 84:666-669
6. Steele G, Jr (1993) Standard postoperative monitoring of patients after primary resection of colon and rectum cancer. Cancer 71:4225-4235
7. Kievit J (2002) Follow-up of patients with colorectal cancer: numbers needed to test and treat. Eur J Cancer 38:986-999
8. Verwaal VJ, Boot H, Aleman BM et al (2004) Recurrences after peritoneal carcinomatosis of colorectal origin treated by cytoreduction and hyperthermic intraperitoneal chemotherapy: location, treatment, and outcome. Ann Surg Oncol 11:375-379
9. Verwaal VJ, van Ruth S, de Bree E et al (2003) Randomized trial of cytoreduction and hyperthermic intraperitoneal chemotherapy versus systemic chemotherapy and palliative surgery in patients with peritoneal carcinomatosis of colorectal cancer. J Clin Oncol 21:3737-3743
10. Portilla AG, Sugarbaker PH, Chang D (1999) Second-look surgery after cytoreduction and intraperitoneal chemotherapy for peritoneal carcinomatosis from colorectal cancer: analysis of prognostic features. World J Surg 23:23-29
11. Park CM, Kim SH, Kim SH et al (2003) Recurrent ovarian malignancy: patterns and spectrum of imaging findings. Abdom Imaging 28:404-415
12. Smeenk RM, Verwaal VJ, Antonini N, Zoetmulder FA (2006) Recurrent pseudomyxoma peritonei after combined modality treatment: management and outcome. Ann Surg Oncol, in press
13. Zoetmulder FA, Sugarbaker PH (1996) Patterns of failure following treatment of pseudomyxoma peritonei of appendiceal origin. Eur J Cancer 32A:1727-1733
14. Averbach AM, Sugarbaker PH (1996) Recurrent intraabdominal cancer causing intestinal obstruction: Washington Hospital Center experience with 42 patients managed by surgery and intraperitoneal chemotherapy. Cancer Treat Res 81:133-147

Systemic Chemotherapy in Patients with Peritoneal Carcinomatosis from Colorectal Cancer

G Folprecht, CH Köhne, MP Lutz

Introduction

Chemotherapy of metastatic colorectal cancer is rapidly evolving. For a long time, 5-fluorouracil (5-FU) with or without folinic acid (FA) has been the mainstay of systemic chemotherapy with response rates reaching around 20% - 25% and median survival times rarely exceeding 12 months. This changed after the more recent addition of new chemotherapeutic agents like irinotecan [1-3] and oxaliplatin [4-6] or after the combination with targeted agents like the antibodies cetuximab [7-9] or bevacizumab [10-12]. Response rates in current trials now regularly reach 50% - 70% and median survival often exceeds 24 months [13-16]. Therefore, older studies require a critical review especially if they compare traditional systemic chemotherapy with 5-FU ± FA with local approaches like palliative surgery or regional chemotherapy. In fact, with the increased efficacy of systemic therapy, local or regional therapy became out of focus within most study groups. On the other hand, the better response rates with systemic chemotherapy now allow surgical removal of initially unresectable metastases after chemotherapy-induced downsizing of the tumour. In contrast to treatment with chemotherapy alone, patients after sequential multimodal approaches now have a chance to be cured with 10 year survival rates exceeding 20% [17].

Peritoneal Carcinomatosis in Trials of Systemic Chemotherapy

Most patients with PC (PC) have metastases in several organs and are treated in studies which investigate systemic treatment of advanced colorectal cancer. Peritoneal involvement is difficult to detect by imaging methods like CT scan, MRI or ultrasonography. For this reason the percentage of patients with PC is underestimated

in clinical trials either because it goes undetected or because the lesions do not fulfill the criteria of measurable disease according to the RECIST or WHO criteria [18] and thus are classified as "non-measurable disease" and ineligible for response evaluation. Most authors therefore do not distinguish patients with PC as a separate subgroup. Only few data are available on the efficacy of systemic treatment on PC and there are no prospectively randomized studies in this patient group.

In a retrospective analysis of a large database with 3,825 patients with advanced colorectal cancer in two groups of chemotherapy trials, the presence of PC was reported in 12% [19]. Upon multivariate analysis, peritoneal involvement was an independent negative prognostic factor, as well as ECOG status, number of tumour sites, presence of liver metastases and several laboratory parameters. Upon univariate comparison, the overall survival of patients decreased with known PC from 11.6 months to 7.6 months [20].

One set of trials examined different ways of 5-FU application because chemotherapy with 5-FU can be optimized by changing the infusion time. The switch from an i.v. bolus to prolonged infusion not only allows higher dose-intensities but also shifts the toxicity profile from sometimes severe and life-threatening hematotoxicity and mucositis to manageable erythrosquamous skin toxicity and predictable diarrhea [21]. The increased efficacy of infusional 5-FU in contrast to bolus 5-FU was confirmed in the whole population [22]. If patients with or without PC were examined separately, the absolute increase in response rates with infusional 5-FU was 4.6% for patients with peritoneal involvement vs. 16.3% in patients without peritoneal involvement (Table 1).

Table 1. Efficacy of 5-FU in patients with or without peritoneal carcinomatosis (PC) [19]

	With PC		**Without PC**	
	5-FU bolus	5-FU infusion	5-FU bolus	5-FU infusion
Response rate	12.6%	19.0%	19.9%	36.2%
N	199	116	1584	669
Progression free survival (m)	4.6	3.7	4.8	7.4
95% CI	3.5-5.7	2.1-5.2	4.5-5.2	6.7-8.0
N	139	118	1187	676
	p=0.3		p<0.0001	
Overall survival (m)	7.8	6.9	10.8	14.6
95% CI	6.6-8.9	5.1-8.8	10.2-11.3	13.3-16-0
N	207	119	1661	677
	p=0.44		p<0.0001	

There was no increase in progression free survival or in overall survival in patients with PC in the infusional 5-FU arms, whereas patients without PC had a slight benefit from the optimized application schedule [19]. Thus, patients with peritoneal involvement - in contrast to patients without PC - do not seem to profit from a change in 5-FU application alone.

Another set of randomized studies examined the benefit of adding the topoisomerase 1 inhibitor irinotecan to 5-FU-based chemotherapy. Out of 1594 patients, information on the PC status was available for 472 patients, of whom 71 patients (15%) had peritoneal involvement. The addition of irinotecan significantly increased the response rates in the whole patient population (Table 3) [1-3]. As expected, patients with PC had shorter progression free and overall survival times than the population without PC (Table 2). However, patients in both groups had a similar increase in progression free survival and in overall survival by the addition of irinotecan, which did not quite reach significance due to the low patient numbers [19].

Table 2. Efficacy of 5-FU ± irinotecan in patients with or without peritoneal carcinomatosis (PC) [19]

	With PC		**Without PC**	
	5-FU	Irinotecan + 5-FU	5-FU	Irinotecan + 5-FU
Response rate	14%	39%	32%	56%
N	35	36	202	198
	p=0.03		p<0.001	
Progression free survival	3.4	6.5	6.6	8.8
95% CI	1.2-5.6	4.3-8.7	5.6-7.5	7.9-9.7
N	16	21	203	198
	p=0.07		p<0.0001	
Overall survival	9.8	17.9	17.5	20.0
95% CI	6.4-13.3	8.5-27.3	15.7-19.3	18.2-22.0
N	35	36	203	198
	p=0.17		p=0.19	

Taken together, patients with PC have reduced response rates to systemic chemotherapy when compared to patients without peritoneal involvement. This translates into shortened progression free survival times as well as shorter overall survival. Nevertheless, patients with PC clearly profit from the addition of irinotecan to 5-FU. Whereas the relative increase in response rates is similar in patients with or without PC, the effect of complex chemotherapy on median survival is even higher in patients with PC than in the control population (Table 1 and 2; Fig. 1 and 2). This analysis supports the general notion that patients with large tumour burden profit from 1st line therapy with complex modern chemotherapy regimens like irinotecan + 5-FU/FA, but not from 5-FU/FA alone.

Systemic Therapy with 5-Fluorouracil

Until the late 1980s, the fluoropyrimidine 5-fluouracil (5-FU) had been the only drug with proven efficacy in the treatment of metastatic colorectal cancer. Compared to best supportive care, 5-FU provides a moderate prolongation of the median survival time from 8.5 months (without chemotherapy) to 12 months (with

5-FU) [23]. A series of trials demonstrated that palliative treatment should be started early and not be delayed until symptomatic disease, and that biochemical modulation with folinic acid increases the efficacy of 5-FU [24,25].

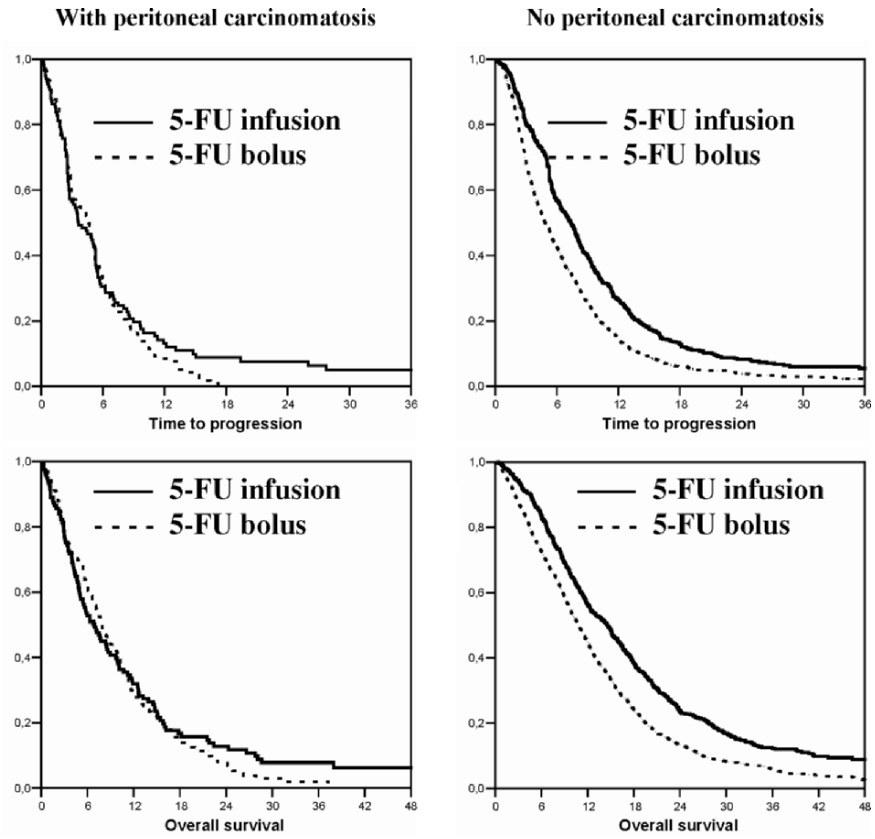


Figure 1. Overall survival and progression free survival in patients with or without peritoneal carcinomatosis from colorectal cancer. Meta-analysis of trials investigating 5-FU as bolus regimen or infusional therapy. In contrast to patients without peritoneal carcinomatosis, patients with peritoneal carcinomatosis did not profit from infusional 5-FU

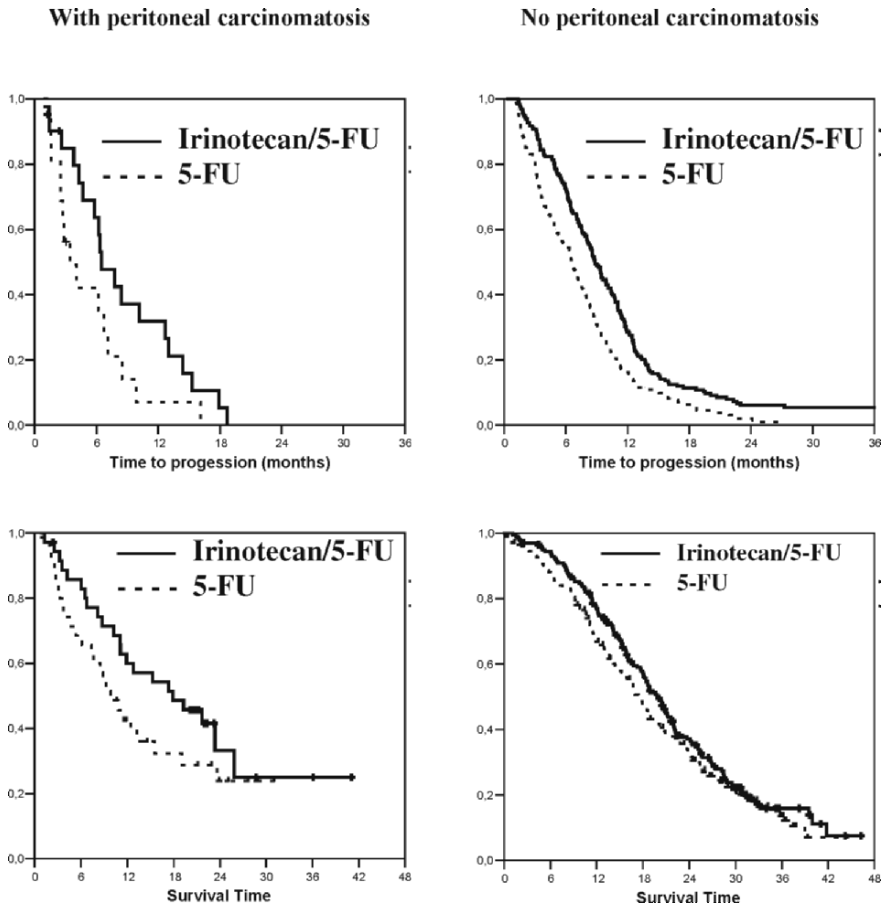


Figure 2. Overall survival and progression free survival in patients treated with 5-FU ± irinotecan. Merged data from a database of three randomized trials (patients with unknown status for peritoneal carcinomatosis excluded). Although the prognosis of patients with peritoneal carcinomatosis is worse compared to that without peritoneal carcinomatosis, the benefit from irinotecan-containing therapy is comparable in both groups

Table 3. 1st line chemotherapy: Combination chemotherapy versus mono chemotherapy

		n	RR	PFS	OS
Irinotecan/5-FU/FA versus 5-FU/FA					
Saltz [1]	irinotecan + bolus 5-FU/FA	231	50*	7.0*	14.8*
	Mayo regimen	226	28	4.3	12.6
	Irinotecan monotherapy	226	29	4.2	12.0
Douillard [2]	Irinotecan + 5-FU(infusion)/FA	198	40.8*	6.7*	17.4*
	5-FU(infusion)/FA	187	23.1	4.4	14.1
Köhne [3]	Irinotecan + 5-FU(infusion)/FA	214	62*	8.5*	20.1
	5-FU (infusion)/FA	216	34	6.4	16.9
Oxaliplatin/5-FU/FA versus 5-FU/FA					
Infusional 5-FU					
de Gramont [4]	Oxaliplatin + 5-FU/FU	210	50.0*	8.2*	16.2
	5-FU/FA	210	21.9	6.0	14.7
Giachetti [5]	Oxaliplatin + 5-FU/FA	100	53*	8.7*	19.1
	5-FU/FA	100	16	6.1	19.4
Bolus 5-FU					
Grothey [6]	Oxaliplatin + 5-FU/FA (Infusion)	125	49.1*	7.8*	21.4
	5-FU/FA (bolus)	124	22.6	5.3	16.1
5-FU/FA → Irinotecan vs. 5-FU/FA → Folfox or Folfiri vs. Folfox → Folfiri or Folfiri → Folfox					
FOCUS trial (RR and PFS for 1st line)					
Seymour [34,51]	5-FU/FU	1413	28.5	6.3-6.7†	13.9-15.2†
	Folfiri	356	51.4*	8.6*	16.3
	Folfox	357	56.2*	8.8*	15.2

RR, response rate (%); PFS, progression free survival (months); OS, overall survival (months); 5-FU, 5-fluorouracil; FA, folinic acid; † 5-FU/FA was followed by different second line treatments (irinotecan, FOLFIRI or FOLFOX); * statistically significant

When 5-FU is infused rather than given as an i.v. bolus, the survival can be further improved by a few weeks [21]. Especially the weekly 24h-infusion (AIO regimen) and the biweekly infusional administration over 48h (LV5FU2) are less toxic and (slightly) increase the efficacy over bolus 5-FU [26-28].

New cytotoxic drugs: Irinotecan and Oxaliplatin

The addition of oxaliplatin and irinotecan to 5-FU markedly improved the efficacy of systemic chemotherapy (Table 3). Oxaliplatin, a modern DACH-platinum compound, is a cross-linking agent; irinotecan a semisynthetic camptothecin derivative which inhibits topoisomerase I. Irinotecan, irinotecan/5-FU/FA (e.g. in the FOLFIRI regimen) and oxaliplatin/5-FU/FA (e.g. in the FOLFOX regimen) are active as 1st and 2nd line therapy. When either of the drugs is added to 5-FU, tumour response rates improve from 16% - 32% with 5-FU/FA to 35% - 64% with the combination therapy [1-6]. The time to tumour progression during the first line of chemotherapy increases from 4.1 - 6.3 months with 5-FU/FA to 7.0 - 8.5

months [1-6]. The median overall survival in the most recent studies with infusional 5-FU plus oxaliplatin or irinotecan is 20 months [3,6,29] if a high percentage of the patients received an effective second line therapy. Median survival times increase further in those patients who receive all drugs at some time during their treatment [30]. There is no clear advantage for either of the combination partners (FOLFOX or FOLFIRI) [29] in the 1st line treatment.

Treatment without 5-FU, i.e. the combination irinotecan plus oxaliplatin is inferior to FOLFOX [31] and is only an option for patients who can not receive fluoropyrimidines, i.e. because of ischemic cardiopathy or mutations in the dihydropyrimidine-dehydrogenase (DPD) gene. FOLFOXIRI - the combination of oxaliplatin, irinotecan *and* 5-FU - has been investigated in only two randomized trials. A Greek study compared a "low dose" FOLFOXIRI (irinotecan 150 mg/m², oxaliplatin 65 mg/m²) to FOLFIRI and found non significant trends for higher response rates (43% vs. 34%) and progression free survival (8.4 vs. 6.9 months) [32]. In contrast, an Italian study group used higher doses for irinotecan (oxaliplatin 85 mg/m², irinotecan 165 mg/m²) and found significantly improved response rates (60% vs. 34%), progression free survival (9.8 vs. 6.9 months) and overall survival (22.6 vs. 16.7 months), but at the cost of considerably higher toxicity [33].

The best choice when starting chemotherapy and the optimal sequence was re-evaluated by the British FOCUS trial study group in a randomized study of monotherapy (e.g. with 5-FU/FA) versus combination therapies at start of therapy (1st line) with defined 2nd and 3rd line regimes. As expected, response rates and progression free survival in the 1st line of therapy were significantly improved with the drug combinations (FOLFOX or FOLFIRI) as compared to the mono-therapy. However, this large trial with 2135 patients failed to demonstrate a significantly prolonged survival with combination therapy upfront [34]. One reason might be the low percentage of patients who received a salvage therapy after failure of one of the combinations. These results support a previous French trial, where the efficacy of FOLFOX and FOLFIRI have been comparable [29], so that either combination can be selected depending on the regimen which has been used as adjuvant postsurgical pretreatment, on the comorbidities of the patient, on the expected side effects, or on the availability of an additional antibody as described below.

The clinically most relevant toxicity of oxaliplatin is a sensory neuropathy which manifests itself either as short-lasting cold-induced paresthesia immediately after oxaliplatin administration, or a dose-dependent cumulative peripheral hypoaesthesia. The hypoaesthesia may be long lasting and is severe in 17% - 18% of patients [4,35,36]. Because the frequency of severe oxaliplatin-induced neuropathy depends on the cumulative dose and increases if more than 800 mg/m² are administered (4.5 months of treatment), and because the median treatment duration is shorter in second line therapy, some investigators prefer a first line therapy with irinotecan [35]. The most relevant toxicities of irinotecan are a delayed acute diarrhea and neutropenia. There have been reports of increased mortality in trials with bolus 5-FU but not with infusional 5-FU. Because of the lower toxicity and higher efficacy of infusional instead of bolus 5-FU [15], both irinotecan and oxaliplatin

are usually combined with infusional 5-FU in biweekly (e.g. FOLFIRI or FOLFOX) or weekly (e.g. AIO) schedules. The rate of severe diarrhea is similar with FOLFIRI and FOLFOX [29,34].

Oral Fluoropyrimidines

Two orally active fluoropyrimidines have been more widely tested. Capecitabine is a prodrug which is metabolized to 5-FU in three steps. UFT is a two-drug combination of ftorafur (also a 5-FU prodrug) and uracil which inhibits the degradation of 5-FU. Both were developed to overcome the need of intravenous injection and to mimic the continuous infusional application. Randomized trials demonstrated that capecitabine and UFT are as active as bolus 5-FU/FA in metastatic colorectal cancer [37-40] and in the adjuvant situation [41,42]. Although hematological toxicity and stomatitis are lower than with bolus 5-FU/FA, patients who take capecitabine have markedly more skin toxicity (hand foot syndrome), and the overall rate of severe toxicity is not lower with that drug than with bolus 5-FU/FA [37,38].

Because a monotherapy with fluoropyrimidines is rarely the treatment of choice for most patients, recent studies focused on combination therapies, i.e. capecitabine/irinotecan or capecitabine/oxaliplatin (Table 4), with mixed results. A randomized EORTC trial was terminated early because of increased mortality in the irinotecan/capecitabine arm [43]. With reduced dose of irinotecan/capecitabine toxicity can be reduced, but the efficacy of this regimen is also inferior to FOLFIRI [15].

Table 4. Randomized trials: Oral fluoropyrimidines in combination with irinotecan or oxaliplatin

		n	RR	PFS	OS
Capecitabine/Oxaliplatin vs. infusional 5-FU/oxaliplatin					
Cassidy [12]	"Xelox" +/- Bevacizumab	1017	n.a.	8.0 †	n.a.
	FOLFOX4 +/- Bevacizumab	1018	n.a.	8.5	n.a.
Arkenau [45]	CapOx	242	47%	7.0	16.3
	FUFOX	234	49%	8.0	17.2
Massuti [44]	"Xelox"	171	37%	8.9	18.0
	FUFOX	171	45%	9.5	21.2
Capecitabine/Irinotecan vs. infusional 5-FU/irinotecan					
De Greve [43]	CapIri +/- Celecoxib	43	35%	5.9	14.8 ‡
	FOLFIRI +/- Celecoxib	39	55%	9.6	19.9
Fuchs [15]	CapIri	145	38%	5.5	18.9
	FUFOX	144	47%	7.6*	23.1

RR, response rate (%); PFS, progression free survival (months); OS, overall survival (months); † HR 1.04 (0.93-1.16), non inferior to FOLFOX; ‡ HR 3.2 (1.4-7.3), no p value because of early termination; * statistically significant

Oxaliplatin together with capecitabine is safe and the response rates are not different to those of FOLFOX or other infusional oxaliplatin/5-FU/FA regimens. However, in several trials a trend towards a shorter time to progression was observed with the oral regime, which was formally not significant in any of the trials [44,45]. Non-inferiority with regard to progression free survival has been demonstrated (8.0 months in controls vs. 8.5 months with capecitabine/oxaliplatin, respectively) in one large trial. Compared to FOLFOX4 (with 5-FU bolus component), less neutropenia grade 3/4 (7% vs. 44%) but more diarrhea grade 3/4 (20 vs. 11%) and severe hand foot syndrome (6 vs. 1%) has been observed [12]. Overall, the difference between capecitabine and infusional 5-FU is rather small. Oxaliplatin/capecitabine is an alternative schedule if intravenous application is not feasible or not desired. At least a metaanalysis of all randomized trials is required before it can be accepted as a standard regimen.

Antibodies in Treatment of Colorectal Cancer

Recently, two antibodies with two different molecular targets were approved. Bevacizumab is a humanized antibody against the vascular endothelial growth factor (VEGF). Cetuximab is a chimeric antibody directed at the epidermal growth factor receptor (EGFR).

Bevacizumab prolongs the progression free survival and the overall survival by 4 months when combined with irinotecan/5-FU/FA [10]. Prolonged overall survival has also been demonstrated in combination with oxaliplatin/5-FU/FA in first line, but the difference in progression free survival was only 1.4 months [12].

Table 5. Randomized trials: Chemotherapy +/- antibody (first line)

		n	RR	PFS	OS
Chemotherapy vs. chemotherapy + bevacizumab					
Hurwitz [10] †	IFL + Bevacizumab	411	45%*	10.6*	20.3
	IFL †	403	35%	6.2	15.6
Kabbinavar [11]	†5-FU/FA + Bevacizumab	104	26%	9.2*	16.6
	5-FU/FA †	105	15%	5.5	12.9
Cassidy [12]	Xelox or FOLFOX + Bevacizumab	699	n.a.	9.4*	n.a.
	Xelox or FOLFOX	701	n.a.	8.0	n.a.
Chemotherapy vs. chemotherapy + cetuximab					
Venook [49]	FOLFOX or FOLFIRI + Cetuximab	108	52%*	8.5	Not reached
	FOLFOX or FOLFIRI	116	38%	9.4	16.9

RR, response rate (%); PFS, progression free survival (months); OS, overall survival (months); † no second line therapy with bevacizumab for patients in the IFL group; * statistically significant

In second line therapy a longer overall survival (12.9 vs. 10.8 months, $p < 0.01$) [46] has been demonstrated if the doubled dose of bevacizumab is added to

FOLFOX. Bevacizumab increases the rate of gastrointestinal perforations and arterial thromboembolic events.

Gastrointestinal perforations occur in 1.3% of patients with colon cancer [10]. However, in patients with ovarian cancer, the rate may increase to 10% [47].
One possible explanation is higher frequency of PC in that disease. Data from patients with colorectal cancer and PC are lacking. Arterial thromboembolic events are relatively rare (3.8% with, 1.7% without bevacizumab), but more probable with increasing age (> 65 years: 7.1%) and if there is a history of arterial thromboembolism (15.7%) [48].

Cetuximab has first been approved in patients with irinotecan-resistant tumours. The overall response rate is 23% with a combination of cetuximab and irinotecan, irrespective of the number of previous chemotherapy lines [7]. Cetuximab also increases the response rates in first line therapy [49]. Reliable results from 1st line phase III trials with regard to progression free and overall survival are still pending. Some phase II trials indicated high response rates in first line therapy (67% - 81%, Table 6).

Table 6. Phase II trials: Chemotherapy + antibodies (first line)

		n	RR	PFS	OS
Bevacizumab					
Kopetz [52]	FOLFIRI + Bevacizumab	23	75%	n.a.	n.a.
Cetuximab					
Rougier [53] / Peeters [57]	FOLFIRI + Cetuximab	42	45%	n.a.	23
Folprecht [13]	Irinotecan/5-FU/FA + Cetuximab	21	67%	9.9	33.0
Seufferlein [14]	FUFOX + Cetuximab	57	54%	8.1	30.6
Diaz-Rubio [54]	FOLFOX4 + Cetuximab	42	81%	12.3	n.a.
Dakhil [55]	FOLFOX6 + Cetuximab	82	61%	n.a.	n.a.
Colucci [56]	FOLFOX4 + Cetuximab	47	68%	n.a.	n.a.

RR, response rate (%); PFS, progression free survival (months); OS, overall survival (months)

Panitumumab is a humanized antibody against the EGFR antibody which is also active in pretreated patients (response rate of 10% as single agent) [50]. In contrast, chemical inhibitors of the EGFR tyrosine kinase like gefitinib, erlotinib and others have not yet shown to be effective in metastatic colorectal cancer. The major toxicities of EGFR-directed agents are acne like rash and other skin toxicities such as dry skin and aseptic periungual inflammation. Of note, the grade of skin toxicity correlates with the response rate and with overall survival. Response rates in pretreated patients are 6% if they have no skin toxicity but increase to 55% if the skin toxicity reaches grade ≥ 3. Similarly, overall survival increases from 3.0 to 9.1 months in patients with skin toxicity [7].

VEGF or EGFR-directed antibodies together with effective polychemotherapy are expected to markedly prolong the overall survival. Currently, several randomized and non-randomized phase II trials observed a median survival of > 24

months median overall survival [13-16]. Confirmation in phase III trials is awaited.

Treatment strategy

For the majority of the patients and definitely for those with symptomatic tumours or with potentially resectable disease, a combination therapy of irinotecan/ infusional 5-FU or oxaliplatin/infusional 5-FU is the chemotherapy of choice with the best chance for response, both combined with an antibody. At the date of print, bevacizumab plus irinotecan/5-FU is approved as first line chemotherapy/antibody combination in Europe. If there is tumour progression, chemotherapy should be changed to oxaliplatin/infusional 5-FU or to irinotecan/cetuximab to prolong survival, each of them followed by the other regimen in case of failure.

In patients without the chance of curative resection after downsizing (which is likely the case for most patients with PC) and with low tumour burden and no tumour-related symptoms, chemotherapy can probably start with a well-tolerated fluoropyrimidine monotherapy (infusional regimen or oral drugs), with or without bevacizumab. These patients should be closely monitored and combination chemotherapy initiated in case of tumour progression.

References

1. Saltz LB, Cox JV, Blanke C et al. (2000) Irinotecan plus fluorouracil and leucovorin for metastatic colorectal cancer. Irinotecan Study Group. N Engl J Med 343:905-914
2. Douillard JY, Cunningham D, Roth AD et al. (2000) Irinotecan combined with fluorouracil compared with fluorouracil alone as first-line treatment for metastatic colorectal cancer: a multicentre randomised trial. Lancet 355:1041-1047
3. Köhne CH, Van Cutsem E, Wils J et al. (2005) Phase III Study of Weekly High-dose Infusional 5-Fluorouracil Plus Folinic Acid With or Without Irinotecan in Patients With Metastatic Colorectal Cancer. EORTC Gastrointestinal Group Study 40986. J Clin Oncol 23:4856-4865
4. de Gramont A, Figer A, Seymour M et al. (2000) Leucovorin and fluorouracil with or without oxaliplatin as first-line treatment in advanced colorectal cancer. J.Clin.Oncol 18:2938-2947
5. Giacchetti S, Perpoint B, Zidani R et al. (2000) Phase III multicenter randomized trial of oxaliplatin added to chronomodulated fluorouracil-leucovorin as first-line treatment of metastatic colorectal cancer. J Clin Oncol 18:136-147
6. Grothey A, Deschler B, Kroening H et al. (2002) Phase III study of bolus 5-fluorouracil (5-FU)/ folinic acid (FA) (Mayo) vs weekly high-dose 24h 5-FU

infusion/ FA + oxaliplatin (OXA) (FUFOX) in advanced colorectal cancer (ACRC). Proc Am Soc Clin Oncol 21:129a
7. Cunningham D, Humblet Y, Siena S et al. (2004) Cetuximab monotherapy and cetuximab plus irinotecan in irinotecan-refractory metastatic colorectal cancer. N.Engl.J Med 351:337-345
8. Saltz LB, Meropol NJ, Loehrer PJ, Sr. et al. (2004) Phase II trial of cetuximab in patients with refractory colorectal cancer that expresses the epidermal growth factor receptor. J Clin Oncol 22:1201-1208
9. Saltz L, Rubin M, Hochster H et al. (2001) Cetuximab (IMC-C225) Plus Irinotecan (CPT-11) is Active in CPT-11-Refractory Colorectal Cancer (CRC) that Expresses Epidermal Growth Factor Receptor (EGFR). Proc Am Soc Clin Oncol 20:3a
10. Hurwitz H, Fehrenbacher L, Novotny W et al. (2004) Bevacizumab plus irinotecan, fluorouracil, and leucovorin for metastatic colorectal cancer. N.Engl.J Med 350:2335-2342
11. Kabbinavar FF, Schulz J, McCleod M et al. (2005) Addition of bevacizumab to bolus fluorouracil and leucovorin in first-line metastatic colorectal cancer: results of a randomized phase II trial. J Clin Oncol 23:3697-3705
12. Cassidy J, Clarke S, Diaz-Rubio E et al. (2006) First efficacy and safety results from XELOX-1/NO16966, a randomised 2x2 factorial phase III trial of XELOX vs.FOLFOX4 1 bevacizumab or placebo in first-line metastatic colorectal cancer (MCRC). Ann Oncol 17:LBA3
13. Folprecht G, Lutz M, Schöffski P et al. (2006) Cetuximab and irinotecan/5-fluorouracil/folinic acid is a safe combination for the first-line treatment of patients with epidermal growth factor receptor expressing metastatic colorectal carcinoma. Ann Oncol 17:450-456
14. Seufferlein T, Dittrich C, Riemann JF et al. (2005) A phase I/II study of cetuximab in combination with 5-fluorouracil (5-FU)/folinic acid (FA) plus weekly oxaliplatin (L-OHP) (FUFOX) in the first-line treatment of patients with metastatic colorectal cancer (mCRC) expressing epidermal growth factor receptor (EGFR). Preliminary results. Proc Am Soc Clin Oncol 24:abstract 3644
15. Fuchs C, Marshall J, Mitchell E et al. (2006) A randomized trial of first-line irinotecan/fluoropymidine combinations with or without celecoxib in metastatic colorectal cancer (BICC-C). J Clin Oncol (Meeting Abstracts) 24:3506
16. Hochster HS, Hart LL, Ramanathan RK et al. (2006) Safety and efficacy of oxaliplatin/fluoropyrimidine regimens with or without bevacizumab as first-line treatment of metastatic colorectal cancer (mCRC): Final analysis of the TREE-Study. J Clin Oncol (Meeting Abstracts) 24:3510
17. Adam R, Delvart V, Pascal G et al. (2004) Rescue surgery for unresectable colorectal liver metastases downstaged by chemotherapy: a model to predict long-term survival. Ann Surg 240:644-657
18. Therasse P, Arbuck SG, Eisenhauer EA et al. (2000) New guidelines to evaluate the response to treatment in solid tumors. European Organization for Research and Treatment of Cancer, National Cancer Institute of the United States, National Cancer Institute of Canada. J Natl Cancer Inst 92:205-216

19. Folprecht G, Lutz MP, Rougier P et al. (2007) Effect of systemic chemotherapy (CTx) in patients with known peritoneal carcinomatosis (PC) from colorectal cancer. Proc Gastrointest Canc Symp (ASCO/AGA/ASTRO/SSO) 2007
20. Köhne CH, Cunningham D, Di Costanzo F et al. (2002) Clinical determinants of survival in patients with 5-fluorouracil- based treatment for metastatic colorectal cancer: results of a multivariate analysis of 3825 patients. Ann Oncol 13:308-317
21. Anon. (1998) Efficacy of intravenous continuous infusion of fluorouracil compared with bolus administration in advanced colorectal cancer. Meta-analysis Group In Cancer. J Clin Oncol 16:301-308
22. Folprecht G, Cunningham D, Ross P et al. (2004) Efficacy of 5-fluorouracil-based chemotherapy in elderly patients with metastatic colorectal cancer: a pooled analysis of clinical trials. Ann Oncol 15:1330-1338
23. Simmonds PC. (2000) Palliative chemotherapy for advanced colorectal cancer: systematic review and meta-analysis. Colorectal Cancer Collaborative Group. Brit Med J 321:531-535
24. Anon. (1992) Modulation of fluorouracil by leucovorin in patients with advanced colorectal cancer: evidence in terms of response rate. Advanced Colorectal Cancer Meta-Analysis Project. J Clin Oncol 10:896-903
25. Thirion P, Michiels S, Pignon JP et al. (2004) Modulation of fluorouracil by leucovorin in patients with advanced colorectal cancer: an updated meta-analysis. J.Clin.Oncol 22:3766-3775
26. de Gramont A, Bosset JF, Milan C et al. (1997) Randomized trial comparing monthly low-dose leucovorin and fluorouracil bolus with bimonthly high-dose leucovorin and fluorouracil bolus plus continuous infusion for advanced colorectal cancer: a French intergroup study. J Clin Oncol 15:808-815
27. Köhne CH, Wils J, Lorenz M et al. (2003) Randomized Phase III Study of High-Dose Fluorouracil Given As a Weekly 24-Hour Infusion With or Without Leucovorin Versus Bolus Fluorouracil Plus Leucovorin in Advanced Colorectal Cancer: European Organization of Research and Treatment of Cancer Gastrointestinal Group Study 40952. J Clin Oncol 21:3721-3728
28. Weh HJ, Zschaber R, Braumann D et al. (1998) A randomized phase III study comparing weekly folinic acid (FA) and high dose 5-Fluorouracil (5-FU) with monthly 5-FU/FA (days1-5) in untreated patients with metastatic colorectal carinoma. Onkologie 21:403-407
29. Tournigand C, Andre T, Achille E et al. (2004) FOLFIRI Followed by FOLFOX6 or the Reverse Sequence in Advanced Colorectal Cancer: A Randomized GERCOR Study. J Clin Oncol 22:229-237
30. Grothey A, Sargent D, Goldberg RM, Schmoll HJ. (2004) Survival of patients with advanced colorectal cancer improves with the availability of fluorouracil-leucovorin, irinotecan, and oxaliplatin in the course of treatment. J Clin Oncol 22:1209-1214
31. Goldberg RM, Sargent DJ, Morton RF et al. (2004) A randomized controlled trial of fluorouracil plus leucovorin, irinotecan, and oxaliplatin combinations

in patients with previously untreated metastatic colorectal cancer. J Clin Oncol 22:23-30
32. Souglakos J, Androulakis N, Syrigos K et al. (2006) FOLFOXIRI (folinic acid, 5-fluorouracil, oxaliplatin and irinotecan) vs FOLFIRI (folinic acid, 5-fluorouracil and irinotecan) as first-line treatment in metastatic colorectal cancer (MCC): a multicentre randomised phase III trial from the Hellenic Oncology Research Group (HORG). Br J Cancer 27:798-805
33. Falcone A, Masi G, Murr R et al. (2006) Biweekly irinotecan, oxaliplatin, and infusional 5FU/LV (FOLFOXIRI) versus FOLFIRI as first-line treatment of metastatic colorectal cancer (MCRC): Results of a randomized, phase III trial by the Gruppo Oncologico Nord Ovest (GONO) [abstract]. Proc Gastrointest Canc Symp (ASCO/AGA/ASTRO/SSO) abstract 227
34. Seymour M. (2004) Optimizing the use and sequencing of fluorouracil, irinotecan and oxaliplatin in advanced colorectal cancer (ACRC): The UK MRC FOCUS (CR08) trial. Ann Oncol 15
35. Cassidy J, Bjarnason GA, Hickish T et al. (2006) Randomized double blind (DB) placebo (Plcb) controlled phase III study assessing the efficacy of xaliproden (X) in reducing the cumulative peripheral sensory neuropathy (PSN) induced by the oxaliplatin (Ox) and 5-FU/LV combination (FOLFOX4) in first-line treatment of patients (pts) with metastatic colorectal cancer (MCRC). J Clin Oncol (Meeting Abstracts) 24:3507
36. Rothenberg M, Meropol NJ, Poplin EA, Van Cutsem E, Wadler S. (2001) Mortality associated with Irinotecan plus bolus Fluorouracil/Leucovorin: Summery findings of an independent panel. J Clin Oncol 19:3801-3807
37. Van Cutsem E, Twelves C, Cassidy J et al. (2001) Oral capecitabine compared with intravenous fluorouracil plus leucovorin in patients with metastatic colorectal cancer: results of a large phase III study. J Clin Oncol. 19:4097-4106
38. Hoff PM, Ansari R, Batist G et al. (2001) Comparison of oral capecitabine versus intravenous fluorouracil plus leucovorin as first-line treatment in 605 patients with metastatic colorectal cancer: results of a randomized phase III study. J Clin Oncol 19:2282-2292
39. Carmichael J, Popiela T, Radstone D et al. (2002) Randomized comparative study of tegafur/uracil and oral leucovorin versus parenteral fluorouracil and leucovorin in patients with previously untreated metastatic colorectal cancer. J Clin Oncol 20:3617-3627
40. Douillard JY, Hoff PM, Skillings JR et al. (2002) Multicenter phase III study of uracil/tegafur and oral leucovorin versus fluorouracil and leucovorin in patients with previously untreated metastatic colorectal cancer. J Clin Oncol 20:3605-3616
41. Twelves C, Wong A, Nowacki MP et al. (2005) Capecitabine as adjuvant treatment for stage III colon cancer. N.Engl.J Med 352:2696-2704
42. Wolmark N, Wieand S, Lembersky B et al. (2004) A phase III trial comparing oral UFT to FULV in stage II and III carcinoma of the colon: Results of NSABP Protocol C-06. J Clin Oncol 22:247s

43. de Greve J, Koehne C, Hartmann J et al. (2006) Capecitabine plus irinotecan versus 5-FU/FA/irinotecan {+/-} celecoxib in first line treatment of metastatic colorectal cancer (CRC). Long-term results of the prospective multicenter EORTC phase III study 40015. J Clin Oncol (Meeting Abstracts) 24:3577
44. Massuti B, Gomez A, Sastre J et al. (2006) Randomized phase III trial of the TTD Group comparing capecitabine and oxaliplatin (XELOX) vs. oxaliplatin and 5-fluorouracil in continuous infusion (FUFOX) as first line treatment in advanced or metastatic colorectal cancer (CRC). J Clin Oncol (Meeting Abstracts) 24:3580
45. Arkenau H, Schmoll HJ, Kubicka S et al. (2005) Infusional 5-fluorouracil/folinic acid plus oxaliplatin (FUFOX) versus capecitabine plus oxaliplatin (CAPOX) as first line treatment of metastatic colorectal cancer (MCRC): Results of the safety and efficacy analysis. Proc Am Soc Clin Oncol abstract 3507
46. Giantonio B, Catalano PJ, Meropol NJ et al. (2005) High-dose bevacizumab improves survival when combined with FOLFOX4 in previously treated advanced colorectal cancer: Results from the Eastern Cooperative Oncology Group (ECOG) study E3200. Proc Am Soc Clin Oncol abstract 2
47. Cannistra SA, Matulonis U, Penson R et al. (2006) Bevacizumab in patients with advanced platinum-resistant ovarian cancer. J Clin Oncol 24:5006
48. Skillings JR, Johnson DH, Miller K et al. (2005) Arterial thromboembolic events (ATEs) in a pooled analysis of 5 randomized, controlled trials (RCTs) of bevacizumab (BV) with chemotherapy. Proc Am Soc Clin Oncol abstract 3019
49. Venook A, Niedzwiecki D, Hollis D et al.(2006) Phase III study of irinotecan/5FU/LV (FOLFIRI) or oxaliplatin/5FU/LV (FOLFOX) {+/-} cetuximab for patients (pts) with untreated metastatic adenocarcinoma of the colon or rectum (MCRC): CALGB 80203 preliminary results. J Clin Oncol (Meeting Abstracts) 24:3509
50. Peeters M, Van Cutsem E, Siena S et al. (2006) A phase 3, multicenter, randomized controlled trial (RCT) of panitumumab plus best supportive care (BSC) vs BSC alone in patients (pts) with metastatic colorectal cancer (mCRC). Proceedings of the AACR abstract CP-1
51. Seymour MT, UK NCRI Colorectal Clinical Studies Group. (2005) Fluorouracil, oxaliplatin and CPT-11 (irinotecan), use and sequencing (MRC FOCUS): A 2135-patient randomized trial in advanced colorectal cancer (ACRC). J Clin Oncol (Meeting Abstracts) 23:3518
52. Kopetz S, Abbruzzese JL, Eng C et al. (2006) Preliminary results from a phase II study of infusional 5-FU, leucovorin, and irinotecan (FOLFIRI) plus bevacizumab as first-line treatment for metastatic colorectal cancer (mCRC). J Clin Oncol (Meeting Abstracts) 24:3579
53. Rougier P, Raoul JL, Van Laethem JL et al. (2004) Cetuximab＋FOLFIRI as first-line treatment for metastatic colorectal CA [abstract]. Proc Am Soc Clin Oncol 23:abstract 3513

54. Diaz-Rubio E, Tabernero J, Van Cutsem E et al. (2005) Cetuximab in combination with oxaliplatin/5-fluorouracil (5-FU)/folinic acid (FA) (FOLFOX-4) in the first-line treatment of patients with epidermal growth factor receptor (EGFR)-expressing metastatic colorectal cancer: An international phase II study. Proc Am Soc Clin Oncol 24:abstract 3535
55. Dakhil S, Cosgriff T, Headley D et al. (2006) Cetuximab + FOLFOX6 as first line therapy for metastatic colorectal cancer (An International Oncology Network study, I-03-002). J Clin Oncol (Meeting Abstracts) 24:3557
56. Colucci G, Giuliani F, Mattioli R et al. (2006) FOLFOX-4 + cetuximab in untreated patients with advanced colorectal cancer. A phase II study of the Gruppo Oncologico dell'Italia Meridionale (prot. GOIM 2402). J Clin Oncol (Meeting Abstracts) 24:3559
57. M. Peeters, JL Raoul, JL van Laethem, et al. (2005) Cetuximab in combination with irinotecan/5-fluorouracil (5-FU)/folinic acid (FA) (FOLFIRI) in the first-line treatment of metastatic colorectal cancer (mCRC). Eur J Cancer Suppl 3, No. 2, p188

Systemic Chemotherapy in patients with Peritoneal Carcinomatosis from Non Colorectal Origin

S Van Lierde, H Denys, M Peeters

Introduction

Peritoneal carcinomatosis (PC) is a malignancy that spreads widely inside the peritoneal cavity, involving mostly the omentum.

Different tumour types can present with peritoneal carcinomatosis. The most common cause of PC in women is ovarian cancer. Approximately 75% of patients with ovarian cancer present with FIGO stage III disease (disease spread throughout the peritoneal cavity or involvement of retroperitoneal or inguinal nodes). Serous epithelial ovarian cancer (EOC) however has histopathological, immunohistochemical and clinical similarities with primary peritoneal carcinomatosis (PPC), a far less common malignancy, that has also be encountered in women following bilateral oophorectomy and even in male patients [1-3]. Since first being described in 1959 by Swerdlow [4], primary PC has been the subject of numerous case reports, case series and retrospective reviews [5-9]. However, interpretation of these data is complicated by confusing and varying terminology. A variation in nomenclature has been used to describe the tumour: peritoneal mesothelioma, peritoneal papillary (serous) carcinoma, extraovarian peritoneal serous papillary carcinoma, serous surface papillary carcinoma, multiple focal extraovarian serous carcinoma and small ovarian carcinoma. This is reflective of the diagnostic dilemma faced by pathologists in cases of müllerian carcinomatosis with minimal ovarian involvement [5].

In an effort to better define this patient population and to develop more organized treatment strategies, the Gynecologic Oncology Group (GOG) developed a concise set of criteria for PPC [10]. First, both ovaries must be either physiologically normal in size or enlarged by a benign process (4.0 cm largest diameter). Second, involvement of extraovarian sites must be greater than that on the surface of either ovary. Third, microscopically, the ovarian component must be one of the following: (a) nonexistent, (b) confined to the ovarian surface epithelium with no evidence of cortical invasion, (c) involving the ovarian surface epithelium and

underlying cortical stroma but any given tumour size must be less than 5 × 5 mm, or (d) tumours less than 5 × 5 mm within the ovarian substance associated with or without surface disease. Fourth, the histological and cytological characteristics of the tumour must be predominantly of the serous type, similar or identical to ovarian serous papillary adenocarcinoma of any grade. Using this clinical definition of PPC, 7-20% of patients previously identified as having primary ovarian papillary serous carcinoma may be reclassified as having PPC [5,7]. However the etiology, pathogenesis, cell of origin, and clinical characteristics of PPC remain obscure. Differential diagnosis may include adenocarcinoma of unknown primary tissue, malignant mesothelioma, and peritoneal adenocarcinoma, as well as metastatic breast cancer (especially lobular cancer of the breast) [11]. In addition to breast and ovarian carcinomas, patients with germline BRCA1 mutations are more likely to develop PPC [12].

Furthermore, PC can be a metastatic site of gastrointestinal malignancies (such as gastric, pancreatic or colorectal carcinomas) [11]. PC is a frequent cause of death in patients with advanced gastric carcinoma. Peritoneal carcinomatosis also commonly originates from colorectal cancer and is present in approximately 10% of patients at the time of first diagnosis and in about 25% of patients with recurrent disease. It is the second most frequent cause of death in colorectal cancer after metastatic disease to the liver. In an estimated 25% of patients, no other tumour locations can be found [13,14].

Another rare tumour type of PC, is peritoneal mesothelioma. In the USA, each year two cases per million are reported. This tumour arises from the serosal lining of the peritoneum. A mesothelioma may involve the pleura, pericardium or peritoneum, and 30-40% has peritoneal manifestations. The tumour can be classified as benign, borderline malignant, or malignant. Benign mesothelioma is a papillary tumour of considerable firmness, while malignant mesothelioma covers the surface of the mesentery and can obliterate the entire peritoneal cavity. Malignant peritoneal mesothelioma is associated with asbestos exposure and is most common in middle age men [15].

Pseudomyxoma peritonei is another distinct subtype of peritoneal carcinomatosis with an approximate incidence of one per million per year. It is caused by rupture of a mucinous cystadenoma of cystadenocarcinoma. The primary tumour usually originates in the ovary or appendix and the mucinous material spreads to the peritoneal surfaces and omentum [16].

Systemic Therapy

Since 1979, cisplatin-based multiagent chemotherapy has been regarded as the standard treatment for patients with *papillary serous ovarian carcinoma* (PSOC). In 1996, a randomized GOG trial demonstrated a significant survival advantage for patients with advanced epithelial ovarian cancer whose residual disease was > 1.0 cm, after cytoreduction, treated with paclitaxel plus cisplatin compared to similar patients who were treated with cisplatin plus cyclophosphamide [17]. As a

result of this study, the combination of paclitaxel and cisplatin is considered first-line chemotherapy for patients with epithelial ovarian cancer.

Platinum analogues remain the most active agents for the treatment of this disease, with the first trials having used cisplatin. The toxic effects associated with cisplatin led to the substitution of cisplatin.for carboplatin. The most commonly used standard regimen consists of a combination of carboplatin at a dose calculated to produce an area under the concentration-time curve of 5.0 to 7.5 with paclitaxel at a dose of 175 mg per square meter of body-surface area over a three-hour period. This regimen produces response rates of about 90%. These data are confirmed in a large randomised study that demonstrated a superior survival to a platinum agent in combination with paclitaxel over single agent platinum [18].
Despite high response rates, relapses occur in most patients. Therefore, efforts have been made to deliver chemotherapy in a more optimal way. Because of the unique pattern of peritoneal spread of epithelial ovarian cancer, the intraperitoneal delivery of cytotoxic drugs is very attractive and it allows a several-fold increase of drug concentration in the abdominal cavity that is not possible to achieve with intravenous drug administration [19]. The Gynecologic Oncology Group conducted a randomised, phase III trial that compared intravenous paclitaxel and cisplatin with intravenous paclitaxel plus intraperitoneal cisplatin and paclitaxel in 415 patients with stage III ovarian cancer who had undergone optimal debulking [20]. With a median follow-up of 50 months, there was a significantly prolongation of progression free survival and overall survival in the intraperitoneal group (5.5 months and 15.9 months respectively) associated with a reduction of 25% in the risk of death. However, intraperitoneal therapy was associated with a high incidence of catheter related complications and more gastrointestinal, metabolic and neurological toxicities. Only 42% of the patients in the intraperitoneal-therapy group completed six cycles of the assigned therapy. In summary, the data from this study demonstrated a significant survival benefit for combined intraperitoneal and intravenously therapy in patients with optimally debulked stage III disease but efforts will be necessary in order to improve the tolerability of intraperitoneal therapy [20].

Histopathological similarities have been drawn between *primary peritoneal carcinomatosis* (PPC) and papillary serous adenocarcinoma of the ovaries [21]. Several authors [6,8,9] have reported that the response of patients with PPC to platinum-based chemotherapy is similar to that of patients with serous ovarian carcinoma and have subsequently recommended treating patients with PPC in a fashion similar to that used in patients with epithelial ovarian cancer. These findings were confirmed in a prospective phase II Study of the Gynecologic Oncology Group [22]. The goal of this study was first, to assess the clinical effectiveness of cisplatin and cyclophosphamide in a well-defined group of women with extraovarian peritoneal serous papillary carcinoma (EPSPC). Secondly, these results were compared with those of a group of patients with papillary serous ovarian carcinoma (PSOC) who received identical therapy. Of note, cisplatin (75 mg/m²) and cyclophosphamide (750 mg/m²) were to be given within 6 weeks after cytoreductive therapy every 21 days for six cycles. Comparing these two prospective trials

confirms the findings of numerous retrospective reports. Patients with extraovarian peritoneal serous papillary carcinoma respond to surgery followed by cisplatin and cytoxan chemotherapy in a manner very similar to that of patients with papillary serous ovarian carcinoma [5,7-9]. This is not only true for clinical response but also, apparently, for pathologically confirmed response determined by a reassessment surgery. Additionally, the incidences of toxicity to treatment in these two groups of patients are similar. However, platinum based chemotherapy in combination with paclitaxel is now the standard of care for advanced ovarian cancer. Therefore, this regimen should also be tested in patients with extraovarian peritoneal serous papillary carcinoma. A recent study compared retrospectively the effect of 6 cycles of paclitaxel and platinum based chemotherapy in the treatment of EPSPC and PSOC [23]. Thirty-two patients with EPSPC and 43 patients with PSOC with FIGO stage III disease were included. The response and overall survival rates were similar in the two study groups. In conclusion, it appears that response to chemotherapeutic agents occurs as frequently in EPSPC patients as in those with PSOC. The reports supporting this notion are fairly consistent, but rather scarce. For example, a recent literature review on EPSPC by Eltabbakh and Pirer [24,25] summarized ten publications that reported the proportion responding to several different front-line chemotherapeutic regimens. The median number of patients evaluated on any specific regimen in any particular study was three (range 1-29). When patients with EPSPC are excluded from clinical trials in which a new regimen is deemed active, clinicians are forced to extrapolate the results without any data on EPSPC patients. It is important, though, that trials are appropriately sized to permit reasonable estimates of treatment effect in both EPSPS and PSOC patients. This approach is not without it own problems, though. First, estimating interactions between treatment and the origin of the disease will often be underpowered even in moderately sized studies. Second, as Scully [26] warned: 'It is likely that some tumours designated as primary peritoneal carcinoma according to the current criteria are actually small ovarian tumours that find the peritoneum a more hospitable site for growth than the ovaries.' Misclassification of patients based on the disease origin can further reduce the statistical power for detecting an important interaction with treatment. However, based on the results of this trial, the GOG has included patients with EPSPC in many of its subsequent ovarian trials.

As already noted, *PC of colorectal origin* is common and the second most frequent cause of death in colorectal cancer after metastatic disease to the liver [13]. Sugarbaker has suggested that PC of colorectal origin should probably not be equated with generalized disease, but can be a first step of dissemination [14]. Based on this concept, attempts have been made to achieve long-term survival in patients with PC by combining surgery and intraperitoneal chemotherapy to eradicate microscopic residual disease. In recent years, several phase II and one randomized phase III study have shown that this therapy improves survival to a median of 2 years and that approximately 20% of patients live longer then 5 years and are probably cured [27-29].

In 2003 a phase III randomized single-institution study of Verwaal et al. from The Netherlands Cancer Institute was published in the Journal of Clinical Oncol-

ogy [29]. The purpose of the study was to confirm the findings from uncontrolled studies that aggressive cytoreduction in combination with hyperthermic intraperitoneal chemotherapy (HIPEC) is superior to standard treatment in patients with PC of colorectal origin. In this study systemic adjuvant chemotherapy after cytoreduction and HIPEC was given, using the modified Laufman regimen [30] (5-Fluorouracil intravenous push-dose of 400 mg/m² and Leucovorin 80 mg/m², weekly). Treatment was not started until at least 6 weeks after HIPEC and was continued for 26 weeks, or until progression, death, or unacceptable toxicity. If patients had been treated with 5-Fluorouracil within a year prior to HIPEC, they were treated with Irinotecan (350 mg/m²) at three weekly intervals for 6 months or until progression or intolerable toxicity, instead of 5-Fluorouracil. This moderately dosed regimen of 5-FU/Leucovorin, was a convenient outpatient regimen with only minimal gastrointestinal toxicity or other toxicity. Recently, somewhat more aggressive schedules of combination chemotherapy have been introduced in advanced colorectal cancer, which are associated with a small survival benefit [31,32]. It is possible that the use of these contemporary chemotherapy schedules would have slightly prolonged survival. The combination of cytoreduction and HIPEC with continuous 5-FU-Leucovorin, irinotecan, and/or oxaliplatin seems certainly promising for further outcome improvements.

In the situation of hyperthermic intraperitoneal chemotherapy for peritoneal carcinomatosis of ovarian or breast origin and for peritoneal mesothelioma frequently cisplatin 50 mg/m² and doxorubicin 15 mg/m² are used as chemotherapeutic agents. In peritoneal carcinomatosis of colorectal or stomach origin mitomycine 12.5 mg/m² is frequently used.

Cytoreductive surgery combined with hyperthermic intra-peritoneal chemotherapy is a novel treatment for patients with peritoneal carcinomatosis. In recent years, several phase II and phase III studies have shown that this therapy improves survival. At this moment it remains to be shown that the encouraging results of uncontrolled studies are not the result of patient selection. The need for a controlled study is particularly urgent because HIPEC is associated with significant morbidity and treatment related mortality. The potential improvement in survival has to be balanced against the side effects of this intensive treatment.

Another urgent question to be answered is the role of adjuvant treatment after cytoreduction and HIPEC. At this moment, available data stem only from retrospective studies including a heterogenous and often quite small study population.

Conclusion

It is difficult to give a standard chemotherapeutic regimen in PC. The small and heterogeneous population, the small number of randomized trials are the main reasons for this lack in uniformity. As a result, every PC patient needs an individualized decision based on multidisciplinary consensus.

Peritoneal carcinomatosis is also an uncultivated area of clinical-scientific work. Targeted therapies such as VEGF or EGFR inhibitors need urgently be investigated in this indication.

References

1. Truong LD, Maccato ML, Awalt H, Cagle PT, Schwarts MR, Kaplan AL. (1990) Serous surface carcinoma of the peritoneum: a clinicopathologic study of 22 cases. Hum Pathol 1990;21:99-110
2. White CD. (1993) Papillary intraperitoneal neoplasia resembling ovarian carcinoma after removal of benign ovaries. W V Med J 89:282-283
3. Shah IA, Jayram L, Gani OS, Fox IS, Stanley TM. (1998) Papillary serous carcinoma of the peritoneum in a man. A case report. Cancer 82:860-866
4. Swerdlow M. (1959) Mesothelioma of the pelvic peritoneum reassembly papillary cystadenocarcinoma of the ovary: case report. Am J Obstet Gynecol 77:197-200
5. Dalrymple JC, Bannatyne P, Russell HJ, Solomon HJ, Tattersall MHN, Atkinson K, et al. (1989) Extraovarian peritoneal serous papillary carcinoma: a clinicopathological study of 31 cases. Cancer 64:110-115
6. Ranson DT, Shreyaskumar RP, Keeney GL, Malkasian GD, Edmonson JH. (1990) Papillary serous carcinoma of the peritoneum. Cancer 1990;66:1091-4
7. Fromm GL, Gershenson DM, Silva EG. Papillary serous carcinoma of the peritoneum. Obstet Gynecol 75:75-89
8. Lele SB, Piver MJ, Matharu J, Tsukadu Y. (1988) Peritoneal papillary carcinoma. Gynecol Oncol 31:315-320
9. Bloss JD, Shu-Yuan L, Buller RE, Manetta A, Berman ML, MC-Meekin S, et al. (1993) Extraovarian peritoneal serous papillary carcinoma: a case-control retrospective comparison to papillary adenocarcinoma of the ovary. Gynecol Oncol 50:347-351
10. Therasse P, Arbuck SG, Eisenhauer EA, et al. (2000) New guidelines to evaluate the response to treatment in solid tumours. European Organization for Research and Treatment of Cancer, National Cancer Institute of the United States, National Cancer Institute of Canada. J Natl Cancer Inst 92:205-216
11. Stafford-Johnson DB, Bree RL, Francis IR, Korobkin M. (1998) CT appearance of primary papillary serous carcinoma of the peritoneum. AJR Am J Roentgenol 171:687-689
12. Schorge JO, Muto MG, Welch WR, Bandera CA, Rubin SC, Bell DA, Berkowitz RS, Mok SC. (1998) Molecular evidence for multifocal papillary serous carcinoma of the peritoneum in patients with germline BRCA1 mutations. J Natl Cancer Inst 90(11):797-799
13. Minsky BD, Mies C, Rich TA, et al. (1988) Potentially curative surgery of colon cancer : Patterns of failure and survival. J Clin Oncol 6:106-118

14. Sugarbaker PH. (1988) Intraperitoneal chemotherapy and cytoreductive surgery for the prevention and treatment of peritoneal carcinomatosis and sarcomatosis. Semin Surg Oncol 14:254-261
15. Warhol MS, Hunter NJ, Corson JM. (1982) An ultrastructural comparison of mesotheliomas and adenocarcinoma of the ovary and endometrium. Int J Gynecol Pathol (1982) 1:125-134
16. Rezkalla MA, Peterson KG, Ryan JJ. (2006) Pseudomyxoma peritonei: a case of mucinous adenocarcinoma of the appendix presenting as inguinal hernia. S D Med 59:54-5,57
17. McGuire WP, Hoskins WJ, Brady MF, Kucera PR, Patridge EE, Look KY, Clarke-Pearson DL, Davidson M. (1996) Cyclophosphamide and cisplatin compared with paclitaxel in patients with stage III and IV ovarian cancer. N Engl J Med 334:1-6
18. Parmer MK, Ledermann JA, Colombo N, du Bois A, Delaloye JF, Kristensen GB, et al. ICON and AGO Collaborators. (2003) Paclitaxel plus platinum-based chemotherapy versus conventional platinum-based chemotherapy in women with relapsed ovarian cancer: the ICON4/AGO-OVAR-2.2 trial. Lancet 21:2099-2106
19. Rothenberg ML, Liu PY, Braly PS, Wilczynski SP, Hannigan EV, Wadler S, Stuart G, Jiang C, Markman M, Alberts DS. (2003) Combined intraperitoneal and intravenous chemotherapy for women with optimally debulked ovarian cancer: results from an intergroup phase II trial. J Clin Oncol 21:1313-1319
20. Armstrong DK, Bundy B, Wenzel L, Huang HQ, Baergen R, Lele S, Copeland LJ, Walker JL, Burger RA; Gynecologic Oncology Group. (2006) Intraperitoneal cisplatin and paclitaxel in ovarian cancer. N Engl J Med 354:34-43
21. Raju U, Fine G, Greenwald KA, Ohrodnik JM. (1989) Primary papillary serous neoplasia of the peritoneum: A clinicopathologic and ultrastructural study of eight cases. Hum Pathol 20:426-436
22. Bloss JD, Brady MF, Shu Yaun Liau, Rocereto T, Partridge EE, Clarke-Pearson DL. (2003) Extraovarian peritoneal serous papillary carcinoma: a phase II trial of cisplatin and cyclophosphamide with comparison to a cohort with papillary serous ovarian carcinoma- a Gynecologic Oncology Group Study. 89:148-154
23. Ayhan A, Taskiran C, Yigit-Celik N, Bozdag G, Gultekin M, Usubutun A, Guler N, Yuce K. (2006) Long-term survival after paclitaxel plus platinum-based combination chemotherapy for extraovarian peritoneal serous papillary carcinoma: is it different from that for ovarian serous papillary cancer? Int J Gynecol Cancer 16:484-489
24. Scully RE. (1998) The Eltabbakh/Piver article reviewed. Oncology 12:820-825
25. Nam JH, Kim YM, Jung MH, et al. (2006) Primary peritoneal carcinoma: experience with cytoreductive surgery and combination chemotherapy. Int J Gynecol Cancer 16:23-28

26. Piver MS, Eltabbakh GH, Hempling RE, Recio FO, Blumenson LE. (1997) Two sequential studies for primary peritoneal carcinoma: induction with weekly cisplatin followed by either cisplatin-doxorubicin-cyclophosphamide or paclitaxel-cisplatin. Gynecol Oncol 67:141-146
27. Sugarbaker PH, Chang D. (1999) Results of treatment of 385 patients with peritoneal surface spread of appendiceal malignancy. Ann Surg Oncol 6:727-731
28. Witkamp AJ, de Bree E, Kaag MM, et al. (2001) Extensive cytoreductive surgery followed by intra-operative hyperthermic intraperitoneal chemotherapy with mitomycine-C in patients with peritoneal carcinomatosis of colorectal origine. Eur J Cancer 37:979-984
29. Verwaal VJ, van Ruth S, de Bree E, et al. (2003) Randomized trial of cytoreduction and hyperthermic intraperitoneal chemotherapy versus systemic chemotherapy and palliative surgery in patients with peritoneal carcinomatosis of colorectal cancer. J Clin Oncol 21:3737-3743
30. Laufman LR, Krzeczowski KA, Roach R, et al. (1987) Leucovorin plus 5-fluorouracil: An effective treatment for metastatic colon cancer. J Clin Oncol 5:1394-1400
31. Andre T, Louvet C, Maindrault-Goebel F, et al. (1999) CPT-11 (irinotecan) addition to bimonthly, high-dose leucovorin and bolus and continuous infusion 5-fluorouracil (FOLFIRI) for pretreated metastatic colorectal cancer – GERCOR. Eur J Cancer 35:1343-1347
32. De Gramont A, Figer A, Seymour M, et al. (2000) Leucovorin and fluorouracil with or without oxaliplatin as first-line treatment in advanced colorectal cancer. J Clin Oncol 18:2938-2947

The Role of Radiotherapy in the treatment of Peritoneal Carcinomatosis

T Boterberg, W Duthoy, W De Neve

Introduction

Despite the fact that radiotherapy (RT) is usually considered and used as a local treatment, it plays an important role in the treatment of disseminated disease too. For painful bone metastases for example, RT is the primary and most effective palliative treatment [1]. Currently, there is growing interest in investigating the role of conformal and stereotactic RT for e.g. liver metastases, even with curative intent [2].

During the past decades, radiation oncologists have used RT to treat peritoneal carcinomatosis (PC) in ovarian cancer, endometrial cancer and gastrointestinal cancer, either as an adjuvant treatment modality, or in palliative situations. Especially in the early days of RT, the results were disappointing because the equipment was not fit for such treatments. With orthovoltage and even with cobalt machines, the energy of the radiation was too low. Another problem was the lack of good dose calculation systems. This resulted in overdosing the superficial tissues and underdosing the actual target, being the peritoneal cavity.

The development of megavoltage linear accelerators and the improving possibilities to calculate dose distributions more accurately generated new interest in the treatment of the whole abdomen with RT. However, the problem of dose-limiting organs like liver and kidneys could not yet be resolved. The dose limits for median dose to kidneys and liver are usually estimated at 20 Gray (Gy) and 30 Gy respectively. Using cerrobend blocks to protect these organs also resulted in a decreased dose to the peritoneum surrounding these organs, while the peritoneum is the actual target for radiotherapy in PC. Apart from this, the discovery of chemotherapeutic agents, which were active in especially ovarian cancer, put RT to the second front. However, recent developments in RT techniques such as intensity modulated arc therapy (IMAT) [3] and improvements in dose calculations may give a new boost to the use of whole abdominal irradiation in the treatment of PC.

Another approach is the use of radioactive isotopes that are instilled in the peritoneal cavity. They can be used as such or bound to antibodies. These techniques belong more to the domain of the nuclear medicine specialists and will not be discussed in this chapter.

PC Originating from Gastrointestinal Cancers

The data on radiotherapeutic treatment of PC of gastrointestinal origin are limited despite the fact that it is a rather common condition in primary tumours originating from the rectum, the colon, the stomach or the pancreas. The presence of peritoneal disease at diagnosis usually reflects the very aggressive nature of the primary tumour. The majority of patients with PC at diagnosis will present with synchronous metastases in liver and/or lungs, requiring systemic chemotherapy or in some cases even requiring no therapy at all, except just supportive care. If PC develops later on in the course of the disease, it is usually also associated with disseminated metastases, again requiring systemic treatment if at all useful. The treatment of isolated PC with surgery and/or intraperitoneal chemotherapy with or without hyperthermia is discussed extensively in other chapters of this book.

Local RT with or without chemotherapy is considered as the standard adjuvant treatment in rectal cancer [4,5], but RT has rarely been used to treat PC of rectal origin. Moreover, as said earlier, in most cases PC of rectal origin is accompanied by systemic disease in lungs and liver, which makes chemotherapy a more appropriate treatment modality. Especially during the last two decades, new chemotherapeutic agents like irinotecan, oxaliplatin and even more recently (immuno)-biological agents like angiogenesis inhibitors, antibodies against epidermal growth factor receptor and several kinase inhibitors have become available. Their use has resulted in a significant increase in survival, even in patients with advanced metastatic disease [6].

For colon cancer, the situation is somewhat different, although no randomized prospective trials with RT as a treatment modality for PC have been published. Theoretically, PC can be expected more readily in colon cancer than in rectal cancer since in transmurally growing tumours the entire peritoneal cavity is at risk for contamination with cancer cells. The extraperitoneal rectum is surrounded by the mesorectal fascia and is not in direct contact with the peritoneal cavity. On the other hand, RT is mostly used for rectal cancer and its role in colon carcinoma is controversial while the benefit of chemotherapy is proven [7]. One of the earliest reports comes from Brenner et al. who published a small series of 42 colon cancer patients treated in the 70s with whole abdominal RT after surgery or treated with surgery alone [8]. In a retrospective analysis, the survival in the RT group was better (65% versus 35% 5-year overall survival) but the difference was not statistically significant. In the 80s, Wong et al. published a retrospective analysis of 55 patients treated with whole abdominal irradiation after surgical excision of colon

cancer [9]. The results for patients with gross residual disease after surgery or peritoneal seeding discovered at surgery were disappointing with all patients dying from local or peritoneal metastases. The results for the patients irradiated on the whole peritoneal cavity following complete resection and considered to be at high risk for peritoneal seeding were encouraging with 55% 5-year survival. Although a prospective randomized trial was announced in this paper, it was never published. Others reported 5-year survival rates of 67% and even 78% in small series where patients were treated with abdominal RT combined with 5-fluorouracil chemotherapy after surgical excision for colon cancer [10,11]. Despite this adjuvant treatment 20% to 30% developed PC afterwards. The dose was usually limited to 30 Gy in 1-1.5 Gy fractions with constraints on the kidneys to 15 Gy and on the liver to 15-20 Gy. Most patients received a boost of 16-20 Gy to the primary tumour site. It is highly unlikely that 30 Gy is sufficient to control microscopic peritoneal disease, let alone gross residual disease, given the knowledge that local control is only achieved with doses of at least 35-40 Gy [12], as derived from the data in rectal cancer. Toxicity was judged as moderate with patients requiring surgical interventions in about 5%.

In PC originating from gastric or pancreatic cancer, no solid data on the use of RT are available. The treatment of this condition is difficult and will often be limited to palliative care. Mostly PC in these tumours has a very poor prognosis with high mortality rates and poor quality of life. In some palliative situations, whole abdominal RT is used to alleviate obstruction or pain. However, the side effects of this treatment should be carefully weighed against the possible benefits. The role of chemotherapy is usually also limited. In some patients, aggressive treatment with cytoreductive surgery followed by intraoperative hyperthermic peritoneal chemotherapy is an option [13] as is extensively discussed elsewhere in this book.

PC Originating from Gynaecological Cancers

Cancers of the uterus and the ovaries are probably conditions with the highest probability for PC in which abdominopelvic RT has been used most frequently. However, once again, almost no published prospective randomized trials are available and the role for RT in both diseases remains controversial and is not clearly defined until now. Already in the 70s a multimodality approach with surgery, chemotherapy, second look surgery and RT was described for the treatment of advanced ovarian cancer [14], as was whole abdominal radiation as a salvage therapy for ovarian cancer [15] in the 80s. In the same period whole abdominal radiation was used for the treatment of endometrial cancer [16]. In all cases the risk for developing PC was recognized as the rationale to treat the whole peritoneal cavity with RT.

Probably one of the first prospective trials in stage II ovarian cancer compared whole abdominal radiation with a pelvic boost (30 Gy and 20 Gy respectively) to pelvic radiation alone combined with oral melphalan [17]. The results were not impressive with the latter treatment actually being better. The introduction of platinum-based chemotherapy radically changed the treatment of this disease. A randomized trial comparing carboplatin with carboplatin followed by RT showed no additional benefit of whole abdominal RT [18]. Reviewing several series, Dembo showed that whole abdominal radiotherapy for ovarian cancer was only useful when microscopic disease was present after surgery [19]. Radiotherapy was not able to control macroscopic residual disease. However, in recurrent ovarian cancer with PC resulting in gastrointestinal obstruction, abdominopelvic RT may be of significant importance as a palliative treatment [20]. However, limiting the dose to the kidneys remains a challenge when performing this type of treatment especially since most patients have been heavily pretreated with nephrotoxic chemotherapy and may have reduced kidney function. Our group developed intensity modulated arc therapy as a possible solution for this problem [21]. Some more details about this technique and its results are given more extensively in the last paragraph of this chapter.

While for ovarian cancer most studies are relatively old, for uterine cancer the role of abdominopelvic RT has been addressed in much more recent studies. In patients with stage III and IV disease, a recent Gynecologic Oncology Group (GOG) phase III randomized study [22] showed that abdominopelvic RT has tolerable toxicity, but that when compared with chemotherapy using cisplatin and doxorubicin, chemotherapy is superior to abdominopelvic RT for progression free and overall survival. Nevertheless, the authors warn, further advances in efficacy and reduction in toxicity are clearly needed, as only 2/3 of the chemotherapy group completed their therapy as scheduled. For stage I and II papillary serous or clear cell carcinoma of the endometrium another study of the GOG was recently published [23]. After hysterectomy, patients were treated with abdominopelvic RT (30 Gy) followed by a pelvic boost to 50 Gy. Over half of the treatment failures were found within the radiation field, suggesting that RT alone is not sufficient to control peritoneal or pelvic disease. The addition of chemotherapy may increase the survival of these patients, as is suggested by a study combining abdominopelvic RT with paclitaxel and platinum-based chemotherapy [24].

PC Originating from Other Cancers

Reports on the use of RT in PC originating from other cancers are very limited and usually anecdotal. Pseudomyxoma peritonei is an unusual condition with massive amounts of mucinous ascites and the presence of mucinous peritoneal and omental implants. Surgical debulking is essential, but the role of intraperitoneal chemotherapy and RT is controversial [25], although RT has been used with success in a case with a survival of 16 years after initial presentation [26] and in a se-

ries of 38 patients treated between 1954 and 1978 at M.D. Anderson Hospital [27]. Once again, surgical debulking was the mainstay of the treatment, but the results in a small number of patients treated with either whole abdominal or limited abdominal RT in addition to surgery suggested that this modality might offer improved survival. In this retrospective analysis, the results for adjuvant chemotherapy were definitely worse with a 5-year survival of 44% compared to 75% with adjuvant RT. Unfortunately, later on randomized prospective trials have not been conducted to compare these modalities and yield statistically significant results. Finally, peritoneal mesothelioma is also a rare disease in which the mesothelium of the peritoneum itself is the origin of the tumour. Some early reports describe long-term survival in patients treated with RT [28], but no large series are available. Another small series of patients treated between 1968 and 1985 describes surgical debulking, combination chemotherapy and whole abdominal irradiation [29]. Six of the ten multimodality treated patients remained free of disease at 19 to 78 months after diagnosis. The four patients that were not treated with this multimodality approach died with disease. Once again, during the 90s, this approach seems to disappear or at least no reports from large trials are published. However, especially in pleural malignant mesothelioma the combined modality approach is again getting more and more attention after the publication of a phase II trial combining extrapleural pneumonectomy with RT to a dose of 54 Gy [30]. Local recurrence was dramatically reduced. These results have been confirmed [31] and currently, trials are running in pleural mesothelioma combining this approach with neoadjuvant chemotherapy.

Conclusions and Future Prospects for Radiotherapy - the Role of Intensity Modulated Arc Therapy

Obviously, the role of RT in the treatment of PC is not obvious, as is the case with many other treatment modalities for this disease lacking good clinical trials. However, some of the drawbacks that make clinicians reluctant to use RT to treat PC may be overcome by using modern RT techniques such as whole abdominopelvic RT with intensity modulated arc therapy (IMAT). Our group described the first clinical experience with this technique in 5 patients with relapsed ovarian cancer [21] after several lines of chemotherapy and repeat surgery in some of them. Additional surgery is often difficult because of the development of adhesions or the poor condition of the patients. Moreover, 10-15% of patients dies within 8 weeks after surgery and 35-38% has no clinical benefit [32,33]. The results of chemotherapy for bowel obstruction are disappointing as well with responses around 5% [34]. On the other hand, RT may have response rates up to 80% in such patients [35]. With the pattern of spread of ovarian cancer, whole abdominopelvic radiation therapy (WAPRT) seems the preferred approach for these patients. However, as already mentioned, the maximum tolerated dose to the kidneys and the liver limits the final dose with conventional techniques. Applying blocks to shield the kidneys and liver allows increasing the dose, but also results

in underdosage of the peritoneum in the shielded regions. Our group showed that combining intensity modulation with arc therapy allows treating the target volume homogeneously while keeping the dose to the kidneys and the liver at a safe level. Intensity modulation gives the possibility to generate concave dose distributions, e.g. when the target volume (the peritoneal cavity) is wrapped around a dose-limiting organ at risk (the kidneys). If this target volume has a large internal radius, an increasing number of beam incidences is necessary to avoid underdosage of the target volume, with an infinite number of incidences being the ideal situation. The fastest way to achieve this is arc therapy. This means that the gantry of the linear accelerator describes an arc (or turns) around the patient while delivering the dose. This technique resulted in a median dose of 33 Gy to the target volume (i.e. the whole peritoneal cavity) delivered in 22 fractions of 1.5 Gy. The median dose to the left kidney, right kidney and liver was 16 Gy, 14 Gy and 24 Gy respectively. The values for a conventional treatment plan (with an anterior and posterior field each with a segment with cerrobend block shielding for the kidneys) were 20 Gy, 19 Gy and 23 Gy respectively. The percentage of the target volume receiving 90% of the prescribed dose was 90% for the IMAT-plan and 80% for the conventional plan. The dose distributions for an IMAT and a conventional plan are shown in Fig. 1.

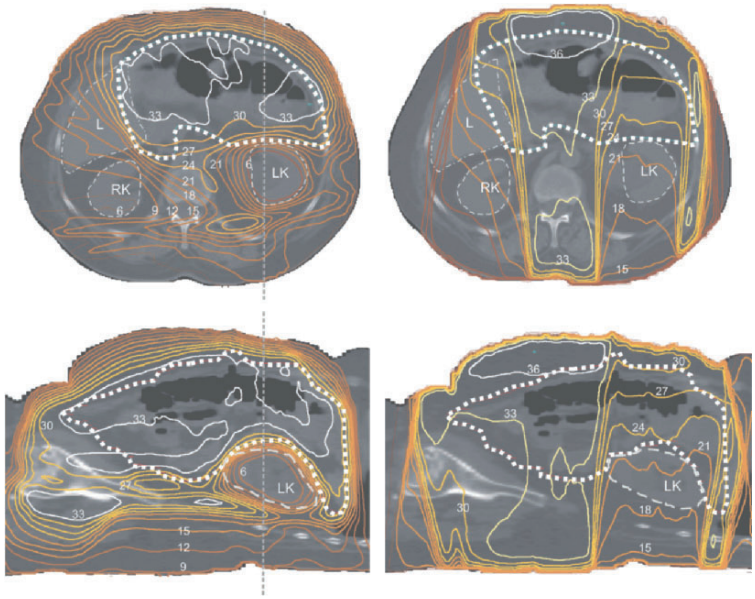


Figure 1. Dose distribution of an Intensity Modulated Arc Therapy (IMAT) and conventional plan. Transversal (upper row) and sagittal (lower row) sections of an IMAT (left) and conventional (right) plan. The white dotted line represents the target volume. The grey dotted line indicates the section level. L, liver, RK, right kidney, LK, left kidney. Figures on the isodoses are expressed in Gy. Note the difference in dose to the kidneys between both plans.

Using a 4-field technique for the conventional treatment plan did not significantly improve these figures as compared to the 2-field technique. IMAT was shown to be deliverable in an acceptable time slot, to produce dose distributions that are significantly more homogeneous than conventional treatment plans and to significantly reduce the dose to organs at risk like the kidneys. A major drawback is still that the planning is quite time-consuming, but improvements to the control software of the linear accelerator and the planning software should reduce this.

In conclusion, IMAT creates new opportunities to treat PC with RT resulting in less dose to organs at risk with the same or potentially higher doses to the target volume. Also, the combination with chemotherapy should be studied. Finally, the use of high resolution biological imaging may guide dose painting to escalate the dose selectively to the largest tumour deposits in order to increase the chances for local control.

References

1. Vakaet LA, Boterberg T. (2004) Pain control by ionizing radiation of bone metastasis. Int J Dev Biol 48(5-6):599-606
2. Schefter TE, Kavanagh BD, Timmerman RD, Cardenes HR, Baron A, Gaspar LE. (2005) A phase I trial of stereotactic body radiation therapy (SBRT) for liver metastases. Int J Radiat Oncol Biol Phys 62(5):1371
3. Duthoy W, De Gersem W, Vergote K, Coghe M, Boterberg T, De Deene Y, De Wagter C, Van Belle S, De Neve W. (2003) Whole abdominopelvic radiotherapy (WAPRT) using intensity-modulated arc therapy (IMAT): first clinical experience. Int J Radiat Oncol Biol Phys 57(4):1019-1032
4. Ortholan C, Francois E, Thomas O, Benchimol D, Baulieux J, Bosset JF, Gerard JP. (2006) Role of radiotherapy with surgery for T3 and resectable T4 rectal cancer: evidence from randomized trials. Dis Colon Rectum 49(3):302-310
5. Ceelen W, Pattyn P, Boterberg T, Peeters M. (2006) Pre-operative combined modality therapy in the management of locally advanced rectal cancer. Eur J Surg Oncol 32(3):259-268
6. Saunders M, Iveson T. (2006) Management of advanced colorectal cancer: state of the art. Br J Cancer 95(2):131-138
7. Mendenhall WM, Amos EH, Rout WR, Zlotecki RA, Hochwald SN, Cance WG. (2004) Adjuvant postoperative radiotherapy for colon carcinoma. Cancer. 101(6):1338-1344
8. Brenner HJ, Bibi C, Chaitchik S. (1983) Adjuvant therapy for Dukes C adenocarcinoma of colon. Int J Radiat Oncol Biol Phys 9(12):1789-1792
9. Wong CS, Harwood AR, Cummings BJ, Keane TJ, Thomas GM, Rider WD. (1984) Total abdominal irradiation for cancer of the colon. Radiother Oncol 2(3):209-214

10. Ben-Josef E, Court WS. (1995) Whole abdominal radiotherapy and concomitant 5-fluorouracil ad adjuvant therapy in advanced colon cancer. Dis Colon Rectum 38:1088-1092
11. Fabian C, Giri S, Estes N, Tangen CM, Poplin E, Vogel S, Goodwin W, Rivkin S, Fleming TR, Macdonald JS. (1995) Adjuvant continuous infusion 5-FU, whole-abdominal radiation, and tumor bed boost in high-risk stage III colon carcinoma: a Southwest Oncology Group Pilot study. Int J Radiat Oncol Biol Phys 32(2):457-464
12. Colorectal Cancer Collaborative Group. (2001) Adjuvant radiotherapy for rectal cancer: a systematic overview of 8,507 patients from 22 randomised trials. Lancet 358(9290):1291-1304
13. Brigand C, Arvieux C, Gilly FN, Glehen O. (2004) Treatment of peritoneal carcinomatosis in gastric cancers. Dig Dis 22(4):366-373
14. Piver MS, Barlow JJ, Lee FJ, Vongtama V. (1975) Sequential therapy for advanced ovarian adenocarcinoma: operation, chemotherapy, second-look laparotomy, and radiation therapy. Am J Obstet Gynecol 122(3):355-357
15. Hacker NF, Berek JS, Burnison CM, Heintz PM, Juillard GJ, Lagasse LD. (1985) Whole abdominal radiation as salvage therapy for epithelial ovarian cancer. Obstet Gynecol 65(1):60-66
16. Potish RA, Twiggs LB, Adcock LL, Prem KA. (1985) Role of whole abdominal radiation therapy in the management of endometrial cancer; prognostic importance of factors indicating peritoneal metastases. Gynecol Oncol 21(1):80-86
17. Piver MS, Lele SB, Patsner B, Krishnamsetty R, Emrich LJ. (1986) Stage II invasive adenocarcinoma of the ovary: results of treatment by whole abdominal radiation plus pelvic boost versus pelvic radiation plus oral melphalan chemotherapy. Gynecol Oncol 23(2):168-175
18. Lambert HE, Rustin GJ, Gregory WM, Nelstrop AE. (1993) A randomized trial comparing single-agent carboplatin with carboplatin followed by radiotherapy for advanced ovarian cancer: a North Thames Ovary Group study. J Clin Oncol 11(3):440-448
19. Dembo AJ. (1992) Epithelial ovarian cancer: the role of radiotherapy. Int J Radiat Oncol Biol Phys 22(5):835-845
20. Corn BW, Lanciano RM, Boente M, Hunter WM, Ladazack J, Ozols RF. (1994) Recurrent ovarian cancer. Effective radiotherapeutic palliation after chemotherapy failure. Cancer 74(11):2979-2983
21. Duthoy W, De Gersem W, Vergote K, Coghe M, Boterberg T, De Deene Y, De Wagter C, Van Belle S, De Neve W. (2003) Whole abdominopelvic radiotherapy (WAPRT) using intensity-modulated arc therapy (IMAT): first clinical experience. Int J Radiat Oncol Biol Phys 57(4):1019-1032
22. Randall ME, Filiaci VL, Muss H, Spirtos NM, Mannel RS, Fowler J, Thigpen JT, Benda JA. (2006) Gynecologic Oncology Group Study. Randomized phase III trial of whole-abdominal irradiation versus doxorubicin and cisplatin chemotherapy in advanced endometrial carcinoma: a Gynecologic Oncology Group Study. J Clin Oncol 24(1):36-44

23. Sutton G, Axelrod JH, Bundy BN, Roy T, Homesley H, Lee RB, Gehrig PA, Zaino R. (2006) Adjuvant whole abdominal irradiation in clinical stages I and II papillary serous or clear cell carcinoma of the endometrium: a phase II study of the Gynecologic Oncology Group. Gynecol Oncol 100(2):349-354
24. Steed H, Manchul L, Rosen B, Fyles A, Lockwood G, Laframboise S, Murphy J, Milosevic M, Chapman W, Oza AM. (2006) Uterine papillary serous carcinoma: evaluation of multimodality treatment with abdominopelvic radiotherapy and chemotherapy. Int J Gynecol Cancer 16 Suppl 1:278-285
25. Sherer DM, Abulafia O, Eliakim R. Pseudomyxoma peritonei: a review of current literature. (2001) Gynecol Obstet Invest 51(2):73-80
26. el Sayed S. Pseudomyxoma peritonei treated by radiotherapy. (1990) Clin Oncol (R Coll Radiol) 2(2):120-122
27. Fernandez RN, Daly JM. (1980) Pseudomyxoma peritonei. Arch Surg 115(4):409-414
28. Rogoff EE, Hilaris BS, Huvos AG. (1973) Long-term survival in patients with malignant peritoneal mesothelioma treated with irradiation. Cancer 32(3):656-664
29. Lederman GS, Recht A, Herman T, Osteen R, Corson J, Antman KH. (1987) Long-term survival in peritoneal mesothelioma. The role of radiotherapy and combined modality treatment. Cancer 59(11):1882-1886
30. Rusch VW, Rosenzweig K, Venkatraman E, Leon L, Raben A, Harrison L, Bains MS, Downey RJ, Ginsberg RJ. (2001) A phase II trial of surgical resection and adjuvant high-dose hemithoracic radiation for malignant pleural mesothelioma. J Thorac Cardiovasc Surg 122(4):788-795
31. Gupta V, Mychalczak B, Krug L, Flores R, Bains M, Rusch VW, Rosenzweig KE. (2005) Hemithoracic radiation therapy after pleurectomy/decortication for malignant pleural mesothelioma. Int J Radiat Oncol Biol Phys 63(4):1045-1052
32. Redman CW, Shafi MI, Ambrose S, Lawton FG, Blackledge GR, Luesley DM, Fielding JW, Chan KK. (1988) Survival following intestinal obstruction in ovarian cancer. Eur J Surg Oncol 14(5):383-386
33. Krebs HB, Goplerud DR. (1983) Surgical management of bowel obstruction in advanced ovarian carcinoma. Obstet Gynecol 61(3):327-330
34. Abu-Rustum NR, Barakat RR, Venkatraman E, Spriggs D. (1997) Chemotherapy and total parenteral nutrition for advanced ovarian cancer with bowel obstruction. Gynecol Oncol 64(3):493-495
35. Tinger A, Waldron T, Peluso N, Katin MJ, Dosoretz DE, Blitzer PH, Rubenstein JH, Garton GR, Nakfoor BA, Patrice SJ, Chuang L, Orr JW Jr. (2001) Effective palliative radiation therapy in advanced and recurrent ovarian carcinoma. Int J Radiat Oncol Biol Phys 51(5):1256-1263

Medical and Palliative Management of Malignant Ascites

Gerhild Becker

Introduction

Malignant ascites is defined as an abnormal accumulation of fluid in the peritoneal cavity as a consequence of cancer [1] and presents a difficult clinical problem causing discomfort and distress to many patients in the advanced stages of their disease. It accounts for around 10% of all cases of ascites and occurs in association with a variety of neoplasms, especially breast, bronchus, ovary, stomach, pancreas and colon cancer [2]. Up to 20% of all patients with malignant ascites have tumours of unknown primary origin [3]. Large amounts of ascites can cause increased abdominal pressure with troublesome symptoms like pain, dyspnea, loss of appetite, nausea, reduced mobility and problems with the body image.

Pathophysiology and Diagnosis

The pathophysiology of malignant ascites is multifactorial and as yet incompletely understood (chapter 6). Ascites may result from obstruction of lymphatic drainage by tumour cells that prevent absorption of intraperitoneal fluid and protein [4] as often seen in lymphomas and breast cancer [5]. Since the ascites of many patients with malignant ascites has a high protein content, alteration in vascular permeability has been implicated in the pathogenesis of ascites production [6]. Hormonal mechanisms are also involved. Due to decreased removal of fluid as a consequence of obstructed lymphatics, the circulating blood volume is reduced and this activates the renin-angiotensin-aldosterone system, leading to sodium retention. Therefore reduced sodium intake together with diuretics is often used to treat malignant ascites, but there is no consensus on effectiveness [7]. Compared to ascites caused by cirrhosis, malignant ascites usually contains more white blood cells and a higher level of lactate dehydrogenase [8, 9]. Diagnosis is confirmed by positive cytology of malignant cells in the fluid. A positive cytology result has a specifity of nearly 100% [2,10,11], but it is not very sensitive, with only about 60% of

malignant aspirates being cytologically positive [2,8]. Paracentesis and diuretics are the most commonly used procedures in management of malignant ascites followed by peritoneovenous shunts, diet measures and other modalities like systemic or intraperitoneal chemotherapy [7,12]. In contrast to the treatment of underlying cancer, there is no generally accepted gold standard for the management of malignant ascites so far.

Symptomatic Management by Paracentesis

Available data show good, although temporary relief of symptoms related to the build-up of fluid in about 90% of patients [13]. There is no consensus on fluid withdrawal speed. Several timings have been reported, varying from 30-90 min [14] to 24 h [15]. Possible complications of paracentesis include secondary peritonitis, pulmonary emboli and hypotension [16]. Repeated large volume paracentesis without plasma volume expansion may be associated with a significantly higher incidence of hypotension and of renal impairment. In the context of benign ascites due to liver disease there are several studies about this topic [2], but in the context of malignant ascites there is only limited evidence available. Fischer reported about 300 cases of abdominal paracentesis for malignant ascites where dextrose 5% was infused intravenously simultaneously and no episodes of severe hypotension were recorded [17]. Studies in patients with benign ascites showed that in paracentesis of a large volume albumin is superior to other plasma expanders in preventing circulatory dysfunction [18]. However, randomized studies showed no significant difference in survival between patients treated with albumin and those treated with other plasma expanders [18]. In patients with malignant ascites no trials of concurrent albumin infusions have been performed. Studies in the context of liver disease showed that up to 5 litres can be removed quickly without risk of significantly affecting plasma volume or renal function [19-21]. Stephenson and colleagues retrospectively analysed 30 paracenteses in 12 patients with malignant ascites after implementing a guideline allowing up to 5 L fluid to drain without clamping and giving intravenous fluids only when specifically indicated. In the analyzed 30 paracenteses intravenous fluids or blood products were given only in 6 procedures and there was no case of symptomatic hypotension [22]. McNamara did a prospective study in the context of malignant ascites observing 48 paracenteses in 44 patients in order to evaluate how much fluid needs to be drained for symptom relief. The results suggest that a significant improvement of the symptoms of abdominal pressure occurs with the removal of a few litres (range 0.8-15 liter, mean 5.3 liter, median 4.9 liter). Severe adverse effects were not reported and apparently patients did not get intravenous fluids, plasma expanders or blood products, but this was not explicitly specified [23]. There are no randomized trials comparing paracentesis with the use of diuretics in the management of malignant ascites.

Symptomatic Management by Diuretics

Diuretic use in managing malignant ascites is inconsistent among physicians. A survey by Lee and colleagues showed that diuretics were used by 61% of physicians treating malignant ascites but by only 45% felt this therapy to be effective [7]. There are no randomized controlled trials assessing the efficacy of diuretic therapy in malignant ascites. Neither efficacy nor effectiveness of diuretics in malignant ascites is sufficiently studied and therefore the evidence for the use of diuretics in malignant ascites is weak. Overall, in patients with different tumours, diuretics seem to be successful in approximately 43% of cases reported in the literature [13]. Phase II data suggest that the efficacy of diuretics in malignant ascites depends on plasma renin/aldosterone concentration [24]. In a study of Greenway and colleagues [25] 13 of 15 patients responded to spironolactone (doses varying from 150 to 450 mg) and plasma renin activity was raised in all of 5 patients in whom it was measured. In the prospective study by Pockros and colleagues [24] a response to diuretics was seen in patients with ascites due to massive hepatic metastases who had a serum-ascites albumin gradient > 1.1 g/dl (congruent to the serum-ascites albumin gradient of patients with benign ascites due to liver cirrhosis) whereas patients with ascites caused by peritoneal carcinomatosis or chylous malignant ascites who had no portal hypertension and a serum-ascites albumin gradient < 1.1 g/dl did not respond to diuretics.

These data suggest that the serum-ascites albumin gradient may provide a useful guide to predict response to diuretics. Up to now there is no approved reliable method for predicting which patients with malignant ascites will respond to diuretics. The renin-angiotensin-aldosterone system can be involved because of a reduction of the circulating blood volume due to decreased removal of fluid as a consequence of obstructed lymphatics. But this is not the case in all tumour patients [24]. This could explain why the data about diuretics in the context of malignant ascites are controversial and why there is no consensus about their effectiveness [7,12]. Further work is needed to identify clearly which patients will benefit from diuretic therapy.

Symptomatic Management by Peritoneovenous Shunts

Initially the peritoneovenous shunt was developed for use in patients with intractable ascites as a result of cirrhosis of the liver [6], but it subsequently became a popular procedure in managing malignant ascites [7]. There are two main types of shunt systems, the Le Veen shunt [26] and the Denver shunt [27]. The Le Veen shunt drains ascitic fluid into the superior vena cava by a one-way valve opening at a pressure of 3 cm H_2O. The Denver shunt works by the same principle. Here the valves open at a positive pressure gradient of about 1 cm H_2O and prevent detectable reflux. There have been no prospective randomized studies comparing the patency rates of the two systems in malignant ascites. One randomized trial has been performed in patients with cirrhotic ascites comparing 12 patients random-

ized to receive Le Veen shunt and 10 to receive Denver shunt. Data showed a superior patency of the Le Veen shunt [28]. Souter and colleagues evaluated 43 patients with malignant ascites, 16 receiving a Denver Shunt, 27 receiving a Le Veen shunt. They observed that shunt occlusion was more common with the Denver Shunt but the two groups of patients were not selected at random and therefore may not be comparable [29]. The objective of using shunts is to achieve symptom relief and prevent the need for distressing paracentesis and the resulting protein and fluid depletion. Hemorrhagic ascites and an ascitic fluid protein content greater than 4.5g/l are considered as contraindications for shunting because of the higher risk of shunt occlusion [6,30]. Loculated ascites, portal hypertension, coagulation disorders and advanced cardiac or renal failure are also contraindications [6]. Although the shunt drains fluid with malignant cells from the peritoneal space to venous system, clinical observations and findings at necropsy indicate that peritoneovenous shunting does not result in the establishment of clinically important haematogenous metastases [6,31]. However, as post-mortem examinations are not performed routinely, this complication may be under-reported [30]. Reported median survival of patients with malignant ascites varies between 52 and 266 days, reflecting patient selection [6]. In all reported studies, patients with ovarian and breast cancer who undergo peritoneovenous shunting have the best response rate ($\geq$ 50%) whereas the response rate in patients with gastrointestinal cancers is far worse (10% to 15%) [6]. Because of the poor prognosis it is agreed by most authors that shunt insertion is contraindicated in patients with malignant ascites due to gastrointestinal cancer [6,16]. An insertion of a shunt is associated with potentially fatal side-effects and costs in terms of time and money, considering that patients need to be monitored closely for at least 24 h after operation with a central venous pressure line to monitor fluid balance. Therefore a shunt should only be used when other treatment options like diuretics have failed and when the life expectancy of the patient is long enough to derive benefit. About the time span there is no consensus, some authors advocate more than one month [6,32] other authors suggest an expected survival of more than 3 months [16,33-34]. The use of shunts has to be balanced by the potential risks of this procedure [13].

New Treatments

There are also novel approaches in the management of malignant ascites. But all these treatment options must be considered as highly experimental, partially investigated in Phase I trials or applied in a limited amount of cases. Some improvement in ascites has been noted in response to immunotherapy with intraperitoneal α or β interferon [35], tumour necrosis factor TNF [36] or with administration of infectious agents in non pathogenic form like *Corynebacterium parvum* [37] or OK-432, a penicillin- and heat-treated powder of Su-strain *Streptococcus pyogenes A3* in the peritoneal cavity [38-39]. Monoclonal antibody therapy has also been used in treating malignant ascites with some success [40-41], as well as intraperitoneal radio-isotopes like ^{198}Au [42] or ^{32}CrP [43]. Octreotide, a somatostatin analogue

known to decrease the secretion of fluid by the intestinal mucosa and to increase water and electrolyte reabsorption [44] was used successfully in reducing ascites in two of three treated patients with malignant ascites [45]. In tumours associated with increased activity of vascular endothelial growth factor (VEGF) a new concept is to reduce the production of ascites by inhibition of neovascularization of the tumour via inhibition of VEGF [46-48] or inhibition of matrix metalloproteinases [49-52]. However, these treatment options must be considered as highly experimental, partially investigated in Phase I trials or applied in a limited amount of cases.

Management of Symptomatic Malignant Ascites

The practice of managing malignant ascites seems to be influenced by the evidence obtained in the context of non-malignant ascites due to liver disease, because approximately 80% of all cases of ascites are caused by chronic liver disease [2]. Although abdominal paracentesis, diuretics and peritoneovenous shunting are commonly used procedures in management of malignant ascites, there are no randomized controlled trials evaluating the efficacy and safety of these therapies.

A recently published systematic review critically summarized the evidence on the effectiveness of abdominal paracentesis, diuretics and peritoneovenous shunting and presented a guideline in order to get evidence to practice [13]. Grading of the evidence and the recommendations in the guideline are based on the revised grading system by the Scottish Intercollegiate Guidelines Network (SIGN) [53].

1. Paracentesis is indicated for those patients who have symptoms of increasing intra-abdominal pressure. Available data show good, although temporary relief of symptoms in most patients. Symptoms like discomfort, dyspnoea, nausea and vomiting seem to be significantly relieved by drainage of up to 5 litres of fluid. (Grade of Recommendation: D)

2. When removing up to 5 litres of fluid, intravenous fluids seem to be not routinely required. (Grade of Recommendation: D)

3. If a patient is hypotensive or dehydrated or known to have severe renal impairment and paracentesis is still indicated, intravenous hydration should be considered. Infusion therapy is not sufficiently studied. The only investigated therapy in malignant ascites is infusion of dextrose 5%. There is no evidence of concurrent albumin infusions in patients with malignant ascites. (Grade of Recommendation: D)

4. To avoid repeated paracenteses a peritoneovenous shunting may be considered. Major complications (pulmonary oedema, pulmonary emboli, clinically relevant disseminated intravascular coagulation, and infection)

have to be expected in about 6% of patients. (Grade of Recommendation: D)

5. There are no randomized controlled trials assessing the efficacy of diuretic therapy in malignant ascites. The available data are controversial and there are no clear predictors to identify which patients would benefit from diuretics. The use of diuretics therefore should be considered in all patients, but has to be evaluated individually. Patients with malignant ascites due to massive hepatic metastasis seem to respond more likely to diuretics than patients with malignant ascites caused by peritoneal carcinomatosis or chylous ascites. (Grade of Recommendation: D)

6. The choice of diuretics is not evaluated. As available data suggest that the efficacy of diuretics in malignant ascites depends on plasma renin/aldosterone concentration, aldosterone antagonists like spironolactone should be used, either alone or in combination with a loop diuretic. (Grade of Recommendation: D)

7. Dose regimens of diuretics are not evaluated in patients with malignant ascites. There is no evidence to diverge from standard clinical practice. Therefore dosage should be performed according to manufacturer's instructions and package inserts. (Grade of Recommendation: D)

References

1. Taber CW (1965) Taber's cyclopedic medical dictionary. Philadelphia (PA), FA Davies Co, A-92
2. Runyon BA (1994) Care of patients with ascites. N Engl J Med 330:337-342
3. Ringenberg QS, Doll DC, Loy TS, Yarbo JW (1989) Malignant ascites of unknown origin. Cancer 64:753-755
4. Garrison RN, Galloway RH, Heuser LS (1987) Mechanisms of malignant ascites production. J Surg Res 42:126-132
5. Olopade OI, Ultmann JE (1991) Malignant effusions. CA Cancer J Clin 41:166-179
6. Adam RA, Adam YG (2004) Malignant ascites: Past, Present, and Future. J Am Coll Surg 198:999-1011
7. Lee CW, Bociek G, Faught W (1998) A Survey of practive in management of malignant ascites. J Pain Symptom Manage 16:96-101
8. Runyon BA, Hoefs JC, Morgan TR (1988) Ascitic fluid analysis in malignancy related ascites. Hepatology 8:1104-1109
9. Salerno F, Restelli B, Incerti P, Annoni G, Capozza L, Badalamenti S, Lampertico P, Mojana E, Moser P, Tommasini M (1990) Utility of ascetic fluid analysis in patients with malignancy-related ascites. Scand J Gastroenterol 25:251-256

10. Castaldo G, Oriani G, Cimino L, Topa M, Mostarda I, Castellano L, Del Veccio-Blanco C, Budillon, G. Salvatore F, Saccetti L (1994) Total discrimination of peritoneal malignant ascites from cirrhosis and hepatocarcinoma-associated ascites by assays of ascetic cholesterol and lactate dehydrogenase. Clin Chem 40:478-483
11. Colli A, Buccino G, Cocciolo M, Parravinci R, Mariani M, Scaltrini G (1989) Diagnostic accuracy of sialic acid in the diagnosis of malignant ascites. Cancer 63:912-916
12. Newman G, Pudney D (2006) A Survey of Current Practice in the Management of Recurrent Malignant Ascites Among Oncologists and Palliative-care Physicians in the UK. Clin Oncol 18:154
13. Becker G, Galandi D, Blum HE (2006) Malignant ascites: Systematic review and guideline for treamtment. Eur J Cancer 42:589-597
14. Gotlieb WH, Feldman B, Feldman-Moran O, Zmira N, Kreizer D, Segal Y, Elran E, Ben-Baruch G (1998) Intraperitoneal pressures and clinical parameters of total paracentesis for palliation of symptomatic ascites in ovarian cancer. Gynecol Oncol 71:381-385
15. Appelqvist P, Silvo J, Salmela L, Kostiainen S (1982) On the treatment and prognosis of malignant ascites: is the survival time determined when the abdominal paracentesis is needed? J Surg Oncol 20:238-242
16. Parsons SL, Watson SA, Steele RJC (1996) Malignant ascites. Br J Surg 83:6-14
17. Fischer DS (1979) Abdominal paracentesis of malignant ascites. Arch Intern Med 139:235
18. Gines P, Cardenas A, Arroyo V, Rodes J (2004) Management of cirrhosis and ascites. N Engl J Med 350:1646-1654
19. Kao HW, Rakov NE, Savage E, Reynolds TB (1985) The effect of large volume paracentesis on plasma volume – a cause of hypovolemia? Hepatology 5:403-407
20. Kellerman PS, Linas SL (1990) Large volume paracentesis in treatment of ascites. Ann Intern Med 112:889-891
21. Reynolds TB (1990) Renaissance of paracentesis in the treatment of ascites. Adv Intern Med 112:365-374
22. Stephenson J, Gilbert J (2002) The development of clinical guidelines on paracentesis for ascites related to malignancy. Palliat Med 16:213-218
23. McNamara P (2000) Paracentesis – an effective method of symptom control in the palliative care setting? Palliat Med 14:62-64
24. Pockros PJ, Esrason KT, Nguyen C, Duque J, Woods S (1992) Mobilization of malignant ascites with diuretics is dependent on ascitic fluid characteristics. Gastroenterology 103:1302-1306
25. Greenway B, Johnson PJ, Williams R (1982) Control of malignant ascites with spironolactone. Br J Surg 69:441-442
26. LeVeen HH, Cristoudias G, Ip M, Luft R, Falk G, Grosberg S (1974) Peritoneovenous shunting for ascites. Ann Surg 180:580-590
27. Lund RH, Newkirk JB (1979) Peritoneovenous shunting system for surgical management of ascites. Contemp Surg 14:31-45

28. Fulenwider JT, Galambos JD, Smith RB 3rd, Henderson JM, Warren WD (1986) LeVeen vs. Denver peritoneovenous shunts for intractable ascites of cirrhosis. A randomized prospective trial. Arch Surg 121:351-355
29. Souter RG, Wells C, Tarin D, Kettlewell MG (1985) Surgical and pathologic complications associated with peritoneovenous shunts in management of malignant ascites. Cancer 55:1973-1978
30. Smith EM, Jayson GC (2003) The current and future management of malignant ascites. Clin Oncol 15:59-72
31. Tarin D, Price JE, Kettlewell MG, Souter RG, Vass AC, Crossley B (1984) Clinicopathological observations on metastasis in man studied in patients treated with peritoneovenous shunts. Br Med J 288:749-751
32. Gough IR (1984) Control of malignant ascites by peritoneovenous shunting. Cancer 54:2226-2230
33. Souter RG, Tarin D, Kettlewell MG (1983) Peritoneovenous shunts in the management of malignant ascites. Br J Surg 70:478-481
34. Wickremesekera SK, Stubbs RS (1997) Peritoneovenous shunting for malignant ascites. NZ Med J 110:33-35
35. Stuart GCE, Nation JG, Snider DD, Thinberg P (1993) Intraperitoneal interferon in the management of malignant ascites. Cancer 71:2027-2030
36. Räth U, Kaufmann M, Schmid H, Hofmann J, Wiedenman B, Kist A, Kempeni J, Schlick E, Bastert G, Kommerell B (1991) Effect of intraperitoneal recombinant human tumour necrosis factor alpha on malignant ascites. Eur J Cancer 27:121-137
37. Mahler F, Rapin CH, Macgee W (1988) Corynebacterium parvum as palliative treatment in malignant ascites. J Palliat Care 4:58-62
38. Katano M, Torisu M (1983) New approach to management of malignant ascites with a streptococcal preparation, OK-432. II. Intraperitoneal inflammatory cell-mediated tumour cell destruction. Surgery 93:365-373
39. Torisu M, Katano M, Kimura Y, Itoh H, Takesue M (1983) New approach to management of malignant ascites with a streptococcal preparation, OK-432. I. Improvement of host immunity and prolongation of survival. Surgery 93:357-364
40. Chen BM, Chan LY, Wang SM, Wu MF, Chern JW, Roffler SR (1997) Cure of malignant ascites and generation of protective immunity by monoclonal antibody-targeted activation of a glucuronide prodrug in rats. Int J Cancer 73:392-402
41. Hird V, Thomas H, Stewart JS, Epenetos AA (1989) Malignant ascites: review of the literature, and an update on monoclonal antibody-targeted therapy. Eur J Obstet Gynecol Reprod Biol 32:37-45
42. Ariel IM, Oropeza R, Pack GT (1966) Intracavitary administration of radioactive isotopes in the control of effusions due to cancer: Results in 267 patients. Cancer 8:1096-1102
43. Jackson, GL Blosser NM (1981) Intracavitary chromic phosphate (^{32}P) colloidal suspension therapy. Cancer 48:2596-2598
44. Twycross R, Wilcock A, Thorp S (1998) Palliative Care Formulary. Oxford, Radcliffe Medical Press

45. Cairns W, Malone R (1999) Octreotid as an agent for the relief of malignant ascites in palliative care patients. Palliat Med 13:429-430
46. Sherer DM, Eliakim R, Abulafia O (2000) The role of angiogenesis in the accumulation of peritoneal fluid in benign conditions and the development of malignant ascites in the female. Gynecol Ostet Invest 50:217-224
47. Xu L, Yoneda J, Herrera C, Wood J, Killion JJ, Fidler IJ (2000) Inhibition of malignant ascites and growth of human ovarian carcinoma by oral administration of a potent inhibitor of the vascular endothelial growth factor receptor tyrosine kinases. Int J Oncol 16:445-454
48. Zucker S, Lysik RM, Zarrabi MH, Moll U (1993) M (r) 92.000 type IV collagenase is increased in plasma of patients with colon cancer and breast cancer. Cancer Res 53:140-146
49. Beattie GJ, Smyth JF (1998) Phase I study of intraperitoneal metalloproteinase inhibitor BB94 in patients with malignant ascites. Clin Cancer Res 4:1899-1902
50. D'Errico A, Garbisa S, Liotta L, Castronovo V, Stetler-Stevenson WG, Grigioni WF (1991) Augmentation of type IV collagenase, laminin receptor and Ki67 proliferation antigen associated with human colon, gastric, and breast carcinoma progression. Mod Pathol 4:239-246
51. Hewitt RE, Leach ICH, Powe DG, Clark IM, Cawston TE, Turner DR (1991) Distribution of collagenase and tissue inhibitor of metalloproteinases (TIMP) in colorectal tumours. Int J Cancer 49:666-672
52. Zebrowski BK, Liu W, Ramirez K, Akagi Y, Mills GB, Ellis LM (1999) Markedly elevated levels of vascular endothelial growth factor in malignant ascites. Ann Surg Oncol 6:373-378
53. Published February 2002, last updated May 2004. Online available: www.sign.ac.uk/guidelines/fulltext/50/index.html (accessed 22.10.2005)

Targeted Intraabdominal Chemotherapy for Peritoneal Carcinomatosis

S Samel, M Löhr

Summary

The prognosis of peritoneal spread from gastrointestinal cancer and subsequent malignant ascites is poor, and current medical treatments available are mostly ineffective. Targeted chemotherapy with intraperitoneal prodrug activation may be a beneficial new approach.

L293 cells were genetically modified to express the cytochrome P450 enzyme 2B1 under the control of a cytomegalovirus immediate early promoter. This CYP2B1 enzyme converts ifosfamide to its active cytotoxic compounds. The cells are encapsulated in a cellulose sulfate formulation (Capcell™; Bavarian Nordic, Martinsried, Germany). Adult Balb/c mice were inoculated intraperitoneally (i.p.) with $1x10^6$ colon cancer cells, previously transfected with GFP to emit a stable green fluorescence, by injection into the left lower abdominal quadrant. Two or five day's later animals were randomly subjected to either i.p. treatment with ifosfamide alone or ifosfamide combined with microencapsulated CYP2B1 expressing cells. Peritoneal tumour volume and tumour viability were assessed 10 days after tumour inoculation by means of fluorescence microscopy, spectroscopy and histology.

Early i.p. treatment with ifosfamide and CYP2B1 cells resulted in a complete response. Treatment starting on day five and single-drug treatment with ifosfamide resulted in a partial response. These results suggest that targeted i.p. chemotherapy using a combination of a prodrug and its converting enzyme may be a successful treatment strategy for peritoneal spread from colorectal cancer.

In summary, by using GFP-transfected colon 26 tumour cells in mice we established a well reproducible animal model of metastatic peritoneal cancer. Fluorescent imaging of GFP-transfected tumour was used to demonstrate tumour distribution in the peritoneal cavity and to estimate tumour growth and tumour response to treatment in this model. The application of Capcell™ and ifosfamide into the peritoneal cavity is a safe and well tolerated procedure in animal models and may help to target chemotherapeutic agents specifically at metastatic peritoneal cancer.

Intentions and Basic Concepts of Targeted Chemotherapy

Peritoneal spread from gastrointestinal cancer is a severely debilitating condition and accounts for a substantial morbidity and mortality among cancer patients [1,2]. The subsequent malignant ascites causes significant pain and discomfort thus dramatically reducing the quality of life in these patients[3,4]. Despite continuous efforts to develop medical and surgical treatments, options are limited and most often palliative [5].

Targeted chemotherapy is a novel approach towards treatment of intraperitoneal cancer either by improving drug delivery from the site of application into the tumour cell [6] or by switching the site of the activation of a prodrug from the liver into the abdominal cavity, thereby enhancing the locoregional antitumour effect. Furthermore, regional application and activation of a prodrug allows a reduction of dosage of the prodrug and minimizes systemic distribution and thus systemic side effects of the drug.

Liposomes were among the first experimental carriers of cytotoxic agents. However, both doxorubicin-loaded streptavidin liposomes [7] and ^{111}In-DTPA-labelled pegylated liposomes [8] failed to demonstrate a significant tumour-specific accumulation of active agents.

Nitrogen mustard N-Oxide (HN2-O) dissolved in lipiodol was given in animals with metastatic VX2 tumours by simultaneous intra-arterial and intraperitoneal administration resulting in complete tumour remission in almost all rabbits [9].

Antibodies too were employed for targeting peritoneal tumour either using specific cytotoxic monoclonal antibodies [10] or by trying to impart site-specific alterations in the disposition of drug molecules using anti-drug antibodies (ADAb) [11].

Finally microencapsulated cells containing a prodrug converting enzyme (Capcell™; Bavarian Nordic, Martinsried, Germany) were introduced in 1998. These Capcell™ served as a site specific prodrug converter when administered intraperitoneally and have shown promising treatment results in experimental models of peritoneal cancer of pancreatic and colorectal origin and went further into clinical evaluation as we will report below.

Clinical Experience

Clinical experience with protocols for targeted chemotherapy is limited. Only a model of microencapsulated cells containing a prodrug converting enzyme (Capcell™; Bavarian Nordic, Martinsried, Germany) has been employed in a clinical phase 1 trail of pancreatic cancer, assessing the safety of local activation of low-dose ifosfamide in 14 patients with advanced pancreatic cancer [12,13].

Encapsulation of allogenic cells in cellulose sulphate allows them to survive in vivo, since they are confined and protected from the host's immune system [14]. Injection of encapsulated CYP2B1-expressing cells into pre-established human pancreatic tumours in nude mice results in complete tumour regression in about

20% of animals and a significant anti-tumour effect in the remainder. Intra-arterial placement of microencapsulated cells in pig pancreas is also feasible [15]. We did an open-label, prospective, single-centre phase I/II study to assess the feasibility and safety of local activation of low-dose ifosfamide in a tumour after intra-arterial placement of encapsulated human 293 cells stably transfected with a CYP2B1 expression construct [16].

Between July, 1998, and April, 1999, 14 patients with advanced and irresectable pancreatic cancer were selected to receive the protocol of targeted intra-arterial chemotherapy with Capcell™.

An historic group of patients with non-resectable pancreatic carcinoma (n=36) served as controls. Seven patients underwent palliative surgery, ten palliative chemotherapy, 24 biliary drainage (endoscopic retrograde cholangio-pancreatography or percutaneous transhepatic cholangial drainage), and 19 best supportive care in addition to biliary drainage or surgery. Although the selection criteria for treated patients could not be fully applied to controls, clinical diagnoses were much the same, as were initial symptoms, age range (treated group: 49–77 years; control group: 39–90 years), and gender (treated group: 64.3% male; control group: 74,3% male).

On day 0, we angiographically placed 300 cellulose sulphate capsules (250 in one patient, Capcell™ (Bavarian Nordic Martinsried, and Q-one, Glasgow, UK), average diameter 0,8 mm, 10^4 cells in each) [15] into a suitable artery feeding the primary tumour. The best approach to the tumour vasculature was through the inferior pancreatoduodenal artery, or the dorsal pancreatic artery, superior pancreatic head branches of the gastroduodenal artery, or both. We were able to suprase-lectively cannulate an appropriate artery leading into tumour in 14 of 17 patients studied. Two patients developed severe infection before the start of the trial and had to be given an alternative treatment, and angiography failed in one individual. There were transient vessel spasms at the site of instillation immediately after Capcell™ placement in most instances.

We monitored patients for the development of adverse reactions and pancreatitis, and measured full blood count and serum concentrations of amylase, lipase, lactate, lactate dehydrogenase, and liver enzymes. On day 2 we started chemotherapy with low-dose (1 g/m^2 body surface) ifosfamide in 250 ml 0,9% sodium chloride as a 1 h infusion given on 3 consecutive days. A 60% dose equivalent of the uroprotective agent mesna was also given as three intravenous injections. This regimen was repeated on days 23–25 (patient numbers five and 17 only received one course of ifosfamide).

Follow-up angiography of target vessels 20 weeks after Capcell™ instillation showed no or only minor changes to tumour vessels, such as reduction of diameter or raised compression, compared with day 0. Two patients had occluded vessels, and in one individual the effects of the tumour on blood vessels were visible. The finding that the vessels of the remaining patients were not appreciably affected by Capcell™ instillation suggests that, in most patients, further instillation of Capcell™ would be possible if required.

None of the 12 serious adverse events recorded were related to treatment. There was no evidence of pancreatitis or allergic response during the study. Although

amylase concentrations in plasma were raised in some patients because of tumour growth, no further increase was seen after angiography and Capcell™ placement. Recorded adverse events were, in most cases, a result of pain, underlying disease, or deteriorating general health. Only one adverse event, increased lipase activity on day 15 after instillation, might have been related to Capcell™. The chemotherapy regimen was well tolerated and no toxicity beyond grade II was detected.

Experimental Evidence

Oxazaphosphorines, e.g. ifosfamide, are pro-drugs that are metabolized into their active compounds by the liver cytochrome P450 system. The enzyme CYP2B1 converts ifosfamide into 4-OH-ifosfamide which in turn results in the active compounds phosphoramide mustard and acrolein [17]. These active compounds have a very short half-life of a few minutes [18,19] in plasma. ifosfamide demonstrates antineoplastic activity against experimentally induced colon cancer [20-22] and it was suggested that it may be useful in the treatment of peritoneal cancer in humans as well [23]. In the case of peritoneal carcinomatosis the administration of ifosfamide-converting enzyme into the abdominal cavity provides activation of ifosfamide directly to the site of tumour development, thus circumventing activation of the prodrug in the liver. This principle has been used by transfection of tumour cells with cytochrome prior to establishing an experimental tumour and subsequent treatment with ifosfamide [24].

Micro-encapsulation of human embryonic kidney cells expressing a transfected ifosfamide-converting enzyme prevents the host immune system from attacking the transfected cells and allows implantation of non-HLA matched cells into patients [25]. Previous studies have already demonstrated the effectiveness of targeted treatment with ifosfamide in combination with encapsulated cells producing ifosfamide-converting enzyme (Capcell™) in pancreatic cancer both in animals [14] and during a phase I study in patients [12]. Ifosfamide, converted to its active derivatives by Capcell™ has been demonstrated to penetrate macroscopic tumour when applied in a distance of up to 10 mm in mice resulting in partial and complete tumour response as well [26]. The therapeutic regimen has also been successfully applied to spontaneously occurring breast carcinoma in dogs [27].

Animals and Experimental Design

Fifteen-week-old male Balb/c mice (Harlan-Winkelmann, Borchen, Germany) were used for our initial experiments [28]. The animals were kept in a temperature and humidity controlled 12 hour light cycle environment and had free access to water and standard pellet food during the entire experimental period. After intraperitoneal administration of syngeneic colon 26 tumour cells, all animals were randomly placed in either treatment or control groups. Each group consisted of eight animals. Tumour growth was assessed 10 days after inoculation at laparotomy.

Cell Culture and Tumour Cell Inoculation

The murine colon tumour cell line colon 26 originally derived from Balb/c mice was transfected with the EGFP-encoding expression vector pEGFP-N1 (Clontech, Heidelberg, Germany) as previously described [29]. We have demonstrated that EGFP fluorescence correlates well to tumour viability and will be effected by cell death only [30]. Cells were grown in DMEM-medium (Gibco BRL, Life Technologies, Scotland) supplemented with 10 percent fetal calf serum (FCS, PAN Biotechnologies GmbH, Germany) and antibiotics (1% penicillin-streptomycin, PAN Biotechnologies GmbH, Germany) and kept in an atmosphere of 100% humidity and 5% CO2 at 37°C. The cells were harvested after having reached confluency and were checked for viability using the tryptan-blue exclusion test.

Metastatic peritoneal tumour growth was induced by intraperitoneal injection of $1x10^6$ tumour cells suspended in 1 ml PBS into the left lower abdominal quadrant.

Intraperitoneal Treatment

The pro-drug ifosfamide (Holoxan®, Baxter Oncology, Frankfurt/Main, Germany) and microencapsulated cells (Capcell™; Bavarian Nordic, Martinsried, Germany) producing the cytochrome P450 subtype of ifosfamide-converting-enzyme CYP2B1, which converts ifosfamide into 4-OH-ifosfamide, were used for intraperitoneal targeted chemotherapy. The synthesis, biological and pharmacological properties of this construct have been described elsewhere [12,14,26,31]. In brief, human L293 cells were transfected with a pCDNA3 vector carrying the murine cytochrome P450 enzyme 2B1 under the control of a CMV early promoter. Cells were microencapsulated in sodium cellulose sulphate as the anion and poly-DADCMAC as cation using an Inotech device (Dottikon, Switzerland). Integrity of capsules was assessed microscopically. Cell viability was measured with the Life Death assay (Molecular probes). Enzymatic function was assessed using the restrain assay and a biological assay measuring the cell killing capacity [12,14,31,32]. Microcapsules were 0.7 mm diameter on average incorporating 3 x 10^5 cells/capsule.

In a first set of experiments (A), a single dose of either ifosfamide 100 mg/kg body weight alone or in combination with 50 Capcell™ in 0.5 ml of saline was administered on day 2 or day 5 after tumour inoculation. In a second set of experiments (B), 50 Capcell™ were administered on day 2 or day 5 followed by a daily administration of ifosfamide on five consecutive days (days 2-6 or days 5-9). Another two groups of animals received daily ifosfamide alone for five consecutive days (days 2-6 or days 5-9). Both Capcell™ and ifosfamide were injected into the left lower abdominal quadrant with a 21 G syringe.

Assessment of Peritoneal Tumour spread, Tumour Growth and Response to Treatment

The animals were anaesthetized by i.p. administration of xylazine hydrochloride 2.5-5 mg/kg (Rompun, Bayer AB, Leverkusen, Germany) and ketamine 100 mg/kg (Hostaket, Parke-Davis, Freiburg, Germany) and placed on a heated operation table (K. Effenberger, Med. Tech. Gerätebau, Pfaffing, Germany) in a supine position. Laparotomy was performed by a standard midline abdominal incision. Intraabdominal tumour spread was documented using photographs and fluorescence microscopy and quantified using a modified Sugarbaker index. This index originally employed to score clinical tumour in patients was defined to score number [n] and diameter of peritoneal tumour nodules in our model as follows: 0= no macroscopic tumour; 1= tumour nodules $\leq$ 2 mm; 2= tumour nodules 2-5 mm; 3= tumour nodules $\geq$ 5 mm. Total tumour volume in each mouse was calculated as the sum of volumes of all peritoneal tumour nodules found in each animal. Peritoneal cancer usually presented as half spherical nodules adjacent to the peritoneum, the serosal surface of organs and in the peri-pancreatic tissue. The volume "V" of each tumour nodule was estimated as half of a spherical volume with a radius "r" using Segner's method [33]: $V=1/2(4/3\ r^3\ \pi)$. Total tumour volume per animal is given as the sum of tumour-nodule volumes (mm^3).

Tumour Growth and Response to Treatment

Macroscopic peritoneal cancer developed in all animals subjected to intraperitoneal administration of colon 26 cancer cells (Fig. 1). The dissemination of colon 26 carcinoma within the peritoneal cavity was described by a modified Sugarbaker-index (Table 1).

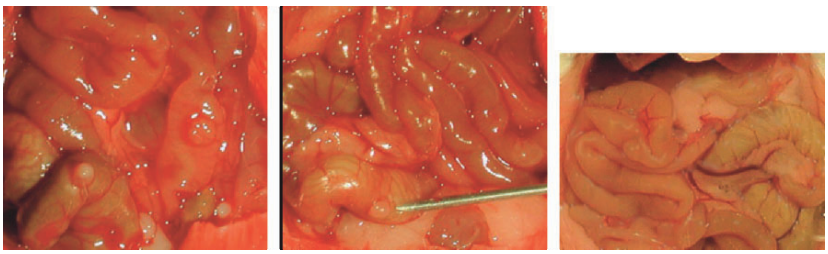


Figure 1. Macro-photograph of the peritoneal cavity on laparotomy ten days after tumor inoculation in (Panel A/left) an animal with untreated metastatic peritoneal cancer with several tumor nodules on the serosal surface, (Panel B/middle) an animal subjected to i.p. administration of Capcell™ and ifosfamide on days 5-9 with only one serosal tumor nodule and (Panel C/right) an animal subjected to i.p. administration of Capcell™ and ifosfamide on days 2-6 with a complete response to treatment

Table 1. Dissemination of tumour nodules within the peritoneal cavity using a modified Sugarbaker-index. The table presents the median number of tumour nodules (total and median number by size) found in mice of each group (8 mice/group)

macroscopic tumour nodules	total	≤ 2 mm	2-5 mm	≥ 5 mm
Capcell™+Ifosfamide day 2-6	none	none	none	none
Capcell™+Ifosfamide day 5-9	6 (3-7)	2 (1-5)	3 (1-5)	none
Ifosfamide day 2-6	5 (3-7)	4 (3-5)	1 (0-2)	none
Ifosfamide day 5-9	8 (8-9)	3 (2-4)	5 (4-6)	0 (0-1)
Capcell™+Ifosfamide day 2	3 (0-5)	2.5 (0-4)	0 (0-2)	none
Capcell™+Ifosfamide day 5	9 (5-11)	4 (3-6)	3 (2-4)	2 (0-3)
Ifosfamide day 2	6 (2-10)	3.5 (0-6)	2 (0-4)	1 (0-3)
Ifosfamide day 5	9 (7-12)	3.5 (2-5)	3 (3-5)	2 (1-4)
no treatment (controls)	14 (10-18)	6 (3-10)	3.5 (2-6)	4.5 (2-6)

Ten days after tumour cell administration untreated animals (controls) had developed macroscopic tumour consisting of several tumour nodules of various sizes (median 14, range 10-18). Most tumour nodules were ≤ 2 mm in diameter (median 6, range 3-10). Larger nodules were less frequent (2-5 mm: median 3.5, range 2-6; ≥ 5 mm: median 4.5, range 2-6). The mean peritoneal tumour volume in this group of untreated animals was 87.75 ± 11.32 mm^3 (Fig. 2).

Peritoneal treatment resulted in an apparent reduction of macroscopic peritoneal tumour with less tumour nodules overall and fewer nodules with a size of 2-5 mm and ≥ 5 mm respectively. Assessment of mean tumour volume showed corresponding results. Early i.p. treatment with ifosfamide+Capcell™ on day 2 resulted in a significant tumour reduction (tumour volume: 9.86 ± 9.60 mm^3, $p<0.05$) and was more effective than starting treatment on day 5 (tumour volume: 67.16 ± 11.17 mm^3, $p<0.05$). Treatment with ifosfamide alone on day 2 was less effective (tumour volume: 35.11 ± 21.95 mm^3, $p<0.05$) than treatment with ifosfamide+Capcell™ on day 2 ($p<0.05$) but more effective than treatment with ifosfamide+Capcell™ on day 5 ($p<0.05$). Neither ifosfamide alone on day 5 (tumour volume: 70.06 ± 10.43 mm^3) nor ifosfamide+Capcell™ on day 5 (tumour volume: 67.16 ± 11.17 mm^3) were effective in reducing tumour volume (Fig. 2).

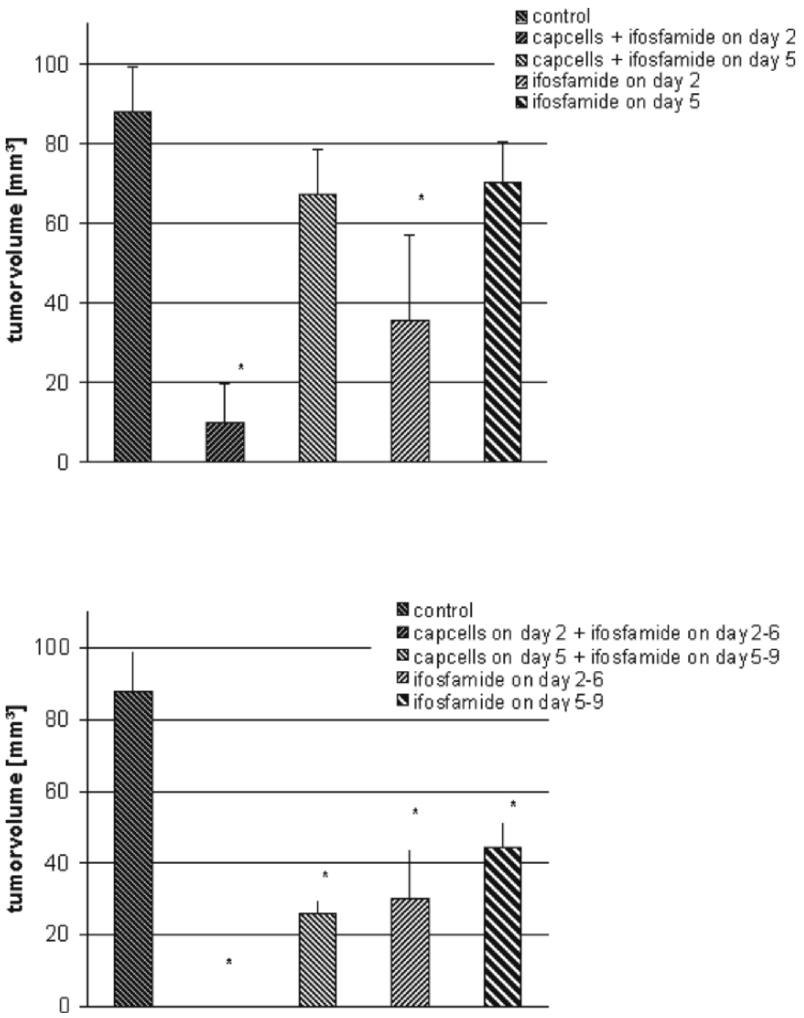


Figure 2. Tumour volume in response to i.p. treatment is represented by vertical bars (mean ± s.d.). Groups were compared by ANOVA and multiple comparisons of means using post-hoc Tukey's test in order to detect significant differences between groups. Significant differences of one group vs. controls (no treatment) and vs. other treatment groups are indicated [*]

Treatment with repeated administration of ifosfamide either without of in combination with Capcell™ resulted in a significant stepwise reduction of tumour volume. Differences were significant between all groups and groups vs. controls as well. Treatment with Capcell™ and repeated administrations of ifosfamide on days 2-6 resulted in complete tumour remission (tumour volume: 0 mm^3, $p < 0.05$) in all animals and treatment with Capcell™ and repeated administrations of

ifosfamide on days 5-9 resulted in a partial tumour response (tumour volume: 26.07 ± 3.66 mm^3, $p < 0.05$). Repeated administrations of ifosfamide alone on days 2-6 (tumour volume: 30.00 ± 14.11 mm^3, $p < 0.05$) and days 5-9 (tumour volume: 44.14 ± 7.33 mm^3, $p < 0.05$) were less effective (Fig. 2). None of the animals developed tumour at the site of injection in the left lower abdominal quadrant.

Complete remission was defined as absent GFP tumour fluorescence on microscopy and spectroscopy and was confirmed using histology in all animals subjected to treatment with Capcell™+ifosfamide on days 2-6. Partial response was indicated by a significant reduction (> 50%) of tumour volume in the other treatment groups.

The characteristic fluorescence emitted by GFP and protoporphyrine IX markedly decreased in these animals. Both, the expression of GFP and the accumulation of delta-aminolevulinic acid by the tumour cell served as indicators of viability of peritoneal metastases. The expression of GFP results from prior transfection of the pEGFP-N1 vector, while intraperitoneal administration of delta-aminolevulinic acid (ALA) is necessary for a tumour-specific accumulation of protoporphyrine IX (PpIX). When excited with light at a defined wavelength and viewed with the appropriate filter, viable tumour cells containing PpIX have a characteristic red fluorescence. However, there were no marked differences in protoporphyrine-fluorescence between treatment groups with complete and incomplete cytoreduction (ifosfamide alone on days 2-6 and 5-9, and Capcell™+ifosfamide on days 2-6 and 5-9). GFP fluorescence was not quantified in these animals.

In comparison to control animals without treatment, the proportion of cancer cells undergoing apoptosis increased significantly to more than 80 percent in animals subjected to i.p. treatment with Capcell™+ifosfamide. There was no increase of apoptosis when ifosfamide alone was given 2 or 5 days or 2-6 or 5-9 days after tumour inocculation (Table 2). In control animals with untreated tumour the spontaneous proportion of apoptotic cancer cells was 14 percent.

Table 2. Proportion of tumour cells undergoing apoptosis after intraperitoneal treatment (means). I.p. treatment with ifosfamide+Capcell™ caused a significant induction of apoptosis (*) without differences between groups. Treatment with ifosfamide alone did not increase tumour cell apoptosis when compared with untreated controls. S.d., standard deviation

treatment	total number of cells (mean ± s.d.)	number of apoptotic cells (mean ± s.d.)	proportion of apoptotic cells (%)
Capcell+Ifosfamide day 2-6	no residual tumour	no residual tumour	no residual tumour
Capcell+Ifosfamide day 5-9	257 ± 3	225 ± 3	87,5 (ns)
Capcell+Ifosfamide day 2	265 ± 3	223 ± 3	84,2 (ns)
Capcell+Ifosfamide day 5	374 ± 4	312 ± 4	83,4 (ns)
no treatment (controls)	411 ± 6	59 ± 4	14,3 (*)

Discussion

Peritoneal carcinomatosis and subsequent malignant ascites represent severe complications of advanced metastatic spread in gastric and colorectal cancer [1,2,34,35] as well as in other peritoneal malignancies [36,37]. Treatment options are few. Although local instillation of cytotoxic drugs is possible, it has not been widely applied due to the lack of significant effectiveness [38]. Intraperitoneal hyperthermic chemotherapy, both as an adjuvant and for palliation following surgical cytoreduction may have some impact on patient's prognosis [4,35]. The application of ifosfamide in combination with CYP2B1 expressing microencapsulated cells serves as a proof of principle for the treatment of experimental peritoneal carcinomatosis. While a single dose of ifosfamide + Capcell™ already resulted in a significant reduction in tumour mass, administration of ifosfamide + Capcell™ for a prolonged period resulted in complete tumour remission in some animals (treatment on days 2-6) and a partial response in the remainder (treatment on days 5-9). No side effects of this chemotherapy could be observed. The application of ifosfamide alone, without the Capcell™, was less effective. The antitumoural effect of ifosfamide + Capcell™, measured by tunnel assay, is mainly apoptotic, as demonstrated before [14,39]. In addition, necrosis may enhance the antitumoural effect of Capcell™ treatment [40].

The liver has the largest pool of the CYP2B1 of cytochrome P450. However, as demonstrated before [31], the availability of a local conversion site such as the Capcell™ containing CYP2B1 expressing cells resulted in an added peak of activated compounds such as 4-OH-ifosfamide. This is of particular importance since the terminal half-lives of active metabolites are less than 20 min in plasma and total clearance is less than 20 ml/min [18,19,41].

Perspectives

In a similar animal model, gene transfer of CYP2B1 to tumour cells prior to the establishment in vivo and subsequent treatment with cyclophosphamide resulted in a significant anti-tumour effect [24,42]. These results are supported by our model of CYP2B1 transfected cells brought into the immediate vicinity of the tumour cells. A further improvement may be the additional expression of cytochrome reductase thus enhancing the effect of the active metabolites [43]. This concept has recently been taken even further by transfection of a retrovirus containing both a cytochrome P450 (CYP2B6) together with the cytochrome reductase P450R and an E1b gene-deleted adenovirus Onyx-017. The use of a tumour-selective, replicating adenovirus to promote the spread of replication-defective gene therapy vectors, such as Adeno-P450, may increase the therapeutic potential of adenoviral delivery systems, and may increase cytochrome P450 activity and enhance tumour selectivity [44].

The rationale of targeted chemotherapy is to enhance antitumour activity by loco-regional activation of anti-tumour agents. Several novel agents carrying pro-drug

activators are at hand for experimental work with animals, e.g. haemagglutinating virus of Japan envelope (HVJ-E; Sendai virus) vector derived from inactivated HVJ particles. It has been reported to deliver DNA, proteins, and drugs into cells both in vitro and in vivo [45].

Besides gene directed enzyme prodrug therapy, other approaches might proof feasible. Circumvening gene therapy, drug eluting beads may present another option. Here, quasi solid microspheres are presoaked with a cytotoxic drug or other antitumoural substances [46]. Such beads will deliver the cytotoxic drug over a prolonged period and eventually resolve themselves. As intellectually stimulating and suggestive gene therapy appears to the scientist [47], a drug delivery system using drug eluting beads has some fundamental advantages over microencapsulated cells. We are currently investigating this approach for peritoneal carcinomatosis.

References

1. Glehen O, Osinsky D, Beaujard AC, Gilly FN (2003) Natural history of peritoneal carcinomatosis from nongynecologic malignancies. Surg Oncol Clin N Am 12:729-39, xiii
2. Glehen O, Gilly FN (2003) Quantitative prognostic indicators of peritoneal surface malignancy: carcinomatosis, sarcomatosis, and peritoneal mesothelioma. Surg Oncol Clin N Am 12:649-671
3. McQuellon RP, Loggie BW, Fleming RA, Russell GB, Lehman AB, Rambo TD (2001) Quality of life after intraperitoneal hyperthermic chemotherapy (IPHC) for peritoneal carcinomatosis. Eur J Surg Oncol 27:65-73
4. McQuellon RP, Loggie BW, Lehman AB, Russell GB, Fleming RA, Shen P, Levine EA (2003) Long-term survivorship and quality of life after cytoreductive surgery plus intraperitoneal hyperthermic chemotherapy for peritoneal carcinomatosis. Ann Surg Oncol 10:155-162
5. Sugarbaker PH (2002) New responsibilities in the management of colorectal cancer with peritoneal seeding. Cancer Invest 20:1118-1122
6. Iinuma H, Maruyama K, Okinaga K, Sasaki K, Sekine T, Ishida O, Ogiwara N, Johkura K, Yonemura Y (2002) Intracellular targeting therapy of cisplatin-encapsulated transferrin-polyethylene glycol liposome on peritoneal dissemination of gastric cancer. Int J Cancer 99:130-137
7. Longman SA, Cullis PR, Choi L, de Jong G, Bally MB (1995) A two-step targeting approach for delivery of doxorubicin-loaded liposomes to tumour cells in vivo. Cancer Chemother Pharmacol 36:91-101
8. Syrigos KN, Vile RG, Peters AM, Harrington KJ (2003) Biodistribution and pharmacokinetics of 111In-dTPA-labelled pegylated liposomes after intraperitoneal injection. Acta Oncol 42:147-153
9. Tabaru K, Konno T, Oda T, Nagamitsu A, Ishimaru Y, Kitamura N (2001) Treatment of VX2 carcinoma implanted in the liver with arterial and intraperitoneal

administration of oily anticancer agents. Cancer Chemother Pharmacol 47:149-154
10. Okamoto K, Yamaguchi T, Otsuji E, Yamaoka N, Yata Y, Tsuruta H, Kitamura K, Takahashi T (1998) Targeted chemotherapy in mice with peritoneally disseminated gastric cancer using monoclonal antibody-drug conjugate. Cancer Lett 122:231-236
11. Balthasar JP, Fung HL (1996) Inverse targeting of peritoneal tumours: selective alteration of the disposition of methotrexate through the use of anti-methotrexate antibodies and antibody fragments. J Pharm Sci 85:1035-1043
12. Löhr M, Hoffmeyer A, Kröger J, Freund M, Hain J, Holle A, Karle P, Knofel WT, Liebe S, Müller P, Nizze H, Renner M, Saller RM, Wagner T, Hauenstein K, Günzburg WH, Salmons B (2001) Microencapsulated cell-mediated treatment of inoperable pancreatic carcinoma. Lancet 357:1591-1592
13. Löhr M, Kröger JC, Hoffmeyer A, Freund M, Hain J, Holle A, Knöfel WT, Liebe S, Nizze H, Renner M, Saller R, Müller P, Wagner T, Hauenstein K, Salmons B, Günzburg WH (2003) Safety, feasibility and clinical benefit of localized chemotherapy using microencapsulated cells for inoperable pancreatic carcinoma in a phase I/II trial. Cancer Ther 1:121-131
14. Löhr M, Müller P, Karle P, Stange J, Mitzner S, Jesnowski R, Nizze H, Nebe B, Liebe S, Salmons B, Günzburg WH (1998) Targeted chemotherapy by intratumour injection of encapsulated cells engineered to produce CYP2B1, an ifosfamide activating cytochrome P450. Gene Ther 1998;5:1070-1078
15. Kröger JC, Bergmeister H, Hoffmeyer A, Ceijna M, Karle P, Saller R, Schwendenwein I, von Rombs K, Liebe S, Günzburg WH, Salmons B, Hauenstein K, Losert U, Löhr M (1999) Intraarterial instillation of microencapsulated cells in the pancreatic arteries in pig. Ann N Y Acad Sci 880:374-378
16. Löhr M, Bago ZT, Bergmeister H, Ceijna M, Freund M, Gelbmann W, Günzburg WH, Jesnowski R, Hain J, Hauenstein K, Henninger W, Hoffmeyer A, Karle P, Kröger JC, Kundt G, Liebe S, Losert U, Muller P, Probst A, Puschel K, Renner M, Renz R, Saller R, Salmons B, Walter I, et al (1999) Cell therapy using microencapsulated 293 cells transfected with a gene construct expressing CYP2B1, an ifosfamide converting enzyme, instilled intra-arterially in patients with advanced-stage pancreatic carcinoma: a phase I/II study. J Mol Med 77:393-398
17. Dirven HA, van Ommen B, van Bladeren PJ (1996) Glutathione conjugation of alkylating cytostatic drugs with a nitrogen mustard group and the role of glutathione S-transferases. Chem Res Toxicol 9:351-360
18. Kurowski V, Wagner T (1993) Comparative pharmacokinetics of ifosfamide, 4-hydroxyifosfamide, chloroacetaldehyde, and 2- and 3-dechloroethylifosfamide in patients on fractionated intravenous ifosfamide therapy. Cancer Chemother Pharmacol 33:36-42
19. Yu LJ, Drewes P, Gustafsson K, Brain EG, Hecht JE, Waxman DJ (1999) In vivo modulation of alternative pathways of P-450-catalyzed cyclophosphamide metabolism: impact on pharmacokinetics and antitumour activity. J Pharmacol Exp Ther 288:928-937

20. Struck RF, Dykes DJ, Corbett TH, Suling WJ, Trader MW (1983) Isophosphoramide mustard, a metabolite of ifosfamide with activity against murine tumours comparable to cyclophosphamide. Br J Cancer 47:15-26
21. Silbermann MH, vd Vecht B, Stoter G, Nooter K, Verweij J (1990) Combination therapy of ACNU and ifosfamide in tumour bearing mice with M2661 breast cancer, B16 malignant melanoma or C38 colon cancer. Eur J Cancer 26:321-325
22. Kusnierczyk H, Pajtasz-Piasecka E, Radzikowski C (1999) Synergistic antitumour effects of chemo-immunotherapy with an oxazaphosphorine drug and IL-2-secreting cells in a mouse colon cancer model. Med Oncol 16:267-278
23. Bunnell CA, Thompson L, Buswell L, Berkowitz R, Muto M, Sheets E, Shulman LN (1998) A Phase I trial of ifosfamide and paclitaxel with granulocyte-colony stimulating factor in the treatment of patients with refractory solid tumours. Cancer 82:561-566
24. Chen L, Waxman DJ (1995) Intratumoural activation and enhanced chemotherapeutic effect of oxazaphosphorines following cytochrome P-450 gene transfer: development of a combined chemotherapy/cancer gene therapy strategy. Cancer Res 55:581-589
25. Okada N, Miyamoto H, Yoshioka T, Sakamoto K, Katsume A, Saito H, Nakagawa S, Ohsugi Y, Mayumi T (1997) Immunological studies of SK2 hybridoma cells microencapsulated with alginate-poly(L)lysine-alginate (APA) membrane following allogeneic transplantation. Biochem Biophys Res Commun 230:524-527
26. Karle P, Müller P, Renz R, Jesnowski R, Saller R, von Rombs K, Nizze H, Liebe S, Günzburg WH, Salmons B, Löhr M (1998) Intratumoural injection of encapsulated cells producing an oxazaphosphorine activating cytochrome P450 for targeted chemotherapy. Adv Exp Med Biol 451:97-106
27. Kammertoens T, Gelbmann W, Karle P, Alton K, Saller R, Salmons B, Günzburg WH, Uckert W (2000) Combined chemotherapy of murine mammary tumours by local activation of the prodrugs ifosfamide and 5-fluorocytosine. Cancer Gene Ther 7:629-636
28. Samel S, Keese M, Lux A, Jesnowski R, Prosst R, Saller R, Hafner M, Sturm J, Post S, Löhr M (2006) Peritoneal cancer treatment with CYP2B1 transfected, microencapsulated cells and ifosfamide. Cancer Gene Ther 13:65-73
29. Sturm JW, Magdeburg R, Berger K, Petruch B, Samel S, Bonninghoff R, Keese M, Hafner M, Post S (2003) Influence of TNF *alpha* on the formation of liver metastases in a syngenic mouse model. Int J Cancer 107:11-21
30. Sturm JW, Keese MA, Petruch B, Bonninghoff RG, Zhang H, Gretz N, Hafner M, Post S, McCuskey RS (2003) Enhanced green fluorescent protein-transfection of murine colon carcinoma cells: key for early tumour detection and quantification. Clin Exp Metastasis 20:395-405
31. Löhr JM, Saller R, Salmons B, Günzburg WH (2002) Microencapsulation of genetically engineered cells for cancer therapy. Methods Enzymol 346:603-618

32. Donato MT, Gomez-Lechon MJ, Castell JV (1993) A microassay for measuring cytochrome P450IA1 and P450IIB1 activities in intact human and rat hepatocytes cultured on 96-well plates. Anal Biochem 213:29-33
33. Beyer WH (1987) CRC Standard Mathematical Table. 28th ed: Boca Raton, FL: CRC Press
34. Bonenkamp JJ, Sasako M, Hermans J, van de Velde CJ (2001) Tumour load and surgical palliation in gastric cancer. Hepatogastroenterology 48:1219-1221
35. Elias DM, Pocard M (2003) Treatment and prevention of peritoneal carcinomatosis from colorectal cancer. Surg Oncol Clin N Am 12:543-559
36. Mohamed F, Sugarbaker PH (2002) Peritoneal mesothelioma. Curr Treat Options Oncol 3:375-386
37. Moran BJ, Cecil TD (2003) The etiology, clinical presentation, and management of pseudomyxoma peritonei. Surg Oncol Clin N Am 12:585-603
38. Markman M (2003) Intraperitoneal antineoplastic drug delivery: rationale and results. Lancet Oncol 4:277-283
39. Schwartz PS, Waxman DJ (2001) Cyclophosphamide induces caspase 9-dependent apoptosis in 9L tumour cells. Mol Pharmacol 60:1268-1279
40. Karle P, Renner M, Salmons B, Günzburg WH (2001) Necrotic, rather than apoptotic, cell death caused by cytochrome P450-activated ifosfamide. Cancer Gene Ther 8:220-230
41. Zheng JJ, Chan KK, Muggia F (1994) Preclinical pharmacokinetics and stability of isophosphoramide mustard. Cancer Chemother Pharmacol 33:391-398
42. Jounaidi Y, Hecht JE, Waxman DJ (1998) Retroviral transfer of human cytochrome P450 genes for oxazaphosphorine-based cancer gene therapy. Cancer Res 58:4391-401
43. Chen L, Yu LJ, Waxman DJ (1997) Potentiation of cytochrome P450/cyclophosphamide-based cancer gene therapy by coexpression of the P450 reductase gene. Cancer Res 57:4830-4837
44. Jounaidi Y, Waxman DJ (2004) Use of replication-conditional adenovirus as a helper system to enhance delivery of P450 prodrug-activation genes for cancer therapy. Cancer Res 64:292-303
45. Mima H, Yamamoto S, Ito M, Tomoshige R, Tabata Y, Tamai K, Kaneda Y (2006) Targeted chemotherapy against intraperitoneally disseminated colon carcinoma using a cationized gelatin-conjugated HVJ envelope vector. Mol Cancer Ther 5:1021-1028
46. Palmer RR, Lewis AL, Kirkwood LC, Rose SF, Lloyd AW, Vick TA, Stratford PW (2004) Biological evaluation and drug delivery application of cationically modified phospholipid polymers. Biomaterials 25:4785-4796
47. Löhr M (2006) Gentherapeutische Ansätze bei gastrointestinalen Tumouren (Gene Therapy of Gastrointestinal Tumours). Z Gastroenterol 44:1-8

Immunotherapy of Peritoneal Carcinomatosis

MA Ströhlein, MM Heiss

Immunological Defence Mechanisms of the Peritoneal Cavity

Undoubtedly, the peritoneum and the associated immunocompetent structures are able to provide a microenvironment, which is potentially able to promote proliferation, differentiation and recruitment of blood cells to generate a state of effective immune defence mechanisms against cellular or viral pathogens. In the normal peritoneal cavity, 45% monocytes/macrophages (CD68+), 45% T-lymphocytes (CD2+), 8% NK-cells and 2% dendritic cells have been reported [1]. A high percentage of the peritoneal CD4+ (92%) and CD8+ (73%) were also found to be CD45RO+, indicating the memory and effector T cell phenotype.

An inversed ratio of CD4+ to CD8+ T-cells with respect to those of the peripheral blood, with a predominance of CD8+ T-cells was described [2]. These findings exhibit the anti-inflammatory Th2 phenotype in human normal peritoneum.

The mesenchymal cell type also offers a considerable immunocompetence. After appropriate stimulation or activation, mesenchymal cells were found to secrete proinflammatory mediators like Interleukin-1, Interleukin-6, Prostaglandin E2 and several growth factors like monocyte colony stimulating factor (MCSF), granulocyte stimulating factor (GCSF), granulocyte monocyte colony stimulating factor (GM-CSF) and vascular epithelial growth factor (VEGF) [3]. Human peritoneal mesothelial cells were also found to express HLA-DR molecules and upregulation of ICAM-1 in tissue culture conditions after Interferon-gamma stimulation. Under these circumstances, mesothelial cells have an extended capacity to present antigens to autologous T-lymphocytes, also promoting anti-CD3 induced T-cell proliferation. These findings were supported by the secretion of IL-2, IL-15 and IFN-gamma in further laboratory experiments.

Taking these findings into account, a high level of immunocompetence in antigen-presentation and T-cell activation characterizes the peritoneal cavity to be attractive for local compartment immunotherapy.

The application of regional intraperitoneal immunotherapy as part of multimodal therapy concepts is conceivable for several reasons. Although peritoneal carcinomatosis represents a far advanced tumour disease, the distribution of single cells

or tumour cell clusters on an extended surface of the peritoneum is another attractive point for locally administered immunotherapy. The same is true for patients with malignant ascites, where single tumour cells or clusters of few tumour cells represent an easily accessible target. In contrast to chemotherapy, the effectiveness of immune therapies is not dependent on the cell cycle or pharmacokinetic parameters, as tumour cells can also be attacked in a dormant cell phase.

Unspecific Immunotherapy/Cytokines

Despite the variety of immunocompetent cells obviously included in the peritoneal cavity, the anti-inflammatory Th2 phenotype was found to be predominant in physiological conditions. Therefore, polarization to a more cellular phenotype by unspecific immunomodulation was tried. The streptococcal preparation OK- 432 was tested in a rat model to have immunostimulatory activity on Natural killer cells, LAK cells und T-lymphocytes. Locoregional administration of OK- 432 was found to be effective in patients with malignant ascites from gastric cancer, and was associated with an up-regulation of Th1 responses [4]. Induction of predominant Th1 type T-helper cells was further increased by combining OK- 432 with Interleukin-2. Another concept is the intraperitoneal application of fms-like tyrosine kinase-3-ligand (Flt3-L), a truncated glycoprotein that increases dendritic cells (DCs) and monocytes. Increased Interleukin-12 as a sign of enhanced cellular immunity together with a maturational shift toward the monocyte-derived Dendritic cell phenotype was observed [5]. Clinical efficacy against peritoneal carcinomatosis was limited to a special group of immunoreactive patients only, who were able to overcome factors like tumour related immunosuppression within OK- 432 or Flt3-L treatment. Nevertheless, these attempts demonstrated the potential of induction of a more predominant Th1 phenotype together with stimulation of innate components of the immune system like natural killer cells and macrophages.

Stimulation of Immunocompetent Cells by Defined Cytokines

During the last two decades, a variety of cytokines and correlating receptors were characterized to dramatically increase the antitumour cytotoxicity of defined subsets of immune cells. The most investigated cytokine is Interleukin-2, which is able to unspecifically stimulate T lymphocytes and to induce lymphokine activated killer cells (LAK). Another concept was the generation of tumour infiltrating lymphocytes (TIL), which represented highly specific T cells expanded out of solid tumour masses. In several animal experiments, application of Interleukin-2 generated lymphokine activated killer (LAK) cells resulted in a significant reduction of intraperitoneal tumour masses [6,7]. Clinical Interleukin-2 regimens were finally limited by severe side effects or technical problems during isolation and

expansion of TIL, but clearly demonstrated the power of T-cell responses in peritoneal carcinomatosis of ovarian and colon carcinoma [8]. Clinical responses in single patients were also reported in patients with malignant ascites due to peritoneal carcinomatosis after intraperitoneal treatment by Interferon alpha [9].

Antibody Constructs

Another promising concept may be based on the possibility to direct immunotherapy against defined molecular targets. Due to the mesenchymal origin of the peritoneal surface, targeting epithelial and not mesenchymal proteins is one of the key concepts of specific immunotherapy. For instance, one of the most attractive structures among several other tumour associated antigens is the 17-1A antigen, which belongs to the family of epithelial cellular adhesion molecules (EpCAM), and is overexpressed as a tumour associated antigen (TAA) in various solid cancers [10]. Since EpCAM is not expressed in lymph nodes, blood vessels or connective tissue, an EpCAM directed immunotherapy enables a form of specific targeting of tumour cells within the peritoneum. The effect of a combined therapy of LAK cells and a monoclonal 17-1A antibody was demonstrated in a SCID mouse model [11].

The major drawback of conventional monoclonal antibodies is the lack of a direct anti-tumour effector component. One possibility to overcome this problem is to use of radionucleotide-conjugated antibodies for locoregional treatment in peritoneal carcinomatosis to direct toxic radiation against tumour cells after specific binding of tumour antigens. For instance, Iodine-131, Indium-111- and yttrium-90-labeled human IgM or IgG constructs exhibited efficacy in animal experiments and clinical models, which were clearly superior to any kind of radiotherapy, illustrating the significance of locoregional intraperitoneal therapy against peritoneal carcinomatosis [12-14].

Presently, the most promising concepts of antibody therapy are based on antibody induced involvement of immune effector cells. Direct activation of immunocompetent cells against defined tumour antigens led to the concept of bispecific antibodies, representing engineered antibody proteins, which are able to bind tumour antigens by one binding site and to bind and activate immune cells by the second binding site (Fig. 1 a-d). Wunderlich et al. demonstrated significant destruction of tumour cells by using an anti-CD3 x anti-FR (fetal receptor of the ovarian cancer cell) bispecific antibody in combination with LAK cells [15]. Clinical efficacy was reported in patients with peritoneal carcinomatosis and malignant ascites by treatment with the bispecific antibody anti-HEA125 x anti-CD3, which was shown to redirect T lymphocytes toward carcinoma cells and to induce tumour cell lysis in vitro. In treated patients, a decrease or stabilisation of ascites accumulation was reported [16].

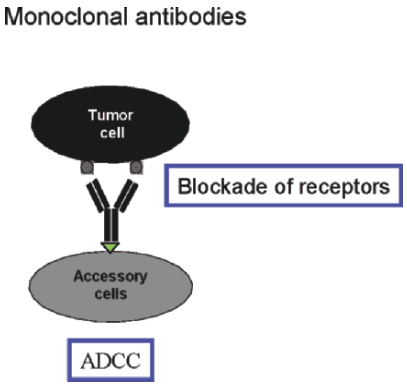


Figure 1a. Conventional monoclonal antibodies (mAb). The anti-tumour efficacy of mAb results from blockade of receptors and signal pathways as well as induction of antibody dependent cellular cytotoxicity by involvement of accessory cells

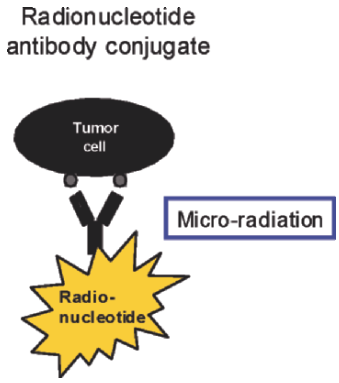


Figure 1b. Radionucleotide antibody conjugates. Radionucleotide antibody conjugates specifically target tumour cells to mediate local micro radiation at the tumour site

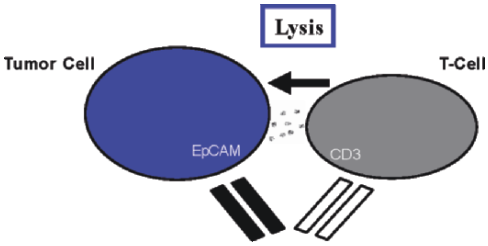


Figure 1c. Bispecific antibodies. Bispecific antibodies are based on Fab2 constructs, which are able to direct T-cell mediated cytotoxicity towards specifically bound tumour cells

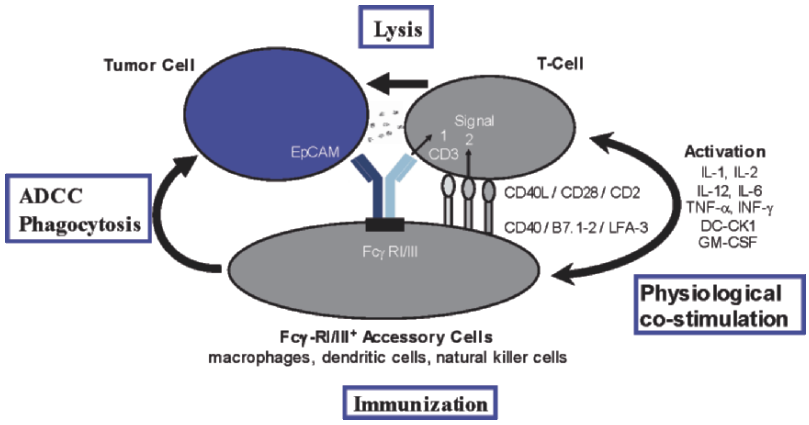


Figure 1d. Mode of action of trifunctional antibodies. Trifunctional antibodies generate a self supporting system of tumour cells, T-lymphocytes, and accessory cells. By this so-called tri-cell-complex, enhanced tumour cell lysis by physiological re-stimulation of T-cells as well as long term immunization by involvement of accessory cells is observed

The advancement of bispecific antibodies is represented by trifunctional antibodies. Trifunctional antibodies are artificially engineered immunoglobulins with two different Fab-binding sites and an intact Fc-region [17]. They effectively enhance the anti-tumour activity not only by induction of T-cells by CD3-binding, but also by simultaneous activation of accessory cells [18,19]. Responsible for this feature is a potent isotype combination (mouse IgG2a and rat IgG2b), which binds and activates FcγRI and RIII positive cells (e.g. dendritic cells, macrophages, granulocytes and NK-cells). The tri-cell complex of T-lymphocytes, tumour cells and accessory cells induces efficient tumour cell killing, which results from

an activating "crosstalk" via cytokines and costimulatory molecules. Involvement and activation of Fcγ receptor type I/III positive professional antigen presenting cells resulted in phagocytosis of tumour cells and subsequent induction of anti-tumour immunity by tumour antigen processing and presentation [20]. This phenomenon was supposed to result in polyclonal humoral and cellular immune responses, including T-cell responses even against unknown, tumour-associated peptides. This hypothesis was confirmed in a syngeneic mouse tumour model, where i.p. treatment with trifunctional antibodies demonstrated striking anti-tumour effects including tumour destruction and long term immunity, which where independent of the primary tumour binding site of the applicated trAb.

Clinical treatment regimens with trifunctional antibodies are presently evaluated in patients with peritoneal carcinomatosis or patients with malignant ascites of a variety of tumour entities. A first pilot study demonstrated clinical efficacy by a stop of ascites accumulation, which was correlated with a complete destruction of tumour cells in the ascitic fluid [21]. Moreover, a first phase I study demonstrated clinical responses in more than 60% of patients together with a prolonged survival [22].

Summary

Intraperitoneal immunotherapy actually is a promising concept for treatment of peritoneal carcinomatosis for several reasons: The use of specifically engineered therapy in terms of antibodies or stimulated T lymphocytes against epithelial tumour antigens offers an elegant way to attack tumours on the peritoneal surface, as peritoneal cells have a mesenchymal origin. This is especially true for modern multimodal treatment concepts, were local compartment treatment together with systemic chemotherapy and (if possible) surgical tumour removal will be individually combined.

Table 1. Overview of immunotherapeutical approaches in peritoneal carcinomatosis

Concept	Mode of action	Clinical effects
Unspecific immunomodulation - OK-432 - Flt3-Ligand	Modulation of cellular immune reactions by induction of the predominant Th1 phenotype	Induction of Th1 cells Decrease of ascites in some patients
Cytokines - Interleukin-2 - Interferon-γ	Direct unspecific stimulation of NK- and T-cells	Significant reduction of tumour mass in animal models and single patients/severe side effects
Monoclonal antibodies	Specific binding of tumour antigens antibody-dependent cell-mediated cytotoxicity (ADCC)	Limited efficacy as single agent
Radionucleotide antibody conjugates	Specific targeting of tumour cells for local micro-radiation	Clinical efficacy in ascites in single patients
Bispecific antibodies	Specific targeting of tumour cells and simultaneous activation of effector cells	Reduction of ascites re-accumulation
Trifunctional antibodies	Specific targeting of tumour cells, stimulation of T-cells, simultaneous activation of accessory cells	Clinical responses in > 60 % of patients with PC Stop of ascites accumulation Induction of long term immunity

PC, peritoneal carcinomatosis

References

1. Kubicka U, Olszewski WL, Tarnowski W, Bielecki K, Ziolkowska A, Wierzbicki Z (1996) Normal human immune peritoneal cells: subpopulations and functional characteristics. Scand J Immunol 44:157-163
2. Tellado JM, Broche F (2001) Defense mechanisms of the peritoneal cavity. Curr Opin Crit Care 7(2):105-116
3. Jayne DG, Perry SL, Morrison E, Farmery SM, Guillou PJ (2000) Activated mesothelial cells produce heparin-binding growth factors: implications for tumour metastases. Br J Cancer 82:1233-1238

4. Yamaguchi Y, Ohshita A, Kawabuchi Y, Hihara J, Miyahara E, Toge T (2004) Locoregional immunotherapy of malignant ascites from gastric cancer using DTH-oriented doses of the streptococcal preparation OK-432: Treatment of Th1 dysfunction in the ascites microenvironment. Int J Oncol 24:959-966
5. Freedman RS, Vadhan-Raj S, Butts C, Savary C, Melichar B, Verschraegen C, Kavanagh JJ, Hicks ME, Levy LB, Folloder JK, Garcia ME (2003) Pilot Study of Flt3 Ligand Comparing Intraperitoneal with Subcutaneous Routes on Hematologic and Immunologic Responses in Patients with Peritoneal Carcinomatosis and Mesotheliomas. Clin Cancer Res 9:5228-5237
6. Eggermont AM, Sugarbaker PH (1987) Lymphokine-activated killer cell and interleukin-2 inhibitors: their role in adoptive immunotherapy. Cell Immunol 107:384-394
7. Ottow RT, Steller EP, Sugarbaker PH, Wesley RA, Rosenberg SA (1987) Immunotherapy of intraperitoneal cancer with interleukin 2 and lymphokine-activated killer cells reduces tumor load and prolongs survival in murine models. Cell Immunol 104:366-376
8. Perrin P, Cassagnau E, Burg C, Patry Y, Vavasseur F, Harb J, Le Pendu J, Douillard JY, Galmiche JP, Bornet F (1994) An interleukin 2/sodium butyrate combination as immunotherapy for rat colon cancer peritoneal carcinomatosis. Gastroenterology 107:1697-1708
9. Sartori S, Nielsen I, Tassinari D, Trevisani L, Abbasciano V, Malacarne P (2001) Evaluation of a standardized protocol of intracavitary recombinant interferon alpha-2b in the palliative treatment of malignant peritoneal effusions. A prospective pilot study. Oncology 61:192-196
10. Litvinov SV, Velders MP, Bakker HA, Fleuren GJ, Warnaar SO (1994) Ep-CAM: a human epithelial antigen is a homophilic cell-cell adhesion molecule. J Cell Biol 125:437-446
11. Piso P, Aselmann H, von Wasielewski R, Dahlke MH, Klempnauer J, Schlitt HJ (2003) Prevention of peritoneal carcinomatosis from human gastric cancer cells by adjuvant-type intraperitoneal immunotherapy in a SCID mouse model. Eur Surg Res 35:470-476
12. Andersson H, Lindegren S, Back T, Jacobsson L, Leser G, Horvath G (2000) Radioimmunotherapy of nude mice with intraperitoneally growing ovarian cancer xenograft utilizing 211At-labelled monoclonal antibody MOv18. Anticancer Res 20:459-462
13. Kinuya S, Li XF, Yokoyama K, Mori H, Shiba K, Watanabe N, Shuke N, Bunko H, Michigishi T, Tonami N (2003) Intraperitoneal radioimmunotherapy in treating peritoneal carcinomatosis of colon cancer in mice compared with systemic radioimmunotherapy. Cancer Sci 94:650-654
14. Muto MG, Finkler NJ, Kassis AI, Howes AE, Anderson LL, Lau CC, Zurawski VR, Jr., Weadock K, Tumeh SS, Lavin P (1992) Intraperitoneal radioimmunotherapy of refractory ovarian carcinoma utilizing iodine-131-labeled monoclonal antibody OC125. Gynecol Oncol 45:265-272
15. Wunderlich JR, Mezzanzanica D, Garrido MA, Neblock DS, Daddona PE, Andrew SM, Zurawski VR, Jr., Canevari S, Colnaghi MI, Segal DM (1992)

Bispecific antibodies and retargeted cellular cytotoxicity: novel approaches to cancer therapy. Int J Clin Lab Res 22:17-20
16. Marme A, Strauss G, Bastert G, Grischke EM, Moldenhauer G (2002) Intraperitoneal bispecific antibody (HEA125xOKT3) therapy inhibits malignant ascites production in advanced ovarian carcinoma. Int J Cancer 101:183-189
17. Lindhofer H, Mocikat R, Steipe B, Thierfelder S (1995) Preferential species-restricted heavy/light chain pairing in rat/mouse quadromas. Implications for a single-step purification of bispecific antibodies. J Immunol 155:219-225
18. Zeidler R, Reisbach G, Wollenberg B, Lang S, Chaubal S, Schmitt B, Lindhofer H (1999) Simultaneous activation of T cells and accessory cells by a new class of intact bispecific antibody results in efficient tumor cell killing. J Immunol 163:1246-1252
19. Zeidler R, Mysliwietz J, Csanady M, Walz A, Ziegler I, Schmitt B, Wollenberg B, Lindhofer H (2000) The Fc-region of a new class of intact bispecific antibody mediates activation of accessory cells and NK cells and induces direct phagocytosis of tumour cells. Br J Cancer 83:261-266
20. Ruf P, Lindhofer H (2001) Induction of a long-lasting antitumor immunity by a trifunctional bispecific antibody. Blood 98:2526-2534
21. Heiss MM, Strohlein MA, Jager M, Kimmig R, Burges A, Schoberth A, Jauch KW, Schildberg FW, Lindhofer H (2005) Immunotherapy of malignant ascites with trifunctional antibodies. Int J Cancer 117:435-443
22. Strohlein MA, Gruetzner KU, Tarabichi A, Jauch KW, Bartelheim H, Lindhofer H, von Roemeling R, Heiss MM (2006) Efficacy of intraperitoneal treatment with the trifunctional antibody catumaxomab in patients with GI-tract cancer and peritoneal carcinomatosis: A matched pair analysis. J Clin Oncol 24:111

Intraperitoneal Photodynamic Therapy

KA Cengel, E Glatstein, SM Hahn

Introduction

Photodynamic therapy (PDT) is a cancer treatment that combines a photosensitizer, oxygen, and visible light [1,2]. PDT cytotoxicity occurs when photosensitizers capture light energy and transfer that energy to oxygen. The excited oxygen species then mediate tumour cytotoxicity through direct mechanisms such as tumour cell apoptosis or necrosis, as well as indirect mechanisms, such as damage to the tumour's blood supply [3-5]. In addition, PDT may help to stimulate an antitumour immune response, although the mechanism for this effect is not well understood [6]. Thus, the response of cancer cells to PDT is complex and depends upon many factors including, the specific photosensitizer used, the predominant subcellular region of photosensitizer localization (e.g. mitochondria), the fluence rate, the timing between photosensitizer and light delivery, the presence of oxygen, and the underlying molecular abnormalities in the neoplastic cell [7].

Clinical trials using PDT have been performed for a variety of malignant and premalignant conditions, including head and neck cancers [8-10], lung cancer [11-14], mesothelioma [15-17], esophageal cancer [18], Barrett's esophagus [19,20], bile duct cancer [21,22], brain tumours [23], breast cancer [24,25], bladder cancer [26], cervical cancer [27,28], prostate cancer [29-31] and malignant and premalignant skin neoplasms [32-34]. In the United States and the European Union, PDT has been approved for the treatment of esophageal, lung and skin cancers. In the European Union, PDT is also approved for treatment of some Head and Neck cancers. However, it should be noted that PDT is not likely to be an appropriate locoregional treatment for all cancers. Nonetheless, PDT may have a role together with other modalities such as surgery and chemotherapy in the treatment of selected malignancies. The critical issue for clinical researchers is to define the situations where PDT has the greatest chance of having a positive impact upon the natural history of cancer.

The appeal of PDT in treatment of peritoneal carcinomatosis is that it has the potential to combine selective destruction of cancerous tissue compared to normal tissues with the ability to treat and conform to relatively large surface areas.

Initial pre-clinical evidence suggested that some photosensitizers, including the first generation photosensitizer, hematoporphyrin derivative (HPD), are retained in tumours to a greater extent than in some normal tissues [35,36]. In patients with peritoneal carcinomatosis, it has recently been shown that uptake ratios, when compared to clinically relevant normal tissues such as bowel, are not nearly as dramatic as the pre-clinical models predicted for the first generation photosensitizer Porfimer sodium and the second generation photosensitizer Motexafin lutetium [37,38]. However, newer technologies have the potential to increase the tumour cell selectivity of PDT by using photosensitizer-anti-tumour antibody conjugates or encapsulation of photosensitizer in molecularly targeted nanoparticles [39]. Tumour cell selectivity can also be obtained by restricting the application of light to the region of the malignancy, thereby avoiding some normal tissues and minimizing damage. The intrinsic, physical limitation in the depth of visible light penetration through tissue limits PDT damage to deeper structures, thereby providing additional potential for tumour cell selectivity and also allowing the treatment of relatively large surface areas with acceptable toxicity. This is especially true after surgical debulking where the residual tumour may be microscopic or less than 5 mm in depth.

Thus, PDT might be ideal for malignancies, such as ovarian and gastric and colorectal cancers, that have the propensity to spread to peritoneal surfaces. However, for disseminated intraperitoneal malignancies, involvement of regional lymph nodes and other micrometastasic disease are clinical concerns. Therefore, a locoregional treatment such as intraperitoneal PDT is not likely to be successful as the sole modality of treatment for these cancers, but rather as a part of a multimodality treatment regimen that includes surgery and/or chemotherapy in addition to intraperitoneal PDT. Nevertheless, recent clinical trials of intraperitoneal PDT (See Clinical Applications of Intraperitoneal PDT, below) and the emerging developments in the field of molecularly targeted PDT show that intraperitoneal PDT remains a highly exciting potential treatment for patients with disseminated intraperitoneal malignancy. In this chapter, the preclinical rationale, the clinical experience to date and the prospects for future improvements in intraperitoneal PDT treatment outcomes will be presented.

Mechanisms of PDT Mediated Cell Death

The anti-tumour efficacy of PDT is thought to stem from a combination of indirect and direct tumour cell killing [7,40]. Indirect tumour cell kill occurs through PDT-mediated changes in tumour microenvironment that are unfavourable for tumour proliferation and survival (e.g. reduction of vascularity). These indirect vascular effects can only be observed and measured in vivo and are likely due to a combination of PDT-mediated vessel leakage, vasoconstriction and vascular thrombosis [41,42]. The anti-vascular effects of PDT are strongly dependent on the identity of the photosensitizer as well as the time interval between the administrations of

photosensitizer and light. In addition, changes in tumour microenvironment such as hypoxia prior to and during PDT administration may have a profound impact on the overall level of PDT-mediated cell killing due to the requirement for molecular oxygen in PDT [43-45].

Other mechanisms for indirect PDT-mediated tumour cell killing include the ability of PDT to stimulate an anti-tumour immune response [6]. The mechanism for this effect has been postulated to involve the release of pro-inflammatory cytokines and fixation of complement to form C3a that occurs during and after PDT as well as PDT-induced infiltration of lymphocytes, monocytes and granulocytes into tumours in response to these pro-inflammatory signals [46-52]. In addition, by producing necrotic cell death, PDT may act to further stimulate host mediated anti-tumour immune responses [53]. The potential immunological component in PDT is perhaps most dramatically demonstrated in experiments by Korbelik and colleagues that compared the efficacy of PDT in Balb/C (immunocompetent) vs. scid (immunocompromised) mice [54]. In these experiments, despite similar efficacy of PDT in initial ablation of EMT6 mammary sarcomas from both Balb/C and scid mice, long term tumour cure occurred in all of the Balb/C and none of the scid mice. Importantly, the long-term efficacy of PDT in scid mice was dramatically improved if bone marrow transplant from Balb/C donors was performed prior to PDT.

Direct tumour cell killing results from PDT-mediated damage to cellular macromolecules that results in cell death from both apoptotic and non-apoptotic mechanisms. In general, apoptotic cell death tends to predominate in the most PDT-sensitive cell lines at lower light/photosensitizer doses and necrotic/non-apoptotic mechanisms tend to predominate at higher light/photosensitizer doses [55-58]. When PDT kills cells by apoptosis, the percent apoptosis achieved, as well as the mechanism of apoptosis (extrinsic vs intrinsic) appears to be both tumour cell line and the photosensitizer-dependent [7,59]. For example, Photosensitizers that localize to the mitochondria, such as Porfimer sodium and benzoporphyrin derivative monoacid (BPD), rapidly and effectively stimulate intrinsic/mitochondrially-mediated apoptosis both in vivo and in vitro in many cell lines [60-68]. However, Porfimer sodium can also stimulate necrotic cell death at relatively low light/photosensitizer doses under certain circumstances, such as when the cells are treated at higher cell densities [69-72]. Importantly, Porfimer sodium-PDT mediated necrotic cell death stimulated a strong bystander effect, killing surrounding tumour cells that had been insufficiently damaged by PDT [69]. This effect was not observed when PDT killed cells by apoptosis and may be due to density dependent alterations in cell signaling [71].

Preclinical Studies of Intraperitoneal PDT

The strong theoretical rationale for intraperitoneal PDT was first tested in animal models by Douglass and colleagues [73]. In these experiments, rabbits with Brown-Pierce epithelioma implants in the serosa of the bowel, liver, pancreas, or bladder were treated with hematoporphyrin derivative (HPD)-mediated PDT (5 mg/kg HPD and 631 nm light). On days 5-7 following HPD-PDT, extensive tumour necrosis was reported. However, these experiments used a single focal spot of light at a very high fluorescence (300 J/cm^2) that most likely resulted in a combination of thermal and PDT-mediated tumour cell killing.

Tochner and colleagues carried out a series of more clinically relevant experiments using ascites tumours in mice. In a mouse model of ovarian peritoneal carcinomatosis, they evaluated HPD-mediated PDT using 50 mg/kg HPD and 514 nm light [74]. In these experiments, mice were injected intraperitoneally with ovarian embryonal cancer cells and then randomly assigned on day 9 following tumour inoculation to receive no treatment, treatment with HPD alone, treatment with light alone or treatment with HPD + light (HPD-PDT). The HPD and light (9.6 J delivered over 16 minutes) were both delivered intraperitoneally and the intraperitoneal tumour burden at the time of treatment was 2-4 g. All of the untreated control animals, as well as the HPD only and light only treated animals died of progressive disease between days 20-23 following tumour inoculation. The mice treated with a single treatment with HPD-PDT on day 9 showed prolonged survival, but only one animal (out of sixteen total) survived past day 34. This animal survived > 50 days and was presumably cured. However, a second group of mice that received two treatments with HPD-PDT on days 9 and 15 showed apparent cure of disease in six out of sixteen animals. It should be noted that in this tumour model, eradication of tumour is difficult to achieve with the administration of intraperitoneal chemotherapy. A 70% cure rate is observed with intraperitoneal doxorubicin but only if the agent is administered two days after tumour inoculation, when the tumour burden is low. If doxorubicin is administered on the same day as the PDT was given (day 9), a cure rate of less than 20% is observed, presumably because of a higher tumour burden [74,75]. Multi-fractionated HPD-PDT has also been studied using a murine ascitic malignant teratoma model. The mice in this study were treated with a total of 4 HPD-PDT treatments using 50 mg/kg HPD delivered intraperitoneally 2 hours prior to therapy and 514 nm light delivered to separate octants (each received 1.2 J in 2 minutes) of the abdomen using a flat cut fiber [76]. 100% of the HPD-PDT treated animals achieved a complete response and 85% showed no evidence of recurrence at necropsy. Taken together, these data suggest that multiple sequential treatments (fractionated PDT) might be necessary in order to achieve a high percentage of cures. These two preclinical studies by Tochner and colleagues provided the impetus for the development of the first Phase I clinical trial of intraperitoneal PDT at the National Cancer Institute (NCI) [77-79].

Preclinical studies have not only helped to define the potential benefits of intraperitoneal PDT, but also have aided in the prediction and evaluation of the potential toxicities of this treatment. Because of the depth of penetration that can be achieved in PDT is greater than the thickness of the bowel wall, toxicity to the bowel, especially bowel perforation, has always been a major concern. In addition to direct bowel toxicity from transmural light penetration, there is also a possibility that PDT could interfere with blood flow in the bowel and thereby indirectly lead to bowel damage through ischemic injury. There has been a suggestion that intraperitoneal PDT with HPD interferes with jejunal blood flow [80]. However, others have found no significant damage to major blood vessels after intraperitoneal treatment [81]. Veenhuizen and colleagues found that the intestines of Wag/RijA rats were the most sensitive organs in a study evaluating intraperitoneal PDT with either Porfimer sodium or meso-tetrahydroxyphenylchlorin (mTHPC) [82]. A steeper toxicity-dose response curve was reported for mTHPC compared to Porfimer sodium but a similar spectrum of toxicities was observed. One bowel perforation in the mTHPC group was reported. Mild reversible damage to the kidneys was seen on histological analysis without functional impairment. Elevations in liver transaminases were also reported in animals treated with Porfimer sodium. Acute lethality caused by intraperitoneal PDT was reported to be the result of toxic shock and rhabdomyolysis leading to circulatory failure.

The normal tissue toxicity of PDT in the peritoneum has also been studied using a dog model [83]. HPD (1.2 mg/kg), was administered intravenously and intraperitoneally. 630 nm light was delivered to the entire peritoneal surface 48 hours after IV injection and 2 hours after intraperitoneal injection of HPD. The doses of light ranged from 0.57-0.74 J/cm^2. Other than a reversible decrease in lymphocyte counts and a modest elevation of liver function tests, no significant toxicities were noted. A mild peritonitis was seen in biopsy specimens of the treated peritoneum.

Given the extensive preclinical evidence that bowel toxicity might be the dose-limiting toxicity of intraperitoneal PDT, concerns were raised regarding the tolerance of bowel anastomoses [84]. Since the initial thoughts were to integrate intraperitoneal PDT with surgical debulking, it was likely that patients would require resection of small bowel as part of the surgical procedure. Small bowel anastomoses were created in New Zealand White rabbits followed by intraperitoneal PDT [84]. HPD was administered in doses of 1.5-2.5 mg/kg twenty-four hours prior to surgery and light doses of 0-20 J/cm^2 were evaluated. No adverse effects on the anastomoses were observed at these doses. Higher doses of HPD (10 mg/kg) administered with light doses of 20 J/cm^2 resulted in a high rate of anastomotic breakdown.

Since the initiation of the Phase I trial at the US National Cancer institute (NCI), other researchers have investigated the preclinical efficacy of fractionated intraperitoneal PDT. Veenhuizen and colleagues have studied intraperitoneal PDT as a treatment for CC531 colon carcinoma implanted in the intraperitoneal fat pad of rats [85]. On day 7 after tumour implantation, rats were treated with Porfimer sodium (5 mg/kg) and 628 nm light (25-75 J/cm^2). All animals treated with Porfimer

sodium-PDT showed significantly longer tumour regrowth time than untreated controls. However, animals treated with multiple fractionations of Porfimer sodium-PDT showed the most prolonged tumour regrowth delays and these results were comparable to or exceeded the results obtained with intraperitoneal cisplatin delivery. Molpus et al. studied PDT with BPD, a second generation photosensitizer, and 690 nm light in a xenograft murine model of the human ovarian cancer, NIH:OVCAR-5 [86]. The light was administered intraperitoneally using a dose of 20 J. Several multi-dose regimens were studied and all led to a reduction in tumour burden at necropsy and a median survival benefit. Despite these apparent benefits of multifraction intraperitoneal PDT in preclinical studies, this concept has yet to be tested in clinical trials, largely due to the inherent difficulty and increased risk of complications associated with repeated surgical procedures (see Clinical Applications of Intraperitoneal PDT). However, recent developments in nanotechnology have the potential to make multi-fraction PDT more feasible [87]. By using nanoparticles comprised of photosensitizer plus a crystal that is capable of up-converting infrared light to visible light, it may be possible to perform intraperitoneal PDT using an external infrared source. This external beam PDT technology is now under evaluation and testing in the preclinical setting at several research centers.

In addition to BPD, several other second generation photosensitizers have been evaluated in preclinical animal studies. 5-aminolevulinic acid (ALA) is converted in vivo into the photosensitizer protoporphyrin IX (PpIX). The ability of ovarian cancer micrometastases to convert ALA to PpIX has been tested in Fischer 344 rats [88]. In these studies, 60-70% of animals with peritoneal ovarian micrometastases showed fluorescence of PpIX on peritoneal surfaces as compared to 0% of the control (no tumour) animals following either intravenous or intraperitoneal ALA delivery. The conversion of ALA to PpIX in ovarian cancer micrometastases has been clinically tested in a study of 17 patients with 36 total biopsies taken from fluorescent and non-fluorescent tissues after intraperitoneal administration of ALA prior to second look laparoscopy [89]. While the sample size is small, this preliminary study showed that for the detection of ovarian cancer micrometastases, the ALA→PpIX conversion has a specificity of 88%, a sensitivity of 100%, a negative predictive value of 100% and a positive predictive value of 91%. However, while the toxicity of ALA-PDT has been evaluated [90], this method has not yet been tested for efficacy in either the preclinical or clinical setting. In addition, multiple groups have demonstrated that the efficiency of a tumour converting ALA→PpIX is inversely proportional to the degree of differentiation of the tumour cells [91-93]. Thus, it is possible that ALA-PDT would be far more effective at killing more highly differentiated intraperitoneal micrometastases and be less effective at killing more poorly differentiated, potentially biologically more aggressive, tumour cells. Motexafin Lutetium (MLu) is another second generation photosensitizer that has an absorbance peak at 732 nm (near-infrared). This peak allows light delivery with less chance of interference from absorption of light by hemoglobin and also allows deeper tissue penetration of MLu-PDT. While this deeper penetration may increase the ability of PDT to treat a greater volume of

residual disease, it also brings with it a greater potential for bowel toxicity. Therefore, the toxicity of MLu-PDT has been tested in canine models of intraperitoneal PDT [94]. In this study, thirteen dogs were treated with 0.2-2mg/kg MLu 3 hours prior to delivery of 0.5-2 J/cm^2 of 732 nm light at laparotomy. Overall this treatment was well tolerated and animals experienced only a mild, transient elevation in liver function tests, but no clinical evidence of significant hepatic or renal impairment. Bowel toxicity was assessed at a second laparotomy 7-10 days after MLu-PDT and histologic evidence of mild enteritis was found in both control and MLu-PDT treated animals. Importantly, in animals that underwent bowel resection at the first laparatomy, there were no anastomotic leaks or other increased bowel toxicities. In another study, similar results and minimal toxicities were observed in dogs undergoing low rectal stapled anastomosis followed by pelvic MLu-PDT [95].

Clinical Applications of Intraperitoneal PDT

A Phase I study of surgery and PDT with laser light and Porfimer sodium was conducted by the Surgery and Radiation Oncology Branches of the NCI for disseminated intraperitoneal malignancies [77-79]. Seventy patients were enrolled on the study, the majority of whom had recurrent ovarian cancer carcinomatosis or peritoneal sarcomatosis. To be eligible for this trial, patients were required to have a work-up that showed no evidence of disease in the liver parenchyma or outside the abdomen and had to be medically fit for surgery. Patients received Porfimer sodium by IV injection prior to laparotomy and an attempt was made to resect all gross disease, where possible, or debulk residual tumour deposits to less than 5 mm in thickness. Any patient with > 5 mm thick residual deposits did not continue on to receive light (PDT), since the effective tissue penetration of 630 nm light is only about 5 mm. Forty-six adequately debulked patients underwent light delivery to all peritoneal surfaces. Real-time light dosimetry was performed using flat photodiodes that were sewn into the right upper quadrant, left upper quadrant, right and left peritoneal gutters, and pelvis. These diodes, along with a mobile diode, measured only incident light and were connected to a computerized on-line dosimetry system. A flat cut fiber was used to illuminate the mesentery, the small bowel and then the large bowel, in that order. Next, the abdominal cavity was filled with dilute intralipid (0.02-0.05%) in order to better scatter the light and improve the homogeneity of light distribution to all areas of the peritoneum. Light to the peritoneal cavity was delivered with a light diffusing wand that was comprised of an optical fiber enclosed in a modified endotracheal tube. This light diffusing wand was moved over anatomic regions that were isolated to ensure uniform delivery of light.

In this phase I study, the PDT dose was sequentially escalated by increasing the sensitizer dose from 1.5-2.5 mg/kg, by shortening the drug-light interval, and by increasing the light dose. Initially 630 nm red light alone was used but later a

combination of 514 nm green light and 630 nm light was used. The reason for this change in wavelengths was that bowel toxicity was initially observed and because of the greater (and presumably transmural) penetration by red light, 514 nm green light was substituted for illumination of the bowel and mesentery. Patients also received boost doses (10-15 joules/cm^2) with 630 nm red light or 5-7.5 joules/cm^2 with 514 nm green light to areas of gross disease on the diaphragms, gutters and/or pelvis.

It should also be pointed out that the distinction between surgical and PDT-related complications was difficult to establish in this study. The patients had advanced refractory disease and often required extensive resections. Some of the complications described above are not atypical of debulking surgery in this patient population. However, most of the complications observed in patients treated on this trial were related to bowel toxicity, and bowel perforation was the PDT-dose limiting toxicity. Four patients developed intestinal fistulae; three patients developed a bowel perforation which was the dose-limiting toxicity. One patient who suffered a colonic perforation, died after multiple procedures and multi-organ failure. All patients who developed a bowel perforation received either 630 nm light to the bowel or a dose of 514 nm light to the bowel of 3.8 J/cm^2 or greater. Even at lower PDT doses, treatment of the entire peritoneum caused intra-abdominal fluid sequestration and small bowel edema that necessitated aggressive fluid resuscitation on the first post-operative day, although this problem was greatest in patients that required more extensive tumour resections or received higher total light doses. In addition to problems with bowel toxicity, seven patients who received light dose of 10 J/cm^2 to the diaphragms developed pleural effusions that caused respiratory compromise and required thoracentesis. Other major (but not dose-limiting) complications included postoperative hemorrhage, necrotizing pancreatitis, splenic rupture, and ureteral leak and urinoma. Sun sensitivity, thrombocytopenia, and asymptomatic liver function test abnormalities were also observed.

Based upon these observed toxicities, the maximally tolerated doses of photosensitizer and light were determined. The maximally tolerated Porfimer sodium dose was 2.5 mg/kg, administered IV 48 hours prior to debulking surgery. The maximally tolerated green light (514 nm) dose to the mesentery, small and large intestine was 2.5 J/cm^2. The maximally tolerated red light (630 nm) dose was 5 J/cm^2 to the stomach, 7.5 J/cm^2 to the liver, spleen, omental bursa, and diaphragm, and 10 J/cm^2 to the retroperitoneal gutters and pelvis. A 15 J/cm^2 boost dose of 630 nm light to limited areas of gross disease in the pelvis, gutters, or diaphragms was also considered tolerable.

While designed to measure toxicity, data on patient outcome were also recorded. Pre and post operative peritoneal cytology was obtained in seventeen patients. Thirteen of seventeen patients with malignant peritoneal cytology were found to have negative follow-up cytologic analysis for an overall peritoneal cytologic response rate of 76%. The median survival of all patients that received PDT was 30 months and there were 3 long term survivors in 25 patients with ovarian cancer. One of these patients died of lymphoma 28 months after treatment and

one patient died of metastatic colon cancer 95 months after treatment. Both patients were free of ovarian cancer recurrence at the time of their deaths. It should be emphasized that these patients had no other treatment after surgical debulking and a single exposure to PDT.

Based upon the results of the Phase I clinical trial, a Phase II clinical trial of intraperitoneal PDT for disseminated intraperitoneal malignancies was initiated in 1997 at the University of Pennsylvania [37,38,44,96-102]. One hundred patients were enrolled, stratified according to cancer type (33 ovarian cancer patients, 37 gastrointestinal malignancy patients, and 30 sarcoma patients) and given doses of Porfimer sodium and light at the maximally tolerated dose a defined in the NCI trial. Of these patients, 29 were not eligible because of the inability to confirm disease status on pathological examination (1 patient), presence of localized disease only (2 patients) or inability to adequately debulk the tumours to <5mm residual disease (26 patients). The primary objective was to define the efficacy of IP PDT in these three groups of patients and the secondary objectives were to report the toxcities of this treatment in each patient population and to assess photosensitizer uptake in tumour and normal tissues. A pathologic restaging of disease was also requested of all patients who were clinically free of disease six months after treatment with PDT.

As in the NCI trial, intraperitoneal PDT was associated with a postoperative capillary leak syndrome that necessitated massive fluid resuscitation in the immediate post-operative period [38,98]. One patient died after suffering a perioperative myocardial infarction that was likely due to a low cardiac output state. A second patient died from sepsis after re-operation for a perioperative bleed. Grade 1 or 2 skin toxicities were observed in 20 patients as a result of skin photosensitization. The remainder of the complications experienced by patients treated on this trial included prolonged intubation secondary to adult respirator distress syndrome (4 patients), bowel fistulae/anastomotic leaks (4 patients) and poor wound healing/infection (4 patients) [38]. Other than the capillary leak syndrome and the skin photosensitivity, these complication rates are not atypical of the complication rates that are observed after similarly extensive surgery in the absence of PDT.

With a 51 month median follow-up, the median failure free survival and overall survival for all enrolled patients by strata were Ovarian: 2.1 months and 20.1 months; gastrointestinal cancers: 1.8 months and 11.1 months; Sarcoma: 3.7 months and 21.9 months. For the patients that received PDT the median failure free survival and overall survival were Ovarian: 3 months and 22 months; gastrointestinal cancers: 3.3 months and 13.2 months; Sarcoma: 4 months and 21.9 months. At six months after therapy, the pathologic complete response rate was 3/33 (9.1%), 2/37 (5.4%), and 4/30 (13.3%) for the patients with ovarian cancer, gastrointestinal cancer and sarcoma, respectively. Although most patients had disease at early follow-up between 3 and 6 months, the median survival of almost 2 years in the ovarian patients and over one year in the gastrointestinal patients suggests some benefit from this treatment. In the patients with sarcoma the prolonged overall survival was primarily due to patients with sarcomatosis from gastrointestinal

stromal tumours who were treated with Gleevec® when it became available. Analysis of the patterns of treatment failure in this study suggests that a significant percentage of patients experienced treatment failure at sites not initially involved by gross disease [96]. Moreover, patients with gross residual disease (that received a PDT boost to these sites) showed similar recurrence kinetics as compared to patients without gross residual disease, suggesting a dose-response relationship in intraperitoneal PDT. However, given the presence of fairly significant toxicities at PDT doses that were not adequate to fully control local disease, the therapeutic window for intraperitoneal PDT would appear to be quite narrow. Thus, undirected PDT dose escalation is unlikely to result in a significant improvement in treatment outcomes.

One of the reasons for this narrow therapeutic window appears to stem from the lower than expected tumour to normal tissue ratios (TNTR) for Porfimer sodium in these studies [37,44]. In normal tissues, drug uptake significantly ($p < 0.0001$) differed as a function of seven different tissue types. In bowel, a toxicity-limiting organ for IP PDT, the mean Photofrin® levels were 2.70 ng/mg and 3.42 ng/mg in full-thickness large and small bowel, respectively. In tumours, drug uptake significantly ($p = 0.0015$) differed as a function of patient cohort: mean Porfimer sodium level was 3.32–5.31 ng/mg among patients with ovarian, gastric or small bowel cancer; 2.09-2.45 ng/mg among patients with sarcoma and appendiceal or colon cancer. Thus, ovarian, gastric, and small bowel cancers demonstrated significantly higher Porfimer sodium uptake than full-thickness large and/or small bowel. However, the ratio of mean drug level in tumour versus bowel was modest at 2.31. In addition, despite multiple analyses, there was no apparent relationship between clinical outcome on this trial and Porfimer sodium uptake. This relatively low TNTR for photosensitizer binding are contrary to the expectations promoted by preclinical studies, but supported by similar results in a phase II trial of intrathoracic PDT using Porfimer sodium [14]. Moreover, the absolute photosensitizer concentrations measured compare favorably with those described in murine models [99]. It has been hypothesized that second generation photosensitizers might show even greater tumour selectivity than first generation photosensitizers such as Porfimer sodium. Indeed, as noted above, ALA appears to concentrate well in peritoneal micrometastases from ovarian cancer, but this sensitizer has not yet been tested clinically in intraperitoneal PDT.

In the processes of completing this trial, great strides were made in measurement of the physical properties that are highly relevant to the potential success of PDT. Real-time, intra-operative light dosimetry included a comparison of incident light measurements (as in the NCI trial) with measurements made from a newer, spherical light dosimetry system that was developed by Starr and colleagues that measures total light dose, including both incident and scattered light [103,104]. The data from the comparison made in patients with peritoneal carcinomatosis demonstrates that the spherical light dosimetry system permits a more accurate measurement of the light dose delivered to superficial tissues [104]. In addition, a specialized broadband infrared spectroscopy system was evaluated for in vivo

measurements of light penetration, blood oxygen saturation, hemoglobin concentration and tissue photosensitizer concentration [102]. Substantial heterogeneity of tissue optical properties were observed giving further credence to the need for real time light dosimetry. In addition, given preclinical results that suggest that correlation of blood flow and tumour oxygenation before, during and after PDT delivery are predictive of the overall efficacy of PDT [105], these dosimetry systems may help to significantly improve PDT outcomes.

New Frontiers in Intraperitoneal PDT: Molecularly Targeted Therapy

These data suggest that improvements in the therapeutic index of intraperitoneal PDT will not be achieved by the use of second generation photosensitizers alone and that other means to increase the therapeutic index such as manipulation of molecular targets involved in PDT response are needed. Multiple lines of evidence have led to the hypothesis that PDT-stimulated signaling through EGFR and post-receptor molecules such as PI3K/AKT and MAPK pathways may lead to cellular resistance to PDT-mediated cytotoxicity. EGFR is a receptor tyrosine kinase that regulates important cellular functions including cell cycle progression and survival mediated through PI3K-AKT, proliferation through MAPK, and protection from apoptosis through STAT3 [106,107]. The interactions between EGFR signaling and PDT are complex and may to some extent be cell line- or photosensitizer-dependent. Some investigators have suggested that PDT-mediated EGFR activation is important for survival of cancer cells following PDT and that EGFR signaling is up-regulated by PDT [108-110]. Others have found that PDT causes a temporary degradation/inactivation of cell surface receptors, including EGFR [111-113]. Interestingly, in either of these cases, inhibition of EGFR signaling might be expected to augment PDT mediated cancer cell killing.

In a recent preclinical publication, Del Carmen, Hasan and colleagues showed that the combination of C225 and BPD-mediated PDT led to a syngergistic response in vivo [114]. In this study, the authors studied the effects of C225-mediated EGFR inhibition on the response to BPD-PDT using a mouse model of ovarian carcinomatosis. C225 was administered in 4 doses of C225 over 9 days (0.5 mg per dose) starting one day after the first treatment with BPD-PDT. An additional dose of BPD-PDT was delivered after completion of the C225 therapy. The combination of C225 + BPD-PDT led to the greatest in vivo tumour response (9.8% tumour burden vs. 38% for PDT alone). Median survival was also greatest in the combination group (80 days vs. 28 days). Importantly, no enhanced normal tissue toxicity was observed in the combination group compared to PDT or C225 alone. This study demonstrates that inhibition of the signal transduction cascade after PDT may improve the therapeutic index of this treatment. Preliminary experiments demonstrate that at least some of this effect likely stems from C225-mediated enhancement of direct cell killing by PDT [115]. Moreover, autocrine

growth factor signaling networks involving the epidermal growth factor (EGF) receptors have been implicated in the development of malignant phenotype as well as the intraperitoneal spread of tumour in both gastrointestinal and ovarian cancers [116-118]. In this respect, C225 has the potential to impact the efficacy of intraperitoneal PDT both by enhancing direct (and possibly indirect) PDT-mediated cancer cell killing and by directly inhibiting the growth and survival of cancer cells.

Another potential mechanism for enhancing the efficacy of intraperitoneal PDT is through targeted photosensitizer delivery. In the Phase II trial of intraperitoneal PDT conducted at the University of Pennsylvania, tumour hypoxia and photosensitizer uptake were poorly correlated with each other and neither correlated well with the size of tumour nodules [44]. There was also significant intra- and inter-patient variability in photosensitizer uptake in both tumour and normal tissues. Along with the relatively narrow therapeutic window of intraperitoneal PDT, these factors suggest the strong clinical potential of molecularly targeted photosensitizers. Solban, Hasan and colleagues have tested the efficacy of anti-EGFR antibody targeted PDT in a variety of settings [119]. In these experiments, an anti-EGFR antibody (OC125) or the $F(ab')_2$ binding portion of this antibody is linked covalently to $chlorin_{e6}$, a photosensitizer derived from chlorophyll. In one study, the efficacy and toxicity of cationic OC125 $F(ab')_2$ $chlorin_{e6}$ cationic conjugate-mediated PDT and free $chlorin_{e6}$-mediated PDT were compared using an mouse model of ovarian carcinomatosis [120]. Tumour treatment response was seen using both agents, but animals treated with OC125 $F(ab')_2$ $chlorin_{e6}$–mediated PDT showed significantly better initial tumour response to treatment, increased overall survival and lower treatment toxicity than animals treated with $chlorin_{e6}$–mediated PDT. Another potential method to target photosensitizers would be to use nanoparticle technology. Early studies have demonstrated PDT-mediated cancer cell killing using ceramic based nanoparticles as a delivery vehicle for photosensitizer [39]. Theoretically, these nanoparticles could be targeted to cancer cells using a variety of ligands and could also be designed to release other toxic substances upon activation by light.

Summary and Conclusions

Peritoneal carcinomatosis and sarcomatosis are generally incurable problems for which there are few good treatment options. Intraperitoneal PDT is potentially an ideal therapy for peritoneal carcinomatosis because of its relatively superficial treatment effect. A Phase II trial of IP PDT with the first generation photosensitizer, Photofrin, demonstrates that this treatment approach is tolerable clinically but is associated with substantial toxicity suggesting a narrow therapeutic index. Remarkably, responses were observed in heavily pre-treated patients suggesting clinical activity. Correlative studies of photosensitizer uptake in human tumour and normal tissues show little tumour selectivity. This lack of photosensitizer

selectivity for tumour in combination with tumour hypoxia (as opposed to oxic normal tissues) is likely a major reason for the narrow therapeutic index of intraperitoneal PDT. However, the advent of novel and potentially molecularly targeted photosensitizers, combined with enhancement of PDT cancer cell cytotoxicity through inhibition of growth factor signaling should greatly improve the therapeutic index of intraperitoneal PDT. In addition, other approaches, including the use of nanotechnology, may allow the administration of fractionated PDT which may also improve the therapeutic index of this treatment. The clinical implementation of these technologies may allow for highly effective and well tolerated treatment of intraperitoneal carcinomatosis with PDT.

References

1. Dougherty TJ, Grindey GB, Fiel R, Weishaupt KR and Boyle DG (1975) Photoradiation therapy. II. Cure of animal tumours with hematoporphyrin and light. J Natl Cancer Inst 55:115-121
2. Manyak MJ, Russo A, Smith PD and Glatstein E (1988) Photodynamic therapy. J Clin Oncol 6:380-391
3. Weishaupt KR, Gomer CJ and Dougherty TJ (1976) Identification of singlet oxygen as the cytotoxic agent in photoinactivation of a murine tumour. Cancer Res 36:2326-2329
4. Peng Q and Nesland JM (2004) Effects of photodynamic therapy on tumour stroma. Ultrastruct Pathol 28:333-340
5. Nelson JS, Liaw LH, Orenstein A, Roberts WG and Berns MW (1988) Mechanism of tumour destruction following photodynamic therapy with hematoporphyrin derivative, chlorin, and phthalocyanine. J Natl Cancer Inst 80:1599-1605
6. van Duijnhoven FH, Aalbers RI, Rovers JP, Terpstra OT and Kuppen PJ (2003) The immunological consequences of photodynamic treatment of cancer, a literature review. Immunobiology 207:105-113
7. Almeida RD, Manadas BJ, Carvalho AP and Duarte CB (2004) Intracellular signaling mechanisms in photodynamic therapy. Biochim Biophys Acta 1704:59-86
8. Biel MA (1994) Photodynamic therapy and the treatment of neoplastic diseases of the larynx. Laryngoscope 104:399-403
9. D'Cruz AK, Robinson MH and Biel MA (2004) mTHPC-mediated photodynamic therapy in patients with advanced, incurable head and neck cancer: a multicenter study of 128 patients. Head Neck 26:232-240
10. Wenig BL, Kurtzman DM, Grossweiner LI, Mafee MF, Harris DM, Lobraico RV, Prycz RA and Appelbaum EL (1990) Photodynamic therapy in the treatment of squamous cell carcinoma of the head and neck. Arch Otolaryngol Head Neck Surg 116:1267-1270

11. Cortese DA, Edell ES and Kinsey JH (1997) Photodynamic therapy for early stage squamous cell carcinoma of the lung. Mayo Clin Proc 72:595-602
12. Diaz-Jimenez JP, Martinez-Ballarin JE, Llunell A, Farrero E, Rodriguez A and Castro MJ (1999) Efficacy and safety of photodynamic therapy versus Nd-YAG laser resection in NSCLC with airway obstruction. Eur Respir J 14:800-805
13. Furuse K, Fukuoka M, Kato H, Horai T, Kubota K, Kodama N, Kusunoki Y, Takifuji N, Okunaka T, Konaka C et al (1993) A prospective phase II study on photodynamic therapy with photofrin II for centrally located early-stage lung cancer. The Japan Lung Cancer Photodynamic Therapy Study Group. J Clin Oncol 11:1852-1857
14. Friedberg JS, Mick R, Stevenson JP, Zhu T, Busch TM, Shin D, Smith D, Culligan M, Dimofte A, Glatstein E and Hahn SM (2004) Phase II trial of pleural photodynamic therapy and surgery for patients with non-small-cell lung cancer with pleural spread. J Clin Oncol 22:2192-2201
15. Baas P, Murrer L, Zoetmulder FA, Stewart FA, Ris HB, van Zandwijk N, Peterse JL and Rutgers EJ (1997) Photodynamic therapy as adjuvant therapy in surgically treated pleural malignancies. Br J Cancer 76:819-826
16. Hahn SM, Smith RP and Friedberg J (2001) Photodynamic therapy for mesothelioma. Curr Treat Options Oncol 2:375-383
17. Pass HI, DeLaney TF, Tochner Z, Smith PE, Temeck BK, Pogrebniak HW, Kranda KC, Russo A, Friauf WS, Cole JW et al (1994) Intrapleural photodynamic therapy: results of a phase I trial. Ann Surg Oncol 1:28-37
18. Lightdale CJ, Heier SK, Marcon NE, McCaughan JS, Jr., Gerdes H, Overholt BF, Sivak MV, Jr., Stiegmann GV and Nava HR (1995) Photodynamic therapy with porfimer sodium versus thermal ablation therapy with Nd:YAG laser for palliation of esophageal cancer: a multicenter randomized trial. Gastrointest Endosc 42:507-512
19. Kelley DJ and Shah JP (1996) Management of Barrett's oesophagus. Lancet 348:561-562
20. Overholt BF, Lightdale CJ, Wang KK, Canto MI, Burdick S, Haggitt RC, Bronner MP, Taylor SL, Grace MG and Depot M (2005) Photodynamic therapy with porfimer sodium for ablation of high-grade dysplasia in Barrett's esophagus: international, partially blinded, randomized phase III trial. Gastrointest Endosc 62:488-498
21. Ortner ME, Caca K, Berr F, Liebetruth J, Mansmann U, Huster D, Voderholzer W, Schachschal G, Mossner J and Lochs H (2003) Successful photodynamic therapy for nonresectable cholangiocarcinoma: a randomized prospective study. Gastroenterology 125:1355-1363
22. Zoepf T, Jakobs R, Arnold JC, Apel D and Riemann JF (2005) Palliation of nonresectable bile duct cancer: improved survival after photodynamic therapy. Am J Gastroenterol 100:2426-2430
23. Muller PJ and Wilson BC (1987) Photodynamic therapy of malignant primary brain tumours: clinical effects, post-operative ICP, and light penetration of the brain. Photochem Photobiol 46:929-935

24. Schuh M, Nseyo UO, Potter WR, Dao TL and Dougherty TJ (1987) Photodynamic therapy for palliation of locally recurrent breast carcinoma. J Clin Oncol 5:1766-1770
25. Sperduto PW, DeLaney TF, Thomas G, Smith P, Dachowski LJ, Russo A, Bonner R and Glatstein E (1991) Photodynamic therapy for chest wall recurrence in breast cancer. Int J Radiat Oncol Biol Phys 21:441-446
26. Marijnissen JP, Star WM, in 't Zandt HJ, D'Hallewin MA and Baert L (1993) In situ light dosimetry during whole bladder wall photodynamic therapy: clinical results and experimental verification. Phys Med Biol 38:567-582
27. Keefe KA, Tadir Y, Tromberg B, Berns M, Osann K, Hashad R and Monk BJ (2002) Photodynamic therapy of high-grade cervical intraepithelial neoplasia with 5-aminolevulinic acid. Lasers Surg Med 31:289-293
28. Barnett AA, Haller JC, Cairnduff F, Lane G, Brown SB and Roberts DJ (2003) A randomised, double-blind, placebo-controlled trial of photodynamic therapy using 5-aminolaevulinic acid for the treatment of cervical intraepithelial neoplasia. Int J Cancer 103:829-832
29. Verigos K, Stripp DC, Mick R, Zhu TC, Whittington R, Smith D, Dimofte A, Finlay J, Busch TM, Tochner ZA, Malkowicz S, Glatstein E and Hahn SM (2006) Updated results of a phase I trial of motexafin lutetium-mediated interstitial photodynamic therapy in patients with locally recurrent prostate cancer. J Environ Pathol Toxicol Oncol 25:373-388
30. Zhu TC, Dimofte A, Finlay JC, Stripp D, Busch T, Miles J, Whittington R, Malkowicz SB, Tochner Z, Glatstein E and Hahn SM (2005) Optical properties of human prostate at 732 nm measured in mediated photodynamic therapy. Photochem Photobiol 81:96-105
31. Zhu TC, Hahn SM, Kapatkin AS, Dimofte A, Rodriguez CE, Vulcan TG, Glatstein E and Hsi RA (2003) In vivo optical properties of normal canine prostate at 732 nm using motexafin lutetium-mediated photodynamic therapy. Photochem Photobiol 77:81-88
32. Morton C, Horn M, Leman J, Tack B, Bedane C, Tjioe M, Ibbotson S, Khemis A and Wolf P (2006) Comparison of topical methyl aminolevulinate photodynamic therapy with cryotherapy or Fluorouracil for treatment of squamous cell carcinoma in situ: Results of a multicenter randomized trial. Arch Dermatol 142:729-735
33. Lui H, Hobbs L, Tope WD, Lee PK, Elmets C, Provost N, Chan A, Neyndorff H, Su XY, Jain H, Hamzavi I, McLean D and Bissonnette R (2004) Photodynamic therapy of multiple nonmelanoma skin cancers with verteporfin and red light-emitting diodes: two-year results evaluating tumour response and cosmetic outcomes. Arch Dermatol 140:26-32
34. Rhodes LE, de Rie M, Enstrom Y, Groves R, Morken T, Goulden V, Wong GA, Grob JJ, Varma S and Wolf P (2004) Photodynamic therapy using topical methyl aminolevulinate vs surgery for nodular basal cell carcinoma: results of a multicenter randomized prospective trial. Arch Dermatol 140:17-23
35. Gomer CJ and Dougherty TJ (1979) Determination of [3H]- and [14C] hematoporphyrin derivative distribution in malignant and normal tissue. Cancer Res 39:146-151

36. Young SW, Woodburn KW, Wright M, Mody TD, Fan Q, Sessler JL, Dow WC and Miller RA (1996) Lutetium texaphyrin (PCI-0123): a near-infrared, water-soluble photosensitizer. Photochem Photobiol 63:892-897
37. Hahn S, Putt M, Metz J, Shin D, Rickter E, Menon C, Smith D, Glatstein E, Fraker D and Busch T (2006) Photofrin uptake in the tumour and normal tissues of patients receiving intra-peritoneal photodynamic therapy. Clin Cancer Res, *submitted*
38. Hahn SM, Fraker DL, Mick R, Metz J, Busch TM, Smith D, Zhu T, Rodriguez C, Dimofte A, Spitz F, Putt M, Rubin SC, Menon C, Wang HW, Shin D, Yodh A and Glatstein E (2006) A phase II trial of intraperitoneal photodynamic therapy for patients with peritoneal carcinomatosis and sarcomatosis. Clin Cancer Res 12:2517-2525
39. Roy I, Ohulchanskyy TY, Pudavar HE, Bergey EJ, Oseroff AR, Morgan J, Dougherty TJ and Prasad PN (2003) Ceramic-based nanoparticles entrapping water-insoluble photosensitizing anticancer drugs: a novel drug-carrier system for photodynamic therapy. J Am Chem Soc 125:7860-7865
40. Oleinick NL, Antunez AR, Clay ME, Rihter BD and Kenney ME (1993) New phthalocyanine photosensitizers for photodynamic therapy. Photochem Photobiol 57:242-247
41. Dolmans DE, Fukumura D and Jain RK (2003) Photodynamic therapy for cancer. Nat Rev Cancer 3:380-387
42. Abels C (2004) Targeting of the vascular system of solid tumours by photodynamic therapy (PDT). Photochem Photobiol Sci 3:765-771
43. Busch TM, Hahn SM, Evans SM and Koch CJ (2000) Depletion of tumour oxygenation during photodynamic therapy: detection by the hypoxia marker EF3 [2-(2-nitroimidazol-1[H]-yl)-N-(3,3,3-trifluoropropyl)acetamide]. Cancer Res 60:2636-2642
44. Busch TM, Hahn SM, Wileyto EP, Koch CJ, Fraker DL, Zhang P, Putt M, Gleason K, Shin DB, Emanuele MJ, Jenkins K, Glatstein E and Evans SM (2004) Hypoxia and photofrin uptake in the intraperitoneal carcinomatosis and sarcomatosis of photodynamic therapy patients. Clin Cancer Res 10:4630-4638
45. Busch TM, Wileyto EP, Emanuele MJ, Del Piero F, Marconato L, Glatstein E and Koch CJ (2002) Photodynamic therapy creates fluence rate-dependent gradients in the intratumoural spatial distribution of oxygen. Cancer Res 62:7273-7279
46. Gollnick SO, Lee BY, Vaughan L, Owczarczak B and Henderson BW (2001) Activation of the IL-10 gene promoter following photodynamic therapy of murine keratinocytes. Photochem Photobiol 73:170-177
47. Gollnick SO, Liu X, Owczarczak B, Musser DA and Henderson BW (1997) Altered expression of interleukin 6 and interleukin 10 as a result of photodynamic therapy in vivo. Cancer Res 57:3904-3909
48. Gollnick SO, Musser DA, Oseroff AR, Vaughan L, Owczarczak B and Henderson BW(2001) IL-10 does not play a role in cutaneous Photofrin photodynamic therapy-induced suppression of the contact hypersensitivity response. Photochem Photobiol 74:811-816

49. Korbelik M (1996) Induction of tumour immunity by photodynamic therapy. J Clin Laser Med Surg 14:329-334
50. Korbelik M and Cecic I (1999) Contribution of myeloid and lymphoid host cells to the curative outcome of mouse sarcoma treatment by photodynamic therapy. Cancer Lett 137:91-98
51. Korbelik M and Dougherty GJ (1999) Photodynamic therapy-mediated immune response against subcutaneous mouse tumours. Cancer Res 59:1941-1946
52. Cecic I, Sun J and Korbelik M (2006) Role of complement anaphylatoxin C3a in photodynamic therapy-elicited engagement of host neutrophils and other immune cells. Photochem Photobiol 82:558-562
53. Edinger AL and Thompson CB (2004) Death by design: apoptosis, necrosis and autophagy. Curr Opin Cell Biol 16:663-669
54. Korbelik M, Krosl G, Krosl J and Dougherty GJ (1996) The role of host lymphoid populations in the response of mouse EMT6 tumour to photodynamic therapy. Cancer Res 56:5647-5652
55. Luo Y and Kessel D (1997) Initiation of apoptosis versus necrosis by photodynamic therapy with chloroaluminum phthalocyanine. Photochem Photobiol 66:479-483
56. Vantieghem A, Assefa Z, Vandenabeele P, Declercq W, Courtois S, Vandenheede JR, Merlevede W, de Witte P and Agostinis P (1998) Hypericin-induced photosensitization of HeLa cells leads to apoptosis or necrosis. Involvement of cytochrome c and procaspase-3 activation in the mechanism of apoptosis. FEBS Lett 440:19-24
57. Kamuhabwa AR, Agostinis PM, D'Hallewin MA, Baert L and de Witte PA (2001) Cellular photodestruction induced by hypericin in AY-27 rat bladder carcinoma cells. Photochem Photobiol 74:126-132
58. Wyld L, Reed MW and Brown NJ (2001) Differential cell death response to photodynamic therapy is dependent on dose and cell type. Br J Cancer 84:1384-1386
59. Oleinick NL, Morris RL and Belichenko I (2002) The role of apoptosis in response to photodynamic therapy: what, where, why, and how. Photochem Photobiol Sci 1:1-21
60. Murant RS, Gibson SL and Hilf R (1987) Photosensitizing effects of Photofrin II on the site-selected mitochondrial enzymes adenylate kinase and monoamine oxidase. Cancer Res 47:4323-4328
61. Woodburn KW, Vardaxis NJ, Hill JS, Kaye AH and Phillips DR (1991) Subcellular localization of porphyrins using confocal laser scanning microscopy. Photochem Photobiol 54:725-732
62. Ricchelli F, Gobbo S, Jori G, Salet C and Moreno G (1995) Temperature-induced changes in fluorescence properties as a probe of porphyrin microenvironment in lipid membranes. 2. The partition of hematoporphyrin and protoporphyrin in mitochondria. Eur J Biochem 233:165-170
63. Peng Q, Moan J and Nesland JM (1996) Correlation of subcellular and intratumoural photosensitizer localization with ultrastructural features after photodynamic therapy. Ultrastruct Pathol 20:109-129

64. Granville DJ, Levy JG and Hunt DW (1997) Photodynamic therapy induces caspase-3 activation in HL-60 cells. Cell Death Differ 4:623-628
65. Granville DJ, Jiang H, An MT, Levy JG, McManus BM and Hunt DW (1998) Overexpression of Bcl-X(L) prevents caspase-3-mediated activation of DNA fragmentation factor (DFF) produced by treatment with the photochemotherapeutic agent BPD-MA. FEBS Lett 422:151-154
66. Carthy CM, Granville DJ, Jiang H, Levy JG, Rudin CM, Thompson CB, McManus BM and Hunt DW (1999) Early release of mitochondrial cytochrome c and expression of mitochondrial epitope 7A6 with a porphyrin-derived photosensitizer: Bcl-2 and Bcl-xL overexpression do not prevent early mitochondrial events but still depress caspase activity. Lab Invest 79:953-965
67. Granville DJ, Jiang H, An MT, Levy JG, McManus BM and Hunt DW (1999) Bcl-2 overexpression blocks caspase activation and downstream apoptotic events instigated by photodynamic therapy. Br J Cancer 79:95-100
68. Granville DJ, Carthy CM, Jiang H, Levy JG, McManus BM, Matroule JY, Piette J and Hunt DW (2000) Nuclear factor-kappaB activation by the photochemotherapeutic agent verteporfin. Blood 95:256-262
69. Dahle J, Bagdonas S, Kaalhus O, Olsen G, Steen HB and Moan J (2000) The bystander effect in photodynamic inactivation of cells. Biochim Biophys Acta 1475:273-280
70. Dahle J, Kaalhus O, Moan J and Steen HB (1997) Cooperative effects of photodynamic treatment of cells in microcolonies. Proc Natl Acad Sci U S A 94:1773-1778
71. Dahle J, Mikalsen SO, Rivedal E and Steen HB (2000) Gap junctional intercellular communication is not a major mediator in the bystander effect in photodynamic treatment of MDCK II cells. Radiat Res 154:331-341
72. Dahle J, Steen HB and Moan J (1999) The mode of cell death induced by photodynamic treatment depends on cell density. Photochem Photobiol 70:363-367
73. Douglass HO, Jr., Nava HR, Weishaupt KR, Boyle D, Sugerman MG, Halpern E and Dougherty TJ (1983) Intra-abdominal applications of hematoporphyrin photoradiation therapy. Adv Exp Med Biol 160:15-21
74. Tochner Z, Mitchell JB, Harrington FS, Smith P, Russo DT and Russo A (1985) Treatment of murine intraperitoneal ovarian ascitic tumour with hematoporphyrin derivative and laser light. Cancer Res 45:2983-2987
75. Ozols RF, Locker GY, Doroshow JH, Grotzinger KR, Myers CE, Fisher RI and Young RC (1979) Chemotherapy for murine ovarian cancer: a rationale for ip therapy with adriamycin. Cancer Treat Rep 63:269-273
76. Tochner Z, Mitchell JB, Smith P, Harrington F, Glatstein E, Russo D and Russo A (1986) Photodynamic therapy of ascites tumours within the peritoneal cavity. Br J Cancer 53:733-736
77. DeLaney TF, Sindelar WF, Tochner Z, Smith PD, Friauf WS, Thomas G, Dachowski L, Cole JW, Steinberg SM and Glatstein E (1993) Phase I study of debulking surgery and photodynamic therapy for disseminated intraperitoneal tumours. Int J Radiat Oncol Biol Phys 25:445-457

78. Sindelar W, Sullivan F, Abraham E, DeLaney T, Smith P, Friauf W, Thomas G, Smith J and Okunieff P (1995) Intraperitoneal Photodynamic Therapy shows efficacy in Phase I Trial. Proc Am Soc Clin Oncol 14:447
79. Sindelar WF, DeLaney TF, Tochner Z, Thomas GF, Dachoswki LJ, Smith PD, Friauf WS, Cole JW and Glatstein E (1991) Technique of photodynamic therapy for disseminated intraperitoneal malignant neoplasms. Phase I study. Arch Surg 126:318-324
80. Selman SH, Kreimer-Birnbaum M, Goldblatt PJ, Anderson TS, Keck RW and Britton SL (1985) Jejunal blood flow after exposure to light in rats injected with hematoporphyrin derivative. Cancer Res 45:6425-6427
81. Suzuki S, Nakamura S and Sakaguchi S (1987) Experimental study of intra-abdominal photodynamic therapy. Lasers Med Sci 2:195-203
82. Veenhuizen RB, Ruevekamp-Helmers MC, Helmerhorst TJ, Kenemans P, Mooi WJ, Marijnissen JP and Stewart FA (1994) Intraperitoneal photodynamic therapy in the rat: comparison of toxicity profiles for photofrin and MTHPC. Int J Cancer 59:830-836
83. Tochner Z, Mitchell JB, Hoekstra HJ, Smith P, DeLuca AM, Barnes M, Harrington F, Manyak M, Russo D, Russo A et al (1991) Photodynamic therapy of the canine peritoneum: normal tissue response to intraperitoneal and intravenous photofrin followed by 630 nm light. Lasers Surg Med 11:158-164
84. DeLaney TF, Sindelar WF, Thomas GF, DeLuca AM and Taubenberger JK (1993) Tolerance of small bowel anastomoses in rabbits to photodynamic therapy with dihematoporphyrin ethers and 630 nm red light. Lasers Surg Med 13:664-671
85. Veenhuizen RB, Marijnissen JP, Kenemans P, Ruevekamp-Helmers MC, t Mannetje LW, Helmerhorst TJ and Stewart FA (1996) Intraperitoneal photodynamic therapy of the rat CC531 adenocarcinoma. Br J Cancer 73:1387-1392
86. Molpus KL, Kato D, Hamblin MR, Lilge L, Bamberg M and Hasan T (1996) Intraperitoneal photodynamic therapy of human epithelial ovarian carcinomatosis in a xenograft murine model. Cancer Res 56:1075-1082
87. Kapoor R, Friend C, Biswas A and Prasad P (2000) Highly efficient infrared-to-visible energy upconversion in Er^{3+}:Y_2O_3. Opt Lett 25:338
88. Major AL, Rose GS, Chapman CF, Hiserodt JC, Tromberg BJ, Krasieva TB, Tadir Y, Haller U, DiSaia PJ and Berns MW (1997) In vivo fluorescence detection of ovarian cancer in the NuTu-19 epithelial ovarian cancer animal model using 5-aminolevulinic acid (ALA). Gynecol Oncol 66:122-132
89. Loning MC, Diddens HC, Holl-Ulrich K, Loning U, Kupker W, Diedrich K and Huttmann G (2006) Fluorescence staining of human ovarian cancer tissue following application of 5-aminolevulinic acid: Fluorescence microscopy studies. Lasers Surg Med 38:549-554
90. Major AL, Rose GS, Svaasand LO, Ludicke F, Campana A and van Gemert MJ (2002) Intraperitoneal photodynamic therapy in the Fischer 344 rat using 5-aminolevulinic acid and violet laser light: a toxicity study. J Photochem Photobiol B 66:107-114

91. Li G, Szewczuk MR, Pottier RH and Kennedy JC (1999) Effect of mammalian cell differentiation on response to exogenous 5-aminolevulinic acid. Photochem Photobiol 69:231-235
92. Ortel B, Chen N, Brissette J, Dotto GP, Maytin E and Hasan T (1998) Differentiation-specific increase in ALA-induced protoporphyrin IX accumulation in primary mouse keratinocytes. Br J Cancer 77:1744-1751
93. Ortel B, Sharlin D, O'Donnell D, Sinha AK, Maytin EV and Hasan T (2002) Differentiation enhances aminolevulinic acid-dependent photodynamic treatment of LNCaP prostate cancer cells. Br J Cancer 87:1321-1327
94. Griffin GM, Zhu T, Solonenko M, Del Piero F, Kapakin A, Busch TM, Yodh A, Polin G, Bauer T, Fraker D and Hahn SM (2001) Preclinical evaluation of motexafin lutetium-mediated intraperitoneal photodynamic therapy in a canine model. Clin Cancer Res 7:374-381
95. Ross HM, Smelstoys JA, Davis GJ, Kapatkin AS, Del Piero F, Reineke E, Wang H, Zhu TC, Busch TM, Yodh AG and Hahn SM (2006) Photodynamic Therapy with Motexafin Lutetium for Rectal Cancer: A Preclinical Model in the Dog. J Surg Res, in press
96. Wilson JJ, Jones H, Burock M, Smith D, Fraker DL, Metz J, Glatstein E and Hahn SM (2004) Patterns of recurrence in patients treated with photodynamic therapy for intraperitoneal carcinomatosis and sarcomatosis. Int J Oncol 24:711-717
97. Hendren SK, Hahn SM, Spitz FR, Bauer TW, Rubin SC, Zhu T, Glatstein E and Fraker DL (2001) Phase II trial of debulking surgery and photodynamic therapy for disseminated intraperitoneal tumours. Ann Surg Oncol 8:65-71
98. Canter RJ, Mick R, Kesmodel SB, Raz DJ, Spitz FR, Metz JM, Glatstein EJ, Hahn SM and Fraker DL (2003) Intraperitoneal photodynamic therapy causes a capillary-leak syndrome. Ann Surg Oncol 10:514-524
99. Menon C, Kutney SN, Lehr SC, Hendren SK, Busch TM, Hahn SM and Fraker DL (2001) Vascularity and uptake of photosensitizer in small human tumour nodules: implications for intraperitoneal photodynamic therapy. Clin Cancer Res 7:3904-3911
100. Bauer TW, Hahn SM, Spitz FR, Kachur A, Glatstein E and Fraker DL (2001) Preliminary report of photodynamic therapy for intraperitoneal sarcomatosis. Ann Surg Oncol 8:254-259
101. Dimofte A, Zhu TC, Hahn SM and Lustig RA (2002) In vivo light dosimetry for motexafin lutetium-mediated PDT of recurrent breast cancer. Lasers Surg Med 31:305-312
102. Wang HW, Zhu TC, Putt ME, Solonenko M, Metz J, Dimofte A, Miles J, Fraker DL, Glatstein E, Hahn SM and Yodh AG (2005) Broadband reflectance measurements of light penetration, blood oxygenation, hemoglobin concentration, and drug concentration in human intraperitoneal tissues before and after photodynamic therapy. J Biomed Opt 10:14004.
103. Van Staveren H, Marijnissen J, Aalders M and Star W (1995) Construction, quality control and calibration of spherical isotropic fibre-optic light diffusers. Lasers Med Sci 10:137-147

104. Vulcan TG, Zhu TC, Rodriguez CE, Hsi A, Fraker DL, Baas P, Murrer LH, Star WM, Glatstein E, Yodh AG and Hahn SM (2000) Comparison between isotropic and nonisotropic dosimetry systems during intraperitoneal photodynamic therapy. Lasers Surg Med 26:292-301
105. Wang HW, Putt ME, Emanuele MJ, Shin DB, Glatstein E, Yodh AG and Busch TM (2004) Treatment-induced changes in tumour oxygenation predict photodynamic therapy outcome. Cancer Res 64:7553-7561
106. Silva CM (2004) Role of STATs as downstream signal transducers in Src family kinase-mediated tumourigenesis. Oncogene 23:8017-8023
107. Hynes NE and Lane HA (2005) ERBB receptors and cancer: the complexity of targeted inhibitors. Nat Rev Cancer 5:341-354
108. Fanuel-Barret D, Patrice T, Foultier MT, Vonarx-Coinsmann V, Robillard N and Lajat Y (1997) Influence of epidermal growth factor on photodynamic therapy of glioblastoma cells in vitro. Res Exp Med (Berl) 197:219-233
109. Tong Z, Singh G and Rainbow AJ (2002) Sustained activation of the extracellular signal-regulated kinase pathway protects cells from photofrin-mediated photodynamic therapy. Cancer Res 62:5528-5535
110. Moor A, Rizvi I, Savellano M, Yu W, Del Carmen M and Hasan T (2000) Photoimunotargeting of the epidermal growth factor receptor for the treatment of ovarian cancer. 13th International Congress on Photobiology and 28th Annual Meeting ASP 166
111. Ahmad N, Kalka K and Mukhtar H (2001) In vitro and in vivo inhibition of epidermal growth factor receptor-tyrosine kinase pathway by photodynamic therapy. Oncogene 20:2314-2317
112. Wong TW, Tracy E, Oseroff AR and Baumann H (2003) Photodynamic therapy mediates immediate loss of cellular responsiveness to cytokines and growth factors. Cancer Res 63:3812-3818
113. Liu W, Oseroff AR and Baumann H (2004) Photodynamic therapy causes cross-linking of signal transducer and activator of transcription proteins and attenuation of interleukin-6 cytokine responsiveness in epithelial cells. Cancer Res 64:6579-6587
114. del Carmen MG, Rizvi I, Chang Y, Moor AC, Oliva E, Sherwood M, Pogue B and Hasan T (2005) Synergism of epidermal growth factor receptor-targeted immunotherapy with photodynamic treatment of ovarian cancer in vivo. J Natl Cancer Inst 97:1516-1524
115. Cengel K, Voong K and Hahn S. Unpublished Observations
116. Mills GB and Moolenaar WH (2003) The emerging role of lysophosphatidic acid in cancer. Nat Rev Cancer 3:582-591
117. Agarwal R and Kaye SB (2003) Ovarian cancer: strategies for overcoming resistance to chemotherapy. Nat Rev Cancer 3:502-516
118. Yagi H, Miyamoto S, Tanaka Y, Sonoda K, Kobayashi H, Kishikawa T, Iwamoto R, Mekada E and Nakano H (2005) Clinical significance of heparin-binding epidermal growth factor-like growth factor in peritoneal fluid of ovarian cancer. Br J Cancer 92:1737-1745
119. Solban N, Rizvi I and Hasan T (2006) Targeted photodynamic therapy. Lasers Surg Med 38:522-531

120. Molpus KL, Hamblin MR, Rizvi I and Hasan T (2000) Intraperitoneal photoimmunotherapy of ovarian carcinoma xenografts in nude mice using charged photoimmunoconjugates. Gynecol Oncol 76:397-404

Intraperitoneal Gene Therapy

S Madhusudan, TS Ganesan

Introduction

Peritoneal carcinomatosis is a part of the evolution of several gynaecological and non-gynaecological cancers. A combination of surgery and intraperitoneal (IP) chemotherapy may improve outcomes for some patients. IP chemotherapy has distinct pharmacokinetic advantages such as high local drug concentration and prolonged half-life of the drug in the peritoneal cavity. In addition, IP chemotherapy may provide an opportunity to selectively target cancer cells [16,31,40,42,].

Advances in cancer biology have identified several new targets for therapy. Among approaches to modulate these 'novel targets', gene therapy offers an opportunity to selectively modulate cancer cells. The main objective is to target cancer cells and minimize systemic normal tissue toxicity. To achieve this objective a variety of strategies have been attempted in the laboratory and in the clinic in recent years. Replacement of defective tumour suppressor genes, inactivation of oncogenes, suicide gene therapy, gene-based vaccines, anti-angiogenic gene therapy, viral gene therapy and bacterial gene therapy strategies are some of the approaches under investigation [5,8,9,12,23,26,28,37,54]. Gene therapy strategy has been attempted in several human tumours such as gastric [45], breast [47], prostate [25], lung [48], head & neck [17], thyroid [35], oesophageal [8] and ovarian cancer [41]. Intraperitoneal gene therapy is a promising strategy to treat cancer confined predominantly to the peritoneal cavity [32,33]. The main focus of this chapter will be on the current status of IP gene therapy using ovarian cancer as a model.

Vectors

Delivery of therapeutic gene to tumours requires vectors which must efficiently mediate transgene expression and specifically target cancer cells. In addition, vectors must be easy to manufacture with a large capacity for transgene inserts and be non-immunogenic [11,39,43].

Current vectors can be broadly classified into non-viral and viral vectors. Naked DNA and DNA complexed with lipids or polymers are the non-viral techniques that have been used in several pre-clinical and clinical studies. Non-viral vectors are non-immunogenic, easy to produce with large gene insert size [37]. Viral vectors integrate into host genome and have long-term gene expression capacity. However viral vectors may be immunogenic and carry a risk of insertional mutagenesis. Viral vectors under investigation include retrovirus, lentivirus, adenovirus and adeno-associated virus vectors [37,59]. Adenovirus based vectors are the most extensively investigated. The ability of bacteria to mediate gene transfer has also been investigated recently [51].

Gene Therapy Strategies

Ovarian cancer has been the model system used for several intraperitoneal gene therapy clinical studies and as such will be the focus of this article [41]. The role of debulking and the effect on survival has been clearly shown in ovarian cancer and more recently IP Chemotherapy is associated with increased survival. In addition, we will also discuss evolving strategies in preclinical xenograft models.

Targeting p53 tumour suppressor gene

The p53 tumour suppressor gene is centrally involved in the regulation of growth and apoptosis. Mutations in p53 are commonly seen in cancer and hence p53 gene replacement therapy is an attractive anti-cancer treatment strategy. Intraperitoneal gene therapy in the ovarian cancer nude mouse model with the recombinant adenoviral-mediated wild-type p53 tumour suppressor gene (Avp53) produced impressive anti-tumour responses [27,53]. A phase I study of intraperitoneal recombinant adenoviral-mediated wild-type p53 tumour suppressor gene (Avp53, INGN 201; ADVEXIN) for patients with platinum- and paclitaxel-resistant epithelial ovarian cancer was published recently [56]. A replication-deficient adenovirus containing human p53 c DNA was used in this study. Eligible patients underwent laparoscopy, washings, biopsies, and placement of an IP catheter. Adp53 was given daily for 5 days every 3 weeks at one of the following four dose levels: 3 x 10^{10}, 3 x 10^{11}, 1 x 10^{12}, or 3 x 10^{12} viral particles. No dose-limiting toxicities (DLT) were observed. Two of 17 patients (12%) had a mixed response. Four patients (24%) had stable disease for up to four courses.

Assessment of p53 gene transfer and biological activities was investigated in another clinical study for recurrent ovarian cancer [55]. Gene transfer and expression were documented in tumour biopsies (four of five patients) and upregulation of p21/WAF1, bax and mdm-2, and down regulation of survivin were observed in tumour samples, suggesting biological activity of p53 gene replacement in tumours [55].

In another phase I/II trial of rAd/p53 (SCH 58500) gene replacement in recurrent ovarian cancer, SCH 58500 was delivered into the peritoneal cavity alone and sequentially in combination with platinum-based chemotherapy, of patients with recurrent ovarian, primary peritoneal, or fallopian tube cancer with confirmed p53 mutation. There were no dose-limiting toxicities. Vector-specific transgene expression in tumour was documented in tumour tissue. In addition, there were extensive adenoviral-induced inflammatory changes in the peritoneum [7].

Given the promising preclinical and clinical data, a multicentre randomized phase II/III p53 gene-therapy study for first-line treatment of patients with ovarian cancer was initiated. Replication-deficient adenoviral vectors carrying wild-type p53 was given IP in combination with standard chemotherapy to patients with confirmed p53 mutations in tumours. Unfortunately this study was closed after the first interim analysis because of lack of therapeutic benefit with addition of p53 gene therapy. Epigenetic changes leading to silencing of several genes and multiple genetic changes that drive the cancer phenotype may account for the failure of this study indicating that targeting one gene may not be a viable anti-cancer strategy. In addition, cross talk between ectopic wild type p53 and mutant p53 may negatively impact on the effectiveness of p53 gene therapy [61].

Targeting HER-2/neu proto-oncogene

HER-2/ neu proto-oncogene encodes a 185 kDa transmembrane receptor tyrosine kinase, whose activation causes initiation of complex signalling pathways involved in the regulation of growth, differentiation, adhesion, migration and apoptosis [57]. Overexpression of HER-2 /neu has been shown to be a rate limiting factor for ovarian cancer growth in preclinical studies [20,24]. HER-2/neu is associated with a poor prognosis in ovarian cancer [6]. The predictive value of HER-2 /neu overexpression and chemoresistance has been demonstrated in ovarian cancer patients [34]. The human adenovirus-5 E1A gene product is known to bind to transcriptional co-activators, co-repressors and cell- cycle regulatory proteins involved in gene regulation and cell growth [14,15]. E1A reverses the malignant phenotype and suppresses the growth of human tumour xenografts [13]. This tumour suppressing effect is achieved by several mechanisms including repression of HER-2/neu overexpression [14]. Cationic liposome based IP E1A gene therapy (E1A-lipid complex) caused significant suppression of tumour growth and improved survival in mice bearing peritoneal tumours produced by IP inoculation of SKOV-3 ovarian cancer cells that overexpress HER-2/neu [60].

Several early phase human studies have been completed in recurrent breast, head & neck and ovarian cancer [21,52,58]. We have recently conducted a phase I trial involving IP administration of E1A-lipid complex in ovarian cancer patients to assess biological activity (E1A gene transfer/ transcription/ translation and HER-2/neu expression) and to determine the maximum tolerated dose. Successive cohorts received E1A-lipid complex at doses of 1.8, 3.6 and 7.2 milligrams DNA/ m^2, given as weekly IP infusions for 3 out of 4 weeks (each course) up to a maxi-

mum of six courses. Peritoneal fluid was sampled at baseline and twice monthly for cellularity, cytology, CA-125 and biological activity. Fifteen patients were recruited. Median age was 57 years (range 43-81). Three, four and eight patients received 1.8, 3.6 and 7.2 milligrams DNA/ m^2 respectively. A total of 91 infusions (range 1-18) were administered. Abdominal pain was the near dose limiting toxicity at 7.2 milligrams DNA/ m^2. E1A gene transfer and expression was seen in all patients and at all dose levels. Two patients (18%) demonstrated HER-2/neu downregulation. There was no correlation between dose and biological activity [30].

In another phase I study involving administration of E1A gene therapy into the pleural or peritoneal cavity, results very similar to ours were reported. A maximum tolerated dose of 3.6 mg DNA/m^2 was defined in this study [21]. These early phase trials in patients with advanced cancer suggest biological and possible clinical benefit to patients who receive loco-regional single agent E1A gene therapy. However, impressive responses seen in animal studies were not achieved mainly because patients recruited into these studies had very advanced cancers with heavy disease burden. Future studies either alone or in combination with chemotherapy particularly in patients with minimal residual disease are likely to produce more promising results.

Other adenovirus based approaches

Gene-directed enzyme prodrug therapy (GDEPT) offers the possibility of a targeted treatment with the ability to selectively kill tumour cells and not the normal tissues. In GDEPT, or suicide gene therapy, the gene encoding an enzyme is delivered to tumour cells, followed by administration of a pro-drug, which is converted locally to a cytotoxic agent by the activating enzyme. According to the phenomenon of 'by stander effect', not only the producer cells but also the surrounding cells are killed, thus enhancing the anti-cancer activity. GDEPT systems investigated in clinical studies have included herpes simplex virus thymidine kinase/gancyclovir, bacterial cytosine deaminase/5-fluorocytosine, bacterial nitroreductase/CB1954 and cytochrome P450/cyclophosphamide. GDEPT systems in preclinical development includes P450 reductase/tirapazamine, carboxypeptidase/CMDA, horseradish peroxidase/indole-3-acetic acid or paracetamol [9].

Several phase I studies of adenoviral-mediated suicide gene therapy in women with recurrent ovarian cancer have been reported. In one study, patients with recurrent ovarian cancer received debulking surgery followed by adenovirus-mediated herpes simplex virus thymidine kinase gene therapy, and systemic application of acyclovir or valacyclovir and topotecan. Biopsies were taken at the time of secondary debulking about 1 month after gene therapy and chemotherapy and were analyzed for expression of coxsackie-adenovirus receptor (CAR) and integrins alphaVbeta3 and alphaVbeta5. Tumour CAR expression was confirmed in all tumours after gene therapy and Integrin alphaVbeta3 was found in all tumours before and after gene therapy [18]. In a similar study, it was reported that a combination of secondary optimal debulking, adenovirus-mediated thymidine kinase gene therapy, and

topotecan, could improve median overall survival than in previously reported second-and third-line trials [19]. In another study, patients were treated intraperitoneally with herpes simplex virus-thymidine kinase (HSV-TK)-encoding adenovirus (AdHSV-TK) and 2 days later, ganciclovir (GCV) was administered intravenously for 14 days. Transient vector-associated fever, abdominal pain and gastro-intestinal symptoms were the commonly reported side effects. 38% of patients achieved stable disease in that study. [2]. Similar studies in ovarian cancer have been reported by other investigators [1,29].

The first clinical trial of replication-competent adenovirus administered IP in ovarian cancer patients was reported recently [50]. The adenovirus dl1520 (ONYX-015) with the E1B 55-kd gene deleted selectively replicates in and causes lysis of p53-deficient tumour cells. Preclinical efficacy has been demonstrated in p53-deficient nude mouse-human ovarian carcinomatosis xenografts. A phase I trial of IP injection of the E1B-55-kd-gene-deleted adenovirus ONYX-015 (dl1520) given on days 1 through 5 every 3 weeks was recently reported in patients with recurrent/refractory epithelial ovarian cancer [50]. Sixteen patients received 35 cycles of dl1520 delivered on days 1 through 5 in four dose cohorts: 1 x 10^9 plaque forming units (pfu), 1 x 10^{10} pfu, 3 x 10^{10} pfu, and 1 x 10^{11} pfu. Flu-like symptoms, abdominal pain and vomiting were the predominant side effects. The presence of virus up to 10 days after the final (day 5) infusion of dl1520 was suggestive of continuing viral replication [50].

A phase I trial of retroviral BRCA1sv gene therapy in ovarian cancer has been reported [46]. Gene transfer of BRCA1sv, a normal splice variant of BRCA1, has been shown to produce growth inhibition in ovarian cancer xenografts models. To assess the pharmacokinetics and toxicity of intraperitoneal BRCA1sv retroviral vector therapy, a dose escalation study was performed. Three of 12 patients developed an acute sterile peritonitis, which spontaneously resolved over 2 days. Plasma and peritoneal antibodies to the retroviral envelope protein were detected in patients treated with the highest dose levels. Tumour responses were reported with 8 patients showing stable disease and three patients showed tumour reduction with diminished miliary tumour implants or radiological response. The vector-related complication of peritonitis was observed in three patients but resolved quickly as in preclinical mouse studies [46].

Experimental Approaches

Transcriptional targeting is another new approach for ovarian cancer gene therapy. Transcriptional control elements (promoters) of genes are commonly upregulated or specifically expressed in tumours compared to non-transformed cells. This differential expression can be exploited to drive expression of therapeutic genes in targeted gene therapy strategies in ovarian cancer. Telomerase plays an important role in cellular immortalization. Telomerase is selectively active in cancer cells compared to normal cells. Adenovirus-mediated suicide gene therapy using the human telomerase catalytic subunit (hTERT) gene promoter induced apoptosis of

ovarian cancer cell line. In this study, hTERT promoter was cloned in place of the CMV promoter and HSV-TK gene was sub-cloned to be controlled by hTERT gene promoter in adenovirus shuttle plasmid. Recombinant adenovirus Ad-hT-TK, was infected into normal and ovarian cancer cell lines. Selective tumour specific cell death by Ad-hT-TK was seen in this study [44]. Similarly, secretory leukoprotease inhibitor (SLPI) promoter was exploited for targeted ovarian cancer gene therapy in another study [3].

Intraperitoneal instillation of an adenoviral vector encoding the mouse interferon-beta gene (Ad.muIFN-beta) was shown to eradicate established mesothelioma tumours in the peritoneal cavity of immune competent, but not in immunodeficient mice [36]. In another preclinical study, interferon-alpha gene therapy by lentiviral vectors was shown to inhibit ovarian cancer growth through inhibition of angiogenesis [22]. Bcl-2 antisense oligonucleotide has been shown to overcome resistance to E1A gene therapy in a low HER2-expressing ovarian cancer xenograft model [4]. Intraperitoneal therapy of ovarian cancer using an engineered replicating measles virus has been reported [38].

The ability to deliver drugs into the peritoneal cavity for cancers such as ovarian cancer allows innovative approaches. Now it is possible to deliver small interfering RNA (siRNA) into cells by chemical modifications such as complexing with polyethylamine. This approach has been used to stabilize siRNA and target "neu" by IP treatment in a mouse model [49]. More recently, siRNA have been coated with reconstituted influenza viral envelopes (virosomes) to deliver them intracellularly [10].

Conclusion

Intraperitoneal gene therapy is an attractive strategy for targeting ovarian cancer. Several reports suggest that this approach is feasible and safe. However, clinical studies reported so far have failed to provide convincing evidence of significant anti-cancer activity. This may be due to multiple genetic and epigenetic dysregulations that drive the cancer phenotype. Therefore targeting a single gene may not translate into meaningful clinical activity. In addition, current gene therapy approaches have serious limitations with regards to their ability to deliver therapeutic gene into cancer cells. However, the recent development of efficient vector systems, gene-directed enzyme pro-drug therapy (GDEPT) and transcriptional targeting provide exiting opportunities for future clinical research.

References

1. Alvarez RD and DT Curiel. (1997) A phase I study of recombinant adenovirus vector-mediated intraperitoneal delivery of herpes simplex virus thymidine kinase (HSV-TK) gene and intravenous ganciclovir for previously

treated ovarian and extraovarian cancer patients. Hum Gene Ther 8: 597-613
2. Alvarez RD, J Gomez-Navarro, et al. (2000) Adenoviral-mediated suicide gene therapy for ovarian cancer. Mol Ther 2: 524-530
3. Barker SD, CJ Coolidge, et al. (2003) The secretory leukoprotease inhibitor (SLPI) promoter for ovarian cancer gene therapy. J Gene Med 5: 300-310
4. Bartholomeusz C, H Itamochi, et al. (2005) Bcl-2 antisense oligonucleotide overcomes resistance to E1A gene therapy in a low HER2-expressing ovarian cancer xenograft model. Cancer Res 65: 8406-8413
5. Belzile JP, SA Choudhury, et al. (2006) Targeting DNA repair proteins: a promising avenue for cancer gene therapy. Curr Gene Ther 6: 111-123
6. Berchuck A, A Kamel, et al. (1990) Overexpression of HER-2/neu is associated with poor survival in advanced epithelial ovarian cancer. Cancer Res 50: 4087-4091
7. Buller RE, IB Runnebaum, et al. (2002) A phase I/II trial of rAd/p53 (SCH 58500) gene replacement in recurrent ovarian cancer. Cancer Gene Ther 9: 553-566
8. Buskens CJ, WA Marsman, et al. (2005) The current state of cancer gene therapy and its application in esophageal carcinoma. Dig Surg 22: 222-233
9. Dachs GU, J Tupper, et al. (2005) From bench to bedside for gene-directed enzyme prodrug therapy of cancer. Anticancer Drugs 16: 349-359
10. de Jonge J, M Holtrop, et al. (2006) Reconstituted influenza virus envelopes as an efficient carrier system for cellular delivery of small-interfering RNAs. Gene Ther 13: 400-411
11. Dong JY and J Worathanadharm. (2005) Gene therapy vector design strategies for the treatment of cancer. Future Oncol 1: 361-373
12. Dougherty GJ, PD Davis, et al. (2004) Vascular-targeted cancer gene therapy. Expert Opin Biol Ther 4: 1911-1920
13. Frisch SM. (1991) Antioncogenic effect of adenovirus E1A in human tumour cells. Proc Natl Acad Sci U S A 88: 9077-9081
14. Frisch SM. (2004) E1A as a tumour suppressor gene: commentary re S. Madhusudan et al. A multicenter Phase I gene therapy clinical trial involving intraperitoneal administration of E1A-lipid complex in patients with recurrent epithelial ovarian cancer overexpressing HER-2/neu oncogene. Clin Cancer Res 10: 2905-2907
15. Frisch SM and JS Mymryk. (2002) Adenovirus-5 e1a: paradox and paradigm. Nat Rev Mol Cell Biol 3: 441-452
16. Harmon RL and PH Sugarbaker. (2005) Prognostic indicators in peritoneal carcinomatosis from gastrointestinal cancer. Int Semin Surg Oncol 2: 3
17. Harrington KJ, CM Nutting, et al. (2005) Gene therapy for head and neck cancer. Cancer Metastasis Rev 24: 147-164
18. Hasenburg A, DC Fischer, et al. (2002) Adenovirus-mediated thymidine kinase gene therapy for recurrent ovarian cancer: expression of coxsackie-adenovirus receptor and integrins alphavbeta3 and alphavbeta5. J Soc Gynecol Investig 9: 174-180
19. Hasenburg A, XW Tong, et al. (2001) Adenovirus-mediated thymidine kinase gene therapy in combination with topotecan for patients with recurrent ovar-

ian cancer: 2.5-year follow-up. Gynecol Oncol 83: 549-554
20. Hellstrom I, G Goodman, et al. (2001) Overexpression of HER-2 in ovarian carcinomas. Cancer Res 61: 2420-2423
21. Hortobagyi GN, NT Ueno, et al. (2001) Cationic liposome-mediated E1A gene transfer to human breast and ovarian cancer cells and its biologic effects: a phase I clinical trial. J Clin Oncol 19: 3422-3433
22. Indraccolo S, V Tisato, et al. (2005) Interferon-alpha gene therapy by lentiviral vectors contrasts ovarian cancer growth through angiogenesis inhibition. Hum Gene Ther 16: 957-970
23. Izquierdo M. (2005) Short interfering RNAs as a tool for cancer gene therapy. Cancer Gene Ther 12: 217-227
24. Juhl H, SG Downing, et al. (1997) HER-2/neu is rate-limiting for ovarian cancer growth. Conditional depletion of HER-2/neu by ribozyme targeting. J Biol Chem 272: 29482-29486
25. Kaliberov SA and DJ Buchsbaum. (2006) Gene delivery and gene therapy of prostate cancer. Expert Opin Drug Deliv 3: 37-51
26. Kanduc D, J Geliebter, et al. (2005) Gene therapy in cancer: the missing point. J Exp Ther Oncol 5: 151-158
27. Kim J, ES Hwang, et al. (1999) Intraperitoneal gene therapy with adenoviral-mediated p53 tumour suppressor gene for ovarian cancer model in nude mouse. Cancer Gene Ther 6: 172-178
28. Libermann TA and LF Zerbini. (2006) Targeting transcription factors for cancer gene therapy. Curr Gene Ther 6: 17-33
29. Link CJ, Jr., D Moorman, et al. (1996) A phase I trial of in vivo gene therapy with the herpes simplex thymidine kinase/ganciclovir system for the treatment of refractory or recurrent ovarian cancer. Hum Gene Ther 7: 1161-1179
30. Madhusudan S, A Tamir, et al. (2004) A multicenter Phase I gene therapy clinical trial involving intraperitoneal administration of E1A-lipid complex in patients with recurrent epithelial ovarian cancer overexpressing HER-2/neu oncogene. Clin Cancer Res 10: 2986-2996
31. Mansfield PF. (2003) Management of peritoneal carcinomatosis: is an answer at hand? Ann Surg Oncol 10: 827-828
32. Markman M. (2003) Role of intraperitoneal chemotherapy in the front-line setting. J Clin Oncol 21: 145-148
33. Markman M and JL Walker. (2006) Intraperitoneal chemotherapy of ovarian cancer: a review, with a focus on practical aspects of treatment. J Clin Oncol 24: 988-994
34. Marx D, A Fattahi-Meibodi, et al. (1998) Detection of p105 (c-erbB-2, HER2/neu) serum levels by a new ELISA in patients with ovarian carcinoma. Anticancer Res 18: 2891-2894
35. Nagayama Y. (2004) Gene therapy for thyroid cancer. Cancer Treat Res 122: 369-379
36. Odaka M, R Wiewrodt, et al. (2002) Analysis of the immunologic response generated by Ad.IFN-beta during successful intraperitoneal tumour gene therapy. Mol Ther 6: 210-218
37. Palmer DH, LS Young, et al. (2006) Cancer gene-therapy: clinical trials.

Trends Biotechnol 24: 76-82
38. Peng KW, CJ TenEyck, et al. (2002) Intraperitoneal therapy of ovarian cancer using an engineered measles virus. Cancer Res 62: 4656-4662
39. Pereboeva L and DT Curiel. (2004) Cellular vehicles for cancer gene therapy: current status and future potential. BioDrugs 18: 361-385
40. Piso P, MH Dahlke, et al. (2004) Cytoreductive surgery and hyperthermic intraperitoneal chemotherapy in peritoneal carcinomatosis from ovarian cancer. World J Surg Oncol 2: 21
41. Rocconi RP, TM Numnum, et al. (2005) Targeted gene therapy for ovarian cancer. Curr Gene Ther 5: 643-653
42. Sadeghi B, C Arvieux, et al. (2000) Peritoneal carcinomatosis from non-gynecologic malignancies: results of the EVOCAPE 1 multicentric prospective study. Cancer 88: 358-363
43. Seth P. (2005) Vector-mediated cancer gene therapy: an overview. Cancer Biol Ther 4: 512-517
44. Song JS, HP Kim, et al. (2003) Adenovirus-mediated suicide gene therapy using the human telomerase catalytic subunit (hTERT) gene promoter induced apoptosis of ovarian cancer cell line. Biosci Biotechnol Biochem 67: 2344-2350
45. Sutter AP and H Fechner. (2006) Gene therapy for gastric cancer: is it promising? World J Gastroenterol 12: 380-387
46. Tait DL, PS Obermiller, et al. (1997) A phase I trial of retroviral BRCA1sv gene therapy in ovarian cancer. Clin Cancer Res 3: 1959-1968
47. Takahashi S, Y Ito, et al. (2006) Gene therapy for breast cancer. --Review of clinical gene therapy trials for breast cancer and MDR1 gene therapy trial in Cancer Institute Hospital. Breast Cancer 13: 8-15
48. Toloza EM. (2005) Gene therapy for lung cancer. Semin Thorac Cardiovasc Surg 17: 205-212
49. Urban-Klein B, S Werth, et al. (2005) RNAi-mediated gene-targeting through systemic application of polyethylenimine (PEI)-complexed siRNA in vivo. Gene Ther 12: 461-466
50. Vasey PA, LN Shulman, et al. (2002) Phase I trial of intraperitoneal injection of the E1B-55-kd-gene-deleted adenovirus ONYX-015 (dl1520) given on days 1 through 5 every 3 weeks in patients with recurrent/refractory epithelial ovarian cancer. J Clin Oncol 20: 1562-1569
51. Vassaux G, J Nitcheu, et al. (2006) Bacterial gene therapy strategies. J Pathol 208: 290-298
52. Villaret D, B Glisson, et al. (2002) A multicenter phase II study of tgDCC-E1A for the intratumoural treatment of patients with recurrent head and neck squamous cell carcinoma. Head Neck 24: 661-669
53. Von Gruenigen VE, JD O'Boyle, et al. (1999) Efficacy of intraperitoneal adenovirus-mediated p53 gene therapy in ovarian cancer. Int J Gynecol Cancer 9: 365-372
54. Wei MQ, P Metharom, et al. (2005) Search for “weapons of mass destruction” for cancer - immuno/gene therapy comes of age. Cell Mol Immunol 2: 351-357

55. Wen SF, V Mahavni, et al. (2003) Assessment of p53 gene transfer and biological activities in a clinical study of adenovirus-p53 gene therapy for recurrent ovarian cancer. Cancer Gene Ther 10: 224-238
56. Wolf JK, DC Bodurka, et al. (2004) A phase I study of Adp53 (INGN 201; ADVEXIN) for patients with platinum- and paclitaxel-resistant epithelial ovarian cancer. Gynecol Oncol 94: 442-448
57. Yarden Y and MX Sliwkowski. (2001) Untangling the ErbB signalling network. Nat Rev Mol Cell Biol 2: 127-137
58. Yoo GH, MC Hung, et al. (2001) Phase I trial of intratumoural liposome E1A gene therapy in patients with recurrent breast and head and neck cancer. Clin Cancer Res 7: 1237-1245
59. Young LS, PF Searle, et al. (2006) Viral gene therapy strategies: from basic science to clinical application. J Pathol 208: 299-318
60. Yu D, A Matin, et al. (1995) Liposome-mediated in vivo E1A gene transfer suppressed dissemination of ovarian cancer cells that overexpress HER-2/neu. Oncogene 11: 1383-1388
61. Zeimet AG and C Marth. (2003) Why did p53 gene therapy fail in ovarian cancer? Lancet Oncol 4: 415-422

Index

D

E

Cancer Treatment and Research

Steven T. Rosen, M.D., *Series Editor*

Miller, A.B. (ed.): *Advances in Cancer Screening*. 1996. ISBN 0-7923-4019-1.
Hait, W.N. (ed.): *Drug Resistance*. 1996. ISBN 0-7923-4022-1.
Pienta, K.J. (ed.): *Diagnosis and Treatment of Genitourinary Malignancies*. 1996. ISBN 0-7923-4164-3.
Arnold, A.J. (ed.): *Endocrine Neoplasms*. 1997. ISBN 0-7923-4354-9.
Pollock, R.E. (ed.): *Surgical Oncology*. 1997. ISBN 0-7923-9900-5.
Verweij, J., Pinedo, H.M., Suit, H.D. (eds): *Soft Tissue Sarcomas: Present Achievements and Future Prospects.* 1997. ISBN 0-7923-9913-7.
Walterhouse, D.O., Cohn, S. L. (eds): *Diagnostic and Therapeutic Advances in Pediatric Oncology.* 1997. ISBN 0-7923-9978-1.
Mittal, B.B., Purdy, J.A., Ang, K.K. (eds): *Radiation Therapy*. 1998. ISBN 0-7923-9981-1.
Foon, K.A., Muss, H.B. (eds): *Biological and Hormonal Therapies of Cancer*. 1998. ISBN 0-7923-9997-8.
Ozols, R.F. (ed.): *Gynecologic Oncology*. 1998. ISBN 0-7923-8070-3.
Noskin, G. A. (ed.): *Management of Infectious Complications in Cancer Patients*. 1998. ISBN 0-7923-8150-5.
Bennett, C. L. (ed.): *Cancer Policy*. 1998. ISBN 0-7923-8203-X.
Benson, A. B. (ed.): *Gastrointestinal Oncology*. 1998. ISBN 0-7923-8205-6.
Tallman, M.S., Gordon, L.I. (eds): *Diagnostic and Therapeutic Advances in Hematologic Malignancies*. 1998. ISBN 0-7923-8206-4.
von Gunten, C.F. (ed.): *Palliative Care and Rehabilitation of Cancer Patients*. 1999. ISBN 0-7923-8525-X
Burt, R.K., Brush, M.M. (eds): *Advances in Allogeneic Hematopoietic Stem Cell Transplantation*. 1999. ISBN 0-7923-7714-1.
Angelos, P. (ed.): *Ethical Issues in Cancer Patient Care* 2000. ISBN 0-7923-7726-5.
Gradishar, W.J., Wood, W.C. (eds): *Advances in Breast Cancer Management*. 2000. ISBN 0-7923-7890-3.
Sparano, J. A. (ed.): *HIV & HTLV-I Associated Malignancies*. 2001. ISBN 0-7923-7220-4.
Ettinger, D. S. (ed.): *Thoracic Oncology*. 2001. ISBN 0-7923-7248-4.
Bergan, R. C. (ed.): *Cancer Chemoprev*ention. 2001. ISBN 0-7923-7259-X.
Raza, A., Mundle, S.D. (eds): *Myelodysplastic Syndromes & Secondary Acute Myelogenous Leukemia* 2001. ISBN: 0-7923-7396.
Talamonti, M. S. (ed.): *Liver Directed Therapy for Primary and Metastatic Liver Tumors*. 2001. ISBN 0-7923-7523-8.
Stack, M.S., Fishman, D.A. (eds): *Ovarian Cancer*. 2001. ISBN 0-7923-7530-0.
Bashey, A., Ball, E.D. (eds): *Non-Myeloablative Allogeneic Transplantation*. 2002. ISBN 0-7923-7646-3.
Leong, S. P.L. (ed.): *Atlas of Selective Sentinel Lymphadenectomy for Melanoma, Breast Cancer and Colon Cancer.* 2002. ISBN 1-4020-7013-6.
Andersson , B., Murray D. (eds): *Clinically Relevant Resistance in Cancer Chemotherapy.* 2002. ISBN 1-4020-7200-7.
Beam, C. (ed.): *Biostatistical Applications in Cancer Research*. 2002. ISBN 1-4020-7226-0.
Brockstein, B., Masters, G. (eds): *Head and Neck Cancer*. 2003. ISBN 1-4020-7336-4.
Frank, D.A. (ed.): S*ignal Transduction in Cancer*. 2003. ISBN 1-4020-7340-2.
Figlin, R. A. (ed.): *Kidney Cancer*. 2003. ISBN 1-4020-7457-3.
Kirsch, M.; Black, P. McL. (ed.): *Angiogenesis in Brain Tumors.* 2003. ISBN 1-4020-7704-1.
Keller, E.T., Chung, L.W.K. (eds): *The Biology of Skeletal Metastases.* 2004. ISBN 1-4020-7749-1.
Kumar, R. (ed.): *Molecular Targeting and Signal Transduction*. 2004. ISBN 1-4020-7822-6.
Verweij, J., Pinedo, H.M. (eds): *Targeting Treatment of Soft Tissue Sarcomas*. 2004. ISBN 1-4020-7808-0.
Finn, W.G., Peterson, L.C. (eds.): *Hematopathology in Oncology*. 2004. ISBN 1-4020-7919-2.
Farid, N. (ed.): *Molecular Basis of Thyroid Cancer*. 2004. ISBN 1-4020-8106-5.
Khleif, S. (ed.): *Tumor Immunology and Cancer* Vaccines. 2004. ISBN 1-4020-8119-7.
Balducci, L., Extermann, M. (eds): *Biological Basis of Geriatric Oncology*. 2004. ISBN
Abrey, L.E., Chamberlain, M.C., Engelhard, H.H. (eds): *Leptomeningeal Metastases*. 2005. ISBN 0-387-24198-1
Platanias, L.C. (ed.): *Cytokines and Cancer*. 2005. ISBN 0-387-24360-7.
Leong, S. P.L., Kitagawa, Y., Kitajima, M. (eds): *Selective Sentinel Lymphadenectomy for Human Solid Cancer.* 2005. ISBN 0-387-23603-1.
Small, Jr. W., Woloschak, G. (eds): *Radiation Toxicity: A Practical Guide*. 2005. ISBN 1-4020-8053-0.
Haefner, B., Dalgleish, A. (eds): *The Link Between Inflammation and Cancer*. 2006. ISBN 0-387-26282-2.
Leonard, J.P., Coleman, M. (eds): *Hodgkin's and Non-Hodgkin's Lymphoma.* 2006. ISBN 0-387-29345.
Leong, S. P.L. (ed): *Cancer Clinical Trials: Proactive Strategies*. 2006. ISBN 0-387-33224-3.
Ceelen, W.P. (ed): *Peritoneal Carcinomatosis: A Multidisciplinary Approach.* 2007. ISBN 978-0-387-48991-9.