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Part I

Protein Kinase Inhibitors

Imatinib Mesylate

1

Cornelius F. Waller

Abstract IMATINIB MESYLATE (Gleevec, Glivec [Novartis, Basel, Switzerland], formerly referred to as STI571 or CGP57148B) represents the paradigm of a new class of anticancer agents, the so-called small molecules. They have a high selectivity against a specific molecular target known to be the cause for the establishment and maintenance of the malignant phenotype. Imatinib is a rationally designed oral signal transduction inhibitor that specifically targets several protein tyrosine kinases, Abl, Arg (*Abl*-related gene), the stem-cell factor receptor (c-KIT), platelet-derived growth factor receptor (PDGF-R), and their oncogenic forms, most notably Bcr-Abl. Imatinib has been shown to have remarkable clinical activity in patients with chronic myeloid leukemia (CML) and malignant gastrointestinal stroma tumors (GIST) leading to its approval for treatment of these diseases.

Treatment with imatinib is generally well tolerated with a low incidence of severe side effects. The most common adverse events (AE) include mild to moderate edema, muscle cramps, diarrhea, nausea, skin rashes, and myelosuppression.

Several mechanisms of resistance have been identified. Clonal evolution, amplification, or overexpression of Bcr-Abl as well as mutations in the catalytic domain, P-loop, and other mutations have been demonstrated to play a role in primary and secondary resistance to imatinib, respectively. Improved understanding of the underlying mechanisms of resistance has led to the development of new second-generation tyrosine kinase inhibitors (see Chaps. 7–9).

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1.1 Introduction

CML is a clonal disorder of the hematopoietic stem cell. The clinical presentation often includes granulocytosis, a hypercellular bone marrow, and splenomegaly. The natural course of the disease involves three sequential phases – chronic phase (CP), progressing often through an accelerated phase (AP) into the terminal blast crisis (BC). The duration of CP is several years, while AP and BC usually last only for months. In the past, median survival was in the range of 4–5 years (Hehlmann et al. 2007b; Sawyers 1999).

CML is characterized by the presence of the Philadelphia chromosome (Ph), a unique reciprocal translocation between the long arms of chromosomes 9 and 22, t (9:22), which is present

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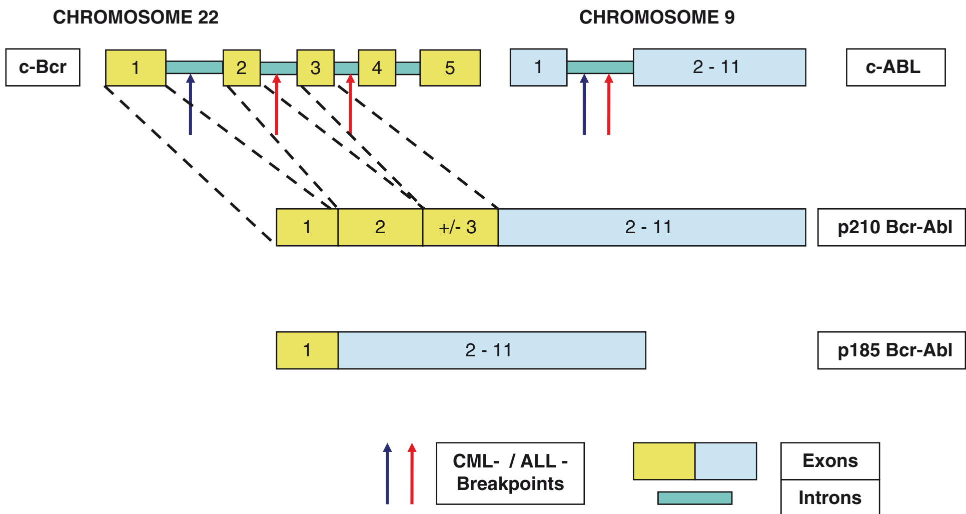

Fig. 1.1 Common breakpoint in chronic myeloid leukemia *(CML)* and Ph+ *ALL*: In *CML*, *bcr* breakpoints occur after the second or third exon, whereas in Ph+ *ALL*, breaks can occur after the first exon. In *c-Abl,* a break occurs between the first and second exon (*CML* and Ph+ *ALL*)

in >90% of patients with CML and approximately 15–30% of ALL (Nowell and Hungerford 1960; Rowley 1973). On the molecular level, t(9;22) results in the generation of an oncogene, the bcr-abl fusion gene, encoding the Bcr-Abl protein, which has constitutive tyrosine kinase activity (Konopka et al. 1984) (Figure 1.1).

Its causal role in the development of CML has been demonstrated in vitro as well as in several animal models (Daley et al. 1990; Heisterkamp et al. 1990; Lugo et al. 1990; Voncken et al. 1995).

The pathological effects of Bcr-Abl include increased proliferation, protection from programmed cell death, altered stem cell adhesion, and possibly genetic instability that leads to disease progression (Deininger et al. 2000).

Before the introduction of imatinib, the standard therapy of CML was interferon-α alone or in combination with cytarabine (ara-C) leading to hematological remissions in the majority of patients, but major cytogenetic responses (MCyR) – i.e., <35% Ph+ metaphases – were only seen in 6–25% of patients (Hehlmann et al. 2007b). The only curative treatment of CML is allogeneic stem cell transplantation from an HLA-compatible donor. However, it is only an option for a part of the patients and is still associated with considerable morbidity and mortality (Gratwohl et al. 1998; Hehlmann et al. 2007a).

The presence of Bcr-Abl in >90% of CML patients and the identification of its essential role in the pathogenesis of the disease provided the rationale for targeting this fusion protein for the treatment of CML.

In the last decade of the twentieth century, the first data for compounds with an effect on tyrosine kinases were published (Levitzky and Gazit 1995). Tyrphostins and other similar compounds were shown to inhibit the ABL as well as the BCR-ABL tyrosine kinase at micromolar concentrations, but had only limited specificity (Anafi et al. 1993a, b; Carlo-Stella et al. 1999). This led to the rational design of further TKI with selective activity against the ABL tyrosine kinase, one of which was a 2-phenylaminopyrimidine called CGP57148B, later called STI571 or imatinib

mesylate (Buchdunger et al. 1995, 1996; Druker and Lydon 2000; Druker et al. 1996).

After demonstration of specificity in vitro, in cell-based systems as well as in different animal models, this compound was tested in several phase I- and phase II-studies (Druker et al. 2001a; Kantarjian et al. 2002). Imatinib was shown to have very high rates of hematological remissions in CP-CML patients previously treated with interferon-α as well as in advanced stages of the disease. Cytogenetic remissions were achieved in a considerable portion of patients. Based on these good results, imatinib was approved for treatment of CML patients in CP after treatment failure with interferon-α and the advanced stages, i.e., AP and BC (Cohen et al. 2002).

The phase III (IRIS) trial led to establishment of imatinib as the standard for first-line therapy of CP-CML.

Other molecular targets of imatinib are the stem-cell factor receptor (c-KIT) and platelet-derived growth factor receptor (PDGF-R) (Buchdunger et al. 2000, 1995; Heinrich et al. 2002).

c-KIT is expressed in a variety of human cancers, including germ cell tumors, neuroblastoma, melanoma, small cell lung cancer, breast and ovarian cancer, acute myeloid leukemia, mast cell disorders as well as malignant gastrointestinal stroma tumors (GIST). While in most of these diseases the exact role of c-KIT expression is not defined, in mastocytosis and GISTs, activating mutations of c-KIT have been identified.

Based on data of a single open-label phase II trial and two large phase-III-trials by the EORTC and SWOG, imatinib received approval for treatment of metastatic/unresectable GIST (Cohen et al. 2009; Dagher et al. 2002).

Furthermore, imatinib has been successfully used in diseases with aberrant PDGF-R. They have been shown to deregulate the growth of a variety of cancers, such as myeloproliferative disorders (Pardanani and Tefferi 2004), e.g., in hypereosinophilic syndrome (FIP1L1/PDGFRα-rearrangement), chronic myelomonocytic leukemia (CMML) harboring the actiating translocations involving the PDGFRβ receptor locus on chromosome 5q33 (FIP1/PDGFR-translocation), carcinomas, melanoma, gliomas, and sarcomas, including dermatofibrosarcoma protuberans (Barnhill et al. 1996; Greco et al. 2001).

1.2 Chemical Structure

Imatinib mesylate is designated chemically as 4-[(4-methyl-1-piperazinyl)methyl]-*N*-[4-methyl-3-[[4-(3-pyridinyl)-2-pyrimidinyl] aminophenyl] benzamide methanesulfonate. Its molecular formula is $C_{29}H_{31}N_7O{\cdot}CH_4SO_3$, and its relative molecular mass is 589.7 (Fig. 1.2).

Imatinib functions as a specific competitive inhibitor of ATP. It binds with high affinity at the ATP binding site in the inactive form of the kinase domain, blocks ATP binding, and thereby inhibits kinase activity by interrupting the transfer of phosphate from ATP to tyrosine residues on substrate proteins (Cohen et al. 2002, 2005; Lyseng-Williamson and Jarvis 2001; Mauro et al. 2002).

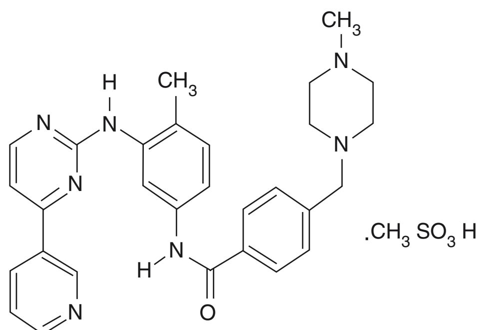

Fig. 1.2 Structure of imatinib mesylate (formerly STI 571 bzw. CGP57148)

1.3 Clinical Pharmacology

After oral administration to normal volunteers, imatinib was well absorbed with an absolute bioavailability of 98%. Peak plasma concentrations were attained between 2 and 4 h. At clinically relevant concentrations, total binding of imatinib to plasma proteins is approximately 95%, mostly to albumin and α1-acid glycoprotein. The elimination half-lives of imatinib and its major active metabolite CGP74588 from plasma are approximately 18 and 40 h, respectively.

Imatinib AUC is dose-proportional at the recommended daily dose range of 400 and 600 mg. Within 7 days, approximately 81% of the dose is eliminated, 68% in feces and 13% in urine.

Cytochrome P450 (CYP3A4) is the major enzyme responsible for imatinib metabolism and both imatinib and CGP74588 appear to be potent in vitro CYP2D6 inhibitors. Imatinib plasma concentrations may be altered when the drug is administered with inhibitors or inducers of CYP3A4. When CYP3A4 inhibitors, e.g., itroconazole, ketoconazole, erythromycin, or clarithromycin, are co-administered with imatinib, its metabolization may be decreased. CYP3A4 inducers, such as dexamethasone, phenytoin, rifampicin, carbamazepine, phenobarbital, and others, may increase imatinib metabolism. Furthermore, increased plasma concentrations of drugs that are substrates of CYP3A4, e.g., simvastatin, cyclosporine, and others, may be the result of imatinib use (Cohen et al. 2002, 2005; Lyseng-Williamson and Jarvis 2001; Mauro et al. 2002).

In a small number of children with Ph+ ALL, imatinib plasma levels as well as those of its metabolite CGP74588 were measured. Imatinib plasma levels were similar to those in adult patients. However, AUC of CGP74588 was only 5–24% of the parent drug's AUC, and it was eliminated much faster than in adults, indicating a lesser role of the metabolite in antileukemic activity (Marangon et al. 2009).

In the phase –III-(IRIS) trial, the correlation of imatinib pharmacokinetics and the response to treatment as well as to side effects could be shown (Larson et al. 2008).

1.4 Drug Targets

Imatinib selectively inhibits all the ABL tyrosine kinases, including BCR-ABL, cellular homologue of the Abelson murine leukemia viral oncogene product (c-ABL), v-ABL, TEL-ABL, and Abelson-related gene (ARG). In addition, it was found to potently inhibit the tyrosine kinase activity of the α- and β-PDGF-R and the receptor for stem cell factor (c-KIT; CD117). The concentrations required for a 50% kinase inhibition were in the range of 0.025 μM in in vitro kinase assays and approximately 0.25 μM in intact cells. Extensive screening did not show activity against other tyrosine kinases or serine/threonine kinases (Buchdunger et al. 1995, 1996, 2000, 2001; Deininger et al. 2005; Druker and Lydon 2000; Druker et al. 1996; Heinrich et al. 2002; Okuda et al. 2001) (Table 1.1).

1.5 Preclinical Studies

In vitro studies demonstrated specific inhibition of myeloid cell lines expressing Bcr-Abl without killing the parental cell lines from which they were derived (Deininger et al. 1997; Druker et al. 1996; Gambacorti-Passerini et al. 1997). Continuous treatment with imatinib inhibited tumor formation in syngeneic mice as well as in a nude mouse model after inoculation of Bcr-Abl-expressing cells in a dose-dependent manner, treated intraperitoneally or with oral administration of STI571, respectively (Druker et al. 1996; le Coutre et al.

Table 1.1 Inhibition of protein kinases by imatinib mesylate (formerly STI 571 bzw. CGP57148) (adapted from Deininger et al. 2005)

Protein kinase	Substrate phosphorylation IC50 (μM)	Cellular tyrosine phosphorylation IC50[a] (μM)
c-abl	0.2; 0.025[a]	ND
v-abl	0.038	0.1 – 0.3
p210 $^{BCR-ABL}$	0.025[a]	0.25
p185 $^{BCR-ABL}$	0.025[a]	0.25
TEL-ABL	ND	0.35
PDGF-Rα and β	0.38 (PDGF-Rβ)	0.1
Tel-PDGF-R	ND	0.15
c-KIT	0.41	0.1
FLT-3	>10	>10
Btk	>10	ND
c-FMS	ND	>10
v-FMS	ND	>10
c-SRC	>100	ND
v-SRC	ND	>10
c-LYN	>100	ND
c-FGR	>100	ND
LCK	9.0	ND
SYK (TPK-IIB)	>100	ND
JAK-2	>100[a]	>100
EGF-R	>100	>100
Insulin receptor	>10	>100
IGF-IR	>10	>100
FGF-R1	31.2	ND
VEGF-R1 (FLT-1)	19.5	ND
VEGF-R2 (KDR)	10.7	ND
VEGF-R3 (FLT-4)	5.7	ND
TIE-2 (TEK)	>50	ND
c-MET	>100	ND
PKA	>500	ND
PPK	>500	ND
PKC α, β1, γ, δ, ε, ξ, η	>100	ND
Proteinkinase CK-1, CK-2	>100	ND
PKB	>10	ND
P39	>10	ND
PDK1	>10	ND
c-RAF-1	0.97	ND
CDC2/cyclin B	>100	ND

Imatinib concentrations causing a 50% reduction in kinase activity (IC50) are given
ND not done; *PDGF-R* platelet-derived growth-factor receptor; *Btk* Bruton tyrosine kinase; *TPK* tyrosine-protein kinase; *EGF-R* epidermal growth factor receptor; *IGF-IR* insulin-like growth factor receptor I; *FGF-R1* fibroblast growth factor receptor 1; *VEGF-R* vascular endothelial growth factor receptor; *PKA* cAMP-dependent protein kinase; *PPK* phosphorylase kinase; *PKC* protein kinase C; *CK* casein kinase; *PKB* protein kinase B (also known as Akt); *PKD1* 3-phosphoinoside-dependent protein kinase 1.
[a]IC50 was determined in immunocomplex assays

1999). Activity on primary CML cells could be demonstrated and a >90% reduction of Bcr-Abl expressing colonies in colony-forming assays from peripheral blood or bone marrow from CML patients was achieved at a concentration of imatinib of 1 µM while normal colonies did not show growth inhibition (Deininger et al. 1997; Druker et al. 1996; Gambacorti-Passerini et al. 1997).

1.6 Clinical Data in CML

1.6.1 Phase I Trials

In 1998, a phase I clinical trial with imatinib was initiated. This study was a dose escalation trial designed to determine the maximally tolerated dose, with clinical benefit as a secondary endpoint. 83 patients with CP CML who had failed standard therapy with interferon-α (IFN-α) or were intolerant to it, were enrolled. One-third of patients had signs of early progression to AP. They received escalating oral doses of imatinib, ranging from 25 to 1,000 mg/day. Clinical features of patients were typical of the disease. Dose-limiting toxicity was not reached, although a higher frequency of severe toxicities was encountered at imatinib doses >750 mg/day. The most common adverse events (AE) were nausea (43%), myalgia (41%), edema (39%), and diarrhea (25%). After 29 patients were enrolled, therapeutic doses of 300 mg or more per day were reached. 53 of 54 patients achieved a complete hematologic response (CHR), reaching normal blood counts typically within 4 weeks of treatment. Fifty-one of these 53 patients maintained normal blood counts after 1 year of therapy. Furthermore, these patients had a 31% rate of MCyR (MCyR; <35% Ph$^+$ metaphases) and a 13% rate of complete cytogenetic responses (CCR; eradication of Ph$^+$ bone marrow cells) (Druker 2008; Druker et al. 2001b).

In another phase-I-trial, patients with myeloid and lymphoid BC and patients with relapsed or refractory Ph$^+$ lymphoblastic leukemia (ALL) were treated with daily doses of 300–1,000 mg of imatinib. Fifty-five of patients with myeloid BC responded to therapy (45% of patients with <5% blasts in the bone marrow, and 11% reaching a complete remission with full recovery of peripheral blood counts, respectively), but only in 18% response was maintained longer than 1 year.

Of 20 patients with Ph$^+$ ALL or lymphoid BC, 70% responded, 20% reaching a complete hematologic remission. Nevertheless, all but one relapsed between days 45 and 117 (Druker et al. 2001a).

Based on the results of the phase I trials, the use of imatinib was expanded to large phase-II and phase-III clinical trials.

1.6.2 Phase II Studies

Three open-label, single-arm phase-II studies using imatinib as a single agent were conducted in patients with Ph$^+$ CML in three clinical settings: CML-CP after IFN-α failure or with intolerance to the drug, CML-AP, and CML-BC. Imatinib was administered orally once daily. Initially, all patients received 400 mg/day. Early in the study, however, the imatinib dose was increased to 600 mg daily for CML-AP and CML-BC trials. Patients with resistant or progressive disease receiving a dose of 400 or 600 mg/day could receive doses of 600 or 800 mg daily (administered as 400 mg twice daily).

In 532 patients with CP CML who had failed IFN-α therapy, 95% of patients reached a CHR, with complete cytogenetic response rates of 41% and major cytogenetic remission (MCR) of 60%. The estimated rates of freedom from progression to accelerated or blastic phase and overall survival at 6 years were 61 and 76%, respectively (Druker 2008; Hochhaus et al. 2008; Kantarjian et al. 2002).

For patients in BC and with Ph+- ALL, the studies confirmed the results of the phase – I trial. Response rates were also high; however, relapses were seen frequently. The majority of patients in BC relapsed during the first year of treatment. Hematologic responses were observed in 52% of patients ($n=260$) with myeloid BC, with a median response duration of 10 months. Interestingly, 48% of patients in this trial developed new cytogenetic abnormalities during treatment, demonstrating clonal evolution (Ottmann et al. 2002; Sawyers et al. 2002).

The efficacy in patients with AP CML was intermediate between CP and BC. Of 181 patients with AP, 82% showed a hematologic response, 53% reaching a CHR, which was sustained in 69%. MCRs were seen in 24% of patients with a complete cytogenetic responses rate of 17% (Talpaz et al. 2002).

The treatment results in advanced-phase CML and Ph+ ALL underline the necessity of combination therapies with conventional chemotherapy as well as the use of second-generation tyrosine kinase inhibitors.

The results of the phase-I and phase-II trials led to the approval by the Food and Drug Administration (FDA) of imatinib for the treatment of CML in advanced phase and after failure of IFN therapy (Cohen et al. 2002; Deininger et al. 2005; Druker 2008).

1.6.3 Phase III Study (IRIS-Trial)

In a landmark phase III study, the International Randomized Study of Interferon and STI571 (IRIS) trial, imatinib and the combination of IFN plus cytarabine were compared in newly diagnosed CP CML patients. More than 1,000 patients were accrued in less than 7 months. Five hundred and fifty three patients were randomized to each of the two treatments, imatinib at 400 mg/day or interferon-α plus Ara-C. There were no significant differences in prognostic or clinical features between the two treatment arms. After a median follow-up of 19 months, patients randomized to imatinib had significantly better results for CHR, MCR, and complete cytogenetic responses, as well as progression-free survival than patients treated with interferon-α plus Ara-C (O'Brien et al. 2003).

The remarkable superiority of imatinib led to early disclosure of study results. Thereafter, most patients were crossed over from interferon-α plus Ara-C to the imatinib arm.

The IRIS trial is now a long-term follow-up study of patients who received imatinib as initial therapy. After a follow-up of 5 years, the overall survival for newly diagnosed CP patients treated with imatinib is 89%. An estimated 93% of imatinib-treated patients remain free from disease progression to the AP or BC. The estimated annual rate of treatment failure was 3.3% in the first year, 7.5% in the year 2, 4.8% in year 3, 1.5% in year 4, and 0.9% in year 5. The progression rate did not increase over time (Druker et al. 2006) (Fig. 1.3).

Most of the side effects of imatinib were mild to moderate, with the most common being edema, muscle cramps, diarrhea, nausea, skin rashes, and myelosuppression (Druker et al. 2006). Quality of life was far better in patients treated with imatinib (Hahn et al. 2003). Rates of hematologic and cytogenetic responses are shown in Table 1.2. The most recent update at 7 years showed an estimated overall survival of 86% and of 94% considering only CML-related deaths, respectively. The estimated EFS at 7 years was 81% and the estimated rate without progression to AP or BC 93%. A complete cytogenetic responses was achieved by 456 of 553 (82%) of patients on first-line imatinib (O'Brien et al. 2008).

Monitoring of residual disease by quantitative RT-PCR in complete cytogenetic responders showed that the risk of disease progression was inversely correlated with the reduction of BCR-ABL mRNA when compared with pretherapeutic levels (Hughes et al. 2003). The rates of major molecular remissions as well as

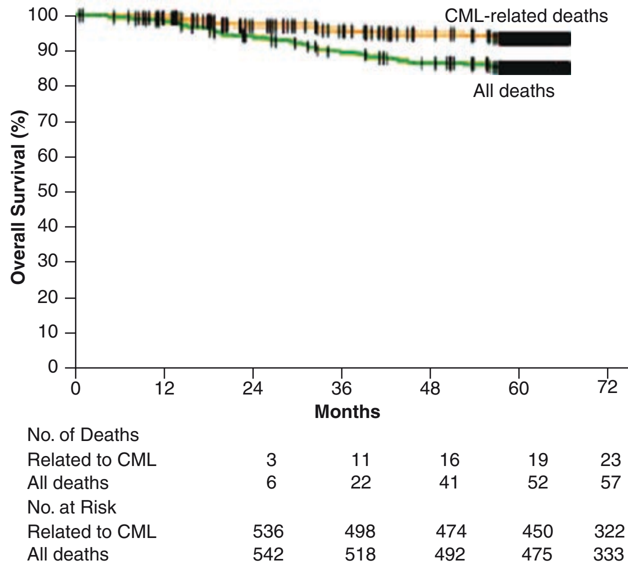

Fig. 1.3 *IRIS-trial: Overall survival for newly diagnosed patients with CML treated with imatinib at 5 years follow-up* (Druker et al. 2006). The estimated overall survival rate at 60 months was 89%. After the censoring of data for patients who died from causes unrelated to CML or transplantation, the estimated overall survival was 95% at 60 months (adapted from Druker et al. 2006 with permission)

the depth of molecular responses increase over time with a downward trend of relapse (O'Brien et al. 2008).

Investigation of pharmacokinetics in the imatinib-treated patients showed a correlation between imatinib trough plasma concentrations with clinical responses, EFS and AEs. Patients with high imatinib exposure had better rates of complete cytogenetic responses, major molecular responses, and event-free survival, respectively (Larson et al. 2008).

The results of the IRIS trial have led to FDA approval of imatinib for first-line treatment of patients with CP CML in 2002 (Cohen et al. 2002, 2005; Druker et al. 2001b).

1.6.4 Side Effects/Toxicity

Hematological side effects of imatinib are shown in Tables 1.3 and 1.4. Grade 3 or 4

Table 1.2 Results from the IRIS-trial (Druker NEJM 2006; O'Brien NEJM 2003)

Timepoint of follow-up	First-line treatment	Estimated cumulative rate of CHR (%)	Estimated cumulative rate of MCR (%)
18 months	IFN + Ara-C $n = 553$	55.5	22.1
	Imatinib $n = 553$	95.3*	85.2*
60 months	Imatinib	98	92

*Statistically significant difference to treatment with IFN + Ara-C p = 0.001
[a]OAS 94% considering only CML-related deaths

neutropenia, thrombocytopenia, or anemia was seen in all phase II – trials and the phase III study. While grade 3/4 neutropenia occurred in first-line treatment of CP CML in about 17%, in accelerated and blastic phase, it could be detected in approximately 60% of patients. In addition, in advanced phase, CML thrombocytopenia and anemia occur more frequently than in CP-CML (first or second line).

Typical nonhematological side effects in phase II-trials of imatinib in CML are shown in Table 1.3 (Cohen et al. 2005, 2002; Guilhot 2004). In the IRIS trial, most of the side effects of imatinib were mild to moderate, with the most common being edema, muscle cramps, diarrhea, nausea, skin rashes, and myelosuppression (Table 1.4) (Druker et al. 2006; O'Brien et al. 2003).

Recently, it has been suggested that imatinib may cause cardiotoxicity (Kerkelä et al. 2006). However, a preexisting condition predisposing to congestive heart failure (CHF) could not be excluded in these patients. Furthermore, a follow-up examination of the Novartis database of imatinib clinical trials including >5,600 years of exposure to imatinib found an incidence of CHF in imatinib recipients of 0.2% cases per year with a possible or probable relationship to the drug. In the IRIS trial, the incidence of cardiac failure and left ventricular dysfunction was estimated at 0.04% per year in the imatinib arm when compared with 0.75% in interferon-α and ara-C-treated patients (Hatfield et al. 2007).

1.7 Disease Progression and Imatinib Resistance

Resistance to imatinib includes de novo resistance and relapse after an initial response. The frequent and durable responses in CP-CML are caused by the selective inhibition of Bcr-Abl by imatinib. In accelerated and blastic phase CML as well as in Ph^+ ALL, the combination of high numbers of proliferating tumor cells and genomic instability may lead to secondary genetic alterations, independent of Bcr-Abl (von Bubnoff et al. 2003). In the majority of patients who respond to imatinib and then relapse, reactivation of the BCR-ABL tyrosine kinase could be shown. This indicates that Bcr-Abl-dependent mechanisms either prevent imatinib from reaching its target or render the target insensitive to Bcr-Abl. In the former category are mechanisms such as increased drug efflux through the multidrug resistance gene or protein binding of imatinib, while the latter include mutations in the catalytic domain, the P-loop, and other mutations (Druker 2008; Gorre et al. 2001). Over 70 point mutations have been demonstrated to play a role in primary and secondary resistance to imatinib, respectively.

Estimated cumulative rate of CCR (%)	Progression-free survival (PFS) (%)	Freedom from progression to AP or BC (%)	[a]OAS (%)	Reference
8.5	73.5	91.5		O'Brien 2003
73.8*	92.1	96.7		O'Brien 2003
87	83	93	89	Druker 2006

Table 1.3 Adverse events ≥10% in the phase II CML trials (Guilhot 2004; Cohen et al. 2002)

Reported or specified term	CML-CP[a] after IFN-failure/intolerance $N>532$ Dosage: 400 mg		CML-AP[b] $N>235$ Dosage 600 mg: $n>158$ Dosage 400 mg: $n>77$		CML-myeloid BC[b] $N>260$ Dosage 600 mg: $n>223$ Dosage 400 mg: $n>37$	
	All grades (%)	Grades 3/4 (%)	All grades (%)	Grades 3/4 (%)	All grades (%)	Grades 3/4 (%)
Hematologic AEs						
Anemia		4		36		50
Neutropenia		33		58		62
Thrombocythemia		16		42		58
Non-hematologic AEs						
Nausea	60	2	71	5	70	4
Fluid retention	66	3	73	6	71	12
Superficial edema	64	2	71	4	67	5
Other fluid retention	7	2	7	2	22	8
Muscle cramps	55	1	42	0.4	27	0.8
Diarrhea	43	2	55	4	42	2
Vomiting	32	1	56	3	54	4
Hemorrhage	22	2	44	9	52	19
GI hemorrhage	2	0.4	5	3	8	3
CNS hemorrhage	1	1	2	0.9	7	5
Musculoskeletal pain	35	2	46	9	43	9
Skin rash	42	3	44	4	35	5
Headache	34	0.2	30	2	27	5
Fatigue	40	1	41	4	29	3
Arthralgia/joint pain	36	1	31	6	25	4
Dyspepsia	24	0	21	0	11	0

Myalgia	25	0.2	22	2	8	0
Weight gain	30	5	14	3	5	0.8
Pyrexia	17	1	39	8	41	7
Abdominal pain	29	0.6	33	3	31	6
Cough	17	0	26	0.9	14	0.8
Dyspnea	9	0.6	20	7	14	4
Anorexia	6	0	17	2	14	2
Constipation	6	0.2	15	0.9	15	2
Nasopharyngitis	18	0.2	16	0	8	0
Night sweats	10	0.2	14	1	12	0.8
Pruritus	12	0.8	13	0.9	8	1
Epistaxis	5	0.2	13	0	13	3
Hypokalemia	5	0.2	8	2	13	4
Petechiae	1	0	5	0.9	10	2
Pneumonia	3	0.8	8	6	12	6
Weakness	7	0.2	9	3	12	3
Upper respiratory tract infection	15	0	9	0.4	3	0
Dizziness	13.0	0.2	12	0	11	0.4
Insomnia	13	0.2	13	0	10	0
Sore throat	11	0	11	0	8	0
Ecchymosis	2	0	6	0.9	11	0.4
Rigors	8	0	11	0.4	10	0
Asthenia	6	0	11	2	5	2
Influenza	10	0.2	6	0	0.8	0.4

CP chronic phase; *AP* accelerated phase; BC blast crisis; *AE* adverse event
[a]AEs considered possibly related to treatment.
[b]All AEs regardless of relationship to treatment

Table 1.4 Most frequently reported AEs: first-line imatinib at 7-year follow-up: (Druker NEJM 2006; O'Brien ASH 2008)

Most common AEs (by 5 years)	All grade AEs patients (%)	Grade 3/4 AEs patients (%)
Superficial edema	60	2
Nausea	50	1
Muscle cramps	49	2
Musculoskeletal pain	47	5
Diarrhea	45	3
Rash/skin problems	40	3
Fatigue	39	2
Headache	37	<1
Abdominal pain	37	4
Joint pain	31	3
Elevated liver enzymes	5	5
Hematological toxicity		
Neutropenia	60.8	17
Thrombocytopenia	56.6	9
Anemia	44.6	4

Only serious AEs (SAEs) were collected after 2005. Grade 3/4 AEs decreased in incidence after years 1–2

Table 1.5 Treatment goals for CP-CML patients treated with imatinib (Baccarani et al. Blood 2006)

Timepoint	Suboptimal response	Treatment failure
3 months	No CHR	No HR
6 months	No PCgR (Ph+ >35%)	No CHR No CgR (Ph+ >95%)
12 months	No CCR (Ph+ >0%)	No PCgR (Ph+ >35%)
18 months	No MMR (BCR-ABL/ABL >0, 10%)	No CCR (Ph+ >0%)
Any time point	Loss of MMR Mutation with high imatinib sensitivity Additional cytogenetic aberrations in Ph+ cells	Loss of CHR/CCR Mutation with low imatinib sensitivity

HR hematologic response; *CHR* complete hematologic response; *CgR* cytogenetic response (Ph+ <95%); *PCgR* partial cytogenetic response (Ph+ <35%); *CCR* complete cytogenetic response (absence of Ph+); *MMR* major molecular response (ratio BCR-ABL/ABL >0, 10%)

Figure 1.4: Point mutations in Bcr-Abl (adapted from Branford 2006).

Gene amplification or overexpression of Bcr-Abl as reason for resistance are seen occasionally (Shah et al. 2008; Shah and Sawyers 2003).

Understanding the underlying mechanisms of resistance has led to the development and investigation of new second-generation tyrosine kinase inhibitors (Mueller 2009; Schiffer 2007) (see Chaps. 7–9).

1.8 Treatment Recommendations for the Use of Imatinib in Chronic Phase CML

Based on the results achieved in the phase I-, II-, and III-trials with imatinib expert panels of the European Leukemia Net and the NCCN have developed guidelines for monitoring and treatment of CP-CML with imatinib (Table 1.5).

(Baccarani et al. 2006; NCCN_Practice_Guidelines_in_Oncology, – v.3 (2008 http://www.nccn.org/professionals/physician_gls/PDF/cml.pdf).

In case of suboptimal response, imatinib dosage should be increased and the option of allogeneic stem cell transplantation should be considered. In addition, in patients with failure of imatinib therapy, second-generation tyrosine kinase inhibitors, such as dasatinib or nilotinib, have been approved. Other TKI, such as bosutinib, and new third-generation TKIs are currently under investigation.

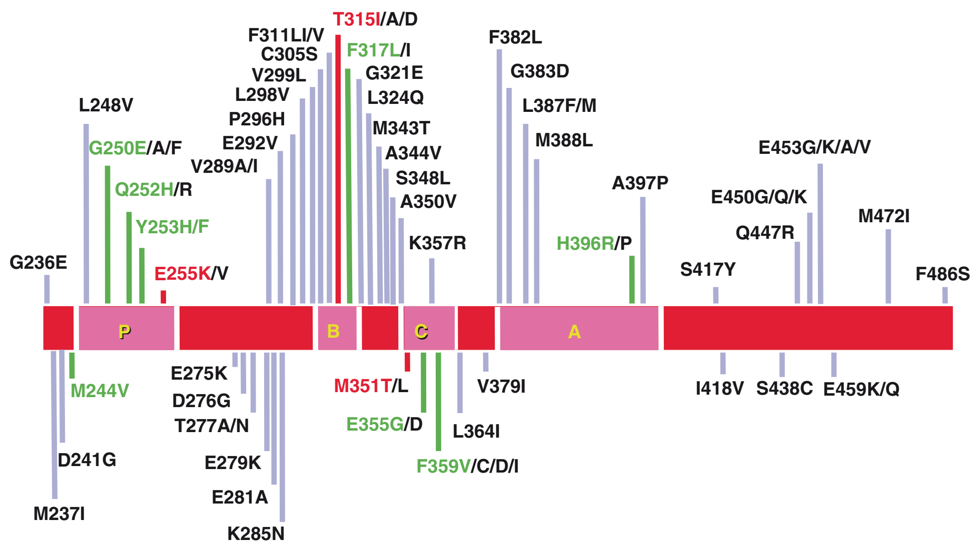

Fig. 1.4 Map of Bcr-Abl kinase domain mutations associated with clinical resistance to imatinib *(adapted from* Branford *2006). P* P-loop; *B* imatinib binding site; *C* catalytic domain; *A* activation loop. Amino-acid substitutions in *green indicate* mutations detected in 2–10% and in *red in* >10% of patients with mutations. (Adapted with permission from Susan Branford, Adelaide, Australia

1.9 Imatinib in Combination with Other Drugs

To further optimize the efficacy of imatinib in CML, a number of approaches have been investigated in phase II-trials. Increase in the dose of imatinib monotherapy to 800 mg/day in CP-CML has shown earlier, complete cytogenetic responses, but is associated with more side effects. However, the importance of faster responses has not been clear, yet (Cortes et al. 2003). In addition, imatinib in combination with other agents, such as interferon-α, cytarabine, and homoharringtonine, has been examined. Patients treated with combination therapy not only reached faster cytogenetic remission, but also experienced higher rates of toxicity, in particular myelotoxicity (Baccarani et al. 2004; Gardembas et al. 2003). Several major phase-III trials have been initiated, which compare standard dose imatinib with increased doses and combinations with cytarabine or interferon.

1.10 Imatinib – Other Targets

Other molecular targets of imatinib are the PDGF-R and the stem-cell factor receptor (c-KIT) (Buchdunger et al. 1995, 2000; Heinrich et al. 2002).

Aberrant PDGF-R have been shown to deregulate the growth of a variety of cancers, such as myeloproliferative disorders (Pardanani and Tefferi 2004), e.g., in hypereosinophilic syndrome (FIP1L1/PDGFR-rearrangement) (Jovanovic et al. 2007), CMML involving the 5q33 translocations (Jovanovic et al. 2007), carcinomas, melanoma, gliomas, and sarcomas, including dermatofibrosarcoma protuberans (Barnhill et al. 1996; Greco et al. 2001).

c-KIT is expressed in a variety of human malignancies, including germ cell tumors, neuroblastoma, melanoma, small cell lung cancer, breast and ovarian cancer, acute myeloid

leukemia, mast cell disorders, as well as malignant GIST.

While in most of these diseases, the exact role of c-KIT expression is not defined in mastocytosis and GISTs, activating mutations of c-KIT have been identified (Heinrich et al. 2003).

In approximately 60% of cases of GIST, there are mutations in *c-kit*[105] in the juxtamembrane domain. In most of the remaining cases, mutations in exon 13 and exon 9 have been found. The mutations lead to constitutive activation of the receptor without its ligand (Lux et al. 2000). Imatinib was investigated in 147 patients with histologically and immuno-histochemically confirmed metastatic and/or unresectable GIST in a single, open-label trial involving one European center and three centers in the United States. Seventy-three patients were randomized to receive 400 mg of imatinib daily, and 74 patients received 600 mg daily. An objective response was confirmed in 56 patients with an overall response rate for the combined study arms of 38% (95% confidence interval, 30–46%). All responses were partial responses. AEs were similar to CML patients and included edema, fluid retention, nausea, vomiting, diarrhea, myalgia, skin rash, bone marrow suppression, bleeding, and elevations in aspartate aminotransferase, alanine aminotransferase, or bilirubin. Gastrointestinal bleeding or intratumoral hemorrhage occurred in seven patients (5%) and was not correlated with thrombocytopenia or tumor bulk. The pharmacokinetics of imatinib in GIST patients were similar to those of CML patients (Demetri 2002; Demetri et al. 2002). Imatinib mesylate at a recommended dose of 400 or 600 mg daily was approved by the United States Food and Drug Administration for the treatment of malignant metastatic and/or unresectable GISTs in 2001 (Dagher et al. 2002).

Following approval, two open-label, controlled, multicenter, randomized phase III studies were performed by the EORTC ($n=946$) and the other by SWOG ($n=746$). Both studies compared imatinib at a dosage of 400 and 800 mg/day, respectively. Combined analysis of the two studies was prospectively defined by both groups. Objective responses were achieved in >50% of patients receiving either imatinib dose. Median PFS was approximately 20 months and median OS was approximately 49 months, respectively. In the combined analysis, 347 patients crossed over to imatinib 800 mg/day at the time of progression according to the protocol. Median OS after crossover was 14.3 months. The most common AEs were fluid retention, nausea, fatigue, skin rash, gastrointestinal complaints, and myalgia, which were usually mild to moderate. The most common laboratory abnormality was anemia. Fluid retention and skin rash were reported more often in patients treated with 800 mg/day. Based on these data, escalation of imatinib dosing up to 800 mg/day for patients with progressive disease was approved (Blanke et al. 2008; Heinrich et al. 2008).

1.11 Conclusion and Future Perspectives

The development of imatinib mesylate resembles the progress made in molecular biology over the past 30 years and has changed the landscape of cancer treatment leading toward causative treatment not only of CML and GIST but also for other malignancies.

After identification of the critical role of Bcr-Abl in the pathogenesis of CML, less than 15 years went by until the development of imatinib, which is now the standard of care for patients in CP CML. It has specific activity against a limited number of targets and has been shown to be highly effective not only in CML but also in other hematologic malignancies and solid tumors such as GIST. Side effects of treatment are mild to moderate. The understanding of mechanisms of resistance and disease progression has further led to the development of second- and third-generation tyrosine kinase inhibitors for this disease.

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2 Erlotinib

M. Steins, M. Thomas, and M. Geißler

Abstract The epidermal growth factor receptor (EGFR) has been implicated in a multiplicity of cancer-related signal transduction pathways like cellular proliferation, adhesion, migration, neoangiogenesis, and apoptosis inhibition, all of them important features of cancerogenesis and tumour progression. Its tyrosine kinase activity plays a central role in mediating these processes and has been intensely studied to exploit it as a therapeutic target. Inhibitors of this pathway have been developed and assessed in trials with significant efficacy in clinical applications. The current review focuses in particular on the clinical data of EGFR tyrosine kinase inhibition in different tumour entities, preferably non-small cell lung cancer (NSCLC) and pancreatic cancer with emphasis on the approved small molecule *erlotinib*. Its clinical applications, evidence-based efficacy, and toxicity as well as predictive markers of response are discussed.

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2.1 Introduction

The development of small molecule inhibitors like erlotinib, gefitinib, sorafenib, sunitinib, or lapatinib evoked a new era of anti-neoplastic agents in cancer therapy to supplement conventional cytotoxic drugs. The principle of this novel anti-cancer treatment is based on the inhibition of receptor tyrosine kinases, which are essential components of the intracellular signalling apparatus. Several cellular receptors on the cell surface regulate their signalling via the extracellular binding of ligands with a consecutive activation of intracellular tyrosine kinase domains and tyrosine phosphorylation. One of these receptors, the epidermal growth factor receptor (EGFR), has gained considerable interest as a possibly useful therapeutic target of tumour cells. EGFR is frequently overexpressed in solid tumours (Arteaga 2002) and plays a pivotal role in signal transduction pathways involved in cell proliferation, migration, adhesion, angiogenesis induction, and apoptosis inhibition. Its overexpression correlates in some tumour entities with disease progression and poorer prognosis (Brabender et al. 2001).

In clinical practice, the use of the EGFR tyrosine kinase inhibitors (EGFR-TKI) erlotinib

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and gefitinib have been approved so far for patients with non-small cell lung cancer (NSCLC) for selected indications. In addition, erlotinib combined with gemcitabine has also gained approval for systemic treatment in advanced, non-operable pancreatic carcinoma. The TKI benefit is mainly based on tumour control and overall survival (OS) rather than rapid tumour responses and complete remission rates. In contrast to cytotoxic agents, these responses have been achieved by a specific molecular mechanism disturbing the enzyme-mediated signal pathways in cancerogenesis.

2.2 Mechanism of Action

EGFR, the primary therapeutic target for erlotinib, belongs to the human epidermal growth factor receptor (HER) family 1, also known as erbB. The structure of this 170-kDa membrane-spanning glycoprotein consists of an extracellular cysteine-rich ligand-binding region, a transmembrane part, and the cytoplasmatic tyrosine kinase domain, which is the binding site for kinase inhibitors like erlotinib. Extracellular binding of ligands like the epidermal growth factor (EGF) and transforming growth factor-alpha (TGF-α) transforms the receptors from inactive monomers to active homo- or heterodimers through confirmational changes, with subsequent phosphorylation of tyrosine residues. These phosphorylated tyrosine residues serve as binding sites for signal transducers with the initiation of a cascade of signalling pathways resulting in tumour growth and progression (Salomon et al. 1995; Alroy and Yarden 1997). In contrast, the small molecule TKIs inhibit the intracellular tyrosine kinase of EGFR by competitive and reversible docking at the ATP binding site of the catalytic domain. Subsequently, the autophosphorylation of the receptor is prevented, resulting in weakening of the downstream signalling pathways (Hynes and Lane 2005). Therefore, signals induced by extracellular ligand binding cannot be conveyed to the tumour cell nucleus where genes involved in cellular differentiation, proliferation, and apoptosis are regulated. The consequences are reduced potency for tumour cell migration and invasiveness on the one hand, and induction of apoptosis on the other. This TKI mechanism differs from the active principle of anti-EGFR antibodies like cetuximab or panitumumab, which function via a competitive binding to the extracellular domain. But it explains the striking efficacy of EGFR-TKIs in patients with somatic mutations of the EGFR kinase domain, as it targets a key protein in the tumorigenesis of these patients.

2.3 Non-Small Cell Lung Cancer

Lung cancer does not only belong to the most frequent tumour entities in Western countries, it is also in cancer mortality statistics on the first range in men, and on the third (after breast and colorectal cancer) in women. This is the consequence of late detection due to delayed and unspecific symptoms in patients with locally advanced or metastasized disease at the time of first diagnosis. But also in earlier and locally limited tumour stages, the risks for relapse are quite high. Altogether, only 15% of all lung cancer patients survive 5 years after diagnosis despite multi-modal therapeutic concepts and new chemotherapeutic agents. Prognosis of the disease still remains serious. Therefore, new agents with efficacy mechanisms, that are different from conventional chemotherapy, are necessary to expand the arsenal of systemic therapy. In the last years, these efforts have led to the emergence of the new group of TKIs with approvals of the EGFR inhibitors erlotinib and gefitinib in advanced

NSCLC. In unselected patients, these inhibitors have shown objective tumour responses in 8–19% and prolongation of OS of 2 months (Fukuoka et al. 2003; Kris et al. 2003; Pérez-Soler et al. 2004; Shepherd et al. 2005). Especially, this last trial, the BR.21 study of Shepherd et al., has led to the approval of erlotinib in the United States and the European Community in the year 2004 and 2005, respectively, as a TKI for patients with advanced NSCLC who did not respond sufficiently to systemic chemotherapy or suffered a tumour relapse. Approval was based on the data of 731 patients in this randomised, placebo-controlled, multi-center phase III trial performed by the National Cancer Institute of Canada. Oral erlotinib was used as single agent in the second or third therapy line in patients with stage IIIb or IV according to UICC/AJCC. It demonstrated substantial advantage in terms of OS and significant release of disease-related symptoms like dyspnoea, pain, and cough (Bezjak et al. 2006). Whereas response rates in the erlotinib group comprises only 8.9% with 0.4 month difference in progression-free survival, the OS – previously defined as the study's primary end point – was 2 months longer when compared with the placebo group (6.7 vs. 4.7 months, hazard ratio 0.70, $p<0.001$). According to the prolongation in median survival, 31% of patients treated with erlotinib in this study were alive at 1 year vs. 22% in the placebo group. As independent clinical predictors for survival non-smoking status, female gender, adenocarcinoma histology, and Asian ethnicity have been identified in the BR.21 trial (Tsao et al. 2005), which are often related to the presence of activating EGFR gene mutations. EGFR mutations of the tyrosine kinase domain have been found in 10% up to 17% of NSCLC patients, preferably with adenocarcinoma and non-smoking status (Marchetti et al. 2005; Pao and Miller 2005; Zhu et al. 2008).

These mutations, mainly within the exons 19 and 21 (exon 19 deletion, L858R mutation), are the most relevant biologic factors associated with an improved response to erlotinib (Zhu et al. 2008). Various studies also with gefitinib have demonstrated that the presence of EGFR gene mutations within the kinase domain of the receptor correlates with TKI sensitivity (Lynch et al. 2004; Paez et al. 2004; Pao et al. 2004). In addition, analyses of EGFR copy numbers by fluorescence in situ hybridisation (FISH) in the BR.21 study revealed high EGFR gene copy as a predictive marker of survival benefit from erlotinib.

On the other hand, erlotinib's efficacy for OS has also been described in patients not presenting the reported clinical characteristics that are associated with the greatest degree of benefit like non-smoking status, female gender, or adenocarcinoma histology. Subset analyses of ever-smokers revealed significant survival advantages also for men and patients with squamous cell histology in the second or third therapy line despite very low response rates under erlotinib treatment (Clark et al. 2006).

Gefitinib, another EGFR-TKI, was positively associated with clinical benefits, such as tumour response, health-related quality of life, and increased survival, in two large randomised phase II studies (IRESSA Dose Evaluation in Advanced Lung Cancer IDEAL-1 and IDEAL-2) in pretreated NSCLC patients (Fukuoka et al. 2003; Natale 2004). However, it did not result in a statistically significant improvement in OS time in comparison with best supportive care in pretreated NSCLC patients of the ISEL (Iressa Survival Evaluation in Lung Cancer) trial, although in preplanned subgroup analyses, a significant survival benefit was shown in never-smokers and Asian patients. Recently, the INTEREST trial (Iressa Non-small cell lung cancer Trial Evaluating REsponse and Survival against Taxotere) and the INVITE trial (open-label, parallel-group study compared gefitinib with vinorelbine in chemotherapy-naïve elderly patients) met the primary endpoint of demonstrating non-inferiority in terms of OS for gefitinib in comparison with docetaxel or vinorelbine.

Moreover, patients treated with gefitinib experienced a lower treatment-related toxicity and better improvement in quality of life.

On the other hand, small molecule EGFR-TKIs have class-specific adverse effects mainly including skin reactions like xerosis, acneiform eruption, and eczema or mucosa-associated toxicity like diarrhoea. Rash has been reported in up to 75% of patients treated with these agents in phase II/III clinical trials. The rash that occurs with EGFR-targeted agents is generally mild to moderate; severe (grade 3/4) rash is rare (<10–15% in NSCLC trials). In a number of clinical trials, a positive correlation between severity of rash (grade ≥2) and clinical outcome with EGFR-targeted therapy has been demonstrated (Dudek et al. 2006; Pérez-Soler 2006; Cedrés et al. 2009) suggesting rash as a surrogate marker for response. Other side effects have been reported rarely like liver dysfunction or interstitial lung disease (Sandler 2006).

For the first-line treatment of metastatic NSCLC, several phase II and III trials have been conducted utilising EGFR-TKIs in this setting. Patients with advanced NSCLC who are life long never-smokers, those with EGFR mutations and/or with bronchioloalveolar cell carcinoma histology seem to have promising efficacy with EGFR-TKI first-line therapy when compared with unselected patients receiving the same agents. In fact, based on the data of the I-PASS (Iressa PanASia Study, Mok et al. 2008), the European Medicines Agency (EMEA) has recommended the approval of gefitinib for mutation-positive NSCLC patients in all treatment lines including upfront therapy. This study performed in never or light former smokers yielded a statistically significant progression-free survival (PFS) for the gefitinib-treated patient group when compared with carboplatin/paclitaxel in first-line therapy of EGFR-mutated NSCLC (HR 0.48, $p < 0.0001$).

In contrast, no improvement in survival could be demonstrated in phase III trials when EGFR-TKIs were combined with conventional platinum-based doublets, with the exception of subset analysis in non-smokers (Giaccone 2004; Herbst et al. 2005; Gatzemeier et al. 2007).

The results have initiated further investigative activity to determine alterations in the EGFR signalling pathway, but also to analyse clinical, immunohistologic, molecular, and genetic issues to predict benefit from an EGFR tyrosine kinase inhibition. In general, the therapeutic aim should be to offer a personalised systemic therapy for NSCLC patients dependent on individual predictive parameters. Furthermore, in case of ineffectiveness against secondary mutations and acquired resistance, development of the first generation of TKIs with their reversible receptor binding should be continued along with new agents with irreversible tyrosine kinase inhibition.

2.4 Pancreatic Adenocarcinoma

Pancreatic cancer is the 13th most common cancer and the 8th leading cause of cancer death worldwide (Parkin et al. 2005). Only few patients with pancreatic cancer (15–20%) present with resectable disease, where surgery offers a chance of cure. Following resection for operable pancreatic cancer, the median disease-free survival interval is 13.4 months for patients treated with adjuvant gemcitabine and 6.9 months for untreated patients. The longer median disease-free survival time associated with adjuvant gemcitabine has translated into a significant 5-year OS advantage (21 vs. 9%) (Neuhaus et al. 2008). A much higher percentage of patients, however, present with metastatic disease (40–45%) or unresectable locally advanced disease (40%). These disease stages are characterised by median survival times of 3–6 months or 8–12 months, respectively. In locally advanced, unresectable disease, patients typically receive 5-fluorouracil (5-FU)-based chemoradiation or gemcitabine chemotherapy alone. The benefits of chemoradiation over

chemotherapy alone in locally advanced disease have not been well established. Erlotinib has been evaluated in two phase I studies using a multi-modal chemoradiation approach. One study examined erlotinib plus gemcitabine and paclitaxel plus radiation followed by maintenance with erlotinib and reported a partial response rate of 46% and median survival time of 14 months (Iannitti et al. 2005). These results are supported by the other trial of erlotinib plus gemcitabine and radiation for patients with locally advanced, unresectable pancreatic cancer (Duffy et al. 2008). Single-agent gemcitabine is the standard first-line agent for the treatment of advanced inoperable pancreatic cancer with a marginally superior clinical benefit and survival when compared with fluorouracil (FU) approximately 10 years ago (Burris et al. 1997). A number of randomised controlled trials performed over the last decade have aimed to demonstrate superiority of alternative cytotoxic agents and cytotoxic combinations over gemcitabine alone with mostly disappointing results. A recent meta-analysis, however, suggested a survival benefit with a reduction of 9% in risk of death for gemcitabine-based combination chemotherapy (14 trials, 4.060 patients; HR = 0.91; 95% CI, 0.85–0.97) (Sultana et al. 2007). In parallel, our understanding of the underlying genetic and molecular abnormalities that drive the development of pancreatic cancer has expanded significantly over the last decade (Schneider et al. 2008). Alterations to oncogenes and tumour suppressor genes, such as *KRas*, *TP53,* and *p16INK4,* are thought to play a critical role in the development of pancreatic cancer. In addition, expression of the human EGFR (HER-1/EGFR) in pancreatic cancer cells is associated with the stimulation of tumour cell proliferation, poor disease outcomes, and lower sensitivity to chemotherapy (Birk et al. 1999; Nicholson et al. 2001; Xiong and Abbruzzese 2002). These observations have allowed for the rational development of targeted therapies for this hard-to-treat disease. However, with the exception of erlotinib, the completed phase III trials have not confirmed an important clinical benefit (Van Cutsem et al. 2004; Moore et al. 2003, 2007; Bramhall et al. 2002; Kindler et al. 2007; Philip et al. 2007; Shapiro et al. 2005). Based on a phase III randomised, placebo-controlled trial (NCIC-CTG study), erlotinib in combination with gemcitabine received U.S. Food and Drug Administration approval as treatment for chemotherapy-naïve locally advanced and metastatic pancreatic cancer in 2005 (Moore et al. 2007). The EMEA subsequently licenced erlotinib in combination with gemcitabine restricted for the treatment of patients with metastatic pancreatic cancer only because there was no survival benefit in the locally advanced stage (HR 0.94; 0.63–1.39). In total, 569 patients were randomly assigned in a 1:1 ratio to receive standard gemcitabine plus erlotinib (100 mg/day orally) or gemcitabine plus placebo in this double blind, international phase III trial. The primary endpoint of a longer OS time was achieved statistically with an HR of 0.82 (95% CI, 0.69–0.99; $p = 0.038$) and a median survival duration of 6.24 vs. 5.91 months. Secondary endpoint results from this trial showed a 1-year survival rate of 23% in the erlotinib plus gemcitabine arm, vs. 17% with gemcitabine monotherapy ($p = 0.023$). The PFS duration was also significantly longer with the combination regimen (3.75 vs. 3.55 months; HR, 0.77; $p = 0.004$). Objective response rates were not significantly different between the arms, although more patients on erlotinib had disease stabilisation. The clinical significance of these efficacy results has been questioned by several investigators and treating physicians. A review of toxicities may further discourage the use of gemcitabine plus erlotinib. Patients receiving erlotinib and gemcitabine experienced higher frequencies of rash (72%), diarrhoea (56%), infection (43%), and stomatitis (23%), generally grade 1 or 2. Grade 3 or 4 toxicities were similar, except for diarrhoea and cutaneous rash, which were more frequent with the two-drug combination (6% each). The six

protocol-related deaths were all in the erlotinib–gemcitabine arm. Two were attributed to treatment complications (interstitial pneumonitis and sepsis) and four were attributed to a combination of cancer and protocol treatment complications (interstitial pneumonitis, sepsis, cerebrovascular accident, and neutropenic sepsis). Interstitial lung disease was observed in seven patients receiving erlotinib plus gemcitabine and in one patient receiving placebo plus gemcitabine. In fact, there may be an interaction between gemcitabine and erlotinib contributing to increased pulmonary toxicity (Boeck et al. 2007).

An unplanned analysis of the NCIC-CTG study suggested the development of rash as a predictive marker for response to therapy with erlotinib. Patients with advanced pancreatic cancer who experienced grade 2 rash or higher ($n=102$) had a reported median survival time of 10.5 months and a 1-year survival rate of 43%. Rash development was linked to overall and progression-free survival and these correlations increased with grade (grade 1 vs. no rash: hazard ratio (HR) 0.47, $p<0.001$; grade 2 or more vs. no rash: HR 0.29; $p<0.001$). These data were supported by a combined analysis from two large phase III studies (National Cancer Institute of Canada Clinical Trials Group Studies BR.21 in NSCLC and NCIC-CTG PA.3 in pancreatic cancer). Presence of rash strongly correlated with OS in both studies. Similar results were observed for PFS (Wacker et al. 2007). In addition, a retrospective exploratory analysis of the phase III AVITA study (gemcitabine+erlotinib+placebo vs. gemcitabine+erlotinib+bevacizumab) confirmed the results of the NCIC-CTG study (Van Cutsem et al. 2009). In the placebo arm, OS was only 4.3 months in patients without rash when compared with 7.1 and 8.3 months in patients with grade 1 and grade >1 rash, respectively ($p<0.0001$). In the NCIC-CTG study, however, rash was also present in 18% of placebo taking patients with median survival 8.2 months (Moore et al. 2007). Placebo-taking patients who did not develop rash had a median survival of 4.7 months. In the combined treatment arm (gemcitabine plus erlotinib), 81% of the patients developed a rash, compared with 30% of patients in the control group. Since no reliable molecular predictive biomarker exists for the medical treatment of pancreatic cancer, physicians and patients should view rash development as a positive event indicative of greater likelihood of clinical benefit. It is important to understand that the development of rash following erlotinib treatment is not an intrinsic effect of erlotinib itself but more likely correlated to individual differences in drug exposure, the integrity of the immune system, or EGFR polymorphisms (Saif et al. 2008; Lynch et al. 2007). Further studies are required to identify patients most likely to develop rash and to determine if dose escalation to induce rash can improve efficacy.

How shall we use rash in daily practice? It has been suggested that the rash clinically improves with continuation of treatment. Nevertheless, severe rash development may be a determining cause of treatment discontinuation by patients on erlotinib outside clinical trials. If rash development is in fact a surrogate marker for treatment success, then patients discontinuing treatment are potentially stopping a life-prolonging treatment. This is why it is crucial to exploit all means available in the treatment of the erlotinib-induced skin rash, to discourage patients from stopping it. Assessing the tumour response according to RECIST or WHO criteria remains the standard of care independent on the development of rash because there may exist responders without rash and, contrary, patients with a tumour progress despite the development of rash.

Since it is unclear if every patient with advanced pancreatic cancer has to be treated with a combination chemotherapy of gemcitabine and erlotinib, there may be a rationale for sequential therapeutic strategies. Several drugs have been examined as a second-line therapy (Kulke et al. 2007). The most promising chemotherapeutic regimen may be the OFF-protocol consisting of Oxaliplatin, 5-FU, and FA. In a randomised phase III study, this combination

chemotherapy resulted in a significant survival advantage when compared with 5-FU/FA alone (Pelzer et al. 2008). Another option in gemcitabine pre-treated patients would be the combination of erlotinib and capecitabine. In one single arm phase II study with 32 patients, the median PFS time was 3.4 months, and the median OS time was 6.5 months (Kulke et al. 2007). One-year OS was 26%. In contrast, disappointing results were reported in a retrospective analysis of 13 patients treated with single-agent erlotinib (Epelbaum et al. 2007). No responses and a median time to progression (TTP) of only 1 month were observed.

At the current time, gemcitabine, either alone or in combination with erlotinib, remains the only approved first-line treatment for advanced pancreatic carcinoma. Multiple trials are planned that will employ new and novel targeted and biological agents together with the search for predictive biomarkers.

2.5 Hepatocellular Carcinoma

Hepatocellular carcinoma (HCC) is the third largest cause of cancer-related death third to lung and colon cancers (Parkin et al. 2005). The incidence has increased in the Western world over the past 20 years primarily as a result of the prevalence of hepatitis C virus infection (El-Serag and Mason 1999). Management of HCC is complex and is guided by the Barcelona Liver Clinic (BCLC) staging system, which has important prognostic value (Llovet et al. 1999). The BCLC system is conceptually useful, because it helps to integrate liver function and tumour features into a classification that is meaningful from a standpoint of treatment options. For example, BCLC C patients are those best suited for systemic therapies or clinical trials. Systemic chemotherapy, however, has largely been disappointing in terms of palliation or cure. Cytotoxic chemotherapy has been shown to provide no survival benefit. With that background in mind, the multi-targeted tyrosine kinase inhibitor sorafenib was studied in HCC. Patients with advanced-stage HCC, who were not candidates for or who had disease progression after locoregional therapy, were enrolled in the Sorafenib Hepatocellular Carcinoma Assessment Randomised Protocol (SHARP) trial (Llovet et al. 2008). The 1-year survival for the sorafenib group was 44 and 33% for the placebo group. The median survival for the sorafenib group was 10.7 months from enrolment compared to 7.9 months for those who received placebo. The survival benefit appeared to be correlated to a 2.7-month delay in radiologic progression (5.5 months for the sorafenib group vs. 2.8 months for the placebo group). A recent phase 3 study of sorafenib vs. placebo in Asian patients reported a similar increase in survival (6.2 vs. 4.1 months) (Cheng et al. 2009). Sorafenib is now considered to be the standard medical treatment for patients with Child-Pugh stage A cirrhosis within the BCLC stage 3 group.

EGFR is frequently overexpressed in HCC (Buckley et al. 2008). In a phase II study, erlotinib was evaluated in 38 patients with unresectable or metastastic HCC (Philip et al. 2005). Most frequent grade 3–4 toxicities were skin rash (13%), diarrhoea (8%), and fatigue (8%). There was a correlation between the severity (grade 3 or higher) of toxicity and Child-Pugh classification: only 22% of the Child-Pugh A patients experienced severe toxicity when compared with 70% of Child-Pugh B patients ($p = 0.02$). Thirty-two of the patients were progression-free after 24 weeks. The overall confirmed response rate was only 9%. Seventeen patients (50%) achieved stabilisation of disease for a median of 3.8 months. There was no correlation between response and EGFR status. The median OS time was 13 months, with a probability of 33% of patients alive at 18 months from entry into the study. In a second phase II study, 40 HCC patients were treated with erlotinib 150 mg daily for 16 weeks (Thomas et al. 2007). There were no complete or partial

responses; however, 17 of 40 patients achieved stable disease at 16 weeks of continuous therapy. The PFS at 16 weeks was 43%, and the median OS was 43 weeks (10.75 months). No patients required dose reductions of erlotinib. Again, no correlation between EGFR expression and outcome was found.

In contrast to lung cancer, the gain of function in EGFR signalling in HCC seems mediated through increase in ligand–receptor interaction, rather than by point mutations or amplifications (Llovet and Bruix 2008). Erlotinib treatment of HCC might inhibit the mitogen activated protein (MAP)-kinase pathway and signal transducer of activation and transcription (STAT)-mediated signalling resulting in an altered expression of apoptosis and cell cycle regulating genes (Huether et al. 2006). Overexpression of proapoptotic factors like caspases and gadds associated with a downregulation of anti-apoptotic factors like Bcl-2, Bcl(XL), or jun-D might account for erlotinib's potency to induce apoptosis. In addition, downregulation of cell cycle regulators promoting the G_1/S-transition and overexpression of cyclin-dependent kinase inhibitors and gadds might contribute to the induction of a G_1/G_0-arrest of HCC cells in response to erlotinib. Together, erlotinib alone appears to have only modest activity against HCC and further randomised studies are needed to evaluate the potential benefit of erlotinib in HCC patients.

There is scientific rationale for combining bevacizumab and erlotinib in HCC (Llovet and Bruix 2008). Overexpression of proangiogenic factors, such as vascular endothelial growth factor (VEGF), platelet-derived growth factor, and angiopoietin-2, has been demonstrated in HCC (Llovet and Bruix 2008; Villanueva et al. 2007; Chiang et al. 2008). As mentioned above, there is also a rationale to abrogate EGFR signalling in HCC. Thomas et al. (2009) reported a single-arm phase II study with 40 HCC patients treated with the combination of bevacizumab (10 mg/kg every 14 days) and erlotinib (150 mg daily). Regarding efficacy, objective response rate was 25%, and the median PFS and OS times were 9 and 15.6 months, respectively. The results are encouraging, but have to be interpreted with caution due to patient selection bias and the small sample size and short follow-up time.

Together, sorafenib is the standard of care in patients with advanced HCC as a result of robust data obtained in the setting of phase III investigations both in the West and Asia. The role of erlotinib and erlotinib combinations has to be explored in randomised phase II and III studies. In fact, a phase III study of erlotinib plus bevacizumab against sorafenib is under consideration within the North American GI Steering Committee Hepatobiliary Task Force.

2.6 Other Tumour Entities

Erlotinib has been examined in phase I and II studies in malignant glioma and colorectal, biliary, gastric, breast, ovarian, endometrial, and renal cell cancer. Efficacy with respect to OS and response rates, however, was low. In contrast, single-agent erlotinib or erlotinib-based polychemotherapy may be promising in recurrent or metastatic squamous cell cancer of the head and neck. These studies are discussed in detail elsewhere (Tang et al. 2006).

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Axitinib (AG-013736) 3

Ronan Joseph Kelly and Olivier Rixe

Abstract The vascular endothelial growth factor (VEGF)/VEGF receptor tyrosine kinase (RTK) signaling pathway plays a pivotal role in tumor angiogenesis. Neovascularization promotes increased tumor cell proliferation, survival and metasasis. Many antiangiogenic agents including multi-RTK inhibitors are either approved or are undergoing testing in clinical trials. Axitinib is a potent and selective inhibitor of VEGF RTK 1, 2, and 3. This chapter discusses the stucture of axitinib as well as its toxicities and drug interactions. Important preclinical and clinical data for axitinib are presented including findings from phase II studies in many tumor types including malignant melanoma and renal, pancreatic, thyroid, breast, lung and colorectal carcinomas. Ongoing phase III studies in pancreatic and metastatic renal cell carcinoma will ultimately define the therapeutic role of this targeted agent.

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3.1 Introduction

Tumors require angiogenesis to grow beyond 1–2 mm in size (Folkman and Engl 1971). Newly formed blood vessels provide nutrients for growing tumors and serve as an escape route for metastatic cells. When a tumor acquires the ability to establish its own vasculature, its behavior becomes more aggressive (Weidner et al. 1991). The inhibition of new blood vessel growth has, therefore, become an important cancer control target and represents a major advancement in the treatment of various solid tumors.

Vascular endothelial growth factor (VEGF) is a potent endothelial cell mitogen, which is normally seen in certain physiologic situations (fetal development, menstruation, wound healing). Overexpression of VEGF has been associated with tumor progression and poor prognosis in several tumor types, including renal cell carcinoma (RCC), colorectal carcinoma, gastric carcinoma, pancreatic carcinoma, breast cancer, prostate cancer, lung cancer, and melanoma.

There are four known VEGFs (VEGF-A to -C and placental growth factor (PLGF) and four platelet-derived growth factors (PDGF-A to -D) (Ferrara et al. 2003). Growth factors of these families function primarily in a paracrine manner to promote angiogenesis and vasculogenesis

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(Leung et al. 1989). Both angiogenesis and vasculogenesis play a role in the formation and maintenance of tumor vasculature and the progression of cancer (Hicklin and Ellis 2005). VEGF and PDGF peptides circulate as homo- or heterodimers that regulate cellular processes, such as proliferation and migration, via binding to tyrosine kinase receptors that are expressed on the surface of target cells (Cross et al. 2003). Ligand binding triggers receptor autophosphorylation and the initiation of downstream signaling processes that promote the proliferation, migration, and survival of endothelial cells. In tumor vascularization, these processes form the framework of immature new neoplastic vessels (Hicklin and Ellis 2005). The downstream effects of the majority of VEGF isoforms are mediated by VEGFR-2.

VEGFR-2 (kinase-insert domain receptor (KDR)/fetal liver kinase (Flk)-1) is a type III transmembrane kinase receptor, first isolated in 1991 by Terman and coworkers (Terman et al. 1991). The human VEGFR-2 gene, located on chromosomes 4q11–q12, encodes a full-length receptor of 1356 amino acids (Sait et al. 1995). It consists of an extracellular region composed of seven immunoglobulin (Ig)-like domains, a short transmembrane domain, and an intracellular region containing a tyrosine kinase domain, split by a 70-amino-acid insert. It belongs to the 7/5-Ig protein tyrosine kinase superfamily, and is thus closely related to the platelet-derived growth factor receptors (PDGFRs), fms receptor, and c-Kit receptor (Shibuya et al. 2002). Within the cell, the VEGFR-2 protein is translated as a 150 kDa protein without significant glycosylation. It is then processed by a series of glycosylations to a mature 230 kDa form that is expressed on the cell surface (Takahashi et al. 1997).

VEGF-A binds to the second and third extracellular Ig-like domains of VEGFR-2. Ligand binding induces receptor dimerization and autophosphorylation. A number of studies have shown that VEGFR-2 is the principal mediator of several physiological and pathological effects of VEGF-A on endothelial cells. These include proliferation, migration, survival, and permeability.

The PDGFs play a role in the regulation of cell proliferation, particularly in connective tissues, and are thought to function as growth signals for pericytes, cells that line and stabilize the nascent vessels formed by endothelial cells. PDGFs are also overexpressed in various cancers. Studies involving anti-VEGF and anti-PDGF receptor therapies have demonstrated that these agents can potently inhibit angiogenesis and tumor growth in preclinical models (Hicklin and Ellis 2005).

The indazole derivative axitinib is an oral and selective inhibitor of the VEGFR-1, -2, and -3. Axitinib entered clinical trials in cancer subjects in May 2002, and it is currently being investigated in various tumor types in Phase 2/3 clinical trials.

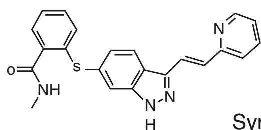
3.2 Structure of Molecule

Structure, chemical characteristics, and interaction with the VEGF receptor are shown in Figs. 3.1 and 3.2.

3.3 Preclinical Data

Axitinib was generated from a structure-based drug design approach and is a potent ATP-competitive inhibitor of receptor tyrosine kinases (RTK) of VEGFR1, 2, 3. It is a weaker inhibitor of PDGFR-β. Axitinib is not a potent inhibitor of the closely related kinases of FGFR-1, Flt-3, or Tie-2, and other kinases of distant families in the kinome.

Axitinib blocks VEGF-mediated endothelial cell adhesion and migration on extracellular matrix proteins and induces endothelial apoptosis as early as 6 h after treatment in cell culture. It has also been shown to produce rapid and potent



Synonyms: AG-013736, AG-13736

Molecular Weight:	386.47 Daltons
Molecular Formula:	$C_{22}H_{18}N_4OS$
Chemical Name:	N-Methyl-2-[3-((E)-2-pyridin-2-yl-vinyl)-1H-indazol-6-ylsulfanyl]-benzamide (IUPAC)
Formulations:	Two strength of tablets (1mg and 5mg), stored at room Temperature and protected from light

Fig. 3.1 Synonyms: AG-013736, AG-13736. Molecular weight: 386.47 Da. Molecular formula: $C_{22}H_{18}N_4OS$. Chemical name: *N*-methyl-2-[3-((E)-2-pyridin-2-yl-vinyl)-1H-indazol-6-ylsulfanyl]-benzamide (IUPAC). Formulations: two strength of tablets (1 and 5 mg), stored at room temperature and protected from light

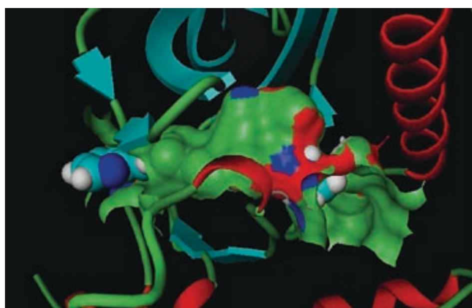


Fig. 3.2 VEGFR-2 and axitinib interaction: image showing axitinib in the kinase domain of the membrane receptor

inhibition of eNOS, Akt, and ERK 1/2 phosphorylation at concentrations that correlate with its potency for VEGFRs (Hu-Lowe et al. 2008).

In vitro, axitinib has limited direct activity against VEGFRs, PDGFR-β, and KIT. In vivo DCE-MRI studies showed that axitinib treatment decreased the overall tumor blood flow/permeability at as early as 2 days after initiation of treatment, with a maximum reduction in K^{trans} (endothelial volume transfer constant, an indicator or vascular leakage of a contrast-enhanced agent) observed on day 7 after dosing. The studies also showed that the changes in vascular K^{trans} correlated with decreased microvessel density, cellular viability, and tumor growth (Wilmes et al. 2007). The antiangiogenesis activity of axitinib was also assessed by measuring tumor microvessel density (MVD, measured by CD-31 staining) after either acute or prolonged treatment in xenograft tumor models. A further study demonstrated that neoangiogenesis occurred as early as 1 day after drug withdrawal and tumors were fully revascularized within 7 days, which still responded to the second cycle of axitinib. Based on this observation, a continuous daily dosing of axitinib would be optimal for antiangiogenesis (Hu-Lowe et al. 2008).

Axitinib has demonstrated additive or synergistic antitumor activity with docetaxel in models of murine lung cancer and human breast cancer, with carboplatin in a model of ovarian cancer, and with gemcitabine in a model of human pancreatic cancer, which resulted in an improvement in antitumor efficacy ranging

from efficacy enhancement to an additive effect and synergism (Rugo et al. 2005). Animal studies have shown that both p.o., b.i.d. dosing (10 and 30 mg/kg), and continuous subcutaneous infusion of axitinib (3, 10 and 30 mg/mL) generated a similar exposure (determined by estimating AUCs), and that the levels of Cmax achieved in the p.o. dosing group were not reached in any of the infusion groups; a greater and/or maximal TGI (tumor growth inhibition) was accomplished by the route of continuous infusion of the compound (Hu-Lowe et al. 2008).

A further study was carried out to evaluate whether, after an initial treatment with axitinib followed by a prolonged dosing break period, tumors would become refractory to axitinib and begin to develop resistance. The results indicated that the regrown tumors after an 11-day dosing break continued to respond to axitinib treatment. However, comparing with continuous daily b.i.d. dosing, cessation of axitinib treatment for 11 days significantly compromised the overall magnitude of TGI in the end. Continuous daily dosing of axitinib seems to be necessary for optimal antitumor efficacy.

In vitro metabolism studies show that axitinib metabolism in the liver is predominantly mediated by CYP3A4, and to a lesser extent by CYP1A2. The pharmacokinetics of axitinib may be affected by CYP3A4 inhibitors and inducers. Systemic exposure of axitinib may be affected by drugs that are substrates or inhibitors of P-glycoprotein.

In vivo metabolism studies show that biotransformation pathways include oxygenation, glucuronidation, glucosylation, and oxygenation followed by either glucuronidation or glucosylation. Axitinib has two major human plasma metabolites, a sulfoxide and an N-glucuronide.

3.3.1 Bioavailability in Humans

Up to early 2007, 21 studies had evaluated the safety, efficacy, and the PK of axitinib. These studies included seven phase 1 studies in healthy subjects, and 14 studies in subjects with cancer.

Axitinib in the fed stage is absorbed rapidly, with peak plasma concentrations occurring within 2–6 h after dosing. The rate and extent of absorption of the drug was higher in the overnight fasted state with peak concentrations occurring 1–2 h after dosing, indicating a significant food effect (Rugo et al. 2005). However, further studies have confirmed that overnight fasting is not required and ongoing studies recommend that subjects take axitinib with food. The plasma elimination half-life ranges between 2 and 5 h. The mean absolute bioavailability for oral axitinib was 58%. This mean estimate indicates that approximately 58% of an administered dose of axitinib reaches the systemic circulation following oral administration (Rugo et al. 2005).

Studies have demonstrated that the effect of pH on absorption of axitinib was not considered to be clinically significant but in patients taking axitinib, antacids, or PPIs should be administered at times other than 2 h before and 2 h after drug dosing (Rugo et al. 2005).

3.4 Phase II Studies

3.4.1 Axitinib in Renal Cell Carcinoma

In recent years, many new promising treatments and investigational drugs inhibiting angiogenesis have been evaluated in RCC. In patients with cytokine-refractory metastatic RCC, median progression-free survival has been reported as 4.8 months in patients treated with high-dose bevacizumab (Yang et al. 2003) and 5.5 months in those treated with sorafenib (Escudier et al. 2007), with objective responses of 10% for both drugs. Sunitinib has shown an objective response rate (ORR) of 40% in patients who have failed cytokine treatment (Motzer et al.

2006), and a high objective response (31%) and longer progression-free survival when compared with interferon alfa in previously untreated patients (Motzer et al. 2007).

Sunitinib and Sorafenib have a broader multi-targeted approach and simultaneously inhibit numerous tyrosine kinase receptors, including VEGF receptor, the platelet-derived growth factor receptor, and the tyrosine kinases c-KIT and FLT3. It was anticipated that the specificity and high picomolar potency of axitinib, against the VEGF receptors 1, 2, and 3-receptors, which play an important role in renal cell cancer pathogenesis, would account for significant antitumor activity.

Axitinib is one of the first tyrosine kinase inhibitors developed in metastatic RCC, and the preliminary results were reported in the 2005 ASCO meeting by Rixe et al. The efficacy of axitinib (5 mg b.i.d.) in patients ($n=52$) with metastatic RCC whose disease was refractory to cytokine treatment was demonstrated in a phase II, nonrandomized clinical trial (Rixe et al. 2007). Patients were treated in 28-day treatment cycles until disease progression or unacceptable toxicity. Analysis of results demonstrated two complete and 21 partial responses, for an ORR of 44.2% (95% CI 30.5–58.7). Median response duration was 23.0 months. Twenty-two patients showed stable disease lasting for longer than 8 weeks, including 13 patients with stable disease for at least 24 weeks. Stable disease was noted in the only patient with papillary histology, who had a 27.3% decrease in tumor diameter (as defined by RECIST) on day 71. Median time to progression was 15.7 months and median overall survival was 29.9 months. In ancillary studies in 13 of the patients (7 responders and 6 nonresponders), decreased tumor perfusion was observed in patients who responded to treatment. Decreased perfusion correlated with improved response in 4 out of 6 patients with stable or progressive disease (Rixe et al. 2005). The results obtained from this phase II study indicate that Axitinib is a potent agent for the treatment of metastatic RCC.

A phase II nonrandomized, open-label, single-group clinical trial evaluated axitinib (5 mg p.o., twice daily) in patients ($n=62$) with advanced and refractory RCC who had also failed sorafenib-based therapy (Rini 2007). A partial response was observed in 13 patients (21%), stable disease in 21 patients (34%), and progressive disease in 16 patients (26%). Tumor shrinkage to some extent was experienced by 57% of patients. A preliminary analysis after a median follow-up of 8.1 months indicated an overall median progression-free survival of 7.4 months. These preliminary results suggest the absence of cross-resistance between axitinib and sorafenib for a limited but significant subset of patients.

3.4.2 Axitinib in Pancreatic Cancer

VEGF promotes tumor growth in pancreatic ductal adenocarcinoma (Korc 2003). High VEGF expression is associated with increased microvessel density (Seo et al. 2000), and is a predictor of early tumor recurrence after curative resection and of poor outcome (Niedergethmann et al. 2002). The addition of bevacizumab to gemcitabine failed to show a survival advantage when compared with gemcitabine alone in advanced pancreatic cancer (Kindler et al. 2007). This prompted studies evaluating the use VEGF inhibitors in pancreatic cancer with a different mechanism of action to bevacizumab.

A Phase II, randomized, open-label clinical trial was conducted to determine the relative survival rates of patients ($n=103$) with metastatic pancreatic cancer receiving either a combination of axitinib and gemcitabine or gemcitabine alone (Spano et al. 2008). Patients were treated with gemcitabine (1,000 mg/m^2 days 1, 8, and 15) and axitinib 5 mg twice daily in 28 day cycles, or gemcitabine 1,000 mg/m^2 days 1, 8, and 15 alone. The primary endpoint was overall survival. The median overall survival with the combination treatment was 6.9 months, compared with 5.6 months for

gemcitabine alone. Progression-free survival results were consistent with those for overall survival. Median progression-free survival with gemcitabine plus axitinib was 4.2 (95% CI 3.6–10.2) months, compared with 3.7 (2.2–6.7) months with gemcitabine alone. The confirmed ORR was 7% for the gemcitabine plus axitinib group when compared with 3% for the gemcitabine alone group. These improvements were not statistically significant (hazard ratio 0.71, 95% CI 0.44–1.13 for overall survival; HR 0.79 CI, 0.43–1.45 for progression-free survival). In a subgroup analysis, patients with locally advanced disease had a greater overall survival advantage when treated with gemcitabine plus axitinib than patients who had metastatic disease (HR 0.54, 95% CI 0.26–1.12 vs. HR 0.96 CI 0.52–1.77). The small, nonstatistically significant gain in overall survival is currently being assessed in a randomized phase III trial using a similar design. This study allows the axitinib dose to be titrated up from the starting dose of 5 mg twice daily to a maximum of 10 mg twice daily.

3.4.3 Axitinib in Metastatic Breast Cancer

A randomized, multicenter, double-blind, placebo-controlled phase 2 study investigated axitinib given in combination with docetaxel vs. docetaxel alone for subjects who have not received prior chemotherapy for metastatic breast cancer (n=168). Patients were eligible if they were ≥12 months from adjuvant chemotherapy, had measurable disease, ECOG PS 0–2, and no uncontrolled brain metastases (Phase 2 study of AG-013736 in combination with docetaxel vs. docetaxel alone for patients with metastatic breast cancer. Clinicaltrials.gov 2006; Rugo et al. 2005). The starting doses were determined to be 80 mg/m^2 of docetaxel (IV, once every 3 weeks) and 5 mg b.i.d. of axitinib (or placebo-equivalent). The primary endpoint of the trial was time to progression. A median of seven cycles were administered in each arm of the trial. The median time to progression was 8.2 months for the combination therapy, compared with 7 months for placebo (p=0.05) (Rugo 2007). In the axitinib arm, the overall response rate was 40% and for the placebo arm, the response rate was 23% (p=0.038). A subgroup analysis revealed that the median time to progression in patients who had previously received anthracycline treatment was 9.0 months in the axitinib arm and 6.3 months in the placebo arm, with a hazard ratio of 0.54 (p=0.012). Within this subgroup, the response rates were 45 and 13% for the axitinib and placebo arms, respectively (p=0.003) (Rugo 2007).

3.4.4 Axitinib in Thyroid Cancer

The prognosis for thyroid cancer is generally favorable when standard paradigms are applied. Radioactive Iodine (RAI) refractory, recurrent, or metastatic disease is therapeutically challenging, and death from thyroid cancer within 3 years under these circumstances is not uncommon. Anaplastic thyroid cancer is relatively rare but it is typically un-resectable at presentation and is resistant to RAI and chemotherapy. Medullary thyroid cancer is derived from parafollicular C cells. RAI does not have a role in the management of medullary thyroid cancer and it has a worse prognosis than the more common papillary thyroid tumors (Cupisti et al. 2007). Many advanced thyroid cancers will eventually develop lack of iodine avidity, making chemotherapy the only viable option for systemic treatment. Doxorubicin is an approved therapy in incurable thyroid cancer based on response rates of 10–37% (Gottlieb and Hill 1974; Shimaoka et al. 1985).

A common element to thyroid cancers is their associated vascularity, with elevated levels of VEGF compared with normal thyroid tissue (Viglietto et al. 1995). Microvessel density is also higher in papillary thyroid cancer than in normal thyroid (Kilicarslan et al. 2003). In

human thyroid tumor specimens, VEGF levels are correlated with stage, large tumor size, nodal involvement, extrathyroidal invasion, and distant metastasis (Klein et al. 1999). These observations support evaluating axitinib in this disease.

In a phase II multicenter clinical trial in patients ($n=60$) with measurable metastatic or unresectable locally advanced thyroid cancer that was refractory to or unsuitable for ^{131}iodine treatment, patients received axitinib at an oral dose of 5 mg twice daily (Cohen et al. 2008). Partial responses were observed in 18 patients, yielding an ORR of 30% (95% CI, 18.9–43.2). Stable disease lasting ≥16 weeks was reported in another 23 patients (38%). Objective responses were noted in all histologic subtypes. Median PFS was 18.1 months (95% CI, 12.1 to not estimable). Axitinib was generally well tolerated with the most common grade ≥3 treatment-related adverse event (AE) being hypertension ($n=7$; 12%). Eight patients (13%) discontinued treatment because of AEs. Axitinib selectively decreased sVEGFR-2 and sVEGFR-3 plasma concentrations vs. sKIT, demonstrating its targeting of VEGFR.

3.4.5 Axitinib in Other Solid Tumors

A phase II, nonrandomized, open-label, uncontrolled clinical trial of axitinib was conducted in patients ($n=32$) with metastatic NSCLC or advanced NSCLC with malignant pleural effusion (Schiller 2007). Patients were orally administered axitinib (5 mg b.i.d.), with doses up to 10 mg permitted, until unacceptable toxicity or disease progression. Three responses were confirmed, with a median duration of response of 9.4 months. There were 10 patients with stable disease and 9 with progressive disease. Median survival and progression-free survival were 12.5 and 5.8 months, respectively. Treatment was discontinued for 26 patients mostly due to a lack of efficacy.

A phase II, single-arm, multicenter, open-label, clinical trial in patients ($n=32$) with metastatic melanoma was presented by Fruehauf et al. at ASCO 2008 (Fruehauf et al. 2008). The primary objective of this study is ORR by RECIST. An investigator report shows an ORR of 19% (95% CI: 7–36%) including one durable CR. Median duration of response was 7.9 months, median PFS was 2.3 months (95% CI 1.8–5.7), and median OS for all patients was 6.8 months (95% CI 5.2–10.4). These results compare favorably with other agents developed in the same indication, and support further evaluations.

Two phase II, multicenter, nonrandomized, open-label clinical trials to study the effect of axitinib in combination with bevacizumab and standard chemotherapy regimens have been initiated in patients with metastatic colorectal cancer. In the first trial, 123 patients receive FOLFOX, and either axitinib (5 mg b.i.d.), bevacizumab (5 mg/kg every 2 weeks), or axitinib (5 mg b.i.d.) plus bevacizumab (2 mg/kg every 2 weeks) (Phase 2 study with AG-013736 combined with chemotherapy and bevacizumab in patients with metastatic colorectal cancer. Clinicaltrials.gov 2008). This trial is currently ongoing. In a second, ongoing study, patients who have previously failed treatment with irinotecan or oxaliplatin-based therapy were to be administered axitinib in conjunction with either FOLFOX or FOLFIRI or bevacizumab with FOLFIRI or FOLFOX. (A study combining FOLFOX or FOLFIRI with AG-013736 or avastin in patients with metastatic colorectal cancer after failure of one first-line regimen. Clinicaltrials.gov 2008).

3.5 Phase III Studies

A phase III, randomized, double-blind, active-controlled clinical trial has been initiated to compare treatment with axitinib plus gemcitabine with gemcitabine plus placebo, in patients ($n=596$) with advanced pancreatic cancer. Patients receive gemcitabine (1,000 mg/m^2 i.v.) on days 1, 8, and

3

Table 3.1 Summary of results from phase II trials

r type	*n*	Phase II/III	Response rate (%)	PFS (months)	Reference
RCC	52	II	44.2	15.7	(Rixe et al. 2007)
Pancreatic	103	II	7	4.2	(Spano et al. 2008)
Breast	168	II	40	8.2	(Phase 2 study of AG-013736 in combination with docetaxel versus docetaxel alone for patients with metastatic breast cancer. Clinicaltrials.gov 2006; Rugo et al. 2005)
Thyroid	60	II	30	18.1	(Cohen et al. 2008)
NSCLC	32	II	9	5.8	(Schiller et al. 2007)
Melanoma	32	II	19	2.3	(Fruehauf et al. 2008)

15 of every 4 weeks, either with or without oral axitinib (5 mg b.i.d.), until disease progression or unacceptable toxicity. The primary endpoint in this trial is overall survival (randomized study of gemcitabine plus AG-013736 vs. gemcitabine for advanced pancreatic cancer. CLINICALTRIALS. GOV 2007).

3.6 Toxicity

AEs reported in axitinib clinical studies are considered manageable, generally reversible and expected for this class of agents. For single-agent axitinib, the most common AEs reported are hypertension, fatigue, and gastrointestinal toxicity.

In phase 1 studies, the dose limiting toxicity (DLT) was hypertension, which was responsive to medications and was reversible with drug cessation. None of the patients receiving 5 mg b.i.d. had hypertension that could not be managed with standard antihypertensive medications. In ongoing clinical programs, subjects receive a starting dose of 5 mg b.i.d. with home monitoring of blood pressure (before each dose) and in-clinic monitoring at the time of scheduled visits. Those subjects who tolerate axitinib with no AEs above CTCAE grade 2 for 2 consecutive weeks increase their dose step-wise to 7 mg b.i.d. and then to 10 mg b.i.d., unless BP is >150/90 mmHg or the subject is receiving antihypertensive medication. Bleeding events that have occurred among the phase 1 studies have included one fatal case of hemoptysis in a subject with lung adenocarcinoma, epistaxis, breast hemorrhage, hematochezia, rectal hemorrhage, and vaginal hemorrhage. Asymptomatic proteinuria was seen in early studies and consequently, the phase 1 protocol was amended to exclude subjects with proteinuria at baseline(>500 mg/24 h) and to require dose modifications of axitinib on the basis of proteinuria. The maximum tolerated dose was defined as a 5 mg b.i.d. starting dose.

In the phase II study conducted in metastatic RCC (Rixe et al. 2007), Axitinib was given as a single agent and toxicities are reported as follows:

The most commonly reported treatment related AEs of severity grade 3 or higher were hypertension (14%), fatigue (10%), diarrhea (4%), palmar plantar erythrodysesthesia syndrome (3%), hypertension aggravated (2%), and stomatitis (2%) (Table 3.2).

Laboratory abnormalities for subjects with solid tumors who received single-agent axitinib were grade 3/4 hyperglycemia in 5.5%, hyponatremia or elevation in creatinine in 4.6%, elevations in AST in4.0%, and proteinuria in 0.8%.

Hematological abnormalities were grade 3 or 4 neutropenia in 0.8% and thrombocytopenia in 1.0%. Grade 3/4 lymphopenia was reported in 19%.

Hypertension is commonly observed during treatment with axitinib and other VEGFR inhibitors. Increases in blood pressure have been proposed as an efficacy marker for VEGF pathway inhibitors. Post hoc, combined analyzes of data from two phase II studies of axitinib in mRCC were performed to explore the possible association between diastolic blood pressure ≥90 mmHg and efficacy endpoints (Rixe et al. 2008). The two studies included 111 patients (59 and 52 with sorafenib and cytokine refractory mRCC, respectively) evaluable for changes in diastolic BP. Seventy patients (63.1%) had ≥1 diastolic BP measurement ≥90 mmHg. The ORR was 48.4% for patients with diastolic BP measurement ≥90 mmHg vs. 12.2% for patients without. Median OS (30.0 vs. 9.8 months; $p<0.0001$) and PFS (17.6 vs. 7.1 months; $p<0.0001$) were longer in patients with diastolic BP measurement ≥90 mmHg than in those without. The frequencies of most commonly reported AEs were greater in patients with diastolic BP measurement ≥90 mmHg than in those without, including fatigue (80.0 vs. 41.5%), diarrhea (72.9 vs. 41.5%), hypertension reported as an AE (67.1 vs. 24.4%), nausea (52.9 vs. 43.9%), and anorexia (51.4 vs. 34.1%). Further studies are required to prospectively validate the occurrence of diastolic BP measurement ≥90 mmHg as a biomarker of axitinib activity. A post hoc exploratory analysis in the phase II pancreatic study (Spano et al. 2008) also showed an improvement in overall survival in patients with at least one diastolic blood pressure measurement of 90 mmHg or more during treatment. Dose-escalation in pancreatic trials, adapted to the blood pressure level, is promising but remains to be validated

Table 3.2 Treatment-related adverse events (AEs) occurring in at least 10% of patients (*n*= 52) (Rixe et al. 2007)

	All grades, *n*	Grades 3–4, *n*
Diarrhea	31	5
Hypertension	30	8
Fatigue	27	4
Nausea	23	0
Hoarseness	19	0
Anorexia	18	1
Dry skin	17	0
Weight loss	14	0
Dyspepsia	12	0
Vomiting NOS	11	0
Limb pain	10	2
Stomatitis	9	1
Headache	8	0
Dry mouth	8	0
Nail disorder	7	0
Arthralgia	7	1
Constipation	7	0
Abdominal pain NOS	6	0
Rash	6	0
Dysgeusia	6	0
Myalgia	6	1

Preliminary evidence suggests that axitinib is safe and has a side-effect profile that gives an advantage over other antiangiogenic drugs. The continuous administration and the constant dose appear to be safe, and compatible with long-term administration. In the phase II RCC study, patients have received Axitinib for more than 3 years, with the absence of cumulative toxicity (Rixe et al. 2007).

3.7 Drug Interactions

As mentioned previously, metabolism of axitinib is primarily mediated by the CYP3A4 drug-metabolizing enzyme, and to a lesser extent by CYP1A2 as determined by in vitro studies with human liver microsomes. There is a mechanistic potential for elevated concentrations of axitinib in plasma in the presence of drugs that are CYP3A4 inhibitors.

3

Ketoconazole is a potent CYP3A4 inhibitor. Ketoconazole causes a twofold increase in the plasma exposure of axitinib and a 1.5-fold increase in peak plasma concentration. Rifampin is a potent CYP3A4 inducer. Rifampin causes a 79% decrease in axitinib plasma exposures and a 71% decrease in axitinib peak plasma concentrations.

3.8 Future

Axitinib has demonstrated outstanding activity in the treatment of solid tumors including RCC and pancreatic tumors. Axitinib presents original characteristics and advantages in comparison with the other antiangiogenic compounds: a favorable profile of toxicity with the absence of cumulative dose-limiting toxicity, a large spectrum of activity, a constant and manageable schedule of administration, the occurrence of complete responses in RCC, the emergence of long-term survivors. From these early phases of development, large randomized phase II and III clinical trials are ongoing with axitinib in several types of cancer and should definitively demonstrate its efficacy and define its future indications. It will not be surprising if this agent gains approval for the treatment of metastatic RCC, pancreatic and thyroid cancers due to the significant activity that was observed in phase II trials in these malignancies.

Cross-resistance between antiangiogenic compounds needs to be addressed. Preliminary preclinical studies tried to demonstrate the putative mechanisms involved in acquired resistance to antivascular agents. They underline the heterogeneity of the endothelial cell, angiogenic factors, and tumor cells, the role of the microenvironment, the potential angiogeno-independence, or the implication of pharmacokinetic parameters. Based on the Rini study in metastatic RCC, axitinib (Rini 2007) has demonstrated a different spectrum of activity from other VEGFR inhibitors, suggesting a potential absence of cross-resistance in a subset of patients.

The definitive role of Axitinib for the treatment of solid tumors will be determined in the two ongoing phase III studies conducted in pancreatic and renal carcinoma. Based on initial positive phase II studies, results from these randomized studies are expected by the community. The pursuit of reliable predictive factors of VEGR inhibitor activity is ongoing. Biomarkers in addition to clinical parameters (such as blood pressure or erythropoietin level) are currently being evaluated with promising preliminary results.

Future developments with Axitinib include combination studies with cytotoxics. Based on preclinical evidence (tumor vasculature normalization induced by VEGF modulation) and a favorable toxicity profile, clinical studies are ongoing in breast, colon, and bladder carcinoma.

Finally, the role of a specific inhibitor of VEGFR-1, -2, and -3 in the adjuvant setting should be addressed. Identification of molecular and cellular pathways that constitute the “premetastatic niche phenomenon,” which is driven by the expression of VEGFR-1 on bone marrow-derived hematopoietic progenitors will most likely lead to a prevention of metastasis at an earlier stage (Kaplan et al. 2006). Axitinib represents an ideal drug to test this hypothesis.

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4 Lapatinib

Tanja Schneider-Merck and Martin Trepel

Abstract The human epidermal growth factor receptor (HER) family of growth factor receptor tyrosine kinases (RTKs) plays an important role in the biology of many cancers. In breast cancer, HER2 and its homo- or heterodimerization with HER1 or HER3 are essential for cancer cell growth and survival. Patients overexpressing HER2 have a poor prognosis, which can be substantially improved upon HER2-targeted therapy using the monoclonal antibody trastuzumab. Lapatinib is a novel dual tyrosine kinase inhibitor, blocking HER1 and HER2 tyrosine kinase activity by binding to the ATP-binding site of the receptor's intracellular domain. This results in inhibition of tumor cell growth. The drug is relatively well tolerated in patients, with few and mostly low-grade adverse effects. In particular and unlike to trastuzumab, it has very little, if any, adverse effects on cardiac function. In patients with advanced HER2-positive breast cancer, lapatinib has shown substantial antitumor activity, particularly in combination with capecitabine upon progressive disease following standard therapy with antracyclines, taxanes, and trastuzumab. Ongoing and future studies will explore its role in the adjuvant therapy setting, in drug combinations other than capecitabine, and in the treatment of HER2-positive tumors other than breast cancer.

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4.1 Introduction

4.1.1 The Epidermal Growth Factor Receptor Family of Tyrosine Kinases

More than 400,000 women die from breast cancer per year, making this the most common cause of death from cancer among women worldwide (Parkin et al. 2005). Breast cancers often display overexpression or constitutive activation of the human epidermal growth factor receptor (HER, EGFR, ErbB) tyrosine kinases, which are also involved in normal breast development (Atalay et al. 2003; Yarden 2001). Aberrant receptor activation or overexpression of HER1 (27–30% of cases) and HER2 (20–25% of cases) in human breast cancers is associated with poor clinical outcome (Nahta et al. 2006; Witton et al. 2003; Yarden and Sliwkowski 2001). These RTKs provide specific docking sites for various adapter proteins

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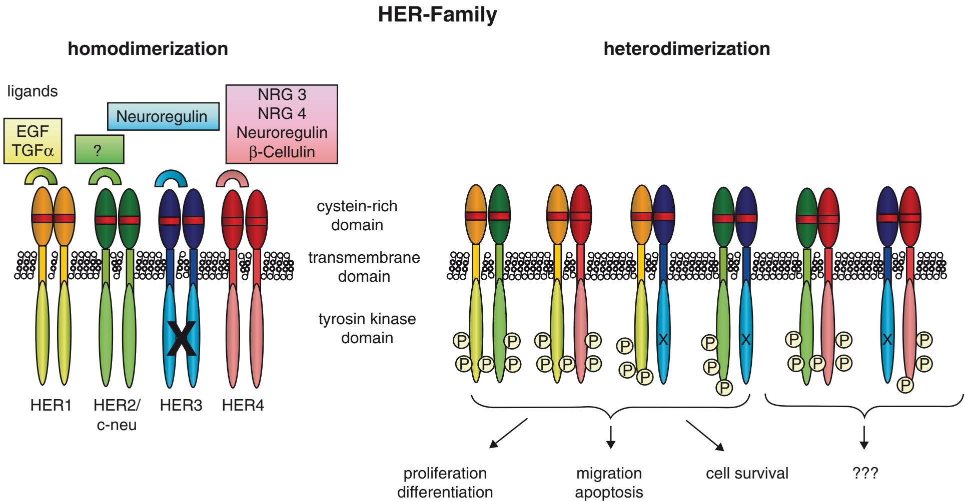

Fig. 4.1 Organizational principle of the epidermal growth factor receptor family and some dimerization possibilities with corresponding downstream biological events. The *left half* of the graph shows the names of the HER family members, depicted as homodimers. The *right half* of the graph shows heterodimers and downstream effects upon dimerization. *P* symbolizes phosphorylation. Ligands are shown as *semicircles* (name in *rectangles*) and in the color corresponding to the suitable receptor. Note, that HER2 does not have a known ligand; it presumably acts mostly as a combination partner for heterodimers. Also note, that HER3 homodimers lack tyrosine kinase activity (indicated by *X*), but upon ligand binding, the receptor can initiate signal transduction as heterodimer (mainly with the preferred dimerization partner HER2) through the other HER-family member's intracellular domain, resulting in multiple downstream effects influencing cell growth and survival

and signaling enzymes, which, upon binding, activate various downstream signaling pathways. These signaling events are linked to cell proliferation, survival, and apoptosis (Atalay et al. 2003; Danielsen and Maihle 2002; Stern 2000). The HER family of RTKs is comprised of four members: HER1 (=EGFR1 or ErbB1), HER2 (=HER2/c-neu or ErbB2), HER3 (=ErbB3), and HER4 (=ErbB4) (Citri and Yarden 2006; Yarden and Sliwkowski 2001). RTKs consist of an extracellular ligand-binding domain, a transmembrane domain, and an intracellular cytoplasmic domain containing the tyrosine kinase catalytic site. The receptors are not fixed in the lipid bilayer of the plasma membrane. Therefore, dimerization can and does occur upon ligand binding to the extracellular domain. Such dimers can be homodimers or heterodimers comprised of two different members of the same RTK family (Fig. 4.1) (Mendelsohn and Baselga 2003). When HER2 is overexpressed in cancers, it appears to be the preferred dimerization partner for all members of the HER-family (Graus-Porta et al. 1997). While homodimers are either inactive (like HER3 homodimers) or provide only weak signaling, HER2-containing heterodimers have attributes, which prolong and enhance downstream signaling (Sliwkowski 2003). Upon dimerization, the intracellular cytoplasmic tyrosine kinase is activated and autophosphorylated. The type of dimerization (homo- or heterodimerization) has an important impact on the downstream signaling pathways in terms of growth, proliferation and transformation (Olayioye et al. 2000; Prenzel et al. 2001).

4.1.2 Human Epidermal Growth Factor Receptors and Breast Cancer

Evidence from in vitro and in vivo studies pointed at the functional importance of the HER-family in a wide range of cancers (Gridelli et al. 2008; Kim et al. 2008; Klapper et al. 2000; Milanezi et al. 2008; Nahleh 2008; Rapidis et al. 2008). This prompted the development of agents that target these receptors, including monoclonal antibodies such as cetuximab and trastuzumab, or small molecule inhibitors of the RTK such as erlotinib and gefitinib (Cappuzzo et al. 2007; Lin and Winer 2004; Rivera et al. 2008; Toschi and Cappuzzo 2007; Valabrega et al. 2007). In breast cancer, the outcome of both early and advanced HER2-positive breast cancer patients could be substantially improved by the addition of trastuzumab, a monoclonal antibody binding to the extracellular domain of HER2 and therefore inhibiting heterodimerization of HER2 and subsequent activation of growth and survival signals in cancer cells. For instance, using trastuzumab in combination with chemotherapy in the adjuvant setting, relapse rates were substantially reduced and overall survival improved (Piccart-Gebhart et al. 2005; Romond et al. 2005). In view of the downstream signaling characteristics within the HER-family, it is reasonable to assume that agents inhibiting both HER1 and HER2 may result in more effective inhibition of cancer cell growth and survival. The rationale for development of such dual HER tyrosine kinase inhibitors (TKIs) such as lapatinib is based on several reasons. First, as opposed to a drug that targets only one member of the HER-family, simultaneous inhibition of HER1 and HER2 may overcome escape mechanisms mediated by redundancy in cell signaling pathways, a form of resistance observed in single tyrosine kinase inhibition, in which upregulation of other members of the HER-family occurs (Lin and Winer 2004; Milanezi et al. 2008). Second, synergistic inhibition of cancer cell growth has been demonstrated upon simultaneous targeting of HER1 and HER2, resulting in a more potent repression of cell growth or greater apoptotic effect compared with targeting either HER1 or HER2 alone (Burris 2004). Third, a dual HER1/HER2 tyrosine kinase inhibitor may be a useful substrate in a wider range of patients, in view of the impact of heterodimerization in the progression of a variety of cancer types and in addition to breast cancer (Kim et al. 2008; Nahta et al. 2006; Olayioye et al. 2000; Xia et al. 2004). Therefore, the dual HER-TKI lapatinib is expected to have superior activity compared with mono-target TKIs, and even though lapatinib has been primarily developed for and evaluated in breast cancer, its efficacy may reach well beyond this disease.

4.2 Structure and Mechanism of Action

Lapatinib ditosylate (GW572016, Tykerb®, Fig. 4.2) is an orally applicable, dual RTK inhibitor targeting two members of the HER-family receptors: HER1 (EGFR1/ErbB1) and HER2/c-neu (ErbB2) (Bilancia et al. 2007; Medina and Goodin 2008; Nelson and Dolder 2006).

Lapatinib acts intracellularly, interacting with the tyrosine kinase domain of the receptor (Fig. 4.3). There, it binds reversibly to the cytoplasmic ATP-binding site of the kinase domain, blocking phosphorylation and activation of the receptor (Moy and Goss 2006). This results in the inhibition of various downstream signaling cascades such as extracellular signal-related kinase1/2 (ERK1/2) and phosphatidylinositol 3'-kinase (PI3K)/AKT, involved in cell proliferation and survival (Burris 2004; Nahta et al. 2003; Okano et al. 2000) and apoptosis (Xia et al. 2005). The kinase inhibition is very effective with IC_{50} (50% inhibitory concentration) values of <0.2 μM. Consequently, application

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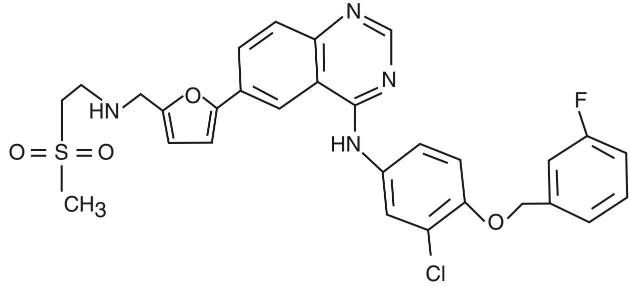

Fig. 4.2 Chemical structure of lapatinib. Lapatinib is a large 4-anilinoquinazoline derivative, distinguishing it from the small head group quinazolines tyrosine kinase inhibitors such as erlotinib and gefitinib

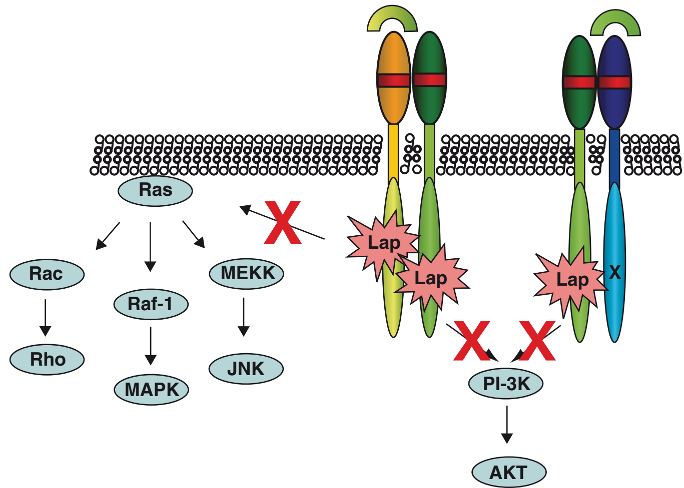

Fig. 4.3 Intracellular action of lapatinib. Lapatinib binds to the tyrosine kinase domain of HER1 and HER2, blocking the ATP-binding site and thus preventing the activation of downstream cascades. HER1 is depicted in *yellow*, HER2 in *green*, HER3 in *blue*. Ligands are shown as *semicircles*. *Lap* lapatinib; *JNK* Jun-N-terminal kinase; *MAPK* mitogen-activated protein kinase; *MEKK* MAPK/extracellular signal-related kinase (ERK); *PI3K* phosphatidylinositol-3-kinase

of the drug in vivo results in a strong inhibition of tumor growth in xenograft models (Rusnak et al. 2001; Xia et al. 2002; Zhou et al. 2006).

Lapatinib binds the *inactive* form of EGFR, and by doing so, it differs from other EGFR tyrosine kinase inhibitors such as erlotinib or gefitinib, which bind the *active* EGFR conformation. Lapatinib also has a slower dissociation rate compared with other TKIs. Both could contribute to a greater duration of effect at the target site (Wood et al. 2004). There are several theoretical advantages of small molecule inhibitors of both HER1 and HER2 compared with the monoclonal antibody cetuximab, which targets the extracellular domain of HER1, or trastuzumab, which targets the extracellular domain of HER2. One such advantage of the dual kinase inhibition activity of lapatinib is that it is active even in truncated mutated forms of the HER1- and HER2-receptors in tumors. While still exhibiting tyrosine kinase activity, these truncated forms lack the extracellular domain of the receptors and therefore cannot be bound by antibodies (Xia et al. 2005). In breast cancer cells expressing such receptor versions, truncated HER2, like the wild type HER2, preferentially

forms heterodimers with HER3. Such truncated HER2-positive cells can still signal (and often do so constitutively). They are necessarily resistant to the treatment with antibodies binding the extracellular HER2-domain (e.g., trastuzumab). However, truncated HER2 is still sensitive to the TKI lapatinib (Xia et al. 2004). Another unique feature of lapatinib over antibody-based anti-HER strategies is its biodistribution. Lapatinib is the first approved small molecule inhibitor with the ability to cross the blood–brain barrier (BBB), making it suitable for targeting brain metastases, a common feature in patients with breast cancer, particularly the ones expressing HER2 (Gril et al. 2008; Lin et al. 2008).

4.3 Clinical Data

4.3.1 Pharmacology

Lapatinib can be administered orally, but resorption rates vary, depending on food effects, which can increase bioavailability rates substantially (Rahman et al. 2007; Ratain and Cohen 2007; Singh and Malhotra 2004). In the blood, lapatinib is bound to 99% to proteins, mainly albumin and acidic alpha1 glycoprotein. Oral administration of lapatinib in healthy volunteers demonstrated the good tolerability of the drug (Bence et al. 2005). Commonly reported side effects included diarrhea, rush, nausea, vomiting, fatigue, and headache (see later). The peak plasma level of lapatinib occurs 3–6 h after administration, and the half life is approximately 17–24 h. Therefore, the drug is administered at a once daily schedule. The drug accumulates upon repetitive administration, and equilibrium plasma levels are reached after 6–7 days of administration. Lapatinib is eliminated by hepatic metabolism, primarily through cytochrome P450(CYP)3A4 and biliary excretion. Therefore, inducers or inhibitors of CYP3A4 may alter the metabolism of lapatinib, and *vice versa*, lapatinib may increase the level of other CYP3A4 substrates (e.g., benzodiazepines and calcium channel blockers) and also CYP2C8 substrates (e.g., amiodarone and pioglitazone) (Burris et al. 2005; Medina and Goodin 2008; Ulhoa-Cintra et al. 2008). Also, administration of the drug in patients with impaired liver function such as liver cirrhosis or diffuse hepatic metastases may be problematic, and if at all, has to be done with particular care and in a dose-reduced schedule, even though it has not been systemically investigated in this setting so far. The recommended daily dose of lapatinib is 1,250 mg as a single dose. To decrease variability in bioavailability, lapatinib intake is recommended no less than one hour before or at least one hour after food intake.

4.3.2 Results from Clinical Trials

4.3.2.1 Efficacy

As lapatinib has been approved solely for the use in breast cancer so far, this chapter is focused on this tumor entity only.

Several preclinical data provided the biological rationale to evaluate lapatinib in patients with HER2-positive breast cancer (Chu et al. 2005; Geyer et al. 2006a; Konecny et al. 2006; Medina and Goodin 2008; Montemurro et al. 2007; Nelson and Dolder 2006; Storniolo et al. 2008). A number of phase I–III clinical trials were conducted or are ongoing in healthy probands or patients with cancers of various origins and stages, evaluating lapatinib both as single agent and in combination with other therapeutics, including chemotherapy, hormone therapy, or monoclonal anti-HER2 antibodies. Several of these trials are listed in Table 4.1. Some exemplifying trials revealing the clinical efficacy of lapatinib are discussed in more detail below.

Table 4.1 Lapatinib in phase I–III clinical trails

Indication	Treatment	Patients (*n*)	Phase	Response (*n*)			References
				CR	PR	SD	
Phase I monotherapy							
Healthy volunteers	Lapatinib (10–175 mg) in single and multiple doses	43	I	NA	NA	NA	Bence et al. (2005); DeSimone et al. (2002)
Second line, solid tumors	Lapatinib (175–1,800 mg) once or twice daily	137	I	1	6	58	Burris (2004); Burris et al (2005); Dees et al (2004); Minami et al (2004); Pandite et al (2004); Spector et al (2005); Versola et al (2004)
Phase I combination therapy (second line)	Lapatinib (750–1,500 mg) **plus**[a]						
Various solid tumors	Capecitabine (1,500 mg/m^2)[b]	11	I	–	1	NR	DeBono et al. (2003)
Breast, ovarian, endometrial	Letrozole (2.5 mg/day)	36	I	–	1	4	Chu et al. (2005)
Various solid tumors	FOLFOX4 (−20% standard dose)	13	I	–	2/7	2/7	Lakhai et al. (2004)
Various solid tumors	Paclitaxel (135–225 mg/m^2)[c]	26	I	–	3	7	Jones et al. (2004)
Various solid tumors	FOLFIRI (60–80% standard dose)[d]	25	I	–	4	10	Midgley et al. (2005)
Metastatic breast cancer	Trastuzumab (2 mg/kg)[e]	48	I	1/27	5/27	10/27	Storniolo et al. (2005)
Pancreatic and biliary cancer	Gemcitabine (1 mg/m^2) or GEMOX (1 g/m^2)[f]	21	I	5/20			Safran et al. (2006)

Phase II and III						
Metastatic breast cancer	Lapatinib	II	12		24	Blackwell et al. (2005); Blackwell et al. (2004); Johnston (2005)
	Lapatinib vs. Capecitabine	III	1	37		Burstein et al. (2004)
	Lapatinib vs. Trastuzumab	III				Geyer et al. (2006b)
	Lapatinib in brain metastases	II		5		Geyer et al. (2006b); Stemmler et al. (2006)
First-line advanced breast cancer	Lapatinib mono ([e] vs. [f])	II	0	35	35	Gomez et al. (2005)
	Lapatinib vs. Paclitaxel	III	d.n.a.			Di Leo et al. (2008)
	Lapatinib vs. Letrozole	III	5		15	Safran et al. (2006)
IBC	Lapatinib in refractory IBC	II	0	70	38	Ingle et al. (2006)
Breast cancer adjuvant	TEACH	III	d.n.a.			Moy and Goss (2007b)

CR complete response; *PR* partial response; *SD* stable disease; *NA* not applicable, healthy volunteer; *NR* not reported; *d.n.a.* data not available; *IBC* inflammatory breast cancer; *TEACH* Tykerb® evaluation after chemotherapy

[a]Once daily

[b]Twice a day

[c]Every 3 weeks

[d]Every 14 days

[e]Loading dose followed by weekly infusions

[f]Weekly

[g]Response to lapatinib treatment

Phase I clinical trials suggested a favorable side effect profile of lapatinib, revealing good tolerability for the majority of patients treated ((Bence et al. 2005; Moy and Goss 2007a), see later for more details on tolerability). Phase II and III studies demonstrated that lapatinib has substantial clinical activity in HER2-positive breast cancer patients, but showed only moderate, if any, activity in cancers with predominant HER1 expression such as colorectal cancer, squamous cell cancer of the head and neck, lung or renal cancer (Montemurro et al. 2007; Ravaud et al. 2008; Reuter et al. 2007; Rocha-Lima et al. 2007).

Lapatinib is moderately effective in frontline therapy as a single agent in metastatic breast cancer (Gomez et al. 2008). Its major strength, however, is probably the combination with other cytotoxic agents. So far, the most important study in this regard has been the EGF100151 trial (Geyer et al. 2006a), forming the basis for approval of the drug (see later). This open label phase III trial included patients with advanced HER2-positive breast cancers, who had been treated before with antracyclines, taxanes, and the anti-HER2 antibody trastuzumab. Patients had to have measurable disease so as to evaluate tumor response following RECIST criteria. Patients were randomized to either receive capecitabine alone (201 patients) or a reduced dose of capecitabine and lapatinib 1,250 mg/day (198 patients). Time to disease progression (TTP) was the primary endpoint of this study. Secondary endpoints were overall survival, event-free survival, and overall response rate as well as safety and tolerability. A planned interims analysis (Geyer et al. 2006a) revealed 49 events in the lapatinib group vs. 72 events in the control group, resulting in a 51% risk reduction in time to progression. Based on these data, randomization within this trial was stopped, and patients in the control arm could also receive lapatinib in addition to capecitabine. A recent update analysis of the trial confirmed the positive results of the interims analysis (Cameron et al. 2008) with TTP improvement from 4.5 to 6.2 months upon addition of lapatinib to capecitabine. Also, there was a thus far statistically nonsignificant trend toward improved overall survival. Importantly, central nervous system (CNS) relapse rates were also significantly reduced in the lapatinib arm compared with the control arm (2% vs. 6%). This confirms the assumption of a high risk for CNS metastases in this group of patients and the ability of lapatinib to cross the BBB and therefore inhibit growth of micrometastases in CNS tissue before they can grow to a size upon which BBB function is disrupted.

Meanwhile, lapatinib in combination with chemotherapy has also been proven effective as first line treatment in HER2-positive metastatic breast cancer (Di Leo et al. 2008). In this large phase III trial, patients were included irrespective of HER2-expression. They were randomly assigned to receive either paclitaxel alone or in combination with lapatinib. While patients without HER2 overexpression did not benefit from the addition of lapatinib, HER2-positive patients had significantly improved response rates and event-free survival rates upon additional treatment with lapatinib (Di Leo et al. 2008).

As stated earlier, brain metastases are a major problem among patients treated with trastuzumab for metastatic HER2-positive breast cancer with incidence rates of 28–43%. The efficacy of lapatinib in breast cancer patients with brain metastases was specifically addressed in a phase II trial (EGF105084) (Lin et al. 2007). Patients included in this trial had brain metastases and had already received trastuzumab therapy as well as brain irradiation, i.e., the study addressed a prognostically particularly unfavorable group of patients. Tumor response (>50% size reduction) was the primary endpoint. Patients received lapatinib monotherapy. Approximately 8% (in a smaller series published recently by the same group: 3%,

(Lin et al. 2008)) achieved a partial response, and 16% (smaller series: 18%) achieved stable disease. These are admittedly moderate clinical benefit rates; however, they were achieved in a group of patients with very little treatment options and an extremely high risk of disease progression. To date, it is unknown, whether lapatinib can actually decrease the risk of *new formation* of brain metastases and therefore prolong survival. A phase III trial comparing lapatinib with trastuzumab treatment in this regard is currently being planned.

A pertinent question concerns the benefit of combining TKIs with monoclonal antibodies directed to HER-family members as compared with either therapy alone. A recent two-armed phase II trial included advanced HER2-positive breast cancer patients progressive after previous standard therapies with antracyclines, taxanes, and trastuzumab. Patients were treated either with a combination of trastuzumab and lapatinib or with lapatinib alone. Progression-free survival was 12 weeks in the combination treatment arm, while it was only 8 weeks in the lapatinib mono arm (O'Shaughnessy et al. 2008). Even though these data are very promising, a randomized phase III trial is needed to comprehensively evaluate the value of this drug combination vs. either therapy alone in advanced breast cancer.

In a very recent phase III trial (Johnston et al. 2008), lapatinib has been shown to be of benefit, when used as first-line treatment in advanced breast cancer. The trial included 1,286 postmenopausal women with hormone receptor-positive metastatic breast cancer, irrespective of HER2-expression status. Patients were treated with the aromatase inhibitor letrozole and were randomized to receive or not receive lapatinib in addition. The addition of lapatinib increased progression-free survival to 11.9 months, compared with 10.8 months for letrozole alone in the overall patient population. In HER2-positive patients, the combination of lapatinib plus letrozole increased progression-free survival even by 29% compared with letrozole alone. Also, the response rate upon the combination treatment was 28%, compared with 15% with letrozole alone. The clinical benefit rate was 48% compared with 29%. These data also confirm the importance of HER2-targeted treatment to overcome resistance to hormonal therapy, as previously suggested by a phase III trial (Mackey et al. 2006), which compared an aromatase inhibitor plus or minus trastuzumab and yielded comparable results. It remains as yet unclear, which HER-inhibiting combination partner for hormone therapy is better and whether a combination of antibody and RTK-inhibitor may have yielded even better results than either drug alone as an addition to hormone therapy.

Markers predicting response to targeted therapy will be one of the most important things to be addressed in future clinical trials. A recent study investigated lapatinib response in refractory patients with inflammatory breast cancer and its dependence on HER-expression to elucidate a molecular signature predictive of lapatinib sensitivity (Kaufman et al. 2008; Spector et al. 2006). This phase II trial (EGF103009) assigned patients to cohorts A (HER2-positive) or B (HER2-negative, but HER1-positive). Patients received lapatinib 1,500 mg once daily. In cohort A, 50% had clinical responses to lapatinib compared with 7% in cohort B. Within cohort A, phosphorylated HER3 and lack of p53 expression predicted for response to lapatinib. Tumors coexpressing phosphorylated HER2 and phosphorylated HER3 were more likely to respond to lapatinib (90% vs. 29%). Prior trastuzumab therapy and loss of phosphate and tensin homolog 10 (PTEN) did not correlate with response to lapatinib.

Based on the results of the pivotal EGF100151 trial, the drug was approved in 2007 by the FDA and in 2008 by the EMEA for its use in patients with advanced HER2-positive breast cancer after progression upon therapy with antracyclines, taxanes, and trastuzumab.

4.3.2.2 Tolerability

Diarrhea, hand–foot syndrome, nausea, vomiting, fatigue, and skin rashes were the most frequently reported adverse events in the EGF100151 trial (Cameron et al. 2008). Of these, diarrhea and skin rashes were more frequent in the lapatinib plus capecitabine group vs. the capecitabine group. These were mainly grade I toxicities. Toxicity-related interruption of therapy (43%), the need for dose reduction (42–43%), or complete stop of treatment due to intolerance (14%) was approximately equal in both treatment arms. Cardiac events were slightly more frequent in the lapatinib group (4 events) than in the control group (2 events), but were asymptomatic. Importantly, cardiac function as defined by left ventricular ejection fraction was observed at an equal rate (2.1%) in both treatment arms of the EGF100151 trial and was asymptomatic in >90% of cases. Cardiac failure is therefore considerably less prominent than upon treatment with trastuzumab, in which reduction in left ventricular output has been a significant concern, prevents simultaneous treatment with antracyclines and excludes patients with coexisting cardiac failure. Nevertheless, a routine evaluation of left ventricular output is usually recommended before initiating treatment with lapatinib. Additional reported, but very infrequent, adverse events observed upon treatment with lapatinib were hepatotoxicity and interstitial pneumonitis. Therefore, routine laboratory evaluation of liver function and clinical observation of pulmonary function are recommended before and during treatment with lapatinib. Altogether, however, life-threatening events (grade 4) or death (grade 5) attributable to lapatinib treatment seem to be very rare and most adverse effects are of grade 2 or 3 in severity (Moy and Goss 2006; Moy and Goss 2007a). Of note, lapatinib does not seem to add to the myelosuppressive adverse effects of chemotherapy.

4.4 Conclusion and Future Perspectives

Lapatinib has been a valuable advancement in the treatment of HER2-positive breast cancer. This group of cancer patients has a high risk of disease progression even upon treatment with conventional chemotherapeutic drugs but benefits enormously of the targeted treatment with trastuzumab. Failure to respond to trastuzumab-containing therapeutic regimens in advanced breast cancer has posed a serious therapeutic dilemma to both patients and clinicians, which can now be addressed by the treatment with lapatinib, since a phase III trial has provided clear evidence of its efficacy in this situation in combination with capecitabine. In particular, it may overcome the primary trastuzumab resistance associated with the inefficiency of antibodies in the treatment of cancers expressing truncated versions of HER2 and in the treatment of brain metastases.

Open questions regarding its future role in the treatment of HER2-positive cancers remain, however, and need to be addressed in current or future clinical trials. Such questions include:

1. What is the role of lapatinib in the adjuvant situation in combination with (which is currently being investigated) or as an alternative to trastuzumab in early breast cancer?
2. What is the role of lapatinib as a single agent in advanced breast cancer compared with combination therapy?
3. Since a recent trial has shown that trastuzumab may be effective in alternative combination regimens after disease progression upon previous trastuzumab treatment (Von Minckwitz et al. 2008), it is unclear whether trastuzumab may have been equally effective as lapatinib in clinical settings such as the ones in the EGF100151 trial. This is particularly important as lapatinib has theoretical advantages over trastuzumab, but also disadvantages (e.g., the lack of immune-mediated cytotoxicity).

4. What is the exact value of the combination of trastuzumab with lapatinib vs. either drug alone in advanced breast cancer?
5. Is lapatinib a better first-line-therapy drug in HER2-positive breast cancers than trastuzumab?
6. Are the drug combinations with capecitabine or paclitaxel ideal or are there superior combination partners with lapatinib?

Many of these questions will be addressed in ongoing or future clinical trials the results of which are eagerly awaited, but may not be available for quite some time. Until then, the combination of lapatinib with capecitabine in HER2-positive, trastuzumab-resistant breast cancer is the only approved application of this novel agent and as such is a valuable and well-tolerable treatment perspective for patients with this dismal disease.

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5 Sorafenib

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Abstract Sorafenib (BAY 43–9006, Nexavar®) is a novel oral kinase inhibitor that targets multiple tyrosine kinases in vivo and in vitro. Main targets are receptor tyrosine kinase pathways frequently deregulated in cancer such as the raf–ras pathway, vascular endothelial growth factor (VEGF) pathway, and FMS-like tyrosine kinase 3 (FLT3). Sorafenib was approved by the FDA in fast track for advanced renal cell cancer and hepatocellular cancer and shows good clinical activity in thyroid cancer. Multiple clinical trials are undertaken to further investigate the role of sorafenib alone or in combination for the treatment of various tumor entities.

5.1 Introduction

Cancer cells exhibit multiple changes in cell cycle control, apoptosis, proliferation, and invasion. In many cases, excessive growth factor signaling leads to increased proliferation of cells. Growth factor receptors (GFRs) function as cell surface receptors for circulating growth factors, cytokines, and hormones. A majority of these receptors possess unique tyrosine kinase domains, so-called receptor tyrosine kinases (RTKs). They consist of an extracellular ligand-binding domain and an intracellular catalytic domain. Activating mutations within the RTK domains important for signal transduction result in constitutive activation of downstream signaling pathways found in many cancers (McInnes and Sykes 1997). These pathways include Raf kinase, PDGF (platelet-derived growth factor), vascular endothelial growth factor receptor (VEGFR) 2 and 3 kinases, and c-Kit, the receptor for stem cell factor. More than 15 different classes of RTK have been identified to date (Fig. 5.1). They function during normal growth and development but are also closely connected to tumorigenesis. Protein kinases are constitutively activated in many molecular pathways that contribute to malignant transformation and growth factor independent growth. Thus, GFRs and their RTKs have become attractive targets for tumor therapy.

There are three general approaches to target RTKs: (1) to target the ligand before it binds to the receptor, (2) to target the extracellular domain of the receptors, and (3) to

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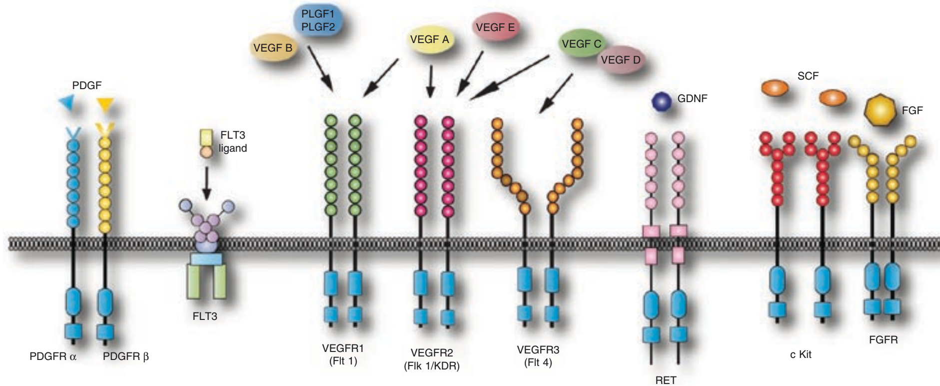

Fig. 5.1 Growth factor receptor and associated protein kinases targeted by sorafenib. *PDGFR* platelet-derived growth factor receptor; *FLT3* FMS-like tyrosine kinase 3; *VEGFR* vascular endothelial growth factor receptor; *PLGF* placental growth factor; *RET* rearranged during transfection; *GDNF* glial-derived neurotrophic factor; *SCF* stem cell factor; *FGFR* fibroblast growth factor. Modified from (Hicklin and Ellis 2005; Sebolt-Leopold and Herrera 2004; Stirewalt and Radich 2003)

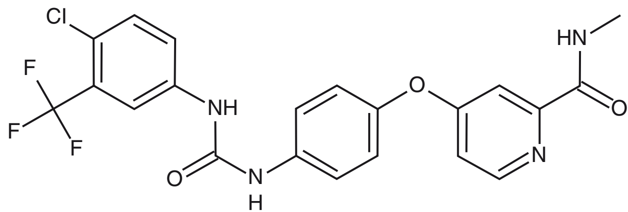

Fig. 5.2 Structure of sorafenib (4-[4-[[4-chloro-3-(trifluoromethyl)-phenyl]-carbamoyl-amino]-phenoxy]-*N*-methyl-pyridine-2-carboxamide) (Wishart et al. 2008)

target the intracellular RTK domain. While (1) and (2) can be achieved by antibody technologies or decoys, small molecule inhibitors, so-called receptor tyrosine kinase inhibitors (RTKIs), have been designed to target the intracellular RTK domains. The first RTKI approved for the treatment of chronic myelogenous leukemia (CML) was imatinib (STI-571, Glivec®) (Savage and Antman 2002). Sorafenib (BAY 43–9006, Nexavar®) is a small molecular inhibitor of multiple protein kinases. Sorafenib was approved by the US Food and Drug Administration (FDA) on December 20, 2005, for the treatment of advanced renal cell cancer (RCC) and shortly after receiving marketing authorization in the EU. (Wilhelm et al. 2006).

5.2 Structure and Mechanism of Action

Sorafenib (Fig. 5.2) is an inhibitor of multiple RTK, including vascular endothelial growth factor (VEGF) receptor 2, FMS-like tyrosine kinase 3 (FLT3), platelet derived growth factor (PDGF) receptor, and fibroblast growth factor receptor-1 (FGFR1). It was originally designed as inhibitor of the Raf kinases A-Raf, B-Raf, and C-Raf (Raf1) by chemical optimization (Wilhelm et al. 2006) and codeveloped by Bayer Pharmaceuticals and Onyx Pharmaceutical.

Raf kinases are the initial kinases in the Ras/Raf/MEK pathway/mitogen-activated protein kinase (MAPK) pathway (Friday and Adjei

2008) and are frequently deregulated in human cancers resulting in altered cellular growth and survival. Apart from Raf1 kinase, sorafenib effectively inhibits wild-type and oncogenic B-raf, VEGFR 1–3, PDGFR, FGFR1, c-kit, Flt-3, and RET (Carlomagno et al. 2006; Wilhelm et al. 2004) (Fig. 5.3). In contrast, sorafenib did not inhibit MEK1, ERK1, epithelial growth factor receptor 1 (EGFR1/HER1/ErbB-1), HER2/neu (ErbB-2), or insulin-like growth factor receptor 1 (IGFR1) (Wilhelm et al. 2004; 2006) in tissue culture experiments. These results could be confirmed in a series of murine xenograft tumor models, including ras-mutant tumors where sorafenib showed broad-spectrum antitumor activity in colon, breast, and non-small cell lung cancer (NSCLC), melanoma, thyroid cancer, hepatocellular cancer (HCC), and RCC and Flt-3 mutant leukemia (Auclair et al. 2007; Carter et al. 2007; Chang et al. 2007; Kim et al. 2007; Liu et al. 2006; Sharma et al. 2005; Smalley et al. 2009) (Table 5.1).

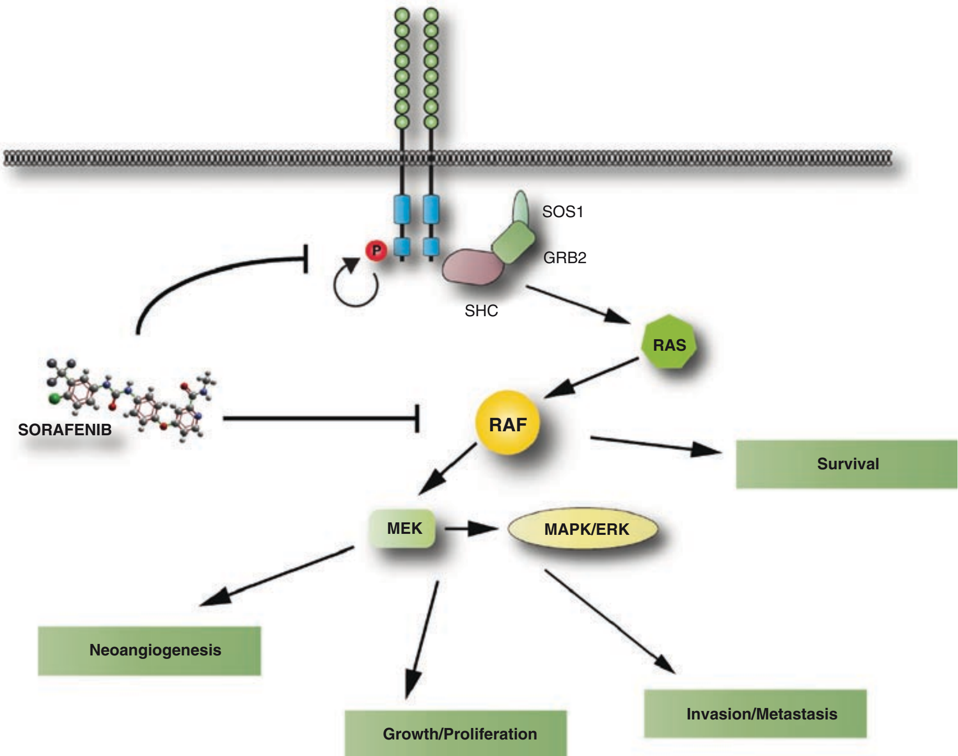

Fig. 5.3 Intracellular targets of sorafenib. Sorafenib blocks signal transduction and (auto)phosphorylation of receptor tyrosine kinases and inhibits raf activity. Inhibition of raf results in decreased tumor angiogenesis and proliferation, reduced invasiveness, and facilitates apoptosis. *ERK* extracellular signal-regulated kinase; *SOS1* son of sevenless homolog; *GRB2* growth factor receptor-bound protein 2; *SHC* Src homology 2 domain containing; *MEK* MAPK/ERK kinase; *MAPK/ERK* mitogen-activated protein kinase/extracellular signal-regulated kinases. Modified from (Wilhelm et al. 2006). Structure of sorafenib modified from DrugBank (Wishart et al. 2008)

Table 5.1 Xenograft tumor models

Tumor type	Reference
Colon cancer	Wilhelm et al. (2004)
Breast cancer	Wilhelm et al. (2004)
Non-small cell lung cancer	Carter et al. (2007); Wilhelm et al. (2004)
Renal cell cancer	Chang et al. (2007)
Thyroid cancer	Carlomagno et al. (2006); Kim et al. (2007); Salvatore et al. (2006)
Melanoma	Karasarides et al. (2004); Smalley et al. (2009)
Hepatocellular carcinoma	Liu et al. (2006); Wang et al. (2008)
Leukemia	Auclair et al. (2007); Zhang et al. (2008)

5.3 Clinical Data

5.3.1 Phase I

Starting from these interesting preclinical data, several phase I studies in patients with solid tumors alone or in combination with standard cytotoxic chemotherapy were launched (Awada et al. 2005; Hotte and Hirte 2002; Lyons et al. 2001; Richly et al. 2003, 2004; Strumberg et al. 2002). These trials proved the safety and efficacy of sorafenib in the treatment of solid tumors (Clark et al. 2005; Kupsch et al. 2005; Moore et al. 2005; Strumberg et al. 2005). Most frequent adverse events observed were hypertension, rash, diarrhea, hand–foot syndrome, and fatigue, whereas hematologic side effects were mild. Side effects improved promptly after sorafenib was stopped. From these trials, the standard dose of sorafenib was determined as 400 mg twice daily. Although resorption is influenced by concomitant food intake, bioavailability is not altered. Plasma half-life is approximately 36 h. Maximum plasma levels are reached 3 h after ingestion, and steady-state levels are reached after 1 week. 99.5% of sorafenib is protein-bound. It is hardly metabolized and excreted in feces (ca. 80%) and urine (ca. 20%). Metabolism of sorafenib is independent of age and gender. Plasma levels in patients with reduced liver function (Child-Pugh A and B) or reduced kidney function (creatinine clearance > 30 mL/min) do not differ from healthy subjects. There are no data about sorafenib in patients with severe liver disease (Child-Pugh C) or severely reduced kidney function (creatinine clearance < 30 mL/min) or patients on hemodialysis. Up to October 2008, 247 trials using sorafenib for the treatment of solid tumors and hematologic malignancies have been registered (Table 5.2) (http://www.cancer.gov/CLINICALTRIALS, http://www.cinicaltrials.gov). A majority of these are phase I and II trials. The greater part of phase II and III trials are in RCC, NSCLC, HCC, malignant melanoma, breast and ovarian cancer, prostate cancer, and head and neck cancer (HNC).

5.3.2 Sorafenib in the Treatment of Renal Cell Cancer (RCC)

Primary treatment of RCC is surgical. Once metastasized or inaberable, RCC had been regarded as chemoresistant. The introduction of TKIs improved the prognosis tremendously. RCC is characterized by dense vascularization, most likely due to upregulation of VEGF and VEGFR activity, resistance to conventional cytotoxic chemotherapy, and upregulation of Raf1 and EGFR (Oka et al. 1995). Thus, a drug targeting the VEGF pathway and the ras pathway such as sorafenib seemed perfect for the treatment of RCC. In phase I trials, some patients with RCC showed significant and sustained disease stabilization (Clark et al. 2005; Strumberg et al. 2002). Based on

Table 5.2 Registered clinical trials with sorafenib

Indication	Phase I	Phase I/II	Phase II	Phase III	Phase IV	Other
RCC	3	8	23	11	8	
NSCLC	1	4	15	3		1
SCLC	1		2			
Advanced cancer, lymphoma	22		1	1		
HCC	1	3	11	5		
Melanoma	1	2	12	3		
Breast cancer		3	11	1		1
Prostate cancer	2	1	8			
Brain tumors	2	3	4			
Head and neck cancer	1		4			
Ovarian cancer		1	7			
Pancreatic cancer	1	1	4	1		
Sarcoma	1	1	4			
Bladder cancer	1		4			
Gastric cancer	1	1	2			
Lymphoma		1	3			
AML/MDS	1	2				
Multiple myeloma		2	1			
Biliary tract cancer			2			
CML			2			
Colorectal cancer		1	1			
GIST			2			
MDS		2				
Neuroendocrine tumors		2				
Thyroid cancer			2			
AML			1			
Cervical cancer		1				
Childhood malignancies	1					
Esophageal cancer						1
Leukemia	1					
Mesothelioma			1			
SCLC	1					
Testicular cancer			1			

these observations, sorafenib was tested as monotherapy for RCC in a phase II randomized discontinuation trial (Ratain et al. 2006). Of 202 patients, 36% had an objective tumor response and 32% showed disease stabilization. Median progression-free survival was increased from 6 to 24 weeks ($p=0.0087$). These results were substantiated by the TARGET (Treatment Approaches in Renal Cancer Global Evaluation Trial) phase III trial (Escudier et al. 2007). Here, 903 patients with renal-cell carcinoma after treatment failure (mostly cytokine) were randomized to receive sorafenib (400 mg b.i.d.) or placebo. Primary study endpoint was overall survival. Because a planned interim analysis showed significant

progression-free survival in the sorafenib arm (5.5 months vs. 2.8 months, $p<0.01$), crossover was allowed. Patients receiving sorafenib experienced a 39% improvement in overall survival compared with placebo ($p<0.018$; hazard ratio 0.72), which did not reach significance (Escudier et al. 2007), and 10% had an objective tumor response. These results led to the approval of sorafenib for treatment of RCC. Currently sorafenib is tested in multiple trials in various combinations and indications for treatment of RCC (Table 5.2).

5.3.3 Sorafenib in the Treatment of Lung Cancer

In addition to 20 phase I and II trials, there are currently 3 phase III trials investigating the efficacy of sorafenib in combination with standard chemotherapy for advanced NSCLC (Table 5.2). In a phase II trial in 52 patients with advanced NSCLC treated with 400 mg sorafenib per day, no objective response was noted. Nevertheless, 59% patients achieved stable disease (Gatzemeier et al. 2006). Sorafenib is evaluated in phase III trials in combination with paclitaxel and carboplatin, and gemcitabine and cisplatin (www.clinicaltrials.gov, (Gutierrez and Giaccone 2008)). The first has been recently closed because of missing activity and a higher risk in patients with squamous cell carcinoma (Scagliotti et al. 2008); the latter is still accruing.

5.3.4 Sorafenib in the Treatment of Hepatocellular Cancer (HCC)

Like in RCC, preclinical and early clinical studies had suggested activity in RCC. In a phase II study in patients with chemo-naive advanced HCC were treated with 400 mg sorafenib b.i.d. Of 137 patients treated, 2.2% patients achieved an objective response, 39.4% had stable disease for at least 16 weeks. Median time to progression was 4.2 months, and median overall survival was 9.2 months. Side effects were generally tolerable and comparable with other trials (Abou-Alfa et al. 2006). This led to a multicenter randomized, double-blind, placebo-controlled phase III trial testing efficacy of 400 mg sorafenib b.i.d. in patients with advanced chemo-naive HCC (Llovet et al. 2008). Only patients with Child-Pugh Class A cirrhosis were included. The primary endpoint was overall survival, which showed an improvement from 7.9 to 10.7 months in patients receiving sorafenib compared with placebo (hazard ratio, 0.69; 95% CI, 0.55–0.87; $p=0.0001$). Likewise, time to radiologic progression increased from 2.8 to 5.5 months ($p<0.001$) (Llovet et al. 2008). Because of this trial, Sorafenib was approved by the FDA for the treatment of advanced HCC.

5.3.5 Sorafenib in the Treatment of Breast Cancer

In breast cancer, there are 16 phase I and phase II trials with sorafenib alone, in combination with antihormonal therapy or in combination with standard cytotoxic chemotherapy (Table 5.2). No results are available yet.

5.3.6 Sorafenib in the Treatment of Malignant Melanoma

B-raf is a target of sorafenib and has been identified as therapeutic target in melanoma (Karasarides et al. 2004). Unfortunately, sorafenib as single agent failed to show clinical efficacy in a phase II trial (Eisen et al. 2006). Nevertheless, when tested in combination with carboplatin and paclitaxel or dacarbacin objective responses were noted (Flaherty et al. 2008; McDermott et al. 2008). Currently

18 trials are investigating sorafenib in combination with chemotherapy in phase I and II trials (Table 5.2).

5.3.7 Sorafenib in the Treatment of Prostate Cancer

To date results from 4 phase II studies of sorafenib in hormone-independent prostate cancer have been published (Chi et al. 2008; Dahut et al. 2008; Safarinejad 2008; Steinbild et al. 2007). In summary, these studies failed to show convincing activity of sorafenib in prostate cancer. More phase I and II trials are investigating sorafenib alone or in combination with chemotherapy, but so far no results have become available yet.

5.3.8 Sorafenib in the Treatment of Head and Neck Cancer

Like in prostate cancer, single agent sorafenib reached only modest results in patients with squamous cell carcinoma of the head and neck (Elser et al. 2007). Thus, five trials are investigating sorafenib in combination with chemotherapy in this tumor entity.

5.3.9 Sorafenib in the Treatment of Ovarian Cancer

Dual inhibition of the VEGF pathway with sorafenib (200 mg b.i.d.) and bevacizumab (5 or 10 mg/m^2) showed promising tumor response in a phase I dose-escalation trial (Azad et al. 2008). Objective responses were seen in 6 (43%) of 13 patients with ovarian cancer. Unfortunately 74% required sorafenib dose reduction, indicating that the combination of sorafenib with bevacizumab might be too toxic for routine use. Other trials are addressing the question of the optimal combination partner for sorafenib in ovarian cancer (Table 5.2).

5.3.10 Sorafenib in the Treatment of Brain Tumors

Two case reports described good response of cerebral metastases of RCC (Ranze et al. 2007; Valcamonico et al. 2009), demonstrating efficacy of sorafenib in the brain. Accordingly, 10 phase I and II trials are investigating the efficacy of sorafenib alone or in combination for brain metastases and primary brain tumors (glioblastoma, gliosarcoma). No results have been presented yet.

5.3.11 Sorafenib in the Treatment of Thyroid Cancer

One open-label phase II trial of sorafenib in 30 patients with advanced thyroid carcinoma recorded 23% objective response lasting for 18–84 weeks and disease control in 53% of patients. Seventeen of 19 patients showed a rapid decrease in thyroglobulin levels. The median PFS was 79 weeks. Compared with standard chemotherapy patients with metastatic, iodine-refractory thyroid carcinoma seem to benefit tremendously from sorafenib (Gupta-Abramson et al. 2008). Four other phase II trials are currently recruiting.

5.3.12 Sorafenib in the Treatment of Hematologic Diseases

Mutations of the Fms-like tyrosine kinase 3 (FLT3) gene have been identified in approximately one third of acute myelogenous leukemia (AML) patients. FLT3-mutations are associated with a poor prognosis in these patients. In this setting, sorafenib might be a new compound to improve therapeutic options of FLT3 mutant AML. In a mouse model, sorafenib reduced the tumor burden of FLT3 mutated blasts, and a phase I study showed clinical activity of sorafenib in FLT3 mutant patients

5

(Zhang et al. 2008). A total of 13 phase I and II trials are using sorafenib in combination with other agents for treatment of leukemia and lymphomas (Table 5.2).

5.4 Conclusion and Future Perspectives

Identification of tumor-specific pathways led to the development of sorafenib as a rationally designed pathway-specific drug. Positive trials in RCC and HCC led to the rapid approval of sorafenib for the treatment of RCC and HCC by the FDA. Because sorafenib targets not only angiogenesis (VEGF pathway) but also tumorigenesis (raf, Flt-3, RET), it might have a much broader activity than currently known. Multiple clinical trials in various tumor entities are on their way and will hopefully show clinical benefit of sorafenib alone or in combination with standard chemotherapy or other targeted agents.

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Sunitinib

6

Daniel Y. C. Heng and Christian Kollmannsberger

Abstract Sunitinib is an oral multikinase inhibitor that blocks the vascular endothelial growth factor receptor (VEGFR), platelet-derived growth factor receptor (PDGFR) alpha and beta, c-kit, and other receptors. These attributes have proven to be efficacious in the treatment of metastatic renal cell carcinoma (RCC) and unresectable gastrointestinal stromal tumors (GIST). Most side effects, including hypertension, hand–foot syndrome, and diarrhea are generally well manageable. Clinical trials are underway to determine the efficacy of sunitinib in other tumor types including metastatic breast, colorectal, and lung cancers. This chapter will detail the preclinical data leading to the results of the pivotal phase III clinical trials that have led to the widespread use of sunitinib in metastatic RCC and advanced GIST.

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6.1 Introduction

Drugs that target the vascular endothelial growth factor (VEGF), platelet-derived growth factor (PDGF), and c-kit pathways have revolutionized the treatment of advanced renal cell carcinoma (RCC) and gastrointestinal stromal tumors (GIST). Sunitinib is an oral tyrosine kinase inhibitor that blocks the VEGF receptor (VEGFR), PDGF receptor (PDGFR) alpha and beta, and c-kit, amongst other targets (Christensen 2007). By interfering with these pathways, sunitinib is able to inhibit the downstream cellular signaling cascades that otherwise would have driven angiogenesis and cellular proliferation. This chapter will detail the development of sunitinib leading to the results of pivotal phase III clinical trials that have made it a standard of care in the treatment of metastatic RCC and advanced GIST.

6.2 Sunitinib

Sunitinib is an oral multikinase inhibitor that blocks VEGFR-1, VEGFR-2 (IC_{50} 4 nM), VEGFR-3, PDGFR alpha (IC_{50} 69 nM) and beta (IC_{50} 39 nM), c-kit (IC_{50} 1–10 nM), FLT-3 (IC_{50}

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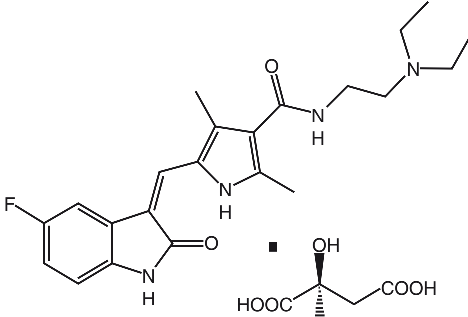

Fig. 6.1 Molecular structure of sunitinib malate

250 nM), RET (IC_{50} 50 nM), fibroblast growth factor receptor-1 (FGFR-1) (IC_{50} 880 nM), and colony stimulating factor 1 (CSF-1) (IC_{50} 50–100 nM) (Christensen 2007; Mendel et al. 2003; Chow and Eckhardt 2007). Note that IC_{50} values must be interpreted with caution, as there is a great variation among the laboratories using different cell lines, and in vitro activity may not reflect the same magnitude as in vivo activity. The molecular formula of sunitinib is $C_{22}H_{27}FN_4O_2$, and its structure is represented in Fig. 6.1 (Sun et al. 2003).

Sunitinib attaches to the adenosine triphosphate (ATP) binding pocket of these receptor tyrosine kinases. By acting as a competitive inhibitor of ATP, sunitinib prevents its activation and downstream cellular signaling (Christensen 2007). Receptor tyrosine kinases play an integral role in the signaling cascade of VEGF and PDGF. Each receptor has an extracellular domain that binds its respective ligand. The transmembrane region spans the membrane into the cytoplasm, and the intracellular domain holds the tyrosine kinase responsible for downstream signal activation. Upon ligand binding, the receptor tyrosine kinases dimerize or multimerize to induce a conformational change that allows ATP binding, resulting in autophosphorylation and transphosphorylation. These phosphorylated tyrosine domains are then able to activate downstream signal transduction by phosphorylation of various other proteins.

Mouse xenograft studies have demonstrated that sunitinib can cause tumor regression, growth arrest, and reduced tumor growth in a dose-dependent manner. Videomicroscopy has shown evidence of tumor vessel density reduction when compared with controls (Laird et al. 2000). These studies suggest that sunitinib has antiangiogenic properties that may explain, at least in part, its antitumor activity.

Phase I dose-finding studies were performed in healthy individuals and those with solid malignancies. A single dose of 50 mg of sunitinib given to healthy individuals was shown to be well tolerated and safe. The time to maximal concentration was 8 h, and the estimated half-life was 60 h (Houk et al. 2005).

Phase I repeat-dosing studies (Houk et al. 2005; Faivre et al. 2006) have been performed investigating the daily or every 2-day schedule of sunitinib administered in 3-week cycles (2 weeks on, 1 week off), 4-week cycles (2 weeks on, 2 weeks off), and 6-week cycles (4 weeks on, 2 weeks off). Daily dosing of 50 mg of sunitinib produced target plasma concentrations above the 50 ng/mL required to inhibit PDGFR and VEGFR. Plasma concentrations declined to predose levels during the 14-day rest period. Dose-limiting toxicities of fatigue, asthenia, and thrombocytopenia were observed, and thus, the final dose of 50 mg, 4 weeks on, and 2 weeks off was adopted as the standard for future clinical trials.

6.3 Renal Cell Carcinoma

6.3.1 Targets for Renal Cell Carcinoma

Metastatic RCC portends a poor prognosis and is estimated to have caused 13,010 deaths in the United States in 2008 (Jemal et al. 2008). Previously, immunotherapy agents, such as interleukin-2 and interferon (IFN) alpha, were the only treatments

available that demonstrated low response rates of approximately 15% (McDermott et al. 2005; Yang et al. 2003; Negrier et al. 1998, 2007; Coppin et al. 2005, 2008). Based on the increasing knowledge of the biology underlying RCC, agents targeting relevant biologic pathways have been investigated (Cohen and McGovern 2005). This initially developed from the understanding of patients with von Hippel Lindau (VHL) syndrome, which is an inherited, autosomal dominant genetic disorder that commonly manifests by the development of clear cell RCC in most of the affected patients.

Clear cell RCCs, which account for 85% of all RCCs, commonly demonstrate aberrations of the VHL gene (Kovacs et al. 1997; Rini and Small 2005) in both hereditary and nonhereditary forms. A single VHL allele deletion occurs in approximately 78.4–98% of sporadic tumors (Banks et al. 2006; Gnarra et al. 1994; Shuin et al. 1994; Kondo et al. 2002; Brauch et al. 2000; Kenck et al. 1996). For the remaining allele, VHL gene mutations are seen in 34–57%, while gene inactivation via hypermethylation of CpG-rich DNA islands occurs in about 5–20.4% of clear cell RCC (Banks et al. 2006; Kondo et al. 2002; Brauch et al. 2000; Clifford et al. 1998; Foster et al. 1994). Thus, it is clear that in both hereditary and sporadic cases of clear cell RCC, VHL abnormalities are a key factor in pathogenesis.

When the VHL gene is mutated or inactivated, the VHL gene product can no longer regulate the degradation of the hypoxia inducible factor (HIF) alpha, which is a transcription factor (Fig. 6.2). Normally, low oxygen conditions cause HIF alpha

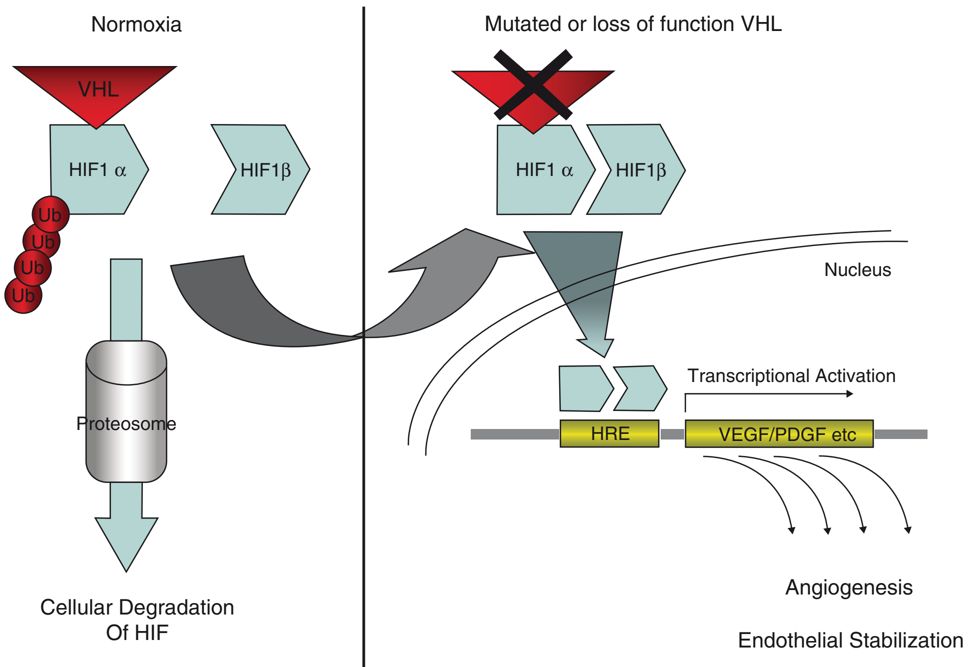

Fig. 6.2 Normal function of von Hippel Lindau (VHL) in the normoxic state when compared with the aberrant VHL state. Under normal conditions, VHL binds to HIFα and polyubiquinates it to mark it for destruction in the cellular proteosome. When VHL function is lost, HIFα binds to HIFβ and then translocates into the nucleus to activate HIF responsive elements (HRE). This results in transcriptional activation of genes important in angiogenesis and endothelial stabilization

to accumulate and bind to HIF beta, thereby creating a complex that transcriptionally activates genes related to glucose metabolism, apoptosis, angiogenesis, and endothelial stabilization. In patients with aberrant VHL, HIF alpha is not destroyed and thus, is left to freely accumulate without degradation even under normal oxygen conditions. There are over 100 HIF-responsive genes, including growth factors and their receptors, such as VEGF and PDGF (Cohen and McGovern 2005; Rini and Small 2005). These pathways are targeted by sunitinib and have been studied in phase II/III studies to determine the drug's efficacy.

6.3.2 Phase II/III Studies in Metastatic RCC

Two multicenter phase II trials treated cytokine-refractory metastatic clear cell RCC patients with sunitinib (Motzer et al. 2006a, b). They demonstrated that 34–40% of the patients receiving oral sunitinib at 50 mg daily for 4 weeks out of a 6-week cycle achieved a partial response, while 27–29% maintained stable disease according to the response evaluation criteria in solid tumors (RECIST) guidelines. The median time to progression in the combined analysis of these two studies was 8.2 months. These data led the Food and Drug Administration (FDA) to provisionally approve sunitinib in the treatment of advanced RCC pending confirmation in a randomized controlled trial.

The resulting pivotal phase III trial (Motzer et al. 2007) enrolled 750 patients to compare first-line sunitinib with first-line interferon. This demonstrated a statistically significant difference in progression-free survival (PFS) (11 vs. 5 months) with a hazard ratio of 0.42 ($p<0.001$). The overall survival data for sunitinib was presented recently showing an impressive difference when compared with interferon (26.4 vs. 21.8 months) (Figlin et al. 2008). Owing to the crossover of patients from the interferon group to sunitinib or another VEGF-targeted agent after preliminary results were presented, a dilution of the overall survival benefit may have occurred. It is important to note that this is the first randomized study in which the median overall survival of patients with metastatic RCC exceeded 2 years. Based on this data, this agent has become a standard of care for the first-line treatment of metastatic RCC.

It is important to note that the vast majority of patients enrolled in this trial (94%) had favorable or intermediate risk Memorial Sloan Kettering Cancer Center (MSKCC) prognostic criteria (Motzer et al. 2002), and only patients with clear cell histology were enrolled. Thus, the generalizability of this data to patients with poor prognostic profiles or nonclear cell histologies is uncertain. Retrospective studies and population-based experiences have demonstrated that sunitinib does indeed have activity in poor risk groups and nonclear cell histologies, although response rates and overall survival were shorter in these subgroups when compared with other patients with mRCC (Heng et al. 2009; Choueiri et al. 2008). As papillary (10–15%), chromophobe (5–10%), and collecting duct (<1%) histologies account for a minority of metastatic RCCs, clinical trials investigating sunitinib have excluded these patients with nonclear cell histologies (Heng and Bukowski 2007). A retrospective analysis of 20 patients with nonclear cell mRCC treated with sunitinib demonstrated a response rate of 16% and a PFS of 11.9 months (Choueiri et al. 2008). In an expanded access trial of sunitinib (Gore et al. 2007), 2341 patients were enrolled, among whom 87.8% had clear cell histology, 11.8% had nonclear cell histology, and 0.4% had missing data about their subtype. Of the 276 patients with nonclear cell histology, the overall response rate was 5.4, 41.6% had stable disease, and the PFS for this subgroup was 6.7 months. This was compared with the entire cohort of 2341 patients, in whom the overall response rate was 9.3, 43% had stable disease, and the PFS for the entire cohort was 8.9 months. Although the quality of data obtained from the expanded access trials may not

be as precise or accurate as randomized controlled trials, this study demonstrates that patients with nonclear cell histology appear to have a clinically meaningful response to sunitinib.

6.4 Gastrointestinal Stromal Tumors

6.4.1 Targets for Gastrointestinal Stromal Tumors

GIST are the most common mesenchymal malignancies of the gastrointestinal tract. GISTs are thought to arise from the interstitial cells of Cajal, or a common precursor, and are most commonly found in the stomach and small intestine, with metastases most commonly to the liver and peritoneum (Nowain et al. 2005). Although surgery remains the mainstay curative treatment for GIST, half of all the patients already have metastatic disease at the time of diagnosis. Additionally, 45–90% of the patients will relapse following even a complete resection (Dematteo et al. 2008). Approximately 85% of GISTs manifest a mutation in the KIT receptor tyrosine kinase: 67% in the intracellular domain in exon 11, 18% in the extramembrane domain in exon 9, and a smaller proportion in exons 13 and 17 (Corless et al. 2004; Heinrich et al. 2003a). The constitutive activation of this receptor affects the downstream cellular signaling cascades that promote cellular proliferation and prevent apoptosis. An additional 5–7% of patients with GIST have an activating mutation of PDGFR alpha (Corless et al. 2004; Heinrich et al. 2003a). Largely because of these two types of mutations, imatinib mesylate, which is both a c-kit and PDGFR alpha inhibitor, has become the first-line standard of care in patients with metastatic GIST, as demonstrated in a series of randomized trials (Verweij et al. 2004; Demetri et al. 2002; Blanke et al. 2008).

Imatinib was the first targeted therapy used in the treatment of metastatic GIST and one of the first to be used in any solid tumor. However, 12–14% of patients with GIST have primary resistance to imatinib, and 40% of patients who had initial responses to imatinib develop secondary resistance to the drug after a median of 18–26 months (Verweij et al. 2004). Secondary resistance may develop as a result of secondary mutations in the KIT or PDGFR-alpha kinases, gene amplification, or loss of target expression (Weisberg and Griffin 2003; Van Glabbeke et al. 2005). Thus, second-line therapy for metastatic GIST was greatly needed, and sunitinib has taken up this role.

6.4.2 GIST Clinical Trials

The efficacy and safety of sunitinib was initially evaluated in an open-label phase I/II study, where the optimum dosing was determined to be 50 mg daily for 4 weeks, followed by a 2-week break (6-week cycle). A total of 97 imatinib-resistant or intolerant patients were enrolled in this trial. Of these, 7% had RECIST-defined partial responses and 27% had stable disease for 6 months or longer. The time to tumor progression (TTP) was 34 weeks (Maki et al. 2005).

Subsequently, a phase III double-blinded randomized placebo-controlled trial was performed with a 2:1 randomization scheme. Three hundred and twelve patients with imatinib-refractory or resistant unresectable GIST were accrued (Demetri et al. 2006a). Eight percent of the patients exhibited a partial response in the sunitinib group vs. 0% in the placebo group. The median TTP was 27.3 weeks in those treated with sunitinib vs. 6.4 weeks in those treated with placebo (HR 0.33, $p<0.0001$). The hazard ratio for the overall survival was 0.49 in favor of the sunitinib group ($p>0.007$), though the median survivals had not yet been reached for this analysis. This pivotal study formed the basis for the FDA to approve sunitinib in patients with unresectable GIST with disease

progression or intolerance to imatinib (Goodman et al. 2007).

Upon the presentation of these results, patients in the trial were unblinded, so that all could receive open-label sunitinib (Demetri et al. 2006b). Despite this crossover, improvements in TTP were maintained (28.4 vs. 8.2 weeks, $p<0.0001$). The crossover also allowed for a comparison of overall survival in patients who were treated immediately with sunitinib vs. those who were initially treated with placebo and then crossed over to sunitinib (delayed administration). The overall survival of the delayed (24.3 weeks) vs. the immediate (28.9 weeks) administration groups was comparable. Although this result is interesting, one must be cautious in its interpretation because the delayed administration group is selected from among those patients who survived long enough to eventually cross over.

Response to sunitinib appears to be affected by pre-imatinib tumor genotypes. It appears that those patients with wild-type genotypes or a primary KIT exon 9 mutation have a significantly longer PFS and overall survival than those with exon 11 mutations (Heinrich et al. 2008). This is contrary to the pattern seen with imatinib, wherein exon 11 mutations are associated with greater response rates and improved survival when compared with wild-type or exon 9-mutated KIT (Heinrich et al. 2003b).

Investigations into the appropriate dosing schedule of sunitinib were made after anecdotal experience demonstrated tumor growth while on the 2-week sunitinib break (George et al. 2008). A phase II open-label, multicenter trial randomized 60 imatinib-refractory patients to either morning or evening sunitinib dosing at 37.5 mg daily continuously without the 2-week break. Twelve percent of the patients had a partial response and the median PFS was 32 weeks. Although cross-trial comparisons should be interpreted with caution, these outcomes appeared comparable with those seen in the aforementioned phase III trial of sunitinib dosed at 50 mg daily for 4 weeks, followed by a 2-week break (Demetri et al. 2006a). The continuous dosing trial included the analysis of VEGF, soluble (s) VEGFR-2, sVEGFR-3, and sKIT concentrations in these patients, which confirmed the persistent pharmacologic effect of sunitinib with continuous dosing. There was no rebound in these concentrations which are otherwise observed during off-treatment periods with continuous dosing. Finally, a decrease in the plasma sKIT after the first 3 cycles, and particularly after cycle 5 ($p>0.007$), was associated with a longer overall survival when compared with those without a decrease in plasma sKIT (George et al. 2008). These preliminary results will require further prospective evaluation as to whether continuous dosing is just as efficacious as intermittent dosing, and whether biomarkers such as sKIT can be used to predict prognosis or response.

6.4.3 Side Effects

Most of the side effects increase in intensity as the cycle progresses, but then begin to resolve during the 2-week break. Side effects can be managed with preventative and symptomatic measures, dose reductions, or delays. Patients usually start at a dose of 50 mg orally for 4 weeks and take a 2-week break. If toxicities become an issue, sunitinib can be dose-reduced to 37.5 mg for 4 weeks followed by a 2-week break. If another dose reduction is required, 25 mg for 4 weeks followed by a 2-week break can be considered. Recently published data suggest that a dose-response relationship exists for sunitinib, particularly in RCC (Houk et al. 2007). Therefore, grade 1/2 toxicities should be managed while dose reductions should be reserved for those patients with otherwise intolerable side effects.

The most common side effects of sunitinib include generalized fatigue and anorexia. It is important to rule out other underlying causes of these symptoms. More characteristic side effects include hand–foot syndrome, diarrhea,

Table 6.1 Precautions and side effects of sunitinib

Precautions	Common and major side effects
Caution in preexisting uncontrolled hypertension, left ventricular dysfunction, or arrhythmias	Fatigue Hand–foot syndrome Diarrhea
Avoid pregnancy and breastfeeding	Hypertension Mucositis/stomatitis Hypothyroidism Yellow discoloration of skin (not jaundice) Cardiotoxicity

hypertension, mucositis, and stomatitis (Table 6.1) (Kollmannsberger et al. 2007b).

The hand–foot syndrome is a blistering and potentially ulcerating condition of pressure points that can be quite painful. Patients should use moisturizing lotions for their hands and feet and avoid injuries or overuse which may exacerbate the pressure points. Handguards, tight jewelry and shoes, shaving of the blisters, and exposure to extremes of temperature should be avoided, as these may exacerbate the symptoms.

Diarrhea can be managed with agents, such as loperamide and diphenoxylate, and patients should refrain from taking laxatives. The diarrhea usually resolves once the 2-week break commences. Hypertension should be regularly monitored and treated with standard antihypertensives; however, those drugs that interact with CYP3A4 (see Drug Interactions) should be avoided. Mucositis and stomatitis can be treated with good oral hygiene, nonalcoholic mouthwashes (e.g., with baking soda), viscous lidocaine, nonperoxide toothpastes, and lip creams or balms.

Skin and hair manifestations are also common. A generalized yellow discoloration of the skin due to sunitinib and its active metabolite and can often be confused for jaundice. Occasionally, a maculopapular or seborrheic dermatitis-like rash can appear with sunitinib therapy. Additionally, depigmentation of the hair can occur 5–6 weeks into treatment. All these manifestations are reversible upon discontinuation of the drug.

Sunitinib-induced hypothyroidism is a phenomenon with greater incidence upon progressive cycles of sunitinib. Although the mechanism is not completely understood, there may be some similarities to propylthiouracil in the way it inhibits thyroid peroxidase (Wong et al. 2007). In preclinical models, thyroid capillary regression and an increase in thyroid-stimulating hormone (TSH) have been demonstrated. Patients on sunitinib therapy should have their TSH measured on days 1 and 28 of the first 4 cycles, and if found normal, it should be monitored every 2–3 months (Wolter et al. 2008). When patients develop hypothyroidism, hormone replacement should be initiated to treat the associated symptoms and to achieve biochemical normalization (Rini et al. 2007).

Cardiotoxicity was documented in 10% of the patients receiving sunitinib in the phase III study (Motzer et al. 2007). The majority of these patients were asymptomatic and it appears that the left ventricular dysfunction is reversible upon stopping the drug. In a retrospective analysis from a single institution, 48 patients treated with sunitinib were assessable (Witteles et al. 2008). Eighty-five percent had a diagnosis of RCC. Seven (14.6%) patients experienced symptomatic Grade 3/4 left ventricular dysfunction, 22–435 days after initiation of sunitinib. Three out of five patients with subsequent cardiac evaluations had persistent left ventricular dysfunction after discontinuation of sunitinib and initiation of standard heart failure therapy. The mean age of patients experiencing cardiotoxicity was 67 years. A history of congestive heart failure ($p>0.002$), coronary artery disease ($p>0.05$), and lower body mass index ($p>0.03$) were factors associated with increased risk. As more evidence is emerging on cardiotoxicity, clinicians should consider performing baseline cardiac imaging and monitor for symptoms of congestive heart failure in patients

with predisposing risk factors or a prior history of heart disease.

Macrocytosis has also been documented in patients receiving sunitinib. In a retrospective analysis, macrocytosis was observed to resolve when the drug was stopped for other reasons. In the limited number of bone marrow examinations in these patients, no evidence of metastases was found. Although the long-term clinical implications are unknown, macrocytosis does not appear to cause any deleterious effects (Rini et al. 2008). Other well-recognized hematologic side effects include thrombocytopenia and neutropenia, which occur in approximately 20% of patients, requiring dose delays or reductions (Motzer et al. 2006a, b; 2007).

6.4.4 Drug Interactions

It is important to note that the active metabolite of sunitinib, SU012662, is produced by the cytochrome P450-3A4 system (CYP3A4). Drugs that are inhibitors of the CYP3A4 system such as ketoconazole, aprepitant, diltiazem, fluoxetine, glyburide, grapefruit juice, propranolol, and verapamil could reduce the amount of active metabolite produced. CPY3A4 inducer drugs such as rifampin, carbamazepine, phenytoin, St.John's wort, and troglitazone could potentially increase the active metabolite and associated side effects. Thus, concurrent administration of sunitinib with these drugs, especially those requiring a narrow therapeutic window, should be avoided (Kollmannsberger et al. 2007b). When given together, close observation of toxicity and response with appropriate dose adjustments is mandatory.

6.4.5 Activity in Other Tumor Sites and Ongoing Research

Preliminary evidence has suggested promising activity of sunitinib in other tumor groups. Dose-finding studies of sunitinib, in addition to standard chemotherapy and preliminary efficacy analyses, have also been reported in prostate, bladder, testicular, and colorectal cancers amongst other solid malignancies. Interim results of a phase II study of sunitinib in previously treated patients with recurrent ovarian, fallopian tube, or primary peritoneal carcinomas have exhibited two partial responses and 10 patients with stable disease out of the 17 patients enrolled (Biagi et al. 2008). In a phase II trial of 64 patients with refractory, metastatic breast cancer, sunitinib treatment resulted in an 11% objective response rate, mostly seen in patients with triple negative (ER-, PR-, HER2-) breast cancers or HER2 positive cancers previously treated with trastuzumab (Burstein et al. 2008). These results have led to the opening of a phase III trial comparing sunitinib with the standard of care in patients with advanced triple negative breast cancers. In patients with stage IIIB/IV non small cell lung cancer previously treated with 1–2 chemotherapy regimens, 47 patients were treated on a 37.5 mg/day continuous dosing schedule. One patient (2%) had a confirmed partial response, and 8 (17%) had stable disease lasting longer than 3 months, with an overall median PFS of 12.1 weeks (Brahmer et al. 2007).

Currently, large phase III randomized trials are underway comparing sunitinib with standard of care therapy in metastatic breast, lung, and colorectal cancers (SUN trials). In two separate trials for advanced breast cancer, the addition of sunitinib to either docetaxel or capecitabine is being compared with docetaxel or capecitabine alone, respectively. The combination of sunitinib with paclitaxel is being compared with bevacizumab (another VEGF inhibitor) plus paclitaxel in patients with metastatic breast cancer. The interim results of some of these trials will be available in 2010. In advanced lung cancer, a second-line standard of care, erlotinib, is being compared with erlotinib plus sunitinib. In metastatic colorectal cancer, the standard of care FOLFOX

(5-FU, leucovorin, oxaliplatin) plus bevacizumab is being compared with FOLFOX plus sunitinib.

In RCC, sunitinib is being investigated in the adjuvant setting where high-risk localized disease has been resected. The Adjuvant Sorafenib or Sunitinib for Unfavorable Renal Carcinoma intergroup trial randomizes high-risk nephrectomized patients to 1 year of sorafenib, sunitinib, or placebo.

Studies combining the targeted therapies are being performed with the known caveat that combination therapies are associated with high financial cost and possibly increased toxicity. A phase I trial of bevacizumab and sunitinib in a variety of solid tumors led by the Cleveland Clinic reported one unconfirmed partial response in a patient with papillary RCC out of nine evaluable patients (Cooney et al. 2007). Another phase I trial of this combination given exclusively to patients with metastatic RCC reported 4/13 patients with partial responses (Feldman et al. 2007). Using a different combination, a randomized phase II trial studying bevacizumab and erlotinib (an inhibitor of the epidermal growth factor receptor (EGFR) pathway) vs. bevacizumab and placebo revealed no benefit to the combination in terms of overall response rate or PFS (Bukowski et al. 2007). Currently, combinations of targeted therapy still remain experimental,and they should only be employed in the context of a clinical trial.

6.5 Conclusion

Sunitinib and other VEGF-targeted therapies have revolutionized the treatment of advanced RCC and unresectable GIST. Through the identification of relevant pathways associated with tumor growth and angiogenesis, we now have effective tools to treat these patients more effectively. Sunitinib has become a first-line standard of care for patients with metastatic RCC and it is a standard of care for patients with GIST after progressing on or being intolerant to imatinib. Research is underway to determine if sunitinib will have efficacy in other tumor types.

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Dasatinib 7

Markus Lindauer and Andreas Hochhaus

Abstract Dasatinib, (former BMS 354825), is an orally available small-molecule multikinase inhibitor. It potently inhibits BCR-ABL and SRC-family kinases (SRC, LCK, YES, FYN), but also c-KIT, PDGFR-α and β, and ephrin receptor kinase.

Dasatinib is about 300 times more potent than imatinib in cells expressing unmutated BCR-ABL in vitro. The drug has demonstrated activity against clinically relevant mutations, including those associated with poor prognosis during ongoing imatinib therapy.

Dasatinib is approved for the treatment of patients with BCR-ABL-positive chronic myeloid leukemia (CML), resistant or intolerant to imatinib in chronic, accelerated, and blast phase. It also is approved for the treatment of Philadelphia Chromosome positive (Ph^+) acute lymphoblastic leukemia (ALL) resistant or intolerant to imatinib.

A single daily dose of 100 mg in chronic phase CML results in high hematologic and molecular remission rates and prolongation of survival. In accelerated and blastic phase as well as in ALL, 70 mg twice daily is recommended. Complete hematologic and cytogenetic remissions (CR) frequently occur even in this patient group with poor prognosis. Remissions however are very short.

Side effects of dasatinib are frequent but mostly moderate and manageable and include cytopenias and pleural effusions. The role of dasatinib in other diseases, including solid tumors, has to be identified.

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7.1 Introduction

CML is a clonal disease of the hematopoietic stem cell. It has been considered as a model for other cancers based on its multistep evolution with three stages, its association with a defined cytogenetic translocation t(9;22)(q34; q11), the elucidation of molecular pathogenesis, and the successful development of a molecular therapy.

The Philadelphia chromosome (Ph) was recognized as the first constitutive chromosomal abnormality in cancer in CML in 1960 (Nowell and Hungerford 1960). Subsequent studies revealed that Ph originates from a t(9;22)(q34;q11) translocation. The translocation involves the ABL-tyrosine kinase on chromosome 9 and a gene on chromosome 22 named breakpoint cluster region (BCR). Transcription of the fusion gene results in

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the chimeric protein BCR-ABL, which is a constitutively active form of the c-ABL tyrosine kinase.

BCR-ABL plays a key role in the pathogenesis of CML and in the 15–30% of acute lymphoblastic leukemias (ALL), which are Ph^{+} (Faderl et al. 2000). The pathogenetic relevance of BCR-ABL has been proven by its capacity to transform hematologic cells in vitro and in vivo (Daley and van Etten 1990; Druker et al. 1996). However, expression of BCR-ABL is not sufficient to produce CML in humans, and additional changes are necessary. This is supported by the observations of clonal Ph-negative precursors of CML (Copland et al. 2006); moreover, with very sensitive PCR-methods, BCR-ABL can be found in about 30% of healthy adults (Bose et al. 1998).

The estimated incidence of CML is approximately one case per year in 100,000 people. The clinical course of the disease is characterized by a chronic phase, which eventually develops into an accelerated phase and subsequently into fatal blast crisis. Median survival of untreated patients is approximately 3–5 years (Hochhaus 2007).

Treatment was palliative during the first century of CML, and included splenic irradiation, oral cytotoxic drugs busulfan and hydroxyurea, and interferon alpha (IFNα). Allografting, introduced in the 1970s, despite its risks for mortality and morbidity has been the initial treatment of choice for younger patients, as it is the only proven, potentially curative treatment. However, because of donor and demographic characteristics, it is an option only for about 40% of the patients (Gratwohl et al. 1998). IFN induces major cytogenetic responses (MCyR) in 6–19% of patients with chronic phase CML (Hehlmann et al. 1994). This rate increases with the addition of cytarabine (Guilhot et al. 1997), and complete cytogenetic responders have longer remission duration and survival compared with nonresponders (The Italian Cooperative Study Group on ChronicMyeloid Leukemia 1998).

With the introduction of the first ABL tyrosine kinase inhibitor, imatinib (formerly STI571), in 2001, the therapy of CML has changed dramatically. Response rates in imatinib treated patients are high in chronic phase disease with complete cytogenetic response rates (CCyR) of up to 87% as reported by the International Randomized Study of Interferon and STI571 (IRIS) (Druker et al. 2006). In accelerated or blast phase, however, only 21 and 7% achieved MCyR, respectively, and the responses were only transient (Giles et al. 2008).

Relapse occurs annually in about 4% of the patients in chronic phase treated with imatinib and is more frequent in advanced phases of the disease. Criteria for suboptimal response and failure to imatinib have been defined, depending on treatment duration (Baccarani et al. 2006).

In the pivotal IRIS reporting imatinib treatment of CML with a follow-up of 5 years, failure to achieve a CCyR at 12 months was 31%, and at 5 years was 13% (Druker et al. 2006). Unsatisfactory therapeutic effect was the most common reason to discontinue treatment in this study. In accelerated phase and blast crisis, rates of resistance to imatinib and disease relapse were even higher (Giles et al. 2008).

Mechanisms of resistance to imatinib can be divided into BCR-ABL-dependent and BCR-ABL-independent mechanisms (Ritchie and Nichols 2006). Most common are kinase domain mutations in the BCR-ABL gene that either impair the ability of the kinase to adopt the inactive conformation to which imatinib binds, or directly interfere with imatinib-binding to the BCR-ABL protein, which account for 50–90% of all cases.

More than 60 different BCR-ABL mutations have been identified, conferring clinical resistance to imatinib. Occasionally, resistance is caused by overexpression of BCR-ABL due to BCR-ABL-gene amplification (Shah 2005; Apperley 2007).

BCR-ABL-independent mechanisms include clonal evolution and activation of alternative signaling pathways, like the SRC-pathway. Rarely, resistance to imatinib may occur if the intracellular concentration of the drug is too

low. Reasons could be an increased efflux of the drug, mediated by multi drug resistance gene 1 (MDR1), but also diminished influx caused by interactions with the organic cation transporter 1 (hOCT1) (Hochhaus et al. 2007a).

Strategies to overcome Imatinib failure include application of higher doses of the drug, but also the administration of structurally different BCR-ABL tyrosine kinase inhibitors such as Dasatinib (Sprycel™) or Nilotinib (Tasigna™). Other tyrosine kinase inhibitors in earlier stages of clinical development include Bosutinib (SKI-606), a SRC-ABL, but not PDGFR or c-KIT inhibitor and INNO-406, a LYN-ABL inhibitor (Jabbour et al. 2007).

Dasatinib is approved for patients with all phases of CML and also patients with Ph^+ ALL, who are intolerant or resistant to Imatinib. It induces marked remissions even in pretreated patients.

Dasatinib is a thiazole-carboxamide compound that is structurally unrelated to Imatinib. As an ATP-competitive small-molecule multikinase inhibitor, it inhibits BCR-ABL, but also other kinases including the SRC family of kinases (SFK). When compared with imatinib, dasatinib exhibits increased potency to inhibit BCR-ABL, but with reduced selectivity. Dasatinib binds to both active and inactive conformations of BCR-ABL with less stringent conformational requirements than Imatinib.

The drug has demonstrated activity against clinically relevant mutations, including those associated with poor prognosis during ongoing imatinib therapy. However, it is not active against the T315I BCR-ABL mutation. Inhibition of the SFK allows dasatinib to overcome resistance conferred by SFK activation.

Inhibition of SRC is an attractive target in oncology, as elevated levels and activities of Src and SFK have been shown in numerous human cancer cell lines and tumor tissues. Dasatinib as an inhibitor of SRC has been shown to inhibit growth, migration, cell adhesion, and induces cell cycle arrest in many different cell lines. There are clinical studies ongoing on the effect of dasatinib in solid tumors and Ph-negative myeloproliferative disorders.

7.2 Structure and Mechanism of Action

Dasatinib, (former BMS 354825), or N-(2-chloro-6-methyl-phenyl)-2-(6-(4-(2-hydroxyethyl)-piperazin-1-yl)-2-methylpyrimidin-4-ylamino)thiazole-5-carboxamide monohydrate ($C_{22}H_{26}ClN_7O_2S$) is an orally available small-molecule multitargeted kinase inhibitor (Fig. 7.1). It potently inhibits BCR-ABL and SRC-family kinases (SRC, LCK, YES, FYN), as well as c-KIT, PDGFR-α and β, and ephrin receptor kinase at nanomolar concentrations (Lombardo et al. 2004; Huang et al. 2007). In addition, it has activity against most imatinib resistant isoforms of BCR-ABL. Dasatinib has been shown to block G1/S transition and inhibit cell growth in normal and neoplastic cells (Fabarius et al. 2008).

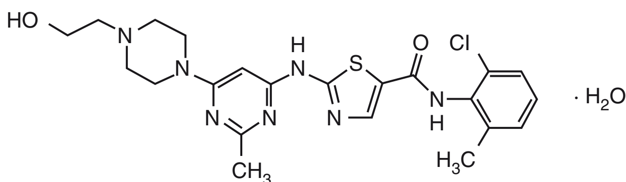

Fig. 7.1 Dasatinib

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7.2.1 Inhibition of ABL

Dasatinib was designed as an ATP-competitive inhibitor of SRC and ABL. Abelson kinase (ABL) is the constitutively active tyrosine-kinase of the BCR-ABL fusion protein. It is a cytoplasmic nonreceptor tyrosine kinase. Human c-ABL has a number of structural domains critical for its activity. The major isoform of c-ABL has three SRC-homology (SH) domains. SH1 domain contains the tyrosine kinase activity, while SH2 and SH3 domains allow interaction with other proteins. Under normal conditions, the activity of the ABL tyrosine kinase is tightly regulated.

Like many tyrosine kinases, ABL regulates its catalytic activity *via* conformational changes, switching between active and inactive forms by opening and closing an activation loop. The sequence available for binding in the inactive conformation varies dramatically between different kinases and provides a potential for binding specificity.

As demonstrated by X-ray crystallography, Dasatinib binds the active conformation of the kinase. The compound has been shown to bind the ATP-binding site of the SH domain 1 of ABL (Lombardo et al. 2004; Tokarski et al. 2006). In addition, molecular docking studies (Gambacorti-Passerini et al. 2005) showed that dasatinib is likely to bind to the inactive form of ABL as well, requiring a lower conformational stringency than imatinib, which selectively targets only an inactive conformation of the ABL-kinase domain (Fig. 7.2).

Dasatinib has been shown to be 325-fold more potent than Imatinib for inhibiting unmutated BCR-ABL. The concentration required for 50% inhibition [IC_{50}] is 0.6 nmol/L for dasatinib and 280 nmol/L for imatinib (O'Hare et al. 2005). It is suggested that the stronger binding activity of dasatinib over imatinib is at least partially due to its ability to bind to active and inactive conformations of the ABL protein.

Owing to the different structural requirements for binding of dasatinib when compared

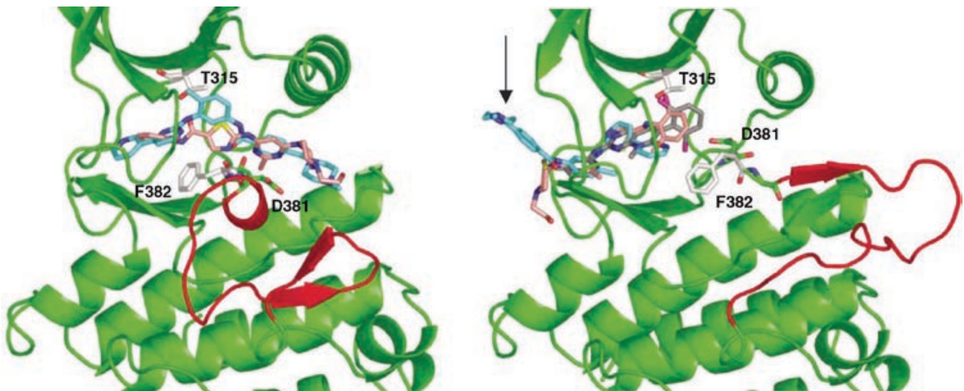

Fig. 7.2 Binding of dasatinib (*pink*) and imatinib (*cyan*) to the ATP-binding site of the ABL kinase domain of BCR-ABL (*green*). On the *left*, the inactive conformation is shown with the activation loop (*red*) in the closed position with the experimentally determined orientation of imatinib (*cyan*) and the docked orientation of dasatinib. On the *right*, the active conformation is shown with the docked orientations of imatinib and dasatinib together with the experimentally determined orientation of PD173955, a further inhibitor of ABL. The *arrow* indicates the moiety of imatinib failing to interact with the protein in the active conformation (adapted from Gambacorti-Passerini et al. 2005, with kind permission)

with imatinib, the drug inhibits various imatinib-resistant BCR-ABL mutations, except the T315I type (O'Hare et al. 2005; Shah et al. 2004). This is the basis for the activity of the drug in imatinib resistant disease, caused by mutated BCR-ABL.

7.2.2 Inhibition of SRC

SRC is a member of a nine-gene family that includes YES, FYN, LYN, LCK, HCK, FGR, BLK, and YRK. SRC family kinases (SFK) consist of a unique NH2-terminal region, two SRC homology domains (SH2 and SH3), a highly conserved kinase domain, and a COOH-terminal tail containing a negative regulatory tyrosine residue. SRC and SFK cooperate in several cellular processes including migration, adhesion, invasion, angiogenesis, proliferation, differentiation, and immune function. They play a major role in the development, growth, progression, and metastasis of a wide variety of human cancers (Kopetz et al. 2007).

Elevated levels of SRC kinase activity and/or protein expression levels have been found in a variety of human epithelial cancers, including colon, breast, pancreatic and lung carcinomas, brain tumors, as well as in osteosarcomas and Ewing sarcomas. The levels of expression or activation generally correlate with disease progression.

Dasatinib inhibits SRC with an IC_{50} of 0.5 nmol/L (Lombardo et al. 2004). Inhibition of SRC activation by dasatinib can suppress tumor growth in human breast cancer cell lines, human prostate cancer cells, head and neck, lung cancer, and osteosarcoma cell lines (Johnson et al. 2005; Finn et al. 2007; Shor et al. 2007). However, preclinical studies suggest that dasatinib induces apoptosis in only a small subset of cell lines. Inhibition of migration, invasion, and cell adhesion by dasatinib is reported more frequently (Johnson et al. 2005; Nam et al. 2005; Serrels et al. 2006). In a nude mouse model of prostate cancer, tumor growth and the development of lymph node metastasis were inhibited by dasatinib (Park et al. 2008).

Therefore, from in vitro and animal data, as single agent, dasatinib can be predicted to have only modest effect in most tumors. Recent phase I and phase II trials on dasatinib in several solid tumors confirm this prediction (Johnson et al. 2007; Evans et al. 2005; Yu et al. 2008).

7.2.3 Inhibition of c-KIT

KIT (CD117) is a receptor tyrosine kinase. Normally, KIT is activated when bound to its ligand, the stem cell factor (SCF). Gain of function mutations of c-KIT plays a crucial role in several malignancies, including gastrointestinal stromal tumors (GIST) (Hirota et al. 1998), systemic mastocytosis (SM), acute myeloid leukemia (AML), lymphomas, and germ cell tumors (Schittenhelm et al. 2006). Imatinib, which is a potent inhibitor of c-KIT has become the treatment of choice for advanced GIST. Comparable with its binding properties to ABL, imatinib only binds to the inactive conformation of c-KIT.

Imatinib-resistant c-KIT mutants are frequent and often occur in the activation loop of c-KIT, resulting in a constitutive active conformation of c-KIT, to which imatinib cannot bind.

These mutations have relevance in mast cell disorders, seminoma, and AML, and are always resistant to imatinib. Dasatinib inhibits c-KIT 10–20-fold stronger than imatinib with an IC_{50} for inhibition of autophosphorylation and cellular proliferation of 5–10 nmol/L (Schittenhelm et al. 2006). In addition, dasatinib is a potent inhibitor of many clinically relevant mutated forms of c-KIT, including imatinib-resistant KIT activation loop mutations in vitro (Shah et al. 2006). Despite these promising in vitro data, dasatinib failed to induce significant remission rates in a phase II study in patients with SM (Verstovsek et al. 2008).

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7.2.4 Inhibition of Platelet-Derived Growth Factor Receptor (PDGFR)-α and β Tyrosine Kinases

PDGFR-α and β are receptor tyrosine kinases. They are activated by binding of platelet-derived growth factor (PDGF). PDGF-signaling has a significant role in the formation of connective tissue and is also important during wound healing in the adults. PDGFR-α and β are expressed mainly on fibroblasts and smooth muscle cells (Heldin and Westermark 1999).

PDGFR-α tyrosine kinase activating mutations have been described in the pathogenesis of some GIST tumors (Heinrich et al. 2003). Fusion proteins consisting of the fibroblast growth factor receptor 1 (FGFR1) and PDGFR-α and β receptor tyrosine kinases have constitutive transforming activity. They are found in a subgroup of myeloproliferative disorders associated with eosinophilia (Cross and Reiter 2008).

In addition, PDGFR-β is involved in the formation of restenosis following coronar angioplastie, and, in combination with ABL, in the pathogenesis of dermal fibrosis. Dasatinib, which inhibits PDGFR-β with an IC_{50} of 28 nmol/L, is active in in vitro models for these diseases (Chen et al. 2006; Akhmetshina et al. 2008).

7.2.5 Inhibition of Ephrin Receptor Tyrosine Kinases

The Ephrin family of receptor tyrosine kinases constitutes the largest subfamily of receptor tyrosine kinases. They are divided into two subclasses (Ephrin A and Ephrin B) based on sequence similarity and their preferential binding to ligands, which are tethered to the cell surface either by a glycosylphosphatidylinositol-anchor (Ephrin A) or by a single transmembrane domain (Ephrin B) (Kullander and Klein 2002). Eph receptor tyrosine kinases have important functions in development and diseases. In tumorigenesis, they have been implicated in cellular transformation, metastasis, and angiogenesis. EphA2 is frequently overexpressed and functionally altered in many invasive cancers including metastatic melanoma, as well as cancers of the mammary gland, cervix, ovary, prostate, colon, lung, kidney, esophagus, and pancreas.

Dasatinib was shown to be a potent inhibitor of ephrin A2 receptor kinase with an IC_{50} of 17 nmol/L in various cell lines (Huang et al. 2007; Chang et al. 2008).

7.2.6 Additional Effects

It has been demonstrated that dasatinib induces defects in spindle generation, cell cycle arrest, and centrosome alterations in leukemic cells, tumor cell lines, as well as normal cells. These effects are not attributable to the inhibition of a single kinase; rather it is the expression of non specific effects on multiple kinases (Fabarius et al. 2008).

7.3 Clinical Data

7.3.1 Pharmacokinetic Profile

Dasatinib is administered orally. The drug is rapidly absorbed, and peak plasma concentrations occur 0.5–3 h after administration. The intake of food is not relevant for pharmacokinetics of dasatinib. In a dose range of 25–120 mg twice daily, the area under the plasma concentration-time curve (AUC) increased proportionally. The drug is extensively metabolized in the liver, predominantly by cytochrome P 450 (CYP) 3A4, only 30% remain unchanged. The metabolites of the compound are unlikely to play a

pharmacologic role. There are linear elimination characteristics over the abovementioned dose range with a terminal elimination half life of 5–6 h.

Elimination occurs mostly in the feces (85%) and only little in urine (4%). Dasatinib is excreted as metabolites, only 19% of a dose was recovered as unchanged drug in the feces (Bristol-Myers Squibb 2007).

Dasatinib is a substrate and an inhibitor of CYP3A4. Therefore, there is a potential for interaction with other concomitantly administered drugs that are metabolized primarily by or modulate the activity of CYP3A4.

Systemic exposure to dasatinib is increased if it is co-administered with drugs that are inhibitors of CYP3A4 (e.g., clarythromycin, erythromycin, itraconazole, ketoconazole).

If co-administered with drugs that induce CYP3A4 (e.g., dexamethasone, phenytoin, carbamazepine, rifampicin, Phenobarbital or *Hypericum perforatum*, also known as St. John's Wort), dasatinib AUC is reduced. It was reduced by 82% when co-administered with rifampicin.

Dasatinib AUC was reduced when co-administered with H2-blockers/proton-pump inhibitors or antacids. Concomitant administration of famotidin reduced dasatinib AUC by 61%, while co-administration of aluminium hydroxide reduced dasatinib AUC by 55% (Bristol-Myers Squibb 2007).

7.3.2 Clinical Studies with Dasatinib in CML and Other Diseases

Most clinical data have been reported on the treatment of patients with all phases of CML and Ph^{+} ALL. There are few reports on the properties of the drug outside CML, in Ph-negative myeloproliferative disorders, in GIST-tumors, in hormone refractory prostate cancer, and in various other solid tumors.

7.3.3 CML and Ph^{+} ALL – Overview

The clinical efficacy of dasatinib in CML patients resistant or intolerant to imatinib was assessed in a phase I trial (Talpaz et al. 2006). Five phase II trials, termed START (**S**RC-ABL **T**yrosine kinase inhibition **A**ctivity **R**esearch **T**rials), were consecutively performed in all phases of CML in patients resistant or intolerant to imatinib (Kantarjian et al. 2007; Hochhaus et al. 2007 b; Ottmann et al. 2007; Guilhot et al. 2007a; Guilhot et al. 2007b; Cortes et al. 2007). Dose optimization Phase III trails have been performed in chronic phase CML (Shah et al. 2008) and in advanced phases of the disease (Pasquini et al. 2007). First-line treatment of CML patients with dasatinib has been assessed in an ongoing Phase II trial (Quintas-Cardama et al. 2007b; Borthakur et al. 2008).

7.3.3.1 Phase I Dose Escalation Study

Patients with chronic-phase CML, accelerated- and blast-phase CML, and also patients with Ph^{+} ALL were enrolled in this study (CA180002). The study population consisted of 84 patients, 72 of them resistant to imatinib. They were treated with dasatinib, ranging from 15 to 240 mg/day. The drug was administered with different treatment schedules, from once daily in a weekly schedule for 5 days on, 2 days off, up to continuous administration of the drug twice daily (Talpaz et al. 2006). Hematologic remissions (HR) and cytogenetic remissions (CR) were seen in all phases of CML and in patients with Ph^{+} ALL. Remissions were also seen with all BCR-ABL mutations, except T315I. On the basis of pharmacokinetic and pharmacodynamic results, a twice-daily dosing schedule was chosen for subsequent dasatinib clinical trials.

7.3.3.2
Phase II: Chronic Phase CML (START C)

The START C trial (CA 180013), an international multicenter trial, investigated the efficacy and safety of dasatinib in chronic phase CML with imatinib resistance or intolerance. A median follow-up of 8.3 months has been published for 186 patients (Hochhaus et al. 2007b); this review includes all 387 patients with a follow-up of 15.2 months (Hochhaus et al. 2008a).

Median time from diagnosis was 61 months. Previous treatment included IFN (65%), stem cell transplantation (10%), prior imatinib of >600 mg/day (55%), and /or imatinib for >3 years (53%). BCR-ABL mutations were present at baseline in 40% of the patients. Of the 387 patients treated, 91% achieved or maintained a complete hematologic remission (CHR). Major and complete cytogenetic responses were observed in 59 and 49% of the treated patients. Response rates were higher in patients intolerant to imatinib when compared with those with imatinib resistance. Cytogenetic response rates were achieved irrespective of the presence of BCR-ABL mutations. Median progression-free survival and overall survival were not reached (Table 7.1).

Grade 3/4 cytopenias were observed mostly in the initial phase of therapy, and included leukocytopenia (27%), neutropenia (49%), and thrombocytopenia (48%). Nonhematological toxicities were generally mild to moderate. Grade 3 pleural effusions occurred in 6% of patients.

7.3.3.3
Accelerated Phase CML (START A)

Patients with accelerated phase CML, resistant or intolerant to imatinib were enrolled into a multicenter study (START A, CA 180005). Results of 107 patients with a minimum follow-up of 8 months have been published (Guilhot et al. 2007a); this review focuses on data with a median follow-up of 14.1 months for the full cohort of 174 patients (Guilhot et al. 2007b). The median time from diagnosis was 82 months, previous treatment consisted of IFN (72%), stem cell transplantation (13%), imatinib of >600 mg/day (52%), and/or imatinib for >3 years (59%). Durable hematological and CR were observed in a high proportion of patients. Response rates were achieved irrespective of prior allogeneic hematologic stem cell transplantation and presence of BCR-ABL mutations at baseline. Estimated 2-year progression-free survival was 46% and estimated 2-year overall survival was 72% (Table 7.1). Grade 3/4 cytopenias were common (leukocytopenia 58%, neutropenia 75%, thrombocytopenia 81%). Grade 3/4 pleural effusions occurred in 5% of patients.

7.3.3.4
Myeloid Blast Phase CML (START B) and Lymphatic Blast Phase CML (START L)

The START B and START L studies were multicenter single arm phase II trials with identical designs, enrolling patients with myeloid blast crisis (START-B) and patients with lymphoid blast crisis (START L) resistant or intolerant to imatinib (Cortes et al. 2007; Cortes et al. 2008; Gambacorti et al. 2007). The majority of patients (90%) were imatinib resistant. Median time from diagnosis of CML was 44 months.

In the START B trial (CA 180006), 109 patients were treated with a follow-up extending 20 months. Previous treatment included stem cell transplantation (14%), imatinib of >600 mg/day (50%), and/or imatinib for >3 years (41%). A complete hematologic response was achieved in 27% of patients; MCyR occurred in 34%, with a complete cytogenetic response in 26%, irrespective of baseline BCR-ABL mutation status (Table 7.1). Median duration of CHR and MCyR had not been reached. Median progression-free survival was 6.7 months and overall survival was 11.8 months. Grade 3/4 toxicities were again common, mainly

Table 7.1 Efficacy of dasatinib in phase II single-arm clinical studies in CML and Ph^+ ALL (START-trials)

Trial	Disease/phase	*n*	Follow up (months)	Mutated BCR-ABL	Response (% of patients)				Survival (% of patients)	
				% of patients	MHR	CHR	MCyR	CCyR	2 year PFS	2 year OS
Start C	Chronic phase	387	15.2	40	–	91	59 ImI 80 ImR 52	49 ImI 75 ImR 40	80	94
Start A	Accelerated phase	174	14.1		85	45	40	33	46	72
Start B	Myeloid blast phase	109	>12	42	34	27	34	26	20	38
Start L	Lymphoid blast phase	49	>12	65	35	29	52	46	5	26
Start L	Ph^+ ALL	46	>12	78	41	35	57	54	12	31

CHR complete hematologic response: normal white blood cell count, platelets <450,000/μL, no blasts or promyelocytes in peripheral blood, <5% myelocytes, and metamyelocytes in peripheral blood, normal basophil count, no extramedullary involvement

Advanced diseases: *MHR* major hematologic response: CHR or neutrophil count between 500 and 1,000/μL, and/or platelets between 20,000 and 100,000/μL

MCyR Major cytogenetic response: ≤35% Ph^+-cells in metaphase in bone marrow

CCyR complete cytogenetic response: 0% Ph^+-cells in metaphase in bone marrow

PFS progression free survival; *OS* overall survival; *ImI* imatinib intolerant patients

ImR Imatinib resistant patients

hematologic (leukocytopenia 61%, neutropenia 80%, thrombocytopenia 89%, anemia 75%). Pleural effusions CTC grade 3/4 occurred in 15% of patients (Cortes et al. 2008).

The START L study (CA 180015) included 48 patients with lymphoid blast phase CML. Previous treatment included stem cell transplantation (31%), imatinib of >600 mg/day (52%), and/or imatinib for >3 years (23%). After a 20-month extended follow-up, 29% of patients had a CHR and 52% had a MCyR (Table 7.1). Remissions were short with a median duration of 4.9 months for HR and 5.6 months for MCyR. Higher response rates were attained in patients with unmutated BCR-ABL (50% CCyR) when compared with those with any mutation (27% CCyR). Median progression-free survival was 3.0 months and overall survival was 5.3 months. Grade 3/4 hematologic toxicities were frequent (leukocytopenia 71%, neutropenia 81%, thrombocytopenia 88%, anemia 50%). Pleural effusions occurred in 6% of patients (Cortes et al. 2008).

7.3.3.5 Philadelphia Chromosome Positive Acute Lymphoblastic Leukemia (ALL)

In the START-L trial (CA 180015), a cohort of 46 adult patients with ph^+ ALL were treated with dasatinib. Follow-up data at 8 months in 36 patients have been published (Ottmann et al. 2007), an abstract is available with follow-up data of 12 months in the full cohort of 46 patients (Porkka et al. 2007). Prior treatment included stem cell transplantation (37%), imatinib of >600 mg/day (46%), or imatinib for >12 months (52%). Median time from diagnosis of Ph^+ ALL was 18 months. BCR-ABL mutations were reported in 78% of patients.

Complete hematologic responses occurred in 35% of patients. A MCyR and CCyR was achieved in 57 and 54% of the patients, respectively (Table 7.1). Responses were similar in patients irrespective of baseline BCR-ABL mutation status. The median duration of CCyR was 6.9 months. A median overall survival was 8 months.

Most common was hematologic toxicity with grade 3/4 cytopenias occurring in 78% of the patients. Grade 3/4 diarrhea was reported in 9% of patients, pleural effusion in 7%, and pyrexia in 2%. Dasatinib dose had to be reduced in 30% of patients; treatment was interrupted in 43% of patients, primarily attributable to nonhematologic toxicities.

7.3.3.6 Central Nervous System Disease of Ph^+ Blast Phase CML or Ph^+ ALL

A recent report has shown substantial activity of dasatinib in patients with Ph^+ ALL or blast phase CML and central nervous system (CNS) involvement. Eleven adult and pediatric patients were treated with dasatinib as first-line treatment for CNS leukemia, whereas three patients experienced a CNS relapse while on dasatinib therapy for other reasons. All of the 11 patients responded, with seven complete responders, four after dasatinib monotherapy. Three patients achieved a partial response. Responses were generally durable, and response durations of more than 26 months have been reported (Porkka et al. 2008).

7.3.3.7 Randomized Comparison of Dasatinib vs. High-Dose Imatinib (START R)

An international, randomized, open-label, phase II trial (START R) investigated the efficacy and safety of dasatinib of 70 mg b.i.d. (n>101) vs. high-dose imatinib of 800 mg (n>49) in patients with imatinib-resistant chronic-phase CML (Kantarjian et al. 2007). Cross over was permitted after 3 months of therapy. Data at a median follow-up of 15 months are available in 150 patients.

Median time from diagnosis of CML was 59 months. All patients had been treated with imatinib, 67% of patients had been treated with doses of >400 mg/day, and 40% of patients had received the drug for 3 years or longer. Further pretreatment included hydroxyurea (95%), IFN (71%), and stem cell transplantation (6%). At baseline, BCR-ABL mutations were present in 45% of patients randomized to receive dasatinib, and in 22% of those in the high-dose imatinib arm.

After 12 weeks, a cytogenetic evaluation was performed and patients either continued therapy or crossed over if there was progression, lack of MCyR, or intolerance to the randomized therapy. After 12 weeks, a higher proportion of patients on dasatinib had already achieved a CHR (93 vs. 82%, $p=0.034$), MCyR (36 vs. 29%, $p>0.4$), and CCyR (22 vs. 8%, $p=0.041$). At a median follow-up of 15 months, major (52 vs. 33%, $p<0.023$) and complete (40 vs. 16%, $p<0.004$) cytogenetic responses were significantly better with dasatinib, when compared to high-dose imatinib (Kantarjian et al. 2007). In addition, in all the subgroups of prognostic interest, namely, prior treatment with 600 mg of imatinib daily, no prior CyR with imatinib, and mutated BCR-ABL, MCyRs and CCyRs were consistently higher with respect to dasatinib (Table 7.2).

Dasatinib was superior to high-dose imatinib in terms of progression-free survival ($p<0.001$). Grade 3/4 cytopenias were frequent and more common in the dasatinib treatment group (neutropenia 61 vs. 39%, thrombocytopenia 56 vs. 14%) Pleural effusions were reported only with dasatinib (16 vs. 0%).

7.3.3.8 Dasatinib in Previously Untreated Chronic Myelogenous Leukemia Patients

Patients with previously untreated chronic-phase CML were randomized in an ongoing phase II trial to either dasatinib of 100 mg once daily or 50 mg twice daily (Quintas-Cardama et al. 2007b; Borthakur et al. 2008). Out of the 40 patients enrolled, 21 patients were randomized to receive dasatinib once daily, and 19 to the twice daily arm. Dose escalations to 140 or 180 mg/day for poor response, and dose reductions to 80 or 40 mg/day for toxicity were allowed. After 12 months, all evaluable patients (100%) had achieved a CCyR (26 out of 26) and eight of the 25 evaluable patients (32%) had a major molecular response. According to the study investigators, these data are significantly better than historical data from a similar group of patients treated in studies at the MD Anderson Cancer Center with imatinib of 400 or 800 mg/day.

Transient Grade 3–4 thrombocytopenia was reported in 10%, neutropenia in 5%, and anemia in 3% of patients. The most common nonhematological adverse events of Grade 3/4 included fatigue (5%), headache (3%), and rash (3%). Pleural effusions, all Grade 1/2, occurred in five patients (13%). Treatment interruption and dose reduction was required in 18 patients (46%).

7.3.3.9 Dose Optimization Trial in Chronic Phase CML

Randomized dose optimization phase III noninferiority trials were performed comparing once daily vs. twice daily administration of dasatinib. Patients with chronic-phase CML, resistant or intolerant to imatinib were enrolled into an open label phase III trial (CA 180034) (Shah et al. 2008). A total of 670 patients were randomized 1:1:1:1 to receive dasatinib of 100 or 140 mg once daily, and 50 or 70 mg twice daily. Approximately 41% of the patients had received imatinib for >3 years, 34% had received high-dose imatinib of >600 mg/day, 30% had mutated BCR-ABL, 74% were considered imatinib resistant, and 26% imatinib intolerant. After a median duration of treatment of 8 months, response rates were similar across groups (CHR 86–92%, MCyR 55–59%, CCyR 41–45%). The

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Table 7.2 Comparison of dasatinib with high-dose imatinib: results of a randomized phase II trial

	Dasatinib ($n=101$)	High-dose imatinib ($n=49$)
Mutated BCR-ABL (% patients)	45	22
Response after 15 months (% patients)		
CHR[a]	93	82
MCyR[a]	52	33
CCyR[a]	40	16
TTF (months)	n.r.	3.5
Treatment characteristics		
Median duration (months)	13.7	3.1
Discontinued (% of patients)		
Reason for discontinuation	28	81
No response	5	61
Intolerance	16	18

Patients with disease progression, lack of major cytogenetic response at 12 weeks of treatment or intolerance (≥Grade 3 nonhematological toxicity) or hematologic toxicity requiring multiple dose reductions were permitted to cross over to the other treatment.
TTF time to treatment failure; *n.r.* not reached
[a]$p<0.05$

100 mg once-daily arm was associated with a reduced incidence of Grade 3/4 thrombocytopenia and pleural effusions, when compared with the other three arms combined. In addition, there were fewer interruptions or reductions, and fewer patients discontinued treatment due to drug-related toxicity in the 100 mg, once-daily arm. The results from this study were the basis for the new starting dose regimen of 100 mg once daily in patients with chronic-phase CML resistant or intolerant to prior therapy including imatinib. The advantage of a once-daily schedule is most probably based on a reduction of off-target inhibition associated with the short half-life of dasatinib in vivo.

7.3.3.10 Dose Optimization Phase III Trial in Advanced Phase CML

In a second, open label, prospective trial (CA 180035), patients with advanced phase CML or Ph$^+$ ALL resistant or intolerant to imatinib were randomized to receive dasatinib of 140 mg once daily vs. 70 mg twice daily (Pasquini et al. 2007). Dose escalation was allowed for inadequate response, and dose reductions for toxicity. In total, 611 patients were treated and had a median follow-up of roughly 6 months. More than one-third of these patients had previously received imatinib therapy for more than 3 years, 43% had received high-dose imatinib, and 78% were imatinib resistant. Response rates were similar between the once-daily and twice-daily schedules. In the accelerated phase and myeloid blast phase subgroups, the duration of response was similar between the dosing schedules, whereas in the lymphoid blast phase and Ph$^+$ ALL subgroups, the duration of response was improved in the 70 mg twice-daily arm. With once-daily dasatinib administration, the duration of major hematological response was 10.2 months, whereas with twice-daily administration, it was 12.2 months. Similarly, progression-free survival was improved in the twice-daily group (7.9 vs. 11.7 months), and twice as many

patients in the once-daily schedule relapsed after achieving a hematological response as in the twice-daily regimen (30 vs. 16%). There was a trend for improved tolerability with the 140 mg of once-daily schedule. The recommended starting dose for advanced phase CML or Ph^+ ALL remains 70 mg twice daily.

7.3.4 Dasatinib and Other Diseases

Up to now, there is no approved indication for dasatinib outside Ph^+ CML and Ph^+ ALL. There are ongoing studies in several different tumors and leukemias.

7.3.4.1 Phase I Study in GIST

A phase I clinical trial assessed the effect of dasatinib in GIST and in other solid tumors (Evans et al. 2005). The study included 14 patients, nine with treatment-resistant GIST and five with other refractory solid tumors. Dasatinib was administered at dose levels of 35, 50, and 70 mg twice daily, 5 days on, 2 day off in a weekly schedule. Toxicity included one CTC Grade 3 lymphopenia, one CTC Grade 3 anorexia, and elevation of alkaline phosphatase Grade 3. Of note, no clinical significant hematologic toxicity was reported. CT scans were performed every 8 weeks, and no objective response was reported in CT scans. The complete resolution of ascites in one patient with GIST was reported, with some mixed responses in FDG PET-CT.

7.3.4.2 Phase I Study in Solid Tumors

This study enrolled 26 patients, 14 with epithelial tumors, 12 with other solid tumors. Patients were treated with dasatinib for 7 days per week at three dose levels: 90 mg twice daily, 140 mg once daily, and 180 mg once daily. Six patients had stable disease with continued treatment for 2–10 months. Toxicity included pleural effusions in the 180 mg cohort (Johnson et al. 2007).

7.3.4.3 Phase II Study of Dasatinib in Philadelphia Chromosome Negative Acute and Chronic Myeloid Diseases, Including Systemic Mastocytosis

The study included a total number of 67 patients, with various hematologic disorders including 33 patients with SM, nine patients with AML, six patients with myelodysplastic syndromes, five patients with hypereosinophilic syndrome (HES), three patients with chronic eosinophilic leukemia (CEL), and 11 patients with primary myelofibrosis (PMF) (Verstovsek et al. 2008).

Most patients with SM presented with the D816V c-KIT mutation, which confers imatinib resistance. As dasatinib has been shown to be active against the c-KIT D816V mutation in vitro, activity of the drug in SM was expected. The D816V was present in 28 of the 33 patients with SM. Patients were treated with dasatinib with different doses and schedules. In SM patients, an overall response rate of 33% was reported, mostly symptomatic improvements including two complete responses, none of them with the D816V.

Complete remissions were also reported for a patient with HES, and one patient with AML. The patient with HES had a complex karyotype with an aberrant signaling *via* PDGFR-β. The patient with AML was c-KIT-positive.

The authors concluded that it is questionable, whether the use of dasatinib provides any advantage over other treatment options in SM and that dasatinib therapy does not seem to have significant activity in patients with AML, MDS, PMF, and HES/CEL.

7.3.4.4 Phase II Study with Dasatinib in Patients with Hormone Refractory Progressive Prostate Cancer

A recent phase II study enrolled 46 patients with metastatic hormone refractory prostate cancer and no prior chemotherapy. The starting dose of dasatinib was changed from 100 mg twice daily to 70 mg twice daily after the first 25 patients for improved tolerability. A composite endpoint of PSA, bone scan, and disease control by RECIST was applied.

An improved PSA doubling time was seen in 29 of 36 patients (80.1%). One patient had a PSA decrease from 19.3 to 3.3 ng/mL. Bone scans were stable in 16 of 27 patients, and one was improved. Only 15 patients were evaluable for RECIST, and 10 of them (67%) had stable disease.

Pleural effusions were reported in 17 of the 46 patients, and mostly Grade 1–2, Grade 3 leucopoenia occurred in 2% of patients (Yu et al. 2008).

7.3.5 Safety and Tolerability

Nearly all patients treated with dasatinib experience adverse reactions at some time. Most reactions are mild to moderate. The tolerability of the compound has been investigated in the above-mentioned phase I, II, and III studies in patients with CML. Data of 2,182 patients enrolled in these studies have been reported in the prescribing information of dasatinib (Bristol-Myers Squibb 2007). Of the 2,182 patients treated, 25% were ≥65 years of age, while 5% were ≥75 years of age. The median duration of the therapy was 11 months (range 0.03–31 months). Owing to adverse reactions, dasatinib had to be discontinued in 11% of patients in chronic phase, and in 8–15% in advanced disease.

Most patients with imatinib intolerance in chronic phase-CML tolerated treatment with dasatinib well. In the Phase II single-arm study in chronic-phase CML, three of the 99 imatinib-intolerant patients had the same Grade 3/4 non-hematological toxicity with dasatinib, similar to the prior imatinib; all the three patients continued dasatinib treatment after dose reduction.

The most frequently reported adverse reactions were fluid retention (including pleural effusion), diarrhea, headache, nausea, skin rash, dyspnea, hemorrhage, fatigue, musculoskeletal pain, infection, vomiting, cough, abdominal pain, and pyrexia (Table 7.3).

7.3.5.1 Hematological Toxicity

Myelosuppression is the most common toxicity occurring under treatment with dasatinib. Hematological toxicity is dependent on the disease phase, as it is more severe in advanced phase disease. It is generally reversible and managed by withholding treatment or dose reduction.

7.3.5.2 Fluid Retention

Dasatinib is frequently associated with fluid retention. In all clinical studies, fluid retention occurred in 20–30% of the patients. Severe fluid retention was reported in 9% of patients, including severe pleural (6%) and pericardial effusion (1%). Severe ascites and generalized edema were each reported in <1% of patients. Severe noncardiogenic pulmonary edema was reported in 1% of patients.

In a smaller series, pleural effusions occurred with higher frequency in patients treated for advanced CML (Quintas-Cardama et al. 2007a). Up to 36% of treated patients experienced

Table 7.3 Adverse drug reactions reported ≥5% in clinical trials ($n > 2.182$) percent (%) of Patients

	All grades	Grades 3/4
Gastrointestinal disorders		
Diarrhea	32	4
Nausea	22	1
Vomiting	13	1
Abdominal pain	10	1
Gastrointestinal bleeding	8	4
Mucosal inflammation (including mucositis/stomatitis)	7	<1
Dyspepsia	5	0
Abdominal distension	5	0
Respiratory, thoracic, and mediastinal disorders		
Pleural effusion	25	6
Dyspnoea	21	4
Cough	10	<1
Nervous system disorders		
Headache	25	1
Neuropathy (including peripheral neuropathy)	6	<1
Skin and subcutaneous tissue disorders		
Skin rash	22	1
Pruritus	7	<1
General disorders and administration site conditions		
Superficial edema	21	<1
Fatigue	21	2
Pyrexia	13	1
Pain	7	<1
Asthenia	9	1
Chest pain	5	1
Vascular disorders		
Hemorrhage	15	2
Musculoskeletal and connective tissue disorders		
Musculoskeletal pain	14	1
Arthralgia	8	1
Myalgia	8	<1
Infections and infestations		
Infection (including bacterial, viral, fungal, nonspecific)	10	3
Metabolism and nutrition disorders		
Anorexia	9	<1
Blood and lymphatic system disorders		
Febrile neutropenia	5	5

pleural effusion, with Grade 3/4 in approximately 15% (Cortes et al. 2008).

The pathomechanism for edema, pleural effusion, and other fluid-retention adverse events under dasatinib treatment is conflicting. It can be speculated whether this might be related to the off-target effect on PDGFR-β kinase. Mice deficient in PDGFR-β develop edema (Lindahl and Johannsson 1997), and a clinical trial assessing a humanized pegylated PDGFR-β blocking FAB-fragment in patients with ovarian and colorectal cancer resulted in significant edema and pleural effusion (Jayson et al. 2005; Quintas-Cardama et al. 2007a).

Treatment of patients in chronic phase with once-daily application of dasatinib had a significant lower incidence of pleural effusions when compared to twice-daily administration (Shah et al. 2008).

Management of edema, pleural effusion, and fluid retention often requires stopping of dasatinib treatment. About 25% of patients require therapeutic thoracocentesis. After lung manifestations have resolved, the drug can be reintroduced cautiously. Corticosteroids may assist in management, diuretics are also recommended, however, with only limited success (Quintas-Cardama et al. 2007a).

7.3.5.3 Bleeding

Despite the high rate of thrombocytopenia, bleeding episodes were rare. Severe CNS hemorrhage occurred in <1% of patients. Eight cases were fatal and five of them were associated with CTC Grade 4 thrombocytopenia.

Severe gastrointestinal hemorrhage occurred in 4% of patients and generally required drug interruptions and transfusions. Other severe hemorrhage occurred in 2% of patients. Most bleeding-related events were typically associated with severe thrombocytopenia.

7.3.5.4 QT-Prolongation

Of the 2,182 patients who received dasatinib in Phase II/III clinical trials, 18 had QTc prolongation reported as an adverse event. Seventeen patients (<1%) experienced a QTcF of >500 ms.

Dasatinib should be administered with caution to patients who have or may develop prolongation of QTc. These include patients with hypokalemia or hypomagnesemia, patients with congenital long QT syndrome, patients taking antiarrhythmic medication or other drugs which lead to QT prolongation, and cumulative high-dose anthracycline therapy.

Hypokalemia or hypomagnesemia should be corrected prior to dasatinib administration.

7.4 Conclusion and Further Perspectives

Dasatinib is approved for the treatment of patients with Ph+ CML or Ph+ ALL, intolerant or resistant to imatinib. Clinical studies have shown activity in patients with most imatinib-resistant mutations of BCR-ABL, except the T315I mutation. High rates of hematologic and cytogentic remissions, together with the prolongation of survival have been demonstrated. Starting dose in chronic phase is 100 mg daily, while in advanced phases, 70 mg twice daily is recommended. Most patients experience side effects, which are mostly mild to moderate and manageable.

Dasatinib, like imatinib, does not have the potential to eradicate the quiescent fraction of stem cells, despite the fact that it targets earlier BCR-ABL-positive stem cells than imatinib (Copland et al. 2006). Therefore, it is unlikely that treatment with dasatinib and other tyrosine kinase inhibitors will cure CML, and rather treatment has to continue lifelong. Since there are no real long-term data till date for tyrosine

kinase inhibitors, even mild toxicity has to be of concern. Kinase inhibition occurs in all cells, and it is not clear whether there will be some kind of late toxicity.

In addition, emergence of resistance and relapse poses a further concern, and indicates that adaptation plays a role in the complex interaction between ABL tyrosine kinase inhibition and CML progression, a challenge that cannot be solved solely by the development of new BCR-ABL inhibitors.

The outlook for long-term treatment is likely to involve combination therapies with cytotoxic agents, agents that target multiple sites within the BCR-ABL signal transduction pathway, or even the reintroduction of IFN as immunotherapy. It has been shown in a small cohort of patients that, after remission induction in CML patients with imatinib, IFN can safely be administered as maintenance treatment (Hochhaus et al. 2008b).

Dasatinib outside CML and Ph^+ ALL is still under investigation. Treatment of patients with Ph-negative myeloproliferative disorders with dasatinib generally induced only minimal improvements, however, there were individual patients experiencing very good remissions (Verstovsek et al. 2008).

In solid tumors, preclinical studies in numerous tumor cell lines with dasatinib showed growth retardation or cell cycle arrest rather than induction of apoptosis and cell death. Therefore, the preliminary results of clinical phase I and II studies in solid tumors with ‘stable disease’ as best results were to be expected.

Better responses to dasatinib in vitro have been reported in selected cell lines or under certain circumstances. For example, triple negative breast cancer cell lines responded better to dasatinib when compared to other types of breast cancer (Finn et al. 2007); in lung cancer cell lines, dasatinib activity was dependent on the epithelial growth factor status (Song et al. 2006), and dasatinib induced apoptosis in SRC-dependent osteosarcoma cell lines (Shor et al. 2007).

Hence, identification of predictive markers for treatment response to dasatinib in solid tumors will be of great importance. The results of preclinical and clinical studies till date provide hope that dasatinib in biologically relevant combination with other anticancer agents will find a place in the treatment for at least some tumors.

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Nilotinib

8

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Abstract Therapy with imatinib mesylate is a standard of care for most patients with Philadelphia chromosome-positive chronic myeloid leukemia (CML). However, resistance or intolerance to imatinib develops in a considerable number of patients leading to relapse or discontinuation of treatment. Nilotinib is a rationally designed second-generation tyrosine kinase inhibitor (TKI) with improved affinity and specificity against the BCR-ABL kinase, when compared with imatinib. Considerable efficacy after imatinib failure has been demonstrated in clinical trials leading to nilotinib's current approval as second-line therapy for CML in chronic and accelerated phase (AP). The role of nilotinib as first-line treatment for CML, in combinatorial strategies and in the context of specific BCR-ABL mutations, requires future studies.

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8.1 Background

Targeted therapy has revolutionized the treatment of Philadelphia chromosome-positive (Ph-positive) chronic myeloid leukemia (CML). Imatinib mesylate (formerly STI571 or CGP 5148B) was specifically developed to inhibit the oncogenic activity of the BCR-ABL tyrosine kinase, the molecular correlate of the Philadelphia chromosome (Druker et al. 1996). Significant activity in clinical trials led to the rapid approval of imatinib for Ph-positive CML in chronic phase (CP), accelerated phase (AP), and blast phase (BP) (summarized in reference (Cohen et al. 2005)). Based on the phase III IRIS study (International Randomized Study of IFN and STI571) which showed a 5-year estimated overall survival (OS) rate of 89%, imatinib at a daily dose of 400 mg is the standard first-line treatment of CML in CP (Druker et al. 2006).

Despite its considerable activity, a substantial portion of patients will develop acquired or secondary resistance to imatinib with prolonged treatment. The overall failure rate was 17%, and more than 30% in the IRIS study had discontinued imatinib for various reasons after 5 years (Druker et al. 2006). Several mechanisms of resistance have been identified (Apperley 2007), but point mutations within the kinase domain of

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BCR-ABL are probably the most prevalent (Gorre et al. 2001; O'Hare et al. 2007). Nearly 100 different mutations that confer varying degrees of resistance to imatinib have been described in vitro and in vivo (Hughes et al. 2006; Shah et al. 2002). Recurrence of disease is common with discontinuation of imatinib therapy (Cortes et al. 2004; Rousselot et al. 2007), indicating that real cure is not achieved by imatinib. Likewise, identification of residual BCR-ABL positive cells by molecular analysis during imatinib treatment is frequently observed (Hughes et al. 2003). Moreover, some patients treated with imatinib will have a suboptimal response as defined by failure to fulfill certain response criteria at specified timepoints (Baccarani et al. 2006). In patients in accelerated (AP) and BP, the activity of imatinib is considerably lower than in patients in CP (Sawyers et al. 2002; Talpaz et al. 2002). Furthermore, even in responding patients, adverse effects can impair the quality of life and result in treatment discontinuation in approximately 10% of patients undergoing imatinib therapy (Deininger et al. 2003).

The need for treatment options after imatinib failure led to the development of second-generation tyrosine kinase inhibitors (TKIs), of which nilotinib (Tasigna®) and dasatinib (Sprycel®) are currently approved for the treatment of patients with CML resistant or intolerant to imatinib therapy.

8.2 Preclinical and Pharmacokinetic Data

8.2.1 Pharmacological Design

Nilotinib (formerly AMN107) was rationally designed on the basis of crystallographic studies on the interaction between imatinib and the ABL kinase domain (Manley et al. 2004). Nilotinib is an orally active, high-affinity aminopyrimidine-based ATP-competitive inhibitor of the ABL kinase domain. Reducing the number of hydrogen bonds with the ABL kinase domain from six to four led to higher specificity and approximately 20–30-fold higher inhibitory potency against unmutated BCR-ABL kinase, as well as most imatinib-resistant point mutations. Nilotinib binds to an inactive conformation of the ABL kinase domain and prevents the enzyme to adopt a catalytically active conformation.

8.2.2 Drug Targets

Like imatinib, nilotinib is a selective TKI and inhibits the tyrosine kinases BCR-ABL, KIT, and PDGFR (platelet-derived growth factor receptor) with an IC50 of 25, 160, and 57 nmol/L, respectively (Weisberg et al. 2005). The modifications leading to nilotinib's structure did not largely affect the affinities for KIT or PDGFR. Recently, a chemical proteomics approach has identified novel nilotinib targets including the receptor tyrosine kinase DDR1 (discoidin domain receptor 1) and the nonkinase target NAD(P)H:quinone oxidoreductase NQO2 (or quinone reductase QR2), against which nilotinib is highly active (Rix et al. 2007). The role of the inhibition of these novel targets on the clinical activity of nilotinib in patients with CML is currently unknown.

8.2.3 Preclinical Activity

In vitro, nilotinib showed considerably higher antiproliferative activity and inhibition of BCR-ABL autophosphorylation of murine and human cell lines, when compared with imatinib (Weisberg et al. 2005; Golemovic et al. 2005). In vivo, tumor burden was reduced and survival was prolonged after challenge with Ph-positive cells (Weisberg et al. 2005; Golemovic et al. 2005). Nilotinib's higher activity against wild-type BCR-ABL was

Table 8.1 Categorized sensitivity to nilotinib of clinically known and through mutational screens recovered BCR-ABL point mutations

Sensitivity	IC_{50} (nM)	BCR-ABL mutations
High	100	M237I, M244V, K247N, G250A, G250E[a], G250V, Q252H, E255D, E255R, L273F, E275K, D276G[a], E281K, E285N, K285N, V289L, E292K[a], N297T, F311L, F317C, F317L[a], F317V[a], D325N, S348L, M351T, E355A, E355G, H375P, V379I, L387F, M388L, L387F, L387M, H396P, H396R, T406I, W430L, E431G
Medium	200	L248V[a], G250E[a], Y253F, E255K[a], D276G[a], E282K, E292K[a], F311V, F317L[a], F359V, A380S, F486S
Low	1,000	L248V[a], Y253C, Y253H[a], E255K[a], E255V, K285N, F317V, [a]F359C
None	>2,000	T315I

Of note, the extent of sensitivity depends not only on the position of the mutation but also the specific substitution

[a]Denotes mutations that fall into two different categories, based on different values reported. Values are from references (O'Hare et al. 2005; Weisberg et al. 2006; Bradeen et al. 2006; Ray et al. 2007; von Bubnoff et al. 2006)

preserved in 16 (O'Hare et al. 2005) and 32 (Weisberg et al. 2006) of the most common kinase domain mutants conferring imatinib-resistance with the notable exception of the T315I mutation (Table 8.1). This resistance conferred by the T315I mutant against all currently approved TKIs stems from the fact that this residue governs the access to a hydrophobic pocket located in the ABL kinase within which most TKIs used in the clinic are required to bind.

8.2.4 Pharmacokinetics and Metabolism

Pharmacokinetic analyses in healthy volunteers (Kagan et al. 2005; Tanaka et al. 2006) and patients from the phase I study (Kantarjian et al. 2006) (see below) showed a dose-dependent increase in steady-state levels and higher steady-state levels, with 400 mg twice daily than with a single daily dose of 800 mg and further increase with twice-daily 600 mg dosing. Steady-state serum levels were achieved by day 8, and peak serum concentrations were observed 3–4 h after administration with a terminal elimination half-life of about 16 h. With administration of 400 mg twice daily, peak-trough plasma levels were 3.6 and 1.7 µM, respectively, with the trough level exceeding the 50% inhibitory concentration (IC_{50}) of cellular phosphorylation of wild-type (20–57 nM) and 32 of 33 kinase domain mutants (19–709 nM) of BCR-ABL. Bioavailability was increased by 82% when administered with a high-fat meal when compared to fasting. Nilotinib appeared to be a weak inhibitor of CYP3A4, whereas CYP3A4 inhibition increased systemic nilotinib exposure (Tanaka et al. 2006). Complete recovery (4.4% in urine and 93.5% in feces) of mostly unchanged nilotinib within 7 days of administration indicated the absence of retention of the drug or its metabolites with chronic treatment and incomplete oral absorption (Kagan et al. 2005).

8.3 Clinical Efficacy

Phase I and II studies in patients with CML resistant or intolerant to imatinib led to the accelerated approval of nilotinib for CML in CP or AP after imatinib failure. In addition to three

ongoing phase II studies, a three-armed randomized open-label multicenter phase III study comparing nilotinib with imatinib as a first-line treatment in CML in CP (ENESTnd) already completed recruitment.

8.3.1 Nilotinib Phase I Study

One-hundred and nineteen patients with imatinib-resistant CML in CP, AP or BP, and Ph-positive ALL were assigned to receive nilotinib in a dose-escalating phase I study (Kantarjian et al. 2006). Dose-limiting toxic effects were reported in 18 patients at doses higher than 600 mg daily, and a dose of nilotinib of 600 mg twice-daily was established as the maximum tolerated dose due to dose-related and dose-limiting myelosuppression. The similar response rate with a better safety profile established nilotinib at 400 mg twice-daily for phase II studies, with the possibility of dose escalation to 600 mg twice-daily in patients with an inadequate response. Among patients with CML in CP, AP, or BP (whose disease was resistant to a median dose of 800 mg of imatinib), hematological and cytogenetic response rates were 92/53, 72/48, and 39/27%, respectively.

8.3.2 Nilotinib After Imatinib Failure

An open-label multicenter phase II study was conducted in patients with CML in CP, AP, or BC with imatinib resistance or intolerance to assess efficacy (Table 8.2).

Study design, inclusion criteria, and the results of an interim analysis of the first 280 patients with CML in CP have been described in detail (Kantarjian et al. 2007). The last update included 321 patients with at least 19 months of follow-up (Kantarjian et al. 2008a). Median age was 58 years (range 21–85 years) with a median CML duration of 58 months (range 5–275 months), and a median duration of imatinib therapy of 32 months (0.3–94 months). Seventy percent had imatinib resistance and 30% were imatinib intolerant. The median dose intensity of 790 mg/day (151–1,110) indicates excellent tolerability. Overall, a complete hematologic response (CHR) was attained by 76% of patients who did not have a CHR at baseline. A major cytogenetic response (MCyR), the primary endpoint of the study which was chosen because of its correlation with long-term survival and clinical benefit, was achieved in 59% of patients with comparable rates in imatinib-resistant and –intolerant patients. A complete cytogenetic response (CCyR) was seen in 44% of patients. The median time to CHR and MCyR was 1 and 2.8 months, respectively. Responses were durable, and after 24 months, 78% were still in MCyR. Progression-free (PFS) and estimated OS after 24 months were 64 and 88%, respectively. Currently, 59% of patients have discontinued nilotinib therapy, mainly due to disease progression (27%) and drug-related adverse events (15%).

The activity of nilotinib therapy in patients with CML CP who failed prior imatinib and dasatinib therapy has been recently reported. Patients have been followed for at least 4 months, at which point 56% of patients remained on nilotinib therapy. Patients had discontinued imatinib due to resistance in 85% of cases and dasatinib due to intolerance in 67% of cases. Overall, 79% of patients achieved a CHR and 43% a MCyR (24% CCyR), with an OS of 86% at 18 months. The main reasons for discontinuation were disease progression in 28% and drug-related adverse events in only 10% of patients, further emphasizing the tolerability of nilotinib even in heavily pretreated patients (Giles et al. 2008a).

An interim analysis of the first 119 patients with CML in AP with at least 6 months of follow-up has been described in detail (le Coutre et al. 2008a). In a recent update, a total of 137 patients have been included (le Coutre et al. 2008b). Median age was

Table 8.2 Efficacy of nilotinib in chronic phase (CP), accelerated phase (AP) and blast-crisis (BC) of Ph-positive CML (chronic myeloid leukemia) with imatinib-resistance or -intolerance

	CP (*n*>321)	AP (*n*>137)	BC (*n*>136)
Overall HR (%) (CHR+MR/NEL+RTC)	–	56	20–24
CHR (%)	76	31	12–13
MR/NEL (%)	–	12	<1
RTC (%)	–	13	7–10
MCyR (CCyR+PCyR) (%)	59	32	38–50
CCyR (%)	44	20	28–33
PCyR (%)	15	12	9–17
Continous HR (%)	–	74 (12 m)	–
Continous MCyR (%)	84 (18 m)	74 (18 m)	–
Progression-free survival (%)	73 (18 m)	56 (12 m)	–
Overall survival (%)	91 (18 m)	82 (12 m)	42 (12 m)

Numbers are based on references (Giles et al. 2008b; Kantarjian et al. 2008b; le Coutre et al. 2008d). *HR* hematologic response; *CHR* complete hematologic response; *MR/NEL* marrow response/no evidence of leukemia; *RTC* return to chronic phase; *MCyR* major cytogenetic response; *CCyR* complete cytogenetic response; *PCyR* partial cytogenetic response; *m* months; – not reported/applicable

57 years (range 22–82) with a median CML duration of 71 months (range 2–298 months) and a median duration of imatinib therapy of 28 months. Eighty percent had imatinib resistance and 20% had imatinib intolerance. The median exposure of nilotinib was 272 days (2–910 days) with a median dose intensity of 775 mg/day (150–1,149). The overall rate of hematologic response (HR), the primary endpoint of the study, was 56% with a CHR rate of 31% and similar rates in imatinib-resistant and -intolerant patients. The median time to first HR was 1 month. MCyR was seen in 32% of patients and CCyR in 20% of patients. The median time to MCyR was 2.8 months. Of 77 patients achieving a HR, 54% continued to maintain a HR at 24 months. Seventy percent of the 44 patients who achieved a MCyR maintained this response at 24 months, and the estimated OS at 24 months was 67%. At the time of analysis, 75% had discontinued treatment, mostly for disease progression (38%), drug-related (9%), and nondrug-related adverse events (7%). Grade 3–4 adverse events were rare, with the most common being serum lipase elevation in 18% of patients receiving nilotinib.

Results from 136 patients with CML in BP have been presented (Giles et al. 2008b). Median age was 54 years (range 18–79 years) with 21% in lymphoid and 78% in myeloid BP. Median duration of imatinib therapy was 16 months (0–107 months). Eighty-two percent had imatinib resistance and 18% were imatinib intolerant. The median duration of nilotinib therapy was 84 days (3–666 days), with a median dose intensity of 800 mg/day (3.7–1,113 mg). The rate of best-confirmed HR was 24% for myeloid and 20% for lymphoid BP. CHR, the primary endpoint of the trial, was attained by 12 and 13%, respectively. The rate of best cytogenetic response was 38% for myeloid and 50% for lymphoid BP, with 28 and 33% CCyR, respectively. OS was 42% after 12 months. At the time of analysis, 93% of all patients had discontinued treatment, mainly for disease progression (54%).

In conclusion, nilotinib has shown significant activity in a series of phase II studies in patients with CML after failure or intolerance of imatinib therapy, particularly, in those in CP or AP. These

studies provided the bases for the approval of nilotinib in those settings. The evidence which led to the approval of nilotinib for CML in CP and AP after imatinib failure has recently been reviewed elsewhere (Hazarika et al. 2008).

8.3.3 Nilotinib First-Line Therapy

Given the outstanding results obtained with nilotinib in the post-imatinib setting, the next question to be answered is whether this activity might translate into similar results when this TKI is used in the frontline setting. Three independent phase II studies are currently evaluating this question in patients with Ph-positive CML CP. Preliminary results from two of these studies are already available (Cortes et al. 2008a; Rosti et al. 2008). Results from an M.D. Anderson Cancer Center study (Cortes et al. 2008b) involving 49 patients who received a median nilotinib dose intensity of 800 mg/day (range, 200–800) have been recently reported. Overall, 46 (96%) of 48 evaluable patients achieved a CCyR, with 93, 100, 96, and 91% of patients being in CCyR by 3, 6, 12, and 24 months of therapy, respectively, which compares favorably with CCyR rates reported for historical controls treated with imatinib 400 or 800 mg daily. A major molecular response (MMR), the primary endpoint of the study, was achieved by 45% at 6 months and in 52% of patients at 12 months, respectively. In a similar study, 73 patients with CML CP have been enrolled in an open-label multicenter phase II study from the Gruppo Italiano Malattie Ematologiche dell'Adulto (GIMEMA) investigating 400 mg nilotinib twice-daily dosing and the rate of CCyR at 1 year (Rosti et al. 2008). With a short follow-up, a CCyR was achieved in 97% of patients, and a MMR was reported in 75% at 6 months.

It is worth noting that although the studies described above suggest an important activity of nilotinib in newly diagnosed patients with CML CP, none of them are randomized trials and all comparisons are made with historical controls, and therefore, subject to multiple biases. However, a randomized open-label multicenter phase III study comparing nilotinib with imatinib including 771 newly diagnosed Ph-positive patients with CML CP has recently finished accrual (ENESTnd; Evaluating nilotinib efficacy and safety in clinical trials in newly diagnosed Ph^+ CML in CP). In this three-armed trial, nilotinib of 400 mg twice-daily or nilotinib of 300 mg twice-daily are compared with imatinib 400 mg once-daily. The primary endpoints are the rate and duration of major molecular remission (defined as ≥3 log reduction of BCR-ABL transcript levels). The lower dose of nilotinib was chosen to minimize toxicity while maintaining trough levels necessary for efficacy, assuming that the untreated population has a much lower rate of mutations. An extension study protocol permits patients to switch treatment arms. Preliminary results are awaited in 2009.

In conclusion, preliminary results from clinical trials with nilotinib as first-line therapy for CML in CP show a high rate of cytogenetic responses that compares favorably with the historical controls with high-dose imatinib. Responses are generally achieved faster than with imatinib. Results from the ongoing phase III randomized study will certainly help to better define the role of nilotinib in comparison with imatinib in this setting, as well as the clinical importance of more rapid achievement of responses observed with nilotinib.

8.3.4 Nilotinib After Dasatinib Failure

A subgroup of patients in the phase II registration study received nilotinib because of resistance or intolerance to both imatinib and dasatinib, under the assumption that the differing target spectrum will be reflected in the clinical efficacy and the occurrence of adverse events (Hantschel et al. 2008).

The outcome of 78 patients has been reported (Giles et al. 2008a; Abruzzese et al. 2008). Primary endpoints were the rate of MCyR in CP and the rate of confirmed HR in AP and BC. Eighty-five evaluable patients were analyzed. Thirty-nine patients were in CP, 20 in AP, and 28 in BP. The median time since diagnosis of CML was 89, 80, and 31 months, respectively, and the median duration of dasatinib treatment was 6.5, 7.7, and 3.7 months, respectively. Whereas the majority of patients with CML in CP discontinued dasatinib therapy because of an adverse event (67%), most patients in AP or BC discontinued due to disease progression (60 and 75%, respectively). The median duration of nilotinib exposure for all patients was at least 4 months. Among patients in CP, CHR, MCyR, and CCyR rates were 79, 43, and 24%, respectively. In AP, overall HRs were seen in 39 patients, and 6% of patients achieved a CHR. A MCyR was attained by 11% of patients with a median time to first MCyR of 2.3 months and a median duration of MCyR of 2.1 months. In BP, 8% had evidence of HR and 4% attained a MCyR. OS in CP and AP at 12 months was 97 and 63%, respectively. Hematologic and nonhematologic adverse events were comparable with patients who had not received dasatinib before.

Patients receiving nilotinib on a compassionate use program after failure of imatinib alone or both imatinib and dasatinib were analyzed for the occurrence of pleural or pericardial effusions, the two adverse events reported with the use of dasatinib. Results from 621 patients have been reported (le Coutre et al. 2008c). At the last follow-up, 725 CML patients from 66 countries were evaluated. Fifty-nine percent of the patients were in CP; 24% were in AP, and 17% were in BP. Five hundred and seventy-seven patients had failed imatinib, with the majority (67%) being resistant and 148 had failed imatinib and dasatinib with the majority (57%) being intolerant. Effusions were rare in patients who failed to respond to imatinib alone. However, effusions, mostly pleural, were reported in 33% of patients who failed to respond to imatinib and dasatinib, and pleural effusions were the primary reason for treatment discontinuation in 16 of 38 patients treated with dasatinib who developed pleural effusion. Pleural or pericardial effusions occurred at all dose levels of dasatinib, including doses <140 mg, and the development of pleural effusions were independent of the phase of CML. Of note, none of the patients treated with nilotinib in CP developed pleural or pericardial effusion.

In conclusion, nilotinib has significant clinical activity in patients with CML in CP, AP, and BP after previous failure of a second-generation TKI, such as dasatinib, representing a valuable option in this heavily pretreated patient population with limited therapeutic options. Of note, common adverse events after dasatinib failure were rarely seen with nilotinib and were not dose-limiting.

8.3.5 Toxicity

The high dose-intensity that was generally achieved, the low occurrence of Grade 3 or 4 adverse events leading to discontinuation of treatment, and the overall adverse events reported from the phase I and II studies confirm that nilotinib is endowed with a very favorable toxicity profile (Table 8.3) (Kantarjian et al. 2006, 2007, 2008b; le Coutre et al. 2008a; Giles et al. 2008b; le Coutre et al. 2008d).

The most frequently reported nonhematologic adverse events of any grade possibly related to nilotinib were rash, pruritus, nausea, fatigue, headache, diarrhea, vomiting, and constipation. Grade 3 or 4 toxicities were uncommon and observed in ≤2% of patients. Of note, drug-related fluid retention syndromes (e.g., pleural effusion, pericardial effusion, pulmonary edema) and bleeding events were uncommon with Grade 3–4 events being extremely rare.

Myelosuppression was common, and the most-frequent Grade 3–4 hematologic abnormalities were neutropenia and thrombocytopenia.

Table 8.3 Adverse events with nilotinib (400 mg twice daily) and imatinib as reported from patients with CML in CP treated in phase II trials (Kantarjian et al. 2002, 2007, 2008b; Hochhaus et al. 2008)

	Nilotinib ($n>321$)		Imatinib ($n>532$)	
	All grades (%)	Grades 3–4 (%)	All grades (%)	Grades 3–4 (%)
Hematologic toxicity				
Anemia	53	10	–	7
Neutropenia	54	31	–	35
Thrombocytopenia	58	31	–	20
Nonhematologic toxicity				
Rash	31	2	47	<1
Pruritus	26	<1	9	0.4
Nausea	25	<1	63	3
Fatigue	20	1	48	<1
Headache	18	2	36	<1
Diarrhea	12	2	48	3
Vomiting	13	<1	36	3
Constipation	13	<1	–	–
Weight gain	–	–	32	–
Muscle cramps	–	–	62	<1
Myalgia	8	1	20	0.2
Arthralgia	–	–	40	<1
Abdominal pain	–	–	32	2
Dyspepsia	–	–	17	0
Musculoskeletal pain	5	<1	38	2
Cough	–	–	–	–
Dyspnea	–	–	–	–
Pyrexia	–	–	–	–
Asthenia	–	–	–	–
Anorexia	–	–	–	–
Peripheral edema	6	0	67	1
Pericardial effusion	0.6	0.3	–	–
Pleural effusion	1.2	0.6	–	–
Pulmonary edema	0.3	0.3	–	–
Hemorrhages	–	–	30	5
CNS bleeding	0.3	0.3	–	–
GI bleeding	0.9	0.3	–	–
Biochemical abnormalities				
Lipase elevation	46	17	–	–
Hypophosphatemia	54	16	–	–
Hyperglycemia	70	12	–	–
Bilirubin (total)	71	8	–	0.6
ALT	68	4	–	3
AST	55	2	–	3
Hypocalcemia	50	1	–	–
Creatinine	24	2	–	0.2
Hypomagnesemia	17	<1	–	–

ALT alanine aminotransferase; *AST* aspartate aminotransferase

Their incidence was related to the stage of CML with Grade 3–4 neutropenia of 30% in CP, 40% in AP, and 66% in BC, and Grade 3–4 thrombocytopenia in 28, 40, and 60%, respectively. The median time to onset of anemia, neutropenia, and thrombocytopenia were 57, 55, and 42 days, respectively. The median duration of Grade 3–4 myelosuppression was 9, 15, and 23 days, respectively. Myelosuppression was generally easily manageable with few dose interruptions, rare dose reductions, or occasional support with hematopoietic growth factors or platelet transfusions.

Hyperbilirubinemia was the most common adverse event observed (71% in CML CP), but was usually short-lived and asymptomatic. Evidence that a polymorphism in the promoter of the UGT1A1 is associated with nilotinib-induced hyperbilirubinemia underlines the benign character of this adverse event (Singer et al. 2007). Common Grade 3 or 4 biochemical laboratory abnormalities observed with nilotinib were lipase elevations, hypophosphatemia, and hyperglycemia. Elevations in biochemical parameters were generally self-limited and frequently resolved spontaneously. There were four cases of pancreatitis in the phase II study resulting in one of these patients discontinuing therapy. Grade 3–4 elevations of transaminases were rare and did not lead to discontinuation of treatment. No patients discontinued nilotinib due to increased bilirubin or transaminase.

Preclinical data indicated that nilotinib prolongs cardiac ventricular repolarization. Nilotinib appeared to increase the corrected QT interval by Fridericia's formula (QTcF) in a dose-dependent manner, but less than 1% of treated patients showed an absolute QTcF interval exceeding 500 ms. No episodes of Torsades de Pointes tachycardia have been reported so far. A possible causal relationship of rare sudden cardiac deaths (0.6%) with nilotinib could not be excluded. Therefore, this risk is described in a boxed warning in the labeling. Electrolyte abnormalities should be corrected before instituting nilotinib therapy, and close electrocardiographic monitoring is mandatory. Caution is warranted when used with other drugs interfering with CYP3A4 metabolism. Nilotinib should be taken on an empty stomach to avoid high plasma peak levels.

Cross-intolerance between imatinib and nilotinib, defined as the occurrence of the same grade 3–4 toxicity, regardless of causality, that led to discontinuation of imatinib, was assessed in 122 patients in CP ($n>95$) or AP ($n>27$) to evaluate the safety of nilotinib (Jabbour et al. 2008a). Most patients had discontinued imatinib because of Grade 3–4 events, 76 and 78% of those in CP or AP, respectively. Cross-intolerance of nonhematologic adverse events between nilotinib and imatinib was rare, and never led to dose reduction or the discontinuation of nilotinib. Cross-intolerance of hematologic adverse events was infrequent with fewer events observed during nilotinib therapy. Thrombocytopenia was the only event that led to discontinuation of nilotinib in a minority of patients, being Grade 3–4 or persistent Grade 2 in 17%, which led to nilotinib discontinuation in 7% of patients with CML CP. Overall, cross-intolerance was minimal in imatinib-intolerant patients. However, as this subgroup analysis only included imatinib-intolerant patients, data obtained in an imatinib-resistant population is needed.

To obtain additional information and evaluate the safety of nilotinib and expand access prior to regulatory approval, an open-label multicenter study was performed in patients with imatinib-resistant or -intolerant CML in CP, AP, and BP (Expanding Nilotinib Access in Clinical Trials, ENACT). Dosing was 400 mg twice daily with no dose escalation allowed. More than 2,100 patients have been enrolled in 42 countries at 375 centers. Results for the first 1,056 patients have been presented recently (Nicolini et al. 2008). Results from this and other studies (Rosti et al. 2008; Abruzzese et al. 2008; Cortes et al. 2008c) confirm the tolerability and the favorable safety profile in all phases of CML after imatinib failure or intolerance, even in heavily pretreated patients.

8.3.6 Resistance to Nilotinib

The detailed analysis and prediction of resistance to nilotinib is of increasing importance and may guide treatment decisions with other TKIs to choose from in the future.

In vitro screening assays were applied to predict potential resistance mechanisms against nilotinib (Bradeen et al. 2006; Ray et al. 2007; von Bubnoff et al. 2006). Nilotinib expectedly produced fewer resistant clones and a reduced spectrum of mutations when compared with imatinib in a cell-based assay (Table 8.1) (von Bubnoff et al. 2006). In a mutagenesis screen with imatinib, nilotinib, and dasatinib at various concentrations, resistance was almost exclusively confined to mutations mapping to the P-loop of BCR-ABL kinase, with a small subset of partly overlapping spectrum of mutants for each TKI (Bradeen et al. 2006). This is in agreement with the clinical observation of nilotinib's substantial efficacy after failure of imatinib and dasatinib (Abruzzese et al. 2008).

The mutational status of BCR-ABL before and during treatment with nilotinib, and its influence on the clinical outcome was assessed in a substantial portion of the patients included in the phase II study in CML in CP (Kantarjian et al. 2007; Radich et al. 2008). At the last follow-up, 281 of 321 patients had baseline mutational data available. Forty-one percent had a BCR-ABL mutation at baseline with a higher prevalence in imatinib-resistant patients (55%) than in imatinib-intolerant patients (10%). Patients with secondary resistance had a slightly higher incidence of baseline mutations than patients with primary resistance (58 vs. 46%, respectively). Most of the baseline mutations in imatinib-resistant patients had a high sensitivity with an IC_{50} ≤150 nM. Mutations with a medium sensitivity and an IC_{50} >150 nM (Y253H, E255K/V, and F359C/V) were detected in 17% of imatinib-resistant patients. The T315I mutation with complete resistance and an IC_{50} >10,000 nM occurred in 3% of patients. Overall, response and progression rates were similar in patients with or without baseline mutations, but responses were less frequent in patients with Y253H, E255K/V, or F359C/V, and most patients with E255K/V and F359C/V progressed. During nilotinib therapy, newly detectable mutations were found in 17% of all patients with a higher incidence in patients with (29%) than without (9%) baseline mutations. Most mutations belonged to the group with medium (Y253H, E255K/V, F359C/V) or no (T315I) sensitivity. At progression, most patients with baseline mutations also had either the same baseline or newly detectable mutations, whereas most patients with no baseline mutation did not have newly detectable mutations, suggesting the presence of alternative mechanisms of resistance.

The T315I mutation is resistant against all currently approved TKIs, including nilotinib and dasatinib, and was consistently selected in all in vitro screens (Bradeen et al. 2006; Ray et al. 2007; von Bubnoff et al. 2006). Although the T315I mutation is not invariably selected during progression (Willis et al. 2005) and prognosis is mostly dependent on stage of disease (Jabbour et al. 2008b), its complete resistance against the available targeted therapy leads to increased effort to develop inhibitors that are specifically effective against this mutation (Quintas-Cardama et al. 2007). The F317L mutation is relatively resistant to imatinib and seems to be selected after dasatinib failure (Deguchi et al. 2008; Jabbour et al. 2008c). Patients with this mutation did not seem to have a higher rate of progression with nilotinib therapy (Radich et al. 2008) and may, therefore, be preferentially treated with nilotinib.

In cases of CML in AP, where BCR-ABL mutational status was available for evaluation, response rates seemed to be comparable in patients with either unmutated or mutated BCR-ABL, but also depended on the type of mutation (le Coutre et al. 2008a; Saglio et al. 2007).

An alternative mechanism of resistance against nilotinib may be the relative insensitivity of CML stem cells to nilotinib (Jorgensen et al. 2007; Konig et al. 2008) similar to what has been observed with imatinib (Bhatia et al. 2003; Graham et al. 2002; Holtz et al. 2002), therefore suggesting that nilotinib may not be a definite cure for the disease.

In conclusion, comparable hematologic and cytogenetic responses to nilotinib are observed both in patients with and without BCR-ABL mutations. However, while some mutations with reduced sensitivity in vitro may be associated with lower response rates and higher rates of progression (Y253H, E255K/V, F359C/V), some that are selected with other TKIs (e.g., V299L and F317L with dasatinib) are responsive to nilotinib. The T315I mutation is completely resistant to all available TKIs, and therefore, patients with this mutation should be referred to alternative treatment options, such as allogeneic stem cell transplantation or investigational agents. For the few BCR-ABL mutations where detailed knowledge about sensitivity is available, this information should be incorporated into treatment decisions.

8.4 Outlook

Nilotinib has proven to be efficacious and safe in patients with CML after imatinib failure (Kim et al. 2008). However, with the increasing number of available effective treatments and improved knowledge about mechanisms of resistance to TKIs, therapeutic decisions are already becoming increasingly complex and will have to be more individualized. Currently, there are no guidelines or recommendations to guide treatment with second-generation TKIs similar to those established for imatinib (Baccarani et al. 2006). Recently, data from a single institution suggests that failure to achieve early cytogenetic response after 3–6 months with a second-generation TKI may identify those patients who should be started on alternative agents (Tam et al. 2008).

Similarly, the role of second-generation TKIs as first-line therapies in CML is scarce. Several phase II studies are currently addressing this question, including one phase III randomized study comparing the activity of nilotinib with that of nilotinib in that setting. Early use of more potent second-generation TKIs may potentially induce faster responses and reduce the selection of resistant clones. To this end, second-generation TKIs, nilotinib and dasatinib, have been approved for the treatment of CML after imatinib failure. Evidence to favor one over the other is lacking. In addition, switching therapy from dasatinib to nilotinib and from nilotinib to dasatinib has been shown to be clinically effective, with minimal cross-reactivity with imatinib. A major player in deciding which second-generation TKI to choose after imatinib failure may be the presence of BCR-ABL mutations selected for by specific TKIs, but amenable to inhibition by others. For instance, whilst the Y253H, E255K/V, and F359C/V mutations confer important degrees of resistance to nilotinib, they remain sensitive to dasatinib, and the contrary is the case with the V299L and F317L mutations. Unfortunately, the T315 mutation remains completely resistant to all approved TKIs and requires alternative forms of treatment. Thus, the presence and type of BCR-ABL mutations as well as clinical judgment to balance patients' comorbidities against specific TKIs' toxicity profiles are elements that ultimately will guide the type of TKI to use in particular scenarios.

Finally, longer follow-up of patients treated with TKIs is warranted to determine whether TKI therapy can be safely stopped. Current evidence indicates that while long-lasting molecular remissions may occur after discontinuation of imatinib (Rousselot et al. 2007), relapse ensues in a high proportion of patients (Cortes et al. 2004; Rousselot et al. 2007).

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8.5 Conclusion

Nilotinib is a second-generation TKI with enhanced specificity for BCR-ABL kinase when compared with imatinib. In clinical trials, nilotinib has shown considerable activity in Ph-positive CML, which has led to its approval for patients with CML CP or AP after failure of previous treatments including imatinib. Adverse events are generally mild to moderate with nilotinib, and cross-intolerance with imatinib is rare. Specific BCR-ABL mutations may result in decreased activity (e.g., P-loop mutations) or complete insensitivity to nilotinib (i.e., T315I) and should be taken into consideration when selecting nilotinib for the treatment of CML after imatinib failure. Ongoing studies will determine whether nilotinib therapy improves the results obtained with imatinib as frontline therapy for CML.

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Bosutinib

9

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Abstract Bosutinib (SKI-606) is a 7-alkoxy-3-quinolinecarbonitrile, which functions as a dual inhibitor of Src and Abl kinases. In biochemical and proliferation assays, the compound was shown to be active against src family kinases and Bcr-Abl at IC50s of 100 and 90 nM, respectively. The bcr-abl fusion gene product, a consecutively activated tyrosine kinase, which is crucial for the development of chronic myeloid leukaemia (CML), is highly sensitive to bosutinib. Interestingly, distinctly lower concentrations of the dual src/abl inhibitor are required to ablate Bcr-Abl phosphorylation when compared to first-generation tyrosine kinase inhibitor imatinib (IM). Bosutinib is a potent inhibitor of CML cell proliferation *in vitro* and *in vivo* experiments and has demonstrated promising harbouring results in CML patients resistance or intolerance to IM in ongoing phase I/II clinical trials. Remarkably, bosutinib has been found to be capable of overcoming the majority of IM-resistant bcr-abl mutations. A randomised open label phase III clinical study to compare the efficacy of bosutinib and IM in first-line therapy of Ph^{+} chronic phase (CP) CML has recently been initiated. In a phase I/II clinical study with subjects suffering from advanced stages of solid tumours, long-term responses have also been reported. In conclusion, Bosutinib is a promising novel small molecule inhibitor for targeted therapy of CML and solid tumours.

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9.1 Chemical Structure

Bosutinib (SKI-606), 4-[(2, 4-dichloro-5-methoxyphenyl)amino]-6-methoxy-7-[3-(4-methyl-1-piperazinyl)propoxy]-3-quinolinecarbonitrile monohydrate, is a competitive inhibitor of both Src and Abl tyrosine kinases. It was originally synthesised as an inhibitor of the src kinase family and its preparation started with methyl vanillate. The small molecule inhibitor is of low weight (548.46 kDa) and is orally bioavailable (Boschelli et al. 2001).

9.2 Mechanism of Action

Bosutinib is a potent inhibitor of the Src and Abl tyrosine kinases with distinct lower activity

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against few other serine–threonine/tyrosine kinases (Puttini et al. 2006).

9.2.1 SRC Kinase Inhibition

The tyrosine kinase Src is a member of a family of related kinases known as the SFKs (Src family kinases) that share a common structural organisation and function as key regulators of signal transduction pathways triggered by a wide variety of surface receptors, including receptor tyrosine kinases, integrins, G-protein-coupled receptors and antigen receptors (Thomas and Brugge 1997). Various studies and clinical observations point to a key role of Src kinases in malignant cell transformation, tumour progression and metastatic spread as a consequence of changes in protein expression and/or kinase activity (Summy and Gallick 2003; Johnson and Gallick 2007; Li 2008). Indeed, overexpression of src kinases has been detected in several human malignancies, including carcinomas of the breast, lung, colon, oesophagus, skin, pancreas, cervix as well as gastric tissues (Mazurenko et al. 1992; Ottenhoff-Kalff et al. 1992; Verbeek et al. 1996; Lutz et al. 1998; Jallal et al. 2007; Zhang et al. 2007). Bosutinib is capable of inhibiting Src kinase at nanomolar concentrations. Thus, an IC50 of 1.2 nM has been reported in the src enzymatic assay inhibition of Src-dependent protein tyrosine phosphorylation can be detected at comparable or lower concentrations. In addition, bosutinib successfully inhibited growth of Src-transformed fibroblasts and src overexpressing HT29 colon tumours subcutaneously transplanted into athymic nu/nu mice (Compound 31a) (Boschelli et al. 2001).

9.2.2 Abl and bcr-abl Inhibition

c-Abl belongs to an evolutionary conserved family and encodes a ubiquitously expressed non-receptor protein tyrosine kinase localised in the cytoplasm and the nucleus (Laneuville 1995; Pendergast 1996). Oncogenic transformation mediated by different genomic alterations of the c-abl protooncogene results in abnormal cellular development and a suppression of apoptosis (Chung et al. 1996). Bosutinib inhibits bacterially expressed Abl kinase with an IC50 of 1 nM and growth of Abl-MLV-transformed fibroblasts at an IC50 of 90 nM, which is in the range of the IC50 obtained for tyrosine kinase inhibitor imatinib (IM). Tyrosine phosphorylation is by bosutinib in whole cell extracts of Abl-MLV-transformed fibroblasts in a manner consistent with the attained anti-proliferative activity. In addition, incubation of Abl-MLV-transformed Rat 2 fibroblasts with comparable concentrations of bosutinib and IM results in quantitatively similar reductions of tyrosine phosphorylation of cellular proteins (Golas et al. 2003).

In chronic myeloid leukaemia (CML) and Philadelphia chromosome positive (Ph^+) acute lymphocytic leukaemia (ALL), a reciprocal translocation of the proto-oncogene *c-ABL* from chromosome 9 to the breakpoint cluster region *(BCR)* of chromosome 22 results in activation of the c-*ABL* oncogene. This hybrid *BCR-ABL* fusion gene encodes a consecutively activated tyrosine kinase, which phosphorylates a broad range of substrates, many of which are crucial in cellular signal transduction (Sattler and Griffin 2003). In *BCR-ABL* positive CML cells, Bosutinib shows a similar pattern of phosphorylation inhibition like IM. However, while the efficacy between IM and bosutinib as inhibitors of v-abl phosphorylation is within the same range distinct lower concentrations of the dual src/abl inhibitor are required to ablate bcr-abl phosphorylation. In addition, bosutinib virtually abolishes tyrosine phosphorylation of bcr-abl at concentrations between 25 and 50 nM, whereas v-Abl phosphorylation in the immunoprecipitates does not decrease to this extent until a concentration of 200 nM. This indicates that tyrosine phosphorylation of v-Abl is less sensitive to bosutinib than bcr-abl (Golas et al. 2003).

9.3 Bosutinib in Chronic Myeloid Leukaemia (CML)

9.3.1 Preclinical Data

The anti-proliferative activity of bosutinib has been demonstrated in different bcr-abl expressing leukaemia cell lines. In fact, efficacy of bosutinib has been found to be superior to IM with IC50 values ranging from 1 to 20 nM when compared to IM with 51–221 nM, respectively (Golas et al. 2003; Puttini et al. 2006). In addition, bosutinib successfully inhibits growth of IM-resistant human cell lines, such as Lama84R, KCL22R and K562R. In line with these findings, inhibition of proliferation of murine pro-B Ba/F3 cells stably transformed by p210 Bcr-Abl WT or four imatinib-resistant point mutants (D276G, Y253F, E255K and T315I) is more pronounced under bosutinib treatment than under IM therapy. Thus, WT, D276G and Y253F transfectants are inhibited in the low nanomolar range of the dual src/abl kinase inhibitor. However, the T315I bcr-abl mutant requires one to two order higher concentrations of bosutinib when compared with wt bcr-abl cells (Puttini et al. 2006), suggesting inactivity against this mutation since these levels are unlikely to be achieved in patients. In *in vivo* experiments, bosutinib administered at 75 mg/kg twice daily or 150 mg/kg once daily results in a complete regression of human K562 xenografts for up to 40 days (Golas et al. 2003). Remarkably, while IM is unable to eradicate KU812 human tumour xenografts with a relapse rate of 30% at experimental day 8, bosutinib treatment initiated at day 8 and 15 after leukemic cell injection leads to a complete eradication of disease with all animals remaining tumour-free for up to experimental day 210 (Puttini et al. 2006). In mice injected s.c. with Ba/F3 Bcr-Abl+xenografts containing WT or mutant Bcr-Abl (E255K, Y253F and D276G) and treated with bosutinib 1 day after tumour cell injection, the dual src/abl kinase inhibitor induces a statistically significant decrease of tumour growth and a prolonged event-free survival. However, in animals with a delayed initiation of bosutinib therapy, relapse of disease is found in the majority of mice. Furthermore, bosutinib does not influence proliferation of highly IM-resistant T315I xenografts (Puttini et al. 2006).

9.3.2 Clinical Trials

A phase I/II clinical trial in Philadelphia chromosome positive leukaemia is currently being carried out (3160A4-200-WW, www.clinicaltrials.gov). The phase I part of this trial has identified a treatment dose of 500 mg daily, for which clinical efficacy has been demonstrated while a dose-limiting toxicity has been found at 600 mg daily. The phase II part of the study investigating the efficacy and safety of bosutinib in CML patients (pts) with chronic phase (CP), accelerated phase (AP) or blast crisis (BC) or PH$^+$ ALL who have failed IM therapy is currently ongoing.

9.3.2.1 CML Chronic Phase (CP CML)

Preliminary data of IM-resistant or -intolerant CML CP pts treated with bosutinib 500 mg daily have recently been presented at the 2008 annual meeting of the American society of clinical oncology (ASCO) in Chicago (Bruemmendorf et al. 2008). The study population includes IM-resistant or -intolerant patients: IM resistance has been defined by no complete haematologic response (CHR) after 3 months, no cytogenetic response after 6 months and/or no major cytogenetic response (MCR) after 12 months of therapy with an IM dose of ^{3}600 mg daily. Individuals have been considered to be intolerant to IM if toxicities ^{3}grade 3 or persistent grade 2 not

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Table 9.1 Patient characteristics of chronic phase (CP) CML patients

Characteristics	CP CML (*n*>257)
Median age: years (range)	54 (18–91)
Median time from diagnosis: months (range)	52 (0–264)
Mutations[a]	44 (42)
Prior therapy (other than IM)	
Interferon	86 (33)
Dasatinib	60 (23)
Nilotinib	7 (3)
SCT	13 (5)

[a]104 patients tested for mutations

responding to adequate management and/or dose adjustments appear. Patients' characteristics are listed in Table 9.1. In total, at the time of this analysis, 278 patients have been included in the study with 65% sharing resistance and 28% intolerance to IM. In addition to prior treatment with IM, a subset of patients received interferon (86 pts), dasatinib (60 pts), or nilotinib (7 pts). Thirteen patients had undergone stem cell transplantation. At the point of presentation, median duration of bosutinib treatment was 5.3 months (range 0.23–26.4). Haematologic, cytogenetic and molecular responses were evaluated for 59, 77 and 57 IM-resistant patients, respectively. Eighty percent (47 pts) had a CHR, 40% (31 pts) achieved an MCR with a complete cytogenetic response (CCR) in 26% (20 pts). In addition, in 35%, a major molecular response (MMR) was observed of which 18% were complete. Remarkably, in patients with dasatinib resistance, a 100% (11/11) overall haematologic response was found. Response in IM-intolerant patients with no prior exposure to other TKIs than IM revealed a CHR in 80% (20/25), an MCR in 60% (12/20) with a CCR in 45% and MCR in 28% (7/25) with 20% CMR (5/25) of the patients. In 104 individuals, mutation status was assessed before initiation of bosutinib therapy and 18 different mutations were identified. Response analysis by individual mutations revealed haematologic and cytogenetic responses over a broad spectrum of mutations including both P-loop and non-P-loop mutations.

The most common non-haematologic adverse events included diarrhoea, nausea, rash, abdominal pain and vomiting. Diarrhoea was of low grade in the majority of the cases, restricted to the first 2 weeks and typically self-limiting. Fluid retention was observed in only 10% of the patients. The events of haematologic toxicity were moderate with grade 3/4 neutropenia, thrombocytopenia and anaemia in 10, 19 and 6%, respectively.

9.3.2.2 Accelerated Phase (AP CML), Blast Phase (BP CML) and Ph+ ALL

An update of data of an ongoing phase II trial with an open label continuous daily dosing schedule (bosutinib 500 mg/day) in patients with AP (*n*>41) and BP CML (*n*>35) and Ph+ ALL (*n*>21) was presented at the 2008 ASCO Annual Meeting (Gambacorti-Passerini et al. 2008). All patients included were previously treated with IM plus/minus other TKIs and exhibited IM resistance or intolerance. Patients characteristics are summarised in Table 9.2. Haematologic, cytogenetic and molecular response data are shown in Table 9.3. Fifty-seven patients were evaluated for mutational status before bosutinib therapy; 14 different mutations were detected with 18% of the patients harbouring P-Loop mutations and 37% harbouring non-P-Loop mutations. In addition, 14% of the individuals had the highly IM-resistant T315I-mutation while 46% did not have a utation prior to bosutinib therapy. Remarkably, bosutinib has been found to be capable to overcome the majority of mutations except the mutation T315I (see Table 9.4). In accordance to the other clinical trials with bosutinib, most common treatment emerging adverse events included diarrhoea, vomiting, nausea, pyrexia, rash and abdominal pain. Fluid retention occurred in 19% of all patients

Table 9.2 Patient characteristics of CML patients in accelerated phase (AP) or blast crisis (BC) and patients suffering from Philadelphia positive (Ph^+) ALL

Characteristics		AP (n=41)	BC (n=35)	Ph^+ ALL (n=21)	Total (n=98)
Sex: *n* (%)	Male	25 (61)	21 (60)	10 (48)	57 (58)
	Female	16 (39)	14 (40)	11 (52)	41 (42)
Median age: years (range)		53 (18–83)	49 (22–80)	58 (24–81)	51 (18–84)
Median time from diagnosis: months, (range)		71 (0–202)	49 (4–173)	12.5 (2–92)	48 (0–202)
Prior therapy: (other than IM) n (%)					
Interferon		21 (51)	12 (34)	0 (0)	33 (34)
Dasatinib		19 (46)	13 (37)	5 (24)	37 (38)
Nilotinib		8 (20)	5 (14)	1 (5)	14 (14)
SCT		3 (7)	3 (9)	4 (19)	10 (10)

Table 9.3 Response to bosutinb treatment in AP/BC CML and Ph^+ ALL

Response	Patients exposed to imatinib only *n* (%)	Patients exposed to IM andother TKIs *n* (%)
Haematologic response		
Evaluable	25	13
Overall	12 (48)	4 (31)
Complete	12 (48)	3 (23)
Cytogenetic response		
Evaluable	21	19
Major	15 (71)	7 (37)
Complete	9 (43)	3 (16)
Molecular response		
Evaluable	25	20
Major	10 (40)	3 (15)
Complete	4 (16)	1 (5)

Table 9.4 Response by mutation status in AP/BP CML and Ph^+ ALL

Mutation type	Response, *n*/evaluable (%)		
	OHR	CHR	MCR
P-Loop	1/3 (33)	1/3 (33)	2/4 (50)
Non-P-Loop	2/6 (67)	1/6 (17)	3/8 (38)
No mutation	6/15 (40)	6/15 (40)	8/16 (50)

OHR overall haematological response

with 3% being of grade 3 and 4. Two of latter patients suffered from pleural effusion. Haematologic toxicity was detected in the majority of patients and grade 3/4 anaemia was reported in 38%, thrombocytopenia in 70% and neutropenia in 49% of the treated individuals.

A worldwide clinical phase III randomised open label trial to compare the rate of CCRs 1 year after treatment initiation with bosutinib or IM in individuals with newly diagnosed CP Ph+ CML has started recruitment (3160A4-3000-WW, www.clinicaltrials.gov).

9.4 Bosutinib in Solid Tumours

9.4.1 Preclinical Data

9.4.1.1 Breast Cancer

Bosutinib causes a decrease in cell proliferation, migration and invasion of breast cancer cell lines accompanied by an increase of cell-to-cell adhesions and a membrane localisation of beta-catenin, a phosphoprotein that functions as both, a structural component of the cell adhesion/actin cytoskeleton network and a signalling molecule when localised in the nucleus. Analysis of downstream effectors of Src reveals an inhibition of mitogen-activated protein kinase (MAPK) and Akt phosphorylation as well as a reduced phosphorylation of focal adhesion kinase (FAK), proline-rich tyrosine kinase 2 (Pyk2) and Crk-associated substrate (p130Cas). Thus, bosutinib inhibits signalling pathways involved in cell proliferation and malignant transformation as well as tumour cell motility and invasion (Jallal et al. 2007; Vultur et al. 2008). Proliferation of MDA-MB-231 cells inoculated into the mammary fat pads of female BALB/c nu/nu mice is significantly suppressed secondary to bosutinib therapy when compared with control animals receiving only the vehicle solution. In addition, analysis of lungs, liver and spleen have shown a significant reduction of metastatic spread in animals treated with the small molecule inhibitor at a well-tolerated dose.

9.4.1.2 Colorectal Cancer

Bosutinib decreases tumour growths of subcutaneous colorectal cancer xenograft models generated with different tumour cell lines (HT29, Colo205, HCT116 and DLD1) and causes substantial reduction of Src autophosphorylation at Tyr418 (Golas et al. 2005). In addition, it prevents Src-dependent activation of beta-catenin. While, however, protein levels of beta-catenin remain substantially unchanged by bosutinib, a cytosolic/membranous retention of beta-catenin is promoted instead. The bosutinib mediated relocalisation of beta-catenin increases its binding affinity to E-cadherin and adhesion of colorectal cancer cells resulting in a reduced cell motility (Coluccia et al. 2006). A decreased cell motion as well as the ability of bosutinib to reduce VEGF-mediated vascular permeability and tumour cell extravasation combined with the effect of Src inhibition in stromal cells may be responsible for the superior activity of bosutinib *in vivo* when compared with the attained effects in cell culture experiments.

9.4.1.3 Non-Small Cell Lung Cell Cancer (NSCLC)

Immunohistochemical analyses of NSCLC biopsy samples reveal an upregulation of src kinase in 33% of the tumours. In NSCLC cell lines with elevated src kinase activity, treatment with bosutinib induces apoptosis and causes a cleavage of caspase-3 and PARP (Zhang et al. 2007).

9.4.2 Clinical Trials

At the annual ASCO meeting in 2007, Messersmith et al. presented preliminary results of a phase I study (3160A1-100-US) carried out in patients with advanced solid tumours to assess tolerability, safety, pharmacokinetics as well as preliminary anti-tumour activity of bosutinib. The compound was generally well tolerated with predominant gastrointestinal adverse events (see also above). Six patients with breast, colorectal or non-small cell lung cancer were reported to have a stable disease for more than 15 weeks while one subject suffering from pancreatic cancer did not experience profession on disease for more than 52 weeks (Messersmith et al. 2007). Bosutinib is currently evaluated in Phase II clinical trials for the treatment of solid tumours (metastatic breast cancer; Protocol 3160A2-201-WW).

9.5 Conclusion and Future Directions

In conclusion, bosutinib is a novel dual src/abl kinase inhibitor with high activity against IM-resistant CML as well as solid tumours overexpressing src kinase. Its profile of activity is specific with an only limited number of targets outside the abl and src kinase family. Therefore, a reduced number of off-target effects potentially resulting in a decreased toxicity profile can be expected when compared with other second-generation tyrosine kinase inhibitors and maybe also compared with IM (Fig. 9.1). Indeed, presumably

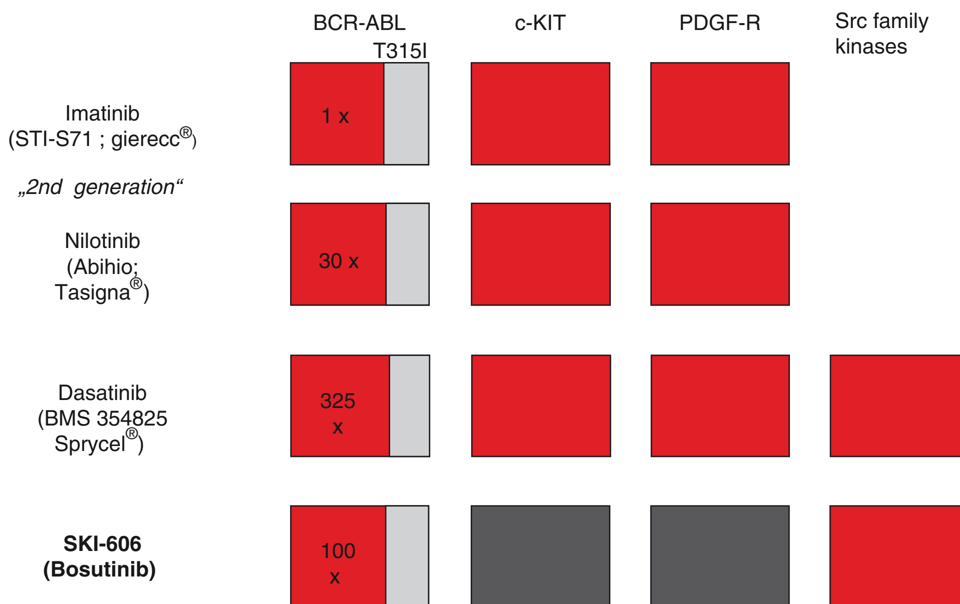

Fig. 9.1 *Activity profile of imatinib and second-generation tyrosine kinase inhibitors.* Bosutinib selectively targets bcr-abl and src kinase with no activity against PDGFR or c-KIT. This new inhibition profile with very limited target effects has been held responsible for the decreased number of side effects observed under bosutinib therapy

PDGFR and/or KIT-mediated side effects such as oedema, muscle cramps, skin rash and inhibition of normal haematopoiesis typically observed under the TKIs used in bcr-abl-positive leukemias may possibly occur less frequent in patients treated with bosutinib. Decreasing toxicity at maintained efficacy is of particular interest once second-generation TKIs make their way into frontline therapy of CP CML.

The reciprocal translocation of chromosome 9 and 22 resulting in the *BCR-ABL* fusion gene is a key event in the malignant transformation of CML. However, different studies point to an important role of SFKs in disease progression. Thus, overexpression and/or activation of Hck and Lyn has been observed during CML progression (Donato et al. 2003). In addition, the transition of CP CML to lymphoid blast crisis (BC) in mice requires the presence of Lyn, Hck and Fgr (Hu et al. 2006). Remarkably, downregulation of Lyn expression by siRNA induces apoptosis in bcr-abl positive blasts, in particular when lymphoid in nature (Ptasznik et al. 2004). With respect to these findings, the dual inhibition of BCR-ABL and SFK seems a promising strategy, which may delay or even potentially avoid the transition of CP CML to (lymphoid) BC. Additional clinical studies will have to be carried out to further clarify role of bosutinib in treatment of CP and advanced CML as well as in solid tumours in the future.

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Part II

Epigenetic Modifiers

10 Decitabine

Michael Daskalakis, Nadja Blagitko-Dorfs,
and Björn Hackanson

Abstract The pyrimidine analogs, 5-azacytidine (azacitidine, Vidaza®) and its deoxy derivative, 5-aza-2'-deoxycytidine (decitabine, Dacogen®), are the most widely used inhibitors of DNA methylation which trigger demethylation leading to a consecutive reactivation of epigenetically silenced tumor suppressor genes in vitro and in vivo.

Although the antileukemic capacity of decitabine has been known for almost 40 years, its therapeutic potential in hematologic malignancies is still under intensive investigation. Multiple clinical trials have shown the promising activity of low-dose decitabine in AML, MDS, CML, and hemoglobinopathies, whereas its efficacy in solid tumors is rather limited.

Clinical responses appear to be induced by both epigenetic alterations and the induction of cell-cycle arrest and/or apoptosis. Recent clinical trials have been investigating new dosing schedules, routes of administration, and combination of decitabine with other agents, including histone deacetylase (HDAC) inhibitors.

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10.1 Introduction

Epigenetic changes play an important role in the development and progression of malignant diseases (Baylin et al. 1998; Baylin 2002; Toyota and Issa 2005). Epigenetic deregulation is defined as changes in gene expression mediated through mechanisms other than alterations in the DNA sequence itself. This includes modifications of core histone proteins, alterations in the methylation status of DNA, and RNA interference (Bhalla 2005).

DNA methylation refers to the addition of a methyl group to a CpG site (Jones and Baylin 2007). These sites cluster together in areas known as CpG islands and are frequently localized in the gene promoter regions. Physiologic and aberrant DNA methylation of gene promoter regions can result in gene silencing. Hypermethylation-induced gene silencing of tumor suppressor and other cancer-related genes is one of the essential mechanisms in tumorigenesis (Esteller 2007, 2008). At present, two hypomethylating agents are widely used: 5-azacytidine (azacitidine, Vidaza®) and 5-aza-2'-deoxycitidine (decitabine, Dacogen®). The rationale for the application of DNA methyltransferase (DNMT) inhibitors is their ability to revert hypermethylation-induced gene silencing and to restore proliferation control and apoptosis sensitivity in the malignant clone (Mund et al. 2006).

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In vitro studies using cell lines have shown a time- and dose-dependent inhibition of proliferation by decitabine. At high concentrations, there is a cytotoxic effect, which may be related, in part, to the synthesis of alkali-labile DNA strands (D'Incalci et al. 1985). At lower concentrations, decitabine acts as a weak inducer of differentiation in myeloid leukemic cell lines (Creusot et al. 1982; Momparler et al. 1985a). Primary leukemic myeloid cells were shown by Pinto et al. (1984) to have a propensity for in vitro granulocytic or monocytic differentiation induced by decitabine.

One of the early studies focused on the p15 tumor suppressor gene, which is often hypermethylated in MDS and AML patients, and can be demethylated and reactivated in patients undergoing decitabine therapy (Daskalakis et al. 2002). Several clinical trials investigating different drug dosing schedules have shown significant clinical benefit in the treatment of patients with MDS and AML (Mund et al. 2005; Yang et al. 2006). In 2007, the drug received FDA approval for the treatment of patients with MDS. Studies are ongoing to identify and develop new generations of DNMT inhibitors (Lyko and Brown 2005; Yoo and Jones 2006). However, none of the currently available new compounds has shown comparable potency in demethylating activity (Chuang et al. 2005; Stresemann et al. 2006) and none of them has reached advanced clinical trials yet.

10.2 Structure and Mechanism of Action

Decitabine was synthesized in 1964 by Sorm and coworkers (Piml and Sorm 1964) as a classical cytostatic agent. It is a ring analog of the pyrimidine nucleoside 2'-deoxycytidine (Fig. 10.1). The drug is widely considered to be unstable, and has therefore been handled with care. Owing to deamination, the plasma half-life of decitabine is approximately 35 min (Rivard et al. 1981). Recently, the in vitro stability of decitabine in a neutral aqueous solution was determined at different temperatures. The results indicated a considerable chemical stability (half-life time of 7 days at 4°C, of 96 h at 20°C, and of 21 h at 37°C), and even storing the solution at room temperature showed an effective inhibition in cytosine methylation (Stresemann and Lyko 2008).

After cellular uptake by a nucleoside-specific transport mechanism (Groeningen et al. 1986; Hubeek et al. 2005), decitabine is phosphorylated by the deoxycytidine kinase and metabolically converted into the active nucleotide for DNA methylation inhibition, 5-aza-2'-deoxycytidine-5'-triphosphate (Momparler and Derse 1979). Following incorporation of decitabine into DNA, newly hypomethylated DNA strands are synthesized (Wilson et al. 1983). Upon incorporation into DNA, decitabine forms a covalent complex with the DNMT Dnmt 1, thereby depleting the cells of its enzymatic activity (Bouchard and Momparler 1983; Santi et al. 1983). Inactivation of the drug occurs through deamination by cytidine deaminase not only in the human liver and spleen, but also in granulocytes, intestinal epithelium, and plasma (Momparler et al. 1997). At equimolar concentrations, decitabine

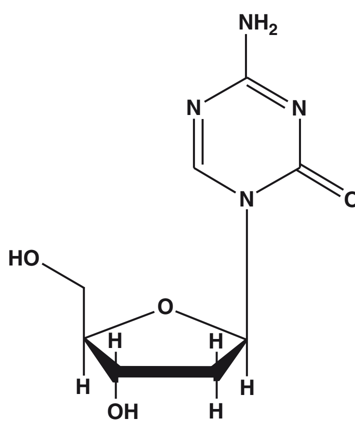

Fig. 10.1 Chemical structure of 5-aza-2'-deoxycytidin (decitabine, Dacogen™)

is at least twice as potent as azacitidine in inhibiting methylation (Creusot et al. 1982).

In a recent study, the pharmacokinetics of low-dose decitabine (15 mg/m^2 i.v. over 3 h, every 8 h, on 3 consecutive days, repeated every 6 weeks) was evaluated in sixteen patients with MDS or AML (Cashen et al. 2008). They concluded that the pharmacokinetics of low-dose decitabine remained unchanged from cycle to cycle. Despite repeated dosing, no systemic accumulation of the drug was observed, and the toxicity profile (transient myelosuppression) was predictable and manageable.

Regarding new compounds, Lavelle and colleagues investigated an oral decitabine formular recently (Lavelle et al. 2007), and Yoo and colleagues developed a decitabine-containing dinucleotide S110 to improve plasma stability (Yoo et al. 2007).

Figure 10.2 schematically summarizes the regulation of gene expression by the promoter methylation status and the regulatory mechanism of a demethylating agent.

10.3 Studies of Single-Agent Decitabine in MDS and Acute Leukemias

5-azacytidine (azacitidine) and 5-aza-2'-deoxycytidine (decitabine) were developed in parallel as antileukemic agents. Antitumor activity of decitabine has been shown in mouse models of acute leukemia (Sorm and Vesely 1968).

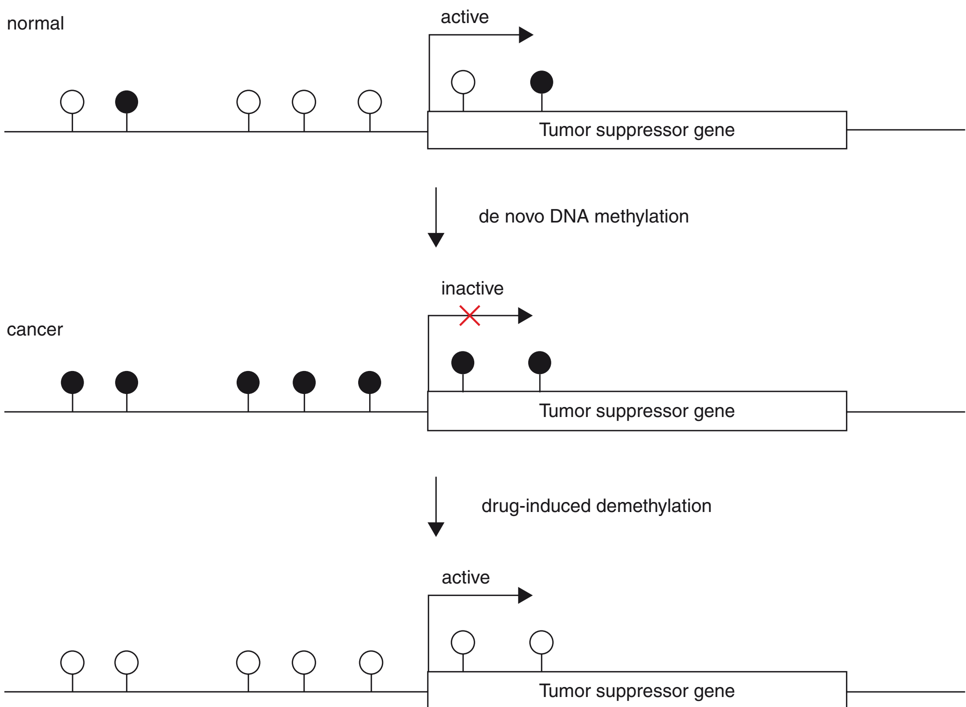

Fig. 10.2 Promoter methylation and gene expression. De novo methylation of the promoter region results in inactivation of the expression of a growth regulatory gene. Demethylating agent, 5-aza-2'-deoxycytidine (decitabine, Dacogen™), reactivates the expression of the epigenetically silenced gene by blocking the DNA methyltransferase Dnmt 1. *Open circles* represent unmethylated CpGs, *closed circles* represent methylated CpGs

Decitabine was first used as a single agent in a phase-I study in children with relapsed or refractory acute leukemia. The dose schedule of decitabine was 0.75–80 mg/kg (Rivard et al. 1981). The authors reported a significant reduction of circulating blasts. In a continuation of this study, Momparler and colleagues (Momparler et al. 1985b) treated 27 children with acute leukemia (21 acute lymphoblastic leukemia (ALL), 6 AML) with a continuous infusion of decitabine at doses ranging from 37 to 80 mg/kg over 36–60 h. In six patients (22%), a CR was induced, and in four patients (15%), a PR was noted. The overall objective response rate was 37% (33% in ALL patients, and 50% in AML patients).

However, the development of the drug at high doses was later discontinued due to the high degree of hematologic (delayed and prolonged myelosuppression) and nonhematologic toxicities (Lübbert 2000).

Pinto and coworkers (Pinto et al. 1989) published the results of a phase I/II trial with low doses of decitabine in elderly AML/MDS patients (median age, 74 years). This group as well as others had shown that in vitro, decitabine induced moderate differentiation in AML cell lines and primary blasts. Twenty-seven patients were treated with decitabine at 15–30 mg/m² for MDS and 30–90 mg/m² for AML, and the drug was given as a 4-h intravenous infusion 3 times daily for 3 days. After a median of two courses, three (15%) of 20 evaluable patients had a CR, and six patients (30%) achieved a PR, resulting in an overall response rate of 45%. The median response duration was 12 weeks (range: 2–58 weeks). The median survival time of the responding patients was 19 weeks (range: 7–64 weeks). A reduction in peripheral blasts ("antileukemic effect"), together with a gradual increase in the absolute numbers of mature cells in the peripheral blood and the bone marrow, compatible with differentiation, were seen.

Since the early 1990s, Wijermans and coworkers have conducted several large low-dose decitabine studies in elderly high-risk MDS patients. In a phase II study, a schedule of continuous infusion (72 h) with total doses of 125–225 mg/m² was used in 29 patients (Wijermans et al. 1997). An overall response rate of 54% with 28% CRs and 25% PRs and a median response duration of 7.3 months was reported. This was followed by a multicenter phase II trial ($n = 66$), also using a 3-day intravenous administration, but this time given over 4 h, 3 times a day; a total dose of 135 mg/m² achieved comparable results (Wijermans et al. 2000). According to the International Prognostic Scoring System (IPSS) three of five patients (60%) with low-risk MDS, 6 of 30 patients (20%) with intermediate, and 10 of 26 patients (38%) with high-risk MDS showed cytogenetic responses; cytogenetic response was associated with longer survival. Complete remissions were associated with cytogenetic remissions (Lübbert et al. 2001). After 2 cycles of decitabine treatment, an improvement in thrombocytopenia was noted in 63% of the MDS patients (van den Bosch et al. 2004).

In a randomized phase II study at the University of Texas M.D. Anderson Cancer Center (MDACC), dose intensity and the subcutaneous route of administration were tested in three schedules of low-dose decitabine in patients with higher risk myelodysplastic syndrome and chronic myelomonocytic leukemia (CMMoL) (Kantarjian et al. 2007a). They used total doses of 100 mg/m² repeated every 4 weeks, with the drug given either as 1-h infusions intravenously over 5 days, in 10 daily infusions over 1 h or 5 daily dosing of subcutaneous drug. By a Bayesian adaptive design, one of the three schedules (5 days, 1-h intravenous schedule) was selected as optimal. The arms scored as inferior contained only 14 and 17 patients, respectively. This study has been criticized for its overall statistical design that may have underestimated the efficacy of the two arms it deemed inferior (Giagounidis 2007), and not noted the well-established preferential response of poor-risk cytogenetic patients, which occurred with

the continuous dosing of decitabine (Rüter et al. 2007). At the ASH meeting 2007, Kantarjian and et al. presented an update of the 5-day schedule from MDACC (Kantarjian et al. 2007b).

Recently, a North American phase III trial of low-dose decitabine was published by Kantarjian and coworkers (Kantarjian et al. 2006). Of the 170 patients randomized, 89 patients received DAC in a schedule of 15 mg/m^2 for a total of 9 doses over 72 h, repeated every 6 weeks, and 81 patients received best supportive care (BSC). The overall response rate in the study group was 30% (9% CR, 8% PR, 13% HI), with a median response duration of 10.3 months when compared with 7% HI in the BSC group. Notably, DAC-treated patients had a prolonged median time to progression to AML or death, when compared with patients receiving BSC, but this was statistically significant only for IPSS high-risk patients (all patients: 12.1 months overall survival (OS) vs. 7.8 months (p>0.16); patients with IPSS int-2/high-risk disease: 12.0 months vs. 6.8 months (p>0.03)). Decitabine-treated patients (IPSS "high-risk" subgroup) had a median time to AML or death of 9.3 months when compared with 2.8 months when receiving BSC alone, which is more than a threefold difference (p>0.01). Quality of life measures were performed, demonstrating a statistically superior quality of life during decitabine treatment regarding global health status, fatigue, and dyspnea. Severe adverse events were noted in 69% of decitabine patients when compared with 56% of patients receiving supportive care. Specifically, 87 and 85% of decitabine treated patients had grade 3/4 neutropenia or thrombocytopenia, respectively, when compared with 50 and 43% in the supportive care arm. Cytopenias appeared to diminish in incidence over the first four courses of decitabine, but still remained frequent. Gastrointestinal toxicities were generally mild and infrequent.

In another study, Lübbert and colleagues addressed the question of whether retreatment with decitabine was beneficial in patients with MDS and disease recurrence. Twenty-two patients were retreated with low-dose decitabine after a median of 11 months (range: 3–27 months) from the last course of initial therapy (Lübbert et al. 2004). Decitabine dosing was administered according to the European and North American phase III trials (15 mg/m^2 i.v. over 4 h, 3 times per day, on 3 consecutive days, repeated every 6 weeks). The overall response rate was 45% (ten patients), including 1 CR, 2 PRs, and 7 HIs. The data also suggested the superiority of prolonged treatment with low-dose decitabine (Rüter et al. 2006).

In MDS patients, even after failure of previous azacitidine treatment, decitabine can still induce responses. Borthakur and colleagues treated 14 patients with low-dose decitabine (20 mg/m^2 i.v. per day over 5 days) after the failure of previously administered azacitidine therapy. The overall response rate was 28% (four patients), including a CR in three patients (21%) and an HI in one patient (7%) (according to the IWG criteria). The median duration of remission was 5.3 months, and the median survival time was 6 months (Borthakur et al. 2008).

Table 10.1 summarizes the clinical phase-I and -II trials of 5-aza-2'-deoxycytidine (decitabine) in acute leukemia and chronic myelogenous leukemia (CML), and Table 10.2 depicts the clinical phase-II and -III trials of decitabine in patients with MDS.

10.4 Combination Treatment in AML, MDS, and Other Diseases

Two epigenetic processes, DNA methylation and histone deacetylation, are linked dynamically together and result in the silencing of genes in cancer (Cameron et al. 1999). Thus, the combination of DNMT inhibitors and histone deacetylase (HDAC) inhibitors in AML, MDS, and other malignancies, might result in enhanced antitumor activity.

Table 10.1 Phase-I and -II trials of 5-aza-2'-deoxycytidine (decitabine) in acute leukemia and chronic myelogenous leukemia

Patients	*n*	Age (range) years	Drug schedule	% ORR (%CR)	Reference
AML, ALL (relapsed/refractory)	22 (7, 15)	9 (2–15)	DAC 0.75–80 mg/kg (12–44 h c.i.v.)	14 (9)	Rivard et al. 1981
AML, ALL (relapsed/refractory)	27 (6, 21)	NA (1–20)	DAC 37–80 mg/kg (36–60 h c.i.v.)	37 (22)	Momparler et al. 1985b
AML, ALL (relapsed/refractory), CML (myeloid blast crisis)	26 (21, 2, 3)	55 (24–74)	DAC 300–500 mg/m^2 (24–120 h c.i.v.)	26 (4)	Debusscher et al. 1990
De novo AML, AML from MDS, CML (not previously treated)	22 (12, 8, 2)	74 (62–83)	DAC 45–180 mg/m^2/day × 3i.v. (>15–60 mg/m^2/4 h t.i.d.)	68 (11)	Zagonel et al. 1990
AML (not previously treated)	12	64 (47–77)	DAC 270–360 mg/m^2/day × 3 i.v.(>90–120 mg/m^2/4 h t.i.d.)	40 (30)	Petti et al. 1993
AML (relapsed/refractory)	5	26 (22–48)	DAC 250–500 mg/m^2 b.i.d. × 6–12	20 (0)	Richel et al. 1991
AML (relapsed/refractory)	11	51 (20–63)	DAC 125–250 mg/m^2 b.i.d. × 12, amsacrine 120 mg/m^2/day × 2	82 (73)	Richel et al. 1991
AML (relapsed/refractory)	49	52 (18–65)	DAC 125–250 mg/m^2 b.i.d. × 12, amsacrine 120 mg/m^2/day × 2	41 (41)	Willemze et al. 1993,1997
AML (relapsed/refractory)	36	57 (NA)	DAC 125–250 mg/m^2 b.i.d. × 12, idarubicin 12 mg/m^2/day × 3	44(44)	Willemze et al. 1993,1997
De novo AML (not previously treated)	8	44 (30–59)	DAC 90 mg/m^2/day × 5, daunorubicin 50 mg/m^2/day × 3	100 (100)	Schwartsmann et al. 1997
CML (myeloid blast crisis)	31	52 (23–78)	DAC 50–100 mg/m^2 b.i.d. × 10	84 (10)	Kantarjian et al. 1997a, 1997b; Sacchi et al. 1998, 1999

CML (accelerated phase)	17	NA	DAC 50–100 mg/m^2 b.i.d. × 10	53 (0)	Kantarjian et al. 1997a, 1997b
CML (Ph-positive) blastic phase (BP), acceler. phase (AP), chronic phase (CP)	123	55 (16–78)	DAC 50–100 mg/m^2 b.i.d. × 10	BP: 28 (10) AP: 55 (23) CP: 63 (13)	Kantarjian et al. 2003
De novo AML, AML from MDS	27	69 (61–87)	DAC 20 mg/m^2 day × 5 i.v.	67(26)	Cashen et al. 2006

ALL acute lymphoblastic leukemia; *AML* acute myeloid leukemia; *b.i.d.* twice daily; *c.i.v.* continuous intravenous infusion; *CML* chronic myeloid leukemia; *CR* complete remission; *DAC* decitabine; *i.v.* intravenous; *MDS* myelodysplastic syndrome; *NA* not available; *t.i.d.* 3 times daily

Table 10.2 Phase-II and -III trials of 5-aza-2'-deoxycytidine (decitabine) in patients with MDS

Reference	Phase	*n*	Age (range) years	Schedule	% responses (% CR/PR/HI)	RD (mo)	OS (mo)
Zagonel et al. 1993	II	10	68	DAC 45 mg/m²/day × 3 (i.v.) (> 15 mg/m²/4 h t.i.d.); DAC 50 mg/m²/day × 3 (c.i.v.)	50 (40/10/0)	11	NA
Wijermans et al. 1997	II	29	72 (58–82)	DAC 40–75 mg/m²/day × 3 (c.i.v.)	54 (28/18/7)	7.3	10.5
Wijermans et al. 2000	II	66	68 (38–84)	DAC 45 mg/m²/day × 3 (i.v.) (> 15 mg/m²/4 h t.i.d.)	49 (20/5/24)	7	15
Wijermans et al. 2002	II	169	70 (38–89)	45–50 mg/m²/day × 3 (i.v.) every 6 wk	49	40 wk	15
Rüter et al. 2006	II (retreatment)	22	71 (51–81)	DAC 45 mg/m²/day × 3 (i.v.) (> 15 mg/m²/4 h t.i.d.)	14 (4/9/32)	4	27, 5[+]
Kantarjian et al 2007a	II	95	66 (39–90)	20 mg/m²/day i.v. q 5d every 4 wk; 20 mg/m²/day s.c. q 5d every 4 wk; 10 mg/m²/day i.v. q 10d every 4 wk q 5d every 4 wk; 10 mg/m²/day i.v. q 10d every 4 wk	73 (34/1/28)	NA	19
Kantarjian et al. 2006	III	89[a]	70 (65–76)	15 mg/m²/3 h t.i.d. q 3d every 6 wk	17 (9/8/13)	10.3	14
Wijermans et al. 2008	III	133	70 (60–90)	DAC 45 mg/m²/day × 3 (i.v.) (> 15 mg/m²/4 h t.i.d.)	34 (13/6/15)	8, 6	10

Table notes:
n number of patients; *CR* complete response; *PR* partial response; *HI* hematologic improvement; *RD* median response duration; *OS* overall survival; *mo* months; *wk* weeks; *DAC* decitabine; *i.v.* intravenous infusion; *c.i.v.* continuous intravenous infusion; *q* for; *t.i.d.* three times daily; *NA* not available; + OS calculated from start of initial decitabine treatment
[a]patients randomized to receive decitabine

In a multicenter phase II study, 51 AML patients (median age, 72 years) received low-dose decitabine treatment (135 mg/m^2 c.i.v. over 72 h) repeated every 6 weeks for up to four courses. Decitabine-sensitive patients received an additional treatment of all-trans retinoic acid during course two of decitabine (at a dose of 45 mg/m^2/day for 28 days) (Lübbert et al. 2005). Additionally, maintenance therapy of decitabine (at a dose of 20 mg/m^2 i.v. over 1 h for 3 days every 8 weeks) was offered to patients completing all four decitabine courses. Four patients (14%) received a CR, and five patients (17%) received a PR (out of 29 evaluable patients). The median survival time was 7.5 months, and the 1-year survival rate was 24%.

Several preclinical studies support the view that pharmacologic targeting of both DNMT and HDAC may result in synergistic anticancer activity (Cameron et al. 1999; Boivin et al. 2002; Garcia-Manero and Issa 2005). As a single agent, valproic acid (VPA) has shown HDAC inhibitory activity in lower risk MDS (Kuendgen et al. 2004). Recently, several phase I/II studies using decitabine or 5-azacytidine, in combination with HDAC inhibitors, have reported encouraging results (Garcia-Manero et al. 2006; Gore et al. 2006; Maslak et al. 2006; Soriano et al. 2007; Blum et al. 2007). Garcia-Manero and et al. (2006) treated 54 patients (AML or high-risk MDS) with low-dose decitabine (15 mg/m^2, i.v., daily for 10 days) administered concomitantly with escalating doses of VPA (50 mg/kg/day, orally, 10 days). The overall response rate (CR and PR) was 22% (12 pts), including 10 (19%) CRs, and 2 (3%) CRs with incomplete platelet recovery. The median duration of remission was 7.2 months, and responding patients showed a median survival time of 15.3 months. Six out of eight responding patients also showed a cytogenetic response. It is notable that responses were achieved after a median of only one cycle of treatment, which is earlier than was expected with decitabine monotherapy.

A smaller phase I study (Blum et al. 2007) of decitabine plus VPA in 25 AML patients could not verify this beneficial effect of VPA, although responses appeared to occur earlier with the combination treatment vs. single agent decitabine, as well. In this trial, 14 patients received decitabine alone to determine the optimal biologic dose, which was 20 mg/m^2/day (d1–10). Only 11 patients received the combination with dose-escalating VPA (d5–21). Dose-limiting encephalopathy occurred in two patients at 25 mg/kg/day. The responses included 2 CRs, 2 CRis, and 2 PRs (ORR 54%), but the authors concluded that VPA might be associated with too much toxicity in this elderly patient population.

In all combination studies, the acetylation status of histone H3 and/or H4 was assessed and increased in response to treatment, but this was not associated with clinical remission. The induction of DNA hypomethylation and global histone (H3 and H4) acetylation was associated with p15 demethylation and gene reactivation. Global DNA methylation was not associated with response (Garcia-Manero et al. 2005, 2006). Gore and et al. (2006) described reversed methylation of the p15 promoter in responding patients, and Garcia-Manero and coworkers found lower pretreatment levels of p15 methylation associated with response.

Recently, the very potent HDAC inhibitor, vorinostat (suberoylanilide hydroxamic acid, SAHA) has been approved by the FDA for treatment of cutaneous T-cell lymphoma (Duvic et al. 2007); clinical studies investigating the combination treatments of this drug with potent DNMT inhibitors like decitabine are still awaited.

Steele and colleagues (2009) investigated the combination of decitabine with a clinically relevant HDAC inhibitor belinostat (PXD 101) in a cisplatin-resistant human ovarian cell line A2780/cp70. This cell line has the hMLH1 and MAGE-A1 genes methylated, and is resistant to cisplatin in vitro and in a xenograft in mice. When compared with decitabine alone, the combined treatment resulted in a higher expression

of hMLH1 and MAGE-A1 in vitro and in vivo, as well as in better cisplatin sensitivity of the xenografts.

Fiskus and colleagues (2009) demonstrated that the combination treatment of decitabine with the pan-HDAC-inhibitor panobinostat (LBH589) targeted multiple epigenetic mechanisms resulting in antileukemic activity in AML cells in vitro.

One obstacle for the curative potential of decitabine is its rapid in vivo inactivation by the enzyme cytidine deaminase, the key enzyme in catabolism of cytosine nucleoside analogs. Besides azacytidine and decitabine, the therapeutic activity of a third cytosine nucleoside analog, pyrimidin-2-one β-ribofuranoside (zebularine, Zeb) has been investigated (Driscoll et al. 1991; Zhou et al. 2002; Marquez et al. 2005; Stresemann et al. 2006; Flotho et al. 2009). Zebularine demonstrated antitumor (Cheng et al. 2003, 2004) and antileukemic activity (Herranz et al. 2006; Scott et al. 2007), and is also a competitive inhibitor of the enzyme cytidine deaminase (Laliberté et al. 1992). It has been shown in murine and human leukemic cell lines, that the inhibition of cytidine deaminase by zebularine enhanced the antineoplastic activity of decitabine (Lemaire et al. 2005, 2009).

Further clinical studies exploring the combinations of decitabine with different HDAC inhibitors or Zebularine are needed to achieve higher antitumor efficacy, find optimal dose schedules, and overcome acquired drug resistance due to DNA methylation and gene silencing.

10.5 Decitabine as a Preparative Agent in Allogeneic Stem Cell Transplantation

Giralt and colleagues at the M.D. Anderson Cancer Center conducted two studies to determine the safety and efficacy of decitabine, as a single agent or as part of a combination preparative regimen, prior to allogeneic peripheral-blood progenitor cell (PBPC) transplantation. The phase I/II study protocol of single-agent decitabine prior to allogeneic PBPC retransfusion included patients with early relapse after a first allogeneic bone-marrow transplantation for AML (nine patients), CML (three patients), and ALL (two patients), respectively (Giralt et al. 1997, 1998a; Ravandi et al. 2001). Median age was 35 years (range: 22–50 years), the median time from previous transplant was 177 days (range: 49–540 days), and all but two patients had active disease at the time of decitabine administration. Median time to relapse from initial transplantation was 6 months (range: 2–31 months). Eight patients received a total dose of 1,000 mg/m^2 decitabine (administered over 5 days), three patients received 1,250 mg/m^2, and three a total dose of 1,500 mg/m^2. In the first three patients, donor cell transfusion was performed 2 days after the end of decitabine infusion. Eight of 14 patients achieved either a complete remission or a remission with partial hematologic recovery. Median time to neutrophil recovery was 13 days from donor cell transfusion (range: 10–30 days). Two of the first three patients treated required additional donor cell transfusions at day 21, because of lack of neutrophil recovery. In subsequent patients, donor stem cells were given 5 days after the last decitabine infusion, and no delayed engraftment has been observed in the responding patients. Four patients developed acute graft-vs.-host disease (GVHD) ≥ grade 2. Two patients developed grade 2 hepatic toxicity, one in association with GVHD.

Seven of eight patients achieving a response relapsed. Median survival of all patients was 190 days (range: 11–1,245 + days). In patients achieving a response, the median disease-free survival was 60 days (range: 29–368 + days). Overall, five patients were alive 176–1,245 + days after transplantation, with two of them in remission. Six patients died from progressive disease, two patients from infection, and one patient from GVHD and associated infections. Thus, salvage

therapy with decitabine followed by allogeneic progenitor cell support is feasible, well-tolerated, and induces CRs or hematologic responses in the majority of patients. Combinations with other treatment modalities, such as donor-lymphocyte infusions or cytokine administrations, are necessary to prolong these remissions.

The other phase I/II study protocol included 23 leukemia patients prior to *first* allogeneic transplantation (Giralt et al. 1998b; de Lima et al. 2003), 12 patients with high-risk AML, one patient with CMMoL, one with ALL, and nine patients with CML. At the time of study enrollment, 20 patients had advanced phases of their disease, only two patients with AML were in first remission and one patient had a late chronic phase CML. The conditioning regimen contained decitabine intravenously (3 dose levels: 400 mg/m^2 (ten patients), 600 mg/m^2 (eight patients) and 800 mg/m^2 (five patients)), in combination with cyclophosphamide (100 mg/kg (four patients) or 120 mg/kg (19 patients)) and busulfan (12 mg/kg, orally). In four patients transplanted according to this protocol (one with AML, three with CML in accelerated phase), the median time from leukemia diagnosis to transplantation was 5 months (range: 4–25 months). The patients' median age was 36 years (range: 18–53 years). The first four patients achieved decitabine on days 7 and 8, but because of delayed neutrophil recovery beyond day 21 in three of them, the drug was given on days 11 and 10 for the subsequent patients. Two of the three patients who had graft failure needed additional donor stem cell transfusions at days 28 and 21, and recovered on days 31 and 37, respectively. Twenty-one patients achieved disease remission (CR or remission with partial hematologic recovery). Six of the 23 patients (26%) were alive at a median of 3.3 years from transplantation. The median survival was 17.2 months, and the disease-free survival 8.9 months. Treatment-related mortality rate at 3 years was 35%, nine patients died of disease recurrence, four patients of chronic GVHD, three patients of infections, and one patient of acute GVHD. No decitabine dose-limiting toxicity was documented, and no dose-response correlation of the three decitabine dose levels was observed.

Recently, a study group from the MD Anderson Cancer Center in Houston (De Padua Silva et al. 2009) and a group from the University Hospital of Freiburg, Dept. of Hematology and Oncology, (Lübbert et al. 2009) again demonstrated the feasibility of an allogeneic transplantation after a hypomethylating therapy with low-dose decitabine in MDS and AML patients. The outcome of 17 MDS patients with a median age of 55.5 years (range: 36–66 years) and a study group of 15 patients with MDS ($n=10$) or AML ($n=5$) with a median age of 69 years (range: 60–75 years) were reported, respectively. All the patients received a Fludarabin-containing conditioning regimen. The group from MD Anderson treated eight patients with a myeloablative regimen, and nine patients with a reduced-intensity conditioning regimen, whereas all patients at the University Hospital of Freiburg received a reduced-intensity conditioning FBM regimen containing fludarabine, BCNU and melphalan. At the MD Anderson Cancer Center, after a median follow-up of 12 months (range: 3–35 months), 11 patients were alive (eight in CR and 2 in PD) and six patients have died (four due to disease progression, one from acute GVHD and one from sepsis). Successful engraftment was achieved in 14/15 patients at the University Hospital of Freiburg. All 14 patients received a CR, with a median duration of 5 months (range: 1–51+ months). Six of these 14 patients are alive, four patients died from relapse, and four from treatment-related complications while in CR. No increased toxicity due to the low-dose decitabine treatment has been described in neither group. Both groups suggested that decitabine may be a valid alternative to standard chemotherapy, especially in elderly MDS/AML patients, and that this drug might improve the outcome of allogeneic transplant in MDS and AML.

10.6 Immunomodulation with Decitabine

By adopting different strategies, cancer cells are able to evade the host's immune surveillance. Epigenetic changes play an important role in the down-regulation of different antigens (i.e., tumor antigens, HLA class I antigens, co-stimulatory molecules), which are involved in the immunological recognition of neoplastic cells. Among the different groups of cancer-associated antigens, cancer testis antigens (CTA) are attracting growing interest as immunotherapeutic targets. CTAs represent a family of immunogenic proteins (i.e., MAGE, BAGE, GAGE, LAGE, and NY-ESO-1) expressed in various neoplastically transformed cells, and are absent in normal tissues, except testis and placenta. CTAs are recognized by autologous, cytotoxic CD8(+) T-lymphocytes (CTL) (Knuth et al. 2000; Bodey 2002). The critical factor for regulating CTA expression in cancer cells is the promoter methylation status, suggesting epigenetic drugs as therapeutic modulators for CTA expression in neoplastic cells (De Smet et al. 1999; Sigalotti et al. 2002). Several studies have shown that decitabine was consistently able to induce or up-regulate CTA expression in solid tumors and hematologic malignancies, resulting in their efficient immunological recognition and lysis by CTA-specific CTLs (Weber et al. 1994; Coral et al. 2002; Gattei et al 2005; Sigalotti et al. 2004, 2005; Natsume et al. 2008).

Regarding combination therapy, in vitro studies with the sequential application of decitabine and the HDACi depsipeptide showed a modest increase of CTA-expression, but no significant enhancement of cancer cell recognition by CTA-specific CTLs, when compared with decitabine treatment alone (Weiser et al 2001a, 2001b). Recently, Oi and colleagues demonstrated that the anticonvulsant VPA, also acting as an HDACi, enhanced the expression of NY-ESO-1 in synergy with decitabine. They observed a significant DNA demethylation, histone H3 Lys9 demethylation, and acetylation (Oi et al. 2009).

Besides regulation of CTA expression, decitabine has been shown to increase expression of HLA class I antigens and other co-stimulatory molecules, and to restore antigen-specific CTL response in vitro and in vivo (Coral et al. 1999, 2006; Calabro et al. 2005; Guo et al. 2006).

Schrump and colleagues (2006) designed a phase I study to investigate the maximum tolerated dose (MTD) of decitabine in patients with thoracic malignancies. No objective responses were observed, but re-expression of CTAs (NY-ESO-I, MAGE-A3, p16) was seen and antibodies to NY-ESO-I were detected post treatment in three patients exhibiting expression of NY-ESO-I in their tumor tissues. The MTD of decitabine in this investigation was 60–75 mg/m^2.

The observation that decitabine induces or up-regulates expression of different CTAs and might be able to generate anti-CTA-antibodies gave impact for investigations of combined chemo immunotherapeutic regimens. Gollob and colleagues (2006) conducted a phase I trial in patients with melanoma or renal cell carcinoma to investigate the efficacy of a decitabine pretreatment followed by a high-dose IL2 immunotherapy. Decitabine was administered subcutaneously (daily for 5 days on weeks 1 and 2 of a 12-week cycle, escalating dosage from 0.1 to 0.25 mg/kg) before high-dose intravenous bolus IL-2. Major responses were observed in 3 of 13 melanoma patients (23%; one complete response and two partial responses). Regarding decitabine immunomodulation, up-regulation as well as down-regulation of genes which may favor the IL2 immunotherapy was observed.

Cotreatment of decitabine with gefitinib in two breast cancer cell lines (CAMA 1 and MB453) resulted in re-expression of the epidermal growth factor receptor (EGFR) and showed a significant effect on the induction of apoptosis in these cell lines (Montero et al. 2006).

After HLA-matched stem cell transplantation (SCT), a graft-vs.-tumor effect is observed to

lead to regression of metastatic solid tumors, but is often associated with GVHD. GVHD is directed mainly against the multiple mismatched minor histocompatibility antigens (mHags). HA-1 is currently the best characterized mHag and particularly attractive for immunotherapy because of its restricted expression on hematopoietic cells and on some solid tumors, but not on cells involved during GVHD (Hambach et al. 2008). Decitabine treatment of HA-1 negative tumor cells induced HA-1 expression and sensitized them for recognition by HA-1-specific cytotoxic T-lymphocytes (Hambach et al. 2009).

Thus, epigenetic drugs are gaining increasing attention on account of their immunomodulatory activity. The decitabine-induced expression of epigenetically silenced CTAs or mHags in hematopoietic malignancies and solid tumors presents a new strategy for a tumor immunochemotherapy or as an immunotherapeutic target after allogeneic SCT.

10.7 Decitabine Treatment in Other Diseases

10.7.1 Activity of Decitabine in Patients with Acute Lymphoblastic Leukemia

The prognosis of refractory ALL after allogeneic hematopoietic SCT is very poor and novel therapeutic agents are warranted to change this situation. Hypermethylation of multiple promoter-associated CpG islands has been frequently identified in ALL patients (Garcia-Manero et al. 2002a, b, 2003; Roman-Gomez et al. 2004, 2007a; Hoshino et al. 2007). Moreover, aberrant methylation is associated with poor prognosis in childhood and adult ALL (Shen et al. 2003; Roman-Gomez et al. 2007b; Kuang et al. 2008; Garcia-Manero et al. 2009).

Treatment of ALL-derived cell lines with decitabine results in hypomethylation and re-expression of putative tumor suppressor genes (Yang et al. 2005).

Residual DNA methylation at the time of morphologic remission of ALL might predict for worse prognosis. Based on this hypothesis, Yang and colleagues (2009) analyzed the methylation levels of p73, p15, and p57(KIP2) at the time of initial remission in 199 patients with ALL (Philadelphia chromosome-negative and MLL-negative). In 123 patients, pretreatment samples were available and were compared with the remission ones. The presence of residual p73 methylation was associated with a significantly lower disease-free survival and OS.

Recently, Yanez and colleagues (2009) reported a successful induction therapy with decitabine in a 10-year-old girl with refractory common B-cell ALL. At diagnosis, the leukemic blast immunophenotyping revealed no additional expression of T-cell or myeloid markers. The ALL was negative for BCR-ABL and TEL-AML1 translocations, as well as for MLL rearrangements in molecular studies. The ALL was classified into standard risk and treated with the Spanish protocol for ALL (SHOP-99). One year after the end of treatment, the first relapse occurred and the girl was treated with the same induction and consolidation therapy followed by an autologous peripheral blood SCT (PBSCT) in second CR. The second relapse was again treated with the same drugs in low doses, followed by an allogeneic haploidentical PBSCT from her mother in third CR. The patient never developed a GVHD. Upon third relapse, the decision was made to treat the girl with decitabine (15 mg/m^2, 3 h continuous infusion, 3 times per day for 3 days) combined with dexamethasone (20 mg/m^2 i.v. days 1–4, 10 mg/m^2 i.v. day 5, 5 mg/m^2 i.v. day 6, and 2.5 mg/m^2 i.v. day 7). Again, a CR was achieved and the girl underwent a second allogeneic PBSCT from her mother. Cyclosporine alone was used for GVHD prophylaxis, and the girl developed an extensive chronic GVHD and remained in CR 8 months after PBSCT.

Based on these data, further results of additional phase I and II trials investigating the activity of decitabine in patients with recurrent or refractory ALL are pending.

10.7.2 Activity of Decitabine in Patients with Chronic Myeloid Leukemia

Prognosis is very poor once CML accelerates and progresses from the chronic phase to blast crisis. If myeloid blast crisis develops, however, remission rates with standard AML-induction chemotherapy regimens are below 20% (Kantarjian et al. 1993).

From 1986 to 1997, at the M.D. Anderson Cancer Center, decitabine (500–1,000 mg/m^2 administered over 5 days) was used in the treatment of 31 patients with CML in myeloid blast crisis (Sacchi et al. 1998) and 17 patients with CML in accelerated phase (Kantarjian et al. 1997a, b). Objective responses were observed in 26% of the patients in blast crisis, with a median survival of 29 weeks. One of the patients with a complete response had suppression of the Philadelphia chromosome (Ph) to 25% of metaphases. Of the 17 patients with accelerated phase of CML, nine (53%) responded to high-dose decitabine, with six patients achieving a second chronic phase of CML, and two showing Ph suppression. Prolonged myelosuppression was the major side effect, but no severe nonhematological toxicity was observed. During these studies, the initial decitabine dose of 1,000 mg/m^2 was, therefore, subsequently lowered to 750 mg/m^2 and 500 mg/m^2 in order to ameliorate the prolonged myelosuppression.

During the same period, a total of 162 adult patients with the diagnosis of CML in nonlymphoid blastic phase (BP) were treated at the MD Anderson Cancer Center either with intensive chemotherapy ($n=90$), with other single agents ($n=41$), or with decitabine ($n=31$), as described earlier. Decitabine showed similar objective response rates when compared with intensive chemotherapy (26 vs. 28%), whereas other single agents showed objective response rates of 7%. The median survival times were 29 weeks with decitabine, 21 weeks with intensive chemotherapy, and 22 weeks with other agents. In elderly patients, survival was significantly better with decitabine when compared with the other treatment options. Decitabine treatment revealed as an independent significant prognostic factor for survival (Sacchi et al. 1999).

Another study investigated the toxicity and activity of decitabine in all different phases of CML (Kantarjian et al. 2003). One hundred and twenty-three patients with Ph-positive CML (64 blastic, 51 accelerated, 8 chronic) and seven patients with Ph-negative CML were treated. In the first 13 patients, decitabine was given at 100 mg/m^2 over 6 h c.i.v. every 12 h for 5 days (1,000 mg/m^2 per course). Owing to severe prolonged myelosuppression, the dose of decitabine was reduced to 75 mg/m^2 in the next 33 patients and to 50 mg/m^2 in the remaining 84 patients. Objective response rates were 28% ($n=18$) in patients with BP (six patients achieved a complete hematologic response (CHR), two achieved a partial hematologic response (PHR), seven achieved a hematologic improvement (HI), and three returned to a second chronic phase (second CP)), 55% ($n=28$) in patients with accelerated phase (12 CHR, 10 PHR, 3 HI, and 3 s CP), and 63% ($n=5$) in the chronic-phase patients. Four of the seven patients with Ph-negative CML had objective responses (57%). The estimated 3-year survival rate was less than 5% for patients with BP and 27% for patients in accelerated phase. The only significant toxicity was severe and prolonged myelosuppression; febrile episodes have been described in 37% of the patients and documented infections in 34%.

In a phase II study, 35 patients with imatinib-resistant CML (12 pts in chronic phase, 17 pts in accelerated phase, and 6 pts in BP) received low-dose decitabine treatment (15 mg/m^2 i.v. over 1 h daily) for a total of 10 doses (Issa et al.

2005). Thirty-four of the patients achieved a CHR and 20% of the patients a PHR, resulting in an overall response rate of 54%. Complete cytogenetic responses were seen in six patients.

In another phase II trial, the combination of low-dose decitabine and imatinib was investigated (Oki et al. 2007). Low-dose decitabine (15 mg/m^2 i.v. for 1 h daily over 5 days a week for 2 weeks) and imatinib (600 mg/day, orally) were given in combination to 28 patients with CML (25 of whom had already known imatinib resistance). Nine patients (32%) achieved CHR, one patient (4%) achieved PHR, and two patients (7%) had HI. Five patients (18%) achieved major cytogenetic responses and three patients minor (11%) cytogenetic responses.

Decitabine appears to have significant activity in all CML phases; additional studies should evaluate decitabine dose schedules in Tyrosine-Kinase-Inhibitor (TKI)-resistant CML, as well as combinations of decitabine and TKIs in different CML phases.

10.7.3 Activity of Decitabine in Patients with Idiopathic Myelofibrosis (IMF)

Several investigators have shown that epigenetic changes are implicated in the pathogenesis of IMF (Wang et al. 2002; Jones et al. 2004; Bogani et al. 2008).

In a recently published small phase II study, Odenike and coworkers (2006) demonstrated the activity of decitabine given subcutaneously (0.3 mg/kg/day on days 1–5 and days 8–12; cycles were repeated every 6 weeks) in seven patients with myelofibrosis. One patient achieved a hematological CR; a second patient showed a HI in platelet counts and a decrease in peripheral circulating blasts.

Shi and colleagues (2007) investigated the treatment of peripheral blood CD34+ cells from patients with IMF with a sequential therapy of decitabine followed by a HDAC inhibitor, trichostatin A. Exposure to this combination therapy resulted in a reduction in the number of circulating malignant hematopoietic progenitor cells (HPCs). The proportion of JAK2V617F-positive HPCs was reduced in 83% of the IMF patients. In two JAK2V617F-negative IMF patients, the sequential treatment led to a dramatic reduction in the number of HPCs that contained chromosomal abnormalities. Treatment of CD34+ cells of IMF patients resulted in the up-regulation of CXCR4 expression restoring the migration of these CD34+ cells in response to SDF-1alpha.

Recently, Danilov and colleagues (2009) reported on the successful decitabine treatment in a 65-year-old white male with symptomatic transfusion-dependent IMF. Despite treatment with hydroxycarbamide and lenalidomide, the patient's transfusion requirement increased further. The peripheral blood smear and a repeat bone marrow biopsy showed a disease progression, whereas the cytogenetic studies showed no evidence for t(9;22) and for the JAK2V617F-mutation. After 6 cycles of decitabine treatment (20 mg/m^2 for 5 days every 4 weeks), the patient's splenomegaly as well as his transfusion requirement decreased markedly. He tolerated the decitabine treatment well, and there was a significant increase up to 90% of his performance status.

10.7.4 Clinical Effects of Decitabine in Severe β-Thalassemia and Sickle Cell Disease

The regulation of globin gene expression in β-thalassemia and sickle cell disease (SCD) has been investigated during the past decades. Epigenetic mechanisms, such as DNA methylation and histone modifications, play an essential role in globin gene expression (Lavelle 2004; Fathallah and Atweh 2006; Saunthararajah 2007; Fathallah 2008). Increasing, the γ-globin chain synthesis leads to a lower globin chain imbalance in β-thalassemia. In SCD, the reactivation of HbF expression interferes with the

polymerization of the sickle hemoglobin. In phase I/II studies in patients with hemoglobin disorders, decitabine has shown a clinically significant increase in total and fetal hemoglobin, as well as reduced red cell adhesion and endothelial damage (Koshy et al. 2000; DeSimone et al. 2002; Saunthararajah et al. 2003, 2008). The main toxicity was transient neutropenia.

Clinical studies investigating the efficacy of hypomethylating agents in children with SCD are still lacking (Trompeter and Roberts 2009). Therefore, larger and longer term studies in adults are needed to confirm the short-term promising results and safety aspects of decitabine, as well as to investigate new drug formulations, like an oral formulation of decitabine, which has been tested in animal models recently (Lavelle et al. 2007).

Saunthararajah and colleagues (2008) described an additional benefit by adding erythropoietin to the decitabine treatment in four adult SCD patients. Choi and colleagues (2007) showed in vitro (HL-60 and T24 cancer cell lines) that hydroxycarbamide inhibits the hypomethylating activity of decitabine when given in combination, suggesting that these drugs should be used sequentially rather than concurrently.

Phase III studies are awaited to further evaluate the activity of decitabine in patients with SCD and to investigate different drug combinations modifying this severe chronic anemia (Wang 2008).

10.7.5 Efficacy of Decitabine in Patients with Solid Tumors

Antitumor activity of decitabine has also been explored in phase I/II trials of patients with previously treated and metastasized solid tumors.

In the first phase I study of decitabine, Rivard and coworkers (1981) also included three children with metastasized solid tumors. Patients received decitabine by continuous infusion; however, even at doses achieving antileukemic effects, only very limited antitumor effects were seen.

A phase I study of 21 adult patients with advanced solid tumors was performed by Pinedo and colleagues using three 1-h infusions of decitabine over 24 h repeated every 3–6 weeks, with doses ranging between 50 mg/m^2 and 300 mg/m^2 per course (Groeningen et al. 1986). The dose-limiting toxicity was myelosuppression. A partial response at the highest dose level was noted in one patient with metastasized, undifferentiated carcinoma of the ethmoidal sinus, with complete regression of the local recurrence and marked decrease in the size of a single abdominal lymph node. No disease recurrence was observed in a 15-month follow-up after resection of the metastasis and continuation of decitabine treatment. The remaining 20 patients had either short-lasting stabilization or progression of disease.

Using three 1-h infusions of 75 mg/m^2 over 24 h, repeated every 5 weeks, the EORTC Early Clinical Trials Cooperative Group conducted a total of seven phase II trials with 153 patients with solid tumors. Tumor types included malignant melanoma (20), head and neck cancer (29), colorectal carcinoma (43), testicular cancer (15), renal cell carcinoma (16), non-small cell lung cancer (NSCLC) (8), ovarian cancer (27), and cervical cancer (17). Evaluable responses were seen in 133 patients. Of these, only two patients showed a PR; one of the 17 patients with malignant melanoma and one of the eight patients with NSCLC (Dodion et al. 1990). The same schedule was also used in a phase II study in cervical cancer by Vermorken and coworkers (1991).

Two studies have revised the concept of activity of DNA methylation inhibitors in solid tumors. Momparler et al. (1997) performed a phase I/II study of decitabine in patients with metastatic NSCLC. Fifteen patients were treated with a single 8-h intravenous infusion of 200–660 mg/m^2 of decitabine. The major side effect was hematopoietic toxicity, necessitating a 5–6-week recovery period before the next treatment course. Steady-state plasma concentrations of decitabine were measured in some of the patients and were in the same

range as those resulting in in vitro demethylation of a gene hypermethylated in lung cancer cell lines. The median survival of these patients was 6.7 months, and three patients survived beyond 15 months, which suggests some clinical activity of relatively high doses of decitabine with an 8-h infusion schedule against metastatic lung cancer.

The second study was performed in 14 patients with progressive metastatic prostate cancer that was refractory to standard treatment (Thibault et al. 1998). The 1-h infusion schedule of 75 mg/m^2 i.v. 3 times daily, which had also been used in the EORTC studies in solid tumors, was applied. Treatment courses were repeated every 5–8 weeks to allow full recovery from myelotoxicity. Stable disease, with time to progression of over 10 weeks, was noted in two of 12 patients with evaluable responses, both of them of African descent.

Regarding combination treatment of decitabine plus chemotherapy in solid tumor patients, Schwartsmann and colleagues (2000) conducted a phase I trial ($n=21$) with four dose-escalations of decitabine (45, 67, 90–120 mg/m^2, respectively) and a fixed dose of cisplatin (33 mg/m^2). Both agents were given on days 1–3, and the cycle was repeated every 3 weeks. The recommended doses for phase II trials in good and poor-risk patients were 90 and 67 mg/m^2, respectively. Decitabine was given as a 2-h intravenous infusion, followed immediately by intravenous cisplatin after the end of decitabine infusion. One patient with cervical cancer showed a short-lasting partial response, and two minor regressions were described in a patient with NSCLC and cervical cancer, respectively. Based on these data, 14 patients with inoperable NSCLC were included in a phase II trial using the decitabine dose of 67 mg/m^2 and cisplatin with 33 mg/m^2. Only three minor responses could be described, and the median survival of the patients was 15 weeks (range: 4–38 weeks).

The same combination regimen was used in another phase II trial in patients with advanced squamous cell carcinoma of the cervix. Twenty-one of the 25 patients selected were evaluable for tumor response, and eight patients (38.1%) achieved a partial response, whereas stable disease was documented in five patients (23.8%). In nonirradiated metastatic tumor sites, the objective response rate was more frequent. The median progression-free survival was 16 weeks, and the median OS was 19 weeks (Pohlmann et al. 2002).

Samlowski and colleagues (2005) evaluated a 7-day continuous intravenous infusion of decitabine in ten patients with refractory solid tumors. Decitabine was administered at 2 mg/m^2 as a continuous infusion for 168 h. Transient grade III/IV neutropenia and grade II thrombocytopenia were the only toxicities that have been observed. By measuring the promoter-specific and global DNA methylation in peripheral-blood cells before and after treatment, they were able to show significant MAGE-1 promoter hypomethylation and significant genomic DNA hypomethylation just 14 days after the start of the treatment. Genomic DNA methylation reverted to baseline levels by 28–35 days after the start of the treatment. Regarding the clinical effects, no objective responses were seen and seven patients progressed after two cycles of decitabine treatment. One woman with metastatic ovarian cancer received four cycles of decitabine and had stable disease for 4 months before progression, and one man with renal carcinoma had stable disease for 6 months before progression.

Venturelli and colleagues (2007) investigated the monotherapy of decitabine or vorinostat (suberoylanilide hydroxamic acid, SAHA), and the combination treatment of both the drugs in human hepatocellular carcinoma (HCC)-derived cell lines and in primary human hepatocytes (PHH). They examined the cell lines for cellular damage, proliferation, histone acetylation pattern, and DNA methylation, and investigated in vivo activities in a xenograft hepatoma model. The combined treatment showed more enhanced antiproliferative effects in HCC-derived cells,

whereas in PHH, there was no impairment of cellular integrity. The authors suggested this combination therapy to be considered for further investigation in clinical trials.

In a recent phase I and pharmacodynamic trial, Appleton and colleagues demonstrated the feasibility of combining decitabine with carboplatin at a dose and at a schedule that was able to cause epigenetic changes in peripheral blood cells, buccal cells, and tumor biopsies (Appleton et al. 2007). They used two separate dose escalations of decitabine, the first with carboplatin fixed at area under the concentration time curve (AUC) 5, and the second with carboplatin at AUC 6. Thirty patients were assessable for response, and one patient with melanoma showed a partial response and three other patients had stable disease. The comparison of methylation status showed significantly less hypomethylation in tumor tissue after decitabine treatment, when compared with the buccal cells and peripheral blood mononuclear cells. There was only a 3% decrease of methylation in the tumor biopsies, which was below the threshold for resensitization to chemotherapy in other preclinical studies (Plumb et al. 2000). Finally, they recommended a phase II dosing of decitabine of 90 mg/m^2 administered on day 1 followed by carboplatin AUC 6 on day 8 of a 28-day cycle. A phase II trial investigating the combination of decitabine and carboplatin in ovarian cancer is ongoing.

Recent in vitro studies have shown a significant increase in susceptibility of transitional cell carcinoma cell lines to cisplatin with the addition of decitabine (Shang et al. 2008).

In summary, most of the trials investigating the activity of decitabine alone or in combination with chemotherapeutics in solid tumors have shown rather disappointing response rates. However, one should keep in mind that in MDS and AML, low-dose, multiday, and multicycle decitabine treatment schedules are achieving promising response rates, whereas many of the solid tumor studies used dose and schedule combinations which were till recently being recognized as suboptimal treatment. Future studies in solid tumors should investigate low-dose schedules of decitabine, by keeping toxicity low and allowing a longer exposure to the drug (several days, several cycles). This might lead to immunomodulatory effects, making the solid tumor cells more sensitive to regular chemotherapeutics.

10.8 Conclusion and Future Perspectives

Epigenetic drugs represent a major improvement in our treatment modalities against hematologic malignancies. As one of the most widely used demethylating single agents, decitabine has shown significant activity in MDS and AML at lower dose schedules in many clinical trials. To improve this activity in MDS and AML, further clinical studies investigating combination regimens with other agents, such as HDAC inhibitors, growth factors, cytarabine, and other chemotherapeutic agents, are needed. While DNMT inhibitors are already an integral part of the treatment, especially, in high-risk disease, HDAC inhibitors, particularly the newer substances, have until now mainly been tested in phase I trials.

Further investigation of decitabine in CML after treatment failure of TKI, as well as the use in ALL and other hematologic diseases should be undertaken.

For all epigenetic drugs, the optimal treatment schedules still have to be determined in monotherapy, and also in combination regimens. Further analysis is needed to determine which patients, e.g., cytogenetic subgroups, benefit most from the different approaches.

The development of new compounds with more potent hypomethylating activity is of clinical importance. And hopefully, these new compounds and drug combinations will translate into longer OS.

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5-Azacytidine/Azacitidine

11

Antonia Müller and Mareike Florek

Abstract: 5-Azacytidine is a pyrimidine nucleoside analog that has been discovered more than 40 years ago. Despite remarkable responses in the treatment of acute myeloid leukemias in the 1970s no earlier than 2004 has this agent been approved by the US FDA for the treatment of all subtypes of myelodysplatic syndromes (MDS). For the first time a drug was proven to alter the natural course of MDS, as demonstrated in three clinical trials conducted by the CALG B. Complete remission rates ranged between 10–17%, and more recently, a significant survival benefit for MDS patients treated with 5-Azacytidine could be established. The antineoplastic activity is due to incorporation into RNA with disruption of RNA metabolism, and inhibition of DNA methylation.

Strategies of combining epigenetic manipulation with other 'new' drugs aim at increasing the efficacy of the hypomethylating agents. Particularly histone deacetylase inhibitors have been deemed useful therapeutic partners, and preliminary results are promising.

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11.1 Introduction: 5-Azacytidine – Novel or Almost Historic?

5-Azacytidine (Azacitidine, Vidaza®; Pharmion Corporation) and its deoxy derivative 5-Aza-2'-Deoxycitidine are pyrimidine nucleoside analogs that were chemically synthesized and characterized in Czechoslovakia by František Šorm and his fellow investigators in the 1960s (Sorm et al. 1964). Shortly after, 5-Azacytidine was also microbiologically isolated from the fermentation beer of *Streptoverticillium ladakanus* (Hanka et al. 1966). The new agent was shown to possess a wide range of biological effects, including antimicrobial, abortive, mutagenic, leukopenic, immunosuppressive, cytotoxic, and antineoplastic activity (von Hoff et al. 1976). Particular interest was evoked when the antitumor activity in leukemia cell lines was established (Li et al. 1970a; Sorm and Vesely 1964), and in vivo studies confirmed the cytotoxicity by demonstrating a prolonged survival of mice with L1210 leukemias after administration of 5-Azacytidine (Presant et al. 1975).

In the 1970s, the clinical efficacy of 5-Azacytidine was tested in a wide range of solid tumors and leukemias. While treatment results in solid tumors were generally discouraging, consistent antitumor activity was observed in patients

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with acute myeloid leukemia (AML) and myelodysplastic syndromes (MDS) (von Hoff et al. 1976).

Because of its capability to induce differentiation of erythroleukemic cells in vitro, and thereby alter malignant cell phenotypes (Jones and Taylor 1980), 5-Azacytidine was tested in hemoglobinopathies. Treatment attempts in sickle cell anemia patients demonstrated an increase of fetal hemoglobin (HbF) and a decline of HbS. Overall, this resulted in a slight increase in total hemoglobin concentration and less hemolysis. Patients with *β*-thalassemia showed increased γ-chain synthesis with significantly improved erythropoiesis, but this was not invariably accompanied by an enhanced hemoglobin concentration (Stamatoyannopoulos 1992).

In 1980, Jones and Taylor discovered that 5-Azacytidine could inhibit DNA methyltransferase activity (Jones and Taylor 1980). Accordingly, when it was recognized that aberrant DNA methylation is critically involved in the development of many neoplasias, including MDS (Aoki et al. 2000; Herman and Baylin 2003; Jones and Baylin 2002), the demethylating agents attracted new attention. Since there was no satisfactory treatment option for the majority of MDS patients, and early studies had shown responses to 5-Azacytidine, MDS offered an appropriate disease entity to study the effects of the drug on DNA methylation, gene transcription, and cell differentiation. In the mid-1980s, trials exploring the usefulness of 5-Azacytidine in MDS were initiated (Silverman 2001; Silverman et al. 1993), and confirmed the clinical efficacy, safety, a reduced risk for transformation into AML, and a beneficial impact on quality of life over best supportive care (Kornblith et al. 2002; Silverman et al. 2002). Thus, in May 2004 5-Azacytidine was approved by the US Food and Drug Administration (FDA), and it has been postulated that it should be considered as the first-line therapy for MDS (Kaminskas et al. 2005a, b).

11.2 Agent

11.2.1 Chemical Structure

5-Azacytidine (4-amino-1-*β*-D-ribofuranosyl 1-1,3,5 triazine-2-one or 1-*β*-D-ribofuranosyl-5-azacytosine; $C_8H_{12}N_4O_5$; molecular weight 244) is a ring analog of the naturally occurring pyrimidine nucleoside cytidine, from which it differs only by a nitrogen in place of the fifth carbon (Bergy and Herr 1966) (Fig. 11.1).

5-Azacytidine is a white to off-white solid that is stable at 25°C, not light sensitive, sparingly soluble in water, and unstable when reconstituted in aqueous solution. Hydrolytic degradation results in a 21–36% loss over 8 h at 25–30°C, and a 2–3% loss at 5°C (Kaminskas et al. 2005a).

11.2.2 Mode of Action

Two main mechanisms of antineoplastic action have been identified for 5-Azacytidine, namely the capacity to [a] incorporate directly into RNA

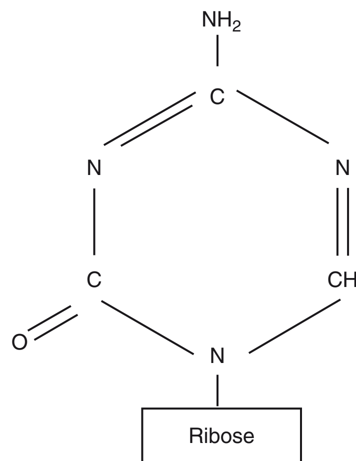

Fig. 11.1 Molecular structure of 5-Azacytidine

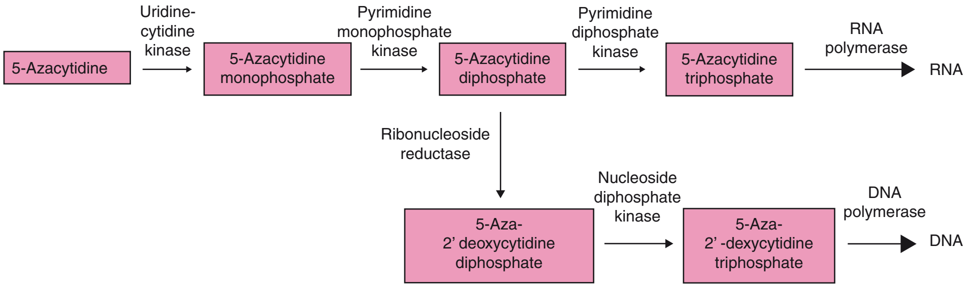

Fig. 11.2 Two main pathways of intracellular 5-Azacytidine metabolism: a) Phosphorylation and incorporation into RNA with disruption of RNA metabolism. b) Phosphorylation and metabolization by ribonucleotide reductase (RNR) with subsequent integration into DNA

with subsequent disruption of RNA metabolism, and [b] to inhibit DNA methylation (Fig. 11.2).

Upon uptake into cells, 5-Azacytidine is phosphorylated by several kinases (uridine cytidine-, pyrimidine monophosphate-, and diphosphate-kinases) to 5-aza-2'deoxycytidine di-, and subsequently triphosphate. The ribose structure needs to be metabolized by ribonucleotide reductase (RNR) first to be integrated into DNA. Incorporation of 5-Azacytidine triphosphate into RNA occurs directly, and causes a disruption of nuclear and cytoplasmic RNA metabolism with subsequent inhibition of protein synthesis (Li et al. 1970b).

The second mechanism of action is the inhibition of DNA methylation by trapping DNA methyltransferases It inhibits the enzyme in its progression along the DNA duplex and functionally depletes it from the cell.. In general, DNA methylation refers to the addition of a methyl group to the cytosine residue of a CpG site. So-called CpG islands are genomic regions with a high frequency of CG dinucleotides (the "p" in CpG notation refers to the phosphodiester bond between the cytosine and the guanine), that are typically located in proximity to promoters. The degree of methylation of CpG islands plays a role in the control of gene transcription. Usually, fully methylated sites are associated with suppression of gene expression, while hypo-methylated or unmethylated CpG islands are linked to active transcription. Forming a tight-binding complex 5-Azacytidine irreversibly binds to methyltransferase. It inhibits the enzyme in its progression along the DNA duplex and functionally depletes it from the cell. Consequently, unmethylated DNA can lead to the transcription of previously quiescent genes (Jones and Taylor 1981; Taylor and Jones 1982). Already minor substitution of cytosine residues (~0.3%) suffices to inactivate more than 95% of methyltransferase activity in the cell (Creusot et al. 1982).

DNA (hyper-)methylation is believed to contribute to cancer initiation and progression by silencing tumor suppressor genes and other genes critical for regulation of the cell cycle, cell growth, differentiation, and apoptosis (Bird 1996). In this setting, 5-Azacytidine can restore the expression of potentially important genes by demethylating such pathologically hypermethylated regions (Silverman 2001).

In addition to these modes of action, 5-Azacytidine has been reported to inhibit DNA histone acetylation, another regulatory mechanism in gene silencing (Chiurazzi et al. 1999).

11.3 Pharmacology

11.3.1 Route of Administration and Dosage

Both subcutaneous (s.c.) and intravenous (i.v.) routes have been tested. The bioavailability of s.c. relative to i.v. 5-Azacytidine is approximately 89%.

Oral formulations are under investigation (Garcia-Manero et al. 2008b). However, their development has been hampered by the instability of the compound.

The recommended starting dose of 5-Azacytidine is 75 mg/m^2 s.c. or i.v. daily for 7 days, regardless of baseline hematology laboratory values. Cycles should be repeated every 28 days. Dose adjustments for consecutive cycles should be based on nadir counts and bone marrow (BM) cellularity. An increase of the dose to 100 mg/m^2 can be considered if no beneficial effect is notable after two cycles and the drug is tolerated well. Response may be delayed. Therefore, therapy should be given at least for 4–5 cycles, and may be continued as long as the positive effect persists. The maximal dose tolerated has not been formally determined, however, some early trials used daily i.v. doses of 150–200 mg/m^2 for 5 days, and even a maximum dose of 500 mg/m^2 has been given on a weekly basis to patients with solid tumors (von Hoff and Slavik 1977).

11.3.2 Bioavailability, Half-Life, Elimination, Drug–Drug Interactions

5-Azacytidine is rapidly absorbed after s.c. administration with peak plasma concentrations after 30 min and a mean half-life of 41 ± 8 min. Urinary excretion is the primary route of elimination of 5-Azacitidine and its metabolites, but presumably additional extrarenal pathways for elimination, such as deamination in the liver and spleen, exist (Chabot et al. 1983; Stresemann and Lyko 2008). Fecal excretion appears to be minimal (Marcucci et al. 2005).

A formal assessment of drug–drug interactions has not been conducted as of yet, and whether the metabolism of 5-Azacytidine is affected by microsomal enzyme inducers or inhibitors remains to be clarified (Marcucci et al. 2005). Of note, ribonucleotide reductase (RNR), which metabolizes 5-Azacytidine into the active metabolite, is a known target of hydroxyurea. Therefore, concomitant use of both drugs could lead to diminished efficacy of 5-Azacytidine and should be avoided, while sequential administration may be possible (Choi et al. 2007).

11.3.3 Safety, Side Effects, and Contraindications

Dose toxicology studies have identified BM, liver, kidney, and lymphoid tissues as target organs of 5-Azacytidine (Kaminskas et al. 2005a). While treatment-related mortality has been consistently low (<1%), severe adverse side effects have been reported in about 60% of 5-Azacytidine patients, largely consisting of thrombocytopenia, febrile neutropenia, fever, and pneumonia. However, safety evaluations from the MDS trials were somewhat confounded by the pathophysiology of this disease, which overlaps to a great extent with the toxicities of the drug. Other common, less serious side effects included injection site events, arthralgia, cough, dyspnea, headache, weakness, dizziness, and insomnia. Usually, adverse events occurred within the first two therapy cycles, and diminished subsequently. Discontinuation of 5-Azacytidine was mostly related to myelosuppression (Silverman et al. 2002).

11.3.3.1 Hematologic Toxicity/Myelosuppression

Several phase I studies pointed to leukopenia (<1,500/µL) as a dose-limiting toxicity. Leukopenia was dose-related, and occurred in approximately 34% of patients, while thrombocytopenia (<100,000/µL) has been reported in 17%. Only 4% of patients had greater than 3 g/dL drop in hemoglobin directly attributable to the drug (von Hoff

et al. 1976). In the CALG B trials myelosuppression, either BM hypoplasia or drug-related cytopenias, required dose reduction in a third of patients (Silverman et al. 1993).

11.3.3.2 Gastrointestinal Toxicity

Initially, the usefulness of 5-Azacytidine was hampered by severe nausea and vomiting that accompanied rapid i.v. injection of the instable drug, and constituted a dose-limiting toxicity. Only when it became clear that the half-life of the drug at 25°C in buffered solutions is significantly longer, infusion time could be extended and gastrointestinal toxicity could be reduced (Israili et al. 1976; Vogler et al. 1976). Split doses and s.c. administration decreased side effects slightly, and subsequent continuous infusions (150 mg/m^2/day over 120 h with fresh preparations every 4 h) were able to further improve tolerability (Lomen et al. 1975). Newer trials using the current standard dose regimen (75 mg/m^2/day over 7 days every 28 days) still revealed mild to moderate nausea and/or vomiting as the most common side effect (63%) (Silverman et al. 1993). Diarrhea occurred in a substantial proportion of patients, but was not dose-limiting (von Hoff et al. 1976).

11.3.3.3 Hepatotoxicity

Liver damage appears to be unrelated to dose, schedule, or route of administration. Liver function abnormalities have been documented in 7–16% of patients receiving 5-Azacytidine, particularly those with preexisting liver cirrhosis (Silverman et al. 2002; von Hoff et al. 1976). Hepatic comas have been reported in context with extensive liver metastasis and low baseline serum albumin levels (0.5%) (Bellet et al. 1973). Therefore, 5-Azacytidine is contraindicated in patients with advanced hepatic malignancies.

11.3.3.4 Nephrotoxicity

Renal dysfunction and failure have been observed in patients receiving combination chemotherapy and/or those with renal impairment (von Hoff et al. 1976), particularly during periods of sepsis and hypotension (Silverman et al. 2002). Since 5-Azacytidine and its metabolites are primarily excreted by the kidneys, dosage needs to be adjusted based on renal function and serum electrolytes, especially in elderly patients and those with renal impairment.

11.3.3.5 Other

Sporadically, in <3% of patients neuromuscular side effects have been documented. The myalgicasthenic syndrome involved generalized muscle tenderness, weakness, and lethargy. Other unspecific symptoms reported were fever (6%), skin rash (2%), stomatitis, phlebitis, and hypotension (von Hoff et al. 1976).

11.3.3.6 Teratogenicity

In animal studies, 5-Azacytidine caused congenital malformations, and was found to be mutagenic, clastogenic, and embryotoxic when females were dosed during gestation. It decreased male fertility, and preconception treatment of male rodents resulted in increased embryofetal loss in mated untreated females. Therefore, women should avoid pregnancy and men should not father a child while receiving treatment with 5-Azacytidine (Kaminskas et al. 2005b).

11.4 Clinical Use of 5-Azacytidine

11.4.1 Early Studies

Clinical trials using 5-Azacytidine were begun in 1967 in Europe, and in the late-1970s in the United States. They investigated the application of the new agent in patients with metastatic cancer and leukemia refractory to conventional chemotherapies. In 1976, von Hoff et al. provided a comprehensive review on all preclinical and clinical data before 1975, encompassing a total of 58 protocols and reviews received at the Investigational Drug Branch of the National Institutes of Health. Eight hundred and twenty one patients who had been treated with 5-Azacytidine, 207 of them within phase I studies were re-evaluated. Promisingly, 5-Azacytidine revealed consistent antitumor activity in patients with AML and achieved an overall response rate of 36% (20% complete remission (CR), 16% partial remission (PR)) in 200 patients with AML refractory to previous treatment. The median duration of remission was between 15 and 19 weeks (von Hoff et al. 1976). Although these remarkable responses verified the activity of 5-Azacytidine as a single agent in AML, it never advanced through the U.S. FDA review process as a leukemia therapy.

Both European and US experiences with 5-Azacytidine for treatment of patients with acute lymphatic leukemia (ALL), chronic myeloid leukemia (CML), and multiple myeloma were disappointing. While only sporadic responses were achieved in ALL, no favorable outcome was denoted in CML or multiple myeloma. Also, unambiguously, clinical results of 5-Azacytidine for treatment of solid tumors were not encouraging at all. Few favorable responses occurred, usually of poor quality, short duration, and associated with significant toxicity (von Hoff et al. 1976).

11.4.2 5-Azacytidine in Myelodysplastic Syndromes (MDS)

MDS comprise a group of several chronic diseases of BM dysfunction characterized by decreased counts of one or more blood cell types and/or an increase in BM blasts. Progression of MDS is often characterized by transformation into AML. Because of their advanced age, most MDS patients are not candidates for aggressive curative therapies, such as high-dose chemotherapy and hematopoietic cell transplantation, and previously had no treatment option superior to best supportive care (Kaminskas et al. 2005a). The discovery of the hypermethylation of the $p15^{INK4B}$ gene in MDS (Christiansen et al. 2003; Uchida et al. 1997) provided the rationale for the effectiveness of 5-Azacytidine in MDS that had been observed already in the early trials of the 1970s and 1980s.

When 5-Azacytidine was the first therapeutic agent approved by the FDA in May 2004 for the treatment of all subtypes of MDS, this decision based on three clinical studies conducted by the Cancer and Leukemia Group B (CALG B). Two of them were single-armed (Silverman 2001; Silverman et al. 1993), the third was a controlled, randomized phase III trial (Silverman et al. 2002). 5-Azacytidine was administered at a starting dose of 75 mg/m^2/day for 7 days with 28-day cycles in all three trials.

The first phase II study (protocol 8421) of the CALG B was initiated in 1984, and 49% of 43 patients receiving 5-Azacytidine as a continuous i.v. infusion responded (12% CR, 25% PR, 12% improved). The overall survival was 13.3 months, median duration of remission was 14.7 months, requirement of RBC transfusions was eliminated in 82%, and the agent was tolerated well (Silverman et al. 1993). In the second trial (protocol 8921), 5-Azacytidine given as a s.c. bolus injection to 67 patients with high-risk MDS yielded comparable results with regard to safety and efficacy (overall response rate 53%;

CR 12%, PR 15%, 27% improved) (Silverman 2001). "Improved" described a response with less than 50% restoration of normal blood counts and less than 50% decreases in RBC or platelet transfusion requirements.

These promising results prompted the initiation of a randomized, open-label phase III trial (protocol 9221) to compare the clinical efficacy and impact on quality of life of 5-Azacytidine with best supportive care. In 191 patients with MDS, an overall response (CR plus PR) was achieved in about 16% (11.8–18.8%), while there was no response in the control group. This difference between both arms was statistically highly significant. Incidence of transformation to AML decreased, and time to AML or death was considerably longer for the 5-Azacytidine group than for the supportive care group (median 21 vs. 12 months, respectively) (Silverman et al. 2002). Generally, overall response rates were similar in females and males, all age groups, and all MDS subtypes. The most evident benefit of a response was in transfusion-dependent patients, which lost their need for transfusion of RBC and/or platelets during CR or PR. Indicators of response, such as decrease in blast counts or increase in platelets, hemoglobin or WBC were observed by the fifth treatment cycle in more than 90% of patients, and responses were long lasting (Silverman et al. 2006).

As part of the phase III study, quality of life was assessed. In contrast to patients in the supportive care group, those receiving 5-Azacytidine experienced significant improvement in fatigue, dyspnea, physical function, positive effect, and psychological distress, which coupled with greater treatment response and delayed time to transformation to AML or death (Kornblith et al. 2002).

In 2006, Silverman et al. reanalyzed the combined data from all 270 patients treated within the three CALG B trials and confirmed previous results of a CR rate of 10–17%. The median number of cycles to first response was three, and 90% of responses were seen by cycle 6. The overall response rate for patients with the retrospective diagnosis of an AML, according to the new WHO classification system, was encouraging. While the CR rate of 9% was rather moderate (vs. 0% in the observation group), the prolongation in survival time to 19.3 months when compared with 12.9 months without specific treatment was remarkable (Silverman et al. 2006).

The CALG B trials could not establish a survival benefit or delay in progression to AML as a treatment benefit for 5-Azacytidine because crossover of observation arm patients to treatment was permitted, and because the trial was insufficiently powered to detect a survival benefit. Just recently, the large, international, randomized Phase III 5-Azacytidine survival trial (AZA-001) demonstrated a statistically significant superior median overall survival (24.4 months) for 179 higher-risk MDS patients receiving 5-Azacytidine as compared to 179 patients under conventional care regimen (15 months). The 5-Azacytidine group experienced a twofold overall survival advantage of 51 vs. 26% at 2 years, a median time to AML transformation or death of 13 months vs. 7.6 months, and a CR and PR rate of 29 vs. 12% when compared with the conventional care group (Fenaux et al. 2007). Subgroup analysis of the AZA-001 trial revealed a particularly favorable response to 5-Azacytidine in patients with alterations of chromosome 7, while those with del 5q had a poorer response rate than other high-risk MDS and AML patients (Fenaux et al. 2007; Itzykson et al. 2008). 5-Azacytidine was comparably effective in patients who had been enrolled into the trial as FAB RAEB-T, but now meet the WHO criteria for AML (Fenaux et al. 2008). Likewise, the subpopulation of elderly high-risk MDS patients (over 75 years) tolerated the agent well and experienced a significantly prolonged 2-year overall survival and reduced risk of death (Seymour et al. 2008). In all patients who responded to 5-Azacytidine (51% CR, PR,

or hematologic improvement), the median number of cycles to first response was 3 (range 1–22), 81, and 90% of patients achieved a first response by cycle 6 and 9, respectively (Silverman et al. 2008a).

Due to the results of the AZA-001 trial, the FDA authorization was extended in August 2008, and 5-Azacytidine became the first drug approved to reflect unprecedented overall survival in patients with higher-risk MDS.

11.4.3 New Therapeutic Approaches

Combination strategies: Aiming at increasing response rates, regimens combining epigenetic manipulation with other conventional therapies are under development. Since alterations in histones, specifically hypoacetylation plus subsequent chromatin remodeling, are also involved in regulating transcription and gene silencing, histone deacetylase (HDAC) inhibitors have been deemed useful combination partners for methyltransferase inhibitors (Griffiths and Gore 2008; Silverman 2001). Indeed, phase I and early phase II trials using 5-Azacytidine and HDAC inhibitors reported overall responses in the range from 20 to 50% in patients with AML and higher-risk MDS. Time to response has been consistently one course (1–3) and appeared to be faster than the four to six courses required with single-agent 5-Azacytidine for primary response (Kuendgen et al. 2004; Soriano et al. 2007). A phase I trial testing 5-Azacytidine plus Vorinostat showed that the synergistic effect is sequence-dependent, requiring exposure to the demethylating agent first followed by the HDAC inhibitor. The combination was well tolerated in repetitive cycles, active in both lower and higher risk MDS/AML patients with a response superior to 5-Azacytidine alone (Silverman et al. 2008b). Studies, such as the combination of 5-Azacytidine plus SNDX-275 (former MS275) or MGCD0103 (both selective HDAC inhibitors with activity in AML and potentially MDS (Beckers et al. 2007)) are ongoing (Garcia-Manero et al. 2008a; Gore and Hermes-DeSantis 2008).

Other therapeutic approaches used the combination of 5-Azacytidine with Thalidomide or Lenalidomide for treatment of MDS and AML, and were able to demonstrate that this combination was effective and well tolerated without additive toxicity (phase I) (Raza et al. 2008; Sekeres et al. 2008).

Of particular interest appears the combination with the anti-CD33 immunotoxin gemtuzumab ozogamicin (Mylotarg®), which is active as a single agent in AML (Larson et al. 2002; Sievers et al. 2001). Preliminary results on the combined approach of 5-Azacytidine, gemtuzumab ozogamicin, and hydroxyurea revealed a CR rate of 70% in 20 elderly patients with AML (Nand et al. 2008).

Maintenance therapy: Another conceivable application for hypomethylating agents is the continuous use of low doses as a maintenance strategy in patients with remissions after more intensive types of therapy. The significance of this approach has not been fully determined, since preliminary results from ongoing trials did not have appropriate control groups (Grövdal et al. 2008), or did not provide information on the relapse rate in their cohort of patients with refractory AML/MDS after hematopoietic cell transplantation (De Lima et al. 2008). However, the safety profile of the maintenance regimen was confirmed.

11.5 Future Perspective, Experimental Studies, and Conclusion

Although 5-Azacytidine is not exactly novel anymore, it recently obtained new attention, when its beneficial influence on survival of patients with high-risk MDS became evident.

For the first time, an agent was proven to alter the natural course of this disease. Particularly, the combination with other new drugs, such as HDAC inhibitors, raises hope that MDS can ultimately be controlled more successfully.

Next to pathological hypermethylation, also physiologically methylated CpG sites may be targets for methyltransferase inhibitors. Recent data imply that a stable and permanent expression of the human transcription factor forkhead box P3 (FOXP3) in regulatory T cells might be crucial in the prevention of autoimmunity, allergy, and graft-vs.-host disease after allogeneic hematopoietic cell transplantation. Apparently, DNA methylation patterns in the FOXP3 locus can serve to discriminate $FOXP3^+$ regulatory T cells with suppressive capacity (demethylated promotor region) from activated $FOXP3^+$ conventional T cells that lack this protective function (methylated CpG sites) (Floess et al. 2007; Polansky et al. 2008). Experimental data support the hypothesis that inhibition of methyltransferases stabilizes transcription of FOXP3, which could result in an increase of suppressive $FOXP3^+$ regulatory T cells. It is conceivable that in the future demethylating agents might be used as a therapeutic tool for immune modulation (Nagar et al. 2008).

Lately, further potential capabilities of 5-Azacytidine in unexpected off-target fields have been discovered, and are subject of preclinical investigations. 5-Azacytidine appears to inhibit the antiapoptotic transcription factor NFκB, presumably via decreased phosphorylation of the upstream regulator IKKα/β, which results in apoptosis and cell death (Fabre et al. 2008). Moreover, a significant inhibition of Wnt-signaling by 5-Azacytidine has been proposed. The Wnt-signaling pathway is known to be involved in oncogene expression in AML (Chim et al. 2007; Jawad et al. 2008).

Almost for several decades, 5-Azacytidine remained fairly unobtrusive in the rank of second-line and salvage treatment options for AML and MDS. Just recently, when the significance of epigenetics in tumorigenesis became clear, 5-Azacytidine also attracted great attention. It was the first drug approved for the treatment of all categories of MDS and its survival benefit was confirmed. The combination of hypomethylating agents with other drugs is promising. Moreover, innovative strategies involving off-target sites of 5-Azacytidine hold a broad potential for cancer therapy as well as immune modulation.

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Part III

Cell Cycle Inhibitors

Bortezomib 12

Hermann Einsele

Abstract The ubiquitin-mediated degradation of proteins in numerous cellular processes, such as turnover and quality control of proteins, cell cycle and apoptosis, transcription and cell signaling, immune response and antigen presentation, and inflammation and development makes the ubiquitin-proteosome systems a very interesting target for various therapeutic interventions.

Proteosome inhibitors were first synthesized as tools to probe the function and specificity of this particle's proteolytic activities. Most synthetic inhibitors rely on a peptide base, which mimics a protein substrate, attached at a COOH terminal "warhead". Notable warheads include boronic acids, such as Bortezomib and epoxyketones, such as carfilzomib. A variety of natural products also inhibit the proteosome that are not peptide-based, most notably lactacystin, that is related to NPI-0052, or salinosporamide A, another inhibitor in clinical trials.

The possibility that proteosome inhibitors could be drug candidates was considered after studies showed that they induced apoptosis in leukemic cell lines.

The first proteasome inhibitor in clinical application, Bortezomib showed activity in non small cell lung and androgen-independent prostate carcinoma, as well as MM and mantle cell and follicular Non-Hodgkin's lymphoma. It is now lincensed for the treatment of newly diagnosed as well as relapsed/progressive MM and has had a major impact on the improvement in the treatment of MM in the last few years.

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12.1 Mode of Action

Intracellular protein degradation occurs predominantly through the proteasome, which is the final common effector for the ubiquitin-dependent and most of the ubiquitin-independent proteolysis (Ciechanover 2005; Demartino and Gillette 2007). In eukaryotic cells, substrate proteins are subjected to polyubiquitination by the ubiquitin-conjugating system.

Thus, the ubiquitin–proteasome system is the major proteolytic system for nonlysosomal degradation of cellular proteins. In 2004, Aaron Ciechanover, Avram Hershko, and Irvine Rose were awarded the Nobel Prize in Chemistry for their original description of ubiquitin-mediated degradation of proteins. This recognition emphasizes the exceptional biological significance of

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ubiquitin-mediated degradation of proteins in numerous cellular processes, such as turnover and quality control of proteins (Goldberg 2003), cell cycle and apoptosis (Jesenberger and Jentsch 2002; Naujokat and Hoffmann 2002), transcription and cell signaling (Fuchs 2002; Muratani and Tansey 2003; Wang 2003; Wojcikiewicz 2004), immune response and antigen presentation (Kloetzel and Ossendorp 2004), and inflammation and development (Bowerman and Kurz 2006; Elliott et al. 2003).

There are two major steps in the ubiquitin–proteasome degradation pathway: proteins are first covalently tagged with polyubiquitin chains and are then degraded by the 26 S proteasome.

The initial step of the degradation pathway, ubiquitination of proteins, involves covalent binding of the ubiquitin molecule to a lysine residue (Lys) of the substrate (Hershko and Ciechanover 1998). Ubiquitination proceeds along a cascade of enzymatic reactions, in which ubiquitin is first activated by the ubiquitin-activating enzyme E1. With the aid of an E2 ubiquitin-conjugating enzyme, ubiquitin is then covalently linked to the substrate by a specific ubiquitin ligase, E3. There is only one E1 enzyme known, several E2s, and multiple classes of E3s (Ciechanover et al. 2000). For polyubiquitination, activated ubiquitin moieties are processively transferred to the Lys 48 residue of the previously conjugated ubiquitin molecule. This process may be facilitated by a polyubiquitination factor, E4 (Koegl et al. 1999).

The second step in the degradation pathway involves proteolysis of ubiquitinated proteins by the 26 S proteasome. The 26 S proteasome is a multicatalytic protease that consists of a 20 S catalytic core and two 19 S regulatory complexes. The 19 S complex is composed of at least 19 different subunits that form a lid- and base-like structure. The lid component provides binding sites for polyubiquitinated substrates, and also contains a deubiquitinating activity, which allows recycling of ubiquitin moieties on substrate degradation. The base component consists of six ATPases that form a ring-like structure and interact with the 20 S proteolytic core (Groll et al. 2000). These ATPases have chaperone function and are required for the ATP-dependent unfolding of substrates (Braun et al. 1999; Strickland et al. 2000) and the opening of the narrow entry pore of the 20 S proteasome (Kohler et al. 2001). The unfolded polypeptide chain is then inserted into the catalytic chamber of the 20 S core complex, where it is degraded into peptides of 3–25 amino acids length. Both 20 S proteasomes and their 19 S regulators are localized within the cytoplasm and nucleus of the cell, and also have been co-localized with the membranes of the endoplasmic reticulum (ER) (Brooks et al. 2000).

In addition to the 19 S regulatory complexes, several components of the ubiquitin system such as polyubiquitin-binding proteins – which presumably serve as substrate shuttles, several deubiquitinating enzymes – which are required for the removal and recycling of ubiquitin moieties, and also several E3 ligases, are associated with the 20 S proteasome, which suggests that the two steps, ubiquitination and degradation, are closely coupled and controlled within the cell (Schmidt et al. 2005).

Proteasome inhibitors were first synthesized as tools to probe the function and specificity of this particle's proteolytic activities (Vinitsky et al. 1992, 1994). Most synthetic inhibitors rely on a peptide base, which mimics a protein substrate, attached to a COOH terminal "warhead." Notable warheads include boronic acids (Adams et al. 1998), such as bortezomib (Adams et al. 1999), and epoxyketones (Sin et al. 1999), such as carfilzomib (Kuhn et al. 2007; Demo et al. 2007; Stapnes et al. 2007). A variety of natural products also inhibit the proteasome that are not peptide-based, most notably lactacystin (Fenteany and Schreiber 1998), that is related to NPI-0052, or salinosporamide A, another inhibitor in clinical trials (Feling et al. 2003; Chauhan et al. 2005).

12.2 Antitumor Effects

The possibility that proteasome inhibitors could be drug candidates was considered after studies showed that they induced apoptosis in leukemic cell lines (Imajoh-Ohmi et al. 1995; Shinohara et al. 1996), including chemotherapy-resistant and radiation-resistant chronic lymphocytic leukemia cells (Delic et al. 1998). This was bolstered by findings that proteasome inhibitors induced apoptosis preferentially in transformed cells (Delic et al. 1998; Orlowski et al. 1998) and were active against an in vivo non-Hodgkin's lymphoma model (Orlowski et al. 1998). One of the early mechanisms of action attributed to proteasome inhibitors was that they repressed nuclear factor-*k*B (NF-*k*B) signaling by stabilizing I*k*B, which binds NF-*k*B and prevents its nuclear translocation (Orlowski and Baldwin 2002). Given the role of NF-*k*B in angiogenesis, cell invasion, oncogenesis, proliferation, and suppression of apoptosis, NF-*k*B inhibition was already an attractive approach to cancer therapy. Moreover, NF-*k*B inhibition induced chemosensitization, because many chemotherapeutics activated antiapoptotic NF-*k*B functions (Wang et al. 1996, 1999; Cusack et al. 2001). An especially strong rationale for targeting NF-*k*B had been worked out in multiple myeloma (MM). Adhesion of myeloma cells to bone marrow stroma induced NF-*k*B–dependent production of the antiapoptotic and growth factor interleukin-6 (Chauhan et al. 1996). Later studies documented the efficacy of proteasome inhibition against preclinical models as a single approach (Hideshima et al. 2001) and in chemosensitization and overcoming resistance (Hideshima et al. 2001, 2002; Ma et al. 2003; Mitsiades et al. 2002), with predominantly synergistic effects when bortezomib was combined with other agents.

Proteasome inhibitors are targeted because they are very potent and selective for the proteasome. Owing to their effect on proteolysis of a wide array of cellular proteins, however, they share characteristics with general cytotoxic agents, such as vinflunine, satraplatin, aurora kinase inhibitors, and epothilones, as discussed in the accompanying reviews and overview (Bennouna et al. 2008; Choy et al. 2008; Gautschi et al. 2008; Lee and Swain 2008; Teicher 2008). In that light, proteasome inhibitors have a number of important mechanisms of action beyond their effects on NF-*k*B that have been validated preclinically in cell line models (Voorhees et al. 2003; Rajkumar et al. 2005). By interfering with timely degradation of cyclins and other cell cycle regulatory proteins, proteasome inhibitors induce cell cycle arrest. Through their ability to stabilize proapoptotic proteins, such as p53 and Bax, while reducing levels of some antiapoptotic proteins, such as Bcl-2, they induce a proapoptotic state. Bortezomib-mediated programmed cell death is accompanied by c-Jun-NH_2 terminal kinase induction, generation of reactive oxygen species, transmembrane mitochondrial potential gradient dissipation, release of proapoptotic mitochondrial proteins, such as cytochrome *c*, and activation of intrinsic, caspase-9–mediated and extrinsic, caspase-8–mediated apoptosis. Other mechanisms include induction of aggresome formation, ER stress, and the unfolded protein response (Hideshima et al. 2005; Nawrocki et al. 2005a, b; Obeng et al. 2006), with the latter possibly having special relevance for MM cells, given their large basal load of immunoglobulin protein substrates. Readers interested in more detailed coverage of the mechanisms of action of proteasome inhibitors are referred to several excellent reviews (Richardson et al. 2006a–e; Nencioni et al. 2007).

Interestingly, the pleiotropic effects of proteasome inhibitors also result in activation of antiapoptotic pathways that may suppress antitumor activity and could be targets for chemosensitization to bortezomib. Heat shock response proteins have been some of the best characterized, including HSP-27 (Hideshima et al. 2004), HSP-70 (Robertson et al. 1999; Voorhees et al. 2007), and HSP-90 (Mitsiades et al. 2006).

Other examples include stress response proteins like mitogen-activated protein kinase phosphatase-1 (Orlowski et al. 2002a, b; Small et al. 2004; Shi et al. 2006) and protein kinase B/Akt (Hideshima et al. 2006).

12.3 Clinical Application of Proteasome Inhibitors

Building on this solid preclinical rationale, a number of phase I studies have documented that bortezomib can be safely given on a variety of schedules (Aghajanian et al. 2002; Orlowski et al. 2002a, b; Papandreou et al. 2004; Cortes et al. 2004; Blaney et al. 2004; Dy et al. 2005). Early indications of activity were seen in nonsmall cell lung (Aghajanian et al. 2002) and androgen-independent prostate carcinoma (Papandreou et al. 2004), as well as MM and mantle cell and follicular nonhodgkin's lymphoma (Orlowski et al. 2002a, b). The most dramatic findings were in myeloma, in which among nine patients, all showed some clinical benefit, including one durable complete remission. Pharmacodynamic studies showed a dose-dependent 20 S proteasome inhibition in peripheral blood mononuclear cells and in limited studies of tumor tissue. However, a correlation between peripheral blood mononuclear cell proteasome inhibition and response could not be established in these small trials, which were not designed with the sample size necessary for such an analysis. Pharmacokinetic studies showed rapid bortezomib plasma clearance and tissue distribution, with an initial $t_{1/2}$ of 0.22–0.46 h followed by a more gradual terminal elimination half-life, with $t_{1/2\beta}$ of >10 h and a large volume of distribution of >500 L. Activity against MM was confirmed with a phase II trial (Richardson et al. 2003) that showed a 27% overall response rate (partial response+complete remission) in heavily pretreated patients, who received what has become the most common dose and schedule, 1.3 mg/m^2 as an i.v. bolus on days 1, 4, 8, and 11 of every 21-day cycle. Further follow-up (Richardson et al. 2006a–e) determined that the median duration of response was 12.7 months, the median time to progression (TTP) was 7 months, and the median overall survival was 17.0 months. A subsequent phase III randomized trial (Richardson et al. 2005a, b, 2007a, b) comparing dexamethasone with bortezomib showed that the latter induced a better overall response rate (43% for bortezomib vs. 18% for dexamethasone), a better response quality, as well as a longer median TTP (6.22 vs. 3.49 months, respectively) and overall survival (29.3 vs. 23.7 months, respectively). Together, these studies led to the approval of bortezomib for relapsed/refractory myeloma in patients who have progressed after at least one prior regimen.

In nonhodgkin's lymphoma, several phase II studies (O'Connor et al. 2005; Goy et al. 2005; Belch et al. 2007) confirmed the activity in follicular, mantle cell, and marginal zone lymphoma. Most recently, a multicenter pivotal trial (Fisher et al. 2006) determined that the overall response rate in relapsed mantle cell lymphoma was 33%, including 8% complete remission/unconfirmed complete remission, with a median duration of response of 9.2 months and TTP of 6.2 months, leading to approval of bortezomib for this indication. Activity has also been described in other B-cell processes, including Waldenström's macroglobulinemia (Treon et al. 2007; Chen et al. 2007; Strauss et al. 2006; Dimopoulos et al. 2005) and amyloidosis (Wechalekar et al. 2006).

When bortezomib was being developed as a drug candidate, there was great concern that it could not be inhibited without direct consequences, because of the proteasome's vital role in cellular homeostasis. Fortunately, an acceptable therapeutic index has been documented, but patients do face the risk of some toxicities. During phase I studies, dose-limiting toxicities included diarrhea, fatigue, fluid retention, hypokalemia,

hyponatremia, hypotension, malaise, nausea, orthostasis, sensory neuropathy, and thrombocytopenia. In the phase II trial of MM patients, adverse events were reported in at least 10% included anemia, anorexia, constipation, dehydration, diarrhea, dizziness, fatigue, headache, limb pain, nausea, neutropenia, peripheral neuropathy, pyrexia, rash, thrombocytopenia, vomiting, and weakness. Subsequent studies have better characterized thrombocytopenia (Lonial et al. 2005) and neuropathy (Richardson et al. 2006a–e), which probably must limit dosing in the clinic. These have elucidated some of the risk factors involved in these transient, reversible effects, but a better understanding of the underlying mechanisms would be of benefit, as would the identification of biomarkers to predict efficacy or toxicity.

12.4 Bortezomib

Proteasome inhibition is a rational therapeutic approach both by itself and as a means to induce chemosensitization and overcome chemoresistance. As noted earlier, many cytotoxic agents activate the antiapoptotic NF-κB pathway, and blockade of this induction by proteasome inhibition enhanced their antitumor efficacy (Ma et al. 2003; Mitsiades et al. 2003). In addition, several strategies by which tumor cells survive the effects of chemotherapy can be similarly abrogated. Overexpression of Bcl-2 is one such mechanism, but proteasome inhibitors induce Bcl-2 phosphorylation and cleavage into proapoptotic fragments (Ling et al. 2002). Selection of cells overexpressing P-glycoprotein is another mechanism, but because proteasome function is needed for P-glycoprotein maturation when the proteasome is inhibited, inactive P-glycoprotein isoforms accumulate and cannot remove chemotherapeutic agents from cancer cells (Loo and Clarke 1998, 1999).

Using these rationales, bortezomib has been combined with a variety of chemotherapeutics, including carboplatin (Aghajanian et al. 2005), docetaxel (Messersmith et al. 2006), irinotecan (Ryan et al. 2006), melphalan (Berenson et al. 2006), pegylated liposomal doxorubicin (Orlowski et al. 2005a, b), and thalidomide (Barlogie et al. 2004), among others. Bortezomib has also been incorporated into more complex regimens, such as paclitaxel and carboplatin (Ma et al. 2007) and gemcitabine and cisplatin (Voortman et al. 2007). From these studies, it seems possible to conclude that bortezomib has generally been successfully combined with other agents without significantly increased toxicity, and without the need for large dose adjustments.

Bortezomib (Velcade®; Millennium Pharmaceuticals Inc., Cambridge, MA, and Johnson and Johnson Pharmaceuticals, Research and Development, L.L.C., Raritan, NJ), a first-in-class proteasome inhibitor, is approved in the U.S. and European Union for the treatment of MM patients who have received at least one prior therapy. Bortezomib was approved based on the results of the randomized, phase III assessment of proteasome inhibition for extending remissions (APEX) trial (Richardson et al. 2005a, b). Compared with high-dose dexamethasone, single-agent bortezomib showed superiority in terms of response rates (including CR rates), median TTP, and survival, and better quality of life (Richardson et al. 2005a, b; Lee et al. 2005). This study also showed that a high quality of response (100% M-protein reduction) to bortezomib was associated with a longer duration of response (Richardson et al. 2005a, b). In addition, bortezomib was well tolerated and retained its superiority over high-dose dexamethasone in elderly patients and patients with high-risk factors such as advanced disease, more prior lines of therapy, and refractoriness to prior therapy (Richardson et al. 2007a, b). A subgroup analysis suggests that bortezomib may be more beneficial when used earlier in the course of treatment; in the APEX trial, patients with one prior line of therapy

appeared to have a higher response rate, longer TTP, and longer survival following bortezomib treatment compared with patients with more than one prior line (Richardson et al. 2005a, b; Sonneveld et al. 2005). In other studies in the relapsed setting, bortezomib has been shown to be active with a similar toxicity profile in patients with chromosome 13 deletion (Sagaster et al. 2007; Jagannath et al. 2007), and in patients with renal dysfunction or renal failure requiring dialysis (Jagannath et al. 2005; Mohrbacher and Levine 2005; Chanan-Khan et al. 2007).

In addition to single-agent studies in the relapsed setting (Richardson et al. 2005a, b; Jagannath et al. 2004; Richardson et al. 2003), bortezomib is also being investigated in a range of combination regimens with other antimyeloma agents (Richardson et al. 2006a–e), including steroids, melphalan, and immunomodulatory drugs (IMiDs) (Palumbo et al. 2007; Kropff et al. 2005a, b; Orlowski et al. 2005a, b; Berenson et al. 2006; Suvannasankha et al. 2006; Zangari et al. 2005; Friedman et al. 2006; Leoni et al. 2006; Hollmig et al. 2004; Biehn et al. 2007; Orlowski et al. 2006; Reece et al. 2006; Richardson et al. 2006a–e; Davies et al. 2006; Terpos et al. 2006; Chanan-Khan, et al. 2006; Popat et al. 2006; Teoh et al. 2006). Encouragingly, despite patients having relapsed or refractory disease, high ORRs (up to 93%) (Teoh et al. 2006) and CR/near complete response (nCR) rates (up to 64%) (Teoh et al. 2006) have been reported.

12.5 Bortezomib-Based Combination Therapy for Multiple Myeloma

Owing to age or concomitant comorbidities at the time of diagnosis, more than half of the patients with MM may not be eligible for transplant; in these patients, melphalan–prednisone (MP) has remained a global standard of care for 40 years (Myeloma Trialists' Collaborative Group 1998; Cavo et al. 2002; Facon et al. 2006). MP typically results in response rates of up to 55%; however, the CR rate is low, typically ≤5% (Cavo et al. 2002; Hernandez et al. 2004; Facon et al. 2006; Palumbo et al. 2006; San Miguel et al. 2008) and the median OS is only 2–3 years (Myeloma Trialists' Collaborative Group 1998; Bladé et al. 2001; Facon et al. 2006). A large body of evidence has shown that the introduction of novel agents into front-line combination therapies improve clinical outcomes for non-transplant-eligible patients. Long-term follow-up of several of these studies is needed to fully assess the duration of response and survival benefit in this patient population.

A multicenter, phase I/II study of 60 elderly patients (aged ³65 years) ineligible for HDT-SCT, evaluated the addition of bortezomib to the MP regimen (VMP) (Mateos et al. 2006). Among 53 evaluable patients, VMP produced an ORR of 89%, including a 32% CR rate and an 11% nCR rate. Among patients achieving CR, half of those assessed had no malignant plasma cells detectable by multiparametric flow cytometry (sensitivity level of 10^{-4}–10^{-5}), representing immunophenotypic minimal residual disease status. These response rates, among the highest reported with conventional therapy, e compare very favorably with those for MP – 35–53% ORR, including a 1–9% CR rate (Palumbo et al. 2006; Facon et al. 2006; Hernandez et al. 2004; Cavo et al. 2002). Response to VMP was rapid, with 70% of patients responding by completion of the first cycle. VMP also compares very favourably with MP historical control data in the context of the PFS rate (91 vs. 66%), event-free survival (EFS) rate (83 vs. 51%), and OS rate (90 vs. 62%) at 16 months (Mateos et al. 2006). Notably, in 33 patients for whom cytogenetic data were available, the response rates appeared comparable among patients with and without retinoblastoma gene deletion or immunoglobulin heavy-chain translocations, suggesting that the mechanism of action of bortezomib may overcome the adverse impact

of these factors (Mateos et al. 2006). A median of seven cycles of therapy was administered (>9 months), indicating good tolerability of the VMP regimen in this elderly population (Mateos et al. 2006). Toxicities were comparable with those seen in other major bortezomib trials.

In the large phase 3 VISTA (Velcade® as Initial Standard Therapy in MM) trial, bortezomib–MP (VMP) demonstrated superiority vs. MP across all efficacy endpoints, including ORR by EBMT criteria (71 vs. 35%, $p<0.001$) and IFx-neg CR rate (30 vs. 4%, $p<0.001$) (San Miguel et al. 2008). Responses were more rapid and durable when compared with MP (Palumbo et al. 2008). VMP therapy significantly prolonged TTP (HR 0·48, $p<0.001$) by approximately 50%, when compared with MP, an improvement similar to that achieved with HDT–SCT vs. conventional chemotherapy (Attal et al. 1996). After a median follow-up of 26 months, VMP offered a substantial survival benefit when compared with MP (HR 0·644, $p>0.0032$) (San Miguel et al. 2008). Importantly, VMP was well-tolerated, with patients remaining on therapy for a median of 46 weeks (vs. 39 weeks with MP), even though patients in the bortezomib-containing arm were reported to have a higher rate of adverse events (San Miguel et al. 2008). Based on VISTA data, bortezomib has recently been approved for the treatment of all patients with MM, thus expanding the indication to include newly diagnosed patients.

12.6 Treatment Options for Patients Eligible for Transplant

HDT–SCT is a standard of care in patients aged up to approximately 65–70 years (Kyle and Rajkumar 2004; Gertz et al. 2006). Standard induction therapies prior to HDT–SCT have included VAD (vincristine, doxorubicin, dexamethasone), DVd (VAD but with liposomal doxorubicin), and high-dose dexamethasone. Response rates are typically 40–61% (Alexanian et al. 1990, 1992; Dimopoulos et al. 2003; Rifkin et al. 2006), and as with MP, CR rates are generally low, ranging from 3 to 13%. A number of combinations are under investigation, utilizing conventional agents such as cyclophosphamide, doxorubicin/liposomal doxorubicin, and steroids in combination with bortezomib and an IMiD, or other novel agent. Emerging data show substantial improvements in response when compared with current standard induction regimens. However, long-term follow-up is required to fully assess the duration of response and survival benefit for these novel-agent-based regimens.

Phase 2 studies with bortezomib–dexamethasone led to the design of the IFM 2005-01 phase 3 trial, where a significantly higher postinduction ORR was achieved with bortezomib–dexamethasone vs. VAD ($p<0.0001$), which translated into a significantly higher ≥VGPR rate posttransplant (57 vs. 38%, $p>0·0003$), with fewer patients requiring a second transplant as a result. CR/nCR rates were (15%) postinduction and (37%) post first-transplant in the bortezomib-containing arm (Harousseau et al. 2007, 2008). Bortezomib–dexamethasone and VAD toxicity profiles were comparable. After a median follow-up of 2 years, no OS advantage was noted; however, 2-year PFS was 69 vs. 60% in the VAD arm ($p>0·0115$) (Harousseau et al. 2008); hence, longer follow-up is needed. A combination with cyclophosphamide (VCD) showed a very good tolerability and high efficacy as an induction regimen prior to ASCT (Knop et al. 2008)

12.7 Next Generation Proteasome Inhibitors

With the validation of the proteasome as a target for cancer therapy, interest has focused on the possibility that other inhibitors could offer some

advantages. Two second-generation agents have entered phase I trials: NPI-0052 (salinosporamide A) and carfilzomib (formerly PR-171). Unlike bortezomib, which binds the proteasome in a slowly reversible manner, NPI-0052 and carfilzomib bind irreversibly, abrogating one mechanism of recovery from proteasome inhibition, namely release of the target by the drug. They both induce depolarization of the transmitochondrial membrane potential and activate caspase-8–mediated apoptosis, whereas carfilzomib also activates caspase-9. Preclinical studies have shown that both (Kuhn et al. 2007; Chauhan et al. 2005) at least partially overcome bortezomib resistance in vitro. Moreover, in a number of models, including MM (Kuhn et al. 2007; Chauhan et al. 2005) and chronic lymphocytic leukemia (Ruiz et al. 2006), these inhibitors have shown enhanced potency when compared with bortezomib, suggesting that they may have a broader spectrum of activity. Early results from phase I studies of carfilzomib indicate that it is well tolerated, even on a dose-intense schedule, and may have less neurotoxicity than bortezomib (Orlowski et al. 2007; Alsina et al. 2007). Evidence of antitumor activity is being seen in MM and Waldenström's macroglobulinemia, including in myeloma patients with previously bortezomib-refractory disease, and phase II studies are being planned. Another interesting target may be the immunoproteasome (Rivett and Hearn 2004), whose expression may be more tissue-restricted than the constitutive proteasome. All of the currently available inhibitors target both the constitutive and immunoproteasome isoforms, but the identification of specific immunoproteasome inhibitors (Orlowski et al. 2005a, b; Ho et al. 2007) may allow for further improvements in the therapeutic index of these drugs. As the immunoproteasome is expressed predominantly in hematopoietic tissues, it is possible that such agents could act without incurring neurotoxicity or gastrointestinal effects, among others, because those tissues express much lower levels of immunoproteasome subunits.

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Temsirolimus 13

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Abstract Temsirolimus, an ester of sirolimus (rapamycin), selectively inhibits the kinase mammalian target of rapamycin and consequently blocks the translation of cell cycle regulatory proteins and prevents overexpression of angiogenic growth factors.

Patients with advanced renal cell carcinoma (RCC) and a poor prognosis who received a once-weekly intravenous (IV) infusion of temsirolimus 25 mg, experienced significant survival benefits when compared with patients receiving standard interferon-α (IFNα) therapy in a large phase III clinical study. In this study, median overall survival was 10.9 vs. 7.3 months and objective response rates were 8.6% in temsirolimus recipients vs. 4.8% IFNα recipient group.

Temsirolimus monotherapy recipients experienced significantly fewer grade 3 or 4 adverse events and had fewer withdrawals for adverse events than patients receiving IFNα.

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13.1 Introduction

Renal cell carcinoma (RCC) accounts for approximately 3% of all adult malignancies and 2% of all cancer-related deaths (Linehan et al. 2001). In USA, RCC accounts for 2–3% of all cancers diagnosed. Nearly 210,000 people worldwide were diagnosed with RCC in 2007 and roughly one-third of these patients presented with metastatic disease, at the time of initial diagnosis, (Jemal et al. 2007) with a median survival time of 10–12 months (Larkin et al. 2007).

RCC may be treated surgically for stage I–III and surgical (laparoscopic or open) resection is the mainstay for tumours that are confined to the kidney.

Most renal cell cancers (85%) are classified histologically as clear cell type. These tumours are typically (>80%) characterised by a loss of expression of a functional von-Hippel–Lindau (VHL) gene. This gene regulates protein stability of hypoxia-inducible transcription factors (HIF) (Alexandrescu and Dasanu 2006; Motzer and Bukowsky 2006). Loss of VHL function prevents the degradation of these factors and leads to their accumulation, with the subsequent increased expression of HIF-regulated proteins such as vascular endothelial growth factor (VEGF) and other angiogenic and growth-stimulating molecules.

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Prior to the introduction of targeted cancer therapies, there were limited options for systemic therapy in patients with RCC. Interleukin-2 (IL-2) and Interferon alfa (IFN-α) were, alone or in combination, the main treatments for metastatic renal cancer. Treatment with these agents resulted in a median survival of 12.0–17.5 months (Aass et al. 2005). Cytokine-based immunotherapy with interleukin-2 (IL-2) and interferon-α (IFNα) are associated with modest objective response rates of 10–15% and substantial toxicity (Motzer and Bukowsky 2006; Rosemberg et al. 1985).

A better understanding of the pathogenesis of RCC, particularly the role of tumour angiogenesis, has led to the development of new therapeutic agents, with VEGF or the mammalian target of rapamycin (mTOR) being targetedas their target. (Escudier 2007).

Temsirolimus is a selective inhibitor of mammalian target of rapamycin (mTOR), a serine–threonine-kinase involved in multiple tumour-promoting intracellular signalling pathways and controlling many cellular functions such as proliferation, survival, protein synthesis and transcription of HIF-α and it has been the first approved mTOR-targeted agent based on a phase III trial (Alexandrescu and Dasanu 2006; Hudes et al. 2007).

13.2 Development

Temsirolimus is a soluble ester of rapamycin, a natural product that was initially developed as an anti-fungal drug and then as an immunosuppressive agent, with anti-cancer activity noted more than 20-years ago. Rapamycin (sirolimus, rapamune) was isolated from the soil bacteria *Streptomyces hygroscopicus* found on Rapa Nui (commonly known as Easter Island) in the South Pacific in 1975, but its development for cancer therapeutics was not prioritised. The immunosuppressant effects of rapamycin were pursued and it resulted in Food and Drug Administration approval in 1999 for prevention of renal allograft rejection. Laboratory studies of rapamycin starting in the early 1980s showed anti-tumour effects in several solid tumours.

Cell cycle inhibitor-779, now known as Temsirolimus, is a derivative of rapamycin and it was identified in the 1990s and subsequently developed as an anti-cancer agent (Peralba et al. 2003).

13.3 Structure and mechanism of action

Temsirolimus (Fig. 13.1) is a serin/threonin kinase involved in controlling many cellular functions and it inhibits the mammalian target of rapamycin (mTOR).

The rapamycin-sensitive complex, also called mTOR complex 1 (mTORC1) (Guertin and Sabatini 2005; Martin and Hall 2005), exists in cytoplasm in a complex with three peptides: the regulatory-associated protein of mTOR (raptor), mLST8 and GhL. Regulation of mTOR pathway activation is mediated through a series of complex signalling interactions linking growth factor receptor signalling and other cell stimuli, phosphatidylinositol

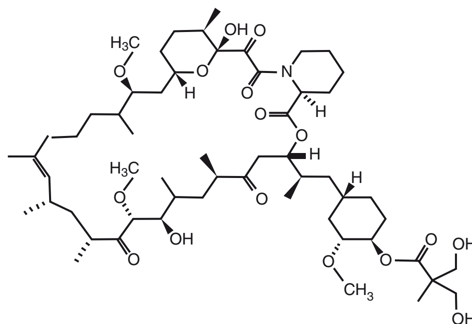

Fig. 13.1 Structure of temsirolimus

3-kinase activation and activation of the Akt/protein kinase B pathway.

mTOR phosphorylates and activates p70 S6 kinase and in this way leads to enhanced translation of certain ribosomal proteins and elongation factors. This process is responsible, among other effects, for the production of hypoxia-inducible factor-1a, which regulates the transcription of genes that stimulate cell growth and angiogenesis, including VEGF (Thomas et al. 2006). When activated, mTOR is linked to increased protein synthesis by modulating elements that are important in a number of cellular processes such as stimulating and regulating the synthesis of several proteins at the translation level through its phosphorylation of S6K1 and 4E-BP1; stimulating cell growth through cyclin D1, and being an important component of a cell cycle checkpoint for DNA replication; increasing production of the HIF-1α protein, a transcriptional regulator of angiogenic growth factors, such as VEGF and PDGF; stimulating an increased expression of glucose and amino acid transporters, allowing the cell to take up additional metabolic fuel and extracellular nutrients. If disregulated, the net result is uncontrolled cell growth (Fig. 13.2).

In cancers, signalling through mTOR is stimulated by defects in one or more of the several pathway components upstream of mTOR (growth factor receptors, PI3-K, Akt, PTEN, TSC1/TSC2) or by stimulation of PI3K by mutant Ras/Raf/MAPK pathway components. In certain types of renal cell cancer and some neuroendocrine

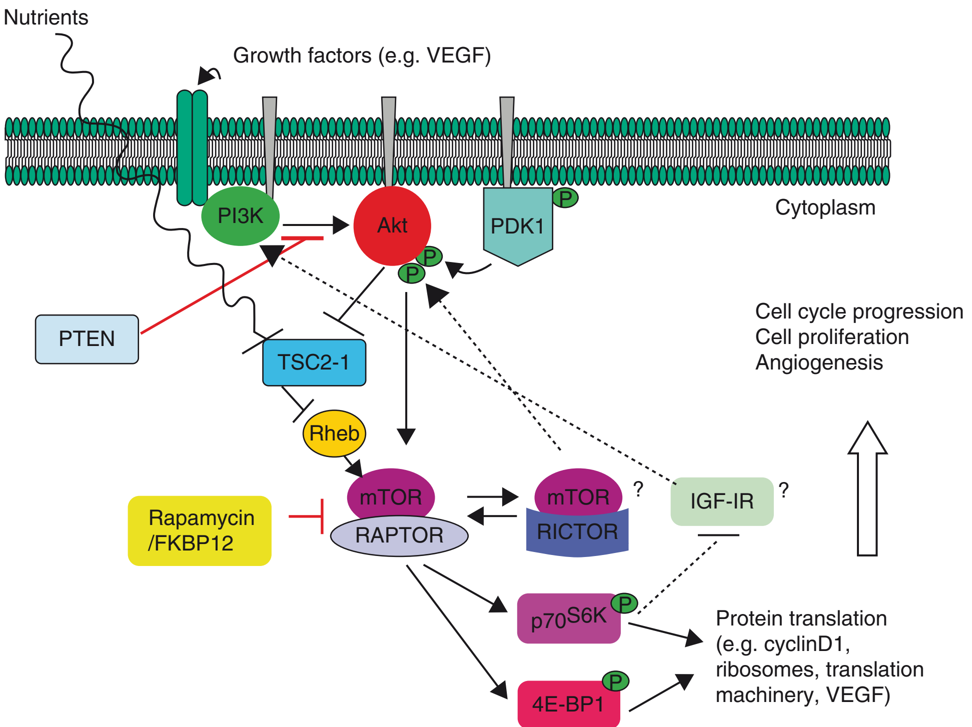

Fig. 13.2 PI3K/AKT/mTOR pathway showing the mTOR protein complexes, mTOR/RAPTOR and mTOR/RICTOR, and the feedback loop involving IGF-IR. *Arrows* indicate activation; *bars* indicate inhibition (Duran et al. 2006)

tumours, loss of function of VHL eliminates the mechanism for clearance of hypoxia-inducible factor 1α (HIF-1α), resulting in the transcription of numerous "hypoxia-associated" proteins, which drive angiogenesis and other cellular functions. HIF-1α translation is controlled by mTOR; inhibiting mTOR may be one approach to overcoming the effects of VHL loss.

Temsirolimus binds to the immuniphilin FK506-binding protein 12 KDa isoform (FKBP12) to form a complex with mTOR (Sabers et al. 1995). When mTOR is bound in this complex, it becomes difficult to phosphorylate protein translation factors, as 4EBP1 and SK6 (also known as p7066 kinase), which are downstream of mTOR in the phosphatidylinsoitol 3-kinase (PI3K)/Akt/mTOR pathway.

The net effect of this class of compounds is inhibition of the translation of several key proteins regulating the cell cycle so that cell is blocked in the G1 phase and angiogenesis is inhibited (Hudes et al. 2007).

13.4 Clinical Data

Temsirolimus showed anti-tumour effects across a wide variety of tumour histotypes in preclinical models (Neshat et al. 2001; Podsypanina et al. 2001; Yu et al. 2001). Over the past 10 years, many authors have carried out a series of randomised phase I–III trials to investigate efficacy of Temsirolimus in advanced RCC and in other tumours as endometrial carcinoma, breast cancer, glioblastoma mutltiforme, melanoma, small cell lung carcinoma, neuroendocrine carcinoma, mantle cell lymphoma. The pivotal Phase III study for temsirolimus in advanced RCC as well as in mantle cell lymphoma (MCL) is being conducted worldwide.

In addition, clinical studies for an oral formulation of temsirolimus in oncology (breast and prostate), multiple sclerosis and rheumatoid arthritis indications have been conducted, but oral formulation is currently not being developed in these indications because of insufficient efficacy observed in the trials.

13.5 Safety and Efficacy

One phase I study evaluated toxicity, pharmacology and preliminary activity of Temsirolimus administrated daily for 5 days every 2 weeks at maximum tolerated dose of 15 mg/m²/day to patients with advanced cancer, (HidalgoM et al. 2006) demonstrating well-tolerated and a preliminary evidence of anti-tumour activity in several advanced solid malignancies. Another phase I trial demonstrated the first evidence of anti-tumour activity in patients with RCC (Raymond et al. 2004).

In a randomised phase II study (Atkins et al. 2004), 111 patients with advanced refractory RCC were retrospectively classified in three groups according to Motzer's criteria (good, intermediate and poor prognosis). They were randomly assigned to receive 25, 75, or 250 mg Temsirolimus weekly to evaluate tumour response, time to tumour progression, survival and adverse events. This study brought up an objective tumour response in 7% of patients. In addition, complete response, partial response, minor response, or stable disease ≥24 weeks was noted in nearly 50% of the patients. Median time to progression was 6.0 months and median survival 15.0 months, with better survival for patients with intermediate or poor prognosis. These data seem to be encouraging when compared to 2.0 months as time to progression and median survival of 10.0 months in non-responding patients who had received IL-2 and/or

IFN-alpha based immunotherapy with no other treatment (Yang et al. 2003). Moreover, results from a phase II trial investigating Temsirolimus in recurrent or metastatic endometrial carcinoma suggest that monotherapy with Temsirolimus could be an option for the treatment of this disease for which no standard of care currently exists (Oza et al. 2005), whereas, treatment of patients with recurrent glioblastoma with 250 mg/week of Temsirolimus does not seem to have good activity as emerging data from phase II trials reveal. (Galanis et al. 2005; Chang et al. 2005).

In a recent phase III, randomised, open label, multi-centre study, 626 patients with metastatic RCC and three or more adverse risk features (indicators of short survival) were randomised in three arms to receive monotherapy with Temsirolimus (25 mg i.v. weekly), monotherapy with IFN-alpha (18 million units 3 times a week) and combination therapy with Temsirolimus (15 mg iv weekly) plus IFN-alpha (6 million units 3 times a week weekly). Overall survival as primary end point was calculated. Patients treated with temsirolimus alone had a statistically longer overall survival than patients in the IFN-alpha monotherapy group (10.9 vs. 7.3 months; $p>0.0069$). Secondary efficacy end points were progression-free survival, the objective response rate and clinical benefits rate, defined as the group of patients with stable disease for at least 24 weeks or an objective response. The median progression-free survival was 3.7 months in the patients treated with temsirolimus (alone or in combination) vs. 1.9 months in the arm treated with IFN-alpha alone and objective response rates of 4.8, 8.6 and 8.1% in patients receiving IFN, temsirolimus and combination therapy, respectively, did not differ significantly. In contrast, a better objective response or a bigger proportion of cases with a stable disease for at least 24 weeks was noted in temsirolimus group (32.1%), in the combination therapy group (28.1%) than in IFN group (15.5%) (Hudes et al. 2007).

13.6 Side Effects

In the same study, tolerability of Temsirolimus was evaluated. Frequently (>30% of the patients) asthenia, rash, anaemia, nausea and anorexia occurred in temsirolimus alone group. The most frequently occurring grade 3 adverse events in the temsirolimus arm were asthenia (11%), anaemia (20%) and dyspnoea (9%). Grade 3 or 4 asthenia were reported in 11% of the patients in the temsirolimus group, in 26% in the interferon group ($p<0.001$) and in 28% in the combination therapy group ($p<0.001$). The proportions of patients who reported dyspnoea, diarrhoea, nausea or vomiting were similar in all three groups. The most frequently occurring temsirolimus-related grade 3 or 4 haematological toxicities included anaemia and thrombocytopenia. Hypercholesterolemia, hyperlipidemia and hyperglycemia were also more common in the temsirolimus arm, reflecting inhibition of mTOR-mediated lipid and glucose metabolism, and generally manageable with dietary or medical management. Immunosuppression is an additional potential toxicity of temsirolimus given the known immunosuppressive effects of sirolimus, but there were not significant differences in the incidence of neutropenia, lymphopenia or infection vs. the IFN control arm.

Temsirolimus demonstrated remarkable antitumour activity in mantle cell lymphoma, a disease driven by cyclin D1 overexpression (Witzig et al. 2005a).

Patients affected by relapse of the Mantle cell lymphoma after conventional therapy or stem cell transplantation have a poor prognosis and are candidates for novel agents. A pathologic hallmark of MCL is the characteristic overexpression of cyclin D1 (CCND1) in the MCL tumour cells. CCND1 is one of the proteins in which translation is under the control of the phosphatidylinositol-3 kinase signal transduction

pathway and is downstream of the mammalian target of rapamycin kinase (mTOR).

A Phase II trial in 35 patients with mantle cell lymphoma that had relapsed after chemotherapy and rituximab treatment indicated that temsirolimus treatment resulted in a remarkable overall response rate of 38%, with a 3% rate of complete remission (CR) and a 35% rate of partial remission (PR) with a median duration of responses of 6 months (Witzig et al. 2005b).

In another phase II study with temsirolimus in mantle cell lymphoma on 27 patients, the overall response rate was 41% and the median time to progression was 6 months (Ansell et al. 2008). Results from a phase III study in 161 patients with relapsed or refractory mantle cell lymphoma has been recently carried out and showed at the Thirteenth Congress of the European Haematology Association (EHA) (Verhoef et al. 2008) and at the Forty Forth Annual Meeting of the American Society of Clinical Oncology (ASCO) (Hess G et al. 2008). In this randomised study, two groups of patients, receiving two different doses of temsirolimus (high dose or low dose temsirolimus), were compared with a third group treated with other chemo or biologic therapies (gemcitabine, fludarabine, etc.).

Objective response was 22, 6 and 2% in the high-dose temsirolimus group, in the low-dose temsirolimus group and in the chemo-biologic treated group, respectively. Progression-free survival was 4.8, 3.4 and 1.9 months in the first, second and third arm. There was no significant difference in overall survival among all patients (Verhoef et al. 2008; Hess G et al. 2008).

13.7 Conclusion and Future Perspectives

The mTOR pathway is likely critical across a broad spectrum of tumour types.

Temsirolimus has shown anti-tumour activity, most notably in poor-risk advanced RCC where a demonstration of overall survival benefit has been observed and promising results have been obtained in mantle cell lymphoma and endometrial cancer.

The proof of principle that mTOR inhibitors can improve cancer patient survival has been recently obtained from a large randomised trial testing temsirolimus in patients with advanced poor prognostic RCC. These data led the Food and Drug Administration (FDA) to approve temsirolimus for advanced RCC in 2007. Temsirolimus is approved in the US for the treatment of patients with advanced RCC and in Europe for first-line treatment of patients with advanced RCC and at least three of six prognostic risk factors (Table 13.1). The drug has shown a significant overall survival benefit, and is associated with fewer withdrawals for adverse events, compared with standard IFN therapy in this patient population. Other targeted agents are now available for metastatic RCC including combination therapy with bevacizumab with IFN-alpha, temsirolimus and sorafenib (Table13.2) and the results are encouraging.

The lack of significant anti-tumour effect of temsirolimus-mediated mTOR inhibition in some tumours, especially those with predicted sensitivity based on alterations such as PTEN mutation, underline the complex interplay of multiple signalling pathways within a single tumour and recent knowledge on the status of PTEN and PI3K/AKT/mTOR-linked pathways might help in the selection of other tumour types that will respond to mTOR inhibitors.

More potent or complete mTOR inhibition (e.g. through agents that inhibit both mTORC1 and mTORC2), inhibition of multiple signalling pathways simultaneously, and/or more precise molecular phenotyping of tumours to define mTOR pathway reliance are needed to build on the clinical benefits of temsirolimus observed to date.

In the future, it will be important to continue the search for factors predictive of resistance or

Table 13.1 Treatment options in metastatic renal cell carcinoma (RCC) (mRCC)

	MSKCC stratification	Treatment options			
		First-line		Second-line	
		Standard of care	Alternatives	Standard of care	Alternatives
mRCC	Favourable risk	IFNα + IL + (5FU) Sunitinib	Bevacizumab + IFN-alpha Cytokines (IL-2)	Sorafenib	Sunitinib
	Intermediate risk	Sunitinib	Bevacizumab + IFN-alpha Sorafenib		mTOR inhibitors
	Poor risk	Temsirolimus	Sunitinib		

Table 13.2 Comparison of efficacy of targeted agents in the first-line treatment in patients with metastatic RCC

	PFS (months)	OS (months)	*p* value
Sunitinib (Motzer et al. 2008)	11.0	Not reached	<0.00001
vs. INF-α	5.1		
Bevacizumab + INF-α (Escudier et al. 2007)	10.2 5.4	Not reached	<0.0001
Bevacizumab + INF-α (Rini et al. 2008)	8.5 5.2	Not reached	<0.0001
Temsirolimus[a] vs. INF-α (Hudes et al. 2007)	5.5 3.1	10.9 7.3	<0.001
Sorafenib (Szczylik et al. 2007)	5.7	Not available	Not available
vs. IFN-α (part 1)	5.6		
Sorafenib (600 mg b.i.d.; part 2)	3.6		
Crossover (IFN- α – Sorafenib 400 mg b.i.d.; part 2)	5.3		

PFS progression-free survival; *OS* overall survival
[a]Three or more of six prognostic risk factors; five risk factors determined by MSKCC (lactate dehydrogenase >1.5 times the upper limit of normal. Haemoglobin <lower limit of normal, correct serum calcium >10 mg/dL, time from diagnosis to first treatment <1 year, Karnofski PS 60–70%) plus the number of metastatic sites

sensitivity to mTOR inhibitors (Temsirolimus and others), and it would be useful to immediately apply existing knowledge that mTOR inhibition can restore sensitivity to some existing chemotherapeutic agents.

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14 Danusertib (formerly PHA-739358) – A Novel Combined Pan-Aurora Kinases and Third Generation Bcr-Abl Tyrosine Kinase Inhibitor

Artur Gontarewicz and Tim H. Brümmendorf

Abstract The Aurora kinases belong to a family of highly conserved serine/threonine protein kinases. They play an essential role as key mitotic regulators, controlling entry into mitosis, centrosome function, chromosome assembly, and segregation. As many other regulators of mitosis, Aurora kinases are frequently found to be aberrantly overexpressed in cancer cells. Therefore, these proteins have become an attractive target for the development of new anticancer therapies. In fact, several small-molecule inhibitors of Aurora kinases have already been developed and some of them have shown promising clinical efficacy in a number of human tumors in Phase I and II clinical trials. Among those, one of the most advanced clinical compound currently is Danusertib (formerly PHA-739358), which exhibits inhibitory activity against all known Aurora kinases as well as other cancer-relevant kinases such as the Bcr-Abl tyrosine kinase, including its multidrug-resistant T315I mutant. This mutation is responsible for up to 25% of all clinically observed resistances in CML patients undergoing Imatinib therapy. However, this particular mutation is predicted to play an even more important clinical role in the future, since in addition to Imatinib, it also confers resistance to second-generation Bcr-Abl inhibitors such as Nilotinib, Dasatinib, and Bosutinib. Therefore, combined Aurora and Bcr-Abl inhibition (the latter including high-grade resistance conferring mutations) with compounds such as Danusertib represents a promising new strategy for treatment of Bcr-Abl positive leukemias, especially those in second and third line of treatment.

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14.1 Introduction

The complete cell cycle is a highly ordered multiphase process culminating in cell division, mitosis, by which the duplicated DNA is precisely segregated into two daughter cells. The main effectors of this critical step are the mitotic microtubule spindle and the centrosomes. Because of the importance of this process for the survival of a cell and the integrity of the organism as a whole, mitosis is under tight control through several biochemical gatekeepers, commonly termed checkpoints, which ultimately ensure the progression and fidelity of

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cell division. Defects in the regulation of these mechanisms can lead to genomic instability, a condition tightly associated with tumorigenesis. In fact, the abnormal function of these regulatory systems is often found in tumor cells (Lengauer et al. 1998). As cancer is a consequence of uncontrolled cell division, great efforts have been made to develop drugs that specifically interfere with the progression of mitosis and therefore stop a cell from dividing. Currently used mitotic inhibitors predominantly impair the normal function of the mitotic microtubule spindle apparatus by targeting tubulins and halting the cell cycle in mitosis. However, in vivo, these compounds exhibit significant side effects, limiting their usage (Jiang et al. 2006). Therefore, it is of particular interest to develop novel chemotherapy agents targeting nonstructural components of the mitotic process (Schmidt and Bastians 2007).

The Aurora kinases have recently become known as key mitotic regulators playing a critical role in many processes during cellular division (Carmena and Earnshaw 2003). This serine/threonine kinase family emerged from a screen for *Drosophila melanogaster* mutants defective in mitotic spindle function and was named Aurora because of the similarity of their disordered mitotic spindles to *aurora borealis*, a phenomenon of the night sky in the polar region (Glover et al. 1995).

In vertebrates, Aurora kinases encompass three known family members, Aurora A, Aurora B, and Aurora C, which are involved in duplication of the centrosome, chromosome condensation, formation of a bipolar mitotic spindle with kinetochore–microtubule interactions, chromosome orientation, and chromatids segregation (Carmena and Earnshaw 2003; Adams et al. 2001a; Nigg 2001). Furthermore, there are clear implications for the involvement of Aurora kinases in tumorigenesis, as they are frequently overexpressed in several human tumors (Adams et al. 2001a, b; Zhou et al. 1998; Katayama et al. 1999; Sorrentino et al. 2004). Based on these data, Aurora kinases have become attractive targets for development of new anticancer drugs. Recently, a new generation of small molecule inhibitors has been developed and importantly, several of them have already entered Phase I and II clinical trials (Warner et al. 2003; Hauf et al. 2003; Ditchfield et al. 2003).

14.2 Structure, Localization, and Functions

The mammalian Aurora paralogues exhibit high sequence homology, particularly within the carboxyterminal catalytic domain, in which human Aurora A and B share 71% identity. The N-terminal domain of the three Auroras differs in the length and sequence, thereby determining selectivity for interactions with different substrates (Carmena and Earnshaw 2003; Bischoff et al. 1998; Giet and Prigent 1999). The evolutionary analysis of Aurora family within vertebrates suggests an early divergence of Aurora A from Aurora B and Aurora C (Cheetam et al. 2000).

In spite of this high level of similarity, the mammalian Aurora kinases show very distinct localizations and functions. Aurora A localizes mainly within the centrosomes from the time of their duplication until mitotic exit. After activation by LIM-Protein Ajuba in the G2 phase of the cell cycle, Aurora A phosphorylates several microtubule-associated proteins and recruits them to the centrosome to promote their maturation. On breakdown of the nuclear envelope, Aurora A is locally activated by its substrate TPX2 and targeted to spindle microtubules at the poles (Carmena and Earnshaw 2003; Bischoff et al. 1998; Fu et al. 2007; Tsai et al. 2003). Aurora A contributes to the process of G2/M transition, though its absence only delays entry into mitosis (Marumoto et al. 2002). This kinase is also involved in cytokinesis, since its overexpression causes aneuploidy exacerbated in cells that lack functional p53 (Meraldi et al. 2002). After exit from mitosis, Aurora A is degraded by the proteasome (Castro et al. 2002a, b).

Aurora B together with three other proteins, inner centromere protein (INCENP), survivin, and borealin, forms the chromosomal passenger complex, which controls the accurate segregation of the chromatids at mitosis, histone modification, and cytokinesis (Vader et al. 2006). This complex localizes to kinetochores until the metaphase–anaphase transition. After chromatid separation, it relocates to the midzone and then remains at the midbody until completion of cytokinesis (Carmena and Earnshaw 2003; Bischoff et al. 1998). Similar to Aurora A, Aurora B is activated by binding to some of its substrates. Activated Aurora B phosphorylates histone H3 at Ser10 and 28, and at Ser7 in the centromere histone variant CENP-A. As these events may be required for chromosome condensation, Aurora B has been linked to chromatin modification (Monier et al. 2007; Hsu et al. 2000; Giet and Glover 2001; Zeitlin et al. 2001). Aurora B phosphorylates also mitotic centromere-associated kinesin (MCAK) and therefore plays a crucial role in the regulation of precise chromatid separation as MCAK is involved in the spindle checkpoint correcting the unproper attachments of microtubules to the kinetochores (Dewar et al. 2004; Ohi et al. 2004). Aurora B has a critical role in a cytokinesis – its downregulation or inhibition of activity results in polyploidy due to the cytokinesis failure (Fu et al. 2007).

Less is known about the third member of the Aurora kinase family. In physiological conditions, Aurora C expression is restricted to the testis (Hu et al. 2000; Bernard et al. 1998). Like other Aurora kinases, Aurora C is activated by its substrates, especially by INCENP, which is also a substrate for Aurora B. Furthermore, Aurora C localizes in a pattern similar to Aurora B, can mimic its function in mitosis, and even rescues Aurora B-depleted cells (Sasai et al. 2004) (Table 14.1).

14.3 Aurora Kinases and Cancer

Human Aurora A is located on chromosome 20q13.2. This region is frequently amplified in many forms of human cancers resulting in protein overexpression (Sen et al. 1997; Bischoff et al. 1998; Zhou et al. 1998; Tanner et al. 2000;

Table 14.1 Human Aurora kinases – substrates, subcellular localization, and associated function

	Putative substrates	Cell localization	Function
Aurora A	BRCA1, p53, NM-23, NDEL-1, Lats2, Eg-5, D-TACC, TPX2, CENP-A, histone H3, Ajuba, PP1, Cdh-1	Duplicated centrosomes; spindle poles; spindle midzone/centrosomes	Centrosome maturation and separation; mitotic entry; bipolar-spindle assembly; cytokinesis
Aurora B	Borealin, Survivin, INCEP, Mad2, GFAP, CENP-A, Myosin II, Desmin, REC-8, GAP1, Vimentin, histone H3, MgcRac/Cyk4, BubR1, MKLP-1	Kinetochores; chromosome arms; spindle midzone/cleavage furrow	Recruitment of centromeric protein; chromosome alignment and segregation; microtubule dynamics and cytokinesis
Aurora C	Aurora B, INCENP	Spindle poles; chromosome arms	Chromosome segregation; cytokinesis; role in spermatogenesis

Jeng et al. 2004). Aurora A can be detected at various levels in breast, colon, pancreatic, bladder, ovarian, and prostate tumors (Bischoff et al. 1998; Zhou et al. 1998; Tanaka et al. 1999; Han et al. 2002; Sen et al. 2002; Gritsko et al. 2003; Li et al. 2003). The degree of Aurora A amplification and overexpression correlates with chromosomal instability, aneuploidy, and clinical aggressiveness in human bladder cancer (Tanaka et al. 1999; Han et al. 2002). In medulloblastoma, Aurora A overexpression was found to be an independent prognostic factor for overall survival (Neben et al. 2004).

Although activating mutations of Aurora kinase genes seem very rare in human tumors (Greenman et al. 2007), several studies revealed commonly occurring polymorphisms of the Aurora A gene and identified it as a candidate low-penetrance tumor susceptibility gene (Ewart-Toland et al. 2003). These polymorphisms are associated with cancer risk and clinical outcome: e.g., isoleucine for phenylalanine in position 31 is preferentially detected in cases of ovarian carcinoma and is also associated with advanced stages of squamous cell carcinoma of the esophagus (DiCioccio et al. 2004; Miao et al. 2004). Another frequent polymorphism of the Aurora A gene – isoleucine for valine in position 57 – is linked to an increased risk of breast and esophagus cancer and to the risk of disease progression in gastric tumors (Egan et al. 2004; Kimura et al. 2005; Ju et al. 2006).

In vitro, overexpression of Aurora A transforms NIH-3T3 and Rat1 fibroblasts, which give rise to tumors when injected into nude mice (Zhou et al. 1998; Bischoff et al 1998; Littlepage et al. 2002). Long-term overexpression of Aurora A in mouse mammary epithelium is also sufficient for induction of genetic instability preceding mammary tumor formation (Wang et al. 2006). So far, it is not clear how the inappropriate expression of Aurora A contributes to the transformation of cells. It has been proposed that it could prevent the chromosomes to achieve their normal spindle orientation. In spite of this, the affected cells can exit mitosis because at the same time overexpressed Aurora A inactivates the spindle-assembly checkpoint, leading therefore to cytokinesis failure and tetraploidy. This phenomenon is enhanced in p53-deficient cells, which insufficiently detect hyperploidy and, as a consequence, proceed through subsequent cell cycles and generate aneuploid progeny (Carmena and Earnshaw 2003).

Aurora B has also been implicated in cancer pathogenesis. Its strong overexpression has been detected in thyroid, colorectal, and prostate carcinoma, in the latter two clearly correlating with the degree of tumor malignancy (Wang et al. 2006; Takahashi et al. 2000; Adams et al. 2001b; Chieffi et al. 2006). In contrast to Aurora A, the chromosomal region encompassing Aurora B gene – 17p13.1 – is not amplified to a high level in tumors (Kimura et al. 1998). Nevertheless, low-level copy number increases have been frequently detected in primary non-small cell lung carcinoma (NSCLC), where the level of Aurora B expression correlates with the degree of genetic instability (Smith et al. 2005). Generally, cells overexpressing Aurora B show defects in chromosome segregation, cytokinesis, and elevated level of phosphorylated histone H3, which, at least in some human colorectal cell lines, correlates with the degree of Aurora B expression (Tatsuka et al. 1998; Ota et al. 2002).

For Aurora C, no defined role in tumorigenesis has been established yet, although it has been found to be expressed in a number of tumors and cancer cell lines (Hu et al. 2000; Kimura et al. 1999).

14.4 Inhibitors

Since the discovery of Aurora kinases, their involvement in the process of mitosis and their implication in tumorigenesis, much effort has been made to identify and develop effective inhibitors. It is estimated that in the last few years, more than 20 drug development programs have been

initiated resulting in the identification of many small molecule compounds with an inhibitory activity toward Aurora kinases. Several of them currently undergo preclinical and clinical assessment (Carpinelli and Moll 2007).

MK-0457 (previously VX680; Merck) is a pyrimidine derivative with affinity for all three Aurora kinases at nanomolar concentrations. This compound inhibits also other kinases like Flt-3 and Abl, including the T315I-Abl mutant (Harrington et al. 2004; Young et al. 2006). In its mechanism of action, MK-0457 prevents cytokinesis, disrupts bipolar spindle formation but allows the cells to progress through mitosis, causing accumulation of polyploid cells and eventually massive apoptosis. In preclinical in vivo models, MK-0457 was able to induce regression in tumor xenografts of leukemia (HL60) and colon cancer (HCT116) (Harrington et al. 2004).

In a first Phase I clinical trial, MK-0457 was given to the patients with previously treated solid tumors in a continuous 5-day intravenous infusion every 4 weeks (Rubin et al. 2006). The main dose-limiting toxicity (DLT) was grade 3 neutropenia and some nonspecific side effects, as low-grade nausea, skin rash, fatigue, and fluid retention. Stabilization of the disease was observed in patients with NSCLC and pancreatic carcinoma. Phase II single-agent efficacy studies in lung and colorectal cancer have been initiated. Furthermore, studies to determine the activity of this compound in hematological malignancies like relapsed or refractory AML, ALL, myelodysplastic syndromes, and CML have been initiated. Because MK-0457 has activity on the T315I-Abl mutant, patients in accelerated or blast phase CML, including those carrying T315I mutation, were treated in Phase I trial according to the following regimen: continuous 5-day infusions every 2–3 weeks at doses of 8–32 mg/m^2/h. All T315I patients responded to the therapy, with one major and four minor hematological as well as with one complete and two partial cytogenetic responses (Hampton 2007).

Clinical responses to MK-0457 therapy were also noted in Ph$^+$ ALL patients resistant to treatment with tyrosine kinase inhibitors (Imatinib, Nilotinib, and Dasatinib) (Giles et al. 2007). Based on these results, Merck has started a new Phase II trial enrolling CML patients in chronic, accelerated, and blast phase as well as Ph$^+$ ALL patients (Carpinelli and Moll 2007). Regrettably, in November 2007 Merck halted enrollment on trials of MK-0457 due to a reported potential heart safety issue (QTc prolongation) in one patient (Keen and Taylor 2009).

AZD-1152 (AstraZeneca) is a phosphate derivative of a quinazoline, a prodrug, which is converted in the plasma into the active metabolite AZD-1152-HQPA. This compound has higher affinity for Aurora B than Aurora A, being also active on Aurora C. Its effects in cancer cells are similar to those of MK-0457. In the ongoing Phase I trial in advanced, pretreated solid tumors this drug has been administered as a weekly 2-h infusion over the 28-day treatment cycle. The DLT was reported to be grade 3 neutropenia with few non-hematologic toxicities. The initial results showed stable disease in three patients (melanoma, nasopharyngeal carcinoma, and adenoid cystic carcinoma) (Schellens et al. 2006). In follow-up studies, the biweekly and continuous infusions have been introduced based on preclinical data suggesting that prolonged AZD-1152 administration results in markedly increased apoptotic effects of AZD-1152-HQPA (Gautschi et al. 2008).

MLN 8054 (Millenium Pharmaceuticals) was the first orally available Aurora kinase inhibitor with activity selectively on Aurora A. In in vitro studies, this compound causes mitotic spindle defects, accumulation of cells in mitosis, and inhibition of proliferation in several cancer cell lines (Manfredi et al. 2007). Moreover, regression of human tumor xenografts in nude mice was observed after oral administration of well-tolerated doses and this effect was sustained even after treatment cessation. In Phase I clinical trial, this compound was orally administered (daily for 7 days, in 21-day intervals) to patients with lymphomas and solid tumors, such as breast, pancreas, bladder, and metastatic colorectal carcinoma, inducing stabilization of the

disease in some cases (Galvin et al. 2006; Jones et al. 2007). The principal DLT observed was the reversible grade 3 somnolence. According to the animal studies, this is due to the ability of MLN 8054 to bind to the structure of the benziodiazepine receptor.

The immunohistochemical analysis of skin biopsies taken from patients treated with the MLN 8054 regimen revealed lower accumulation of mitotic cells than expected from in vitro studies suggesting unsufficient target inhibition in vivo. Therefore, a new study with twice-daily dosing over 14 days has been initiated, with the coadministration of methylphenidase (Gautschi et al. 2008). However, it has recently been announced that MLN-8054 will be replaced by a second-generation follow-up compound showing an increased potency of Aurora kinase A inhibition and a decreased benzodiazepine-like effect (Boss et al. 2009).

AT-9283 (Astex Therapeutics Ltd.) is an Aurora A and B inhibitor, which also inhibits JAK2, JAK3, and Abl kinases, including the T315I-Abl mutant. This compound has entered Phase I/IIa clinical trial, being evaluated in refractory hematologic malignancies such as AML, CML, and myelodysplastic syndromes with a 72-h continuous infusion at doses ranging from 3 to 48 mg/m² daily for three consecutive days (Ravandi et al. 2007).

KW-2449 (Kyowa Pharmaceutical, Inc.) is an oral multikinase inhibitor with a potent activity not only against Aurora A kinase but also Flt-3, FGFR1, Abl as well as T315I-mutated Abl kinases. This compound is now evaluated in Phase I clinical trial in patients with relapsed/refractory AML, treatment resistant/intolerant CML, ALL, and myelodysplastic syndromes being administered in a 14- or 28-day schedule, at daily doses ranging from 50 to 500 mg, divided into 12-h dosing. First results show stabilization of the disease achieved in some patients already after one cycle of therapy (Cortes et al. 2007).

XL228 (Exelixis, Inc.) is also a multikinase inhibitor with activity against kinases such as Aurora A, insulin-like growth factor type-1 receptor (IGF1R), Src, Abl, and its T315I mutant. In vitro studies in CML cell line K562 showed strong dephosphorylation of Bcr-Abl and Stat5 on treatment with XL228, resulting in pronounced inhibition of cell proliferation. In BaF3 cells transduced with BCR-ABL-T315I mutant inhibition of Bcr-Abl phosphorylation due to XL228 treatment was even more effective than on MK-0457 treatment. In an ongoing Phase I study, XL228 is evaluated as a weekly 1-h infusion in patients with CML or Ph⁺ B-ALL after failure of Imatinib or Dasatinib therapy (Shah et al. 2007; Zhang 2006).

Recently, several additional Aurora kinase inhibitors have entered clinical studies. *R763/AS-703569* (Rigel/Serono /Aventis-Sanofi) and *CYC-116* (Cyclacel) are in Phase I clinical trials. R763 is an Aurora B inhibitor, which can be administered orally and i.v. The main compound of Cyclacel – CYC-116 – is an oral Aurora A and B inhibitor with cross-inhibitory activity on VEGFR2.

In 2007, Pfizer introduced a new agent, *PF-03814735*, which is an Aurora A and B inhibitor for oral administration. This compound is being tested now in patients with advanced solid tumors (Carpinelli and Moll 2007).

14.5 Danusertib (formerly PHA-739358)

Danusertib is an Aurora kinase inhibitor, which shows a low-nanomolar activity against all three members of the Aurora kinase family – Aurora A, B, and C – inhibiting them at concentration of 13, 79, and 61 nM, respectively. Danusertib shows also cross-reactivity with other kinases tested, such as Ret, TrkA, FGFR1, and Abl including its T315I mutant. These observed cross-reactivities might have impact on the future role of Danusertib as an anticancer agent since the oncogenic Bcr-Abl tyrosine kinase is responsible for development of CML and *BCR-ABL*-positive ALL (Fig. 14.1).

Expression of TrkA has been reported in thyroid and prostate carcinoma and Ret seems to

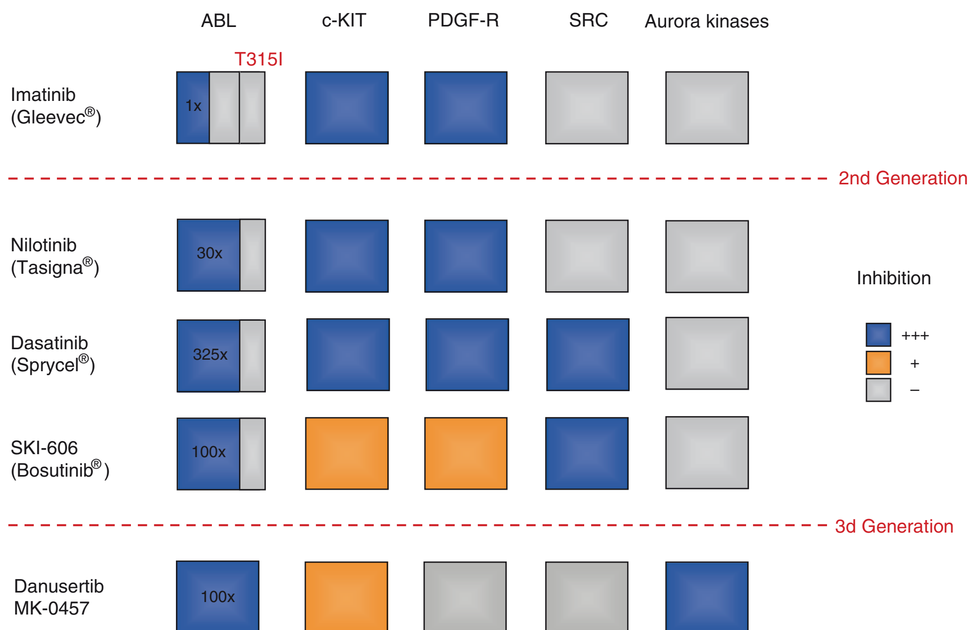

Fig. 14.1 *Simplified view on three generations of targeted Bcr-Abl inhibitors.* The tyrosine kinase inhibitor Imatinib (IM) has become an effective frontline therapy for CP CML. However, primary or acquired resistance to IM, mostly mediated by point mutations in the Abl kinase (shown in *gray*) although occurring at low frequency in CP disease, remain a major therapeutic challenge in the CML therapy, particularly in patients suffering from advanced stages of disease. Second-generation Bcr-Abl inhibitors are capable of overcoming the majority of these mutations, except of the T315I mutation

play an important role in thyroid and breast carcinoma (Lugo et al. 1990; Propp and Lizzi 1970; Bongarzone et al. 1998; Dionne et al. 1998; Sjoblom et al. 2006).

In vitro studies using a broad panel of different human cancer cell lines showed strong antiproliferative effects of Danusertib treatment with accumulation of tetraploid cells in G1-like growth arrest or cells with >4 N DNA as a feature of endoreduplication (Carpinelli et al. 2007; Gontarewicz et al. 2008). These different effects of Danusertib may be cell line specific, reflecting different requirements for Aurora kinase activity. Most probably, they depend on the status of p53-dependent mitotic checkpoint as Danusertib treatment of cells with defective p53 resulted frequently in progression through the cell cycle after failed cytokinesis and accumulation of cells with >4 N DNA. In comparison, cells with wild-type p53 underwent a growth arrest with less tetraploidy observed (Carpinelli et al. 2007).

The efficacy of Aurora kinase inhibition can be monitored by evaluation of changes in Aurora A auto-phosphorylation or in phosphorylation of histone H3. During mitosis, the latter protein is phosphorylated by Aurora B at Serine 10, an event that probably helps to drive mitotic chromatin condensation (Zeitlin et al. 2001; Gurley et al. 1978). As expected, cells treated with Danusertib show decreased autophosphorylation of Aurora A and reduced phosphorylation of histone H3-Ser10, suggesting an effective inhibition of both targets (Carpinelli et al. 2007; Gontarewicz et al. 2008) (Fig. 14.2).

In the biochemical assays testing the inhibitory effects of Danusertib on the kinase activity of Ret and Trk-A, both kinases were effectively inhibited at low-micromolar concentrations.

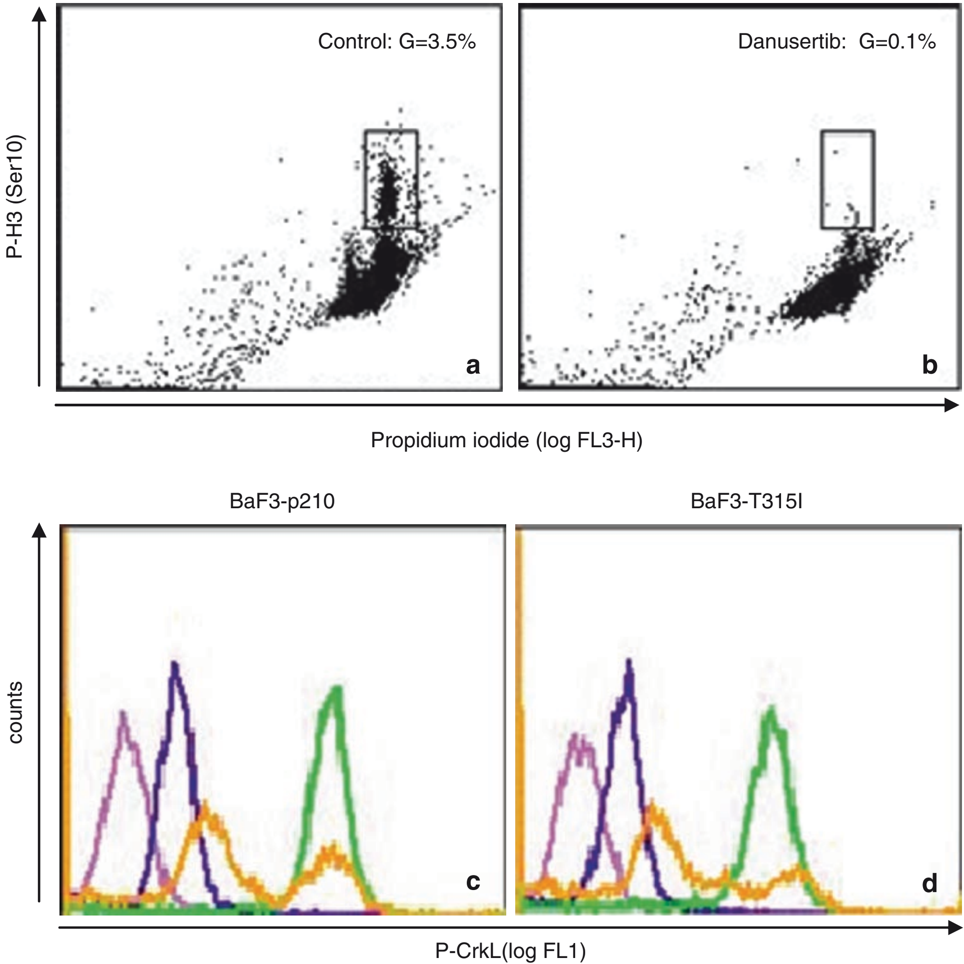

Fig. 14.2 *Danusertib acts in BCR-ABL positive cells via combined inhibition of Aurora and Bcr-Abl kinases. a–b* Danusertib reduces phosphorylation of Aurora B target histone H3. K562 cells were exposed to 5 µM Danusertib for 2 h and flow cytometric analysis of cells double stained with specific phospho-H3(Ser10) antibody and propidium iodide (DNA content) was performed. The gates indicate phospho-H3(Ser10) positive cells in untreated control cells (**a**) or cells exposed to Danusertib (**b**). *c–d* Danusertib treatment reduces phosphorylation of CrkL, a well-known target of Bcr-Abl kinase. Murine wild-type BaF3-p210 (**c**) and IM-resistant BaF3-T315I mutant (**d**) cells were treated with 5 µM of Danusertib for 24 h. The intracellular flow cytometry analysis of CrkL-phosphorylation status revealed that in comparison with untreated control cells (*green lines*) treatment with Danusertib results in strong inhibition of P-CrkL (*yellow lines*), independently of *BCR-ABL* mutational status. "This research was originally published in *Blood*. Gontarewicz et al. 2008. ©The American Society of Hematology"

Furthermore, Danusertib selectively inhibits FGF-dependent activation of MAPK pathway, without having such effects on MAPK activation due to EGF stimulation (Carpinelli et al. 2007). The inhibitory influence of the Danusertib on Bcr-Abl kinase was assessed in CML cell line K562. The treatment with this compound produced distinct inhibition of c-Abl auto-phosphorylation and pronounced inhibition of phosphorylation of CrkL and Stat5, well-known downstream targets of oncogenic Bcr-Abl. Furthermore, strong inhibition of CrkL phosphorylation was observed in murine BaF3 cells transduced with wild-type or mutated *Bcr-Abl*, including the T315I mutation (Gontarewicz et al. 2008) (Fig.14.2).

In vivo, the antitumor activity of Danusertib was evaluated in several solid human tumor xenograft models, clearly showing tumor growth inhibition, which was sustained after discontinuation of treatment (Gautschi et al. 2008; Carpinelli et al. 2007).

The results of the first two Phase I dose escalation studies have been recently presented (De Jonge 2006; Steeghs et al. in press; Cohen et al. in press). In one of them, a 6-h i.v. infusion on day 1, 8, and 15 in a 4-week cycle were administered to the patients with advanced, pretreated solid tumors. The main DLT observed was grade 3–4 neutropenia. No significant objective tumor regressions were observed but 8 of 40 patients achieved disease stabilization for at least 4 months. Monitoring of target inhibition through analysis of histone H3-Ser10 phosphorylation level in skin biopsies taken before and after therapy revealed expected dephosphorylation in eight of nine cases tested. The second study tested a 24-h infusion in a 2-week cycle. As in the previous one, principal DLT was grade 3–4 neutropenia. Additionally, mild, non-hematologic toxicities like fatigue, pyrexia, and diarrhea were observed. Again, 11 of 40 patients showed disease stabilization with no objective tumor remissions, although it is worth to note that in one case of ovarian cancer approx. thirty percent tumor reduction was observed.

Post-therapy skin biopsies showed decreased level of histone H3-Ser10 phosphorylation in four of five cases tested. The recommended dose for Phase II was established at 500 mg/m² without granulocyte colony-stimulating factor (GCSF) support, whereas further dose escalation regimen with filgastrim coadministration have been tested in a few patients.

As already mentioned, Danusertib has strong inhibitory effects on Abl kinase and several of its mutants, including all second-generation tyrosine kinase inhibitors resistant T315I mutant. In vitro studies using CD34+ cells from CML patients at different stages of disease, including those with T315I mutation, revealed time- and dose-dependent inhibition of cell proliferation upon exposure to Danusertib (Fig. 14.3).

Moreover, a significant loss of viability due to apoptosis was observed in Danusertib treated primary CD34+ cells, at least in part caused by inhibition of Bcr-Abl kinase activity as shown by pronounced dephosphorylation of CrkL (Gontarewicz et al. 2008).

The analysis of the crystal structure of T315I-Abl in complex with Danusertib revealed the compound's binding to the active conformation of the mutant kinase in a mode that accommodates the substitution at the "gatekeeper" position 315 (Modugno et al. 2007) (Fig. 14.4).

For the ongoing multicenter Phase II study, seven CML patients at different stages of disease have been enrolled who failed previous therapy with tyrosine kinase inhibitors: one in chronic phase (CP), one in acceleration phase (AP), and five in blast crisis (BC). Among them, six carried the T315I mutation. The compound was administered at two dose levels, 250 or 330 mg/m²/day, as a weekly 6-h infusion for 3 consecutive weeks, every 4 weeks (Paquette et al. 2007). Two patients with T315I mutation achieved a complete hematologic response. The first patient, diagnosed in AP, had also a CCyR durable after >6 months, and a complete molecular response on the 330 mg/m²dose level. The second one was diagnosed in CP and achieved a minor CyR also on the 330 mg/m² dose level.

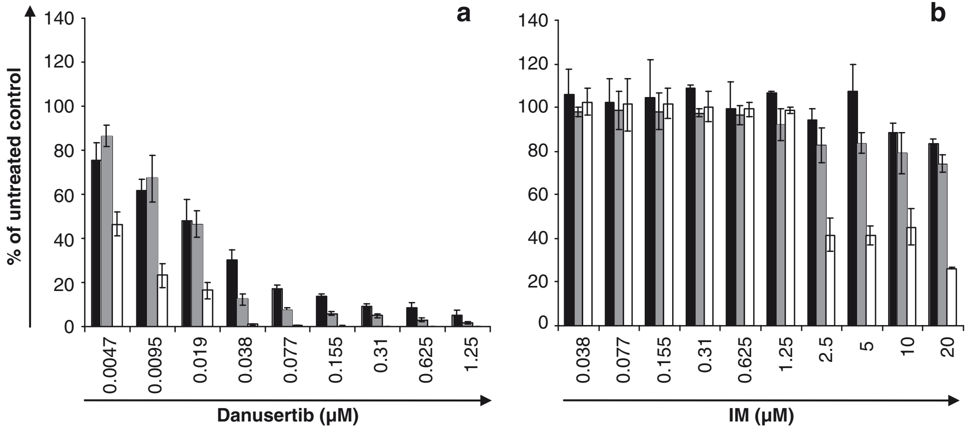

Fig. 14.3 *Exposure to Danusertib leads to growth inhibition of CML progenitor cells with T315I BCR-ABL mutation.* CD34+ cells from a patient diagnosed in IM-resistant CML-BC with a confirmed T315I mutation were exposed to the indicated concentrations of Danusertib (**a**) or IM (**b**) for 9 days in SFM supplemented with growth factors. Cell proliferation was assessed at day 3 (*black bars*), day 6 (*gray bars*), and day 9 (*white bars*). Bar graphs represent the mean % of cellular expansion ± S.D. in relation to untreated control cells. The antiproliferative effects of Danusertib on CD34+ cells were found to be largely unaffected by the T315I *BCR-ABL* mutation. "This research was originally published in *Blood*. Gontarewicz et al. 2008. ©The American Society of Hematology"

The Cmax at the effective dose of 330 mg/m²/day was 4–6 µmol/L/h. All patients tolerated well the Danusertib treatment, with only one who had grade 4 neutropenia and an infusion-related reaction (Paquette et al. 2007).

Thus, the in vitro and in vivo data clearly show that simultaneous targeting of wild-type or mutant Bcr-Abl and Aurora kinases by Danusertib represents a promising new treatment strategy for patients suffering from relapsed/refractory CML, particularly for those harboring the highly resistant T315I mutation.

14.6 Conclusion

Since their discovery, Aurora kinases have changed from almost unknown enzymes involved in the cell cycle regulation to novel, very promising targets of anticancer therapy. Our understanding of their biological properties and functions in normal and neoplastic cells – though still incomplete – has provided a basis for development of a new class of targeted anticancer drugs. The ongoing clinical trials are trying to find answers for many questions: inhibition of which member(s) of the Aurora kinase family is required for sustained tumor responses and an optimal therapeutic window? What is the optimal dosing schedule? Which biological marker can predict treatment response? What combination partners are most promising (other molecular targeted agents aiming crucial cellular rescue pathways, conventional antimitotic chemotherapeutic drugs, angiogenesis inhibitors a.o.). Although still a long way to go, choices of combination treatment in the future will hopefully be made based on individualized molecular profiling of tumors leading to the identification of the most promising target(s) to aim for resulting in the choice of the best compounds to combine with on an individual patient-specific basis.

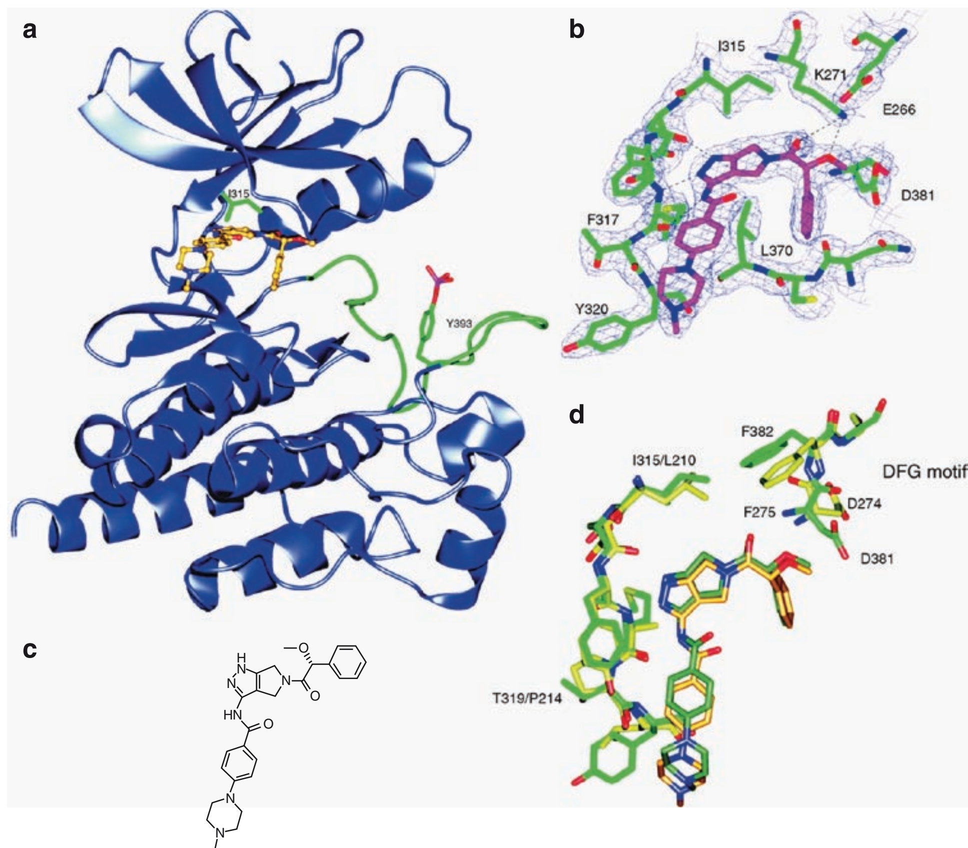

Fig. 14.4 *Structure of Abl-T315I-Danusertib complex.* (**a**) Ribbon representation of the structure of T315I-Abl mutant with Danusertib. The mutated gatekeeper residue Ile[315] and the activation loop with the phosphorylated residue Tyr[393] are highlighted in *green.* (**b**) Close-up view of the binding site of Danusertib showing the final 2Fo-Fc electron density map, contoured at one, associated with the ligand. (**c**) Chemical formula of Danusertib. (**d**) Comparison of Danusertib complexes with the Aurora A structure. Details of the binding of Danusertib to Abl (*green carbon atoms*) and to Aurora A (*yellow carbon atoms*) showing the residues of the hinge region. Published with permission from the AACR Publications Department: Modugno et al. 2007

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15 BI_2536 - Targeting the Mitotic Kinase Polo-Like Kinase 1 (Plk1)

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Abstract Human Polo-like kinase 1 (Plk1) is an essential regulator of mitotic progression. Targeted inhibition of this kinase was effective in killing tumor cells in vitro and in vivo. The Plk1 inhibitor BI_2536 was well tolerated and showed antitumor activity in the first clinical trials enrolling patients with advanced solid tumors and refractory or relapsed acute myeloid leukemia.

15.1 Introduction

The cell division cycle is responsible for duplication of the genetic material and equal distribution to the developing daughter cells. Deregulation of the cell division cycle can lead to unrestrained proliferation and accumulation of genetic defects contributing to cancerogenesis. Chromosome duplication and segregation occur in distinct cell-cycle phases. In the G1 phase the cell prepares for DNA replication, which takes place in the subsequent S phase. In G2 replication errors can be repaired before the cell enters mitosis, where chromosome separation and cytokinesis takes place. In the following G1 phase the cell can either differentiate or enter a new cell cycle. Checkpoints control accurate DNA replication and chromosome separation. DNA damage can activate a checkpoint in the G1 or G2 phase leading to cell cycle arrest and repair of the damaged DNA. The spindle assembly checkpoint controls the correct attachment of the mitotic spindle to the chromosomes to avoid missegregation and aneuploidy. The cell division cycle is regulated by different kinases with the cyclin-dependent-kinases (Cdks) as key enzymes, which are periodically activated by cyclins. The periodic activity is necessary to ensure that Cdks are only active when needed warranting unidirectional cell cycle progression. The periodic cyclin activity is mainly regulated by ordered protein synthesis and degradation. In G1 extracellular signals stimulate cyclin D expression and cyclin D-Cdk complexes phosphorylate the retinoblastoma protein (pRb). This leads to the liberation of E2F transcription factors and expression of other cyclins, such as cyclins E and A and subsequent activation of cyclin B responsible to drive S phase and mitosis. After they have performed their tasks targeted proteolysis of cyclins and other cell cycle regulators by the ubiquitin-proteasome system is required to irreversibly inactivate these

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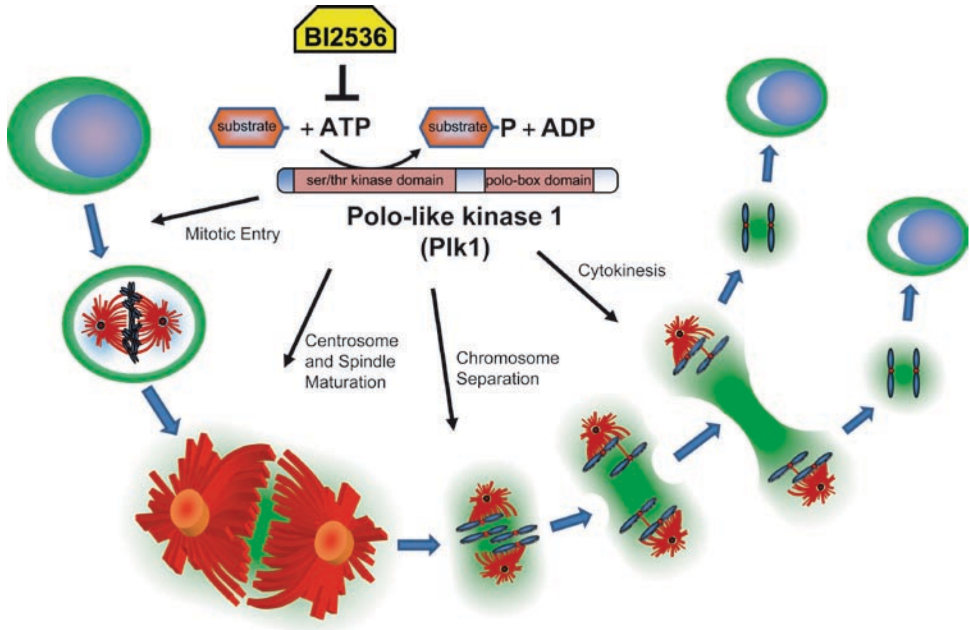

Fig. 15.1 Polo-like kinase 1 (Plk1) is a central regulator of cell division in human cells. Plk1 is important for accurate timing of mitotic entry. In prophase, Plk1 associates with centrosomes to promote mitotic spindle maturation to guarantee the assembly of bipolar spindles. In prometaphase, Plk1 accumulates at the central spindle suggesting a role in the formation of stable microtubule-kinetochore attachments to ensure proper sister chromatid separation. In late mitosis, Plk1 is targeted to the spindle midzone to contribute to the formation of the contractile ring mediating the onset of cytokinesis. The small molecule inhibitor BI_2536 interferes with the catalytic action of this multifunctional mitotic kinase by blocking the ATP-binding site leading to cell cycle arrest and apoptosis in malignant cells

proteins, thereby ensuring for example that the DNA is only duplicated once per cell cycle. Other important regulators are the mitotic kinase families Aurora and Polo-like kinases (Plk) (Morgan 2007; Archambault and Glover 2009). The first identified Plk family member was Polo in *Drosophila melanogaster*(Sunkel and Glover 1988). Subsequently, it has been shown that this mitotic kinase familiy is highly conserved throughout eukaryotes with four family members Plk1–4 in mammalian cells (van de Weerdt and Medema 2006). Plk1 is the best characterized member of the human Plk family. It is a serine/threonine kinase and controls several steps during the progression of cells through mitosis. It is important for entry into mitosis, centrosome separation, and maturation in order to build a bipolar mitotic spindle, metaphase to anaphase transition, mitotic exit, and the onset of cytokinesis (Petronczki et al. 2008). The ordered progression through mitosis is essential to maintain genomic stability and may be deregulated by aberrant Plk1 expression (Engelbert et al. 2008; Garcia-Higuera et al. 2008; Wäsch and Engelbert 2005). Plk1 is transcriptionally regulated in the cell cycle with the highest activity in G2 and M (mitosis) phase (Uchiumi et al. 1997). At the end of mitosis and during G1, Plk1 is inactivated by proteasomal degradation mediated by the E3-ubiquitin-ligase anaphase-promoting complex or cyclosome (APC/C) (Engelbert et al. 2008; Garcia-Higuera et al. 2008). Overexpression of Plk1 can be found

in various human cancers and has prognostic value providing a rationale for its inhibition as a novel and promising therapeutic approach (Strebhardt and Ullrich 2006) (Fig. 15.1).

15.2 Structure and Mechanism of Action

The small molecule inhibitor BI_2536 is a dihydropteridinone derivative and inhibits the enzymatic activity of Plk1 by blocking the ATP-binding site. This compound is highly specific and at least 1,000-fold more selective against Plk1 when compared with a large panel of other tyrosine and serine/threonine kinases (Steegmaier et al. 2007). Inhibition of Plk1 with the small molecule BI 2536 leads to a cell cycle arrest in prometaphase often with abnormal mitotic spindles and subsequent apoptosis of several cancer cell lines including colorectal carcinoma, nonsmall cell lung cancer, acute myeloid leukemia (AML), and B cell lymphoma. Moreover, BI_2536 is able to significantly delay growth of various xenografted human tumors in nude mice (Lenart et al. 2007; Steegmaier et al. 2007).

15.3 Clinical Data

In a Phase I dose escalating and pharmacokinetic study, 40 patients with advanced solid tumors were treated with a median number of three treatment courses. Neutropenia was the predominant dose-limiting toxicity (DLT) and the most frequent adverse event (grade 3–4; 56%). Nausea (52%), fatigue (52%), and anorexia (44%) were also common. The maximum tolerated dose (MTD) was 200 mg. One patient with squamous cell head and neck cancer experienced a transient partial response (PR), and 42% of patients had stable disease (SD) as the best response, which lasted in 23% of patients for 3 months or more (Mross et al. 2008).

BI_2536 was also investigated in lung cancer. BI_2536 can effectively inhibit growth of the human NSCLC cell lines NCI-H460, NCI-H520TC, Calu-6, and A549 in vitro and in vivo in a nude mouse model (Baum et al. 2007). A phase I trial used pemetrexed (500 mg/m^2) in combination with escalating doses of BI_2536 (given d1 q3 weeks) in a standard 3+3 trial design. The primary objective was to determine the MTD of BI_2536 in combination with pemetrexed (Ellis et al. 2008). The secondary endpoints were response rate (RR), overall safety, and PK. Patients not progressing after 6 cycles of combination therapy were eligible to continue BI_2536 maintenance until progression. MTD was defined at 300 mg BI 2536. Two of 22 patients (6%) achieved PRs (Ellis et al. 2008). Although interpreted as encouraging, this antitumor effect might be attributable to pemetrexed and not BI2356. Another phase II trial in patients with relapsed advanced or metastatic NSCLC has recently been presented at ASCO 2008 (Von Pawel et al. 2008). This trial used two dosing schedules of BI2356 (200 mg d1 or 50 mg/60 mg d1–3). The primary endpoint was objective response; the secondary endpoints were progression-free (PFS) and overall survival (OS). PFS was 58 days (95% CI: 48–85), and OS was 189 days (95% CI: 176–304). Four patients (4.2%) showed an objective response (Von Pawel et al. 2008). One phase II trial (ClinicalTrials.gov identifier: NCT00412880) evaluated the role of BI2356 in second-line monotherapy in small cell lung cancer. This trial has been completed, and data are awaited to be presented. Taken together, the role of BI2356 in the treatment of lung cancer remains to be determined.

Another multicenter Phase I/IIa dose escalating and pharmacokinetic study investigated BI_2536 in patients over 60 years with refractory or relapsed AML. The drug was well tolerated in these patients with some responses. Stabilization of the disease by BI_2536 in three of the patients

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enrolled in the trial from our department enabled us to perform an allogeneic transplantation after reduced intensity conditioning with 2/3 of these patients being alive and well.

15.4 Conclusion and Future Perspectives

Cell cycle regulators including Plk1 are promising drug targets for cancer therapy. The better understanding of the biological function of these regulators in cancer is crucial for a more effective therapeutic targeting. Traditional antimitotic drugs including the vinca alkaloids and the taxanes are microtubule inhibitors and are associated with more severe side effects, such as neurotoxicity, due to tubulin function outside mitosis. The generation of more selective compounds targeting cell proliferation and mitosis may thus lead to less toxic but efficient therapeutics used alone or in combination with other anticancer drugs.

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Part IV

Other Novel Agents

Imetelstat (GRN163L) - Telomerase-Based Cancer Therapy

16

Alexander Röth, Calvin B. Harley, and Gabriela M. Baerlocher

Abstract Telomeres and telomerase play essential roles in the regulation of the lifespan of human cells. While normal human somatic cells do not or only transiently express telomerase and therefore shorten their telomeres with each cell division, most human cancer cells typically express high levels of telomerase and show unlimited cell proliferation. High telomerase expression allows cells to proliferate and expand long-term and therefore supports tumor growth. Owing to the high expression and its role, telomerase has become an attractive diagnostic and therapeutic cancer target. Imetelstat (GRN163L) is a potent and specific telomerase inhibitor and so far the only drug of its class in clinical trials. Here, we report on the structure and the mechanism of action of imetelstat as well as about the preclinical and clinical data and future prospects using imetelstat in cancer therapy.

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16.1 Introduction

All human cells are equipped with unique DNA/protein structures at the end of their chromosomes, the so-called telomeres (Moyzis et al. 1988). At birth, telomeres are composed of approximately 12–15 kb of hexameric TTAGGG double-stranded DNA repeats with a single-strand overhang of the 3'-G-rich strand. This single-strand overhang is, together with several telomere-associated proteins (TRF1, TRF2, POT1 etc), important for the 3-dimensional structure of the chromosome end (Fig. 16.1) (Baumann and Cech 2001; Griffith et al. 1999; Smogorzewska and de Lange 2004). Telomeres exist in either an open or a closed form. For the closed state, the chromosome end folds back into a telomere loop structure in order to protect its single-strand overhang from degradation (Griffith et al. 1999). The function of telomeres is to maintain chromosomal integrity, prevent end-to-end chromosome fusion, degradation, and inappropriate recombination (Blackburn 1991; de Lange 2002).

With each round of cell division, however, telomere repeats would theoretically be lost as a result of an incomplete terminal synthesis of the lagging DNA strand (Olovnikov 1973; Watson 1972). It has now been well documented that such a loss occurs both in vitro and in vivo, and that

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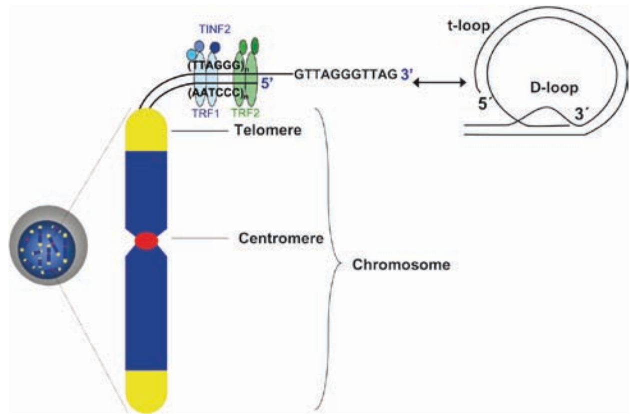

Fig. 16.1 *Structure of human telomeres.* Telomeres are at the end of the linear chromosomes, and consist of repetitive DNA sequences and associated proteins. Most of the telomere is double-stranded DNA, but at the very end, there is a 50–300 base pair of a single-stranded DNA overhang. Telomeres form higher order structures and are folded into a telomere loop structure

other mechanisms can influence the rate of telomere loss with cell aging (Chang and Harley 1995; Harley et al. 1990; Martens et al. 2000; Röth et al. 2003b; Rufer et al. 1998). In order to study telomere dynamics in vivo, a highly sensitive, accurate, and reproducible method to measure telomere length in blood leukocytes was developed (Baerlocher et al. 2002). In addition, this method was combined with immunostaining in order to determine the telomere length in the subsets of leukocytes (Baerlocher and Lansdorp 2003; Baerlocher et al. 2006). The decrease in telomere length in human blood leukocytes over age is not linear, but occurs in different phases with a more pronounced decrease early and late in life (Baerlocher and Mak 2003; Rufer et al. 1998). Based on a study looking at the telomere biology in baboons, it was shown that the telomere length is only maintained for a few weeks after birth before the decrease in telomere length starts to occur (Baerlocher et al. 2003). In addition, the decrease in telomere length with age is not equal for diverse subsets of leukocytes and demonstrates that telomeres in lymphocytes shorten more rapidly over age than in granulocytes. Moreover, telomere length measurements by automated multicolor flow-FISH was able to identify individuals and patients with very short telomeres (telomere length below the first percentile of healthy individuals) in blood leukocytes (Alter et al. 2007). Some of such patients and individuals have a defect in telomere maintenance pathways and are at a high risk to suffer (or potentially going to suffer) from dyskeratosis congenita, idiopathic pulmonary fibrosis, anemia, cirrhosis, or other disorders of proliferative tissues (Garcia et al. 2007). The prototypic human telomerase genetic disease, dyskeratosis congenita, is a rare condition clinically characterized not only by leukoplakia, nail dystrophy, and hyperpigmentation, but also by many other stem cell and organ dysfunctions as well as a shorter life span (Alter et al. 2007; Röth and Baerlocher 2008). The main cause for such short telomeres is a partial deficiency in telomerase or a dysfunction of telomere-associated proteins.

The human telomerase complex is a ribonucleoprotein polymerase that is able to synthesize terminal T_2AG_3 telomere repeats and extend or maintain telomeres (Fig. 16.2) (Blackburn 1992; Collins and Mitchell 2002; Smogorzewska and de Lange 2004). In humans, most of the somatic cells have only low or undetectable telomerase activity, but telomerase expression is important for maintaining telomere length in germ cells and embryonic stem cells, and for slowing the rate of telomere loss or possibly elongating telomeres periodically in organ stem and progenitor cells as

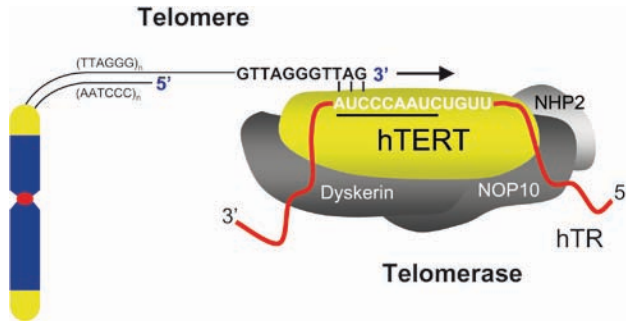

Fig. 16.2 *Structure and function of telomerase.* The human telomerase complex consists of a catalytic subunit (hTERT), an RNA component (hTR), dyskerin, NOP2, NHP2, and additional associated proteins. Here, the de novo synthesis of telomeric repeats by telomerase is illustrated. The template region of hTR is *underlined*. Four additional nucleotides complementary to the 3'-end of imetelstat are also shown

well as for germinal center B- and activated T-cells (Chiu et al. 1996; Lebkowski et al. 2001; Vaziri et al. 1993; Weng et al. 1997). Telomerase activity is suppressed early after birth (Baerlocher et al. 2003) and most likely has evolved as a tumor suppressor mechanism (Verfaillie et al. 2002). The telomerase complex consists of a catalytic subunit with reverse transcriptase activity encoded by the human telomerase reverse transcriptase (hTERT) gene, a RNA template encoded by the hTR gene (human telomerase RNA, TERC), and other associated proteins (e.g., dyskerin, NOP10, NHP2) (McEachern et al. 2000; Nakamura et al. 1997; Vulliamy et al. 2008; Walne et al. 2007). Mutations in at least five telomerase components can lead to a reduction in telomerase expression and consequently to very short telomeres in essentially all cells of the body. When chromosome ends become too short, DNA damage signals induce a state of replicative senescence or cell apoptosis (Campisi 2003; d'Adda et al. 2003). As mentioned earlier, telomeres are extremely short in patients with DC (Alter et al. 2007), leading to premature senescence of especially lymphocytes.

In contrast to low or physiologic levels of telomerase, up to 80–90% of human cancer cells pathologically overexpress telomerase, which is a key element for immortalization (Hahn et al. 1999a). Furthermore, recent studies have suggested that cancer stem or stem-like cells are also telomerase-positive (Armanios and Greider 2005; Ho et al. 2007; Phatak and Burger 2007; Phatak et al. 2007). Overexpression of hTERT leading to high and stable telomerase activity in normal human cells (e.g., fibroblasts, CD4, and CD8 T-cells) proofed the link between telomere elongation and immortalization (Bodnar et al. 1998). However, it also demonstrated that telomeres in T-lymphocytes still get shortened, despite high levels of telomerase activity (Röth et al. 2005). Importantly, telomerase is one of the key components that is needed for the development of a cancer cell capable of long-term growth from a normal human cell (Hahn et al. 1999a). However, how and when telomerase is reactivated in the process of oncogenesis in vivo is not yet clear (Kyo et al. 2008). The difference in telomerase expression and the length of telomeres in healthy and cancer cells makes telomerase a highly attractive diagnostic and therapeutic cancer target, especially as telomerase inhibition could be used in a universal way for most types of human tumors. Telomerase inhibition would mainly affect cancer cells, as these cells in general present high telomerase activity and shorter telomeres.

16.2 Telomerase-Based Approaches of Cancer Treatment

The necessity of maintaining telomeres for replicative immortality and the specificity of high telomerase expression in malignant cells of essentially all types, including the putative cancer stem cells, suggest a universal and critical therapeutic target for specific telomerase-based drugs. Various strategies targeting telomerase-positive tumor cells exist. Today, most of these approaches have proven their principle in animal models, but are still in the preclinical stage of development (Harley 2008). Here, we would like to specifically focus on telomerase inhibitors.

16.3 Telomerase Inhibition

A variety of classes of telomerase inhibitors exist, including small molecules like BIBR1532, antisense oligonucleotides, ribozymes, and dominant-negative constructs (Table 16.1, Fig. 16.3) (Olaussen et al. 2006; Pendino et al. 2006; Rankin et al. 2008). These inhibitors either block the catalytic activity of the enzyme or the access of telomerase to the telomeres (Asai et al. 2003; Mergny et al. 2002; Tauchi et al. 2003). Proof of principle for the therapeutic efficacy of such inhibitors has been demonstrated by in vitro cultures by transfecting dominant-negative hTERT. In various cell types, inhibition of telomerase by dominant-negative hTERT leads to continuous telomere shortening and subsequent cell senescence or apoptosis (Hahn et al. 1999b; Röth et al. 2003a, b; Zhang et al. 1999).

One of the first, initially promising telomerase inhibitors was BIBR1532. BIBR1532 is a non-peptidic, non-nucleosidic, non-competitive inhibitor of the catalytic activity of telomerase, with a drug-binding site distinct from the sites for the deoxyribonucleotide substrates and the DNA primer (Damm et al. 2001; Pascolo et al. 2002). Despite promising inhibitory activity in some model systems, BIBR1532 has not progressed into clinical trials due to unsatisfactory bioavailability (Barma et al. 2003; Pascolo et al. 2002; Ward and Autexier 2005).

16.4 Structure of Imetelstat and Mechanism of Action

One of the newer promising drugs is the short oligonucleotide-based telomerase inhibitor imetelstat (previously named GRN163L). Imetelstat is a novel lipid-based conjugate of the first-generation oligonucleotide GRN163 (Asai et al. 2003), and consists of a 13-mer oligonucleotide N3'–P5' thio-phosphoramidate (NPS oligonucleotide) that is covalently attached to a C16 (palmitoyl) lipid moiety. Owing to the 5' lipid chain and its improved oligonucleotide backbone

Fig. 16.3 *Telomerase inhibitors.* Various telomerase inhibitors and their target on telomerase are illustrated (Table 16.1)

Table 16.1 Telomerase inhibitors

Compound	Class	Target and mechanism	References
Antisense oligonucleotides	Antisense molecule	Blocking hTR or hTERT	(Feng et al. 1995; Koga et al. 2001; Kondo et al. 1998; Kushner et al. 2000; Yatabe et al. 2002)
AZT	Enzyme inhibitor	Blocks dNTP incorporation into DNA	(Fletcher et al. 2001; Melana et al. 1998; Strahl and Blackburn 1996)
BIBR1532	Enzyme inhibitor/ small molecule	Specific inhibition of the active site	(Barma et al. 2003; Damm et al. 2001; El Daly et al. 2005; Pascolo et al. 2002; Röth et al. 2007)
DN-hTERT	Dominant-negative construct	Dominant-negative action on telomerase activity	(Hahn et al. 1999b; Misawa et al. 2002; Röth et al. 2003a, b; Tauchi et al. 2002; Zhang et al. 1999)
GRN163(L)	Antisense molecule	hTR template antagonist	(Akiyama et al. 2003; Asai et al. 2003; Dikmen et al. 2005; Djojosubroto et al. 2005; Gellert et al. 2006; Gomez-Millan et al. 2006; Gryaznov et al. 2007; Hashizume et al. 2008; Herbert et al. 2005; Hochreiter et al. 2006; Jackson et al. 2007; Ozawa et al. 2004; Röth et al. 2008)
Ribozymes	Ribozymes	Cleavage of hTR or hTERT-RNA sequence	(Folini et al. 2000, 2002; Ludwig et al. 2001; Nosrati et al. 2004; Saretzki et al. 2001; Yeo et al. 2005; Yokoyama et al. 1998, 2000)

chemistry, imetelstat has very good cellular and tissue penetration even with lower concentrations and a better biodistribution into both normal and malignant cells. Furthermore, the oligonucleotide, as a larger polyanionic compound, is not likely to be a substrate for common mechanisms of multidrug resistance, a property, which is especially relevant for targeting cancer stem cells. Imetelstat does not exhibit antisense activity, but rather directly binds to the RNA component of telomerase (hTR) with very high affinity and specificity to the template region of hTR in the active site of the telomerase enzyme (Dikmen et al. 2005; Gellert et al. 2006; Herbert et al. 2005). The structure and mechanism of action of imetelstat are demonstrated in Fig. 16.4.

16.5 Preclinical and Clinical Data of Imetelstat

The effects of imetelstat have been well characterized in many preclinical studies. Telomerase inhibition has been shown for diverse human tumor cells (including lung (Dikmen et al. 2005;

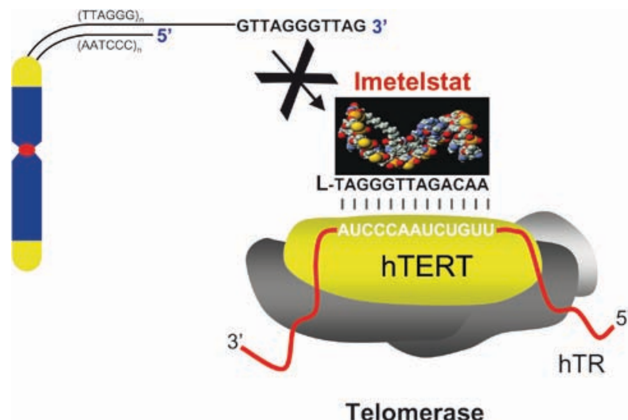

Fig. 16.4 *The telomerase inhibitor imetelstat and its action on telomerase.* Imetelstat is a 13-mer oligonucleotide N3'–P5' thio-phosphoramidate that is covalently attached to a C16 (palmitoyl) lipid moiety. Imetelstat binds to the telomerase RNA component sequence (hTR) in the active site region of telomerase, and hence, is a direct enzymatic inhibitor, a competitive substrate antagonist. Imetelstat blocks the telomere access to telomerase and thereby acts like a conventional pharmaceutical drug

Jackson et al. 2007), breast (Gellert et al. 2006; Gomez-Millan et al. 2006; Hochreiter et al. 2006), prostate (Asai et al. 2003), liver (Djojosubroto et al. 2005), brain (Ozawa et al. 2004), bladder (Dikmen et al. 2008), and hematological malignancies including multiple myeloma and lymphoma (Akiyama et al. 2003; Wang et al. 2004)), in both cell culture systems and mouse xenograft models (Table 16.2). Studies of this agent alone, and in combination with chemotherapeutic agents, indicate the importance of telomerase as a target for the treatment of cancer, and the potential utility of imetelstat in the treatment of patients with hematologic and solid tumor malignancies. Some studies have even shown that imetelstat is additive or synergistic when used in combination with existing cancer drugs or radiation.

Based on in vitro and in vivo efficacy in a series of animal studies, imetelstat has entered six phase I and I/II clinical trials (Table 16.3) for various cancers including hematologic and solid tumors. Three of these trials are single agent studies (chronic lymphoproliferative diseases, multiple myeloma, and solid tumor malignancies), and three are combination studies (breast cancer in which imetelstat is given in conjunction with paclitaxel and bevacizumab, non-small cell lung cancer in which imetelstat is given in conjunction with paclitaxel and carboplatin, and multiple myeloma in which imetelstat is given in conjunction with velcade ± dexamethasone). For all the phase I and I/II trials, the primary outcomes are safety, tolerability, and determination of the maximum tolerated dose (MTD). Dosing is based on 3- or 4-week cycles of once-weekly 2- or 6-h intravenous infusions. Doses are escalated after the first cycle in each cohort until MTD is reached. The once-weekly schedule was based on the pharmacokinetic modeling of the tissue's half-life in animals and humans, and the relatively durable effects of telomerase inhibition for up to 3–7 days were seen in rodent liver or human tumor xenograft tissues in vivo (Dikmen et al. 2005). To determine the relationship between drug exposure and response predictive of antineoplastic activity, pharmacokinetics and pharmacodynamics were included as secondary endpoints. So far, imetelstat has been tolerated and the trials are continuing until an MTD is reached.

Table 16.2 GRN163 and imetelstat (GRN163L) in preclinical studies

Tumor type	Cellular and molecular response	References
Lung cancer	Effective inhibition of telomerase Progressive telomere shortening Reduction of colony formation Prevention of metastasis in xenograft model Altered adhesion with reduced cellular attachment Reduced cell spreading Reduced tumor progression	(Dikmen et al. 2005; Jackson et al. 2007)
Breast cancer	Effective inhibition of telomerase Progressive telomere shortening Reduction of tumor growth, colony formation, tumorgenicity, invasive potential and metastasis Enhanced radiation sensitivity	(Gellert et al. 2006; Gomez-Millan et al. 2006; Hochreiter et al. 2006)
Prostate cancer	Telomere shortening with cellular senescence or apoptosis after lag period correlate with initial telomere length Suppression of tumor growth	(Asai et al. 2003)
Liver cancer	Inhibition of telomerase and tumor cell growth in vitro and in vivo Critical telomere shortening and telomere dysfunction Increased apoptosis Increased sensitivity to doxorubicin	(Djojosubroto et al. 2005)
Brain cancer	Growth delay in tumor size Increased survival times of treated animals	(Ozawa et al. 2004)
Bladder cancer	Telomerase inhibition Morphological changes G_0/G_1 arrest after 2 weeks	(Dikmen et al. 2008)
Multiple myeloma, Lymphoma	Effective inhibition of telomerase Reduction of telomere length Apoptotic cell death after lag period of 2–3 weeks Reduced telomerase levels Rapid telomere shortening Proliferative arrest and apoptosis in cell lines with short but not long telomeres	(Akiyama et al. 2003; Wang et al. 2004)
T-Prolymphocytic leukemia (T-PLL)	Effective inhibition of telomerase Apoptotic cell death without lag period in the situation of short telomeres and high telomerase activity	(Röth et al. 2008)

Table 16.3 Imetelstat in clinical studies

Tumor type	Phase	Trials	Status, references
Chronic lymphoproliferative disease (CLD)	I/II	Safety and dose study of imetelstat in patients with chronic lymphoproliferative disease (CLD)	Recruiting, NCT00124189
Breast cancer	I/II	A study of imetelstat with paclitaxel and bevacizumab to treat patients with locally recurrent or metastatic breast cancer	Recruiting, NCT00732056
Solid tumor malignancies	I	Safety and dose study of imetelstat administered to patients with solid tumor malignancies	Recruiting, NCT00310895
Non-small cell lung cancer	I	Study of imetelstat with paclitaxel and carboplatin in patients with advanced or metastatic non-small cell lung cancer	Recruiting, NCT00510445
Multiple myeloma	I	Safety and dose study of imetelstat administered weekly to treat patients with refractory or relapsed multiple myeloma	Recruiting, NCT00594126
Multiple myeloma	I	Safety and dose study of imetelstat and velcade ± dexamethasone to treat patients with refractory or relapsed myeloma	Recruiting, NCT00718601

Toxicities observed so far have been largely consistent with those seen with oligonucleotides as a class. Prolongation of the aPTT often occurs at higher doses. This effect on intrinsic coagulation has been transient and without resulting in acute bleeding episodes. Neutropenia and thrombocytopenia has also been observed among heavily pretreated patients (Chanan-Khan et al. 2008; Cotter 1999; Jason et al. 2004).

16.6 Conclusion and Future Prospects

One of the challenges regarding the clinical use of telomerase inhibitors is that the effect of telomerase inhibition might depend on the initial telomere length distribution within and between tumor cells in patients (Harley 2008). Even with complete telomerase inhibition many rounds of cell divisions may be required until telomeres become critically short and induce cell apoptosis (the "phenotypic lag"). Furthermore, cancer stem cells potentially exhibit slow kinetics of cell turnover. Thus, telomerase inhibition strategies may not be immediately effective in killing cancer cells, especially those with longer telomeres. The condition of very short telomeres (in which the shortest telomeres in most cells are near to telomere dysfunction) and high telomerase activity, however, might be the ideal target for telomerase inhibition. Prolymphocytic leukemia (T-PLL), a rare aggressive type of leukemia, would be such an example. In prolymphocytic leukemia, the clonal T-cells are characterized by extremely short telomeres and high telomerase activity. The high telomerase activity seems to be required for the maintenance of those critically short telomeres, as inhibition of telomerase in T-PLL cells induced rapid apoptosis of cells (Röth et al. 2007). These findings suggest that telomerase inhibition could be a useful therapeutic strategy especially for this devastating disease (Ohyashiki et al. 2002; Röth et al. 2003a, 2005, 2007).

In addition, it is possible that telomerase has additional functions independent of immortalization, and certain classes of telomerase inhibitors or agents targeting the telomere might therefore trigger more immediate effects on tumor cells, even if telomeres are long (Blackburn 2000; Oulton and Harrington 2000; Shay and Wright 2002). It has been demonstrated that telomerase inhibition can also sensitize cancer cells to radiation, chemotherapy, as well as targeted therapies. Telomerase inhibitors therefore might be the most useful in combination with other therapeutics, especially for drug-resistant tumors (Gomez-Millan et al. 2006; Misawa et al. 2002; Nakajima et al. 2003; Tauchi et al. 2002; Ward and Autexier 2005; Wong et al. 2000).

Moreover, combination therapies could mobilize cancer stem cells, accelerate their telomere loss, or even uncap telomeres in the presence of telomerase inhibitors, possibly leading to an earlier or more durable response in cancers. Inhibition of telomerase could also be combined with telomerase immunotherapy (Harley 2008).

Telomerase is potentially the safest identified cancer target to date, given the differences in telomerase activity, telomere length, and stem cell kinetics in normal cells when compared with cancer cells. However, further studies need to prove whether and how normal tissues could be affected by telomerase-based therapies.

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GDC-0449 - Targeting the Hedgehog Signaling Pathway

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Abstract Hedgehog signaling is activated in a variety of solid tumors and hematologic malignancies by point mutations in hedgehog pathway members or autocrine or paracrine ligand secretion. Several hedgehog inhibitors were developed to block hedgehog pathway activity on the level of the activating receptor, Smoothened (Smo). GDC-0449 is the first systemic Smo-inhibitor entering clinical trials. It was successfully tested in a phase-I clinical trial demonstrating good pharmacodynamic (PD) and pharmacokinetic (PK) properties and showing objective response and clinical benefit in several patients with basal cell carcinoma.

17.1 Introduction

The hedgehog signaling pathway is one of the so-called "developmental pathways" like the Wnt or Notch signaling pathway and plays an important role in the embryonic development of the brain and the pattern formation of various organs (Goodrich and Scott 1998). Hedgehog ligands (Ihh, Shh, Dhh) bind to the seven-transmembrane receptor, Patched (Ptch), inducing a conformational change, that abolishes Ptch-binding to the second transmembrane receptor Smoothened (Smo). Dissociation of these receptors alleviates Ptch1-mediated suppression of Smo activity. Activation of Smo induces a cascade of intracellular events, which results in the activation of the Gli transcription factors (Gli1, Gli2, and Gli3 > Glioma associated protein 1–3) and transcription of specific target genes important for apoptosis, cell cycle regulation, or self-renewal like Ptch1, Gli1, Bcl2, Cyclin D1, Cyclin E, Noggin-1, and Bmi-1 (Marigo and Tabin 1996; Lee 1997; Duman-Scheel et al. 2002; Regl et al. 2004).

In contrast to embryogenesis, the hedgehog signaling pathway is inactivated in differentiated tissues in the adult organism. Tumor cells and especially the tumor stem cell population can reactivate "developmental pathways" like the hedgehog signaling pathway and thereby gain properties specific for embryonic progenitor and stem cell populations. Those include high proliferation rates, specific antiapoptosis mechanisms, and "self-renewal" functions, which contribute to tumor metastasis and resistance of tumor cells and tumor stem cells to conventional

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chemotherapy. Inactivating point mutations in one Ptch1 allele in the germ cell line results in the constitutive activation of the hedgehog signaling pathway and causes the Gorlin syndrome or basal cell nevus syndrome (Hahn et al. 1996; Johnson et al. 1996). The patients develop multiple UV-independent basal cell carcinomas and have a high incidence for the occurrence of medulloblastomas, rhabdomysarcomas (soft tissue carcinomas), and other tumors. Heterozygous loss of Ptch1 in mice results in a similar syndrome, supporting the role of Ptch1 as tumor suppressor (Goodrich et al. 1997; Hahn et al. 1998; Aszterbaum et al. 1999). Somatic mutations, which result in constitutive activation of the hedgehog signaling pathway, were also detected in 67% of sporadic basal cell carcinomas, in 25% of medulloblastomas, and 30% of rhabdomyosarcomas (Gailani et al. 1996; Raffel et al. 1997; Tostar et al. 2006). The mutations include Ptch1 or Sufu (suppressor of Fused) inactivating mutations, Smo activating mutations, and Gli1 amplifications. In other solid tumors like lung cancer, prostate cancer, gastrointestinal tumors, liver cell carcinoma, and pancreatic cancer, activation of hedgehog signaling occurs via autocrine ligand stimulation, and tumor cells mainly produce Ihh (Berman et al. 2003; Thayer et al. 2003; Watkins et al. 2003; Karhadkar et al. 2004). In contrast, B-cell lymphomas are dependent on paracrine hedgehog ligand stimulation provided by stroma cells in lymphoid organs, including the bone marrow (Dierks et al. 2007). Furthermore, oncogenes like Bcr-Abl in CML can activate hedgehog signaling via endogenous upregulation of the activating Smo-receptor, causing an imbalance in between activating and inactivating Hh pathway components (Dierks et al. 2008). Inactivation of hedgehog signaling in animal models of lymphoid malignancies, CML, and various solid tumors by the natural Smo-inhibitor cyclopamine results in tumor regression and loss of the leukemic stem cell population in CML, indicating that Smo inhibitors might be beneficial in the treatment of various cancers.

17.2 Structure and Mechanism of Action

Several companies currently develop small molecule inhibitors to inactivate the hedgehog signaling pathway. GDC-0449 from Curis/Genentech is the first-in-human, first-in-class, potent systemic inhibitor of Hh signal transduction. GDC-0449 binds to the activating receptor Smo and locks it in its inactive conformation. Preclinical studies confirm high specificity of the compound for Smo and good potency in vitro and in vivo to block the hedgehog signaling pathway.

17.3 Clinical Data

GDC-0449 was tested in a phase I dose escalation clinical trial in refractory solid tumor patients to evaluate safety, tolerability, and pharmacokinetics/pharmacodynamics (PK/PD). GDC-0449 was administered orally as a single dose on Day 1 with a 1-week PK break, and then once daily continuously (cycle 1 > 35 days). Altogether, 19 patients were treated at doses 150 mg/day ($n>7$), 270 mg/day ($n>9$), and 540 mg/day ($n>3$). The median patient age was 63 years (range 39–84) with an ECOG performance status 0–2. There have been no dose-limiting toxicities. Reversible drug-related grade 3 hyponatremia and fatigue (one each) were reported. GDC-0449 PK properties showed a prolonged terminal half-life and drug accumulation, which resulted in surprisingly similar mean steady-state plasma concentrations (30–35 mM) across dose levels, resulting in expansion of the patient cohorts receiving the lower doses of GDC-0449. One partial response (150 mg) in basal cell carcinoma (BCC) and two stable diseases (270 mg) in BCC and adenocystic carcinoma were seen. Surrogate tissues were assessed for the expression of the Hh downstream target gene, Gli1. Gli1 was downmodulated more

than twofold in skin biopsies from 11 of 14 patients analyzed, indicating good PD activity of the compound.

In October 2007, Genentech initiated a phase I expansion cohort to enroll additional BCC patients with locally advanced (LA), multifocal (MF), or metastatic disease. These expansion cohort patients were administered 150 mg of GDC-0449 daily continuously beginning on Day 1. In five patients with metastatic BCC to the lungs, two patients had confirmed RECIST partial responses, two have ongoing stable disease, and one had progressive disease. In four patients with clinically evaluable LA or multifocal BCC, two patients exhibited complete response in subcutaneous masses by physical exam and two patients had improvement of skin lesions. Metabolic responses by EORTC positron emission tomography (PET) metabolic response criteria were achieved in five out of five patients that received PET scans. Time on study for all nine patients ranged from 39 days to over 438 days, with a median of over 176 days. The biomarker Gli1 was reduced in all patients.

17.4 Conclusion and Future Perspectives

The Hh antagonist GDC-0449 was evaluated in a phase I clinical trial and continuous oral dosing at 150 mg/day demonstrated safety, effective PK concentrations, PD activity, an objective response, and clinical benefit in patients with BCC. Therefore, several phase II clinical trials are planned or already ongoing.

One phase II study focuses on advanced BCC patients. A second phase II clinical trial is planned on a combination of GDC-0449 with concurrent chemotherapy and bevacizumab as a first-line therapy in colorectal cancer. A third phase II clinical trial will evaluate GDC-0449 in ovarian cancer and further studies are planned in other solid tumors and hematologic malignancies.

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