Bosutinib: A Potent Second-Generation Tyrosine Kinase Inhibitor

Susanne Isfort, Martina Crysandt, Deniz Gezer, Steffen Koschmieder, Tim H. Brümmendorf and Dominik Wolf

Contents

S. Isfort (✉) · M. Crysandt · D. Gezer · S. Koschmieder · T. H. Brümmendorf
Department of Hematology, Oncology, Hemostaseology and Stem Cell Transplantation, University Hospital RWTH Aachen, Pauwelsstraße 30, 52074 Aachen, Germany
e-mail: sisfort@ukaachen.de

D. Wolf
Department of Oncology, Hematology, Immunoncology and Rheumatology, University Hospital Bonn, Sigmund-Freud-Str. 25, 53127 Bonn, Germany

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Abstract

Bosutinib is one of the five tyrosine kinase inhibitors which are currently approved for the treatment of chronic myeloid leukemia. By its dual inhibition of Src and ABL kinase and also targeting further kinases, it creates a unique target portfolio which also explains its unique side effect profile. The approval of bosutinib in 2013 made the drug available for patients previously treated with one or more tyrosine kinase inhibitor(s) and for whom imatinib, nilotinib, and dasatinib are not considered appropriate treatment options. As initially the first-line clinical trial comparing bosutinib with imatinib in CML patients in chronic phase did not reach its primary endpoint and therefore the product was not licensed for first-line therapy, a second first-line trial, the so-called BFORE study, was performed and just recently the promising results have been published predicting a quick expansion of the existing label. In comparison with the other approved TKIs, bosutinib harbors a distinct side effect profile with only very few cardiovascular and thromboembolic events and minimal long-term safety issues with most adverse events happening during the first months of treatment. On the other hand, gastrointestinal side effects are very common (e.g., diarrhea rates in more than 80% of the patients) with bosutinib surprising some of the investigators during the early clinical trials evaluating bosutinib. Until then, several approaches have been used to face this problem resulting in extensive supportive efforts (such as early loperamid treatment) as well as new trials testing alternative dosing strategies with early dose adjustment schedules. This article reports preclinical and clinical data available for bosutinib both in hematologic diseases such as CML or ALL and solid tumours as well as other diseases and envisions future perspectives including additional patient groups in which bosutinib might be of clinical benefit.

Keywords · CML · Tyrosine kinase inhibitor · Bosutinib

1 Structure and Mechanism of Action

1.1 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 (Golas et al. 2003). Originally, it was synthesized as a specific Src kinase family inhibitor. However, target screening demonstrated also potent ABL tyrosine kinase inhibition. The small molecule inhibitor is of low weight (548.46 kDa) and orally bioavailable.

1.2 Mechanism of Action (Target Profile)

Bosutinib inhibits Src with an IC50 of 1.2 nM, inhibits anchorage-independent growth of Src-transformed fibroblasts with an IC50 of 100 nM, and inhibits Src-dependent protein tyrosine phosphorylation at comparable or lower concentrations (Boschelli et al. 2001). Bosutinib however does not inhibit growth factor receptor tyrosine kinases such as platelet-derived growth factor receptor, insulin-like growth factor I receptor, epidermal growth factor receptor, fibroblast growth factor receptor, and serine–threonine kinases such as Akt and Cdk4 (Boschelli et al. 2001). The success of the compound in BCR-ABL positive disease relays on its bosutinib potent dual inhibitory effect on Src and ABL tyrosine kinases (Puttini et al. 2006). In addition to those main target kinases, more than 45 other tyrosine and serine/threonine kinases have been identified as potential targets of bosutinib.

1.3 ABL and BCR-ABL Inhibition

c-ABL belongs to an evolutionary conserved protein family and encodes a ubiquitously expressed non-receptor protein tyrosine kinase localized in both the cytoplasm and the nucleus (Laneuville 1995; Pendergast 1996). Oncogenic transformation leading to ABL-induction is mediated by genomic alterations including genomic rearrangements (e.g. by the Philadelphia (Ph+)-Chromosome in chronic myeloid leukemia (CML) or acute lymphocytic leukemia (ALL) leading to the fusion of the BCR with the ABL genes (Nowell and Hungerford 1961; Heisterkamp et al. 1985) or by enhanced ABL expression [e.g. in solid cancer (Greuber et al. 2013)]. In case of the reciprocal translocation between the proto-oncogene *c-ABL* from chromosome 9 to the breakpoint cluster region *(BCR)* of chromosome 22, high expression levels of a constitutively activated tyrosine kinase are induced which directly or indirectly phosphorylates a broad spectrum of binding substrates. Many of these activated downstream signaling components are a crucial driver of cellular proliferation, migration, and apoptosis (Ren 2005). Interestingly, the efficacy between imatinib and bosutinib as inhibitor of v-ABL phosphorylation is within the same range (approximately, 200 nM are required to inhibit the non-translocated v-ABL), whereas substantially lower concentrations of bosutinib (25 and 50 nM) are required to reduce BCR-ABL phosphorylation (Golas et al. 2003). Concerning IC50 values, it is important to realize that those concentrations substantially depend on the cell system used to address this issue. Exemplarily, bosutinib inhibits BCR-ABL kinase activity at 1 nM in a non-cellular in vitro enzymatic assay, whereas an IC50 of 90 nM is required to inhibit ABL kinase activity and consequently the growth of ABL-MLV-transformed fibroblasts. The extent of tyrosine phosphorylation inhibition by bosutinib in ABL-MLV-transformed fibroblasts correlates with the degree of anti-proliferative activity. In addition, incubation of ABL-MLV-transformed Rat 2 fibroblasts with comparable concentrations of bosutinib and imatinib results in quantitatively similar reductions of tyrosine

phosphorylation of cellular proteins (Golas et al. 2003). In cell line models for Ph+ leukemias, bosutinib inhibited the proliferation of all three cell lines, with IC50s ranging from 5 nm in the KU812 cells to 20 nm in K562 and MEG-01 cell lines. The IC50s for imatinib to inhibit proliferation of these cell lines were higher, ranging from 88 nm (KU812), 180 nm (MEG-01) to 210 nm (K562) (Golas et al. 2005). The emergence of TKI resistance is a major clinical problem during TKI therapy with imatinib (and later nilotinib, dasatinib, or bosutinib) (Patel et al. 2017). Approximately, half of the resistance cases are conferred by specific mutations in the BCR-ABL fusion gene. This may lead to varying degrees of resistance to first-generation (imatinib), second-generation (nilotinib, dasatinib, and bosutinib), and third-generation (ponatinib) TKIs. It is essential for optimized and individualized treatment to screen for BCR-ABL mutations to select (in case one or more mutations are detected) the most appropriate TKI, as all TKIs have a highly specific in vitro resistance profile (shown for bosutinib and the other TKIs, as studied in Ba/F3 cell lines, in Fig. 9.1 (Redaelli et al. 2012)). More advanced structural and spectroscopic analyses revealed the mode of action and explain even efficacy in most imatinib-resistant mutants as well as inefficacy in T315I-mutated CML or ALL (Levinson and Boxer 2012). As mentioned before, the IC50 values are also impacted by the leukemia cells' capability to in- and export TKIs. This is largely mediated by drug transporter such as ATP-binding cassette transporters (ABC transporters). While the mechanisms are not fully understood, Hegedus et al. (2009) were able to identify a significant difference between second-generation TKIs dasatinib and nilotinib in comparison with bosutinib, as neither ABCB1 nor ABCG2 induced resistance to bosutinib. The potential clinical impact of this finding has to be further evaluated, e.g., by quantification of intracellular drug levels in TKI-treated patients.

1.4 Src Kinase Inhibition

The tyrosine kinase Src is a member of a family of related kinases known as the Src family kinases (SFKs) that share a common structural organization 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, tumor 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, esophagus, 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 nM concentrations; accordingly, an IC50 of 1.2 nM has been reported in an enzymatic assay. Inhibition of Src-dependent protein tyrosine phosphorylation can be detected at

		IC50 fold increase (WT = 1)				
		Imatinib	Bosutinib	Dasatinib	Nilotinib	Ponatinib
	Parental	10.8	38.3	568.3	38.4	570.0
	WT	1	1	1	1	1
P-Loop	M244V	0.9	0.9	2.0	1.2	3.2
	L248R	14.6	22.9	12.5	30.2	6.2
	L248V	3.5	3.5	5.1	2.8	3.4
	G250E	6.9	4.3	4.4	4.6	6.0
	Q252H	1.4	0.8	3.1	2.6	6.1
	Y253F	3.6	1.0	1.6	3.2	3.7
	Y253H	8.7	0.6	2.6	36.8	2.6
	E255K	6.0	9.5	5.6	6.7	8.4
	E255V	17.0	5.5	3.4	10.3	12.9
C-Helix	D276G	2.2	0.6	1.4	2.0	2.1
	E279K	3.6	1.0	1.6	2.0	3.0
	E292L	0.7	1.1	1.3	1.8	2.0
ATP-binding region (drug contact sites)	V299L	1.5	26.1	8.7	1.3	0.6
	T315A	1.7	6.0	58.9	2.7	0.4
	T315I	17.5	45.4	75.0	39.4	3.0
	T315V	12.2	29.3	738.8	57.0	2.1
	F317L	2.6	2.4	4.5	2.2	0.7
	F317R	2.3	33.5	114.8	2.3	4.9
	F317V	0.4	11.5	21.3	0.5	2.3
SH2-contact	M343T	1.2	1.1	0.9	0.8	0.9
	M351T	1.8	0.7	0.9	0.4	1.2
Substrate binding region (drug contact sites)	F359I	6.0	2.9	3.0	16.3	2.9
	F359V	2.9	0.9	1.5	5.2	4.4
A-Loop	L384M	1.3	0.5	2.2	2.3	2.2
	H396P	2.4	0.4	1.1	2.4	1.4
	H396R	3.9	0.8	1.6	3.1	5.9
C-terminal lobe	F486S	8.1	2.3	3.0	1.9	2.1
	L248R + F359I	11.7	39.3	13.7	96.2	17.7

Sensitive	≤ 2
Moderately resistant	2.01 - 4
Resistant	4.01 – 10
Highly resistant	> 10

Source: (12)

Fig. 9.1 Resistance profile of bosutinib, imatinib, nilotinib, dasatinib, and ponatinib. *Source* Redaelli et al. (2012)

comparable or lower concentrations (Boschelli et al. 2001). In addition, bosutinib successfully inhibited the growth of Src-transformed fibroblasts and Src overexpressing HT29 colon tumors subcutaneously transplanted into athymic nu/nu mice (Compound 31a) (Boschelli et al. 2001).

2 Preclinical Data

2.1 Malignancies

2.1.1 BCR-ABL-Dependent Cancer Models

The anti-proliferative activity of bosutinib has been demonstrated in different BCR-ABL expressing leukemia cell lines. In line with its higher clinical efficacy compared to imatinib (Cortes et al. 2017), the in vitro efficacy of bosutinib is superior to IM with IC50 values ranging from 1 to 20 nM when compared to imatinib with 51–221 nM, respectively (Golas et al. 2003; Puttini et al. 2006). In addition, bosutinib successfully inhibits the growth of imatinib-resistant human cell lines, such as Lama84R, KCL22R, and K562R (Golas et al. 2003). 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 BCR-ABL point mutants (D276G, Y253F, E255K, and T315I), is more pronounced by bosutinib than by imatinib. However, the T315I BCR-ABL mutant requires excessively high concentrations of bosutinib to be sufficiently inhibited [i.e., one to two orders of magnitude higher when compared with wt BCR-ABL cells (Puttini et al. 2006)]. This is in line with the clinical observation that T315I mutated leukemias cannot be sufficiently be treated by bosutinib. According to these in vitro observations, in vivo experiments demonstrated that 75 mg/kg twice daily or 150 mg/kg once daily bosutinib therapy induces complete regression of human K562 xenografts for up to 40 days (Golas et al. 2003). Remarkably, while imatinib is unable to eradicate KU812 human tumor xenografts with a relapse rate of 30%, bosutinib treatment initiated at day 8 and 15 after leukemic cell injection induces complete disease eradication curing the animals for up to 210 days (Puttini et al. 2006). In mice s.c. injected with Ba/F3 BCR-ABL + xenografts containing WT or mutant BCR-ABL (E255K, Y253F, and D276G) and treated with bosutinib 1 day after tumor cell injection, the dual Src/ABL kinase inhibitor decreased tumor growth and prolonged event-free survival. However, animals with delayed start of bosutinib treatment, relapse of the disease cannot be prevented in the majority of mice. Furthermore, according to the above described in vitro data of almost complete resistance of the T315I mutation, bosutinib does not influence the growth of highly IM-resistant T315I xenografts (Puttini et al. 2006).

2.1.2 Breast Cancer

There is a high medical need to improve breast cancer therapy, particularly in the metastatic setting. Src activation has been implicated in both acquired and de novo

trastuzumab-resistant cells (Zhang et al. 2011). Src regulation involved dephosphorylation by PTEN and increased Src activation conferred trastuzumab resistance in breast cancer cells and correlated with trastuzumab sensitivity in patients. Consequently, targeting Src in combination with trastuzumab re-sensitized multiple trastuzumab-resistant cells lines to trastuzumab and eliminated trastuzumab-resistant tumors in vivo, suggesting the potential clinical application of combining Src inhibitors with trastuzumab (Ocana et al. 2017). Bosutinib has been shown to cause reduced cell proliferation, migration, and invasion of breast cancer cell lines accompanied by an increase of cell-to-cell adhesions and a membrane localization of beta-catenin, a phosphoprotein that functions as both a structural component of the cell adhesion/actin cytoskeleton network and a signaling molecule when localized 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 signaling pathways involved in cell proliferation and malignant transformation as well as tumor cell motility and invasion (Jallal et al. 2007; Vultur et al. 2008). Accordingly, MDA-MB-231 cell in BALB/c nu/nu mice is significantly delayed by bosutinib therapy when compared to control animals. In addition, analysis of lung, liver, and spleen specimen has shown a significant reduction of metastatic spread in animals treated with the small molecule inhibitor at a well-tolerated dose.

2.1.3 Colorectal Cancer

Bosutinib decreases tumor growth in subcutaneous colorectal cancer xenograft models generated with different tumor 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. However, protein levels of beta-catenin remain substantially unchanged by bosutinib, and a cytosolic/membranous retention of beta-catenin is promoted instead. The bosutinib-mediated relocalization of beta-catenin increases its binding affinity to E-cadherin and adhesion of colorectal cancer cells resulting in 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 tumor 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.

2.1.4 Non-small Cell Lung Cell Cancer (NSCLC)

Immunohistochemical analyses of NSCLC biopsy samples reveal an up-regulation of Src kinase in 33% of the tumors. 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). However, monotherapy of bosutinib in solid cancer will probably not exert sufficient efficacy (Daud et al. 2012) and combination approaches have to be tested in the future to define the potential

therapeutic value of additional Src inhibition as add-on to conventional cancer therapeutics.

2.2 Non-malignant Diseases

2.2.1 Polycystic Kidney Disease (PKD)

In polycystic kidney disease, the precise functions of the cystoprotein products remain unknown. Recent data suggest that multimeric cystoprotein complexes lead to aberrant signaling cascades involving c-Src kinases. In two different animal models, greater Src activity was found to correlate with disease progression in PKD. Inhibition of Src activity via bosutinib resulted in amelioration of renal cyst formation and biliary ductal abnormalities in both animal models (Sweeney et al. 2008), suggesting this strategy may provide therapeutic benefit in PKD (Sweeney et al. 2017).

2.2.2 Amyotrophic Lateral Sclerosis (ALS)

Amyotrophic lateral sclerosis (ALS) is a fatal neurological disease causing progressive motor neuron loss. No effective treatment option is available so far. A phenotypic drug screen using ALS motor neuron survival as readout identified the Src/c-ABL signaling pathway a most prominent hit. Motor neurons in this model were generated from induced pluripotent stem cells (iPSCs) derived from an ALS patient with a superoxide dismutase 1 (SOD1) mutation. Src/c-ABL inhibitors (including bosutinib) increased survival of ALS iPSC-derived motor neurons in vitro. Bosutinib boosted autophagy, reduced the amount of misfolded mutant SOD1 protein, and reduced altered expression of mitochondrial genes. Bosutinib also increased survival in vitro of ALS iPSC-derived motor neurons from patients with sporadic ALS or other forms of familial ALS caused by mutations in TAR DNA-binding protein (TDP-43) or repeat expansions in C9orf72. Finally, bosutinib treatment also extended survival of a mouse model of ALS with a SOD1 mutation, suggesting that inhibition of the Src/c-ABL by bosutinib is a potentially useful target for developing new drugs to treat ALS (Imamura et al. 2017).

3 Clinical Data

The initial approval of bosutinib in CML patients in 2013 was based on data published by Cortes et al. (2011) and Khoury et al. (2012) in their phase I/II trial, evaluating bosutinib in second-line and third-/fourth-line treatment upon intolerance or resistance to imatinib and/or intolerance or resistance to a second-generation TKI leading to the conditional approval of bosutinib for treatment in CML in chronic phase (CP), accelerated phase (AP), and blast crisis (BC) in Europe for patients after first-line therapy with first- or second-generation TKI for whom imatinib, nilotinib, or dasatinib are not considered appropriate

treatment options. As mentioned before the approval in first-line CML treatment is still missing, however in addition to the failed first-line CML-CP BELA trial, another first-line trial with a limited starting dose of 400 mg/d was performed (the so-called BFORE trial) and recently published (Cortes et al. 2017). Based on this trial, widening of the approval of bosutinib in CML to first line is expected soon.

The phase I/II clinical trial, testing bosutinib in Philadelphia chromosome-positive leukemia, included 288 patients with imatinib resistance or intolerance between January 2006 and July 2008 where bosutinib was given as second-line treatment. Another 118 patients, which had been pretreated with at least two TKIs (imatinib *plus* one additional second-generation TKI), were recruited. Moreover, 134 patients in later disease phases (accelerated phase (AP) or blast crisis (BC) or Ph+ ALL) were formed the third cohort of this study. Bosutinib (500 mg) was established as current dosing regimen as 600 mg/d lead to dose-limiting toxicities (grade 3 rash, nausea, and vomiting).

Bosutinib has also been tested in several solid tumors and also in non-malignant diseases like polycystic kidney disease (PKD) not yet leading to any approval. The following chapter focuses on the clinical data available in all the different diseases.

3.1 Bosutinib in Treatment-Resistant/-Intolerant CML

3.1.1 Bosutinib as Second-Line Treatment

As mentioned before, the phase I/II trial on which approval of bosutinib was based on included three different cohorts of patients. In this trial, quality of life assessments has also been performed.

The second-line part included patients which were resistant or intolerant to imatinib. The definition of imatinib resistance in this trial (Cortes et al. 2011) applied if a patient did show no hematologic improvement within 4 weeks, no complete hematologic response (CHR) after 12 weeks, no cytogenetic response after 24 weeks, and/or no major cytogenetic response (MCR) after 12 months of therapy with an imatinib dose of at least 600 mg daily. Loss of a MyCR or any hematologic response defined an acquired resistance. Individuals have been considered to be intolerant to imatinib if toxicities grade 4 lasted longer than 7 days, if imatinib-related non-hematological toxicities grade 3 or higher occurred or persistent toxicities grade 2 not responding to adequate management and/or dose adjustments appeared. In addition, patients in whom dose reductions were necessary due to toxicities and who subsequently lost their response to treatment were considered imatinib-intolerant as well. Patients' characteristics are listed in Table 9.1. In total, 288 patients have been included in this part of the study with 69.4% exhibiting resistance and 20.6% intolerance to imatinib. Data on this trial were updated in 2014 by Gambacorti-Passerini et al. (2014) with a longer follow-up of at least 24 months. Response rates were reported as follows: 85% achieved/maintained CHR, 48% CCyR (59% with MyCR), and 35% presented with MMR. Probabilities of overall and progression-free survival were 91% and 81%. Age and cause of imatinib failure (intolerance or resistance) did not lead to

Table 9.1 Patient characteristics of chronic phase (CP) CML patients in the second-line setting

Characteristics	IM resistant (n = 200)	IM intolerant (n = 88)	Total (n = 288)
Median age: years (range)	51	54,5	53 (18–91)
Male sex	116	37	153
Median duration of disease in years (range)	4.0 (0.1–15.1)	2.8 (0.1–13.6)	3.6 (0.1–15.1)
Number of previous treatments			
1 (%)	128 (64)	65 (74)	193 (67)
2 (%)	72 (36)	23 (26)	95 (33)
Previous IFN (%)	69 (35)	23 (26)	92 (32)
Previous SCT (%)	6 (3)	2 (2)	8 (3)
BCR-ABL mutations			
Assessed patients	153	59	212
At least one mutation, *n* (%)	73 (48)	6 (10)	79 (37)

Source Gambacorti-Passerini et al. (2014a)

differential response rates. Brümmendorf et al. (2016) analyzed factors that impact long-term efficacy and safety in the context of the same trial. Prior cytogenetic response on imatinib, baseline MCyR, prior interferon therapy and duration from diagnosis to imatinib treatment initiation of less than 6 months without interferon intake before imatinib were identified as significant predictors of both MCyR and CCyR at 3 and 6 months.

3.1.2 Bosutinib After Failure of Second-Line Therapy

In the same study, 118 patients pretreated with imatinib and at least one other second-generation TKI had been recruited (Khoury et al. 2012). Bosutinib was administered in the 500 mg dose established in the phase I of the same trial. Among those, 118 patients who had previously been treated with IM 37 were dasatinib-resistant and 50 dasatinib intolerant. In addition, 27 were nilotinib resistant, and one patient was intolerant to nilotinib. Three patients had been treated with all three TKIs and failed. Median follow-up was 28.5 months (range 0.3–56.2), and median dose intensity was 478 mg/day (185–563 mg/day). MCyR rate was 32% among all patients with 24% (n = 26) achieving a CCyR; among them was one of the three patients being treated with all three TKIs before. Median time to MCyR among responders was 12.4 weeks (ranges 3.9–88.4 weeks). Molecular responses were assessed in 105 patients; among these, 16 (15%) achieved a MMR, including 12 (11%) with a CMR. Thirty-nine patients had known mutations at the beginning of treatment with bosutinib, and the results of these patients are summarized in Table 9.2.

In 2016, Cortes et al. (2016) published long-term data on this patient cohort proving data with a median follow-up of 32.7 months and a median treatment duration of 8.6 months. Table 9.3 summarizes the long-term probabilities and maintaining of response. Patient-reported outcome assessments in these third and

Table 9.2 Response by mutation status in CP CML after at least two lines of treatment

		Cumulative response, *n*/*n* evaluable (%)	
Mutation status	*n*	CHR	MCyR
No mutation	44	34/44 (77)	15/43 (Khoury et al. 2012)
Any mutation	39	26/39 (67)	11/35 (Sweeney et al. 2008)
>1 mutation	9	3/9 (33)	2/9 (22)
Mutation type			
P-loop	14	9/14 (64)	4/13 (Sweeney et al. 2008)
G250E	6	3/6	0/5
Y253H	6	5/6	4/6
E255 K	1	0/1	0/1
E255 V	1	1/1	0/1
Non-P-loop	29	18/29 (62)	9/26 (Khoury et al. 2012)
M244 V	3	3/3	2/3
V299L	2	1/2	0/2
Q300R	1	1/1	1/1
T315I	7	2/7	0/6
F317L	8	4/8	1/7
N336S	1	1/1	0/1
M351T	1	1/1	0/1
F359C	2	2/2	1/2
F359I	2	2/2	2/2
F359 V	2	0/2	1/2
L387F	1	1/1	0/1
H396R	1	0/1	0/1
E453A	1	1/1	0/0
C475 V	1	1/1	1/1
F486S	1	0/1	0/1

Source Khoury et al. (2012)

Table 9.3 Summary of the long-term response probabilities and rates in the third-/forth-line cohort (Cortes et al. 2016)

	Rate of %	Probability of maintaining at 4 years (%)
cCHR	74	63
MCyR	40	69
Incidence of PD on treatment/ death		24
4-year OS		78

further line patients as well as in the second-line patient cohort did show stabilization of health-related quality of life (QoL) during bosutinib treatment (Kantarjian et al. 2017).

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

Initially, data on patients with AP (n = 77) and BP CML (n = 64) and Ph+ ALL (n = 24) with an open-label continuous daily dosing schedule (bosutinib 500 mg/day) as part of the above-mentioned phase I/II trial were presented at the 2013 ASCO Annual Meeting (2013). All patients included were previously treated with imatinib *plus/minus* other TKIs and exhibited imatinib resistance or intolerance. In this analysis, patients were split into two different cohorts regarding their age (<65 years vs. ≥65 years). Hematologic and cytogenetic response data are shown in Table 9.4.

Long-term efficacy and safety of the whole patient group were analyzed and published in 2015 by Gambacorti-Passerini et al. (2015). Seventy-nine patients with AP, 64 with BP, and 24 patients with Ph+ ALL were treated with bosutinib and followed up for 28.4 (AP), 10.4 (BP), and 3.6 months (Ph+ ALL) (median). All patients had received prior imatinib treatment, and 9% had been treated with three prior TKIs. Median treatment duration was 10.2 months for AP patients, 2.8 months for BP patients, and 0.97 months for ALL patients. Responses were durable in approximately 50% of AP responders at 4 years with 57% maintaining baseline OHR, 40% attained/maintained MCyR by 4 years. For patients with blast crisis as approximately 25% responded at year 1, bosutinib seems to be a feasible treatment to bridge to allogeneic transplantation.

Attila et al. (2015) reported about a case with elderly acute lymphoblastic leukemia transformed from CML with suspected central nervous system involvement. This patient was pretreated with imatinib, dasatinib, and nilotinib as treatment for chronic phase CML, and after 7 years, the sickness transformed into acute B lymphoblastic leukemia occurring with simultaneous suspected central nervous system involvement. The patient was treated with bosutinib 500 mg/day (including

Table 9.4 Response to bosutinib treatment in AP/BC CML and Ph^+ ALL

Response	ADV cohort	
	Aged ≥65 years (N = 30)	Aged <65 years (n = 135)
Hematologic response		
Evaluable patients, n	29	123
MHR, n (%)	8 (28)	38 (31)
CHR	4 (14)	31 (25)
2-year probability of maintaining a MHR	71%	54%
2-year probability of maintaining a CHR	75%	54%
Cytogenetic response		
Evaluable patients, n	26	117
MCyR, n (%)	8 (31)	45 (39)
CCyR	7 (27)	24 (29)
2-year probability of maintaining a MCyR	43%	34%

Source Brummendorf et al. (2013)

several treatment interruptions) and received cerebral radiotherapy and intrathecal chemotherapy with methotrexate and Ara-C. Maintenance therapy could only be performed including bosutinib as the patient could not stand further intrathecal treatment after six rounds of chemotherapy. At 14 months of follow-up, the patient still showed complete hematological and bone marrow response.

Whiteley et al. (2016) reported a significant improvement of several quality of life measurement tools in the AP/BC cohort during bosutinib treatment although the lack of comparison group handicaps the interpretation of these results.

3.2 Bosutinib in CML First-Line Treatment

In the BELA trial published 2012 by Cortes et al. (2012), bosutinib was evaluated in the first-line setting against imatinib in patients with chronic myeloid leukemia in chronic phase. The primary endpoint of this trial was the CCyR rate at 12 months which was the standard primary endpoint in first-line trials at that time, since the standardized molecular analysis was not available in all countries. 502 pts were randomized in a 1:1 manner to each arm, median duration of treatment in both study arms was 13.8 months, and median dose intensity was 489 mg/day for bosutinib and 400 mg/d for imatinib. In the IIT population, the CCyR rate at 12 months was similar in both treatment groups (70% for bosutinib vs. 68% for imatinib; $P = 0.601$). However, time to CCyR was significantly shorter with bosutinib (12.9 weeks vs. 24.6 weeks; $P < 0.001$) with higher rates for CCyR for bosutinib at months 3, 6, and 9. Molecular responses were also significantly higher in the bosutinib group, and in detail MMR rate at 12 months was 41% versus 27% ($P < 0.001$). Transformation to AP/BC CML on treatment occurred less frequently among the bosutinib-treated patients (4.2% vs. 10.4%).

Data from the BELA trial were updated in 2014 regarding safety aspects (Gambacorti-Passerini et al. 2014b) and efficacy in an update after 24 months of follow-up (Brummendorf et al. 2015). The safety update stated clearly the low risk of long-term safety issues and the low amount of cardiovascular events comparable to imatinib treatment. Brümmendorf et al. reported durable responses from the 24-month follow-up of this BELA trial. Between the 12 and the 24 month update no new case of transformation to advanced stages of the disease occurred.

However, the BELA trial did not lead to approval of bosutinib in first-line CML treatment due to the missed primary endpoint; that is why after analysis of efficacy and safety data of individual dose levels of bosutinib, the so-called BFORE was established testing a reduced daily dose of 400 mg bosutinib again in first-line treatment compared to imatinib standard dose which closed recruitment in 2015 (NCT02130557). This hypothesis was supported by as well as based on the experience gained from other second-generation TKIs that first-line treatment requires lower doses of TKI as compared to second and later line treatments. At the end of 2016, the positive study results were announced meaning that bosutinib was superior to imatinib in first-line treatment regarding the MMR rate (and also again, regarding CCyR rate) at 12 months of treatment.

In this trial, patients with newly diagnosed chronic phase CML received 400 mg of bosutinib once daily (n = 268) or imatinib (n = 268) (Cortes et al. 2017). The median dose intensity was 392 mg per day for bosutinib and 400 mg per day for imatinib. The MMR rate at 12 months as mentioned before was significantly higher with bosutinib versus imatinib (47.2% vs. 36.9%; P = 0.02) as was the complete cytogenetic response (CCyR) rate by 12 months (77.2% vs. 66.4%; P = 0.0075). Bosutinib-treated patients achieved faster responses, and less patients discontinued treatment receiving bosutinib because of lack of efficacy in comparison with imatinib, whereas more patients discontinued treatment due to drug-related toxicity (12.7% for bosutinib and 8.7% for imatinib) (Cortes et al. 2017).

At this year´s annual meeting of the American Society of Hematology, a comparison of both the first-line trials regarding exposure and response will be presented including 512 patients (Knight et al. 2017). The amount of side effects seemed to correlate with bosutinib exposure and furthermore the incidence of AEs associated with permanent discontinuation from bosutinib treatment was higher within the BELA trial (21.0% for a starting dose of 500 mg/day (BELA) vs. 14.2% for a starting dose of 400 mg/day (BFORE)). Time on treatment influenced efficacy with both bosutinib exposure and time on bosutinib treatment being significant predictors of MMR. The interpretation of this data might be that staying on treatment could be more important than receiving higher doses and may at least in part explain the suboptimal results achieved by bosutinib therapy in the BELA trial.

3.3 Bosutinib in Solid Tumors

Daud et al. (2012) published their phase I trial in patients with advanced solid tumor malignancies. This trial was conducted in two parts, a dose escalation part where 400 mg/day could be identified as recommended dose for phase II. In the second part, approximately 30 patients each with refractory colorectal, pancreas, or NSCLC were treated. A partial response (breast) and unconfirmed complete response (pancreas) were observed; 8 of 112 evaluable patients had stable disease for 22–101 weeks. However, the primary efficacy endpoints for part 2 were not met.

Campone et al. (2012) performed a phase II study which evaluated single-agent bosutinib in pretreated patients with locally advanced or metastatic breast cancer in 73 patients. The primary endpoint was the progression-free survival (PFS) rate at 16 weeks. For the intent-to-treat population, the PFS rate at 16 weeks was 39.6%. Unexpectedly, all responding patients (n = 4) were hormone receptor positive. The 2-year overall survival rate was 26.4%.

In 2014, Isakoff et al. (2014) published data of a phase I trial testing safety, efficacy, and maximum tolerated dose (MTD) of bosutinib in combination with capecitabine in several solid tumors. Thirty-two patients with locally advanced/metastatic cancer of the breast, pancreas and patients with cholangiocarcinoma, glioblastoma, or colorectal cancer received both drugs in eight different dose combinations with nine of them receiving MTD (300 mg bosutinib/day + capecitabine 1000 mg/m^2 bid). In 6% of the patients (2/31),

dose-limiting toxicities occurred. Efficacy was limited as best overall confirmed PR or SD lasting longer than 24 was only observed in 6 and 13% of the patients, respectively. The safety profile was quite similar to the individual profiles of both drugs, and especially, regarding diarrhea most patients facing this side effect (91%) did only experience low-grade events (grade 1/2).

3.4 Bosutinib in Polycystic Kidney Disease (PKD)

Src kinase overactivation is one of the driving mechanisms in the pathogenesis of autosomal dominant polycystic kidney disease (ADPKD). As mentioned already above, this hypothesis leads to the preclinical and clinical testing of bosutinib in this disease. Tesar et al. (2017) published data from a multicentre phase II trial where 172 patients with ADPKD were randomized 1:1:1 to receive either bosutinib 200 mg/day, bosutinib 400 mg/day, or placebo. Bosutinib 200 mg/day and pooled bosutinib treatment showed a significant reduction (66%/82%) in the rate of kidney enlargement. Annualized eGFR decline was similar in all three arms. Toxicity findings were similar to the side effect profiles established in the hematologic trials.

4 Toxicity

While the general toxicity profile of bosutinib was very similar in hematological trials and studies in solid tumors, there were some expected differences in hematological adverse events.

In an update of the BELA trial (Brummendorf et al. 2015), comparing bosutinib versus imatinib for newly diagnosed patients with CML in CP, grade 1 or 2 side effects like diarrhea occurred in 58%, nausea in 31%, vomiting in 31%, rash in 22%, headache in 12%, and arthralgia in 7% of the patients. The most common cardiovascular AEs were hypertension (6% vs. 4%) and palpitations (2% vs. 2%). Cardiac failure occurred in one (<1%) bosutinib-treated patient and two (1%) imatinib-treated patients. There was no report of peripheral arterial occlusive disease. The most common grade 3/4 treatment-emergent AE was diarrhea (bosutinib, 12%; imatinib, 1%). In the bosutinib arm diarrhea of all grades typically occurred during the first month of treatment and was treated with anti-diarrheal medication. In some patients, temporary interruption was needed to control the side effects. But in most of the cases, it was self-limiting and transient. Hematological adverse events (neutropenia, thrombocytopenia, and anemia) of grade 3 and 4 were lower in the bosutinib arm (10% compared to imatinib 24%). Non-hematological adverse events like elevation of liver enzymes or bilirubin occurred more often in the bosutinib cohort. Side effects could be controlled with concurrent medication and dose modification. None of these effects led to hospitalization or to permanent hepatic injury.

Even in heavily pretreated patients with advanced CML (AP, BP) (Gambacorti-Passerini et al. 2015), the most common AEs were gastrointestinal (96%; 83%), primarily diarrhea (85%; 64%), which was typically low grade (maximum grade 1/2: 81%; 59%) and transient. Serious AEs were pneumonia and pyrexia.

Cortes et al. (2017) analyzed patients receiving bosutinib in first and in later lines regarding renal function. Long-term bosutinib treatment was associated with a reversible decline in renal function; this aspect seems to be similar to long-term imatinib treatment regarding frequency and characteristics. Patients at risk for renal side effects should be monitored closely.

In contrast to the hematological malignancies, myelosuppression in solid tumor studies was minimal. This could be explained by the fact that hematologic toxicity of TKI treatment in CML is not only a reflection of inhibition of normal hematopoiesis but at least in part mediated by suppression of the leukemic population itself by the TKI.

In part one of the solid cancer trial (Daud et al. 2012), dose-limiting toxicities of grade 3 diarrhea (two patients) and grade 3 rash occurred with bosutinib 600 mg/day and the maximum tolerable dose was defined as 500 mg/day. However, the majority of patients treated with 500 mg/day had grade 2 or greater gastrointestinal toxicity. The most common bosutinib-related adverse events were nausea (60% patients), diarrhea (47%), vomiting (40%), fatigue (38%), and anorexia (36%).

In a phase I study of advanced solid tumor patients treated with bosutinib in combination with capecitabine (Isakoff et al. 2014), the most frequent treatment-related adverse events (AEs) were diarrhea, nausea, vomiting, palmar-plantar erythrodysesthesia (PPE), fatigue; most frequent grade 3/4 AEs: PPE, fatigue, and increased alanine/aspartate aminotransferase. Although diarrhea was common, 91% of affected patients experienced maximum grade 1/2 toxicity that resolved. Among breast cancer patients, the main toxic effects were diarrhea (66%), nausea (55%), and vomiting (47%). Grade 3–4 liver aminotransferase elevation occurred in 14 (19%) patients.

5 Drug Interactions

Strong inhibitors of CYP3A4 should be avoided during treatment with bosutinib because of significant increase in bosutinib plasma levels. In this context antifungal treatment with azoles needs to get special attention and should be given with caution (Steegmann et al. 2012, 2016; Ono et al. 2017). Furthermore, some HIV-1 proteasome inhibitors and NNRT inhibitors need to be administered very carefully. In addition to that, grapefruit juice needs to be avoided (Steegmann et al. 2012, 2016; Ono et al. 2017). A full list of CYP3A inhibitors can be found at: http://medicine.iupui.edu/clinpharm/ddis/table.aspx.

Of course, concomitant use of CYP3A4 inducers should be avoided as well. Especially, treatment with rifampicin or anti-epileptic drugs as carbamazepine and phenytoin or the use of St. John´s Worth should not be used as bosutinib plasma levels might be decreased significantly (Steegmann et al. 2012, 2016; Hsyu et al. 2017). A full list of CYP3A4 inducers can be found at: http://medicine.iupui.edu/clinpharm/ddis/table.aspx.

Beside CYP3A4 interactions, special attention needs to be paid in case of usage of any drug with QTc prolongation potential (Steegmann et al. 2012, 2016). If concomitant medication cannot be avoided regularly ECG controls need to be performed. A full list of agents that prolong the QT interval can be found at: https://crediblemeds.org/pdftemp/pdf/CombinedList.pdf.

Resorption of bosutinib is pH dependent; therefore, if medication with proton pump inhibitors is necessary, they should be taken several hours before or after bosutinib medication (Steegmann et al. 2012, 2016; Abbas et al. 2013). Other interactions might be caused by bosutinib and substrates of P-glycoprotein. One in vitro study suggests that plasma levels of P-gp substrates such as digoxin, tacrolimus, some chemotherapeutic agents, and dexamethasone may be increased by bosutinib (Steegmann et al. 2012, 2016; Hsyu et al. 2017).

6 Biomarkers for Response

In CML, BCR-ABL transcript monitoring is essential with any TKI treatment. According to international guidelines (i.e., ELN guidelines (Baccarani et al. 2013), NCCN Guidelines 2.2018), BCR-ABL (expressed as % of housekeeping control gene transcripts) measuring should be performed every 3 months. The goal is to achieve an optimal response, as reflected by CCyR after 6 months and/or a BCR-ABL transcript level of less than 1%, and a reduction in BCR-ABL to equal or less than 0.1% after 12 months of treatment. Early achievement of molecular remission becomes increasingly important; with a decrease in BCR-ABL transcripts to below 10% after three months of treatment is associated with improved 5-year survival as compared to patients who do not achieve this goal (Hanfstein et al. 2012). An even better individual biomarker for response is the slope of the BCR-ABL transcript decline during therapy (Hanfstein et al. 2014; Branford et al. 2014).

In the BELA trial, which tested the efficacy and safety of bosutinib versus imatinib in the first-line setting in patients with newly diagnosed CP CML (Cortes et al. 2012), the rate of molecular response was generally higher at all time points for bosutinib. Bosutinib was also associated with deeper cytogenetic and molecular responses compared with imatinib. For both bosutinib and imatinib, reduction in BCR-ABL/ABL ratio to ≤ 1 or $\leq 10\%$ at months 3, 6, and 9 was associated with higher rates of CCyR and MMR by 12 and 24 months. Overall, these results suggest that early reduction in BCR-ABL/ABL ratio during bosutinib or imatinib therapy is linked to a higher likelihood of experiencing better long-term outcome.

However, the trial failed to show superiority of bosutinib in achieving its primary endpoint, CCyR at 12 months as outlined before (Cortes et al. 2012).

In the second randomized phase III trial (BFORE trial), the superiority of bosutinib over imatinib was validated in patients with CML in chronic phase (Cortes et al. 2017). This trial showed significant improvement of molecular responses in the bosutinib- versus imatinib-treated group, and CCyR by 12 months was also significantly higher in the bosutinib group. It remains to be seen whether these early molecular responses biomarker will translate into superior long-term event-free or even overall survival.

Generally, it is very important to perform these biomarker measurements according to international standards in a well-experienced laboratory following their recommendations for national standardization for quality assurance (Cross et al. 2012, 2015, 2016). Due to the established converting factor to the international scale, follow-up monitoring not necessarily needs to be performed in the same laboratory but in certified laboratories in order to guarantee comparable results.

7 Summary and Perspectives

In conclusion, bosutinib is a novel dual Src/ABL kinase inhibitor with high activity against imatinib-resistant CML as well as solid tumors overexpressing the Src kinase. Its profile of activity is specific with a limited number of molecular targets outside the ABL and Src kinase family. When compared with other second-generation tyrosine kinase inhibitors and with imatinib, bosutinib shows a distinct and very favorable long-term toxicity profile and therefore might be of advantage for a certain cohort of patients based on their pretreatment, toxicities, and/or preexisting comorbidities. Indeed, presumably PDGFR- and/or KIT-mediated side effects such as inhibition of normal hematopoiesis typically observed with other TKIs used in BCR-ABL-positive leukemias (Bartolovic et al. 2004) occur less frequent in patients treated with bosutinib. Furthermore, until now bosutinib treatment seems to be favorable regarding a low rate of long-term toxicity. However, the high rate of gastrointestinal side effects is still a problem that needs to be addressed. A lot of effort has been put in the side management of those events including prophylactic medication and guidelines for patient and physicians how to behave in case of GI toxicity. Furthermore, initial dose reduction has been and is currently tested in different trials with first results showing that time on treatment with bosutinib is important but initial dose decrease can avoid early treatment discontinuation due to side effects. The so-called BODO trial (NCT03205267) is currently recruiting patients and is testing the concept of toxicity-guided intra-individual dose escalation upon starting with a lower dose of bosutinib (300 mg/day) and will hopefully improve GI tolerability by defining the individual maximum tolerable dose while efficacy is preserved due to a more continuous treatment.

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