



Novel Agents in CML Therapy: Tyrosine Kinase Inhibitors and Beyond

Junia V. Melo¹ and Charles Chuah²

¹ Division of Haematology, Institute of Medical & Veterinary Science, Adelaide SA, Australia

² Department of Haematology, Singapore General Hospital; Cancer and Stem Cell Biology Program, Duke-NUS Graduate Medical School, Singapore.

The emergence of resistance to imatinib has become a significant problem despite the remarkable clinical results achieved with this tyrosine kinase inhibitor in the treatment of chronic myeloid leukaemia. The most common cause of imatinib resistance is the selection of leukemic clones with point mutations in the Abl kinase domain. These mutations lead to amino acid substitutions and prevent the appropriate binding of imatinib. Genomic amplification of *BCR-ABL*, modulation of drug efflux or influx transporters, and Bcr-Abl-independent mechanisms also play important roles in the development of resistance. Persistent disease is another therapeutic challenge and may in part, be due

to the inability of imatinib to eradicate primitive stem cell progenitors. A multitude of novel agents have been developed and have shown *in vitro* and *in vivo* efficacy in overcoming imatinib resistance. In this review, we will discuss the current status of the ATP-competitive and non-ATP-competitive Bcr-Abl tyrosine kinase inhibitors. We will also describe inhibitors acting on targets found in signaling pathways downstream of Bcr-Abl, such as the Ras-Raf-mitogen-activated protein kinase and phosphatidylinositol-3 kinase-Akt-mammalian target of rapamycin pathways, and targets without established links with Bcr-Abl.

Introduction

Imatinib was introduced in 1998 into the armamentarium of drugs for the treatment of chronic myeloid leukemia (CML) with remarkable efficacy and has since revolutionized the management of CML. However, the development of resistance and the persistence of minimal residual disease (MRD) have dampened the initial enthusiasm. Since the first reports of resistance appeared in 2000, three major mechanisms have been described. The two most common affect the *BCR-ABL* gene itself, namely mutations in its tyrosine kinase domain and overexpression of the Bcr-Abl protein due to amplification of the *BCR-ABL* gene.^{1,2} The third mechanism is less well characterized and understood, and is represented by phenomena that lead to Bcr-Abl-independent resistance. These include upregulation of the drug efflux pumps,³ downregulation of drug influx transporters^{4,5} and overexpression of Lyn, a Src-family kinase (SFK) protein.^{6,7}

Recently, studies have identified the presence of small numbers of primitive non-dividing stem cells that are refractory to the pro-apoptotic effect of imatinib and conventional chemotherapeutic agents.⁸ The insensitivity of these “quiescent” cells also has important implications for the management of CML with regards to MRD and relapse following imatinib-induced response.

There is an urgency to develop novel compounds to prevent or overcome imatinib resistance and to eradicate MRD. The elucidation of the mechanisms of resistance has enabled the rational development of a plethora of novel agents.

Some of these agents have already been approved for clinical use or are being tested in clinical trials (Table 1).

Second Generation ATP-Competitive Bcr-Abl Inhibitors

Nilotinib

The *N*-methylpiperazine moiety was originally incorporated into imatinib to improve its solubility and oral bioavailability. Substitution of this amide moiety with alternative binding groups, while maintaining H-bond interactions to Glu286 and Asp381, led to the discovery of a more potent compound, nilotinib (AMN107, Tasigna™; Novartis). Nilotinib also inhibits the activity of Arg, Kit, and platelet-derived growth factor receptor (PDGFR), but not Src-family kinases (SFK).⁹ Nilotinib is 10 to 50 times more potent than imatinib in inhibiting the proliferation and autophosphorylation of wild-type Bcr-Abl cell lines and most of the Bcr-Abl mutants, except the T315I mutant.⁹ It is superior to imatinib in reducing leukemic burden and prolonging the survival of mice transplanted with wild-type Bcr-Abl, the M351T and E255V mutants.⁹ However, nilotinib and imatinib produced equivalent reduction in CrkL phosphorylation in primary CD34⁺ CML cells, suggesting that they were equipotent for inhibiting Bcr-Abl activity.¹⁰ Furthermore, nilotinib did not induce apoptosis in the primitive quiescent population. Results from Phase II clinical trials with nilotinib are summarized in Table 2.¹¹⁻¹³ Nilotinib is well tolerated, and common ad-

Table 1. Kinase target profile of inhibitors that can be used for the treatment of chronic myeloid leukemia (CML).

TKI	ABL	ABL _{T315I}	Kit	PDGFR	SRC	Aurora	Others
Imatinib	+	-	++	++	LCK	-	+
Nilotinib	++	-	+	+	-	-	-
Dasatinib	+++	-	++	++	+++	-	+++
Bosutinib	++	-	-	-	+++	-	+
INNO-406	++	-	-	++	LYN	-	NR
MK-0457	+	+	-	-	-	+++	NR
XL228	+++	+++	NR	NR	NR	+++	NR
PHA-739358	++	++	+	NR	+	+++	+
AP24534	+++	+++	NR	NR	NR	NR	NR
SGX393	+++	+++	-	-	LCK	-	+
DC-2036	+++	+++	NR	NR	++	NR	NR

+, weak/moderate inhibition; ++, moderate/strong inhibition; +++, very strong inhibition.

Abbreviations: TKI, tyrosine kinase inhibitor; NR, not reported; LCK, lymphocyte-specific protein tyrosine kinase; LYN, Lck/Yes-related novel tyrosine kinase.

verse events included grade 3-4 myelosuppression, elevated bilirubin and lipase levels.

Dual Src-Family Kinase/Abl Kinase Inhibitors

Dasatinib

Dasatinib (BMS-354825, Sprycel; Bristol Myers Squibb) is a multi-target kinase inhibitor of Bcr-Abl, SFK, ephrin receptor kinases, PDGFR and Kit. In addition, dasatinib binds to other tyrosine and serine/threonine kinases, such as the TEC family kinases, the mitogen-activated protein kinases and the receptor tyrosine kinase, discoidin domain receptor 1.¹⁴ Dasatinib is more potent than imatinib and is effective against the imatinib-resistant active conformation of the kinase domain. It is capable of inhibiting the proliferation and kinase activity of wild-type and most Bcr-Abl mutant cell lines except the T315I mutant. *In vivo* studies in murine models demonstrated the activity of dasatinib

Table 2. Hematologic and cytogenetic responses in nilotinib trials.

Stage of disease	No of patients	CHR, %	MCyR, %	CCyR, %
Chronic phase ¹¹	321	77	57	41
Accelerated phase ¹²	136	26	31	19
Blast crisis ¹³	136	11	40	29

Abbreviations: CHR, complete hematologic response; CCyR, complete cytogenetic response; MCyR, major cytogenetic response.

in inhibiting the leukemic cell growth and prolonging the survival of mice harboring wild-type Bcr-Abl and the M351T, but not the T315I mutant.¹⁵ Phase II clinical trials of dasatinib in imatinib-resistant and -intolerant CML have confirmed its efficacy, and the hematologic and cytogenetic responses are summarized in **Table 3**.¹⁶⁻¹⁸ These are durable in patients with chronic-phase disease, with 59% and 49% achieving a major and complete cytogenetic response (CCyR), respectively, after a median follow-up of 15.2 months.¹⁹ However, responses are generally not durable in the advanced phases. Dasatinib is well tolerated but grade 3-4 myelosuppression is common, especially in the advanced phases. Non-hematological side effects include diarrhea, nausea, headache, peripheral edema and pleural effusion. However, resistance to dasatinib is also an emerging problem. Not surprisingly, the pre-existence

or selection of the T315I mutant is the most frequent mechanism of resistance.²⁰ The emergence of the F317L mutant has also been commonly observed in dasatinib-resistant patients.²⁰ In addition, although dasatinib significantly inhibited CrkL phosphorylation and caused a reduction in the total number of CD34⁺CD38⁻ CML cells compared to imatinib, it did not eliminate the most primitive, quiescent fraction.²¹

Bosutinib

Bosutinib (SKI-606; Wyeth) has potent antiproliferative activity against imatinib-sensitive and -resistant Bcr-Abl-positive cell lines, including the Y253F, E255K and D276G mutants, but not the T315I mutant.²² It is able to bind to both inactive and intermediate conformations of Bcr-Abl.²² Bosutinib inhibited the proliferation of CML progenitors but was moderately effective in inducing apoptosis and was not able to eliminate the primitive, quiescent popula-

Table 3. Hematologic and cytogenetic responses in dasatinib trials.

Stage of disease	No of patients	CHR, %	MCyR, %	CCyR, %
Chronic phase ¹⁶	186	90	52	39
Accelerated phase ¹⁷	107	39	33	24
Myeloid blast crisis ¹⁸	74	26	31	27
Lymphoid blast crisis ¹⁸	42	26	50	43

Abbreviations: CHR, complete hematologic response; CCyR, complete cytogenetic response; MCyR, major cytogenetic response.

tion.²³ Early results from Phase II studies have demonstrated its efficacy and are summarized in **Table 4**.^{24,25} Bosutinib was also effective in patients previously treated with dasatinib or nilotinib. Unlike dasatinib, bosutinib does not significantly inhibit Kit or PDGFR and has a more favorable toxicity profile.²² Adverse events are commonly gastrointestinal in nature and grade 3-4 myelosuppression usually occurs only in the advanced phases.

INNO-406

INNO-406 is a dual Abl/Lyn kinase inhibitor that is up to 55 times more potent than imatinib in Bcr-Abl-positive cell lines.²⁶ INNO-406 inhibited the growth of cells with numerous Bcr-Abl mutants, including the F317L mutant, but not the T315I mutant.²⁶ Unlike the other second-generation tyrosine kinase inhibitors (TKIs), INNO-406 inhibits Lyn kinase but has no or limited activity against the other SFK.²⁶ Since overexpression of Lyn kinase has been implicated in Bcr-Abl independent resistance, INNO-406 may have further importance in imatinib-resistant CML. In a Phase I study, a CCyR was achieved in 2 of 7 CP patients who had failed imatinib and CHR was achieved in 2 of 7 accelerated phase patients who had not responded to multiple TKIs.²⁷ The drug was well tolerated and adverse events included elevation of transaminases.

Other dual SFK/Abl kinase inhibitors include the anilino-quinazoline AZD0530; the purine derivatives, AP23464 and its analogue AP23848; the pyrido-pyrimidines, PD166326, PD173955 and PD180970; the pyrazolo-pyrimidines, PP1 and PP2; and the acetylanes AC22 and K1P. These compounds, however, have not been developed for clinical use.

T315I Kinase Inhibitors

The substitution of the amino acid threonine with isoleucine at position 315 of the Abl protein was the first muta-

tion to be detected in patients with imatinib-resistant CML.¹ Based on the crystal structure of the catalytic domain of Abl complexed to a variant of imatinib,²⁸ this substitution was predicted to reduce the affinity for the drug. The T315I mutant can be detected in 4% to 19% of patients with imatinib-resistant CML and its resistance to the SFK/Abl TKI inhibitors and nilotinib poses a therapeutic challenge.²⁹⁻³¹

A substrate-competitive inhibitor of Bcr-Abl, ON012380, was recently reported to have potent *in vitro* inhibitory activity in cell lines expressing wild-type Bcr-Abl and all the Bcr-Abl mutants, including the T315I mutant. ON012380 was active *in vivo* in mice expressing the T315I mutant and caused a decrease in leukemic cells.³²

Aurora kinases are overexpressed in many cancers and are essential for the regulation of mitotic processes during cell division. MK-0457, formerly known as VX-680, is an Aurora kinase inhibitor that targets Bcr-Abl, FLT3 and JAK2 kinases and induces apoptosis at nanomolar concentrations.³³ MK-0457, unlike the other Abl kinase inhibitors, is able to bind to the kinase domain of the T315I mutant. Clinical responses were achieved in patients with advanced-phase CML with the T315I mutant treated with MK-0457 in a Phase I study, with 3 out of 9 patients attaining a major cytogenetic response.³⁴ Phase II clinical trials are now currently in progress. Another Aurora kinase inhibitor, PHA-739358, also exhibited antiproliferative and proapoptotic activity against CML cell lines and Bcr-Abl mutants, including the T315I mutant.³⁵ Hematologic and cytogenetic responses to PHA-739358 were observed in CML patients harboring the T315I mutant, and Phase II trials are now ongoing.³⁶

SGX393 is an azaindole that inhibits the growth of cells expressing wild-type Bcr-Abl and the T315I mutant, as well as other Bcr-Abl mutants at varying concentrations.³⁷ In addition, SGX393 reduced CrkL phosphorylation in primary hematopoietic cells from patients harboring the T315I mutant and inhibited growth of T315I-driven tumors in mice.³⁷

Other small molecule compounds with *in vitro* or *in vivo* activity against the T315I mutant include the multi-targeted kinase inhibitor XL228,³⁸ the Bcr-Abl kinase inhibitor AP24534,³⁹ and the 2,6,9-trisubstituted purine derivative AP23846.⁴⁰ Phase I studies using XL228 and AP24534 are currently underway.

Allosteric Inhibitors

A recent class of Bcr-Abl inhibitor compounds was uncovered by differential cytotoxicity screen of approximately 50,000 combinatorially derived kinase-directed heterocycles. This is a class of compounds that exert their activity through a newly described allosteric, non-ATP competitive mechanism, potentially involving binding to the myristate pocket in the C-lobe of the Bcr-Abl kinase domain.⁴¹ GNF-2 is the lead compound in this class and has no activity against most kinases including Kit, PDGFR and

Table 4. Hematologic and cytogenetic responses in bosutinib trials.

Stage of disease	CHR, % (n)	MCyR, % (n)	CCyR, % (n)
Chronic phase ²⁴			
Exposed to imatinib only	89 (38)	41 (56)	36 (56)
Exposed to imatinib and other TKIs	77 (13)	20 (10)	—
Accelerated phase ²⁵			
Exposed to imatinib only	50 (8)	44 (9)	22 (9)
Exposed to imatinib and other TKIs	11 (9)	0 (6)	0 (6)
Blast crisis ²⁵			
Exposed to imatinib only	15 (13)	18 (11)	9 (11)
Exposed to imatinib and other TKIs	7 (14)	20 (10)	20 (10)

Abbreviations: CHR, complete hematologic response; CCyR, complete cytogenetic response; MCyR, major cytogenetic response; n, number evaluable; TKI, tyrosine kinase inhibitor.

SFK. GNF-2 inhibited the growth of cells with the Y253F and E255V but not the other P-loop mutants, the T315I or F317L mutants.⁴¹

Heat Shock Protein 90 Inhibitors

Heat shock protein 90 (Hsp90) functions as a molecular chaperone that interacts with proteins such as Raf, Akt, FLT-3 and Bcr-Abl. This interaction is required for maintaining the proteins in a stable and functional conformation. Geldanamycin and its derivative, 17-allylamino-17-demethoxygeldanamycin (17-AAG; National Cancer Institute) bind to the ATP-binding pocket of Hsp90 and inhibit its ability to function as a chaperone, thereby leading to the downregulation of Bcr-Abl and inducing apoptosis in CML cell lines.⁴² Furthermore, geldanamycin and 17-AAG inhibited the growth of cell lines containing the E255K and T315I mutants.⁴³ However, Bcr-Abl-overexpressing CML cell lines remained cross-resistant.⁴⁴ Combination therapy with imatinib and 17-AAG led to synergistic inhibition of growth and induction of apoptosis in the cross-resistant cell lines but not of the imatinib-sensitive counterparts.⁴⁴ In addition, 17-AAG targets the P-glycoprotein multidrug resistance pump and may inhibit imatinib efflux.⁴⁴

Arsenic Trioxide

Arsenic trioxide (As₂O₃, Trisenox; Cell Therapeutics, Inc) induces apoptosis in Bcr-Abl-positive cell lines and reduces the proliferation of CML blasts but not of CD34⁺ progenitors.^{45,46} Combination of As₂O₃ with imatinib induces additive to synergistic inhibition of the growth of Bcr-Abl-expressing cell lines, and induces cell death in imatinib-resistant cell lines that overexpressed Bcr-Abl or had the M351T or Y253F, but not the T315I mutants.^{47,48} A recent report showed that As₂O₃, via the degradation of the promyelocytic leukemia protein, was able to sensitize quiescent CML leukemia-initiating cells to cytosine arabinoside-mediated induction of apoptosis.⁴⁹

Homoharringtonine

Homoharringtonine (HHT) is a plant alkaloid derived from an evergreen tree belonging to the genus *Cephalotaxus*. HHT inhibits protein synthesis and induces apoptosis and, in combination with imatinib, is synergistic or additive in CML cell lines. Clinical responses have been observed with semisynthetic HHT (Omacetaxine; Chemgenex). CHR and cytogenetic responses were attained in imatinib-resistant patients with omacetaxine.⁵⁰ In patients who had achieved a MCyR with imatinib, omacetaxine in combination with imatinib resulted in a 1-log reduction in BCR-ABL transcript levels in 50% of patients.⁵¹ Recently, omacetaxine was shown to cause a reduction or disappearance of the T315I mutant in patients who had developed this mutation while on imatinib.^{52,53} Omacetaxine is currently being tested

in Phase II trials in TKI-resistant patients with or without the T315I mutant.

Histone Deacetylase Inhibitors

Histone deacetylases (HDAC) catalyze the deacetylation of lysine residues at the amino termini of core nucleosomal histones. By inhibiting HDAC, histone deacetylase inhibitors (HDI) such as suberoylanilide hydroxamic acid (SAHA), cause hyperacetylation of histones, leading to transcriptional upregulation of cyclin-dependent kinase inhibitor, p21, cell-cycle arrest and apoptosis in tumor cells.⁵⁴ SAHA also induces expression of p27, a key cell-cycle regulator, and is associated with downregulation of p210Bcr-Abl protein. Combination treatment of CML cell lines with SAHA and imatinib resulted in a greater level of apoptosis than was achieved with either agent alone.^{54,55} This combination also produced synergistic induction of apoptosis in imatinib-resistant CML cell lines that overexpressed Bcr-Abl.⁵⁵ Co-treatment with nilotinib and the HDI LBH589 was synergistic in inducing apoptosis in K562 and LAMA-84 CML cell lines.⁵⁶ LBH589 also induced apoptosis in imatinib-resistant cell lines expressing the T315I and E255K mutants and this was associated with depletion of Bcr-Abl levels.⁵⁶ Recently, the HDI valproate was found to enhance imatinib-induced growth arrest and apoptosis in CML cell lines when combined with this TKI.⁵⁷ In addition, valproate sensitized imatinib-resistant CML cell lines and imatinib-resistant primary mononuclear cells to imatinib and restored its cytotoxic effect.

Proteasome Inhibitors

Proteasome inhibitors target the catalytic 20S core of the proteasome and suppress the proteasomal degradation of numerous cellular proteins.⁵⁸ Inhibition of transcription activated by nuclear factor κ B (NF- κ B) has been implicated as the mechanism responsible for the antitumor effect of proteasome inhibitors. The proteasome inhibitor, bortezomib (PS-341, Velcade; Millennium Pharmaceuticals) was shown to inhibit the proliferation, induce G₂/M phase cell cycle arrest and promote apoptosis of imatinib-sensitive and resistant CML cell lines.⁵⁹ However, the simultaneous treatment of imatinib-sensitive CML cell lines with bortezomib and imatinib produced an antagonistic interaction on growth inhibition, although sequential exposure of CML cell lines to low doses of bortezomib followed by imatinib resulted in additive effects. Synergism between bortezomib and the HDI SAHA and between bortezomib and flavopiridol has been reported in *in vitro* studies of growth inhibition of CML cell lines.^{58,60}

Cyclin-Dependent Kinase Inhibitors

Multiple cyclin-dependent kinases are targeted by the semisynthetic flavone, flavopiridol (L86-8275; National Can-

cer Institute).⁶¹ Treatment with imatinib and flavopiridol led to increased mitochondrial damage, and activation of caspases and apoptosis in CML but not in Bcr-Abl-negative leukemia cell lines.⁶¹ This drug combination also effectively induced apoptosis in an imatinib-resistant CML cell line that overexpressed Bcr-Abl61. A Phase I trial showed that the combination of imatinib and flavopiridol in Bcr-Abl-positive hematologic malignancies was tolerable and was responsible for four objective responses, including two CHR.⁶²

DNA-Methyltransferase Inhibitors

Epigenetic changes are a characteristic feature of human leukemias, and many gene promoters exhibit abnormally high methylation. Methylation of promoter sequences contributes to the malignant phenotype of transformed cells by silencing genes that are essential for differentiation and apoptosis. Decitabine (5-aza-2'-deoxycytidine; SuperGen) is a DNA hypomethylating agent that integrates into DNA and forms irreversible covalent bonds with DNA-methyltransferase (Mtase) at cytosine residues targeted for methylation. DNA synthesis stalls at these covalently modified sites and the DNA-Mtase complexes are eventually degraded. Loss of the Mtase-DNA complexes is associated with depletion of Mtase levels and, when renewed DNA synthesis occurs, the newly synthesized DNA is hypomethylated.⁶³

Hematologic and cytogenetic responses were observed in a study of 130 patients with CML treated with decitabine at doses from 50 to 100 mg/m² over 6 hours every 12 hours for 5 days.⁶⁴ However, these doses were associated with severe myelosuppression that was delayed, prolonged and dose-dependent. In a Phase I trial in relapsed or refractory leukemias, low-dose prolonged exposure schedules of decitabine were given to 50 patients, of whom 5 had CML.⁶⁵ Of these 5 patients with CML, 2 achieved CHR and 2 partial hematologic responses. The combination of decitabine with imatinib may be useful in CML. An *in vitro* study revealed that this combination had additive to synergistic growth inhibitory effects upon cells containing Bcr-Abl with the M351T and Y253F mutants.⁴⁷ However, the combination of imatinib and decitabine was less potent than decitabine alone at inhibiting the growth of cells with the T315I mutant.

Tumor Suppressor PP2A

Bcr-Abl inactivates the protein phosphatase 2A (PP2A) tumor suppressor by enhancing the expression of a PP2A inhibitor, SET, in CML blast crisis progenitors.⁶⁵ The molecular or pharmacologic reactivation of PP2A activity suppresses Bcr-Abl expression and function, resulting in growth inhibition, increased apoptosis, impaired clonogenicity and decreased *in vivo* leukemogenesis in CML cell lines and primary CML cells.⁶⁵ FTY720 is a PP2A acti-

vator that is structurally similar to sphingosine and is being investigated as an immunomodulator in clinical trials for patients with multiple sclerosis or undergoing renal transplantation. FTY720 suppressed the growth, abolished Bcr-Abl phosphorylation and induced Bcr-Abl down-regulation via the activation of PP2A in imatinib-sensitive and T315I-expressing cell lines and in primary CML cells.⁶⁶ FTY720 also suppressed *in vivo* wild type and T315I Bcr-Abl-driven leukemogenesis without exerting side effects.⁶⁶

Targeting Pathways Downstream of Bcr-Abl

The constitutive activation of the Bcr-Abl tyrosine kinase results in several abnormalities in CML cells: altered cell adhesion, inhibition of apoptosis, proteasomal degradation and activation of mitogenic downstream signaling pathways, for example, Ras and mitogen-activated protein kinase (MAPK), Janus kinase-signal transducer and activator of transcription, phosphatidylinositol-3 (PI-3) kinase and Myc pathways. Targeting these downstream pathways may offer a synergistic antiproliferative and pro-apoptotic effect when combined with imatinib in CML cells.

Farnesyl Transferase Inhibitors

The Ras pathway is intimately linked with CML through its activation by Bcr-Abl. Genetic and biochemical data have shown that Ras activation plays a central role in leukemogenic transformation by Bcr-Abl. Bcr-Abl couples to the Ras pathway through protein-protein interactions with components of the Ras-MAPK signaling complex, including Grb2, SHC and CrkL. This is essential for fibroblast and hematopoietic oncogenic transformation. The correct function of Ras is dependent on prenylation, which is a post-translational modification, involving the addition of a 15-carbon farnesyl isoprenoid moiety to a conserved cysteine residue in a carboxy-terminal CAAX motif. Prenylation facilitates membrane targeting and anchors the proteins to the plasma cell membrane. Prenylation is catalyzed by farnesyltransferase (FT) and geranylgeranyl-transferase (GGT) and rational drug design has yielded FT inhibitors (FTI) which interfere with the FT activity. Two FTIs, tipifarnib (R115777; Johnson & Johnson Pharmaceutical) and lonafarnib (SCH66336; Schering-Plough), have demonstrated potential as antileukemic agents in CML.

Tipifarnib

A Phase II trial of tipifarnib involving 22 patients with CML, 8 with myelofibrosis and 10 with multiple myeloma revealed that the drug had modest activity, inducing complete or partial hematological responses in 7 (32%) of the CML patients.⁶⁷ Minor cytogenetic responses were also achieved by 4 of these 7 patients but responses were not sustained and the median duration was only 9 weeks.⁶⁷ Recently, a Phase I trial investigating the combination of

imatinib and tipifarnib in patients with imatinib-resistant CML showed that this combination was active and well tolerated.⁶⁸ Major cytogenetic responses were achieved in 7 of 26 patients and a partial cytogenetic response was attained by a patient harboring the T315I mutant. Toxicities included diarrhea, nausea and grade 3-4 neutropenia and thrombocytopenia.⁶⁸

Lonafarnib

Lonafarnib is another FTI that is a potent and selective inhibitor of the growth of primary cells from patients with CML and demonstrated efficacy against a murine model of CML in BC.⁶⁹ Lonafarnib inhibited the proliferation of imatinib-resistant cell lines and reduced colony formation by primary cells obtained from patients who are resistant to imatinib.⁷⁰ However, the results of a pilot study investigating lonafarnib in patients with imatinib-resistant CML have been discouraging with only 2 of 13 patients achieving a clinical response.⁷¹ A recent report showed that lonafarnib is able to sensitize primitive, quiescent Bcr-Abl-positive progenitors to imatinib.⁷²

Recently, the FTI BMS-214662 was reported to enhance the cytotoxic effect of imatinib or dasatinib in primary CD34⁺ CML cells and significantly reduce the numbers of undivided primitive quiescent CML stem cells, either alone or in combination with imatinib or dasatinib.²¹ This effect was selective and normal stem cells were relatively spared. The cytotoxic action was via apoptosis as evidenced by enhanced caspase-3 activity.²¹ BMS-214662 is currently in Phase I trials in AML and the possibility of clinical trials in CML are being explored.²¹

MEK1/2 Inhibitors

Downstream of Ras, Raf-1 activates the MAPK kinases, MEK1/2 (MAPK or ERK Kinase). Several MEK1/2 inhibitors have been developed, including PD18435273 (Parke-Davis) and U0126 (DuPont Merck). PD184352 or U0126, when combined with imatinib, caused synergistic induction of apoptosis in CML cell lines.⁷³ In addition, the combination of PD184352 and imatinib effectively induced cell death in an imatinib-resistant cell line that overexpressed Bcr-Abl73. PD184352 was recently shown to interact synergistically with dasatinib in inducing apoptosis in human CML cells and imatinib-resistant cells but not cells with the T315I mutant.⁷⁴

PI-3 Kinase-Akt-mTOR Signaling

Bcr-Abl activates PI-3 kinase via a direct association with its 85 kDa regulatory subunit and signaling via the PI-3 kinase is essential for the growth of CML progenitors. The mammalian target of rapamycin (mTOR) is a serine-threonine kinase downstream of PI-3 kinase that is activated upon phosphorylation by Akt.⁷⁵ The macrolide antibiotic rapamycin (Sirolimus; Wyeth Pharmaceuticals) binds to the

immunophilin molecule FKBP12, and the resulting complex inhibits mTOR.⁷⁵ Recently, a derivative of rapamycin, RAD001 (Everolimus; Novartis Pharma), has been developed that has superior oral bioavailability.⁷⁶ Treatment with rapamycin alone inhibited the growth of Ba/F3-Bcr-Abl as well as Bcr-Abl-transformed B lymphoblasts and primary CML cells, and prolonged the survival of mice transplanted with bone marrow that had been retrovirally transduced with Bcr-Abl.⁷⁶⁻⁷⁸ The inhibitory effect of rapamycin on the *in vitro* growth of primary CML cells is due to induction of G₁ cell cycle arrest and subsequent apoptosis.⁷⁷ In addition, the combination of imatinib and rapamycin was effective at suppressing the growth of imatinib-resistant cell lines overexpressing Bcr-Abl.⁷⁵ However, the reports on the effect of this combination on Bcr-Abl mutants have been conflicting.⁷⁵⁻⁷⁸ Recently, signaling via the PI-3K-Akt-mTOR pathway has been implicated as a compensatory mechanism responsible for maintaining the viability of imatinib-naïve cells upon first exposure to imatinib.⁷⁹ Treatment with imatinib led to the activation of the PI-3 kinase/Akt/mTOR pathway, and this activation was important in mediating cell survival during the early development of imatinib resistance before overt resistance developed.⁷⁹ Clinical trials investigating the safety and efficacy of combining imatinib and RAD001 or CCI-779, another mTOR inhibitor, are currently in progress.

Conclusion

Without doubt, imatinib represents a major achievement for the treatment of CML but resistance to this drug has become and will continue to be a therapeutic challenge. Single agent therapy with imatinib may not be the best long-term option in CML, at least for a proportion of patients, and other strategies need to be explored. Many novel compounds are currently being investigated preclinically and clinically, and therapeutic approaches to circumvent the problem of imatinib resistance are now possible. Dasatinib and nilotinib represent the first of the newer generation TKIs which are effective and safe in patients with imatinib-resistant and -intolerant CML. It is likely, however, that subclones with novel Bcr-Abl mutants will again develop in response to these new small-molecule inhibitors. Therefore, alternative therapeutic approaches are required and these may involve the combination of Bcr-Abl TKIs with inhibitors of non-Bcr-Abl targets or targets downstream of Bcr-Abl to achieve a synergistic effect and possibly prevent or overcome resistance.

Disclosures

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Correspondence

Junia V. Melo, MD, PhD, Institute of Medical and Veterinary Science, Ducane Road, Adelaide, W12 0NN, Australia; Phone: 44 (20) 83832167; Fax: 44 (20) 87429335; e-mail: junia.melo@imvs.sa.gov.au

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