

Therapeutic Options Against *BCR-ABL1* T315I-Positive Chronic Myelogenous Leukemia

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Abstract Despite the efficacy of imatinib therapy in chronic myelogenous leukemia, the development of resistance continues to challenge the treatment of this disease. Mutations within the kinase domain of *BCR-ABL1* constitute the most frequent mechanism of resistance in patients with chronic myelogenous leukemia treated with imatinib or the second generation tyrosine kinase inhibitors nilotinib and dasatinib. Of particular concern is the substitution of the threonine residue at the highly conserved gatekeeper residue 315 with a bulkier hydrophobic isoleucine amino acid. This mutation causes steric hindrance precluding the access ATP-competitive inhibitors to the ATP-binding pocket. To expedite the identification of strategies to override the resistance imposed by the T315I mutation, several strategies have been pursued, including the exploitation of *BCR-ABL1* kinase sites distant from the ATP-binding pocket to cripple the kinase activity of the enzyme and inhibiting signaling pathways downstream from *BCR-ABL1*. Recent insights gained regarding the structural biology of T315I have led to the development of a variety of compounds against this mutant. We herein summarize the most clinically promising anti-T315I therapies.

BCR-ABL1 kinase has been shown to play a pivotal role *in vivo* in the development and maintenance of *BCR-ABL1*-mediated leukemogenesis in several experimental models (1–3). The marked dependence of *BCR-ABL1*-positive cells on *BCR-ABL1* kinase spurred the design of agents aiming at blocking the activity of this enzyme and its downstream signaling pathways. One such agent, imatinib mesylate, inhibits the activity of *BCR-ABL1* *in vitro* with IC_{50} values ranging between 100 and 500 nmol/L (4–6). In clinical trials, therapy with imatinib resulted in cumulative best complete hematologic and cytogenetic (CCyR) response rates of 98% and 87%, respectively, after 5 years of follow-up in patients with newly diagnosed chronic phase (CP) chronic myelogenous leukemia (CML; ref. 7). These results notwithstanding, a subset of patients receiving imatinib therapy develop resistance to imatinib, which are frequently associated with single-point mutations within the kinase domain of *BCR-ABL1*. More than 100 different ABL kinase point mutations have been reported in patients with imatinib-resistant CML (8–13). These mutations impair imatinib binding either by changing the identity of residues with

which imatinib makes direct contact within the kinase domain (e.g., T315I) or by blocking the transition of the enzyme to its inactive conformation, to which imatinib binds (e.g., mutations at the A-loop; refs. 14–16). The frequency with which *BCR-ABL1* mutations have been reported in patients who fail imatinib therapy ranges from 40% to 90% depending upon the methodology of detection and the CML phase (8, 17–21). Of note, different mutations confer varying degrees of insensitivity to imatinib and other tyrosine kinase inhibitors (TKI). The *BCR-ABL1* T315I mutation affects a highly conserved threonine residue close to the catalytic domain of the enzyme and confers insensitivity to imatinib and the second generation TKIs nilotinib, dasatinib, and bosutinib (22–26).

This article will review the agents under clinical or preclinical development that hold promise for the management of patients with *BCR-ABL1* T315I-positive leukemia.

The Vexing *BCR-ABL1* T315I Mutation

The highly conserved threonine 315 residue is called the “gatekeeper” because it is located near the ABL catalytic domain controlling the access to a hydrophobic pocket of the enzymatic active site (27). The substitution of the threonine residue at position 315 of the *BCR-ABL1* protein by isoleucine (T315I) places the bulky isoleucine side chain in the center of the imatinib-binding site in ABL, thus causing steric clash with imatinib. The structural constraints posed by this mutation are believed to be responsible for the lack of activity of other ATP-competitive TKIs such as nilotinib, dasatinib, bosutinib, and INNO-406 against *BCR-ABL1* T315I-positive cells (22–26). The T315I mutation has been reported to occur in ~15% of patients who develop resistance to imatinib (18, 28). Although this mutation is more frequently isolated from patients with

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CML in blast phase (BP) or Ph-positive acute lymphoblastic leukemia who relapsed during imatinib therapy (8, 18), it can also be detected in imatinib-naïve CML and in CD34+ and/or mononuclear cells from patients in CCyR receiving imatinib. Furthermore, in a subset of patients, T315I was detected only transiently and had no effect on the maintenance of the CCyR (29, 30). Therefore, T315I may only contribute to CML stem cell persistence and relapse in patients in whom this mutation is expressed at high levels in a persistent manner, which typically occur in patients with BP or accelerated phase (AP) CML. In addition, expression of low levels of T315I may not be selected during imatinib therapy (31) or correlate with residual disease (32). By contrast, T315I was the only mutation recovered in mutagenesis screening assays when high doses of nilotinib were used (33), and in phase II studies of dasatinib for patients with CML after imatinib failure, T315I and F317L/I were the mutations more frequently encountered (34).

To overcome the TKI resistance imposed by T315I mutation, several strategies have been pursued, including the modeling of TKIs able to accommodate the structural constraints imposed by this mutant, and the development of compounds that inhibit the activity of the kinase by targeting key functional motifs distant from the ATP-binding pocket of BCR-ABL1 where mutations occur. Moreover, several TKIs designed to target oncogenic kinases different from ABL1 have shown opportunistic activity against T315I.

Direct BCR-ABL1 T315I Kinase Inhibitors

Dual Aurora/ABL kinase inhibitors

The human Aurora proteins (A, B, and C) are serine/threonine kinases that regulate different steps during mitosis, including the G₂-M transition, mitotic spindle organization, chromosome segregation, and cytokinesis (35). Aurora-A and Aurora-B are overexpressed or gene amplified in a variety of human malignancies, including leukemia. Interestingly, several Aurora kinase inhibitors have been shown to inhibit both wild-type and mutant isoforms of the BCR-ABL1 kinase, and some are currently being tested in clinical trials for patients with BCR-ABL1 T315I-positive leukemia (Table 1).

MK-0457. The Aurora kinase inhibitor MK-0457 (formerly L-001281814; VX-680) is a potent inhibitor of both wild-type (IC₅₀, 10 nmol/L) and T315I BCR-ABL1 kinases (IC₅₀, 30 nmol/L; ref. 16). In cell-based assays in which the pre-B Ba/F3 cell line is engineered to express different BCR-ABL1 mutant isoforms, MK-0457 inhibited the proliferation of cells expressing unmutated, Y253F, or T315I BCR-ABL1 kinase with IC₅₀ values of ~300 nmol/L (36, 37). A high-resolution crystal structure of Aurora-A in complex with MK-0457 has been recently compared with that of imatinib bound to ABL1 kinase, revealing that both drugs exhibit nonoverlapping interactions with their respective kinases (38). MK-0457, however, anchors at the hinge region engaging Asp381 but does not reach as deep into the kinase domain as does imatinib, which allows MK-0457 to avoid the steric constraints imposed by the T315I mutations (39). In a recent phase I study that included 9 patients with BCR-ABL1 T315I-positive refractory CML in either AP (*n* = 4) or BP (*n* = 5), therapy with MK-0457 at 12 to 32 mg/m²/hour given as a 5-day continuous i.v. infusion at 2- to 3-week intervals, rendered 4 CCyRs (1 CCyR, 2 partial, and 1 minor; ref. 40). The main toxicities consisted of

myelosuppression, alopecia, and mucositis. Significant inhibition of CrKL phosphorylation was observed in responders. MK-0457 steady-state plasma concentrations were ≥1 μmol/L at a doses over 20 mg/m²/hour, which are higher than those necessary to inhibit T315I kinase (40).

Dasatinib has also been shown to synergize with MK-0457. Treatment of BCR-ABL1 T315I-positive Ba/F3 cells with MK-0457 (1 μmol/L) and dasatinib (50 nmol/L) resulted in higher attenuation of STAT5 phosphorylation and increased apoptosis compared with treatment with either agent separately, and prolonged survival in athymic nude mice i.v. injected with BCR-ABL1 T315I-positive Ba/F3 cells, compared with either agent alone (41). These results provide the rationale for combination trials of MK-0457 and dasatinib in patients with BCR-ABL1 T315I-positive CML.

XL228. XL228 is an Aurora A inhibitor (IC₅₀, ~3 nmol/L) that has shown potent biochemical activity against ABL1 (K_i, 5 nmol/L), as well as the BCR-ABL1 T315I (K_i, 1.4 nmol/L) kinases (42). *In vitro*, XL228 inhibits phosphorylation of BCR-ABL1 and STAT5 in K562 cells with IC₅₀ values of 33 and 43 nmol/L, respectively, resulting in marked inhibition of cell proliferation (IC₅₀ < 100 nmol/L; ref. 43). When tested against Ba/F3 cells expressing BCR-ABL1 T315I, XL228 was more effective than MK-0457, imatinib, or dasatinib in down-regulating BCR-ABL1 phosphorylation, with IC₅₀ values of 406, 6,912, >10,000, and >10,000 nmol/L, respectively, and in xenografts *in vivo*. In an ongoing multicenter phase I study, XL228 is administered as a weekly 1-hour infusion in patients with CML or BCR-ABL1-positive B-ALL who failed therapy with imatinib and dasatinib.

PHA-739358. PHA-739358 is a pan-Aurora kinase inhibitor with activity against T315I BCR-ABL1 kinase (Fig. 1). Treatment with PHA-739358 of CD34+ cells carrying T315I obtained from imatinib-resistant patients with BP CML significantly decreased phosphorylation of histone H3 Ser¹⁰, a marker of Aurora B activity, and CrKL, indicating that this compound inhibits simultaneously Aurora and BCR-ABL1 (44). The cocrystal structure of BCR-ABL1 T315I with PHA-739358 reveals that the compound binds to the active conformation of the mutant kinase in a mode that accommodates the substitution of isoleucine for threonine, thus avoiding steric clash (45). In an ongoing multicenter phase II study for patients with CML who failed TKI therapy, seven patients (one CP, one AP, and five BP) have been enrolled, including six carrying the T315I mutation. PHA-739358 was administered at 250 or 330 mg/m²/day as a weekly 6-hour infusion for 3 consecutive weeks, every 4 weeks (46). Two BCR-ABL1 T315I-positive patients achieved a complete hematologic response, including 1 in AP who also had a CCyR durable after >6 months and a complete molecular response on the 330 mg/m² dose level. The second patient was treated in CP and achieved a minor CyR at the 330 mg/m² dose level. At 330 mg/m²/day, the C_{max} was 4 to 6 μmol/L/h. PHA-739358 was well-tolerated, with only one patient having grade 4 neutropenia and an infusion-related reaction (46). Dose escalation in patients with advanced-phase CML is ongoing.

KW-2449. KW-2449 is an oral multikinase inhibitor with potent activity against Aurora A (IC₅₀, 48 nmol/L), FLT3 (IC₅₀, 7 nmol/L), FGFR1 (IC₅₀, 36 nmol/L), BCR-ABL1 (IC₅₀, 14 nmol/L), and BCR-ABL1 T315I (IC₅₀, 4 nmol/L) kinases (47). In a phase I study, KW-2449 is administered at daily doses

Table 1. Selection of agents with activity against *BCR-ABL1* T315I-positive cells

Compound	Chemical class	Aurora selectivity	Other targets	Route	Stage	Comments	Clinical trial in CML
MK-0457	Pyrazolo-quinazoline	Pan-Aurora	ABL1, JAK2, FLT3	i.v.	Phase II	Also being tested in patients with JAK2 V617F+ MPDs	NCT00405054
PHA-739358	Pyrrolo-pyrazole	Pan-Aurora	ABL1, RET, TRK-A, FGFR1	i.v.	Phase II	Being tested in CML after failure of imatinib or other antiABL therapy	NCT00335868
XL228	Not disclosed	Aurora A	ABL1, IGF1R, SRC	i.v.	Phase I	Being tested in CML and Ph+ALL after failure of imatinib or dasatinib therapy	NCT00464113
KW-2449	Not disclosed	Aurora A	FGFR1, FLT3, VEGFR	Oral	Phase I	Being tested in acute leukemia, high-risk MDS, and CML	NCT00346632
AT-9283	Not disclosed	Pan-Aurora	ABL1, JAK2, JAK3, FLT3	i.v./Oral	Phase I/II	Being tested in patients with refractory hematologic malignancies	NCT00522990
VE-465	Pyrazolo-quinazoline	Pan-Aurora	Not disclosed	NA	Preclinical	Structurally related to MK-0457	NA
DCC-2036	Not disclosed	None	ABL1, FLT3, SRC	NA	Preclinical	Target switch pocket. Non-ATP competitive ABL1 inhibitor. High ABL1 kinase residency time	NA
17AAG	Benzoquinone	None	HSP90, ABL1, MEK, AKT	i.v.	Phase I	Inhibitor of HSP90. Depletes BCR-ABL1 via proteasome-mediated degradation	NCT00100997
FTY720	Myriocin derivative	None	PP2A, sphingosine-1-phosphate receptors	Oral	Preclinical	Novel immunosuppressant. Active in multiple sclerosis	NA
HHT	Cephalotaxine ester	None	MCL-1	s.c./i.v.	Phase II/III	Synergistic with the BH3 mimetic ABT-737	NCT00375219 NCT00462943
LBH589	Hydroxamic acid derivative	None	HDAC-6, HSP90	Oral	Phase II/III	Synergistic with nilotinib	NCT00451035 NCT00449761

Abbreviations: FLT3, Fms-like tyrosine kinase 3; IGF1R, insulin-like growth factor type-1 receptor; FGFR1, fibroblast growth factor receptor-1; MPD, myeloproliferative disorder; Ph+, Philadelphia chromosome positive; ALL, acute lymphoblastic leukemia; MDS, myelodysplastic syndrome; HSP90, heat shock protein 90; HDAC-6, histone deacetylase 6; HHT, homoharringtonine; NA, not applicable.

ranging from 25 to 500 mg divided into 12-hour dosing either on a 14-day or a 28-day schedule. Twenty-nine patients have been enrolled to date, including four with CML, of whom three carried T315I. The mean half-life of KW-2449 was 2.8 to 3.9 hours. No treatment-related deaths have been reported. After one cycle of therapy, seven patients had stable disease (47). Accrual is ongoing and different dosing schedules will be explored given the short half-life of KW-2449.

Other Aurora kinase inhibitors with anti-T315I activity. VE-465 is an Aurora kinase inhibitor structurally related to MK-0457 with potent activity against Ba/F3 cells expressing either wild-type (IC_{50} , 2.0 μ mol/L) or T315I (IC_{50} , 3.5 μ mol/L) isoforms of BCR-ABL1 kinase. Therapy with VE-465 of athymic nude mice injected with *BCR-ABL1* T315I-positive Ba/F3 cells resulted in improved survival compared with animals treated with imatinib, with a good therapeutic index (48).

AT9283, an inhibitor of Aurora A, Aurora B, JAK2, JAK3, and BCR-ABL T315I kinases (IC_{50} < 5 nmol/L in all case), is undergoing evaluation in a phase I trial for patients with refractory hematologic malignancies, including *BCR-ABL1*

T315I-positive CML, administered as a 72-hour continuous infusion at doses ranging from 3 to 48 mg/m² daily for 3 consecutive days (49). Neither the maximum tolerated dose nor the dose-limiting toxicity have been yet identified.

ABL switch pocket inhibitors

A new class of small-molecule non-ATP-competitive inhibitors designed to target "switch pockets" that regulate conformational changes involved in kinase activity has been recently reported. In doing so, these agents have the ability to avoid steric clash with the gatekeeper mutant T315I at the active site of ABL kinase. Given that ATP-binding pockets are highly conserved structures across human kinases, an additional advantage of these compounds is that the switch pocket structures they target are quite distinct for any given kinase, which significantly increases their specificity (50). The lead compound of this class, DCC-2036, potently inhibited proliferation and induces apoptosis of Ba/F3 cells expressing Y253F, T315I, or M351T with IC_{50} values of 5 to 25 nmol/L (Table 2), while sparing parental Ba/F3 cells. Daily dosing of DCC-2036

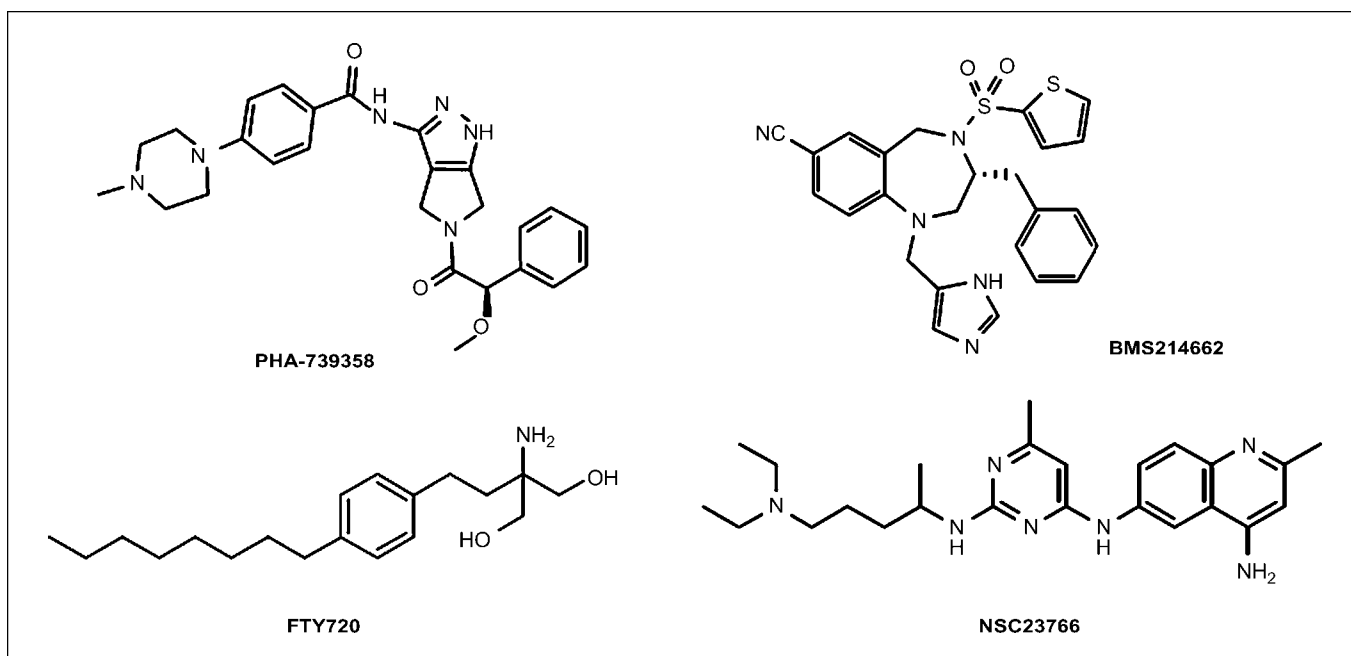


Fig. 1. Selected agents from promising drug classes against T315-positive CML. PHA-739358 (Aurora kinase inhibitor), BMS214662 (farnesyl transferase inhibitor), FTY720 (PP2A activator), and NSC23766 (RAC1/RAC2 GTPase inhibitor).

significantly prolonged survival of BALB/c mice injected with Ba/F3 cells expressing *BCR-ABL1* T315I. In keeping with its long kinase residency time (off-rate of 400 minutes versus 3 minutes for imatinib), one single oral dose of DCC-2036 at 100 mg/kg inhibited ABL1 and STAT5 phosphorylation for >8 hours. DP-2629, with a higher selectivity than DCC-2036, and DP-2494, are other switch pocket inhibitors with high potency against *BCR-ABL1* kinase in preclinical development. These preliminary encouraging results support further investigation of ABL switch pocket inhibitors in clinical trials of patients carrying highly imatinib-resistant *BCR-ABL* kinase isoforms, including T315I (50).

Other direct T315I inhibitors in preclinical development

BIRB-796 is a potent inhibitor of p38 mitogen-activated protein kinase (51, 52) with greater affinity for ABL1 T315I

(K_d , 41 nmol/L), 53 than wild-type ABL (K_d , 1,500 nmol/L) or other imatinib-resistant ABL isoforms (K_d , 2,200 to >10 μ mol/L; ref. 53). However, when tested in assays that directly measure inhibition rather than binding, BIRB-796 was found only moderately effective against Ba/F3 cells expressing *BCR-ABL1* T315I (IC_{50} , 2-3 μ mol/L; refs. 16, 54), which may preclude the clinical development of this compound in patients carrying T315I.

The benzotriazine derivatives TG100598 and TG101114 inhibited T315I with IC_{50} values of ~3.4 nmol/L (55), with TG101114 exhibiting superior pharmacokinetic properties and increased *in vivo* efficacy against *BCR-ABL1* T315I-expressing tumors in a severe combined immunodeficient mouse xenograft model (56). TG101477, a derivative of TG101114, containing the thiazole core of dasatinib, showed equipotency compared with TG101114 against primary *BCR-ABL1* T315I-positive cells

Table 2. IC_{50} values for inhibition of cell proliferation of marketed TKIs and novel small molecule *BCR-ABL1* inhibitors

	Unmutated	T315I	Y253F	Y253H	E255K	E255V	M351T	K562 cells
Imatinib	260	>6,400	3,475	>6,400	5,200	>6,400	880	250-400
Nilotinib	13	>2,000	125	450	200	430	15	30
Dasatinib	0.8	>200	1.4	1.3	5.6	11	1.1	1
DCC-2036	5.8	7.9	25	—	83	—	11	5.5
MK-0457	100-200	100-200	100-200	—	—	—	—	<300
AP24534	2	14	—	—	—	—	—	60*
SGX393	12	7.3	334	<300	77	>500	<25	<25

NOTE: The activity of all compounds on cell proliferation of the *BCR-ABL1* - positive K562 is shown for comparison. IC_{50} , concentration of an inhibitor required for 50% inhibition of cell proliferation. All *BCR-ABL1* constructs were engineered into Ba/F3 cells. All values are expressed in nmol/L.

* IC_{90} value in K562 cells.

with increased selectivity compared with dasatinib, inhibiting only 6 of 76 tested kinases at 500 nmol/L (56).

AP24534 is a novel, orally active small molecule that potently inhibits the proliferation of *BCR-ABL1* T315I-positive Ba/F3 cells (IC₅₀, 8 nmol/L) while exhibiting potent inhibitory activity against FLT3, SRC, VEGFR, and FGFR (IC₅₀, 0.4–58 nmol/L; ref. 57). AP24534 at 50 nmol/L resulted in >50% attenuation of CrKL phosphorylation in mononuclear cells isolated from three patients with BP CML or Ph-positive acute lymphoblastic leukemia carrying the T315I mutation. Notably, twice-weekly administration of AP24534 seemed sufficient to cause complete regression of K562 xenografts in mice (57). A phase I study of AP24534 is planned for patients with CML.

SGX393 (also known as SGX70393) is a potent, selective orally bioavailable azapyridine-based inhibitor of *BCR-ABL1* T315I kinase at low nanomolar concentrations (58). SGX393 proved nontoxic in colony forming assays of normal human mononuclear cells at concentrations up to 2 μmol/L while inhibiting *BCR-ABL1* T315I-driven tumor growth in mice. In a cell-based mutagenesis screen, SGX393 rendered a profile of resistant clones limited to four P-loop residues and position 317. More important, combinations of SGX393 with clinically achievable concentrations of either nilotinib or dasatinib abrogated the emergence of resistant subclones (58), suggesting that combinations of inhibitors containing a potent anti-T315I agent may preemptively avoid the emergence of clinically relevant mutants.

Inhibitors of *BCR-ABL* T315 Kinase Downstream Effectors

An alternative strategy to inhibit the highly TKI-resistant T315I mutant is to develop drugs that target signaling elements important in the pathogenesis of CML downstream from the *BCR-ABL1* T315I kinase, therefore bypassing the need to overcome the structural constraints posed by this mutant at the kinase domain of the enzyme.

HSP90 chaperone complex

The heat shock protein HSP90 is a chaperone protein involved in the proper folding and intracellular disposition of multiple proteins kinases found activated in patients with CML, such as p210^{*BCR-ABL1*}, mitogen-activated protein/extracellular signal-regulated kinase, and AKT. The benzoquinone ansamycin antibiotic 17-allylamino-17-demethoxygeldanamycin (17AAG) disrupts the function of HSP90, resulting in the depletion of multiple proteins via proteasome-mediated degradation (8, 17). AAG inhibited the proliferation of Ba/F3 cells expressing p210 *BCR-ABL1* T315I with values of 2.3 ± 0.4 (59), and has proved amenable to synergistic combinations with other active agents against this mutant such as LBH589 (60), suggesting a role for 17AAG or the more water-soluble analogue, DMAG (61), for the treatment of T315I-positive CML.

RAS/RAF pathway

RAS signaling is part of the *BCR-ABL1* kinase downstream signaling network and, therefore, a potential target in CML therapy. A combination of the farnesyl transferase inhibitor tipifarnib with imatinib showed significant clinical activity in

patients with imatinib-resistant CP CML, with hematologic and CCyRs of 62% and 36%, respectively, including a patient with the T315I mutation (62). A study combining dasatinib and the FTI BMS214662 (Fig. 1), highly active and equipotent against wild-type and T315I-expressing *BCR-ABL1* – positive CML stem cells, (63) is planned.

RAC GTPases

The GTPases RAC1, RAC2, and RAC3 are activated by *BCR-ABL1* in cells isolated from patients with CP CML (64, 65). In a murine model of p210^{*BCR-ABL1*} CML, targeting of RAC1 and RAC2 genes delayed remarkably the development of myeloproliferation, which was accompanied by abrogation of phosphorylation of the *BCR-ABL1* downstream signaling molecules CrKL, extracellular signal-regulated kinase, c-Jun-NH₂-kinase, and p38 (65), suggesting that *BCR-ABL1* signaling network is highly dependent on RAC GTPases (Fig. 2). Indeed, treatment with the specific RAC1/RAC2 inhibitor NSC237664 (Fig. 1) reduced remarkably the growth of primary bone marrow cells from patients with BP CML as well as Ba/F3 cells ectopically expressing *BCR-ABL1* T315I (65). Chronic therapy with NSC23766 was relatively nontoxic in mice, further supporting the clinical testing of this compound in patients with CML.

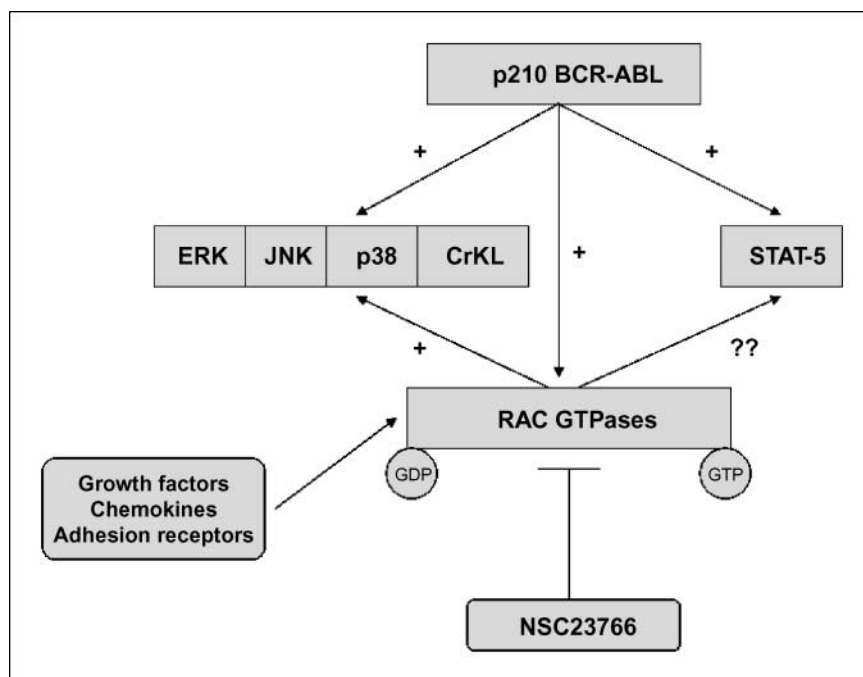
Protein phosphatase 2A

Protein phosphatase 2A (PP2A) is a serine/threonine phosphatase that acts as a tumor suppressor by antagonizing *BCR-ABL1* (66). In CML, *BCR-ABL1* kinase inhibits PP2A by posttranscriptional up-regulation of SET, a phosphoprotein that inhibits PP2A (66). Active PP2A activates protein tyrosine phosphatase 1, which catalyzes *BCR-ABL1* dephosphorylation, leading to *BCR-ABL1* down-regulation through proteosomal degradation (66). Therefore, SET inhibition or PP2A activation are potential strategies for the treatment of CML. Silencing of SET by siRNA or treatment of *BCR-ABL1* – positive cells with forskolin, a pharmacologic activator of PP2A, resulted in decreased expression of *BCR-ABL1* (66). FTY720 (fingolimod), another PP2A activator, has proved efficacious and safe as chronic immunosuppressive therapy in multiple sclerosis (Fig. 1; ref. 67) FTY720 (10 mg/kg/day) suppressed leukemogenesis in severe combined immunodeficient mice transplanted with myeloid or lymphoid progenitor cells transformed with p210^{*BCR-ABL1*} or p190^{*BCR-ABL1*}, respectively. More importantly, 80% and 90% of p210 and p190 mice, respectively, were in molecular remission after 11 weeks of therapy with FTY720, as well as 50% of mice transplanted with progenitors expressing the multiresistant T315I p210^{*BCR-ABL1*} mutant (68). These preclinical observations support the use of FTY720 for the treatment of patients with advanced phase CML as well as those expressing the T315I mutation.

Apoptotic pathways

Homoharringtonine is a cephalotaxine ester that exerts its activity against CML cells through inhibition of protein synthesis and disruption of the mitochondrial membrane potential with subsequent release of cytochrome *c* that results in caspase-9 and caspase-3 activation but not caspase-8 activation nor BID cleavage (69). Homoharringtonine-mediated inhibition of protein synthesis occurs rapidly and is associated with increased MCL-1 turnover (69). A semisynthetic formulation of homoharringtonine (omacetaxine mepesuccinate) was

Fig. 2. RAC GTPases represent novel targets in CML. The RAC GTPases RAC1, RAC2, and RAC3 are enzymes that integrate a variety of exogenous signals (e.g., growth factors, chemokines, adhesion receptors) to coordinate cellular responses. RAC GTPases are activated by BCR-ABL kinase along with other downstream signaling pathways that provide a proliferative advantage to CML cells. The knockdown of *RAC1* and *RAC2* remarkably attenuates the activation of multiple BCR-ABL downstream effectors (STAT5 phosphorylation is variably affected) and inhibits myeloproliferation in mice. NSC23766 inhibits specifically RAC and abrogates BCR-ABL – induced proliferation in a dose-dependent manner, further validating RAC GTPases as therapeutic targets in CML. *ERK*, extracellular-signal regulated kinase; *JNK*, c-Jun – activated kinase; *STAT5*, signal transducer and activator of transcription 5.



administered s.c. at 1.25 mg/m² s.c. twice daily for 14 days every month to 12 patients (2 in CP, 4 in AP, and 6 in BP), including 4 (33%) with *BCR-ABL1* mutations (T315I, F359V, Q252H, and F317L) while receiving imatinib (70). Five (42%) patients had a hematologic response (complete hematologic in 3 cases), including 1 who also achieved a minor CyR and 2 who had a CCyR (70). In an ongoing international trial for patients with CML carrying *BCR-ABL1* T315I (NCT00375219; CML-202), omacetaxine mepesuccinate is administered at 2.5 mg/m² s.c. daily for 14 days every 28 days for up to 6 cycles (71). During the remission-induction phase, patients receive omacetaxine mepesuccinate s.c. at 1.25 mg/m² twice daily for 14 consecutive days every 28 days until complete hematologic response or hematologic improvement. Patients can receive maintenance therapy with omacetaxine mepesuccinate s.c. at 1.25 mg/m² twice daily s.c. for 7 days every 28 days until progression or for up to 24 months. To date, 19 patients have been enrolled: 11 CP, 4 AP, and 4 BP. All patients had failed imatinib therapy, 10 had failed dasatinib, 8 nilotinib, and 3 the multikinase inhibitor MK-0457. Five patients (1 AP and 4 CP) had complete disappearance of T315I transcripts, and in 2 of them, this was associated with meaningful clinical responses (1 complete hematologic and 1 CCyR).

Imatinib-induced killing of *BCR-ABL1* –positive cells relies upon activation of the BCL-2–regulated apoptotic pathway. Imatinib activates several proapoptotic BH3-only proteins such as BIM and BAD, whose loss abrogates imatinib-mediated killing. Imatinib resistance associated with BCL-2 overexpression or loss of BIM could be overcome by cotreatment with ABT-737, a BH3 mimetic that inhibits the antiapoptotic proteins BCL-2, BCL-X_L, and BCL-w but not MCL-1 or A1 (72). The combination of ABT-737 with homoharringtonine dramatically enhanced the killing of CML cells by ABT-737, including those expressing T315I (73), supporting the use of ABT-737 in combinatorial chemotherapeutic approaches for CML.

The multikinase inhibitor sorafenib (BAY 43-9006) has been shown to potently induce apoptosis in Ba/F3 cells expressing T315I, and this activity was associated with rapid and pronounced down-regulation of MCL-1 and inhibition of STAT5 phosphorylation. This activity was independent from inhibition of the MEK1/2/ERK1/2 pathway and was associated with only very modest and delayed (>16 hours) inactivation of CrKL (74). A phase II study is evaluating the activity of sorafenib in patients with imatinib-resistant CP CML (NCT00085007).

Epigenetic Approaches against T315I

Histone deacetylases (HDAC) are enzymes that catalyze the deacetylation of the N termini of the core nucleosomal histone tails at evolutionarily conserved lysine residues, resulting in chromatin condensation and transcriptional repression (75). Treatment with HDAC inhibitors results in depletion of *BCR-ABL1*, induction of apoptosis, and sensitization to imatinib-induced apoptosis, likely through inhibition of HDAC-6 and acetylation of HSP90 that results in polyubiquitylation, proteasomal degradation, and depletion of HSP90-client proteins such as *BCR-ABL1*, c-RAF, and AKT (76). Treatment of primary CML cells expressing the T315I mutation with the combination of LBH589 and nilotinib resulted in synergistic decrements in phosphorylation of STAT5 and ERK1/2, increased levels of p27 and BIM, and apoptotic activity (77). Similar effects were observed with the combination of the pan-HDAC inhibitor suberoylanilide hydroxamic acid and dasatinib (78), providing the rationale for the testing of these combinations in patients with CML carrying the T315I mutation. Ongoing clinical studies of LBH589 in patients with refractory CML in all phases will also address the activity of single-agent LBH589 in patients with CML carrying *BCR-ABL1* T315I. Subtoxic concentrations of vorinostat (0.5-2 μmol/L) were synergistic with MK-0457 (5-100 nmol/L) against primary CD34+ CML cells and Ba/F3 cells expressing *BCR-ABL1* T315I,

while sparing normal bone marrow mononuclear cells (79). Vorinostat strikingly enhanced MK-0457-induced Aurora kinase inhibition, reflected by markedly diminished phosphorylation of histone H3 at residue Ser¹⁰.

Other HDAC inhibitors such as depsipeptide (FK228) and LAQ824 have also shown a preferential proapoptotic effect against cells expressing the T315I mutation *in vitro* and *ex vivo* and hold promise for the treatment of patients with *BCR-ABL1* T315I-positive CML (80, 81).

Concluding Remarks

Merely 5 years ago, hardly any treatment was available for patients with *BCR-ABL1* T315I-positive CML with the exception of allogeneic stem cell transplantation, a treatment modality only available to a selected fraction of patients. Albeit neither imatinib, nor any of the second generation TKIs is active against *BCR-ABL1* T315I, recent insights in the fields of structural and molecular biology have facilitated the development of a variety

of alternatives for the treatment of these patients. The challenge for the years ahead will be to develop the most clinically promising agents. In all likelihood, the development of effective therapies against resistant CML will encompass the combination of several of these agents. Moreover, the outcome of patients carrying highly TKI-resistant *BCR-ABL1* mutants will rely upon the timely detection of these mutants. Thus, the success of active agents against T315I will depend, at least in part, on the efficient application of periodic genotyping with sensitive techniques in the clinic. Finally, the activity of combination therapies containing agents with potent activity against T315I must be investigated to establish whether the emergence of resistance mediated by this and other highly resistant mutations can be prevented.

Disclosure of Potential Conflicts of Interest

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