Targeted therapy and the T315I mutation in Philadelphia-positive leukemias

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The deregulated tyrosine kinase activity of the Bcr–Abl chimeric protein, resulting from the t(9;22)(q34;q11) chromosomal translocation on the 22q- derivative – most commonly referred to as the Philadelphia (Ph) chromosome – is responsible for initiation and maintenance of 95% of chronic myeloid leukemias (CML) and 30% of acute lymphoblastic leukemias (ALL) and represents an extremely attractive target for therapeutic intervention. In recent years this has fostered intensive efforts in screening for compounds capable of interrupting Bcr-Abl oncogenic signaling. Imatinib mesylate, an orally available inhibitor of Abl, c-kit, PDGFR and c-fms kinases, is the first example of targeted therapy of a human malignancy to enter clinical practice. The remarkably successful results of clinical trials with imatinib have rapidly revolutionized CML treatment paradigms establishing imatinib as the first-line therapy for all newly diagnosed patients. However, the leukemic clone may evolve to evade Bcr-Abl inhibition. Amplification of the *BCR-ABL* gene may be observed in about 20% of patients who relapse on imatinib therapy.[1,2] More frequently, point mutations in the Abl kinase domain (KD) are selected, which disrupt imatinib binding by affecting critical residues implicated in direct contact with the inhibitor or preventing Bcr-Abl from effectively adopting the specific inactive conformation to which imatinib binds. Early investigations in advanced phase CML patients who had relapsed on imatinib therapy first indicated a mutation at residue 315 (T315I) as the main determinant of Bcr-Abl reactivation within the leukemic clone.[1] The substitution of threonine with a bulkier and more hydrophobic isoleucine was shown to eliminate a crucial hydrogen bond required for high-affinity inhibitor binding and to create steric hindrance interfering with imatinib placement within the ATP-binding pocket.[1,3] A strikingly identical amino acid substitution was later shown to occur at homologous positions in the KD of c-kit (T670I) and PDGFRα (T674I) kinases in imatinib-resistant gastrointestinal stromal tumors and hypereosinophilic syndromes, respectively,[4,5] further highlighting the central role of this highly-conserved *gatekeeper* threonine in controlling the accessibility of the ATP-binding pocket to inhibitors.

The vast number of mutation reports since published has led to an exponential increase in the number and type of amino acid substitutions found in CML patients who either lost or did not achieve response to imatinib treatment has been reported by Baccarani *et al*.[6] The majority of these Bcr-Abl mutant forms are now well characterized in terms of the extent to which they confer insensitivity to imatinib.[7] Indeed, while some mutations (T315I and mutations falling within the P-loop region, i.e., G250E, Y253F/H and E255K/V) confer a highly resistant phenotype, other are associated with a relatively modest increase in resistance to imatinib which might, therefore, be overcome by a dose increase. However, T315I currently remains the most troublesome mutant. Some authors have suggested that T315I is associated with a highly aggressive disease phenotype and poor outcome if no timely therapeutic reassessment is made.[8,9] The two second-generation inhibitors that have just received, or are awaiting, FDA approval for the treatment of resistant/intolerant CML and Ph chromosome positive (Ph$^+$) ALL patients, dasatinib and nilotinib, respectively, have proven successful in most imatinib-resistant cases – whether resistance is driven by Abl KD mutations or by other mechanisms – with the notable exception of T315I-positive cases.[10–13] In addition, *de novo* emergence of T315I seems to be by far the most frequently observed resistance mechanism in Ph$^+$ patients who relapse after an initial hematologic and even cytogenetic response to dasatinib.[14] A number of other ATP-competitive inhibitors with increased potency and less stringent conformation requirements in Bcr-Abl binding have proved active against several mutant forms (even P-loop mutants) but have failed to show any significant activity against T315I-Bcr-Abl when tested *in vitro (*as reviewed by Martinelli *et al.).*[15] This has earned the T315I mutant the frequent appellation of the *Achilles' heel* of tyrosine kinase inhibitors.

Must the T315I be regarded as the defeat of targeted therapy in CML? No, it must not. In the three largest retrospective studies investigating the frequency of Abl KD mutations in imatinib-resistant patients[8,9,16] the incidence of T315I was 4%, 11% and 19%, respectively, with differences depending on the composition of the patient populations under study in terms of disease phase and type of resistance (for comparison, the frequency of P-loop mutations in the same series of patients were reportedly much higher, being 28%, 46% and 39% respectively).[8,9,16] Of note, the T315I mutation was clearly more frequently found in advanced-phase CML and in Ph$^+$ ALL patients.[8,9] Nowadays, however, the vast majority of CML cases are represented by patients in early chronic phase who receive imatinib as first-line treatment. The most recent update of the IRIS trial[17] showed that at 60 months, 98% of newly diagnosed CML patients treated with imatinib in early chronic phase achieved a complete hematologic remission

(CHR), 92% a major cytogenetic remission (MCR; <35% Ph+ metaphases) and 87% a complete cytogenetic remission (CCR; no Ph+ metaphases) – thus primary resistance is rare. Further, after 60 months, 2.5% of patients had lost CHR, 5.1% had lost MCR, and 6.3% had progressed to accelerated phase or blast crisis[17] – thus acquired resistance is, also, rare. In the case of resistance, only 14-24% of patients in early chronic phase may be predicted to have a detectable mutation[9,16] and it is likely that in very few cases the mutation will turn out to be a T315I.[9] Therefore, the number of T315I-positive CML patients is likely to decrease progressively over time. Much more problematic is the setting of Ph+ ALL patients, in whom responses to tyrosine kinase inhibitor therapy are generally short-lived, mutations account for resistance in more than 80% of patients,[9] most probably because of the high genomic instability of ALL cells, and T315I is quite commonly detected at the time of relapse.[9,14]

For T315I-positive patients, however, effective therapeutic approaches might soon be available. To expedite the identification and the availability of second-line strategies overcoming resistance induced by the T315I mutation, three approaches are being successfully pursued. The first is to design inhibitors binding regions of Bcr-Abl other than the ATP binding pocket. This is the case of ON012380, a substrate-competitive inhibitor which exhibited activity at low nanomolar concentrations against wild-type Bcr-Abl and all imatinib-resistant Bcr–Abl mutants, including T315I, both in biochemical and in cellular assays.[18] Unfortunately, ON012380 has not yet entered clinical trials in which it must prove its safety in use as well as its *in vivo* efficacy in achieving remission and preventing resistance. A second approach is to test targeted agents with a different mode of action, i.e., molecules that target the stability of Bcr-Abl or a Bcr-Abl downstream signal transducer, alone or in combination with imatinib (or another Bcr-Abl kinase inhibitor). Very recently, the histone-deacetylase inhibitor LBH589 has been documented to deplete Bcr-Abl and induce growth arrest and apoptosis in cells expressing T315I-Bcr-Abl, both when administered alone and, even more effectively, when administered in combination with nilotinib.[19] The safety and tolerability of the orally-available LBH589B are being assessed in a phase I trial in patients with advanced solid tumors and cutaneous T-cell lymphomas,[20] and phase II trials in CML are being planned. Similar encouraging results have been obtained with another histone-deacetylase inhibitor, vorinostat (suberoylanilide hydroxamic acid, SAHA), alone or in combination with dasatinib.[21] Combinations of vorinostat with various conventional agents are currently being evaluated in several malignant conditions (*http://www.clinicaltrials.gov*). By inhibiting histone deacetylase 6 and inducing acetylation of heat shock protein 90 (hsp90), LBH589 and vorinostat have been shown to attenuate the ATP-binding and chaperone function of hsp90.[22] This leads to polyubiquitylation, proteasomal degradation and depletion of hsp90-client proteins, including Bcr-Abl itself and its downstream effectors c-Raf and AKT. Moreover, vorinostat and LBH589 are known to induce apoptosis in human leukemia cells via a general mechanism of accumulation of pro-apoptotic proteins (Bax, Bim) coupled with depletion of anti-apoptotic factors (Bcl-2, Bcl-XL, survivin), which may enhance the effects of Bcr-Abl inhibitors.[23]

A third, intriguing approach is to explore the possibility of whether molecules that have been developed as inhibitors for other protein kinases and are already undergoing clinical trials, might include the T315I-Bcr-Abl among their *off-targets*. One such screening has recently revealed that the p38 inhibitor BIRB-796 and the aurora kinase inhibitor MK-0457 (VX-680) are both capable of binding T315I-Bcr-Abl.[24] BIRB-796, currently being evaluated in clinical trials for inflammatory bowel disease, binds T315I-Bcr-Abl with good affinity (K_d=40 nM), but has significantly weaker affinity for wild-type and other imatinib-resistant forms of Abl (K_d values >1 μM). MK-0457 (VX-680) is able to bind both wild-type and mutated Bcr-Abl and has been reported to inhibit T315I-Bcr-Abl in primary patient cells at low micromolar concentrations.[25] Additionally, recent co-crystal studies have shown that this Y-shaped molecule engages the Abl kinase domain in such a way that a close encounter with the gatekeeper residue is avoided, explaining why the compound is able to accommodate the substitution of threonine with isoleucine without any significant decrease in binding affinity.[25] MK-0457 is currently undergoing a phase I trial in leukemias, including advanced phase CML and Ph+ ALL, and encouraging responses in patients harboring the T315I mutation have been reported.[26] A phase II trial in the specific setting of T315I-positive Ph+ leukemias is forthcoming.

Imatinib is nowadays the first-choice treatment of CML.[6] It is remarkably effective, and as more and more patients in early chronic phase will receive it first-line, resistance is likely to become a phenomenon confined to progressively smaller subsets of patients. On the other hand, the search for alternative therapeutic strategies is making tremendous progress. More potent Bcr-Abl inhibitors administered first-line might prove valuable in drying up the pool of residual leukemic cells from which mutant clones may emerge. Clinical trials are being planned in order to evaluate these premises. In addition, combinations of inhibitors with non-overlapping spectra of resistance mutations or, even better, with non-overlapping modes of action, have been predicted to be particularly promising[27] and their assessment in clinical trials is also warranted. These strategies could be particularly suitable for the treatment of Ph+ ALL, in which marked genomic instability rapidly drives the emergence and selection of T315I and other highly resistant Abl KD

mutations. Although it is nowadays emphasized as a clinical emergency, the problem of resistance driven by the T315I mutant is likely to be resolved soon.

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