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Brief Communication

Strategies to circumvent the T315I gatekeeper mutation in the Bcr-Abl tyrosine kinase

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ABSTRACT

Despite the remarkable success of imatinib against Bcr-Abl, development of secondary resistance, most often due to point mutations in the Bcr-Abl tyrosine kinase (TK) domain, is quite common. Of these, the T315I "gatekeeper" mutation is resistant to all currently registered Bcr-Abl TK inhibitors (TKIs) with the notable exception of ponatinib (Iclusig™), which was very recently approved by the United States Food and Drug Administration (FDA). Besides ponatinib, numerous strategies have been developed to circumvent this problem. These include the protein synthesis inhibitor omacetaxine (Synribo®), and "switch-control" inhibitors. Dual Bcr-Abl and aurora kinase inhibitors represent another promising strategy. Finally, several promising synergistic combinations, such as TKIs with histone deacetylase inhibitors (HDACIs), warrant attention.

Introduction

The Bcr-Abl fusion protein, central to the pathogenesis of chronic myelogenous leukemia (CML), interacts with a variety of effector proteins, leading to deregulated cellular proliferation, decreased adherence of leukemia cells to the bone marrow stroma, and a reduced apoptotic response to mutagenic stimuli.[1] The revolutionary results of the International Randomized Study of Interferon and STI571 (IRIS), a phase III randomized trial of imatinib compared with the combination of interferon alfa and cytarabine in newly diagnosed patients with chronic phase CML (CML-CP), dramatically transformed the therapeutic landscape of CML. The second generation Bcr-Abl TKIs dasatinib and nilotinib were initially approved for CML patients intolerant of or resistant to imatinib, and subsequently for previously untreated patients with CML-CP. Recently, another second generation Bcr-Abl TKI, bosutinib (Bosulif®), has been approved for the treatment of patients with all phases of CML who have demonstrated resistance or intolerance to prior therapy.

While resistance to imatinib is rare in patients treated in CML-CP, resistance does eventually develop in the majority of patients treated in the advanced phases.[1] In a high proportion of patients who develop secondary resistance after initial responses, point mutations in the *BCR-ABL* kinase domain have been identified, often associated with reactivation of the dys-regulated enzymatic activity of the Bcr-Abl protein. The first such mutation identified was a C→T nucleotide change that results in a threonine to isoleucine substitution at position 315 in the Abl component of Bcr-Abl (Thr^{315}→Ile; T315I).[2] Thr^{315} forms a critical hydrogen bond with imatinib. The absence of the oxygen atom normally provided by the side chain of Thr^{315} precludes formation of a hydrogen bond with the secondary amino group of imatinib, but does not interfere with adenosine triphosphate (ATP) binding.[2] Although a large number of such mutations have now been identified, the most important one still remains T315I, referred to as the "gatekeeper" mutation, because it uniformly confers resistance to all first and second generation Bcr-Abl TKIs. Allogeneic hematopoietic stem cell transplantation (HSCT), effective in a proportion of cases by virtue of a strong graft-versus-leukemia (GVL) effect has, therefore, been the only recourse for these patients.

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Ponatinib

Ponatinib (AP24534, Iclusig™) is a multi-targeted TKI optimized using structure-based drug design to bind to the inactive conformation of Abl and Abl^{T315I}. It does not form a hydrogen bond with the side chain of Thr^{315} in native Abl. The key structural feature of the molecule is a carbon-carbon triple bond linkage that makes productive hydrophobic contact with the side chain of Ile^{315}, allowing inhibition of the T315I mutant.[3] In the ongoing pivotal phase II PACE trial[4] in patients with refractory CML (all phases) and Philadelphia chromosome-positive acute lymphoblastic leukemia (Ph$^+$ ALL) who were resistant or intolerant to dasatinib or nilotinib, or carried the T315I mutation, it showed substantial activity in all patients, although activity appeared particularly pronounced in CP patients with T315I. In this group, at a median follow-up of 12 months, rates of CCyR and major molecular response (MMR) were 67% and 56%, respectively.[4] In patients with accelerated and blastic phase CML and Ph$^+$ ALL bearing the T315I mutation, respectively, CCyR rates were 22%, 16% and 50%.[5] Response rates continued to improve with longer follow-up. Based on the findings of this trial, the US FDA granted accelerated approval to ponatinib for the treatment of adults with CML (all phases) or Ph$^+$ ALL who are resistant or intolerant to prior TKI therapy on December 14, 2012.

Although unquestionably a major breakthrough in Bcr-Abl TKI therapy, there have been concerns regarding the ability of ponatinib, except at high concentrations, to inhibit compound mutants involving T315I, such as Y253H/T315I or E255V/T315I that sometimes pose a challenge, particularly in later lines of therapy,[3] and that it might actually be relatively less potent against native Bcr-Abl. To answer the latter question, a phase III trial comparing ponatinib with imatinib as initial therapy for newly diagnosed patients with CML-CP (NCT01650805) is currently enrolling patients.

Homoharringtonine/omacetaxine

Homoharringtonine is a natural alkaloid obtained from various *Cephalotaxus* species that exerts antitumor activity through inhibition of protein synthesis and promotion of apoptosis.[6] Among other actions, homoharringtonine down-regulates short-lived anti-apoptotic proteins such as myeloid cell leukemia-1 (Mcl-1) through translational inhibition. Because of its unique mechanism of action, the activity of this agent against CML cells is independent of their *BCR-ABL* mutational status. Omacetaxine mepesuccinate (Synribo®) is a semisynthetic form of homoharringtonine that has excellent bioavailability upon subcutaneous administration. Although known for over three decades, the clinical development of these compounds has been impeded by the success of the Bcr-Abl TKIs. In a very recently reported large phase II trial[7] of omacetaxine in CML patients with T315I and TKI failure, 48 of 62 (77%) patients in CP achieved complete hematologic response (CHR), and 10 (16%) achieved CCyR. Myelosuppression, typically manageable by dose reduction, was the major toxicity. On October 26, 2012, omacetaxine mepesuccinate received accelerated approval from the FDA for the treatment of adults with chronic and accelerated phase CML with resistance and/or intolerance to two or more TKIs.

Conformational control inhibition

Conformational escape resistance is a phenomenon where point mutations (such as T315I) drive the Abl kinase toward the active type I state. "Switch-control inhibitors" bind in a non-ATP-competitive fashion to residues (E282, R386) the Abl protein uses to switch between inactive and active conformations.[8] The lead clinical candidate, DCC-2036 (rebastinib), an orally active TKI, potently inhibits Abl by inducing and stabilizing an inactive, inhibitor-bound (type II) conformation, and retains efficacy against most clinically relevant CML-resistance mutants, including T315I, although "P loop" mutants (E255V, E255K) may be less sensitive. DCC-2036 inhibits Bcr-Abl^{T315I}-expressing cell lines, prolongs survival in mouse models of T315I-mutant CML and Ph$^+$ ALL, and inhibits primary patient-derived leukemia cells expressing T315I both in vitro and in vivo. Sustained inhibition of Bcr-Abl and downstream pathways was observed in patients with refractory CML enrolled on a phase I clinical trial of this agent. In a Ba/F3 cell-based mutagenesis screen, no *BCR-ABL* mutations emerged at higher concentrations of DCC-2036 that are readily achievable clinically.[8] However, rebastinib is currently being developed as an inhibitor of the TRKA, TIE-2 and FLT3 kinases with a clinical focus on various solid tumors and refractory acute myelogenous leukemia.

Dual aurora/BCR-ABL kinase inhibitors

The aurora kinases, which are overexpressed in many cancer types including leukemia, are serine-threonine kinases that regulate different steps during mitosis, including the G_2-M transition, mitotic spindle organization, chromosome segregation, and cytokinesis. MK-0457 (VX-680) and KW-2449 potently inhibit both aurora and Bcr-Abl (including Bcr-Abl^{T315I}) kinases, but neither compound is any longer in clinical development. Other molecules in this category include XL228, PHA-739358, VE-465 and AT9283. AT9283 shows potent anti-proliferative activity on *BCR-ABL*$^+$ cell lines and primary patient samples, both wild type and T315I, as well as in mouse xenograft models.[9]

Combination strategies

HDACIs potently enhance the lethality of the dual aurora/Bcr-Abl TKIs by interfering with the function of chaperone proteins such as heat shock protein 90 (hsp 90), of which Bcr-Abl is a client, and interruption of the mitotic spindle checkpoint (induction of "mitotic slippage"). Indeed, synergistic interactions have been demonstrated between MK-0457 (VX-680) and vorinostat,[10] as well as between KW-2449 and entinostat,[11] both in vitro and in vivo, in human *BCR-ABL*$^+$ cells, including those resistant to imatinib and bearing the T315I and E255K mutations. Accordingly, a phase I clinical trial of AT9283 (which also inhibits fms-like tyrosine kinase 3 (FLT3) and Janus kinases) and entinostat in patients with relapsed/refractory and poor-risk acute leukemias will soon begin enrolling patients.

Conclusion

In this very brief review, an attempt has been made to highlight some of the most promising strategies in development against the recalcitrant T315I "gatekeeper" mutation in *BCR-ABL*. Examples of other agents being studied include inhibitors of hsp 90 and RAC GTPases, protein phosphatase 2A activators, TKI/farnesyltransferase inhibitor combinations, the novel TKIs ON012380 (substrate-competitive) and HG-7-85-01 (ATP-competitive), and nilotinib combined with the allosteric inhibitor GNF-2. Although allogeneic transplantation had thus far been the only available therapeutic option for patients with the T315I mutation, this has finally begun to change.

Authors' contributions

P. B. conducted literature searches and drafted the article. J. A. and H. P. conducted literature searches. S. G. revised the article critically for important intellectual content.

Conflict of interest

None of the authors have any relevant conflicts of interest to disclose.

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