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New drugs in the treatment of chronic myeloid leukaemia

Novas drogas no tratamento da leucemia mielóide crônica

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*The introduction of the BCR-ABL kinase inhibitor, imatinib mesylate (Gleevec®, Novartis) led to significant changes in the treatment of chronic myeloid leukaemia (CML) patients. However, despite the impressive percentage of responding patients, some CML cases, particularly those in advanced phases of the disease, show primary resistance or relapse after the initial response. The second-generation BCR-ABL inhibitors nilotinib (Tasigna®, Novartis) and dasatinib (Sprycel®, Bristol-Myers Squibb) have shown significant activity in clinical trials in patients who failed imatinib therapy, but these agents are still incapable of inhibiting the T315I mutant of Bcr-Abl and present partial activity in advanced phases of CML. The acquired biological notions of the mechanisms of tyrosine kinase inhibitor (TKI) resistance has led to the development of new compounds, some of which have shown encouraging preliminary results in clinical trials, even against T315I mutants. In this paper we discuss the new emerging therapies which may overcome TKI resistance in CML patients. Rev. bras. hematol. hemoter. 2008; **30**(Supl. 2):24-29.*

Key words: *Chronic myeloid leukaemia; BCR-ABL kinase inhibitors; TKI resistance.*

The first BCR-ABL inhibitor to come into the clinical practice, imatinib mesylate, is now the first-choice of treatment for all newly diagnosed CML patients. This drug was tested in phase I and II clinical trials and soon moved to a phase III randomised trial (International Randomised Study of Interferon versus ST1571 [IRIS]) in which the drug was compared with interferon alfa plus cytosine arabinoside (IFNα plus ARA-C).[1] The treatment provides an impressive rate of complete haematological responses (CHR) and complete cytogenetic remissions (CCgR), assessed at 95% and 94%, respectively for imatinib, compared with 55% and 8.5% for IFNα - ARA-C. Progression free survival at 18 months was 96.7% for imatinib compared with 91.5% for IFNα ARA-C . The data coming from the IRIS trial have been recently updated[2] and show that the cumulative incidences of CHR and CCgR at 5 years are 98% and 87%, respectively, for imatinib. For those patients achieving a CCgR at 18 months or for patients obtaining a major molecular response (MMR) (three-log reduction in leukemic cells), the 5-year progression-free survival is 97% and 99%, respectively. Despite the impressive percentage of responding patients, some CML cases, particularly in the more advanced phases of the disease, show primary resistance or relapse after an initial response.[1,2] The most common mechanisms of resistance include: (i) BCR-ABL kinase domain mutations; (ii) BCR-ABL overexpression; (iii) clonal evolution with activation of additional transformation pathways.[3,4]

One of the most studied mechanism of resistance to imatinib therapy is the development of point mutations within the kinase domain of *BCR-ABL*. The frequency of *BCR-ABL* mutations in imatinib resistant patients ranges from 40%-90% depending on the CML phase and on the methodology for the detection.[5-6]

Depending on the region where they are located, mutations can actually act by interrupting critical contact points between the drug and BCR/ABL protein or by inducing a conformational change to which imatinib is unable to bind. At present, approximately 90 different BCR/ABL mutations have been identified in patients with Imatinib-resistant CML through random mutagenesis in vitro. Many

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of these are relatively rare, whereas the most common, which account for 60%-70% of all the mutations, affect residues Gly250, Tyr253, Glu255, Thr315, Met351 and Phe359.[7] Mutations also differ from each other for the kind of resistance they can determine: some mutant clones are completely resistant (Y253F/H, E255K, T315I), others only partially (M244V, F317L, Met351T), depending on whether the bound with the drug following the amino acid substitutions is prevented or only hindered. In this latter case, the sensibility can be restored by simply increasing the imatinib dose. The mutations with a greater level of resistance fall inside the ATP binding site of the KD domain, an highly conserved region responsible for phosphate binding and known as phosphate-binding loop (P-loop) (a.a. 248-256, motif LGGGQYGEV). Initially these mutations have been also associated with an especially poor prognosis[6,8] but this still remains, at the moment, a matter of debate.

Several approaches to overcome resistance have been proposed including the development of more powerful tyrosine kinase inhibitors (TKIs) which are under investigation in clinical or pre-clinical studies on patients with imatinib-resistant or intolerant CML, at any stage of the disease. Classes of these new inhibitors include selective ABL inhibitors (nilotinib), inhibitors of both ABL and Src-family kinases dasatinib, bosutinib, INNO-404, AZD0530), Aurora Kinase inhibitors (MK-0457, PHA-739358), and non-ATP competitive inhibitors of BCR/ABL (ON012380) (Table 1). All these drugs have proved effective in pre-clinical studies against the mutant enzymes, and significal clinical responses in clinical trials have been reported for many of these.[9,10]

Dasatinib (Sprycel®, Bristol-Myers Squibb) has recently been approved by the FDA and by EMEA for the treatment of adults with CP, AP, or myeloid or lymphoid BP CML with resistance or intolerance to prior therapy including imatinib. Dasatinib is also indicated for the treatment of adults with resistant Ph+ positive acute lymphoblastic leukaemia.

Dasatinib is a multikinase inhibitor with potent activity against BCR-ABL kinase (IC50 <1 nM) and Src family kinases (IC50 of 0.2-1.1 nM). Dasatinib inhibits all imatinib resistant kinase domain mutations tested, with the exception of T315I.[11]

This compound is able to bind ABL with greater affinity as compared to imatinib. This is, at least in part, due to the ability of recognizing multiple ABL configurations. This feature provides a potential clinical advantage over imatinib and nilotinib.[12] As a result, the number of *BCR-ABL* mutants that confer resistance to dasatinib is limited almost exclusively to those directly affecting the contact sites.[13]

A potential limitation of these new drugs is the increased degree of toxicity which may be caused by an increased potency but it may be also related to their inhibitory activity against a broader range of protein kinases. For instance, dasatinib acts potently on many members of the Scr kinase family and also on KIT, PDGFR and ephrin receptor (EPHA2) tyrosine kinases, which are directly implicated in many biological processes. These effects may provide the physiological explanation for some of the toxicities observed such as pleural effusion and myelosuppression. However the inhibition of Src kinases may also be crucial and of clinical benefit for the cure of imatinib-resistant CML and Ph-positive B- ALL. In these settings the pathogenetic role of many members of the Src family has been demonstrated, such as the overexpression of Lyn.[14,15] The Src kinases family comprises nine non-receptor tyrosine kinases (SRC, FYN, YES, BLK, YRK, FGR, HCK, LCK, LYN). 16 Some Src members are ubiquitously expressed, whereas others display tissue-specific expression patterns. [16]

Published data support the evidence that Src kinases are involved in the proliferation of *BCR-ABL*-expressing cell lines.[17] The activation of many Src kinases such as LYN and HCK seems to be dependant from the interaction with BCR-ABL, but not from its kinase activity, as demonstrated by the presence of multiple interactions between the BCR-ABL domains and HCK and LYN, finally resulting into their activation.[18,19] This activation is not influenced by the effective block of BCR-ABL kinase activity.

Importantly, the kinases LCK and FYN are linked with the co-receptors CD4 and CD8 and have a crucial role in mediating T-cell-receptor signal transduction in clonal lymphocytes.[20]

Finally, the role of Src kinases in the pathogenesis of chronic phase CML remains to be fully understood.

Table 1. New tyrosine kinase inhibitors (TKI

Compound	Company	Targets	Clinical phase
Dasatinib (BMS-354825)	Bristol-Meyers	ABL, KIT, PDGFR, EPHB4,SRC and SRC family kinases	Approved
Nilotinib (AMN 107)	Novartis	ABL, KIT, PDGFR, EPHB4	II
Bosutinib (SKI-606)	Wyeth	ABL, FGR, LYN, SRC	II
AZD0530	Astra Zeneca	ABL, SRC	II(solid tumors)
MK-0457(VX-680)	Merck	ABL (T315I included), Aurora kinases, FLT3,JAK2,	II
PHA-739358	Nerviano	Aurora A,B and C	II
INNO-406 (NS-187)	Innovive	ABL, LYN, PDGFR,KIT	I
AP23464	Ariad	ABL, SRC	Pre-clinical
CGP76030	Pfizer	SRC	Pre-clinical

Transduction of *BCR-ABL* into bone marrow from mice lacking HCK, LYN and FGR can efficiently induce a CML-like myeloproliferative disorder.[21] In addition, mice with CML-like disease responded to imatinib but not to the treatment with selective inhibitors of Src kinases. However, HCK, LYN and FGR are required for *BCR-ABL*-induced B ALL.

Dasatinib may target an earlier progenitor population than imatinib in CML patient isolates, although the most primitive quiescent cells may be inherently resistant to both drugs.[22] Results from the START programs demonstrated that treatment with dasatinib at a dose of 70 mg twice daily resulted in hematologic, cytogenetic and molecular responses across all phases of CML in both imatinib-intolerant and imatinib-resistant patients, including those harbouring Bcr-Abl mutations with the exception of the T315I.[9]

Nilotinib (Tasigna®, Novartis) is a phenylamino-pyrimidine derivative developed by the reconciliation of the crystal structures of imatinib[23] with increased affinity for the inactive conformation of wild-type BCR-ABL by 20-fold to 30-fold, while similar activity against KIT, PDGFR is maintained. Results from the pivotal phase II studies of nilotinib for patients with CML after failure or intolerance to imatinib therapy indicate that nilotinib has a favorable toxicity profile and is highly efficacious in this setting.[10]

Similarly to imatinib, nilotinib binds Bcr-Abl only in its inactive conformation.[24] Nilotinib inhibits almost all the Bcr-Abl mutants, but, like dasatinib, it is unable to inhibit T315I mutant. Nilotinib at the dose of 400 mg twice a day has been examined in patients with CML resistant or intolerant to imatinib in all phases of disease where it proves able to induce hematologic, cytogenetic and molecular responses. Nilotinib treatment is well tolerated and associated with a very favorable toxic profile which includes myelosuppression, skin rashes, nausea and peripheral oedema represented the most frequent side effects.[24]

Many other Bcr-Abl inhibitors are currently in early clinical development and bosutinib (SKI-606) and INNO-406 (NS-187) have reached Phase I-II trials.[25,26]

Bosutinib, (SKI-606, Whyet) is an orally available 4 anilino 3-quinolinecarbonitrile derivative.[25] It is classified as a dual Src/Abl kinase inhibitor which inhibits Bcr-Abl with a 200-fold increased potency as compared to imatinib and it is active against imatinib-resistant Bcr-Abl mutant proteins and against three different *BCR-ABL*-positive cell lines, including LAMA84R, in which resistance is caused by *BCR-ABL* gene amplification, and K562R and KCL22R in which the mechanism of resistance has not yet been defined. [27]

Unlike imatinib and dasatinib, SKI-606 exhibits no significant inhibition of c-kit or PDGFR thus resulting in a safer toxicity profile *in vivo*.[27] This differential selectivity may result in clinical benefit by decreasing some adverse events, in particular those due to fluid retention. In a phase I study, patients in chronic phase with imatinib relapsed or refractory disease were shown to achieve a consistent percentage of complete hematologic and cytogenetic responses with encouraging evidence of clinical activity in imatinib-resistant patients with BCR-ABL mutations.[27,28] Bosutinib was given as a single dose at 400 mg, 500 mg, or 600 mg daily. Side effects were minimal with the most frequent being mild to moderate diarrhoea (87%), nausea (33%) and vomiting (20%).[28] In contrast to dasatinib, no pleural effusion or pulmonary oedema was observed with bosutinib. The dose-limiting toxicity of bosutinib occurred at 600 mg daily. A dose of 500 mg daily was selected as the dose for the Phase II study, which is currently recruiting patients in all phases of CML and *BCR-ABL*-positive B ALL. [28]

The superior spectrum of activity of bosutinib with respect to imatinib may be attributed to its ability to bind both inactive and intermediate conformations of BCR-ABL.[28] Importantly, bosutinib is significantly more potent than imatinib in inhibiting BCR-ABL tyrosine kinase in both primitive and committed CD34+CD38- progenitors from untreated CML patients.

On the basis of these results, bosutinib is currently being tested in patients with CML who have become resistant to imatinib or to the second-generation TKIs nilotinib and dasatinib.

Quite recently a novel BCR-ABL/LYN dual inhibitor INNO-406 (NS-187) which apparently shows unique profile to overcome imatinib resistance has been introduced in clinical studies. INNO-406 is a is a 3-substituted benzamide derivative, is a dual-specificity ABL and LYN kinase inhibitor that is 25-55-times more potent than imatinib against ABL.[29] In addition, INNO-406 inhibited the *in vitro* growth of cells with different mutant forms of BCR-ABL oncoproteins,[30] but not with T315I.[29,30] INNO-406 inhibited kinases other than ABL including ABL-related gene *ARG* and FYN, but not PDGFRα/β, SRC, BLK or YES. Furthermore, INNO-406 potently inhibited LYN kinase (IC50 of 19 nM), which has been implicated in BCR-ABL independent resistance,[13] without affecting the phosphorylation of Src, Blk or Yes, therefore presenting a further potential in imatinib-resistant CML.[26] In addition, the ability of NS-187 to specifically target the Bcr-Abl and Lyn kinases may result in a better side effect profile than agents that target multiple kinases such as Src/Abl inhibitors.[29]

Approximately 6% of adult patients in lymphoid blast phase CML have evidence of central nervous system (CNS) involvement. The concentration of INNO-406 in CNS is about 10% of that detected in the plasma.[30] Due to the low concentrations reached by nilotinib or dasatinib in the CNS at the dose administered in clinical practice, INNO 406 represents a promising alternative for the treatment of CNS *BCR-ABL1*-positive leukaemia.

None of the described agents however are capable of inhibiting the T315I mutant of Bcr-Abl. MK-0457 (Merck Sharp and Dome, previously VX-680) is a small-molecule which inhibits the aurora kinases A, B, and C, and wild type and

mutant BCR-ABL, including the T315I variant.[31] The human Aurora kinases (AURKs), AURKA, AURKB and AURKC, are essential for proliferation and for the correct progression through the mitotic phase of the cell cycle.[32] Aurora kinase A and B are overexpressed or gene amplified in several human malignancies. In enzyme activity assays, MK 0457 inhibited wild-type ABL and the ABL T315I mutant isoform, with IC50 of 10 nM and 30 nM, respectively.[33] Furthermore, MK 0457 inhibited the viability of Ba/F3 cells transformed by wild-type, Y253F or T315I mutants of BCR-ABL (IC50 ~300 nM).[34] A relevant difference, when compared to imatinib, in terms of mechanism of action is that when penetrates deeply into the ABL kinase domain, MK 0457 anchors itself firmly at the hinge region and engages Asp381 at a much more superficial level within the kinase domain.[31] This allows MK 0457 to overcome the potential steric constraints imposed by the mutant ABL T315I kinase. In a recent Phase I study, eleven patients with *BCR-ABL* T315I-positive refractory CML have been treated with MK-0457. One patients obtained a major haematological response, four minor haematological responses and four cytogenetic responses.[35] Remarkably, no drug-related non-hematological toxicity was observed. Some patients showed an apparent myelosuppression, which is an expected mechanism-based side effect of Aurora kinase inhibition. MK-0457 was very well tolerated. [36]

MK-0457 is therefore the first T315I BCR-ABL inhibitor to show activity against this highly refractory, poor prognostic subpopulation of CML.

All TKIs currently used for the treatment of CML compete with ATP for the binding site of the BCR-ABL oncoprotein. An alternative strategy to target ABL kinase involves the development of small molecules targeting BCR-ABL motifs that are remote from the kinase domain. Such compounds may potentially be unaffected by mutations of the kinase domain that make *BCR-ABL*-positive leukaemic cells resistant to imatinib.

GNF 2 was identified as a highly selective inhibitor of proliferation in BCR-ABL positive cells which is active against most clinically relevant imatinib-resistant BCR-ABL mutants. GNF 2 is the first compound which inhibits BCR-ABL kinase by binding to the autoregulatory myristate binding cleft of BCR-ABL located at the *N* terminus, spatially distant from the active site of ABL kinase, which results in stabilization of the protein in an inactive state.[37] This mode of action probably contributes to the synergistic effect between GNF 2 and imatinib. GNF 2 does not compete with any ABL substrate, and inhibits the proliferation of *BCR-ABL*-expressing cells in a selective, non-ATP-competitive manner with an IC50 of 138 nM, but proved inactive against the catalytic domain of ABL kinase.[38] The selectivity of GNF 2 was further supported by its lack of activity against other kinases such as FLT3, KIT, PDGFR, LCK and SRC.

GNF 2 is the leading compound of a new class of putative allosteric ABL kinase inhibitors. Although the mechanism of action of GNF 2 is not yet fully characterized, its selectivity for BCR-ABL suggests that this agent may not present off-target effects and significant toxicity. Finally, LBH589 (Novartis) is a novel histone deacetylase inhibitor that inhibits proliferation and induces apoptosis in tumor cell lines, including cell lines harbouring the BCR-ABL mutant with T315I. LBH589 induces cell death through an increase in the mitochondrial outer membrane permeability and favous apoptosome formation by inducing cytochrome c release. In addition, LBH589 stimulates a caspase-independent pathway through the release of AIF from the mitochondria, down-regulating Bcl-2 and particularly Bcl-X. All these data indicates that LBH589 could be a useful drug for the treatment of resistant CML patients including those with the T315I mutations.[39]

In summary, several novel tyrosine kinase inhibitors that have been developed to override imatinib resistance mechanisms such as overexpression of Bcr-Abl and point mutations within the Abl kinase domain, are currently competing. Inhibitors of Abl tyrosine kinase can be divided into two main groups, namely, ATP-competitive and ATP non-competitive inhibitors. In addition, ATP competitive inhibitors can be subdivided into two subclasses: the Src/Abl inhibitors, and imatinib-like compounds. Dasatinib, SKI-606 are classified as Src/Abl inhibitors while nilotinib and NS-187 (INNO-406) belong to the latter subclass of inhibitors. Among these agents, clinical studies on dasatinib and AMN107 had started earlier than the others and favorable results are accumulating. Clinical studies of other compounds including SKI606 and INNO-406 are currently ongoing. Because of their strong affinity, most ATP competitive inhibitors may be effective against imatinib-resistant patients, including those harbouring mutations, but are ineffective against the T315I mutation. To address this problem, ATP non-competitive inhibitors such as Aurora kinase inhibitor MK-0457 and LBH589 have been developed. For the improvement of CML and Ph+ALL treatment, maybe a combination therapy with novel ATP-competitive inhibitors and these new non-ATP-competitive agents will be taken into consideration.[40]

Resumo

A introdução do inibidor de tirosino quinase BCR-ABL mesilato de imatinibe (Glivec®, Novartis) levou a significantes mudanças no tratamento da LMC. Entretanto, a despeito de impressionante porcentagem de pacientes que respondem, alguns casos de LMC, particularmente em fases avançadas da doença mostram resistência primaria ou recidivas após terapêutica inicial. Inibidores de tirosino quinases de segunda geração como o nilotinibe (Tasigna®, Novartis) e o dasatinibe (Sprycel®, Bristol Myers Squibb) têm mostrado significante atividade nos estudos clínicos em paciente onde o imatinibe falhou. Porém, estes agentes não são capazes de inibir a mutação T315I do Bcr-Abl e apresentam atividade parcial em fases avançadas da LMC. As noções biológicas adquiridas sobre os

*mecanismos de resistência aos inibidores de TK levaram ao desenvolvimento de novos compostos alguns dos quais têm resultados preliminares encorajadores incluindo a mutação T315I. Neste trabalho nós discutimos os novos agentes emergentes e qual o potencial poderão atingir para ultrapassar a resistência aos inibidores de TK em pacientes com LMC. Rev. bras. hematol. hemoter. 2008; **30**(Supl. 2):24-29.*

Palavras-chave: *Leucemia mielóide crônica; inibidores da quinase BCR-ABL; resistência aos TKI.*

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