

Management of Chronic Myeloid Leukemia Patients Resistant to Tyrosine Kinase Inhibitors Treatment

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ABSTRACT: Chronic myeloid leukemia (CML) is a myeloproliferative disorder associated with a characteristic chromosomal translocation called the Philadelphia chromosome. This oncogene is generated by the fusion of breakpoint cluster region (BCR) and Abelson leukemia virus (ABL) genes and encodes a novel fusion gene translating into a protein with constitutive tyrosine kinase activity. The discovery and introduction of tyrosine kinase inhibitors (TKIs) irreversibly changed the landscape of CML treatment, leading to dramatic improvement in long-term survival rates. The majority of patients with CML in the chronic phase have a life expectancy comparable with that of healthy age-matched individuals. Although an enormous therapeutic improvement has been accomplished, there are still some unresolved issues in the treatment of patients with CML. One of the most important problems is based on the fact that TKIs can efficiently target proliferating mature cells but do not eradicate leukemic stem cells, allowing persistence of the malignant clone. Owing to the resistance mechanisms arising during the course of the disease, treatment with most of the approved BCR-ABL1 TKIs may become ineffective in a proportion of patients. This article highlights the different molecular mechanisms of acquired resistance being developed during treatment with TKIs as well as the pharmacological strategies to overcome it. Moreover, it gives an overview of novel drugs and therapies that are aiming in overcoming drug resistance, loss of response, and kinase domain mutations.

KEYWORDS: CML, BCR-ABL tyrosine kinase inhibitors, resistance, patient management

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Introduction

Chronic myeloid leukemia (CML) is a myeloproliferative disorder associated with a characteristic reciprocal chromosomal translocation between the Abelson leukemia virus (C-ABL) oncogene, present in the long arm of chromosome 9, and the breakpoint cluster region (BCR), present in the long arm of chromosome 22. This translocation results in the creation of a chimeric fusion protein. This protein is a constitutively activated tyrosine kinase causing abnormal activation of various intracellular signal transduction pathways, resulting in genomic instability, pathologic cell proliferation, initiation, and persistence of CML clones.^{1,2}

The discovery of selective ABL tyrosine kinase inhibitor (TKI) in 1996 contributed to a paradigm change in the CML treatment. Druker et al showed *in vitro* a 92–98% decrease in the number of BCR-ABL1 colonies grown from blood or bone marrow of CML patients in the presence of a selective ABL1 TKI. Moreover, the normal colonies were unaffected.³ The clinical efficacy of imatinib in the treatment of CML patients was first reported in 2001.⁴ This led to the approval of imatinib by the US Food and Drug Administration (FDA) as a first-line treatment for Philadelphia (Ph) chromosome-positive

CML, both in adult and pediatric settings. Unfortunately, it soon became apparent that certain point mutations in the kinase domain can result in complete lack of treatment effectiveness even with increased doses of imatinib. Thanks to crystallographic analyses, the development of second-generation TKIs, such as dasatinib and nilotinib, was then possible. Both agents have proven to be effective in imatinib-resistant patients. However, the development of resistance to second-generation TKIs is also common.^{5,6} Getting a deeper insight into the molecular events underlying TKI resistance is needed for optimizing the management of CML and the development of new treatment approaches.^{7–12}

Development of Resistance Under TKI Therapy

The resistance mechanism developed during TKI therapy by CML patients can be categorized as primary and secondary. Insufficient protein binding and aberrant expressions of a drug transporter number belong to primary resistance mechanisms. Secondary resistance mechanisms involve ABL1 kinase domain mutation.

As already stated, one of the reasons for primary resistance may be an insufficient binding of TKI to the plasma proteins



albumin and alpha-1 acid glycoprotein.¹³ The subanalysis of the International Randomized Study of Interferon vs STI571 indicated that plasma levels of imatinib following the first month of treatment may be a significant prognostic factor for long-term clinical response.¹⁴ Also, other analyses showed a correlation between higher plasma levels of imatinib and major and complete molecular response.^{15,16} However, there are also data available suggesting that plasma levels of imatinib in patients receiving different dose schedules had no correlation with response to therapy. Therefore, these results should be interpreted with caution.¹⁷ Although these data might be useful to monitor patient's adherence to therapy, they are so far not sufficient to support the therapeutic decisions based on imatinib plasma levels (National Comprehensive Cancer Network [NCCN] Guidelines Chronic Myelogenous Leukemia, Version 1.2015). Another reason for primary resistance to TKI therapy seems to be associated with aberrant expressions of drug transporters, such as multidrug resistance adenosine triphosphate (ATP)-binding cassette (ABC) transporters (MDR1 or ABCB1 and ABCG2) and human organic cation transporter 1 (hOCT1).^{13,18} It has been shown that overexpression of the multidrug resistance (MDR1) gene is associated with decreased intracellular concentrations of imatinib, and this could explain some cases of imatinib resistance.¹⁴ Moreover, there are some reports suggesting that resistance to imatinib, dasatinib, and nilotinib may be conferred by the presence of ABCB1 and ABCG2.^{15,16} According to the data gathered in the Therapeutic Intensification in De Novo Leukaemia (TIDEL)¹⁷ and Tyrosine Kinase Inhibitor Optimization and Selectivity study (TOPS) trials,¹⁹ the percentage of major molecular responses has been higher in patients with high hOCT1 activity. On the contrary, cellular uptake of dasatinib or nilotinib seems to be independent of hOCT1 expression, suggesting that patients with low hOCT1 expression might have better outcomes with dasatinib or nilotinib.²⁰⁻²³

The most common mechanism for secondary resistance is the reactivation of BCR-ABL1 activity by occurrence of

point mutations within the kinase domain. The mutations cause steric changes in the protein structure that can block TKI binding or stabilize the active conformation of the kinase enhancing its resistance.^{13,24} Many studies suggest that mutations in phosphate-binding loop (P-loop) were associated with a particularly high risk of progression.²⁵ Other studies have reported that mutations in the ATP P-loop are associated with a poor prognosis and a high risk of progression among patients treated with imatinib.^{26,27} Among the mutations in the ABL1 kinase domain, the presence of T315I mutation confers the highest resistance to imatinib, dasatinib, and nilotinib. It results in an amino acid substitution at position 315 in BCR-ABL1, from a threonine (T) to an isoleucine group (I).²⁷ It was suggested that T315I is associated with disease progression and shortened survival rates.²⁸ According to Jabbour et al, survival of patients with T315I is dependent on the stage of the disease, with many chronic-phase patients having an indolent course and patients in the accelerated phase and blast phase having poor prognosis.²⁹ Other secondary resistance mechanisms include BCR-ABL1 gene amplification or increased BCR-ABL1 expression.^{18,30} Activation of the SRC family of kinases or cytogenetic clonal evolutions as characterized by additional chromosomal abnormalities in the Ph-positive cells^{13,18} represents other mechanisms of imatinib resistance.³¹⁻³³

Response Monitoring and Mutational Analysis

According to NCCN Guidelines, mutational analysis should be performed in patients who fail to achieve first-line TKI treatment milestones ($\leq 10\%$ BCR-ABL1 transcript levels or partial cytogenetic response at 3 and 6 months, or complete cytogenetic response at 12 and 18 months) or in patients with disease progression.

Recommendation of European LeukemiaNet (ELN) advises performing mutational analysis in AP/BC patients at only diagnosis and also during the first-line imatinib therapy (a) in case of therapy failure, (b) in case of an increase in

Table 1. A brief summary of the available TKIs, their therapeutic targets, clinical trials in which they have been tested, and main side effects.

	IMATINIB	DASATINIB	NILOTINIB	BOSUTINIB	PONATINIB
Clinical trials	IRIS ⁷ DASISION ⁸ ENESTnd ⁹ BELA ¹⁰	ENESTnd ¹¹	BELA ¹⁰	BELA ¹⁰	PACE ¹²
Therapeutic targets	ABL, Kit, PDGFR, DDR1, NQO2	SRC family, PDGFR, KIT, EPHA2	ABL, Kit, PDGFR, DDR1, NQO2, VEGF	ABL, SRC family	Pan-BCR-ABL kinase and SRC kinase
Main side effects	Myelotoxicity, periorbital edema, rash, nausea, skin pigmentation, elevated liver enzymes, diarrhea, myalgia, headache	Myelotoxicity, thrombocytopenia, pleural effusions, QT prolongation, low phosphate, diarrhea	Elevated liver, pancreatic enzymes and glucose, QTc prolongation, skin rash, myelotoxicity, diarrhea, myalgia, nausea	Diarrhea, elevated liver enzymes, myelotoxicity, edema, nausea, rash	Pancreatitis, hepatotoxicity, hypertension, rash, myelotoxicity, thrombocytopenia, edema, thromboembolism, QTc prolongation



BCR-ABL1 transcript levels leading to MMR loss, and (c) in any other case of suboptimal response. Mutational analysis should also be performed in case of hematologic or cytogenetic failure during second-line dasatinib or nilotinib therapy.³⁴ In order to discover mutant cells, the mutational analysis should be performed while patients remain on their TKI therapy, in order to prevent overgrowth of nonmutated BCR-ABL1 cells and masking of the underlying mutations.

Direct sequencing remains the most popular method for BCR-ABL1 mutational analysis since it makes use of real-time polymerase chain reaction (PCR) amplification of BCR-ABL1 from RNA, which is commonly done for molecular monitoring. The disadvantage of this method is its low sensitivity, which cannot detect a mutant clone, unless it is present in at least 15–25% of the total number of BCR-ABL1-positive cells.³⁵

There are also some other methods, such as denaturing high-performance liquid chromatography,³⁶ allele-specific oligonucleotide PCR,³⁷ pyrosequencing,³⁸ and ultra-deep sequencing,³⁸ for BCR-ABL1 mutational analysis. Despite their high specificity, these have not yet been widely available. Also, it is unclear what clinical significance the low-level mutations may bear.^{39,40}

In summary, direct sequencing remains golden standard for BCR-ABL1 mutational diagnostic.

Usage of Next-Generation TKIs as Possible Treatment Modalities

After appearance of point mutations in the BCR-ABL1 kinase domain, an adequate choice of sequential therapy is crucial. According to NCCN Guidelines, dasatinib is recommended for mutations Y253H, E255K/V (P-loop), and F359V/C/I (substrate-binding region). Nilotinib should be used for mutations V299L, T315A, and F317L/V/I/C (ATP-binding region). Bosutinib has shown potent activity in patients with BCR-ABL1 mutations resistant to dasatinib (F317L) and nilotinib (Y253H and F359).

Another potent TKI that has demonstrated activity in patients with BCR-ABL1 mutations resistant to imatinib, dasatinib, or nilotinib (F317L, E255K, F359V, and G250E), including T315I, is ponatinib.⁴¹

Through usage of its unique carbon-carbon triple bond, ponatinib is able to avoid the steric hindrance caused by T315I, and it inhibits the BCR-ABL kinase. Ponatinib, which is a third-generation multitargeted TKI, also causes inhibition of VEGFR, PDGFR, FGFR and SRC kinase, KIT, RET, and FLT3. Ponatinib had been approved by the FDA on December 14, 2012, for the treatment of patients with resistant or intolerant CML and Ph-positive acute lymphoid leukemia (ALL). Its approval was based on safety and efficacy data generated within a single-arm, multicenter, phase II clinical trial (Ponatinib Ph+ ALL and CML Evaluation [PACE] trial).¹² A total of 449 patients with CML intolerant to prior TKI therapy or those with the resistant disease (dasatinib or nilotinib) or with the

T315I mutation (270 patients with CML in the chronic phase, 85 patients with CML in the accelerated phase, 62 patients with CML in the blast phase, and 32 patients with Ph-positive ALL) were treated with 45 mg ponatinib once daily. In all, 45 of the 64 (70%) patients with TKI resistance or intolerance or T315I mutation achieved major cytogenetic response.⁴² Despite excellent efficacy, ponatinib has to be used with caution, because of its safety profile. The FDA issued a partial clinical hold on new trial enrollment for ponatinib on October 9, 2013, due to an increased number of arterial thromboses. As a consequence, a black box warning for arterial thrombosis and hepatotoxicity has been issued by the manufacturer. Moreover, the European Prospective Investigation into Cancer and Nutrition (EPIC) trial, which evaluated the usage of ponatinib in patients with newly diagnosed CML, was terminated prematurely on October 18 of the same year.⁴³

Targeting Leukemic Stem Cell

Although great progress has already been made in regard to the treatment of CML, there is still a need for approaches that would directly target leukemic stem cells. This is crucial, since there is now more and more evidence that TKIs – by targeting only mature proliferating cells – can enable a leukemic stem cell to secure disease persistence.⁴⁴

Through the activation of BCR-ABL1 in the stem cell compartment, different signaling pathways are being modulated, which allow leukemic stem cells to avoid apoptosis and have an advantage of survival.⁴⁵

Although allogeneic hematopoietic stem cell transplantation is associated with relatively high treatment-related toxicity and is no longer recommended as a frontline treatment, it still remains the only treatment that allows lifelong protection from the disease because it can induce the complete eradication of leukemic stem cells, which is a prerequisite of cure.⁴⁶

Since TKIs have become available, the number of allogeneic transplantations systematically declined for patients in their first chronic phase. However, during the last years, the number of transplants in more advanced stages of CML is continuously increasing. It is now commonly accepted that there can be a clear indication for allogeneic hematopoietic stem cell transplantation in patients who fail to achieve their therapeutic goals after using two to three different TKIs, and patients with disease progression to CML in the blast phase and patients with T315I mutation.⁴⁷ Moreover, ELN recommends consideration of allogeneic stem cell transplantation in the second-line therapy, after failure of nilotinib or dasatinib in the first-line therapy, failure and/or intolerance to the third-line therapy, as well as in patients with T315I mutation.⁴⁸

Recent data show that allogeneic stem cell transplantation can also be an effective treatment modality for patients with T315I mutation, especially in earlier stages of the disease.^{49,50}

Furthermore, some of the contraindications that have prevented stem transplantation after high-dose therapy in patients with age-associated comorbidities are no longer present when



new approaches using nonmyeloablative, reduced-intensity conditioning are applied.^{51–53}

According to the ELN recommendation, the value of using a TKI as maintenance after allogeneic stem cell transplantation is not proven but seems intuitively logical.^{48,54}

Omacetaxine (Homoharringtonine) is a drug derived from the alkaloid *Cephalotaxus harringtonia* and has been successfully applied in patients with intolerance to two or more TKIs or TKI-resistant CML patients who progressed into the accelerated or blast phase and patients with T315I mutation. Omacetaxine inhibits protein translation by preventing the initial elongation step of protein synthesis. It also interacts with the ribosomal A site and prevents the correct positioning of amino acid side chains of incoming aminoacyl-tRNAs.⁵⁵ Moreover, it has been shown in preclinical models that omacetaxine effectively targets BCR-ABL-positive leukemia stem cells *in vivo*.⁵⁶

The FDA approval of this drug in October 2012 was based on a phase II study that included 62 CML patients with resistance and/or intolerance to two previous TKI treatments, 60 patients with three lines of TKI treatment, and patients with T315I mutations. It has been reported that 77% of the patients had complete hematological remission, 23% of the patients achieved major cytogenetic response, and 16% had complete cytogenetic response. In all, 30% of 17 patients in the accelerated phase had a major hematological response. The median progression-free survival was 7.7 months.^{57,58}

Outlook

A lot of effort has been directed into the development of new therapeutic approaches with unique mechanisms of action that can potentially eliminate dormant leukemic stem cells and resistant CML clones.^{59,60}

Rebastinib (also known as DCC-2036) is a multitargeted TKI that is able to inhibit TIE2, VEGFR1, and BCR-ABL kinase. Rebastinib acts via a non-ATP competitive mechanism and prevents the activation of ABL kinase by blocking an conformation change in the switch pockets.⁶¹

A phase I clinical trial evaluated rebastinib in CML patient populations resistant or intolerant to imatinib, nilotinib, or dasatinib and showed some efficacy among T315I mutants. Until now, it is only the preclinical data that have been published.⁶²

Also, utilization of aurora kinase inhibitors, which block various aurora kinases and ABL kinases such as tozasertib (also known as VX680, MK-0457), danusertib, or KW2449, seems to be a promising approach. Especially, tozasertib – by preventing the binding of isoleucine at the gatekeeper area – has, like ponatinib, become relevant for the treatment of CML patients with T315I mutations. It has been reported recently that tozasertib induced hematological responses in 8 of the 18 CML patients with T315I mutation.⁶³ Also danusertib showed very promising results in phase I study in patients with refractory CML, including BCR-ABL T3151 mutants.⁶⁴

GNF-2 and its analog GNF-5 offer an alternative strategy to overcome the gatekeeper T315I mutation and show some promising results in preclinical studies. Unlike approved TKIs, GNF-2/GNF-5 is an allosteric inhibitor of BCR-ABL that enters the myristate-binding pocket at the base of the C-lobe in the Abl kinase domain, favoring the inactive conformation. Moreover, a combination of high concentrations of GNF-5 and nilotinib showed inhibitory activity against this gatekeeper mutant in biochemical and cellular assays.⁶⁵

ABL001 is also a potent, selective BCR-ABL1 inhibitor that binds to a different region of BCR-ABL, forcing a conformational change that disables the protein's active site. In a preclinical CML model, the combination of ABL001 and nilotinib resulted in complete and sustained tumor regression with no evidence of disease relapse. ABL001 is currently being evaluated in a phase I clinical study in patients with CML and Ph-positive ALL (NCT02081378).

Since CML stem cell survival is independent of BCR-ABL, it has been suggested that the JAK/STAT signaling pathway may provide CML leukemic stem cells with prosurvival signals. Chen et al showed that the combination of the JAK2 inhibitor, ruxolitinib, with nilotinib may lead to lower JAK2/STAT5 signaling than either of the single agent. In that way induced apoptosis that leads to the decreased incidence of BCR-ABL-positive stem cells.⁶⁶ The effectiveness of JAK2 inhibitors and TKIs combination therapy is currently being tested in clinical trials (phase I/II: NCT01914484/NCT01751425).

It has been reported that CML leukemic stem cells induce autophagy as a defense mechanism in response to TKI treatment, in order to evade apoptosis.⁶⁷ The idea of targeting the process of autophagy (by using hydroxychloroquine) in combination with TKI treatment (imatinib) among patients with CML who are in major cytogenetic response and have residual disease is currently being tested in the CHlorO-quine and Imatinib Combination to Eliminate Stem cells (CHOICES) trial.^{68,69}

In order to eliminate the leukemic stem cell clone, it may be necessary to target additional sites, such as Hedgehog/Smoothed pathways⁷⁰ or β -catenin.⁷¹ Given the role of these pathways in normal stem cell physiology, it is still unclear whether or not a sufficient therapeutic window exists to distinguish between normal and leukemic stem cells. Substances such as hedgehog pathway inhibitors and Wnt-1/beta catenin pathway inhibitors, as well as HDAC inhibitors (like panobinostat),⁷² PP2A activators,⁷³ or lipoxygenase pathway inhibitors (zileuton)⁷⁴ due to their potential of targeting leukemic stem cell are currently being investigated extensively.

Another treatment approach includes inhibition of promyelocytic leukemia protein (PML), which is highly expressed in hematopoietic stem cell compartment and in CML patients.⁷⁵ It has been reported that inhibition of PML by arsenic trioxide could inhibit CML stem



cells. The results of first clinical studies (phase I study: NCT01397734 and phase II study: NCT00250042) are still being awaited.

Heat shock protein 90 inhibitors, such as geldanamycin and ganetespib (STA-9090), through binding to the ATP-binding domain of Hsp90 and promotion of the degradation of BCR-ABL protein kinase in the cells are also being investigated in phase I trials.³⁰ Clinical responses also included hematological responses in two patients with CML.⁷⁶

Also novel immunotherapeutic approaches, for example, the usage of CML genetically redirected T cells, could be effective in treating refractory CML patients. Promising preclinical data with CAR-modified T cells have already been reported.⁷⁷

Conclusions

The discovery of TKIs and their development reaching into the next generation has added dramatically to the life expectancy of CML patients and has changed their lifetimes from very limited ages into those of healthy age-matched individuals. CML itself has changed from a life-threatening into a chronic disease, which demands true compliance, proper monitoring, and a wise use of those therapies presently available. The focus of modern CML treatment has shifted toward achieving faster and deeper responses that are considered surrogate for long-term outcomes.

Major advances in the drug development bring along with them their own challenges, such as drug resistance, loss of response, kinase domain mutations, and transformations in accelerated and blast phases as well as patient noncompliance. As a consequence, there is now a great need for differentiated, comparative monitoring and intensive research that hopefully will result in final treatment strategies that include the successful targeting of quiescent CML stem cells.

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Wrote the first draft of the manuscript: AW. Contributed to the writing of the manuscript: LU. Agreed with manuscript results and conclusions: AW, LU. Jointly developed the structure and arguments for the paper: AW, LU. Made critical revisions and approved final version: AW, LU. Both authors reviewed and approved the final manuscript.

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