Mutations Associated with Imatinib Mesylate Resistance - Review

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INTRODUCTION

CML is almost unique among human neoplasms in that a specific genetic marker - the chromosomal translocation t(9;22) - is associated with the malignant phenotype. As a result of this translocation, a BCR-ABL fusion gene is formed on the 22q derivative chromosome, which is known as the Philadelphia (Ph) chromosome. The gene encodes a protein with tyrosine kinase activity. Consequently, it initiates and maintains the disease. CML is the first human malignancy for which the promise of targeted therapy has come true.

Imatinib mesylate is a potent and well-tolerated inhibitor of the BCR-ABL tyrosine kinase and has revolutionized the treatment of the patients.

IMATINIB

The phenylaminopyrimidine compound imatinib mesylate (Gleevec) acts as a potent inhibitor of the tyrosine kinases ABL (Fig. 1). It represents the first selective protein kinase-inhibitor to be developed for targeted cancer therapy, and its superior clinical efficacy was first shown in the treatment of CML. At the molecular level, imatinib acts by binding the catalytic domain of the ABL-protein in its inactive conformation, thereby preventing ATP-binding. Thus, it keeps substrates involved in BCR-ABL downstream signal transduction pathways from phosphorylation and activation. Imatinib mesylate is identified as a tyrosine kinase inhibitor because it curbs the activity of BCR-ABL tyrosine kinase. It replaced Interferon alpha as the first line of therapy and achieved durable results. Survival of up to 85% of the patients on imatinib mesylate was confirmed by the International Randomized Study. The BCR-ABL protein kinase is constitutively active. It binds to ATP and tyrosine residues on various substrates receive phosphate from it. This results in increased proliferation of cells. Imatinib is effective as it prevents ATP to bind to BCR-ABL tyrosine kinase arresting its activity. As the subsequent phosphorylation procedure stops, the adverse cellular events
are terminated too. Despite this progress, up to 27% of patients who achieve complete cytogenetic response (CCyR) and a few patients in the advanced phases of the disease show relapse or resistance while undergoing imatinib therapy. Intrinsic resistance is also known as primary resistance and is caused in the absence of initial efficacy of drug. Acquired loss of response or relapse is also known as secondary resistance and is caused when the drug loses its initial efficacy on the patient. Resistance can best be defined using the European Leukemia Net (ELN) criteria for failure to imatinib therapy: less than a complete hematologic response (CHR) at 3 months, no cytogenetic response (CyR) (reduction in Ph bone marrow metaphases) at 6 months, less than a partial CyR (35% Ph metaphases) at 12 months, less than a complete CyR (no Ph metaphases) at 18 months, or loss of a complete CyR or complete hematologic response anytime during therapy. ELN also established the concept of a suboptimal response: the patient may still have a substantial long-term benefit from continuing imatinib, but the chances of an optimal outcome are reduced so that the patient may be eligible for alternative treatments. Suboptimal responders are those who show no CyR at 3 months, less than a partial CyR at 6 months, less than a complete CyR at 12 months, or less than a major molecular response (three-log reduction in BCR-ABL transcript levels) at 18 months, and those who lose a previously achieved major molecular response anytime during treatment. Although it is now well established that several different factors may concur to determine imatinib resistance, the most extensively investigated one is the selection of point mutations in the BCR-ABL kinase domain (KD) that impair inhibitor binding. They were the first and most frequent resistance mechanisms identified in phase II studies of imatinib in advanced phase CML patients and immediately catalyzed researchers’ attention. These mutations were demonstrated to alter the biochemical properties of imatinib contact points and to induce conformational changes in the tertiary structure of the protein that make it incompatible with imatinib binding.

**IMATINIB-RESISTANCE**

Despite the great expectations for imatinib, it has recently been determined that patients with CML, especially those in advanced phases, treated with it often experience relapse due to drug resistance. The main reason for this is the point mutations within the BCR-ABL kinase domain. BCR-ABL mutations can confer high-level resistance to BCR-ABL TKIs and promote the disease progression from chronic phase (CP) to acute phase (AP) and blast crisis (BC). The main course of resistance is considered to be the expansion of subclone with acquired mutations encoding altered BCR-ABL protein sequences, which prevent BCR-ABL inhibitors from binding or favoring conformations with reduced TKI sensitivity.

The mutations induce substitutions of amino acids that either directly interfere with imatinib in the binding pocket (gatekeeper residues), or that are involved in establishing a distinct conformation, which imatinib is unable to bind. Imatinib exclusively binds the inactive conformation of the ABL kinase domain. Thus, if the conformational change of the kinase is hampered by an amino acid change, imatinib-binding may be drastically reduced.

The mutations are mainly found in four regions of the ABL-kinase domain (Fig. 2). The phosphate binding loop (P-loop) that normally surrounds a phosphate group in the depth of the binding pocket, the activation loop (A-loop) that is crucial for the conformation of the kinase, the gatekeeper residues that are directly in contact with imatinib, and other more downstream located residues in the catalytic domain (drug-binding), which may participate to stabilize the A-loop in a certain conformation, like the methionine residue in position 351.

It seems that the majority of mutations target amino acid residues involved in conformational change, and only a few residues seem to directly contact the drug in the ABL binding pocket, like the 315 and 317 residues. The T315I-mutation seems to be particularly important since it is frequently
Mutations Associated with Imatinib Mesylate Resistance

**Figure 2.** Mutations in the BCR-ABL kinase domain that are associated with imatinib-resistance. The mutations are mainly found in the P-loop, at amino acid residues in direct contact with imatinib, close to the catalytic domain or at the activation loop.6

detected and confers resistance also to new drugs developed particularly for imatinib-resistance subjects.7 Fig. 3 shows the ABL kinase domain complexes with imatinib, and illustrates some of the most important mutations associated with resistance.

The most commonly mutated region was the drug-binding site (29%) followed by P-loop region (26%).9 The direct binding site T315 is essential for hydrogen binding between imatinib and ABL; thus, a point mutation of threonine 315 to isoleucine (T315I) interferes with this hydrogen bond. Furthermore, the T315I mutation induces a conformational change in several amino acid residues, which are important for the binding of imatinib and BCR-ABL (the allosteric effect). Accordingly, T315I yields imatinib resistance more strongly than other point mutations. P-loop is called the induced-fit site because of its conformational change accompanied by imatinib binding.10 This induced-fit enables imatinib to make a hydrogen bond with tyrosine 253 (Y253) that is intensified by other hydrophobic amino acids surrounding it. Therefore, the point mutations at Y253 including Y253F and Y253H interfere with imatinib binding to Y253. Imatinib can only bind to inactive ABL which kinaseactive site is closed by the activation-loop (A-loop); this loop is involved in ABL specificity and resistance. Mutations within drug-binding site such as M351T also induce a conformational change of active ABL, while mutations within the A-loop prompt ABL to form a more active conformation. Among these mutations, Y253H, E255K, T315I, and M315T are observed more frequently. Also, multiple mutations causing imatinib resistance may be detected simultaneously. When multiple mutations are detected, mutations may be present in different BCR-ABL molecules (polyclonal mutation) or in a single BCR-ABL molecule (compound mutation). Compound mutations have been reported to often cause stronger resistance to TKIs.5,10,14,15

Considering the latest developments in CML-monotherapy and the introduction of new drugs, detection of mutations in the ABL kinase domain is getting more important. For the most frequent mutations (T315I, V299L, T315A, F317L/V/I/C, Y253H, E255K/V, and F359V/C/I) 50% inhibitory concentration data have been published and the available clinical experience casts little doubt on a causative role in imatinib resistance. When one such mutation is detected, imatinib treatment, at least at a standard dose, is no longer advised.5,11,14,15

Mutation analysis of these specific mutations should be used to choose the type of second or subsequent-line TKI. According to recommendations from an expert panel on behalf of European LeukemiaNet11 the detection of different types of mutations must be accompanied by a specific treatment algorithm, namely:

- In case of V299L, T315A, or F317L/V/I/C mutations, nilotinib is probably more effective than dasatinib.11,15
- In case of Y253H, E255K/V, or F359V/C/I mutations, dasatinib is probably more effective than nilotinib.11,15
**Figure 3.** Ribbon illustration of the ABL kinase domain in complex with imatinib. Imatinib is shown in yellow, and the numbers represent amino acid residues involved in resistance. Red spheres (1-3) symbolize mutations that directly affect the drug-binding of imatinib, green spheres (4-8) are P-loop mutations and blue spheres (9-13) are mutations close to the activation loop (A-loop). The amino acid residues are: 1 F317L; 2 T315I; 3 F359; 4 M244; 5 G250; 6 Q252; 7 Y253; 8 E255; 9 M351; 10 E355, 11 V379; 12 L387; 13 H396.8

- In case of detection of T315I mutation which is highly resistant to imatinib, dasatinib, and nilotinib, shows the mandatory application of the only oral TKI that inhibits the T315I mutant, ponatinib.12,15

Upon disease progression, BCR-ABL1 genotyping is crucial for selection of the optimal TKI as salvage therapy. Recommendations are based on activity comparisons in vitro, typically half maximal (IC50) or 90% of maximal (IC90) inhibitory concentration values determined in BaF/ 3 cells expressing BCR-ABL1 mutants.12 Most commonly, TKI activity against a mutant is semi-quantifiable in relation to the native kinase, which permits a relative ranking of TKI activities despite different dose ranges. Half maximal inhibitory concentration (IC50) values for cell proliferation of the indicated TKIs are shown against BCR-ABL1 single mutants (Table 1). The grayscale demonstrates the IC50 sensitivity for each TKI relative to its activity against cells expressing native BCR-ABL1. Note that clinical activity is also dependent on additional factors, such as the drug concentrations achieved in the plasma of patients.13

BCR-ABL KD mutation analysis is not recommended in newly diagnosed chronic phase patients. Conversely, it can be performed in the rare cases which are in accelerated phase or blast crisis at the time of imatinib start. Mutation analysis is recommended both in case of failure and suboptimal response to imatinib. From a clinical standpoint, ‘failure’ means that continuing a specific treatment is no longer appropriate because favorable outcome is unlikely. ‘Suboptimal response’ means that the patient may still have a substantial long-term benefit from continuing a specific treatment but the chances of optimal outcome are reduced. For this reason, ‘suboptimal responders’ to imatinib may either continue on imatinib at the same dose or may become eligible for alternative approaches, including increase of the dose of imatinib. For all these reasons, it is more reasonable to advise that, in patients showing an increase in BCR-ABL transcript level, only confirmed loss of major molecular response (MMR), loss of CCyR and loss CHR must be the initiate for a mutation analysis. Optimization of CML treatment is a continuous process. Data is still accumulating and the therapeutic scenario is still evolving.11

**CONCLUSION**

Thus we conclude that it is essential to identify the harboring mutations in the patients who show absence of or suboptimal response to first line drugs in order to provide them with the most appropriate targeted therapy. The frequency by which mutations have been implicated in imatinib resistance is different in the different phases of CML ranging from 25% to 30% of early chronic phase patients on first-line imatinib to approximately 70% to 80% of blast crisis patients. Mutational analysis of these patients is mandatory. Although the choice of the second- or subsequent-line strategy must result from a decision algorithm, including several factors, such as patient history, risk factors, and comorbidities, detection of some specific mutations (the T315I above all) should be part of this algorithm.

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### Table 1. Activities of imatinib, bosutinib, dasatinib, nilotinib, and ponatinib against mutated forms of BCR-ABL1

| Location of mutation | Mutation | imatinib | bosutinib | dasatinib | nilotinib | ponatinib |
|----------------------|----------|----------|-----------|-----------|-----------|-----------|
|                      |          | IC50-fold increase (WT=1) | IC50-fold increase (WT=1) | IC50-fold increase (WT=1) | IC50-fold increase (WT=1) | IC50-fold increase (WT=1) |
| Parental             |          | 10.8     | 38.3      | 568.3     | 38.4      | 570.0     |
| WT                   |          | 1        | 1         | 1         | 1         | 1         |
| P-loop               |          |          |           |           |           |           |
| M244V                |          | 0.9      | 0.9       | 2.0       | 1.2       | 3.2       |
| L248R                |          | 14.6     | 22.9      | 12.5      | 30.2      | 6.2       |
| L248V                |          | 3.5      | 3.5       | 5.1       | 2.8       | 3.4       |
| G250E                |          | 6.9      | 4.3       | 4.4       | 4.6       | 6.0       |
| Q252H                |          | 1.4      | 0.8       | 3.1       | 2.6       | 6.1       |
| Y250F                |          | 3.6      | 1.0       | 1.6       | 3.2       | 3.7       |
| Y250H                |          | 8.7      | 0.6       | 2.6       | 36.8      | 2.6       |
| E255K                |          | 6.0      | 9.5       | 5.6       | 6.7       | 8.4       |
| E255V                |          | 17.0     | 5.5       | 3.4       | 10.3      | 12.9      |
| C-helix              |          |          |           |           |           |           |
| D256G                |          | 2.2      | 0.6       | 1.4       | 2.0       | 2.1       |
| E279K                |          | 3.6      | 1.0       | 1.6       | 2.0       | 3.0       |
| E292L                |          | 0.7      | 1.1       | 1.3       | 1.8       | 2.0       |
| ATP binding region   |          |          |           |           |           |           |
| V299L                |          | 1.5      | 26.1      | 8.7       | 1.3       | 0.6       |
| T315A                |          | 1.7      | 6.0       | 58.9      | 2.7       | 0.4       |
| T315I                |          | 17.5     | 45.4      | 75.0      | 39.4      | 3.0       |
| T315V                |          | 12.2     | 29.3      | 73.8      | 57.0      | 2.1       |
| F317L                |          | 2.6      | 2.4       | 4.5       | 2.2       | 0.7       |
| F317R                |          | 2.3      | 33.5      | 114.8     | 2.3       | 4.9       |
| F317V                |          | 0.4      | 11.5      | 21.3      | 0.5       | 2.3       |
| SH2-contact          |          |          |           |           |           |           |
| M343T                |          | 1.2      | 1.1       | 0.9       | 0.8       | 0.9       |
| M351T                |          | 1.8      | 0.7       | 0.9       | 0.4       | 1.2       |
| Substrate binding region |      |          |           |           |           |           |
| F359I                |          | 6.0      | 2.9       | 3.0       | 16.3      | 2.9       |
| F359V                |          | 2.9      | 0.9       | 1.5       | 5.2       | 4.4       |
| A-loop               |          |          |           |           |           |           |
| L384M                |          | 1.3      | 0.5       | 2.2       | 2.3       | 2.2       |
| H396P                |          | 2.4      | 0.4       | 1.1       | 2.4       | 1.4       |
| H396R                |          | 3.9      | 0.8       | 1.6       | 3.1       | 5.9       |
| C-terminal lobe      |          |          |           |           |           |           |
| F486S                |          | 8.1      | 2.3       | 3.0       | 1.9       | 2.1       |
| L248R/F359I          |          | 11.7     | 39.3      | 13.7      | 96.2      | 17.7      |
| Sensitive            |          | ≤ 2      |           |           |           |           |
| Moderately resistant |          | 2.1-10   |           |           |           |           |
| Highly resistant     |          | > 10     |           |           |           |           |
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Мутации, связанные с резистентностью к иматинибу мезилата – обзор

Хронический миелоидный лейкоз (ХМЛ) возникает при слиянии гена BCR с ABL1. Ген BCR (хромосома 22q11.2) и ген ABL1 (хромосома 9q34) сливаются из-за взаимной транслокации хромосом, образуя филадельфийскую хромосому (Ph). Этот ген слияния кодирует тирозинкиназу, которая ускоряет деление клеток и ингибирует восстановление ДНК. Мезилат иматиниба является селективным ингибитором этой тирозинкиназы. Он используется в качестве первой линии лечения пациентов с ХМЛ. Тем не менее, выяснилось, что положительные для филадельфийской хромосомы (Ph +) клетки могут развиваться, чтобы избежать ингибирования из-за точечных мутаций в BCR-ABL-киназной области. До настоящего времени было выявлено более 40 мутаций, и их раннее выявление важно для клинического лечения. С развитием новых ингибиторов тирозинкиназы (TKIs), связанных с этими мутациями, проблема резистентности, по-видимому, ослабевает, поскольку некоторые из новых препаратов менее склонны к резистентности. Цель этого обзора - сосредоточиться на различных мутациях, ведущих к резистентности.