Choosing the Best Second-Line Tyrosine Kinase Inhibitor in Imatinib-Resistant Chronic Myeloid Leukemia Patients Harboring Bcr-Abl Kinase Domain Mutations: How Reliable Is the IC₅₀?

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LEARNING OBJECTIVES

After completing this course, the reader will be able to:

1. Explain the IC₅₀ of a tyrosine kinase inhibitor and the kind of information this parameter provides about its efficacy.
2. List the multiple factors that may be responsible for resistance to a target therapeutic agent.
3. Describe the clinical relevance of Bcr-Abl mutations in chronic myeloid leukemia patients.

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ABSTRACT

Development of drug resistance to imatinib mesylate in chronic myeloid leukemia (CML) patients is often accompanied by selection of point mutations in the kinase domain (KD) of the Bcr-Abl oncoprotein, where imatinib binds. Several second-generation tyrosine kinase inhibitors (TKIs) have been designed rationally so as to enhance potency and retain the ability to bind mutated

forms of Bcr-Abl. Since the preclinical phase of their development, most of these inhibitors have been tested in *in vitro* studies to assess their half maximal inhibitory concentration (IC₅₀) for unmutated and mutated Bcr-Abl—that is, the drug concentration required to inhibit the cell proliferation or the phosphorylation processes driven by either the unmutated or the mutated forms of

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the kinase. A number of such studies have been published, and now that two inhibitors—dasatinib and nilotinib—are available for the treatment of imatinib-resistant cases, it is tempting for clinicians to reason on the IC_{50} values to

guess, case by case, which one will work best in patients harboring specific Bcr-Abl KD mutations. Here, we discuss the pros and cons of using this approach in TKI selection. *The Oncologist* 2011;16:868–876

INTRODUCTION

Chronic myeloid leukemia (CML) is almost unique among human neoplasms in that a specific genetic lesion, the t(9;22) chromosomal translocation, is invariably associated with the malignant phenotype [1]. As a consequence of this translocation, a *BCR-ABL* fusion gene is formed on the 22q-derivative (traditionally known as the Philadelphia [Ph] chromosome) [2, 3] and the deregulated tyrosine kinase activity of the protein encoded by this gene has been shown to be both necessary and sufficient for the initiation and maintenance of the disease [4]. CML is the first human malignancy for which the promise of targeted therapy has come true. Imatinib mesylate, a potent and well-tolerated inhibitor of the Bcr-Abl tyrosine kinase, in 2001 became the first-choice treatment for CML patients [5] and has since revolutionized both the outcome and the quality of life of patients. The long-term efficacy of imatinib therapy, however, may be compromised by the development of drug resistance [6]. Resistance can best be defined using the European LeukemiaNet (ELN) criteria for failure to imatinib therapy: less than a complete hematologic response at 3 months, no cytogenetic response (CyR) (reduction in Ph^+ bone marrow metaphases) at 6 months, less than a partial CyR ($\leq 35\%$ 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 [7]. 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 [7, 8]. 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 [7]. Although it is now well established that several different factors may concur to determine imatinib resistance [9], 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 [10] 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 [11–14].

A number of studies have been published over the last decade that investigated their frequency, their clinical relevance, and the conformational changes they induce in the kinase. As time passed, the list of amino acid substitutions detected in imatinib-resistant patients increased exponentially. More than 70 different amino acid substitutions within the KD have since been described in association with imatinib resistance, although 15 (T315I, Y253F/H, E255K/V, M351T, G250E, F359C/V, H396R/P, M244V, E355G, F317L, M237I, Q252H/R, D276G, L248V, F486S) account for $\sim 85\%$ of mutated cases [9].

Soon after the first reports of imatinib-resistant mutations, in vitro studies interestingly suggested that not all mutations were equally challenging: different mutations could be associated with different levels of resistance [15, 16]. These studies measured the degree of sensitivity to imatinib of the most recurrent Bcr-Abl mutant forms in terms of the half maximal inhibitory concentration (IC_{50}), considered to be a measure of the effectiveness of a compound at inhibiting a biological or biochemical function and experimentally determined by quantifying the amount of a substance required to inhibit the activity of the target by 50%. Two types of IC_{50} exist depending on the in vitro strategy used to assess it—the cellular IC_{50} and the biochemical IC_{50} . The cellular IC_{50} is measured in cell lines (mainly, the Ba/F3 mouse lymphoblastoid cell line) engineered to express either unmutated or mutated Bcr-Abl and can be calculated either as the drug concentration required to reduce cell proliferation/viability by 50% or as the drug concentration required to reduce Bcr-Abl autophosphorylation by 50%. The biochemical IC_{50} can be obtained using an unmutated or mutated synthetic Bcr-Abl KD, and can be derived either as the drug concentration required to reduce the phosphorylation of Crkl, a known substrate of Bcr-Abl, by 50% or, as in the cellular system, the drug concentration required to reduce Bcr-Abl autophosphorylation by 50%. The great majority of published studies report cellular IC_{50} assessed as a function of cellular proliferation [15–24], either as an absolute value or in terms of the fold increase in

IC_{50} , that is, the ratio between the IC_{50} of a specific mutant form of Bcr-Abl and the IC_{50} of unmutated Bcr-Abl, intended as a quantitative estimate of how less sensitive to imatinib the mutant is expected to be.

The use of IC_{50} to measure efficacy in Bcr-Abl inhibition soon extended from imatinib to all second-generation tyrosine kinase inhibitors (TKIs) that were rationally developed to offer clinicians additional pharmacological options to be employed in imatinib-resistant patients. Given that, for years, mutations were considered the tougher enemy to counteract in an attempt to overcome drug resistance, pre-clinical assessment of all newly developed TKIs could not fail to challenge them for their efficacy against as many Bcr-Abl mutant forms as possible. Accordingly, a number of in vitro studies were published reporting the IC_{50} values of dasatinib, nilotinib, and other TKIs for the main Bcr-Abl mutant forms [18, 20–24]. These studies predicted dasatinib, nilotinib, bosutinib, and others to be all equally ineffective against the T315I mutation, given that the IC_{50} values were as high as the one measured for imatinib. In line with these data, crystallographic and molecular modeling studies showed that, despite the different chemical scaffolds of different inhibitors, threonine 315 remained a critical contact residue [13, 25, 26]. The same in vitro studies, however, showed that the IC_{50} values for many mutations were much lower than those known for imatinib, and the difference with respect to unmutated Bcr-Abl was, in most cases, two- to sevenfold, although differences were evident across different inhibitors [18, 20–24].

Bcr-Abl KD mutation analysis, most frequently performed by direct sequencing, is becoming available for a growing number of patients. Knowledge of the Bcr-Abl mutation status is a useful piece of information to tailor the best therapeutic strategy for all those who fail or have a sub-optimal response to imatinib, and for whom an alternative treatment approach is needed or advisable, respectively. Nowadays, both dasatinib and nilotinib are widely available for the second-line treatment of these patients. Thus, clinicians are enjoying the privilege of having two potent pharmacological alternatives to be employed in case a CML patient fails imatinib, but also facing a dilemma: which one to choose?

One way to select the TKI that is most likely to be effective in an imatinib-resistant patient known to harbor a specific Bcr-Abl KD mutation is to check the inhibitor IC_{50} for the mutation. Color-coded tables reporting IC_{50} values [27] or fold differences in IC_{50} [24] values for imatinib, dasatinib, and nilotinib for the most frequent mutant forms have also been published, in a more or less intentional attempt to facilitate the use of IC_{50} values. A straightforward traffic light-like code is superimposed

on experimental data so that these tables look like a sort of “at-a-glance” guide to the choice of second-line TKI: green obviously means that a patient with the mutation is expected to be sensitive to the inhibitor, yellow or orange mean that sensitivity is incomplete or uncertain, and red means that a patient with the mutation is definitely insensitive to the inhibitor.

Reports of phase II studies of dasatinib [28, 29] and nilotinib [30] in imatinib-resistant patients, as well as of the MD Anderson experience [31, 32], have indeed highlighted that a certain degree of correlation between the IC_{50} value for a specific mutation in vitro and the clinical response in patients harboring the same mutation in vivo does exist, in that: (a) patients harboring mutations with higher IC_{50} values had lower hematologic response and CyR rates than those harboring mutations with lower IC_{50} values and (b) mutations selected in patients developing dasatinib or nilotinib resistance were exactly those with the highest IC_{50} values. The first few imatinib-resistant patients positive for the T315I mutation who were enrolled in phase I–II studies were shown to not obtain any significant and/or long-lasting hematological improvement. In addition, T315I soon became the most frequently detected mutation in patients who relapsed after an initial response to dasatinib or nilotinib. Clinical experience has also provided clear evidence that patients with the imatinib-resistant F317L/V/I/C and Q252H mutations are poorly sensitive to dasatinib [28, 31–36], and that patients with the imatinib-resistant Y253H, E255K/V, and F359V/C/I mutations are poorly sensitive to nilotinib [30–32, 35]. Much lower response rates were indeed recorded in imatinib-resistant patients who were positive for any of these mutations. More strikingly, the F317, Y253, E255, and F359 mutations were reported, by several authors, to be newly acquired concomitantly with a loss of response to dasatinib or nilotinib. In addition, the V299L and T315A mutations were indeed shown to occasionally emerge in patients relapsing on dasatinib [37, 38], after some authors recovered these novel mutations, never observed in association with imatinib resistance, in an in vitro saturation mutagenesis screen for dasatinib-resistant Bcr-Abl mutants and associated them with a 13- and 93-fold higher IC_{50} value, respectively, than with unmutated Bcr-Abl [20].

Thus, should we reason in terms of the IC_{50} value also for other imatinib-resistant mutations? Should we take advantage of IC_{50} tables? Although such an approach looks like a captivating help in TKI selection, a series of considerations and drawbacks must be born in mind that warn against the noncritical use of IC_{50} values.

FROM A PRACTICAL POINT OF VIEW, HOW CAN IC₅₀ VALUES BE TRANSLATED INTO INDICATIONS REGARDING DRUG EFFICACY?

To determine whether a specific IC₅₀ value (or fold difference in IC₅₀) will practically translate into sensitivity to the inhibitor, it is necessary to categorize IC₅₀ values in at least three groups, such as “sensitive,” “poorly sensitive,” and “insensitive,” or something similar. Ideally, such categories should unequivocally be defined by a specific range of absolute (or fold difference) values. This has actually been attempted by some authors. Redaelli et al. [24], for example, defined as “sensitive” those mutations with a fold difference in IC₅₀ ≤ 2, as “moderately resistant” those mutations with a fold difference in IC₅₀ of 2–4, as “resistant” those mutations with a fold difference in IC₅₀ of 4–10, and as “highly resistant” those mutations with a fold difference in IC₅₀ > 10. O’Hare et al. [27] reasoned on the absolute IC₅₀ values and used the following cutoffs—imatinib: sensitive, ≤ 1,000 nM; intermediate, ≤ 3,000 nM; insensitive, > 3,000 nM; nilotinib: sensitive, ≤ 50 nM; intermediate, ≤ 500 nM; insensitive, > 500 nM; dasatinib: sensitive, ≤ 3 nM; intermediate, ≤ 60 nM; insensitive, > 60 nM. Neither group clarified what the biological bases for choosing these cutoff values were. For some mutations, such classifications look to be working reasonably well. Patients with the V299L and F317L mutations, for example, are predicted to be “resistant” or “intermediately sensitive” to dasatinib by both approaches [24, 27], and this is in line with what we observe in patients [28, 31–36]. On the other hand, the M351T mutation is indicated as sensitive to imatinib by both classifications [24, 27] despite the fact that it is one of the most frequent mutations we observe in imatinib-resistant patients [9]. This is one of the most striking demonstrations that the IC₅₀ may not always be a reliable indicator of TKI effectiveness in vivo.

HOW TO COPE WITH DISCREPANCIES

Since 2002, there have been at least 10 different experimental studies that independently assessed imatinib, nilotinib, and/or dasatinib cellular IC₅₀ [15–24]. A thorough comparison of these IC₅₀ values across different studies uncovers a worrying degree of variability, especially for some mutations (Table 1). This variability can be attributed to differences in experimental conditions: although the system was the same (a Ba/F3 cell line transfected with an expression vector encoding either wild-type or mutant p210^{BCR-ABL}), different incubation times and different ranges of inhibitor concentration were used in different studies to build the cellular proliferation curves from which the IC₅₀ value, by interpolation, was derived. In addition, a spectrum of different methods was used to assess cellular

proliferation. Whatever the reason(s) may be, these inconsistencies represent a serious caveat—how to base therapeutic decisions on a biological variable that is so profoundly influenced by the experimental methods and conditions used to determine it. An additional, more practical point is: which IC₅₀ value should one choose among the many published?

Additional considerations argue against the general transferability of IC₅₀ data to the in vivo setting.

MUTATIONS MAY NOT ALWAYS BE THE MAIN AND/OR THE ONLY MECHANISM DRIVING RESISTANCE

Other factors exist that may be responsible for drug resistance. They include *BCR-ABL* gene amplification [10, 39–41], clonal cytogenetic evolution [42–45], reduced activity of the human organic cation transporter type 1 (hOCT1) imatinib transporter [46, 47], and activation of additional or alternative signal transduction pathways like that of the Src kinases [48–50] (see [9] for a detailed review of all resistance mechanisms). A concept that is gaining more and more credit is that resistance mechanisms are not always mutually exclusive—different ones can act in synergy to determine insensitivity to inhibition. So, finding a Bcr-Abl KD mutation in a patient who relapsed on imatinib does not necessarily rule out the possibility that something else is contributing to determine the resistant phenotype we observe [51]. It cannot even be excluded that mutations may, in some cases, be innocent bystanders—maybe because another resistance mechanism determines incomplete inhibition of Bcr-Abl, allowing for less sensitive clones harboring mutations to survive. Besides additional cytogenetic abnormalities, whose occurrence can easily be detected by chromosome banding analysis, other factors known, or hypothesized, to determine drug resistance are not routinely evaluated in imatinib-resistant patients, so clinicians have only a few pieces of the puzzle to reason on.

MEASURING IN VITRO ANTIPROLIFERATIVE ACTIVITY IS A RATHER ARTIFICIAL WAY TO JUDGE AND RANK INHIBITORS FOR THEIR ANTILEUKEMIC ACTIVITY IN VIVO

The human body is a much more complicated system than a cell line. Using a cell line as a study model does not allow taking into account the complex interplay of important variables acting in humans, like absorption, metabolism, distribution to target compartments, transport, and excretion, that critically influence the actual delivery of the inhibitor to Ph⁺ cells. In addition, the biology of a cell line artificially engineered to express Bcr-Abl may not be identical to that of a Ph⁺ CML progenitor endogenously expressing Bcr-Abl.

Table 1. Published IC ₅₀ values of IM, NI, and DA for the most common Bcr-Abl mutant forms and fold difference with respect to the IC ₅₀ for WT Bcr-Abl												
	WT			M244V			L248V			G250E		
	IC ₅₀ (nM)			IC ₅₀ (nM)			IC ₅₀ (nM)			IC ₅₀ (nM)		
	Fold difference			Fold difference			Fold difference			Fold difference		
	IM	NI	DA	IM	NI	DA	IM	NI	DA	IM	NI	DA
Shah et al. [16]	600									>10,000		
										>17		
Corbin et al. [15]	500			1,600						4,500		
				3.2						9		
Azam et al. [17]	600			3,100						>20,000		
				5.2						>33		
O'Hare et al. [18]	260	13	0.8	2,000	38	1.3				1,350	48	1.8
				7.7	2.9	1.6				5.2	3.7	2.3
Burgess et al. [20]	323		1.34									
Manley et al. [21]	678	25										
Weisberg et al. [19]	649	25			39			919			219	
					1.6			37			8.8	
Bradeen et al. [22]	320	14								1,350	48	
										4.2	3.4	
Ray et al. [23]	450	<10					10,000	675				
							22	68				
Redaelli et al. [24]	527	18	1.8				1,866	49.5	9.4	3,613	81	8.1
							3.5	2.8	5.1	6.9	4.6	4.4
	E255V			T315I			F317L			M351T		
	IC ₅₀ (nM)			IC ₅₀ (nM)			IC ₅₀ (nM)			IC ₅₀ (nM)		
	Fold difference			Fold difference			Fold difference			Fold difference		
	IM	NI	DA	IM	NI	DA	IM	NI	DA	IM	NI	DA
Shah et al. [16]				>10,000			7,500			4,380		
				>17			12.5			7.3		
Corbin et al. [15]							1,300					
							2.6					
Azam et al. [17]				>20,000			2,300			4,900		
				>33			3.8			8.2		
O'Hare et al. [18]	>6,400	430	11.0	>6,400	>2,000	>200	1,050	50	7.4	880	15	1.1
	>25	33.1	13.8	>25	>150	>250	4	3.8	9.3	3.4	1.2	1.4
Burgess et al. [20]				>10,000		>1,000	810		18			
				>30		>746	2.5		13.4			
Manley et al. [21]	6,111	725		>7,000	>7,000		1,528	91		1682	38	
	9.0	29		>10	>280		2.3	3.6		2.5	1.5	
Weisberg et al. [19]	6,368	681		7,393	>10,000		1,583	80		1285	33	
	9.8	27.2		50	>40		2.4	3.2		2.0	1.3	
Bradeen et al. [22]	>6,400	430		>6,400	>2,000		1,050	50				
	>20	30.7		>20	>140		3.3	3.6				
Ray et al. [23]				10,000	8,000							
				22	>800							
Redaelli et al. [24]	8,953	182	6.3	9,221	697	137	1,370	39.2	8.1	927	7.8	1.6
	17	10.3	3.4	17	39	75	2.6	2.2	4.4	1.8	0.4	0.9
(continued)												

Q252H			Y253H			Y253F			E255K		
<div>IC₅₀ (nM)</div>			<div>IC₅₀ (nM)</div>			<div>IC₅₀ (nM)</div>			<div>IC₅₀ (nM)</div>		
Fold difference			Fold difference			Fold difference			Fold difference		
IM	NI	DA	IM	NI	DA	IM	NI	DA	IM	NI	DA
3120			8,940						>10,000		
5.2			15						>17		
			17,700						12,100		
			29.5						20		
1,325	70	3.4	>6,400	450	1.3	>6,400	430	11.0	5,200	200	5.6
5.1	5.4	4.3	>25	35	1.6	>25	33.1	13.8	20	15	7
			>10,000		10				>10,000		13
			>30		7.5				>30		10
						6,111	725		7,161	548	
						9.0	29		11	22	
						6,368	681		>6,000	566	
						9.8	27.2		>9	23	
1325	70		>6,400	450		>6,40	430		5,200	200	
4.1	5		>20	32		>20	30.7		16	14	
1,300	40		10,000	1,300					10,000	200	
2.9	>4		22	>20					22	>20	
734	46.8	5.6				8,953	182	6.3	3,174	118	10.3
1.4	2.6	3.1				17	10.3	3.4	6	6.7	5.6
F359V			L384M			H396R			H396P		
<div>IC₅₀ (nM)</div>			<div>IC₅₀ (nM)</div>			<div>IC₅₀ (nM)</div>			<div>IC₅₀ (nM)</div>		
Fold difference			Fold difference			Fold difference			Fold difference		
IM	NI	DA	IM	NI	DA	IM	NI	DA	IM	NI	DA
1,400						5,400			4,300		
2.8						11			8.6		
			2,800								
			4.7								
1,825	175	2.2				1,750	41	1.3	850	41	0.6
7.0	13.5	2.8				6.7	3.2	1.6	3.3	3.2	0.8
						39					
						1.6					
1,509	91	2.7	674	41.2	4	2,058	55	3.0	1,280	43	2.0
2.9	5.2	1.5	1.3	2.3	2.2	3.9	3.1	1.6	2.4	2.4	1.1

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EACH PATIENT IS AN INDIVIDUAL

Inhibitor efficacy is likely to vary from patient to patient depending on factors like compliance, interaction with food or other medications, and inherited factors. Nonadherence or intermittent adherence to the prescribed imatinib dose was recently shown, by two independent studies, to be significantly correlated with poorer responses [52, 53]. Food has been shown to influence nilotinib bioavailability [54]. Interactions with cytochrome p450 (CYP) isoform 3A4 and 3A5 (CYP3A4, CYP3A5) enzyme inducers (dexamethasone, phenobarbital, progesterone, rifampin) are known to be able to decrease imatinib (and also nilotinib) plasma concentrations to subtherapeutic levels [55]. Even more importantly, pharmacogenomics postulates that a significant degree of interpatient variability in therapeutic response lies in single nucleotide polymorphisms influencing the expression level and/or activity of drug-metabolizing enzymes and drug transporters. This has been shown to also hold true in patients treated with imatinib, because correlations between specific CYP3A5, ABCB1/MDR1, ABCG2, and hOCT1 genotypes and response have been reported [53, 56–59].

CONCLUSIONS

Taken together, these considerations warn that any IC_{50} table is an imperfect tool to guide second- or subsequent-line TKI selection. An interesting attempt to recalculate values correcting for the pharmacokinetic parameters (plasma concentrations) of dasatinib and nilotinib was recently published [60]; however, the other herein discussed limitations still remain.

Thus, at present, there is a small, definitive number of mutations for which clinical experience has already confirmed that a higher IC_{50} indeed corresponds to a relevant degree of in vivo insensitivity: V299L, T315I, T315A, and F317L/V/I/C mutations for dasatinib; Y253H, E255K/V, T315I, and F359I/V/C mutations for nilotinib.

Detection of one of the mutations listed above at the time of failure or suboptimal response to imatinib is currently an indication for switching to one inhibitor rather than the other. Currently, direct sequencing is the gold stan-

dard for Bcr-Abl KD mutation screening; the fact that these critical mutations are limited to seven residues only theoretically facilitates the development and routine application of highly sensitive mutation detection strategies aimed at early identification of the determinants of resistance to dasatinib and nilotinib. This is currently being attempted and evaluated by some groups [61–65]. If these strategies are proven to increase the diagnostic window for the detection of emerging mutated clones and to reliably anticipate resistance, thus allowing for earlier, more effective therapeutic interventions, they would find their place in routine mutation assessment. So far, however, the clinical impact of high-sensitivity Bcr-Abl KD mutation detection has surprisingly proven questionable. Retrospective studies in newly diagnosed patients [64] as well as in patients in complete CyR [65] have suggested that mutations found in rare Ph^+ cells are not necessarily selected; hence, their detection does not always correlate with subsequent treatment failure, probably because it is impossible to predict whether or not these cells represent a clone capable of sustaining long-term hematopoiesis and outcompeting unmutated one(s).

For many other mutations, physicians should be aware that an IC_{50} -based prediction of whether dasatinib or nilotinib will be more effective may not always lead to the expected clinical response. In these cases, it is wiser to adopt a decision algorithm weighing the Bcr-Abl mutation status no more than other equally important clinical insights, like patient history, risk factors, and comorbidities.

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