Mechanisms of Resistance to Imatinib and Second-Generation Tyrosine Inhibitors in Chronic Myeloid Leukemia

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Abstract Targeted therapy in the form of selective tyrosine kinase inhibitors (TKI) has transformed the approach to management of chronic myeloid leukemia (CML) and dramatically improved patient outcome to the extent that imatinib is currently accepted as the first-line agent for nearly all patients presenting with CML, regardless of the phase of the disease. Impressive clinical responses are obtained in the majority of patients in chronic phase; however, not all patients experience an optimal response to imatinib, and furthermore, the clinical response in a number of patients will not be sustained. The process by which the leukemic cells prove resistant to TKIs and the restoration of BCR-ABL1 signal transduction from previous inhibition has initiated the pursuit for the causal mechanisms of resistance and strategies by which to surmount resistance to therapeutic intervention. ABL kinase domain mutations have been extensively implicated in the pathogenesis of TKI resistance, however, it is increasingly evident that the presence of mutations does not explain all cases of resistance and does not account for the failure of TKIs to eliminate minimal residual disease in patients who respond optimally. The focus of exploring TKI resistance has expanded to include the mechanism by which the drug is delivered to its target and the impact of drug influx and efflux proteins on TKI bioavailability. The limitations of imatinib have inspired the development of second generation TKIs in order to overcome the effect of resistance to this primary therapy. (Clin Cancer Res 2009;15(24):7519–27)

Chronic myeloid leukemia (CML) results from the balanced translocation of *c-ABL* from chromosome 9 and *BCR* on chromosome 22 leading to the formation of BCR-ABL1 chimeric oncoprotein, the product of the *BCR-ABL1* hybrid gene, with constitutive tyrosine kinase activity (1, 2). Deregulated BCR-ABL1 activity results in enhanced cellular proliferation, and resistance to apoptosis and oncogenesis (3, 4). CML naturally progresses through distinct phases from early chronic phase to an intermediate accelerated phase followed by a terminal blast phase. Imatinib, the first tyrosine kinase inhibitor (TKI) approved for the treatment of CML (5), is a phenylaminopyridimine, which principally targets the tyrosine kinase activity of BCR-ABL1, exclusively binding to BCR-ABL1 in the inactive conformation in addition to inhibitory effects on KIT, ARG, and PDGFR kinases (6). The recent update of the phase III randomized IRIS study (International Randomized Study of Interferon-α plus Ara-C versus STI571) prospectively comparing imatinib with interferon-α and cytarabine in previously untreated patients in first chronic phase showed the best observed rate for a complete cytogenetic response [CCyR; or an undetectable number of Philadelphia chromosome positive (Ph+) chromosomes by conventional metaphase analysis] on imatinib of 82% at 6 years (7), with a declining annual rate of progression as the molecular response improved with time.

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Clinical Resistance to TKIs

In order to best determine an individual's response to therapy, an operational set of goals, defined within specific time periods have been established for all patients (Table 1; ref. 8). An initial requirement is the achievement of a complete hematological response (CHR), accepted as a normal peripheral blood count within 3 months of imatinib. Further response to treatment is subsequently monitored by sequential cytogenetic assessments of the bone marrow with the aim to achieve a CCyR by 18 months. Subsequent evaluation of the therapeutic response is recommended by means of molecular analysis, with reverse-transcriptase polymerase chain reaction (RT-PCR). Patients that achieve a major molecular response (MMR) equivalent to a reduction in BCR-ABL1 transcripts to less than 0.1% as defined on the international scale (9), are predicted to have a remarkably low risk of disease progression. Within the framework of recommendations, proposals for the definition of failure and suboptimal response are now recognized (8). Resistance to imatinib encompasses failure to reach CHR, CCyR, and MMR within an allocated duration of time (primary resistance). A number of

Table 1. Response criteria for CML in first chronic phase treated *de novo* with imatinib

Imatinib Response Criteria For CML In First CP			
Time from the Start of Imatinib (Months)	**Failure of Response**	**Suboptimal Response**	**Warning Sign**
At presentation	N/A	N/A	High-risk Sokal score; del 9q+; ACA in Ph+ cells
3	No hematological response (stable disease or disease progression)	<CHR	N/A
6	<CHR, no CyR	<PCyR (Ph+ >35%)	N/A
12	<PCyR (Ph+ >35%)	<CCyR	<MMR
18	<CCyR	<MMR	N/A
At anytime	Loss of CHR	ACA in Ph+ cells	Any increase in BCR-ABL1 transcripts
	Loss of CCyR	Loss of MMR	ACA in Ph- cells
	ABL KD mutation insensitive to imatinib	ABL KD mutation with some sensitivity to imatinib	

NOTE: Adapted from Baccarani et al. (8). © the American Society of Hematology.
Abbreviations: CP, chronic phase; ACA, acquired cytogenetic abnormalities; N/A, not applicable; CyR, cytogenetic response; PCyR, partial cytogenetic response; KD, kinase domain; Ph-, Philadelphia chromosome negative.

patients still do not succeed in obtaining a CHR, 20 to 25% do not achieve a CCyR (10), and fewer than 10% of patients achieve complete molecular response (CMR) (11). Loss of a previously obtained response to imatinib (secondary or acquired resistance) occurs in some 20 to 25% of patients that reach CHR and/or CCyR. Loss of CHR or a cytogenetic response have clear implications, however loss of MMR within the context of a sustained CCyR allows for less precise interpretation, in part because of a lack of standardization of RT-PCR assays and that any increment in transcript number should be confirmed by serial analysis. Although it has been shown that subjects who achieve CCyR, but not MMR have no impact on overall survival, it has been clearly shown that a lack of MMR leads to a loss of CCyR in a proportion of patients (12, 13). It remains important to rationalize these data in order to choose the best therapeutic course for each patient in the context of an increasing number of available therapeutic options aside from imatinib.

The commercially available second generation TKIs (2G-TKI), dasatinib and nilotinib, are both effective in imatinib failure. Dasatinib, a dual SRC/ABL kinase inhibitor that binds to the ABL kinase domain irrespective of the configuration of the activation loop (14) also inhibits KIT and PDGFR receptors, as well as being 325-fold more potent than imatinib against unmutated BCR-ABL1 *in vitro*. Nilotinib, an orally available aminopyrimidine derivative, is a more specific inhibitor of ABL kinase binding to the inactive-closed conformation, and also inhibits KIT and PDGFR receptors and has been noted to have a 20-fold greater potency than imatinib (15). Recommendations for optimal responses to 2G-TKI following imatinib failure are currently in preparation by the European Leukaemia Net.

Mechanisms of Resistance

Clinical observations have shown that durable responses are more easily attainable in the chronic phase of CML in comparison to the more advanced phases. Further work on both primary cells as well as resistant cell lines identified a number of mechanisms by which resistance to imatinib arises, namely amplification of BCR-ABL1, overexpression of the multidrug-resistant P-glycoprotein (MDR-1), BCR-ABL1, and the emergence of mutations in the ABL-kinase domain as well as the development of BCR-ABL1-independent pathways of signal transduction (Table 2). Gene expression profiling at presentation may also be predictive of imatinib response and distinguish primary from secondary resistance (16).

Oral Bioavailability

From a clinical perspective, in order for a drug to be effective it is required to reach its target. Imatinib is an oral medication and influenced in the first instance by the adherence or compliance of the patient to imatinib therapy. Following ingestion, imatinib undergoes gastrointestinal absorption and first-pass metabolism. Imatinib is highly plasma protein bound and is affected by drug influx and drug efflux transporter mechanisms.

A subanalysis of the IRIS study identified wide interpatient exposure variability to imatinib (coefficient of variation, 40-60%), despite positive pharmacokinetic characteristics and complete oral bioavailability of 98% (17). Imatinib is metabolised through the cytochrome p450 system, with the isoenzyme CYP3A4 mainly implicated. Intrinsic variability of CYP enzyme activity and co-medication that may influence CYP3A4 isoenzyme activity can also contribute to the variability in imatinib concentrations. Imatinib trough level plasma samples, at a level at or higher than 1,000 ng/mL, obtained after 29 days of treatment were found to correlate with an improved rate of CCyR, MMR, and event-free survival (17). However, no apparent difference in the frequency of dose-escalation was observed between the extremes of

PK values, and at the present moment, the role of imatinib plasma levels remains exploratory and yet to be fully defined in clinical practice. Similarly CYP3A4 plays a major role in dasatinib and nilotinib clearance (18, 19).

Plasma-protein Binding

Imatinib is approximately 95% bound to plasma proteins, mainly to albumin, as well as α-1 acidglycoprotein (AGP), a hepatic acute-phase protein (20). It was proposed that AGP can bind to imatinib in the plasma and hence decrease the availability of free or active drug. Other investigators have not confirmed the AGP binding as a mechanism for imatinib resistance (21), regardless of the dose of imatinib (22), and currently the influence of AGP as a cause of imatinib resistance remains doubtful. Following ingestion both nilotinib and dasatinib are rapidly absorbed (23). Based on *in vitro* analyses, plasma protein binding is approximately 98% for nilotinib and 96% for dasatinib.

Intracellular Availability of TKIs

ATP-binding cassette transporter family. Multidrug efflux transporters of the ATP-binding cassette (ABC) transporter family, which include the multidrug resistance gene product P-glycoprotein (P-gp; *ABCB1*), and the breast cancer resistance protein (*ABCG2*), may have a significant effect on restricting drug uptake from tumor cells through active efflux (24). In addition to high expression on hematopoietic primitive cells, both ABCB1 and ABCG2 show tissue localization in the small bowel, brain (25), testes, and canalicular membrane of hepatocytes and may contribute to imatinib resistance by causing drug efflux from cells from these sites (26). The ABCB1 transporter or MDR-1 is overexpressed in cells from patients in blast phase and implicated both in the reduced efficacy of chemotherapy in advanced-phase disease (27), as well as resistance to imatinib (28). The significance of the role of ABCB1 in imatinib resistance has yet to be fully clarified, as the efflux of imatinib from ABCB1-expressing cells is less pronounced than the efflux of cytotoxic drugs and observers have not been able to correlate ABCB1 overexpression in cell lines to imatinib resistance (29), although this has not been confirmed in other studies (30). From a clinical perspective, conflicting results are again in evidence as ABCB1 overexpression has been shown to be associated with a lack of MCyR as well as progression to advanced phase (31), however, inhibition of MDR1 has not been proven to enhance the effect of imatinib against BCR-ABL1 activity (32). ABCG2, provides a further mechanism for imatinib efflux, but imatinib seems to be an inhibitor of, but not a substrate for, ABCG2, and, therefore, ABCG2 does not modulate intracellular concentrations of imatinib in primitive CML cells (33). Nilotinib has been identified as a substrate of P-gp (the product of the *MDR-1* gene) in nilotinib-resistant cell lines (34) and of ABCG2 (35), however, resistance through ABCG2 may not be observed at clinically relevant levels of nilotinib (35, 36). *In vitro* studies suggest that cellular delivery of dasatinib is predominantly a passive process, unlike imatinib (37), and is also limited by active efflux because of ABCB1 (38) and ABCG2 (25, 37), but of interest, no significant difference was found in the amount of dasatinib unabsorbed in the gastrointestinal tract in ABCB1 knockout and wild-type mice (39).

OCT-1

The human organic cation transporter (hOCT-1; SLC22A1) has been advocated as a significant factor affecting intracellular drug availability through inhibition of imatinib influx (40, 41), and polymorphisms of *OCT-1* may alter the entry of imatinib into cells through this transporter mechanism (42). Other observers have suggested OCTN2 as a more effective transporter of imatinib and a greater role for the organic anion transporting polypeptide OATP1A2 (43). However, a high OCT-1 activity has been observed to be predictive of a MMR and patients with low OCT-1 activity seem to require a higher imatinib dosage in order to achieve an optimal response (44). Furthermore, there is no significant difference in the proportion of patients achieving MMR based on trough imatinib levels in patients with high OCT-1 activity, indicating that OCT-1 activity is not a surrogate marker of imatinib pharmacokinetic values (45). Importantly, neither dasatinib (37) nor nilotinib cellular uptake is significantly affected by OCT-1 activity (46), which in turn exhibits less interpatient variability. Recently single-nucleotide polymorphisms have been reported in genes involved in the pharmacogenetics of imatinib, and specific genotypes of *ABCB1* (presence of the G allele in 2677G > T/A; ref. 47), ABCG2 (rs2231137), and HOCT-1(rs683369) were associated with poor response to imatinib therapy (48). Examining drug influx and drug efflux properties at presentation prior to therapeutic intervention, may give insight from the start of therapy about an expected response and potentially provide a strategy for the use of a particular TKI in order to achieve the best outcome for an individual patient.

Table 2. Classification of TKI resistance

BCR-ABL1 Independent	BCR-ABL1 Dependent
Patient related	Increased expression of BCR-ABL1
Poor compliance	Mutations in the ABL-kinase domain
Pharmacological	
Poor intestinal absorption	-
Drug interactions	-
Binding with plasma components	-
Leukaemia cell related	
Heterogeneity of CML cells	-
Reduced levels of transporter (hoct1)	-
Increased levels of exporter (ABCB1, ABCG2)	-
QSCs	-
Clonal evolution	-
SRC overexpression	-

Clonal Evolution

Chromosomal abnormalities in the Ph+ population following presentation, defined as clonal evolution, usually indicate transformation to a more advanced phase (49) and are shown in up to 80% of patients. The most common cytogenetic aberrations include, in order, an additional Ph+ chromosome, trisomy 8, and isochromosome 17q (50). Clonal evolution is associated with a reduced response to imatinib with regard to cytogenetic response, increased hematological relapse (51), and subsequent reduction in overall survival (52), and is proposed to reflect the genetic instability of the highly proliferative CML progenitors associated with CML progression (53). Hematological resistance to TK inhibition has been reported to be more common with clonal evolution (58%) rather than the presence of BCR-ABL1 mutations (45%; ref. 54). The incidence of clonal evolution is further emphasized in blast phase disease (73%) in comparison to the frequency of kinase domain mutations (30%). Conversely, BCR-ABL1 kinase domain mutations are more prevalent during imatinib failure in those patients that exhibit clonal evolution (58%) than those with Ph+ metaphases as the sole anomaly (28%; ref. 55).

SRC Overexpression

SRC-family kinases play a pivotal role in signaling through surface receptors on hematopoietic cells, and of the nine members of the SRC family, HCK, FGR, and LYN are primarily expressed on myeloid cells (56) and can also be activated by BCR-ABL1 kinase (57). Imatinib resistance and progression to, in particular, lymphoid blast phase has been suggested to be mediated through LYN and HCK upregulation (58, 59). Imatinib-resistant cell lines have shown greatly increased expression of LYN, which were susceptible to apoptosis following treatment with a SRC inhibitor (60). SRC-family kinases are also implicated in imatinib resistance by virtue of stabilizing the active conformation of BCR-ABL1 to which imatinib is unable to bind (61). Similarly, an increase of LYN expression has accompanied failure of nilotinib treatment in CML patients (34). Targeting both BCR-ABL1 and LYN kinases may be required in resistant CML, and dasatinib has also been shown to be effective in imatinib resistance consequent to BCR-ABL1-independent LYN activation (62).

Quiescent Stem Cells

Despite the remarkable results obtained with imatinib, disease persistence is detected in the majority of patients. The failure of imatinib to eradicate all malignant cells may be as a consequence of the inherent insensitivity of quiescent CML cells to imatinib. These primitive leukemic CD34+CD38- cells, which have entered the G0-phase of the cell cycle and are therefore quiescent, account for less than 1% of total CD34+ cells present at diagnosis (63), and it is this quiescent fraction that is postulated to sustain the disease with the constant potential to escalate. The resistance of quiescent stem cells (QSC) seems multifactorial and includes altered drug influx or efflux mechanisms (a marked reduction in the expression OCT1 and an elevated expression of ABCB1 and ABCG2; ref. 64), increased *BCR-ABL1* transcript levels in the absence of *BCR-ABL1* gene amplification (63), and decreased BCR-ABL1 transcript degradation. Imatinib has recently been found to restore CXCR4 expression, recognized to be associated with cell migration defects in CML and down-regulated by BCR-ABL1 overexpression, thereby promoting the migration of CML cells to bone marrow stroma, causing G0-G1 cell cycle arrest and preserving the survival of quiescent CML progenitor cells (65).

Dasatinib, although able to induce significant inhibition of CrKL phosphorylation in CD34+CD38- cells in comparison to no effect with imatinib and to inhibit an earlier progenitor population, remains ineffective in eradicating the most primitive QSC population (cells that retain maximal carboxyfluorescein succinimidyl ester fluorescence; refs. 63, 66). Similarly, the quiescent fraction shows resistance to nilotinib, and furthermore, is noted to increase with the combination of nilotinib and imatinib secondary to antiproliferative and nonapoptotic effects (67). BMS-214662, a cytotoxic farnesyl transferase inhibitor that preferentially lyses nonproliferating cells, has been found to effectively induce apoptosis of CML progenitor cells and to synergize with imatinib or dasatinib (68), however clinical trials with this agent are not currently forthcoming and other non-BCR-ABL1 kinase approaches to eradicate the stem cell pool continue to be explored.

A number of difficulties remain with the concept of QSC. Residual disease is commonly observed in most patients, and QSC are present in inadequate numbers to account for this level of detection. Point mutations occur commonly during cell proliferation and as such should theoretically become sensitive to targeted therapy as they exit G0. Nevertheless, concerted efforts to target this potential disease reservoir continue and include growth-factor induction of the cell-cycle (69) and immunological approaches (70).

Increased Expression of BCR-ABL1

Amplification of *BCR-ABL1* occurs more commonly in advanced-phase disease (71) and was first reported in 3 of 11 patients with blast phase CML or Ph+ acute lymphoblastic leukemia who developed acquired resistance to imatinib (72). It is unclear whether these findings are as a direct result of increased expression of the BCR-ABL1 protein (73), or as a result of other factors implicated in transformation and imatinibresistance, such as point mutations in the ABL-kinase domain as documented in one of the three cases, or as a consequence of increased genomic instability. However, in a subsequent study of 66 imatinib resistant patients, only 2 patients showed *BCR-ABL1* gene amplification (74) and overexpression of *BCR-ABL1* is understood to account only for the minority of cases of imatinib resistance. Still, it would seem that the level of BCR-ABL1 protein is closely associated with the pace of the emergence of imatinib-resistant mutant subclones. Cells expressing a high level of BCR-ABL1 have been observed to be far less sensitive to imatinib and

more rapidly yield imatinib-resistant mutant subclones than cells with low BCR-ABL1 expression levels (75). Similarly, resistance to nilotinib *in vitro* has also been found consequent to BCR-ABL1 overexpression *in vitro* (34).

ABL Kinase Domain Mutations

The emergence of mutations within the kinase domain of *BCR-ABL1* is regularly associated with resistance to TKI therapy. The most frequently described mechanism of acquired resistance to imatinib is the occurrence of point mutations, representing a single aa substitution in the kinase domain, which impair drug binding by affecting essential residues for direct contact with the TKI or by preventing BCR-ABL1 from assuming the inactive conformation appropriate for imatinib binding. The published incidence of mutations remains variable and in the order of 40 to 90% as a consequence of different methods of detection, nature of resistance, and disease phase examined (76). Mutations were first identified in 2001, in which restoration of BCR-ABL1 signal transduction on imatinib therapy was associated with a T315I mutation (72). Thr315 forms a fundamental hydrogen bond with imatinib, disrupted by a single aa change with a bulkier isoleucine, which prevents imatinib localization within the ATP binding pocket by consequent stearic hindrance. The T315I mutation is one of the most frequent mutations arising in patients on imatinib therapy, occurring between 4 to 19% of resistant cases (55, 77, 78) and is resistant to all ABL kinase inhibitors. Although the T315I mutation is generally accepted as conferring a poor outcome (median survival 12.6 months; refs. 79, 80), sustained cytogenetic responses despite accelerated phase and during therapy with a second TKI have recently been reported (78).

Four categories of mutations have been recognized to correlate with clinical resistance to imatinib affecting the: (i) imatinib binding site, (ii) P-loop (ATP binding site), (iii) catalytic (C) domain, and (iv) activation (A) loop (2). Mutations in the phosphate (P-loop; residues 244-255 of ABL), which account for up to 48% of all mutations in imatinib resistant cases (81), destabilize the conformation required for imatinib binding, and have been associated with an increased transforming potential (82) and a worse prognosis regardless of their sensitivity to imatinib (77, 81, 83, 84). P-loop mutations have been reported to be associated with a worse prognosis in comparison with other categories of mutations (81, 83), however, other observers have not confirmed these findings (55), perhaps because of the nature of the criteria used to select patients for mutation screening. Another potential explanation for this inconsistency may be on account of the M244V mutation, which may not confer a poor outcome and has been variably included in the P-loop categories of mutations (84). A series of mutations are located in the catalytic domain (residues 350-363 of ABL) and can also affect imatinib binding. The activation loop of the ABL kinase is the major regulatory component of the kinase domain and can adopt an open and/or active or closed and/or inactive conformation. Mutations in the activation loop instigate the open and/or active configuration, and as the inactive and/or closed configuration is required for imatinib activity, resistance occurs. Nevertheless, aa substitutions at only seven residues [M244V, G250E, Y253F/H, E255K/V (P-loop), T315I (imatinib binding site), M351T, and F359V (catalytic domain)] account for 85% of all resistance-associated mutations (80).

Although point mutations have been more frequently described in TKI resistance and advanced-phase CML (Table 3), they have also been documented prior to TKI therapy (85), inherently suggesting that pre-existing mutations do not acquire a survival advantage until subjected to a TKI. In addition, investigators have found no difference in mutational status in those patients who have relapsed (74). The relevance of these observations remains unclear, specifically about whether certain mutations are responsible for disease progression or whether they occur as a consequence of the underlying genomic instability linked with advanced phase disease (86). It would seem that gain-of-function mutations may independently contribute to disease progression, whereas

Table 3. Frequency of ABL-kinase domain mutations by disease phase

KD Mutation	No. of Mutations*	No. of CP (%)†	No. of AP (%)*	No. of BP (%)*
P-loop‡				
M244	47	33 (70)	1 (2)	13 (28)
L248	13	10 (77)	2 (15)	1 (8)
G250	63	31 (49)	6 (10)	26 (41)
Q252	14	3 (21)	3 (21)	8 (58)
Y253	68	23 (34)	9 (13)	36 (53)
E255	63	17 (27)	12 (19)	34 (54)
IM binding site				
D276	12	6 (50)	2 (17)	4 (33)
F311	5	2 (40)	1 (20)	2 (40)
T315	56	9 (16)	12 (23)	35 (63)
F317	15	10 (67)	2 (13)	3 (20)
Catalytic domain				
M351	62	33 (53)	12 (19)	17 (28)
E355	22	13 (59)	4 (18)	5 (23)
F359	35	21 (60)	5 (14)	9 (26)
Activation loop				
H396	29	21 (72)	2 (7)	6 (21)
C-terminal lobe				
S417	3	2 (67)	1 (33)	0 (72)
E459	6	2 (33)	0 (72)	4 (67)
F486	8	0 (0)	1 (13)	7 (88)

Note: Adapted from Apperley (100).
Abbreviations: KD, kinase domain; CP, chronic phase; AP, accelerated phase; BP, blast phase; IM, imatinib.
*Number of mutations detected in a pool of patients reviewed in Apperley (100). Infrequently an individual patient harbored more than one KD mutation; any detected- mutation is included in the table.
†Percentage of all KD mutations detected related to disease phase.
‡P-loop mutations have been inconsistently reported to be associated with a worse prognosis in comparison with other categories of mutations (55, 81, 83). Furthermore, the M244V mutation may not confer a poor outcome and has been variably included in the P-loop categories of mutations (84).

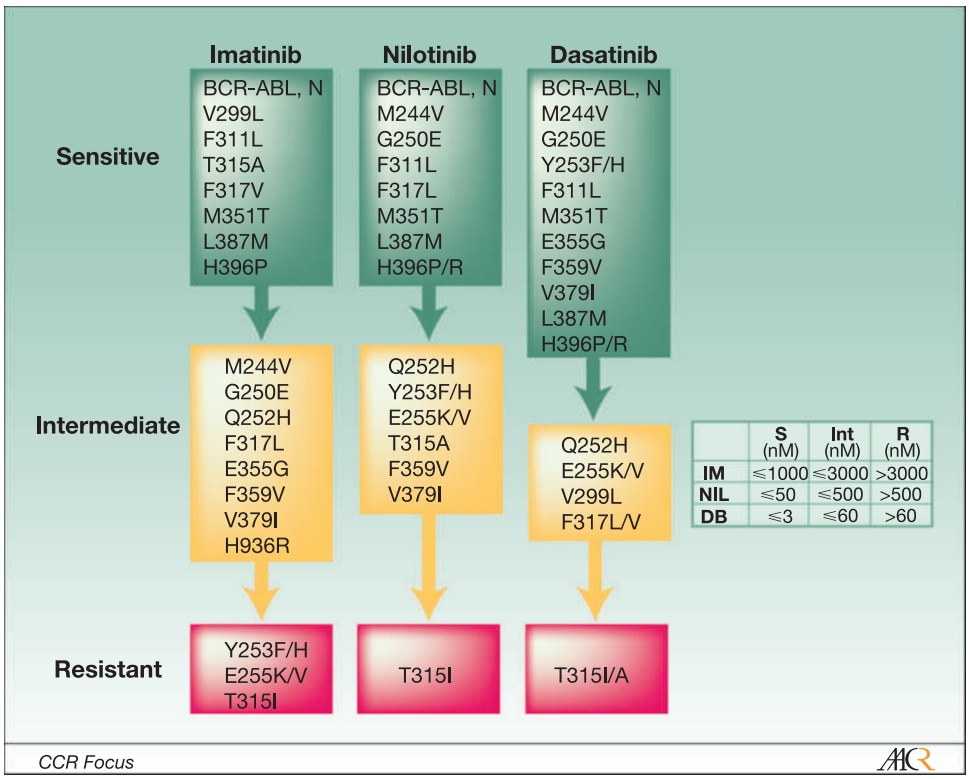

Fig. 1. Although equivalent experimental systems have been employed in a variety of assessments to determine BCR-ABL1 kinase domain mutation sensitivity on the basis of IC_{50} values (89, 98), different incubation times and TKI concentration ranges have been used as well as varying methods to measure cell viability and proliferation. Color-coded schemes to indicate TKI sensitivity based on *in vitro* analyses should be interpreted with clinical caution as *in vitro* findings cannot be directly extrapolated to the clinical setting. (Figure adapted from O'Hare et al. 89, © the American Society of Hematology).

loss-of-function mutations are more often subject to selective pressure by imatinib (82, 87). Specific mutations considerably affect the transformation potency of BCR-ABL1, and *in vitro* studies have indicated relative transformation potencies of mutations from distinct sections of the kinase domain to be: Y253F > E255K (P-loop) > unmutated BCR-ABL1 ≥ T315I (imatinib binding site) > H396P (activation loop) > M351T (catalytic domain; ref. 82). Particular mutations, as in the case of E255K, are noted to have increased oncogenic potency despite reduced kinase activity compared with unmutated BCR-ABL1 (88). The proliferative advantage of a given mutant seems multifactorial and determined by intrinsic kinase activity, substrate specificity, and extrinsic factors including growth factors and cytokines.

Although most of the clinically relevant mutations are inhibited by dasatinib and nilotinib, with the exception of T315I (Fig. 1; ref. 89), the presence of existing mutations after imatinib failure, as well as development of new mutations on a subsequent second TKI is naturally a potential source of resistance to successive TKI (90–93). The influence of baseline BCR-ABL1 mutations on response to nilotinib in patients with imatinib-resistant CML in chronic phase has shown an inferior outcome in patients who harbored mutations that were less sensitive to nilotinib *in vitro* (Y253H, E255V/K, F359V/C; ref. 94). Recently, the selective pressure of sequential TKI therapy has been assessed in the outcome of imatinib-resistant patients already harboring imatinib-resistant kinase domain mutations subsequently treated with an alternative TKI on a second or even third occasion and showed that 83% of cases of relapse after an initial response were associated with the emergence of newly acquired mutations (95). The T315I mutation was most commonly implicated with a frequency of 36% (95). The inability to achieve a sustained cytogenetic response could in part be as a consequence of the development of new therapy-resistant kinase domain mutations as patients are exposed to sequential TKIs, although some of the arising mutations were reported as having a relatively good *in vitro* sensitivity to the concurrent TKI (96).

In summary, the consequence of identifying a mutation remains unclear and seems relevant only according to the disease phase and response, with a greater impact in advanced phase CML in which the mutated clone may be responsible for disease progression, but less certain in cases of on-going response to TKI therapy. Resistance mechanisms may be overcome with imatinib dose escalation (97), alternative therapy with a 2G-TKI (98) to which the mutant has documented sensitivity, withdrawing TKI therapy to allow the mutant clone to recede (99), as well as non-BCR-ABL1-dependent therapies.

Conclusions

Targeted molecular therapy has afforded exceptional clinical responses in the majority of patients with CML to the extent that therapeutic regimens have centered on the achievement of a MMR, early within the start of therapy. As most will continue on imatinib in CCyR, the emphasis has diverted to overcoming imatinib resistance and the

generation of alternative TKI engineered to surmount these clinical challenges. The cause of resistance to TKI therapy is likely to be multifactorial, but ultimately the clinical response is influenced in most part by leukemia-related factors and the phase of the disease. Further investigation into the underlying causes of TKI resistance remain mandatory in order to best direct appropriate therapy in this subset of patients.

Disclosure of Potential Conflicts of Interest

D. Milojkovic, J. Apperley, consultants, honoraria, Novartis, Bristol-Myers-Squibb.

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