Overcoming Resistance in Chronic Myelogenous Leukemia

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OVERVIEW

Resistance in chronic myelogenous leukemia is an issue that has developed in parallel to the availability of rationally designed small molecule tyrosine kinase inhibitors to treat the disease. A significant fraction of patients with clinical resistance are recognized to harbor point mutations/substitutions in the Abl kinase domain, which limit or preclude drug binding and activity. Recent data suggest that compound mutations may develop as well. Proper identification of clinical resistance and prudent screening for all causes of resistance, ranging from adherence to therapy to Abl kinase mutations, is crucial to success with kinase inhibitor therapy. There is currently an array of Abl kinase inhibitors with unique toxicity and activity profiles available, allowing for individualizing therapy beginning with initial choice at diagnosis and as well informed choice of subsequent therapy in the face of toxicity or resistance, with or without Abl kinase domain mutations. Recent studies continue to highlight the merits of increasingly aggressive initial therapy to subvert resistance and importance of early response to identify need for change in therapy. Proper knowledge and navigation amongst novel therapy options and consideration of drug toxicities, individual patient characteristics, disease response, and vigilance for development of resistance are necessary elements of optimized care for the patient with chronic myelogenous leukemia.

The treatment of chronic myelogenous leukemia (CML) has seen unprecedented discoveries and advances in diagnostics, molecular pathogenesis, and therapeutics over the past 50 years. Following the initial discovery of the unique and singular genetic event causative in CML—genisis of the Philadelphia chromosome (Ph), first recognized by Nowell and Hungerford in 1960—a sequence of subsequent discoveries set the stage for such progress. At the cytogenetic level, the subsequent recognition of a 9:22 translocation by Janet Rowley marked the next advance and facilitated the unraveling of the location and oncogenic potential of Bcr-Abl by Heisterkamp, Groffen, Daley, and others. In the therapeutic arena, conceptualization and realization of small molecule inhibitors for Ph+ leukemias, pioneered by Brian Druker and others, gave rise to a dramatic paradigm shift in the prognosis and approach to chronic phase CML. For the majority of patients, chronic phase CML is an extremely treatable disease with an excellent prognosis that is projected to morph into a “functional cure.”

At each point along the way, such discoveries have led to increasingly granular understanding of the means by which Ph+ clonal diseases become resistant to therapy. Once the hallmark cytogenetic event t(9;22) was well understood, classic cytogenetics alone could identify clonal evolution. Genetic instability beyond the discrete Ph+ rearrangement amid the clonal populations in Ph+ leukemias manifest as a “other” non-Ph genetic anomalies in cells that have the Ph chromosome. Common findings include trisomy 8 and isochromosome 17. Clonal evolution within the Ph+ clonal population remains a poor prognostic feature and sign of accelerated or progressive disease.

With the advent of the small molecule inhibitors of Bcr-Abl—of which there are five now United States Food and Drug Administration approved: imatinib, nilotinib, dasatinib, bosutinib, and ponatinib—characterization of resistance has now, like the therapies themselves, turned the focus significantly onto the target Bcr-Abl. Years of research have led to identification of a host of amino acid substitutions, or missense mutations, that limit or prohibit the function of small molecule kinase inhibitors. Also, a number of insertions and deletions within or flanking the Bcr-Abl coding region are known and their effect is uncertain. A select group of such kinase domain mutations, based on their predicted ability to interfere with drug binding and Bcr-Abl confirmation, has taken the center stage with regard to resistance in CML in the tyrosine kinase inhibitor (TKI) era. Most recently, given the use of increasingly potent kinase inhibitors in CML, it is now observed that multiple mutations of the target Bcr-Abl—compound mutations—can be generated and may be the basis of even higher-level resistance than previously observed.

However, resistance in CML should be viewed broadly and encompasses a variety of potential areas for investigation, scrutiny, and solution; this is portrayed conceptually, yet simply, in Fig. 1. At the drug, patient, leukemic cell, and oncogene levels, understanding has increased, and overcoming resistance in CML is possible. Fortunately, response to TKI
therapy for patients with chronic phase disease is astonishingly good with proven first-line options (imatinib, nilotinib, and dasatinib).\textsuperscript{11-13} Salvage is increasingly possible with more alternatives (bosutinib, ponatinib, and omacetaxine), and investigation continues into how to best subvert resistance and how to pursue a path to cure.\textsuperscript{14-16} The fraction of resistance in the short term is decreasing; however, we must prepare for the challenge of increasingly larger numbers of patient with CML thrust into remission and in need of a proper long-term strategy.

**TAKING A STEP BACK AND CONSIDERING DIFFERENT CAUSES OF RESISTANCE**

In CML currently, well into the era of multiple oral small molecule Abl kinase inhibitors, resistance may be driven by a variety of disease-independent factors. Given the ongoing treatment paradigm of chronic maintenance oral outpatient therapy and only speculation on the potential for finite therapy and sustained remission after discontinuation, less than optimal adherence to prescribed therapy is likely an under-recognized cause for resistance. Studies involving monitoring of patient behavior using microelectronic devices set in the TKI bottle tops showed adherence to therapy as a predictor of ability to retain complete cytogenetic response and that modest reduction in adherence (85%; $p = 0.0002$) can be significant.\textsuperscript{17} Perception of stable remission equating to cure, under-management of chronic adverse events, or simply forgetting are all likely contributors to the “human nature” effect on outcome in CML.

Once ingested, delivery of a TKI to its intended target can be hampered by limited absorption, metabolic variance because of concomitant medications, differing efficiency of drug transport into the leukemic cell, and other factors. The use of proton-pump inhibitors and H2 antagonists can significantly affect the absorption of dasatinib in particular and nilotinib to a lesser degree.\textsuperscript{18,19} All of the TKIs are metabolized by the hepatic p450 cytochrome system, and coprescription of medications that induce or inhibit relevant hepatic pathways is high and warrants careful attention.\textsuperscript{20} One example of particular interaction is between azole antifungal therapy—commonly used for prevention of infection in patients with leukemia—and the TKIs used in Ph+ leukemia. The organic cation transporter 1 (OCT-1) is the major influx pump responsible for transport of imatinib into Bcr-Abl+ cells, and its activity has been shown to predict for molecular response and survival.\textsuperscript{21} The second-generation TKIs do not appear to be transported into the leukemic cell by this specific active transport and thus may not be affected by inter-patient differences in activity.\textsuperscript{22,23}

There are therefore a number of known and potential unknown factors that can preclude ideal delivery of small molecule inhibitors to their intended target. It is thus very important to consider such factors first when considering resistance in CML as the remedy. Instillation of proper supportive care to manage toxicity or simply deploying strategies to overcome human nature and forgetfulness common with chronic oral therapies may be quite straightforward.

**KINASE DOMAIN MUTATIONS: HOW, WHEN, AND WHY?**

As described, kinase domain mutations have been in central focus given their frequency and direct link to drug efficacy given their profile. They represent the most important disease-related factor in CML resistance. In multiple clinical studies, it is a consistent fraction (approximately 50% overall) of patients who, in the setting of clinical resistance, demonstrate Bcr-Abl kinase domain mutations.\textsuperscript{9} In turn, another half of patients with chronic phase and smaller proportion of patients with advanced disease Ph+ leukemia do not harbor such kinase domain mutations. It is clear from this consistent finding that resistance based in kinase domain mutation may be a strong phenotype, and selection and dominance of such resistant subclones in patients with CML is logical. However, the “missing piece” may be the genotype or molecular underpinning of clones capable of generating mutations.

Another important observation regarding mutations is the timing of their appearance. Studies have demonstrated that
although potentially expected to be observed only after the application of selection pressure with TKIs, Ph+ leukemias have a history and natural tendency that may include genesis of clones bearing the exact mutations that are TKI resistant before any TKI exposure.24 Also, despite the application of more potent TKIs at CML diagnosis, trials still report rare but rapid transformation of patients into myeloid or lymphoid blast crisis (more often the latter), suggesting that highly resistant clones may preexist and emerge rapidly.11-13,25 Lastly, the prevalence and tendency to develop mutations increase with advancing phases of Ph+ CML and are perhaps highest in Ph+ acute lymphoblastic leukemia, potential proof that the genesis of mutant clones is, in part, a function of the proliferation rate of the leukemia.26 Thus the assumptions regarding resistance based in kinase domain mutations are that (1) it may preexist and become rapidly evident, (2) its manifestation may be a function of the leukemia proliferation rate, (3) its clinical effect is related to effects on TKI sensitivity, and (4) it is only a partial explanation for what is observed clinically.

HIGHLY SELECTIVE RESISTANCE (T315I) AND COMPOUND MUTATIONS
Simultaneous with the report of imatinib’s remarkable efficacy came the first report of a highly selective kinase domain mutation as the basis of resistance in transformed Ph+ leukemia after imatinib, described by M. Gorre and C. Sawyers.27 Fast-forwarding a decade, we now have specific therapy able to overcome highly selective resistance. Rationally designed like its predecessors, AP24534, or ponatinib (iclisug), has been studied in patients with multi-TKI-resistant disease with excellent results, particularly in those with the T315I mutation, and was recently approved for all phases of Ph+ leukemia and resistance and intolerance to prior therapy.15 After a lengthy period of development, the protein synthesis inhibitor omacetaxine was also recently approved and provides another means to treat high-level resistance, albeit with more limited activity and based on an alternative mechanism of action that circumnavigates Bcr-Abl specificity.16 Multivariate analysis of patients on the ponatinib phase II PACE trial revealed that presence of the T315I mutation did not predict for response to ponatinib in Ph+ leukemia, whereas the lower number of prior therapies, younger age, and the dose intensity of ponatinib delivered were associated with higher response rates seen.28 This is consistent with the notion that the T315I mutation represented a highly drug-resistant mutation but not necessarily an unstable or transforming change in the resistant clone. A global study of the natural history of Ph+ leukemia bearing the T315I demonstrated the somewhat indolent nature and transforming potential of such cases.29 Lessons learned from such observations are that resistance can be complicated more from the kinetic potential and instability of the leukemia and the unchecked proliferation that drug-resistant mutations permit the presence of select mutations per se.

Preclinical data using ponatinib in a mutagenesis model of CML showed that at relevant concentrations, outgrowth of predictable mutant clones could be entirely avoided.30 Yet some patients’ disease indeed fail ponatinib, especially in more advanced phases of Ph+ leukemia. The basis of resistance in such heavily pretreated patients remains to be revealed. Although mutation-negative cases may exist, increasing evidence points toward compound mutations for further evolution of Ph+ clonal disease, where the Abl kinase domain may harbor more than one discrete substitution or other alteration. As noted earlier, a recent report analyzing patients with drug-resistant disease with multiple Abl mutations demonstrated that the majority of cases were actually compound mutations, with the T315I missense exchange being involved most frequently.10 Further reports have shown that compound mutations are likely observed increasingly with sequential therapy and may prove to be a new challenge in mutation-based resistance in CML.31

HOW IS RESISTANCE CURRENTLY DEFINED FOR CML?
Resistance in CML can be either primary or secondary and is declared when therapy fails to trigger response milestones or achieved response is lost. Evidence-based guidelines and recommendations have been developed and updated by both the European LeukemiaNet (ELN) and the National Comprehensive Cancer Network (NCCN) to guide in judging response and actions recommended at key times.32,33 Of most significance has been the addition of early molecular response as a crucial milestone and requirement for response. Many years ago, Hughes et al34 recognized and reported that reduction in Bcr-Abl transcripts to threshold levels of 1 or 2 logs below an untreated level at 3 or 6 months, respectively, predicted for the best odds of subsequent deeper molecular response—specifically the key threshold response of major molecular response (MMR). More recent longer-term analysis has shown significant prognostic value of Bcr-Abl transcripts below 10% at 3 months (the same as a one-log reduction from untreated baseline of 100%), with improved progression-free and overall survival in studies of imatinib therapy or optimized imatinib and imatinib combinations, among others.35,36 Early cytogenetic response remains a proper goal for CML therapy, with complete remission by 12 months firmly established and the likelihood that earlier complete cytogenetic remission may be superior and likely expected with greater use of the second-generation TKIs. Previously, failure to achieve cytogenetic response proved more ominous than failure to achieve optimal MMR, and cytogenetic response that was heading in the wrong direction (defined as suboptimal) often converted to a failure classification over time, exposing the patient to greater risk with time.37 Given the increased importance of early molecular response, less invasive (blood-based polymerase chain reaction) testing earlier appears to do what we keenly need to do: identify early the patients in whom likelihood of molecular remission and progression-free survival is severely diminished and in whom a change is needed. Emerging data shows

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3-month transcript levels to be predictive with second-generation TKIs.\(^38,39\) When considering resistance among patients initiated on second-generation TKIs, although the treatment options are more limited, there are significantly fewer patients lacking the 10% or less reduction in transcript.

Better guidelines have emerged regarding the use of mutation analysis and the categories of response (or lack thereof) that are likely to yield relevant fractions of patients with mutations.\(^40\) Although the most common finding in the setting of clinical resistance and associated structurally with kinase inhibitor resistance, Abl kinase domain mutations are limited in their ability to inform and drive treatment choice. Identification of the T315I mutation limits treatment options to ponatinib or less likely omacetaxine. Select mutations have been identified as less responsive to either nilotinib or dasatinib (and bosutinib, given their similar spectrum of activity) and can direct TKI choice. Mutation testing is viewed as being “informative” in approximately 30% of cases, increasing with more advanced stages of Ph\(^+\) leukemia given the greater propensity toward mutations in these cases.\(^26\)

**CURRENT APPROACH TO RESISTANCE: PREVENT IT!**

Increasingly the philosophy of managing CML is to—as practically and as safely as possible—elicit “faster and deeper” response. This is perhaps driven more by the observation that events related to resistance and loss of response appear early in the course of disease. With time, appropriately deep response appears to be increasingly stable and is now viewed as a potential opportunity to test treatment cessation to query for stable response without therapy—the “functional cure” converting perhaps to a real cure. Differences in survival, for example, in the comparative trials of nilotinib and dasatinib compared with imatinib are nascent or absent; it is the surrogate achievement of deep molecular response that has buoyed the front-line use of more potent TKIs.\(^12,13\) Current front-line trials will query if resistance can be further prevented by the use of ponatinib, carefully balanced with information about the toxicity profile of our most evolved TKI in the newly diagnosed CML population.

In the ENESTnd and DASISION trials, both the experimental arms led to lower rates of progression to advanced disease, the most overt form of resistance in CML.\(^12,13\) It is notable that progression events were seen in patients with lower-risk CML as assessed by the Sokal score, which stratifies patients’ disease risk and is predictive for response to TKI therapy. However, progression events were usually not observed in patients who achieved and maintained timely molecular response, lending credence to the philosophy of treating more aggressively to gain early, stable molecular response. The greatest difficulty is the lack of ability to predict which patients need intensified or alternative therapies to gain deep molecular remission and avoid progression. The NCCN guidelines for CML do include the advice to consider more potent TKIs at diagnosis for patients with intermediate- or higher-risk CML by Sokal (or other risk model) score.\(^33\) In the front-line trials of nilotinib and dasatinib, benefit was seen across all risk groups including patients with lower-risk disease. Results from the TIDEII trial recently reported that despite using higher-dose imatinib and early switch to nilotinib, early molecular remission was not as protective for patients who had less efficient drug influx in the leukemic cell (low OCT-1 activity).\(^41\) The trial concluded that drug levels (TKI trough levels), drug influx (OCT-1 activity), and early molecular response were predictive of subsequent molecular response. It remains to be determined how to best risk stratify a patient presenting with newly diagnosed CML to maximize response and minimize chance of resistance, which casts use of newer and more potent TKIs at diagnosis in a favorable light.

**HOW TO NAVIGATE IN THE FACE OF RESISTANCE**

In chronic phase, evidence does not point toward screening patients for resistance, in particular screening for kinase domain mutations. The ability to identify relevant populations amid wild-type Bcr-Abl and the predictive value, with either absence or presence of any detectable mutant clone, remains controversial.\(^42\) After initial treatment choice is made, early molecular monitoring is now well established as a patient-friendly and accurate way to identify patients at high risk for poor response and progression. Current NCCN guidelines and other reports de-emphasize bone marrow cytogenetics in favor of sequential molecular monitoring of patients with CML in early response to determine transcript reduction equivalents of complete cytogenetic response (CCyR), usually agreed to be a 2-log drop below untreated levels, and moreover MMR (3-log drop).\(^43\) If transcript kinetics (or classic cytogenetics) do not show CCyR by 12 months, NCCN guidelines recommend a change in therapy. MMR is a major landmark in the ELN guidelines and is considered optimal if achieved at 12 months and acceptable if achieved by 18 months.

Now with three second-generation TKIs (nilotinib, dasatinib, and bosutinib) and one third-generation TKI (ponatinib) approved for resistance to prior therapy, in the face of resistance deemed worthy of treatment change, there are several important factors to consider: (1) the patient’s previous TKI exposure, (2) the patient’s possible comorbidities and the risk of toxicity specific to the available choices of TKI, and (3) the results of resistance testing, in particular mutation analysis, and any informative mutation results. There are not contraindications to specific TKIs per se, except for a patient with a prolonged QT interval at screening who is being considered for nilotinib therapy. There are suggested avoidance strategies given the specific toxicity profile of available TKIs, such as for patients with a history of pancreatic disease (for nilotinib and ponatinib), cardiopulmonary disease (for dasatinib), vascular disease (for nilotinib), and so on. The emerging toxicities of approved TKIs, such as peripheral arterial occlusive disease for nilotinib and pulmonary arterial hypertension for
dasatinib, raise concern over long-term exposure and require further clarification as to prevalence and association with drug.\textsuperscript{44,45} The risk of such potential adverse events must be contrasted with the benefit of a particular choice of alternative TKI and it is fortunate that there are many choices.

The ability to choose specific therapy based on mutations identified historically has been based on very limited numbers of patients in phase II clinical trials and is hampered by assumptions made from in vitro assessments of IC\textsubscript{50} values, which may not directly translate to in vivo predictability for response.\textsuperscript{46} Nonetheless, select mutations warrant attention and direct therapy choice. These are outlined in Table 1. Transformation to advanced phase disease, even with more than a decade of research and availability of five TKIs, still usually warrants direct consideration (if not done prior) of allogeneic stem cell transplant. Disease stabilization and restoration of chronic phase disease or even better, cytogenetic, or molecular response, are worthy but long-term response with salvage therapy in transformed disease remains limited, and longer-term data with the newest agent ponatinib is not yet available.

Monitoring of patients beyond first line of therapy has usually mirrored the approach of monitoring initial response. There is expectation of rapid cytogenetic and subsequent molecular response, and studies to date show a stark difference between patients with resistance who achieve threshold cytogenetic response early and those who do not.\textsuperscript{47} The notion is perhaps that with more potent inhibitors, response declaration is faster with both initial use and salvage use, and one is able to cull out the sensitive and resistant cases. This is this philosophy driving the acceptance of 3-month molecular response, and studies to date show a stark difference between patients with resistance who achieve threshold cytogenetic response early and those who do not.\textsuperscript{47} The notion is perhaps that with more potent inhibitors, response declaration is faster with both initial use and salvage use, and one is able to cull out the sensitive and resistant cases.

The question of proper management strategies will increase in importance, and visibility of the prevalence of CML—the number of cases alive in remission—continues to grow exponentially with current therapies.\textsuperscript{49}

Many quotable lines have tried to warn us about resistance; perhaps the most famous might be from the Borg creatures of the famed Star Trek series, who routinely stated that “Resistance is futile.” This was borrowed, most likely, from the United Kingdom series Doctor Who, where “Resistance is useless” was the tagline for the extraterrestrial mutants known as the Daleks. Perhaps we should reject summarily the warnings of fictional creatures and be inspired by the quip made famous by The Ohio State University football coach Woody Hayes, “Paralyze resistance with persistence.”

### CONCLUSION

Resistance in CML is recognized as a more narrow but evolving problem for which solutions are increasingly available. With a palette of medications available and data supporting intervention after early diagnostic suggestion of underlying resistance, it may be possible to avoid subsequent clinical consequences, particularly proliferation of disease or transformation to advanced stages of CML. Tools available for the unstable and proliferative case of transformed disease are limited, and the task remains to balance the degree of empiric or preventative intensification of therapy for the purpose of subverting resistance with any risks of more potent therapies. The question of proper management strategies will increase in importance, and visibility of the prevalence of CML—the number of cases alive in remission—continues to grow exponentially with current therapies.\textsuperscript{49}

Table 1. Selected Findings during Treatment of CML and Suggested Action

<table>
<thead>
<tr>
<th>Finding</th>
<th>Action</th>
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<tbody>
<tr>
<td>Bcr-Abl transcripts &gt; 10% at 3 months after IM</td>
<td>Strongly consider switch to DAS/NIL/BOS, or consider PON</td>
</tr>
<tr>
<td>Bcr-Abl transcripts &gt; 10% at 3 months after NIL or DAS</td>
<td>Strongly consider switch to PON, or consider BOS</td>
</tr>
<tr>
<td>Less than CCyR @ 12 months after IM</td>
<td>Switch to DAS/NIL/BOS, or consider PON</td>
</tr>
<tr>
<td>Failure to achieve MMR &gt; 18 months after IM</td>
<td>Consider switch to NIL, or consider DAS/BOS/PON</td>
</tr>
<tr>
<td>Less than CCyR @ 12 months after NIL or DAS</td>
<td>Switch to PON, or consider BOS</td>
</tr>
<tr>
<td>Clinical resistance, T315I mutation</td>
<td>Switch to PON, or consider OMA</td>
</tr>
<tr>
<td>Clinical resistance, V299L mutation</td>
<td>Switch to NIL or PON; consider OMA</td>
</tr>
<tr>
<td>Clinical resistance, F317L/V/I/C</td>
<td>Switch to NIL, BOS, or PON; consider IM or OMA</td>
</tr>
<tr>
<td>Clinical resistance, Y253H or E255K/V or F359 V/C/I</td>
<td>Switch to DAS, BOS, or PON; consider OMA</td>
</tr>
<tr>
<td>Clinical resistance, other mutation</td>
<td>Switch to NIL, DAS, BOS, or PON; consider HD IM or OMA</td>
</tr>
<tr>
<td>Transformation to AP/BC</td>
<td>Evaluate (if not done), consider alloSCT after TKI stabilization</td>
</tr>
</tbody>
</table>

Abbreviations: CML, chronic myelogenous leukemia; IM, imatinib; DAS, dasatinib; NIL, nilotinib; BOS, bosutinib; PON, ponatinib; CCyR, complete cytogenetic response; MMR, major molecular response; OMA, omacetaxine; AP, accelerated phase; BC, blast crisis; SCT, stem cell transplant; HD, high dose.
Disclosures of Potential Conflicts of Interest

Relationships are considered self-held and compensated unless otherwise noted. Relationships marked “L” indicate leadership positions. Relationships marked “I” are those held by an immediate family member; those marked “B” are held by the author and an immediate family member. Relationships marked “U” are uncompensated.

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