Mutant *BCR-ABL* clones in chronic myeloid leukemia

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The management of chronic myeloid leukemia (CML) has been revolutionized by the availability of small molecule tyrosine kinase inhibitors (TKI), such as imatinib (formerly STI571), dasatinib, and nilotinib. These agents work by competing with adenosine triphosphate (ATP) at its binding site on the ABL kinase, leading to inhibition of tyrosine phosphorylation of proteins involved in signal transduction.[1] The updated results of the International Randomized Study of Interferon and STI571 (IRIS) have confirmed high rates of durable cytogenetic remission coupled with a very low risk of progression to advanced disease.[2] At the 6-year follow up, progression-free survival and overall survival rates were 93% and 88%, respectively, for patients treated with imatinib. Nevertheless, some patients never respond to imatinib while some others have an initial response which is then followed by disease progression. Primary resistance refers to a lack of any relevant hematologic or cytogenetic response. Secondary resistance is acquired, and is defined as the achievement of hematologic or cytogenetic response followed by subsequent loss of disease control. Acquired resistance was documented in 24% of the patients in the 5-year follow up of the IRIS data.[3] There appears to be a peak of imatinib resistance in the second and third years of therapy, underscoring the need for second-line options or improved front-line strategies.[4]

A number of factors may be implicated in patients who eventually lose their response to imatinib. The mechanisms may involve *BCR-ABL* gene amplification, *BCR-ABL* overexpression, aberrations in other signaling pathways, and a host of others.[5] Point mutations in the *BCR-ABL* kinase domain are a major cause of imatinib resistance, and may be identified in approximately 50% or more of the cases. Mutations in the kinase domain have the ability to impair the binding of imatinib, leading to drug resistance. Many more than 100 different mutations have so far been identified, with varying degrees of clinical relevance.[6] The particular mutation identified may have therapeutic implications in terms of second- or third-line therapy.[7] One of the most notable mutations is the threonine to isoleucine mutation at codon 315 (T315I), which is known to be resistant to all currently available TKI. Jabbour *et al.* evaluated whether scoring a mutation based on the *in vitro* 50% inhibitory concentration (IC_{50}) at the time of imatinib failure had an impact on outcome in the context of treatment with other TKI.[8] The study demonstrated that patients in chronic phase disease with mutations demonstrating intermediate IC_{50} values had a shorter duration of response to second-line therapy, and inferior survival overall. It is, therefore, important to obtain a mutational analysis for all patients failing to meet prespecified therapeutic benchmarks.[9]

Dasatinib and nilotinib are second generation TKI that were initially developed to treat patients who could not tolerate, or did not respond adequately to imatinib. Recently, the results of large phase III trials comparing these agents to imatinib in treatment-naïve patients have been published, establishing both dasatinib and nilotinib as potentially viable front-line therapeutic options.[10,11] It has previously been shown that most mutations that confer resistance to imatinib retain sensitivity to both dasatinib and nilotinib.[12,13]

Investigators have evaluated the influence of *BCR-ABL* mutations on treatment with dasatinib. Of 805 patients who had suboptimal response or resistance to imatinib, 48% were found to have a *BCR-ABL* mutation.[12] Despite the high prevalence of mutations, relevant outcomes in patients with mutations were similar to those without baseline mutations (complete cytogenetic response 40% *versus* 41%, respectively). However, there were specific mutations identified according to the IC_{50} that appear to predict an inferior outcome in dasatinib-treated patients; these mutations included the phenylalanine to leucine mutation at codon 317 (F317L) and valine to lysine mutation at codon 299 (V299L). These mutations have been consistently associated with lower rates of cytogenetic remission under treatment with dasatinib, making alternative treatments more attractive when faced with this scenario.

Similarly, nilotinib has been shown to be active against most known *BCR-ABL* mutations leading to failure of imatinib treatment.[13] When evaluating data from one of the phase II trials evaluating the use of nilotinib in CML, patients were stratified into three groups based on their mutation status at the time of failure of imatinib treatment: no mutations, sensitive mutations, and those with less sensitive mutations according to the IC_{50}. Rates of response, including major cytogenetic response, complete cytogenetic response, and major molecular response, were all similar between the three groups. However, further evaluation did isolate several low sensitivity mutations that influenced the response to nilotinib. These included the glutamic acid to valine or lysine mutation at codon 255 (E255V/K), the tyrosine to histidine mutation at codon 253 (Y253H), and the phenylalanine to cysteine or valine mutations at codon 359 (F359C/V).

Mutational status should not be the sole factor determining how to treat a patient in whom imatinib treatment has failed or who has disease progression. The patient's medical history must be considered and placed in the context of the known toxicity profile of a given treatment. Apart from displaying different patterns of activity and resistance to various *BCR-ABL* mutations, the currently available TKI also have distinct adverse event profiles. Any patient with uncontrolled diabetes, or a past history of pancreatitis should be followed very closely if nilotinib is prescribed, as the drug has been associated with grade 3/4 elevations of serum glucose and lipase. Likewise, if dasatinib treatment is being considered, caution must be exercised in individuals with a history of gastrointestinal bleeding, asthma, chronic obstructive pulmonary disease, or congestive heart failure, given the high risk of hemorrhage and pleural or pericardial effusions.[14] These pieces of information coupled with the mutational status of a patient may help to optimally tailor and monitor further therapy.

There is also the issue of what happens to the *BCR-ABL* genotype over time, and how it might be influenced by the most recent therapy. The concept of clonal selection, or cytogenetic evolution in CML has been described previously.[15] Small populations of mutated cells have been shown to exist at baseline, and therapy with a TKI may offer these cells a growth advantage, eventually leading to their predominance. Baseline mutations, however, did not have an effect on response to therapy. One question that remains to be thoroughly explored is what happens to the *BCR-ABL* genotype once the selective pressure of TKI therapy is removed? In this issue of *Haematologica*, Hanfstein *et al.* report on the dynamics of *BCR-ABL* mutations in 19 patients after therapy with a TKI was stopped.[16] These patients were switched to non-TKI based therapy upon imatinib failure deemed secondary to the emergence of a well-characterized mutation. Patients were followed over time using highly sensitive methods to determine the size of the mutant clone after cessation of the TKI. The investigators were able to demonstrate consistent regression of most *BCR-ABL* mutations over a period of months, and overall described an 86% relative decline of the size of the mutant clones after cessation of the TKI. Importantly, they were also able to document the complete disappearance of the T315I mutation in two patients, though more commonly this mutation persisted despite manipulation of the therapeutic strategy. The authors also found evidence of repeated deselection and reselection of mutant clones in patients who resumed therapy with TKI after periods of cessation.

There are several points to be made regarding the above findings. First, clinicians should no longer consider the mutational status of a patient as a static event, and screening for *BCR-ABL* mutations should always be placed in the context of where the patient is in terms of therapy. This has implications for the monitoring of the mutational status as patients are switched to an alternative TKI or non-TKI based therapy. One important question that remains concerns the resumption of currently available TKI therapy after the disappearance of the selected mutation. In particular, for patients with the T315I mutation, therapeutic options are limited, and it would be helpful to know whether such patients could safely and effectively resume a TKI. Potentially, non-cross resistant chemotherapy combined with a TKI may be an option for these patients if the mutant clone regresses appreciably. The authors noted that resurgence of the mutant clone after resumption of TKI therapy was possible. Indeed, the patterns of deselection and reselection of mutant clones in a number of the patients described in the report indicate that the clones may not be completely eradicated. It is uncertain whether TKI withdrawal or the effects of non-specific chemotherapy were principally responsible for the disappearance of some mutations. The phenomenon may be multifactorial.

The treatment landscape of CML continues to evolve, as does the capability to characterize and quantify minimal residual disease. Second generation TKI are now moving to the frontline, and it will be important to monitor the impact that this paradigm shift has on *BCR-ABL* mutants. Indeed, the emergence of novel *BCR-ABL* mutants was noted shortly after the introduction of dasatinib and nilotinib into clinical practice as second-line options.[17] Regression of mutations conferring imatinib resistance was also noted in this report, but it was not as pronounced or predictable as described in the present study. Novel TKI, such as bosutinib and ponatinib (formerly AP24534), are now entering into advanced stages of clinical development for the management of CML.[18,19] Soon, mutational information will have to be placed in the context of the availability of these newer options. Innovative strategies, which may include non-TKI-based regimens or a combination approach, continue to be necessary for patients who develop mutations that are resistant to current therapy. More intensive monitoring of the mutational status should be considered for patients who develop mutations, but who subsequently receive therapy that may allow for deselection over time by relieving clonal pressure.

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References

1. Druker BJ, Talpaz M, Resta DJ, Peng B, Buchdunger E, Ford JM, et al. Efficacy and safety of a specific inhibitor of the BCR-ABL tyrosine kinase in chronic myeloid leukemia. N Engl J Med. 2001;344(14): 1031-7.
2. Hochhaus A, O'Brien SG, Guilhot F, Druker BJ, Branford S, Foroni L, et al. Six-year follow-up of patients receiving imatinib for the first-line treatment of chronic myeloid leukemia. Leukemia. 2009;23(6):1054-61.
3. Druker BJ, Guilhot F, O'Brien SG, Gathmann I, Kantarjian H, Gattermann N, et al. Five-year follow-up of patients receiving imatinib for chronic myeloid leukemia. N Engl J Med. 2006;355(23): 2408-17.
4. Deininger M, O'Brien SG, Guilhot F, Goldman JM, Hochhaus A, Hughes TP, et al. International randomized study of interferon vs. STI571 (IRIS) 8-year follow-up: sustained survival and low risk for progression or events in patients with newly diagnosed chronic myeloid leukemia in chronic phase (CML-CP). Blood. 2009;114:462 (abstract 1126).
5. Apperley JF. Part I: mechanisms of resistance to imatinib in chronic myeloid leukemia. Lancet Oncol. 2007;8(11):1018-29.
6. Branford S, Melo JV, Hughes TP. Selecting optimal second-line tyrosine kinase inhibitor therapy for chronic myeloid leukemia patients after imatinib failure: does the BRC-ABL mutation really matter? Blood. 2009;114(27):5426-35.
7. Jabbour EJ, Branford S, Saglio G, Jones D, Cortes JE, Kantarjian HM. Practical advice for determining the role of BCR-ABL mutations in

guiding tyrosine kinase inhibitor therapy in patients with chronic myeloid leukemia. Cancer n/a. doi: 10.1002/cncr.25717.
8. Jabbour E, Jones D, Kantarjian HM, O'Brien S, Tam C, Koller C, et al. Long-term outcome of patients with chronic myeloid leukemia treated with second-generation tyrosine kinase inhibitors after imatinib failure is predicted by the in vitro sensitivity of BCR-ABL kinase domain mutations. Blood. 2009;114(10):2037-43.
9. Baccarani M, Cortes J, Pane F, Niederwieser D, Saglio G, Apperley J, et al. Chronic myeloid leukemia: an update of concepts and management recommendations of European LeukemiaNet. J Clin Oncol. 2009;27(35):6041-51.
10. Kantarjian H, Shah NP, Hochhaus A, Cortes J, Shah S, Ayala M, et al. Dasatinib versus imatinib in newly diagnosed chronic-phase chronic myeloid leukemia. N Engl J Med. 2010;362(24):2260-70.
11. Saglio G, Kim DW, Issaragrsisil S, le Coutre P, Etienne G, Lobo C, et al. Nilotinib versus imatinib for newly diagnosed chronic myeloid leukemia. N Engl J Med. 2010;362(24):2251-9.
12. Muller MC, Cortes JE, Kim DW, Druker BJ, Erben P, Pasquini R, et al. Dasatinib treatment of chronic-phase chronic myeloid leukemia: analysis of responses according to preexisting BCR-ABL mutations. Blood. 2009;114(24):4944-53.
13. Hughes T, Saglio G, Branford S, Soverini S, Kim DW, Muller MC, et al. Impact of baseline BCR-ABL mutations on response to nilotinib in patients with chronic myeloid leukemia in chronic phase. J Clin Oncol. 2009;27(25):4204-10.
14. Quintas-Cardama A, Kantarjian HM, O'Brien S, Ravandi F, Ferrajoli A, Garcia-Manero G, et al. Association of pleural effusion and bleeding in patients with chronic myelogenous leukemia receiving dasatinib. Blood. 2008;11:112 (Abstract 2112).
15. Willis SG, Lange T, Demehri S, Otto S, Crossman L, Niederwieser D, et al. High-sensitivity detection of BCR-ABL kinase domain mutations in imatinib-naïve patients: correlation with clonal cytogenetic evolution but not response to therapy. Blood. 2005;106(6):2128-37.
16. Hanfstein B, Muller MC, Kreil S, Ernst T, Schenk T, Lorentz C, et al. Dynamics of mutant BCR-ABL positive clones after cessation of tyrosine kinase inhibitor therapy. Haematologica. 2011;96(3):360-6.
17. Cortes J, Jabbour E, Kantarjian H, Yin CC, Shan J, O'Brien S, et al. Dynamics of BCR-ABL kinase domain mutations in chronic myeloid leukemia after sequential treatment with multiple tyrosine kinase inhibitors. Blood. 2007;110(12):4005-11.
18. Gambacorti-Passerini C, Kim DW, Kantarjian HM, Brummerdorf TH, Dyagil I, Griskevicius L, et al. An ongoing phase 3 study of bosutinib (SKI-606) versus imatinib in patients with newly diagnosed chronic phase chronic myeloid leukemia. Blood. 2010;21:116 (Abstract 208).
19. Cortes J, Talpaz M, Bixby D, Deininger M, Shah N, Flinn IW, et al. A phase 1 trial of oral ponatinib (AP24534) in patients with refractory chronic myelogenous leukemia (CML) and other hematologic malignancies: emerging safety and clinical response findings. Blood. 2010;21:116 (Abstract 210).

Risk assessment in myelodysplastic syndromes and myelodysplastic/myeloproliferative neoplasms

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Myelodysplastic syndromes (MDS)[1] are included in the World Health Organization (WHO) classification of the myeloid neoplasms[2] together with myeloproliferative neoplasms (MPN), myelodysplastic/myeloproliferative neoplasms (MDS/MPN) and acute myeloid leukemia (AML).

Classifications of myelodysplastic syndromes

MDS were defined and classified in 1982 by the FAB group.[3] The FAB classification included five categories: refractory anemia (RA), RA with ring sideroblasts (RARS), RA with excess of blasts (RAEB), RAEB "in transformation" (RAEB-t), and chronic myelomonocytic leukemia (CMML). This latter is now considered as a myelodysplastic/myeloproliferative neoplasm, while RAEB-t is now classified as AML.[4] Figure 1A provides a Kaplan-Meier analysis of overall survival in MDS patients classified according to the FAB classification. It is apparent that, from a prognostic point of view, this classification was essentially able to identify two risk groups based on the absence or presence of blast excess.

In 2001 the World Health Organization (WHO) classification was developed.[5] This classification,[6] carries relevant prognostic information. Figure 1B provides a Kaplan-Meier analysis of overall survival in MDS patients classified according to the 2008 WHO classification. It is apparent that, among patients without an excess of marrow blasts, the presence of bone marrow multilineage dysplasia is associated with a significantly worse prognosis compared to unilineage dysplasia. Despite some concern regarding the reproducibility of the assessment of multilineage dysplasia, its prognostic value was confirmed in different independent cohorts of patients in both retrospective[7] and prospective[8] studies, clearly indicating that this parameter must be included in the prognostic evaluation of MDS patients. Survival curves of Figure 1 support the conclusion that nowadays clinical decision making in MDS cannot rely upon the FAB classification and must be based on the WHO classification.[9]

Prognostic scoring systems for myelodysplastic syndromes

To overcome the limitations of the FAB classification, Greenberg and co-workers developed the International Prognostic Scoring System (IPSS).[10] Although widely adopted, this scoring system does not consider the severity of anemia, in particular transfusion dependency,[1] which represents one of the most important negative prognostic factors in MDS. Furthermore, it underestimates the negative impact of poor cytogenetics, especially relative to blast count.

The introduction of the WHO classification, excluding patients with 20% blasts or more and those with CMML from the category of MDS, considerably modified the composition of the MDS population and demanded a refinement of prognostic factors in patients diagnosed according to the WHO criteria. We found that WHO categories, cytogenetic pattern and transfusion dependency were the most powerful prognostic indicators, and developed a prognostic model that accounted for these parameters.[11] This WHO