T315I, *more or less*, predicts for major molecular response: the devil is in the details!

Michael J. Mauro

Knight Cancer Institute, Center for Hematologic Malignancies, Oregon Health and Science University, Portland, OR, USA

E-mail: maurom@ohsu.edu *doi:10.3324/haematol.2013.084251*

Initial reports of the presence of a specific mutation in the kinase domain of BCR-ABL[1] as the basis of clinical resistance as well as progression in chronic myeloid leukemia (CML) came on the heels of another first report of Abl kinase inhibitor success,[2] and the former was both a beacon of light and nidus for uncertainty regarding potential resistance to targeted therapy for Philadelphia chromosome positive (Ph$^+$) leukemias. More than a decade later, the same area of discovery has expanded in parallel to the therapy choices, with rational development of next-generation Abl kinase inhibitors increasingly able to remain active in the presence of specific mutations. Fortunately, resistance to kinase inhibitors in Ph$^+$ leukemia has remained a limited and early problem, and the list of culprit kinase domain mutations of greatest concern is still short. Most recently, clinical reports[3] and US Food and Drug Administration registration of the 3rd generation tyrosine kinase inhibitor (TKI) ponatinib have brought us an alternative able to overcome, among others, the threonine-isoleucine substitution at position 315, known as the T315I mutation, seemingly filling the last remaining 'chink in the armor' for the management of mutation-based Abl kinase resistance.

Clinically, the T315I mutation had represented clonal disease with stark and complete drug resistance, although, paradoxically, somewhat indolent with regard to disease course and progression risk.[4] With emerging data on specific (ponatinib)[3] and non-specific (omacetaxine)[5] therapies active in the face of such mutations, as well as the availability of a total of five kinase inhibitors for Ph$^+$ leukemia, screening for this and other select mutations continues to be emphasized. While mutation-based resistance may be viewed simply as a 'lock' and 'key' scenario, where clonal disease bearing a specific mutation evolves to evade drug effect and dominates with eventual clinical resistance, the natural history of Ph$^+$ leukemias and their potential for resistance may be much more complex.

Increased inquiry into the timing of mutations relative to drug exposure, the variability of their detection based on volume of mutated clone, and, perhaps most importantly, the clinical impact of identifying clonal disease harboring Abl kinase mutations have all widened the lens significantly. Several points have emerged. 1) Ph$^+$ leukemia, including chronic phase CML, may generate Abl kinase mutated clones without TKI exposure.[6] 2) Mutations can be observed in patients with minimal residual disease and without overt clinical resistance.[7] 3) Also, most recently, it has been demonstrated that multiple mutations may co-reside in a clone (compound mutations).[8] Naturally, there is also strong curiosity as to the basis of clinical resistance in the *absence* of Abl kinase domain mutations, and thus continued inquiry into a more basic or 'root cause' of resistance. Based on the observations that kinase domain mutations are seen more frequently in advanced forms of Ph$^+$ leukemia and are more often highly drug resistant,[9] and most obviously that kinase inhibitor resistant clones are seen prior to kinase inhibitor exposure,[6] genesis of mutations is thought to be part of the natural history of Ph$^+$ transformed clonal diseases. Other key factors related to clinical resistance appear to include proliferative rate and degree of differentiation, adherence to therapy and dose intensity, and the potential of other Bcr-Abl dependent or independent mechanisms that are yet to be identified.

Current guidelines[10] suggest, and the general practice of managing patients on kinase inhibitor therapy support the view that investigation is warranted into Abl kinase mutations when clinical resistance is observed. Screening for mutations has not been incorporated into routine practice, expect perhaps for patients diagnosed *a priori* with advanced Ph$^+$ disease. In general, mutation testing has been viewed as a means to explain resistance to current therapy or predict resistance to next therapy choice rather than predict response. With the ability to detect 'low-level' mutations, not generally observed with routine clinically available methods, the implication of mutation findings has extended into the area of predicting response in a general sense, not just based on available data of selected kinase inhibitors to treat selected clonal mutation-positive disease. However, the implications of low-level mutations remain controversial with regard to the level detected and, moreover, the point at which the detection occurs: new diagnosis, chronic phase response *versus* resistance, or with progression.

In this issue of *Haematologica*, collaborators from Germany and Norway led by Thoralf Lange of the Universitätsklinikum Leipzig dig deeper into the prognostic significance of low-level mutations.[11] They quantified the burden of Bcr-Abl bearing the T315I mutation in patients with imatinib resistance six months into treatment with either nilotinib or dasatinib to assess its ability to predict major molecular response (MMR) at 12 months, considered an optimal response with initial imatinib therapy and likely considered equally or even more ideal in the salvage setting. After determining the appropriate low-level cut off of 10^{-5} Bcr-Abl$^{T315I\%}$/GUS at six months, subsequent molecular response (MMR at 12 months) was predicted quite nicely with a sensitivity of approximately 93% and specificity of approximately 88% from a mixed learning and validation sample of 80 patients. These results were obtained using ligation polymerase chain reaction (PCR), which permits precise and ultrasensitive quantification of specific mutations based on the joining (ligation) of specific probes to amplified DNA only in the presence of a select mutation, allowing for measurement of Bcr-Abl^{total} *and* Bcr-Abl^{T315I}. In addition, the sensitivity of the ligation PCR technique identified patients harboring clonal disease with the T315I mutation earlier than conventional PCR methods.

What are the implications of these findings? First, it suggests we must take care in interpreting the notion of 'T315I mutation positive leukemia'; it is the *quantification* that may be of greatest significance, and, as the authors speculate, a

threshold amount of clonal disease with a mutation may be necessary for expansion and clinical resistance. Second, should ligation PCR or other similar techniques become more widely available? Incorporation of such an assay into clinical practice might influence the philosophy of salvage for patients with primary imatinib (or possibly other TKI as primary) resistance. Until now, predicting long-term success with salvage therapy in CML has been related to factors at time of switch, such as best response to prior TKI, Sokal risk score, and prior myelosuppression, followed by response over time with subsequent kinase inhibitor therapy. Increasingly, transplant has been called upon, rightly so, for patients without odds of long-term success with salvage therapy.[12] By adding a molecular assessment early in treatment *irrespective of response* to judge future success, as the authors propose, we may be able to see, if you will, the 'devil in the details' and navigate salvage better. Clones with the T315I as seen in this study, with the potential to persist at low levels or expand, may serve as a basis for clinical resistance to 2nd generation TKIs or even more complex disease, such as compound mutations, which may pose a problem for even 3rd generation TKIs such as ponatinib. Earlier identification *and* justification for change in therapy using the T315I clonal burden may clarify how best to use therapies such as ponatinib as we move forward in an era of greater understanding and more powerful diagnostics to combat resistance in CML.

Michael J. Mauro is Associate Professor at the Center for Hematologic Malignancies, Knight Cancer Institute, Oregon Health and Science University, Portland, Oregon, USA. His field of interest is: CML and myeloproliferative neoplasms.

Financial and other disclosures provided by the author using the ICMJE (www.icmje.org) Uniform Format for Disclosure of Competing Interests are available with the full text of this paper at www.haematologica.org.

References

1. Gorre ME, Mohammed M, Ellwood K, Hsu N, Paquette R, Rao PN, Sawyers CL. Clinical resistance to STI-571 cancer therapy caused by BCR-ABL gene mutation or amplification. Science. 2001;293(5531): 876-80.
2. 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.
3. Cortes JE, Kantarjian H, Shah NP, Bixby D, Mauro MJ, Flinn I, et al. Ponatinib in refractory Philadelphia chromosome-positive leukemias. N Engl J Med. 2012;367(22):2075-88.
4. Nicolini FE, Mauro MJ, Martinelli G, Kim DW, Soverini S, Müller MC, et al. Epidemiologic study on survival of chronic myeloid leukemia and Ph(+) acute lymphoblastic leukemia patients with the BCR-ABL T315I mutation. Blood 2009;114(26):5271-8.
5. Cortes J, Lipton JH, Rea D, Digumarti R, Chuah C, Nanda N, et al. Phase 2 study of subcutaneous omacetaxine mepesuccinate after TKI failure in patients with chronic-phase CML with T315I mutation. Blood. 2012;120(13):2573-80.
6. Roche-Lestienne C, Soenen-Cornu V, Grardel-Duflos N, Laï JL, Philippe N, Facon T, et al. Several types of mutations of the Abl gene can be found in chronic myeloid leukemia patients resistant to STI571, and they can pre-exist to the onset of treatment. Blood. 2002;100(3):1014-8.
7. Ernst T, Erben P, Müller MC, Paschka P, Schenk T, Hoffmann J, et al. Dynamics of BCR-ABL mutated clones prior to hematologic or cytogenetic resistance to imatinib. Haematologica. 2008;93(2):186-92.
8. Khorashad JS, Kelley TW, Szankasi P, Mason CC, Soverini S, Adrian LT, et al. BCR-ABL1 compound mutations in tyrosine kinase inhibitor-resistant CML: frequency and clonal relationships. Blood. 2013; 121(3):489-98.
9. Branford S, Melo JV, Hughes TP. Selecting optimal second-line tyrosine kinase inhibitor therapy for chronic myeloid leukemia patients after imatinib failure: does the BCR-ABL mutation status really matter? Blood. 2009;114(27):5426-35.
10. Soverini S, Hochhaus A, Nicolini FE, Gruber F, Lange T, Saglio G, et al. BCR-ABL kinase domain mutation analysis in chronic myeloid leukemia patients treated with tyrosine kinase inhibitors: recommendations from an expert panel on behalf of European LeukemiaNet. Blood. 2011;118(5):1208-15.
11. Lange T, Ernst T, Gruber FX, Maier J, Cross M, Müller MC, et al. The quantitative level of T315I mutated BCR-ABL predicts for major molecular response to second-line nilotinib or dasatinib treatment in patients with chronic myeloid leukemia. Haematologica. 2013;85(5): 714-717.
12. Pavlu J, Szydlo RM, Goldman JM, Apperley JF. Three decades of transplantation for chronic myeloid leukemia: what have we learned? Blood. 2011;117(3):755-63.