Predictive Value of In Vitro Mutation Data to Guide Second-Generation Tyrosine Kinase Inhibitor Selection: Ready for Prime Time?

RICHARD T. SILVER

Weill Cornell Medical College, Department of Medicine, Division of Hematology and Medical Oncology,
New York, New York, USA

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ABSTRACT

Significant advances in treatment and monitoring for patients with chronic myeloid leukemia have occurred over the last decade. With the introduction of the tyrosine kinase inhibitor imatinib, long-term outcomes have improved and new challenges, such as resistance, including mutations, have emerged. Research efforts into mutational analysis have intensified, with emphasis on the potential of using this technique to guide second-generation tyrosine kinase inhibitor selection. Although some data suggest that a small number of mutations may be associated with a less favorable response to treatment with one second-generation tyrosine kinase inhibitor versus another, these data need to be inter-

preted cautiously because they are derived primarily retrospectively from single-institution studies and a small number of patients. More research and clinical experience and a better understanding of the implications of in vitro data are needed before these data can be routinely incorporated into therapeutic decisions. Currently, there is no consensus on when to screen patients for mutations, what technique should be used, or how values should be reported. Selection of a second-generation tyrosine kinase inhibitor should therefore be based upon its toxicity profile in conjunction with the patient's comorbidities and the practitioner's experience. *The Oncologist* 2011;16:554–558

INTRODUCTION

Imatinib, a potent and selective tyrosine kinase inhibitor (TKI), binds to the ATP-binding site of BCR-ABL, suppressing the uncontrolled proliferation and survival of chronic myeloid leukemia (CML) cells [1]. A rationally de-

signed drug, imatinib targets the molecular abnormality of CML and has become the standard treatment for all phases of CML [2]. Since imatinib's approval for initial therapy, patient outcomes have dramatically improved. The 8-year update of the International Randomized Study of Interferon

Correspondence: Richard T. Silver, M.D., F.A.C.P., Weill Cornell Medical College, Department of Medicine, Division of Hematology and Medical Oncology, New York, New York 10065-4896, USA. Telephone: 212-746-2541; Fax: 212-746-8107; email: rtsilve@med.cornell.edu Received August 30, 2010; accepted for publication February 1, 2011; first published online in *The Oncologist Express* on March 30, 2011. ©AlphaMed Press 1083-7159/2011/\$30.00/0 doi: 10.1634/theoncologist.2010-0297

and STI571, the IRIS trial, showed an estimated event-free survival (EFS) rate of 81% and rate of freedom from progression to accelerated or blast phase of 92% [3]. The estimated overall survival (OS) rate was 85%; when only CML-related deaths and those prior to stem cell transplantation were considered, the estimated OS rate was 93% [3]. Despite these successes, relapses can occur early during treatment; the event rate during years 1–3 was 15% but decreased to <2% during years 4–8 [3].

Second-generation TKIs were specifically developed to overcome imatinib resistance. Dasatinib, a multikinase inhibitor with activity against BCR-ABL and SRC, and nilotinib, a selective inhibitor of BCR-ABL, are more potent than imatinib because of enhanced binding to ABL. They are active in patients after imatinib failure, in those who develop mutations responsible for resistance, and in newly diagnosed patients [4–6].

The ability to predict response to TKI therapy would represent another significant advance in “personalized” treatment of CML. Routine mutational analysis and in vitro sensitivity data to support clinical decisions and guide therapeutic selection have been recommended [7–11]. Classifying mutations into high, intermediate, and low sensitivity to TKIs can predict long-term outcomes [12]. However, with few exceptions, such as T315I, the current data are not robust or mature enough to allow therapeutic selection solely on the basis of in vitro inhibition data or mutational analysis [13].

RESISTANCE AND MUTATION

Although the cause underlying primary resistance to imatinib is often unknown, the most common known causes of imatinib resistance are gene amplification resulting in protein overexpression and point mutations in the ABL tyrosine kinase domain [8]. Additional potential mechanisms of resistance and factors that may influence outcomes [2] include intrinsic factors, such as aberrant oncogenic signaling pathways and persistence of leukemic stem cells, and extrinsic factors, such as drug influx and efflux, multi-drug resistance, adherence, and drug–drug interactions.

Kinase domain mutations may arise as a natural consequence of CML, irrespective of imatinib therapy. They are more frequent in the advanced phase (AP) and in blast crisis (BC) than in the chronic phase (CP), and they increase with disease duration: 27%, 52%, 75%, and 83% of patients with CP, AP, BC, and lymphoid blast crisis/Philadelphia chromosome–positive acute lymphoblastic leukemia, respectively [2, 4]. More than 100 *BCR-ABL* mutations have been identified; however, the clinical and prognostic significance of most mutations is not entirely clear [14, 15]. For example, in a study of imatinib-naïve patients, kinase do-

main mutations did not correlate with response to imatinib, EFS, or OS [14].

The European LeukemiaNet and National Comprehensive Cancer Network recommend ABL kinase domain mutational testing before changing therapy [15] and for patients with a suboptimal response or failure to imatinib [15, 16]. However, the utility of such testing in guiding therapeutic decisions is unclear because there are no recommendations for altering the dose of imatinib or selecting a second-generation TKI therapy. Furthermore, these recommendations do not specify the significance of the specific mutations (except T315I) and the predicted response to imatinib, dasatinib, or nilotinib.

LIMITATIONS OF USING IN VITRO SENSITIVITY DATA TO GUIDE TKI SELECTION

Studies have highlighted the complexities, discrepancies, and limitations of using in vitro sensitivity data or mutations to predict clinical outcomes [13, 17, 18]. Because of differing methods, lack of laboratory standardization, and variation in specificity, there is a high degree of variability among the median inhibitory concentration (IC_{50}) values reported in different studies [17]. Moreover, reported IC_{50} values do not correlate with pharmacokinetic data [13]. Although dasatinib was more potent than nilotinib because of a lower concentration leading to 50% growth inhibition (GI_{50}) in vitro, nilotinib resulted in higher peak and trough plasma concentrations based on pharmacokinetic data, relative to the GI_{50} values [13]. Analysis of correlation between in vitro GI_{50} data and clinical responses in imatinib-resistant patients with mutations indicated discordance between in vitro sensitivity data and in vivo response, perhaps because of the contribution of nonmutational resistance [13, 18].

Factors other than mutations can affect response to TKI therapies. For example, in patients whose disease progressed on imatinib therapy, 57% (82 of 145) had *BCR-ABL* mutations whereas 43% (63 of 145) did not [19]. Mutations were more common in patients with secondary resistance than in those with primary resistance (57% versus 30%) [19]. The outcomes of patients in complete cytogenetic remission (CCyR) on TKI therapy who develop mutations are ambiguous. In one study, seven *BCR-ABL* mutations were detected in 38% (five of 13) of patients [20]. Two of those patients subsequently relapsed, whereas the other three remained in CCyR for 21–30 months. In another study of 42 patients in CCyR during imatinib therapy, 19% of patients had mutations [21]. Large, long-term, prospective population-based studies are needed to confirm the association between mutations and clinical re-

lapse and to determine whether mutation analysis provides prognostic value [20, 22].

Data are emerging suggesting that a small number of mutations may be associated with a less favorable response to treatment with dasatinib (e.g., Q252H, E255K/V, V299L, F317L, T315A) or nilotinib (e.g., E255K/V, Y253H, F359C/V) [1]. However, it is important to recognize the variability in mutation patterns among studies, which may be attributable to differing methods of mutational analysis [4]. Factors complicating interpretation of these resistance patterns include derivation of retrospective data from single-institution studies [8], small numbers of patients [10], considerable variation in frequency of mutations based on the disease phase [8], evolution of mutational patterns during TKI therapy [18], unexpected responses to mutations [18], and the presence of multiple mutations that may alter the response to TKI therapy [8]. To date, selecting a second-generation TKI based on a specific mutation has not been evaluated prospectively in an adequately powered study [23]. Hopefully, correlative clinical studies in the future will resolve these issues.

T315I

Although the T315I mutation is resistant to all commercially available TKIs, only 0.16%–0.32% of newly diagnosed patients in the CP receiving therapy are predicted to develop this mutation [23]. Survival of patients with the T315I mutation depends on the stage of disease when it is found [24]. In one study that characterized patients with T315I and assessed their outcomes to second-line therapy after imatinib failure, 87% of patients in CP-CML were alive 2 years after detection of the T315I mutation, whereas the median survival times of patients in AP and blast phase were 14 months and 4 months, respectively, after imatinib failure [24]. Two patients who received a second TKI after imatinib failure and were in the AP had cytogenetic responses. One patient acquired the T315I mutation 5 months after initiating nilotinib but achieved a major molecular response lasting 21 months. The second patient acquired the T315I mutation 6 months after initiating bosutinib and achieved a major cytogenetic response sustained for >8 months. Although T315I is not a favorable prognostic finding [24], it does not predict unequivocal resistance in all cases. A number of drugs that may be more effective against T315I are in various stages of clinical development, including omacetaxine, AP24534, danusertib, XL-228, and DCC-2036 [25–28]. The need for accurate in vitro diagnostic assays for detecting the T315I mutation is reflected in the Food and Drug Administration's decision to delay approval of omacetaxine until such a standardized test is developed [29].

THE ASSESSMENT OF THE AGENCY FOR HEALTHCARE RESEARCH AND QUALITY

Evidence-based medicine, the conscientious application of the current best scientific evidence to guide therapeutic decisions, emerged in the 1990s to improve patient care and involves integrating clinical expertise with clinically relevant scientific research [30, 31]. Its implementation has allowed the development of practice guidelines, such as the Cochrane Reviews, with the ultimate goal of improving patient outcomes [31]. In the late 1990s, the American Society of Hematology recognized this discipline and applied it to evaluating the results of interferon and marrow transplantation in the CP of CML [32].

The Agency for Healthcare Research and Quality conducted an evidence-based assessment evaluating the utility of *BCR-ABL* mutation analysis to predict response to imatinib, dasatinib, and nilotinib [33]. A MEDLINE search identified 3,388 publications relating to *BCR-ABL* testing in patients with CML; 31 publications were selected (three studies of TKI therapy in previously untreated patients, 20 studies of TKI therapy in patients with a suboptimal response to imatinib [intolerant or resistant], one study of TKI therapy in patients with a suboptimal response to imatinib and a second-generation TKI, and seven studies of *BCR-ABL* mutation monitoring). The analyses compared *no* mutation with *any* mutation. Outcomes were categorized by mortality, progression, and treatment failure. In the studies evaluated, clinical outcomes generally focused on short-term hematologic, cytogenetic, or molecular responses versus long-term outcomes such as progression-free survival. Data were sparse and studies varied widely in their methods of measurement and duration of follow-up. Furthermore, there was substantial variation in study techniques, definitions, and outcomes measured.

This evidence-based review of the literature indicated that the T315I mutation predicts TKI treatment failure. However, there was no evidence that any other *BCR-ABL* mutation predicted response to TKI therapy in previously untreated and treated patients with CML. The report did not discuss findings that suggest response rates may be lower for patients with some mutations [7, 10].

CONCLUSION

Currently, there is no consensus on when patients should be screened for mutations, which technique should be used, or how values should be reported [8, 34]. Although there are commercial kits available for *BCR-ABL* mutational analysis, laboratory protocols and baseline values have not been standardized. Variability among laboratories and in vitro assays limits both the comparability and utility of in vitro

data and the ability to extrapolate in vitro sensitivity data to clinical outcomes [13].

Routine application of in vitro mutation data in daily practice appears premature given the lack of evidence of its usefulness. Furthermore, misinterpretation could result in clinical decisions that could lead to adverse outcomes [23]. Although some data suggest that there may be differences among the second-generation TKIs with respect to some mutations, response rates and progression rates in patients with mutations receiving dasatinib and nilotinib are similar [2] and mutations are rarely unresponsive to second-generation TKIs [2].

The selection of second-generation TKI therapy should be based primarily on toxicity profiles in conjunction with

patient comorbidities and practitioner experience and comfort level with a given agent. If these factors do not prove instructive, mutational analysis results can be considered. Although this approach does not guarantee or predict outcomes, it is the most prudent considering the limitations of available studies and current knowledge of CML biology.

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Central Park

Manfred Hauben, MD, MPH