Integrating in vitro sensitivity and dose-response slope is predictive of clinical response to ABL kinase inhibitors in chronic myeloid leukemia

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Running title: Drug-response slope is predictive in resistant CML
Key Points:
1. Decreased in vitro dose-response slope tracks with resistance BCR-ABL mutants to ABL tyrosine kinase inhibitors.
2. Integrating in vitro dose-response slope, IC50 of various BCR-ABL mutants, and clinical PK data can predict CML patients response to TKIs.

Abstract

BCR-ABL mutations result in clinical resistance to ABL tyrosine kinase inhibitors (TKIs) in CML. Although in vitro IC50 values for specific mutations have been suggested to guide TKI choice in the clinic, quantitative relationship between IC50 and clinical response has never been demonstrated. We used Hill’s equation for in vitro response of Ba/F3 cells transduced with various BCR-ABL mutants to determine IC50 and the slope of the dose-response curve. We found that slope variability between mutants tracked with in vitro TKI resistance, providing particular additional interpretive value in cases where in vitro IC50 and clinical response are disparate. Moreover, unlike IC50 alone, higher inhibitory potential at peak concentration (IPP), which integrates IC50, slope, and Cmax, correlated with improved complete cytogenetic response (CCyR) rates in CML patients treated with dasatinib. Our findings suggest a metric integrating in vitro and clinical data may provide an improved tool for BCR-ABL mutation-guided TKI selection.

Introduction

BCR-ABL kinase domain mutations represent a common mechanism of resistance to ABL tyrosine kinase inhibitors (TKIs) in chronic myeloid leukemia (CML). In vitro cellular IC50 values have been proposed to guide TKI treatment selection for specific mutations1. However, using Cmax/IC50 as a measure of potential in vivo activity failed to show correlation with complete cytogenetic response (CCyR) rates in CML patients2.

Importantly, an IC50 value constitutes only one point on the dose-response curve for a given drug. Most dose-response curves can be described by Hill’s equation (equation 1), which incorporates both IC50 and slope (m) parameters:

\[ f_a = \frac{D^m}{D^m + IC_{50}^m} \]  

Here, \( f_a \) and \( f_u \) are cell fractions affected and unaffected by treatment, respectively (\( f_u = 1 - f_a \)), and \( D \) is drug dose. Theoretical and clinical importance of evaluation of the slope in addition to IC50 has already been shown for antiretroviral drug resistance in HIV3.

We report estimation of the slope of in vitro dose-response curves for wild-type and kinase domain-mutant BCR-ABL against clinical ABL TKIs for CML and examine the value of this incorporated parameter for predicting clinical response.

Methods

Ba/F3 cellular data

Dose response curves for imatinib, nilotinib and dasatinib were determined previously by methanethiosulfonate-based cell viability assay in Ba/F3 cells expressing wild-type or kinase
domain-mutant BCR-ABL. Since completely insensitive to all three ABL TKIs tested, the BCR-ABL_T315I mutant was excluded from analysis.

Calculation of inhibitory potential values

Logarithmic transformation of the Hill’s equation reaches:

\[
\log \left( \frac{f_u}{f_u} \right) = m \cdot \log D - m \cdot \log IC_{50}
\]

(2)

The parameters \( m \) and \( IC_{50} \) were determined for each mutation and drug by fitting the equation (2) to the respective dose-response curve using least square sum criterion. Inhibitory potential at peak concentration (IPP) was subsequently calculated as:

\[
\log \left( \frac{1}{f_u} \right) = \log \left( 1 + \left( \frac{D}{IC_{50}} \right)^m \right)
\]

(3)

Here, \( D \) is mean peak concentration (C_max) in plasma as reported.

Comparison with clinical response

IPP and IC_{50} values for each Ba/F3 BCR-ABL mutant were compared with previously reported CCyR rates for nilotinib and dasatinib. Response data for mutations reported in more than 2 patients was included, divided based on mutation IPP and IC_{50} values, and CCyR rates were compared between groups by two-tailed Student’s t-test with unequal variance (p=0.05 significance threshold). Multivariate analysis was performed by linear multiple regression and the Cox proportional hazard model using JMP-SAS version 10 software (see Supplementary material for details).

Results and Discussion

We fitted Hill’s equation to Ba/F3 cell viability dose-response curves for imatinib, nilotinib and dasatinib for wild-type BCR-ABL and each of 15 BCR-ABL kinase domain point mutants (see representative curves in Supplementary Figure 1; all data reported in 4). Excellent goodness of fit (r^2 values 0.94-0.99) was observed for all drug-mutation pairings. Resultant values of IC_{50} and slope for each case are summarized in Table 1, along with calculated IPP values (see equation 3 in Methods). IPP provides a natural way to combine drug efficacy data in vitro (i.e., IC_{50} and slope) with clinical pharmacokinetic data and compare with clinical outcomes.

Imatinib

Most P-loop mutations are reported to render worse response to imatinib. We found that 4 of 7 P-loop mutations tested (G250E, Y253H, E255K, E255V) showed lower dose-response slope relative to wild-type BCR-ABL in addition to high IC_{50} (>1100 nM), while all other mutations showed variably increased slopes (Table 1). Consistent with particularly negative effects of these mutations on drug binding and clinical outcome with imatinib, their lower slopes indicate shallower drug efficacy over a given increase in concentration. Differences in slope values across different resistant mutations likely reflect a varied degree of inhibitor binding destabilization (rather than binding preclusion). Furthermore, the range of IPPs for these mutations was lower than (and not overlapping with) all other mutations (0.084-1.66 vs 2.93-5.59; t-test for range: p=6*10^{-6}).

Additional value of the slope parameter was particularly apparent in cases where increased in vitro IC_{50} does not track with clinical resistance. For example, upon comparing the
G250E and V379I mutants, which feature comparable cellular IC_{50} values for imatinib (1184 and 1140 nM, respectively), only G250E harbors worse clinical prognosis, arguably due to its lower dose-response slope. This is also reflected in a lower IPP value compared to V379I (Table 1). Similarly, the P-loop mutation M244V does not confer marked clinical resistance to imatinib despite increased IC_{50} relative to wild-type BCR-ABL, possibly due to an exceptionally high slope value \((m=5\) vs. \(m=1.87\) for wild-type BCR-ABL) reflecting a very steep dose-response curve (Table 1). While at 6000 nM the IPP values for the M244V mutant and wild-type BCR-ABL become virtually identical (5.70 and 5.77, respectively), this concentration is below imatinib C_{max} for some individuals but not others\(^6\), suggesting patients with this mutation may be particularly vulnerable to consequences of unfavorable imatinib pharmacokinetic profile or reduced compliance. Indeed, one study found clinical resistance to imatinib in 3 out of 6 patients with a M244V mutation was overcome by dose increase\(^7\).

**Nilotinib and Dasatinib**

Particularly high slope values \((m>4)\) were found for M244V and Y253F with nilotinib treatment, in contrast to lower values for both mutations with dasatinib treatment (Table 1).

Comparison of BCR-ABL mutant IPP values between nilotinib and dasatinib revealed favorable, higher IPP for dasatinib over nilotinib against the Y253H mutant; nilotinib demonstrated higher IPP for E255K and F317L compared with dasatinib. For the E255V mutation, both drugs featured low IPPs, consistent with this mutation being reported in clinical failures of both drugs\(^8,9\).

We next examined whether higher IPP values are predictive of better clinical response. Patient response data was initially divided according to the median mutant IPP value for each TKI. Dasatinib-treated patients with mutations resulting in IPP values above the median (IPP=8) had a significantly higher mean CCyR rate than those patients with mutation IPPs below the median (53% [range 39-80%] vs 31% [range 7-56%]; \(p=0.038\)). Notably, using an IPP threshold value of 7 resulted in even better group separation of mean CCyR rate (\(p=0.007\); Figure 1A, see Supplementary Figure 2 for \(p\)-values for a range of threshold values). In contrast, this relationship was not evident for mean CCyR rates when IC_{50} alone was used as the comparator (\(p=0.83\) for IC_{50} values above vs below median IC_{50}; Figure 1B).

Upon comparison of nilotinib-treated patients by either mutation IPP or IC_{50} value, the difference in mean CCyR rate between patients with values above or below the median approached but did not reach statistical significance (\(p=0.055\) for both cases; Figure 1C,D). Notably, however, both the number of mutations and overall number of nilotinib-treated patients with published available mutation-response data were smaller than for dasatinib (7 vs 11 mutations, 65 vs 295 patients with mutations, respectively).

Multivariate analysis by two methods also demonstrated that a model including IPP and TKI type significantly correlates with CCyR (\(p<0.001\)) and outperforms a model that includes IC_{50} and slope as separate variables based on the model significance, covariate significance and Akaike information criterion (Supplementary Table 1).

In conclusion, we show that in vitro dose-response slope provides important additional information regarding efficacy of ABL TKIs beyond in vitro IC_{50} values: lower slopes are indicative of increased resistance, while very high slopes result in a steep dose-response curve presenting potential challenges in cases of low compliance or unfavorable pharmacokinetic profile. Calculation of IPP (based on IC_{50}, slope and clinical plasma C_{max}) allows for separation of patients with respect to rates of CCyR with dasatinib, unlike using in vitro IC_{50} alone\(^2\). Further
implementation of our results for improvement of mutationally-guided TKI treatment selection in CML will require incorporation of additional variables such as plasma protein binding, drug clearance and distribution, as well as validation using independent large patient databases.

**Authorship**

Contribution: V.V. and B.J.D. designed research, T.O. and B.J.D. contributed all in vitro experimental data, V.V. and C.A.E. performed the research, O.S. performed statistical analysis, and V.V., C.A.E. and B.J.D. wrote the manuscript.

**Conflict-of-interest disclosure**

V.V. is employed by Neumedicines, Inc as a clinical advisor.

B.J.D. is currently principal investigator or co-investigator on Novartis and Bristol-Myers Squibb clinical trials. B.J.D.’s institution has contracts with these companies to pay for patient costs, nurse and data manager salaries, and institutional overhead. B.J.D. does not derive salary, nor does his lab receive funds from these contracts. OHSU and B.J.D. have a financial interest in MolecularMD. OHSU has licensed technology used in some of these clinical trials to MolecularMD. B.J.D.’s potential individual and institutional conflict of interest has been reviewed and managed by OHSU.

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**References**


Table1. In vitro dose-response curve parameters, calculated inhibitory potential at peak concentration (IPP), and rate of complete cytogenetic response (CCyR%)⁸,⁹ for 3 ABL TKIs in various BCR-ABL mutants.
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**Slope**

- Baseline: 0.00
- LL: 0.00
- UL: 0.00

**RR**

- Baseline: 0.89
- LL: 0.86
- UL: 0.92
Figure 1. Correlation between inhibitory potential at peak concentration (IPP) or IC$_{50}$ and clinical response for dasatinib and nilotinib. IPP was calculated based on drug IC$_{50}$ and slope of in vitro response of Ba/F3 cells expressing various BCR-ABL mutations and on population pharmacokinetic mean peak concentrations in plasma reported for each drug. Mutations were divided into two groups for dasatinib (panels A & B) and nilotinib (panels C & D) based on cut-off values of IPP (non-dimensional) or IC$_{50}$ (nM) as indicated. For each group, clinical response analysis was based on previously published mutation-specific rates of complete cytogenetic response (CCyR)$^{8,9}$, and the median, range, and 25th and 75th percentiles are shown.
Figure 1

Dasatinib

Nilotinib

Dasatinib

Nilotinib

IC50 < 1.4

IC50 > 1.4

IPP < 7

IPP > 7

IPP < 10

IPP > 10

IC50 < 50

IC50 > 50

IC50 < 7

IC50 > 7
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