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Application of PKPD Principles to Drive the Discovery and Development of Novel BCR-ABL Tyrosine Kinase Inhibitors

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OBJECTIVE

To determine key drivers for anti-chronic myeloid leukemia (CML) activity and to drive the discovery and development of novel tyrosine kinase inhibitors (TKIs) for CML, using ELVN-001 as an example

CONCLUSIONS

- Selection of appropriate *in vitro* potency measurements is important for pharmacokinetic/pharmacodynamic (PKPD) evaluation and for compound selection at the discovery stage
- A high correlation was observed between PK parameter C_{avg} and efficacy outcomes, consistent with the mechanism of action (MOA) of TKI inhibition of BCR::ABL1
  - The model was used to estimate the efficacious dose and to help identify the dose range in early clinical development for ELVN-001
  - The model is unable to reliably predict efficacy outcomes outside of the boundary, such as MMR rate for a drug with 90% patients with C_{avg} > 90%
  - A mechanistic PKPD model should be developed and used to evaluate efficacy in CML when a TKI enters clinical development

BACKGROUND

Chronic Myeloid Leukemia (CML) Overview

- CML is a myeloproliferative neoplasm characterized by the dysregulated production and uncontrolled proliferation of maturing granulocytes with relatively normal differentiation[1]
- Reciprocal translocation between chromosomes 9 and 22 within breakpoint cluster region (*BCR*) and Abelson tyrosine kinase (TK; *ABL1*) genes are in > 99% of patients
- The resultant *BCR::ABL1* oncogene encodes a BCR::ABL1 fusion protein whose constitutive TK activity leads to aberrant activation of downstream signaling pathways, driving abnormal differentiation, growth, and survival of leukemic cells
- Critical for BCR::ABL1 dependent transformation: GAB2, MYC, CRKL, and STAT5 [2,3]
- Challenges associated with current TKI therapies:
  - ~25% of patients switch TKIs within the first year, and ~40% switch within first 5 years due to loss of clinical benefit or intolerance[4]
  - Therapeutic benefit and quality of life are impacted by treatment-related adverse events (TRAEs), due in part to off-target inhibition of other TKs (*eg*, c-KIT, FLT-3, PDGFR, VEGFR2, c-SRC)[5]
  - Loss of disease control are often associated with point mutations in the BCR::ABL1 kinase domain, which impair TKI binding[6]
- ELVN-001: a potent Type I inhibitor of BCR::ABL1
  - Active against the T315I mutation *in vitro* and *in vivo*, while sparing key anti-target kinases, such as c-KIT, FLT-3, PDGFR, VEGFR2, and c-SRC
  - Due to its selectivity, ELVN-001 has the potential to minimize TRAEs and therefore enable greater target engagement and efficacy

Importance of Consideration of PK Properties and Modeling Strategies in Discovery and Early Clinical Development

- Modifying PK profile can help to optimize the safety profile
- Lower C_{max} to reduce C_{max}-related adverse events (AEs) by dosing twice daily (BID) rather than once daily (QD), if the half-life ($t_{1/2}$) allows, for example apixaban
- Reducing dosing frequency can impact outcomes (ponatinib vs. olverembatinib)
- At the discovery stage for small molecule compounds, key parameters for efficacy, safety, and PK should be considered and screened
  - This may address potential resistance mechanisms, including P-glycoprotein (P-gp)- and breast cancer resistance protein (BCRP)-mediated drug resistance
  - Evaluation of key PK attributes together with *in vitro* potency may increase the success rate of candidates
  - In discovery, simple, empirical models are favored over complicated, system pharmacology models
  - In clinical development, more sophisticated models are reserved for more quantitative predictions

METHODS

Phospho-CRKL (pCRKL) ELISA Assay for Measuring BCR::ABL1 Inhibition

- K562 ($2x10^5$cells/100 µL/well) cells were seeded in 96-well plates. Compounds were added, mixed, and incubated in the presence of 10% fetal bovine serum (FBS) or 100% human serum (HS) for 90 min at 37ºC, 5% CO_2
- Plates were then centrifuged 5 min at 3000 rpm and supernatant removed from each well. Cells were washed 3x with 150 µL PBS prior to addition of 100 µL cell lysis buffer supplied with 1x complete ULTRA cocktail inhibitor (Roche, 05892791001) and 1x PhosSTOP Phosphatase Inhibitor Cocktail Tablets (Roche, 04906837001). Cells were incubated with lysis buffer for 1 h at 4ºC. A capture antibody able to detect phosphorylated and non-phosphorylated CRKL was added to Meso Scale Discovery (MSD) standard bind plates at 5 µg/mL and incubated at 4ºC overnight
- Plates were then washed with PBS + 0.05% Tween-20 (PBST) and 150 µL of 5% BSA blocking solution was added for 1 h at room temperature with shaking
- Plates were incubated for 1 h at room temperature with shaking. Plates were washed with PBST prior to addition of 30 µL of a sulfo-tagged goat anti-mouse detection antibody and incubated for 1 h at room temperature. Plates were washed with PBST prior to addition of 150 µL of 1x MSD read buffer T
- Electrochemiluminescence was read on an MSD plate reader

In Vitro Equilibrium Dialysis for Determining Plasma Protein Binding (PPB)

- To investigate *in vitro* binding of TKIs, including ELVN-001, to mouse, rat, dog, monkey, and human plasma: dialysis buffer solution and plasma sample containing 1 µM of compounds, or the positive control ketoconazole, were added to separate chambers of dialysis wells of the HTDialysis device
- The dialysis plate was placed in an incubator at 37°C with 5% CO_2 at ~100 rpm for 6 h. After dialysis samples were treated for bioanalytical assays
- Samples in plate were vortexed for 5 min and centrifuged at 3220 g for 30 min at 4°C. The supernatant was then analyzed by UPLC-MS/MS
- Unbound fraction and bound fraction were calculated using the following equations:

$$\frac{\text{Peak Area Ratio}_{\text{buffer chamber}}}{\text{Peak Area Ratio}_{\text{plasma chamber}}}$$

$$\text{Bound fraction} = 1 - \text{Unbound fraction}$$

PK Parameters and ER correlations

- Steady-state human plasma exposure parameters (C_{max}, AUC, C_{min}) and variability to all approved TKIs were obtained from the literature or NDA review documents[7-11]
- PPB values were obtained from the literature for the approved TKIs
- The proportion of patients with newly diagnosed CML who achieved different levels of molecular response (MR, *BCR::ABL1* transcript level in plasma on an international scale, %) was obtained from the literature[12-14]
- Exposure-response (ER) correlation between steady-state PK parameters and major molecular response (MMR) rate by different time were evaluated using simply linear regression, or hyperbolic type relationship
- Average plasma concentration over a dosing interval at steady state is calculated as: $C_{avg} = \frac{AUC_{tau}}{Tau}$
- Linear equation: $Y = Slope * X + Intercept$
- E_{max} equation: $E = \frac{E_{max} * C}{(EC_{50} + C)}$

RESULTS

In Vitro Assay Selection

- The rank order of PPB adjusted potency values was consistent with those observed with 100% human serum (**Table 1**)
- In the pCRKL assay, IC_{50} values in presence of 100% human serum were most appropriate for potency comparison across TKIs

Table 1. *In Vitro* Assay Selection

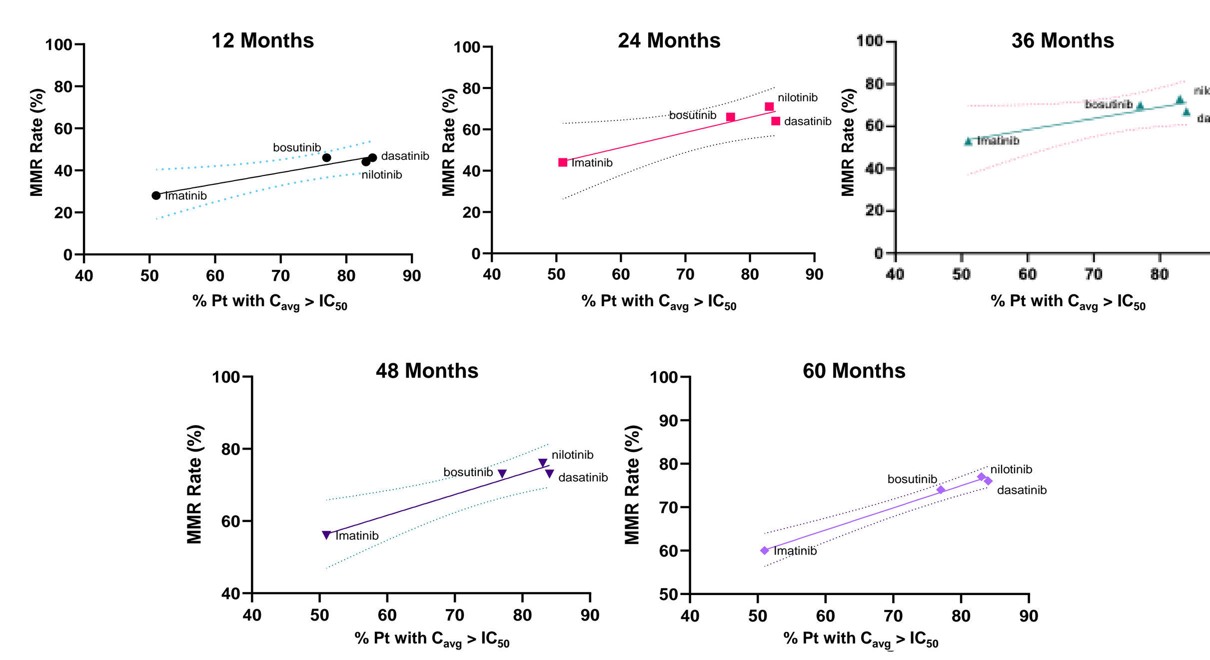
	pCRKL IC_{50} in K562 cells (nM)			
TKI	10% FBS	Unbound Fraction (%)	PPB adjusted IC_{50}	100% HS
Imatinib	503	5%	10068	7000
Nilotinib	53.7	1%	5371	1180
Dasatinib	2.53	4%	63	16
Bosutinib	34.4	6%	573	93

FBS, fetal bovine serum; HS, human serum; IC50, half maximal inhibitory concentration; PPB, plasma protein binding; TKI, tyrosine kinase inhibitor

PKPD Correlations of C_{avg} with MMR

- It is essential to understand the potential efficacy of lead compounds and pick the most efficacious and safe compound for clinical development
- An assumption of the modeling is that the efficacy readouts from different studies can be compared based on the same MOA for all drugs
- As the percentage of patients with C_{avg} > IC_{50} increased, the MMR rate by 12, 24, 36, 48, and 60 months increased (**Figure 1**)
- No clear separation of efficacy was observed between nilotinib and dasatinib, as the percentage of patient with C_{avg} > IC_{50} was similar for both drugs
- Inter-study variability was observed (**Figure 5**), but did not affect the correlation results
- The slopes of most correlation analysis were significantly different from zero, except MMR by 36 months (**Table 2**). R^2 for most correlations were greater than 0.9, except for MMR by 36 months
- A nonlinear E_{max} model could also fit the observed data (**Figure 2**), but the resultant E_{max} was higher than 100%, which was greater than the biologically achievable value

Figure 1. Correlations of C_{avg} with MMR



Note: Symbols represent observed data; line represents model fitted curve; the dotted lines represent 95% CI

Figure 2. Correlation with MMR using E_{max} Model

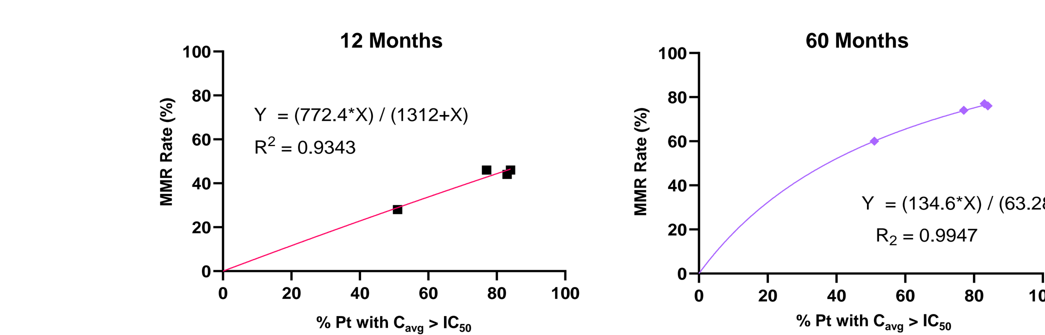


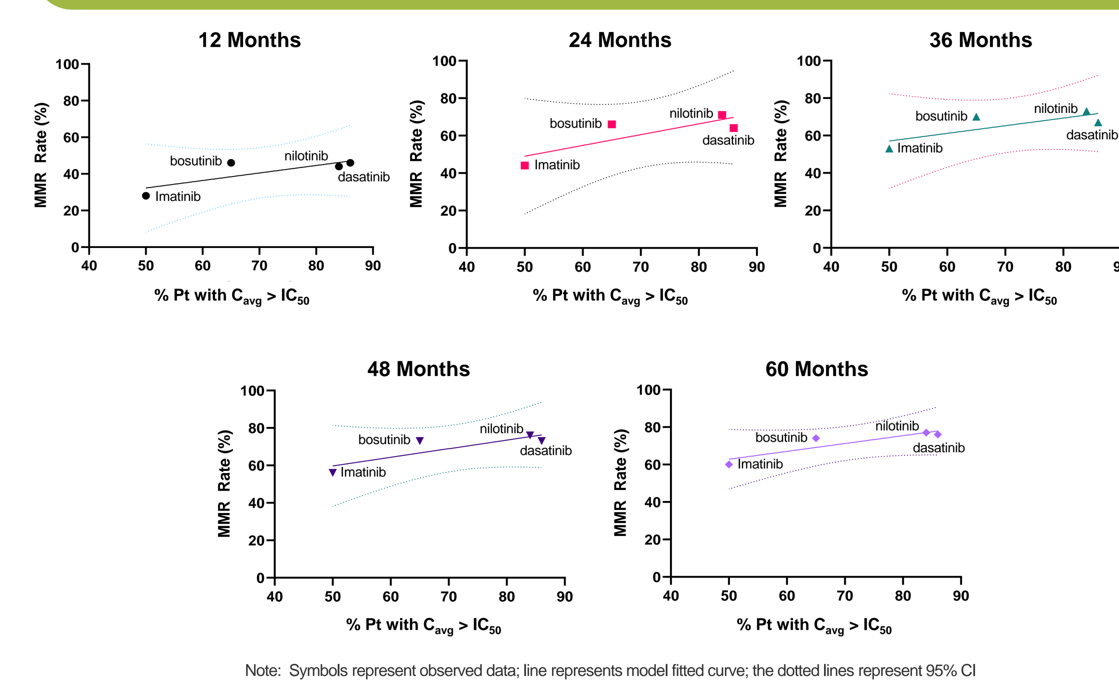
Table 2. Linear Regression Model Fitting Results

Endpoints	MMR by 12 months	MMR by 24 months	MMR by 36 months	MMR by 48 months	MMR by 60 months
Slope	0.103	0.161	0.144	0.0832	0.0335
Y-intercept	7.732	12.10	10.80	6.239	2.510
R-square	0.933	0.911	0.873	0.960	0.992
P value	0.0341	0.0453	0.0658	0.0202	0.0043

PKPD Correlations of C_{min} & MMR

- The linear fitting resulted in R^2 values of 0.642, 0.684, 0.617, 0.736, and 0.812 for MMR by 12, 24, 36, 48, and 60 months, and none of the slopes are significantly different from zero (**Figure 3**)
- The fitting with E_{max} also failed to show significance

Figure 3. Correlations of C_{min} with MMR

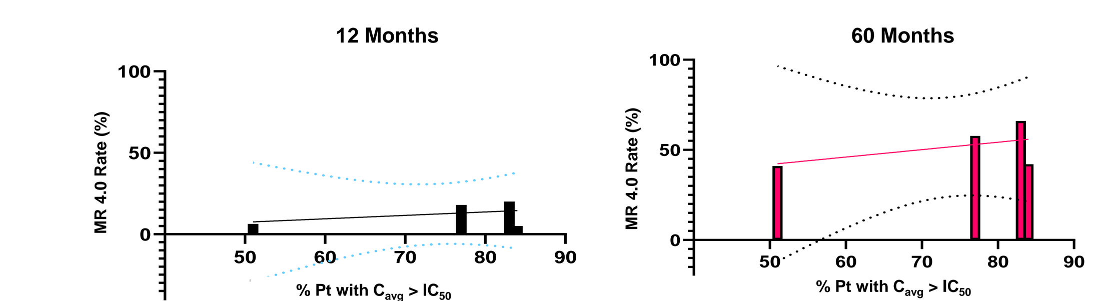


Note: Symbols represent observed data; line represents model fitted curve; the dotted lines represent 95% CI

PKPD Correlations of MR4 with C_{avg}

- The linear fitting resulted in R^2 values of 0.1765, 0.2867, 0.2217, 0.2681, and 0.2710 for MR4.0 by 12, 24, 36, 48, and 60 months, and none of the slopes were significantly different from zero (**Figure 4**)
- The fitting with E_{max} also failed to show significance
- Similarly, no clear correlation was found for MR4.5 by 12, 24, 36, 48, & 60 months

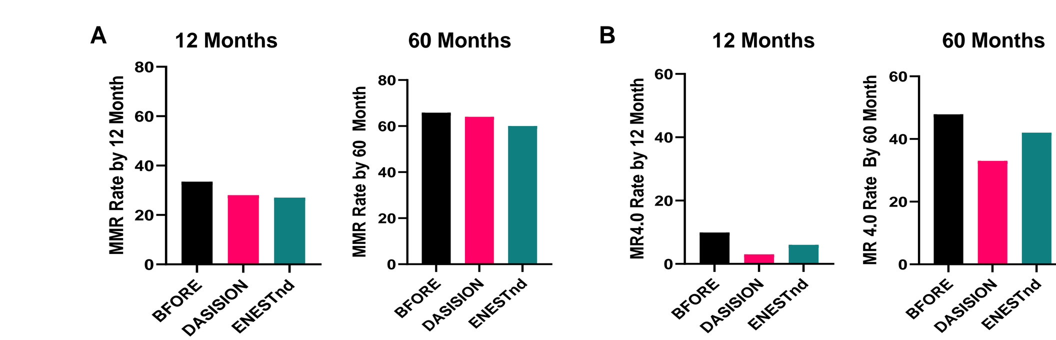
Figure 4. Correlations of C_{avg} with MR4.0



Imatinib MMR Rate Across Studies

- As shown in **Figure 5A**, there were relatively small differences in MMR rates across 3 imatinib studies (BFORE, DASISION, and ENESTnd)
  - Differences were observed between BFORE and the other two studies
  - Differences in MMR rates, however, diminished over time
- Greater magnitude of difference between studies in MR4.0 than MMR (**Figure 5B**)
  - Greater differences were observed at 12 months than at 60 months
  - The difference in MR4.0 rate is still apparent at 60 months
  - This large variability between studies may explain the lack of correlation for MR4.0 or MR4.5 with PK parameters

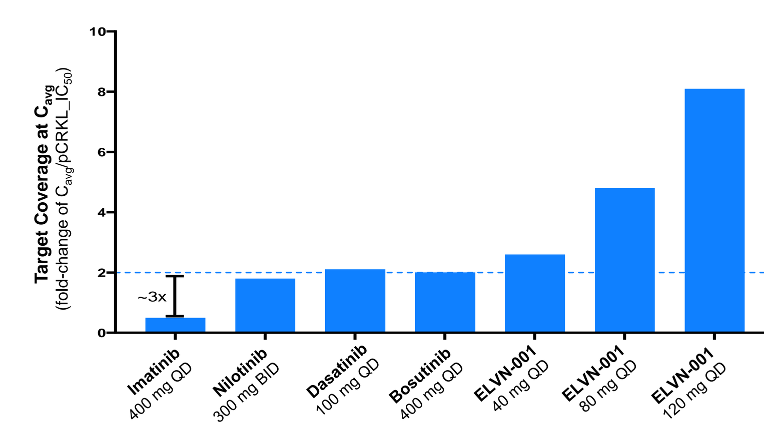
Figure 5. Imatinib MMR Rate Across Studies



Target Coverage for Comparing Drug Activity

- Comparison of the PK parameter C_{avg} by *in vitro* potency between approved TKIs and ELVN-001 are shown
  - Dose-dependent increases in target coverage by ELVN-001 are all numerically higher than comparator TKIs at their approved doses (**Figure 6**)
  - Similar intra-patient PK variabilities were assumed across compounds

Figure 6. Target Coverage



Note: Preliminary plasma PK parameters of ELVN-001 were obtained from ongoing clinical trial

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