Poster Number: 4361

Development and Application of a Mechanistic Pharmacokinetic Pharmacodynamic (PK/PD) Model to Predict Anti-Chronic Myeloid Leukemia (CML) Effects of Tyrosine Kinase Inhibitors

Enliven THERAPEUTICS

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INTRODUCTION

Chronic Myeloid Leukemia (CML):

- CML is a myeloproliferative neoplasm characterized by the dysregulated production and uncontrolled proliferation of maturing granulocytes with relatively normal differentiation[1]
- The BCR::ABL1 oncogene encodes an enzyme, BCR::ABL1 kinase, with constitutive tyrosine kinase activity that activates downstream signaling pathways, leading to abnormal differentiation, growth, and survival of leukemic cells

Current state of the disease:

- The development of tyrosine kinase inhibitors (TKIs) targeting the BCR::ABL1 kinase has improved the outcome for patients with CML
- Life expectancy for newly diagnosed patients with chronic phase (CP) CML now approaches that for the age-matched general population[2]

Unmet medical needs and challenges to standard of care:

- Patients, however, may not be able to respond to TKI therapies due to intolerance or loss of efficacy due to point mutations in the BCR::ABL1 kinase domain
- New therapeutics for CML are needed to improve the efficacy and quality life of patients with CML

BCR::ABL1 Pathways[3,4]

- Critical for BCR::ABL1 dependent transformation:
  - Gab2, Myc, and CrkL and STAT5
- CRKL is an adaptor protein, specifically important for BCR::ABL1 dependent oncogenic transformation
- STAT5 is a transcription factor, critical for leukemia initiation and maintenance

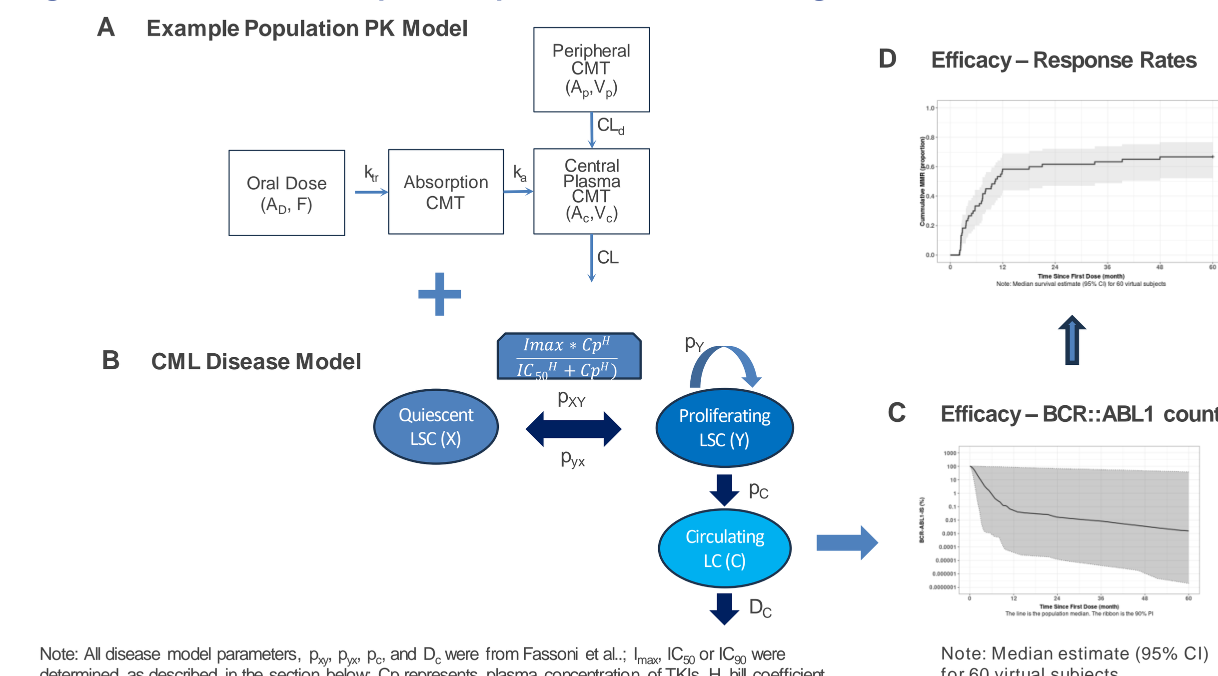
OBJECTIVE

The objective of this study was to develop a mechanistic pharmacokinetic (PK)/pharmacodynamic (PD) model to assess anti-CML activity of TKIs in patients with newly diagnosed CML. This model can then be used to predict efficacy outcomes of TKIs in clinical development.

METHODS

Structure of the Population PK/PD Modeling for BCR::ABL1 TKIs

Figure 1: Illustrative Example of Population PK/PD Modeling for CML



Note: All disease model parameters, p_{xy}, p_{yx}, p_y, p_c and D_c were from Fassoni et al.; I_{max}, IC_{50} or IC_{90} were determined as described in the section below; Cp represents plasma concentration of TKIs, H, hill coefficient

Note: Median estimate (95% CI) for 60 virtual subjects

- The population PK/PD (popPK/PD) model consists of three parts: a population PK for a specific TKI, a systems PD model which is TKI independent, and a TKI effect component. Characteristics are:
  - Fixed mean population parameters for CML disease model for all TKIs
    - Population variability associated with each parameters were from Fassoni et al.[21]
  - Shared inhibition mechanism among TKIs for BCR::ABL1
    - Maximum inhibition (I_{max}) is determined without population variability
    - Hill coefficient is determined without population variability
  - Compound specific parameters of popPK of respective TKIs
    - PK parameters and associated population variability obtained from publications
    - Dosing is according to respective drug labels
  - Inhibition potencies (IC_{50} or IC_{90}) are determined using *in vitro* assays, without population variability

In Vitro Potency Determination

- **pCRKL assay:** K562 cells (2.0x10^5 cells/100µl/well) were seeded in a 96-well plate. Compounds were added, mixed, and incubated in the presence of 10% FBS or 100% HS for 90 min at 37°C, 5% CO_2. Plates were centrifuged (5 min, 3000 RPM) and supernatant removed. Cells were washed 3x (150µl PBS), then incubated for 1 h, 4°C with 100µl cell lysis buffer plus 1x complete ULTRA cocktail inhibitor and 1x PhosSTOP Phosphatase Inhibitor Cocktail Tablets (Roche). A capture Ab (5µg/mL) for detecting CRKL and pCRKL was added and incubated overnight, 4°C. Plates were washed with PBST, then blocked with 150µl 5% BSA blocking solution (1 h, room temp) with shaking. MSD plates were washed, then incubated with 30µl of a pCRKL detection Ab (1µg/mL) for 1 h, room temp, with shaking. Plates were washed, then incubated with 30µl of a sulfo-tagged goat anti-mouse detection Ab for 1 h at room temp. Plates were washed with PBST prior to addition of 150µl of 1x MSD read buffer T, and ECLU measured on an MSD plate reader.
- **pSTAT5 assay:** K562 cells (2.0x10^4 cells/40µl/well) were seeded in a 384-well plate. 40nL of the compound dilution series were transferred to assay plates using liquid handler Echo550. Negative and positive controls were DMSO alone (high control, HC) and 10µM nilotinib (low control, LC), respectively. Plates were removed from the incubator after 4 h, media removed, and cells lysed for 40 min with 1x lysis buffer. Lysates were transferred to a detection plate (Optiplate, APRICOT DESIGNS), and detection procedures were completed at room temp in the dark: 5µl/well 1x acceptor mix was added and incubated for 1.5–2 h, then 5µl/well of 1x donor mix was added and incubated overnight. The signal was detected by the Envision plate reader using standard AlphaLISA settings. Inhibitory activity was calculated as follows:

$$\% \text{ Inhibition} = 100 \times (\text{LumHC} - \text{LumSample}) / (\text{LumHC} - \text{LumLC})$$

- IC_{50} and IC_{90} was determined by curve fitting the resultant dose/response % inhibition using Xlfit (v5.3.1.3):

$$Y = \text{Bottom} + (\text{Top} - \text{Bottom})/(1 + 10^{((\text{LogIC50} - X)*\text{HillSlope})})$$

Population PK Modeling

- PopPK models for imatinib, dasatinib, nilotinib, bosutinib, and asciminib were obtained from literature. If multiple popPK models existed, the most appropriate model was selected after it was validated against literature data.
- The popPK models were then applied to the popPK/PD validation without further modification of parameters
- **Table 1** shows the final selected popPK model and the data sources used for validation. Models which were not selected are not shown.

Table 1: Final Population PK Model

Drug	PopPK model source	Validation literature sources
imatinib	Schmidli[5]	Peng, Arora, le Coutre[6-8]
dasatinib	Wang[9]	Wang, FDA NDA[9,10]
nilotinib	Giles[11]	FDA NDA, Zhou, Yin, Wang[12-15]
bosutinib	Hsyu[16]	FDA NDA, Abbas[17,18]
asciminib	Li[19]	Li, Hoch[19,20]

PD Modeling

- The PD model was adapted from the publication by Fassoni et al. (**Figure 1B**)[21]
- In this model, leukemic stem cells (LSC) may exist in two states: quiescent state or proliferating state.[22] There is no resistant cell pool in this model.
- After proliferation, some differentiated leukemic cells will enter the blood circulation
- TKIs will affect the proliferating LSCs only. TKIs have no direct impact on quiescent LSCs.

Simulation of Molecular Response

- Simulations were conducted using PD parameters listed in **Table 2** for five TKIs. All other PK/PD parameters were not listed in the table.
- A virtual CML patient population was created according to publication[22] (N=200 for all TKI simulations)
- For simplicity, a Hill coefficient of 2.5 was used for all TKIs. This might result in a slight overprediction of efficacy for some TKIs.
- The model simulation results were then compared with literature-reported efficacy results of newly diagnosed patients with CML[23-26]

Table 2: Model Parameters Used for PopPK/PD Simulation

	Imatinib	Nilotinib	Dasatinib	Bosutinib	Asciminib
Dose (mg)	400 QD	400 BID	100 QD	500 QD	80 mg QD
I_{max}	0.0004	0.0004	0.0004	0.0004	0.0004
IC_{50} /IC_{90} (nM)	7000	1180	21	123	183*
Hill	2.5	2.5	2.5	2.5	2.5

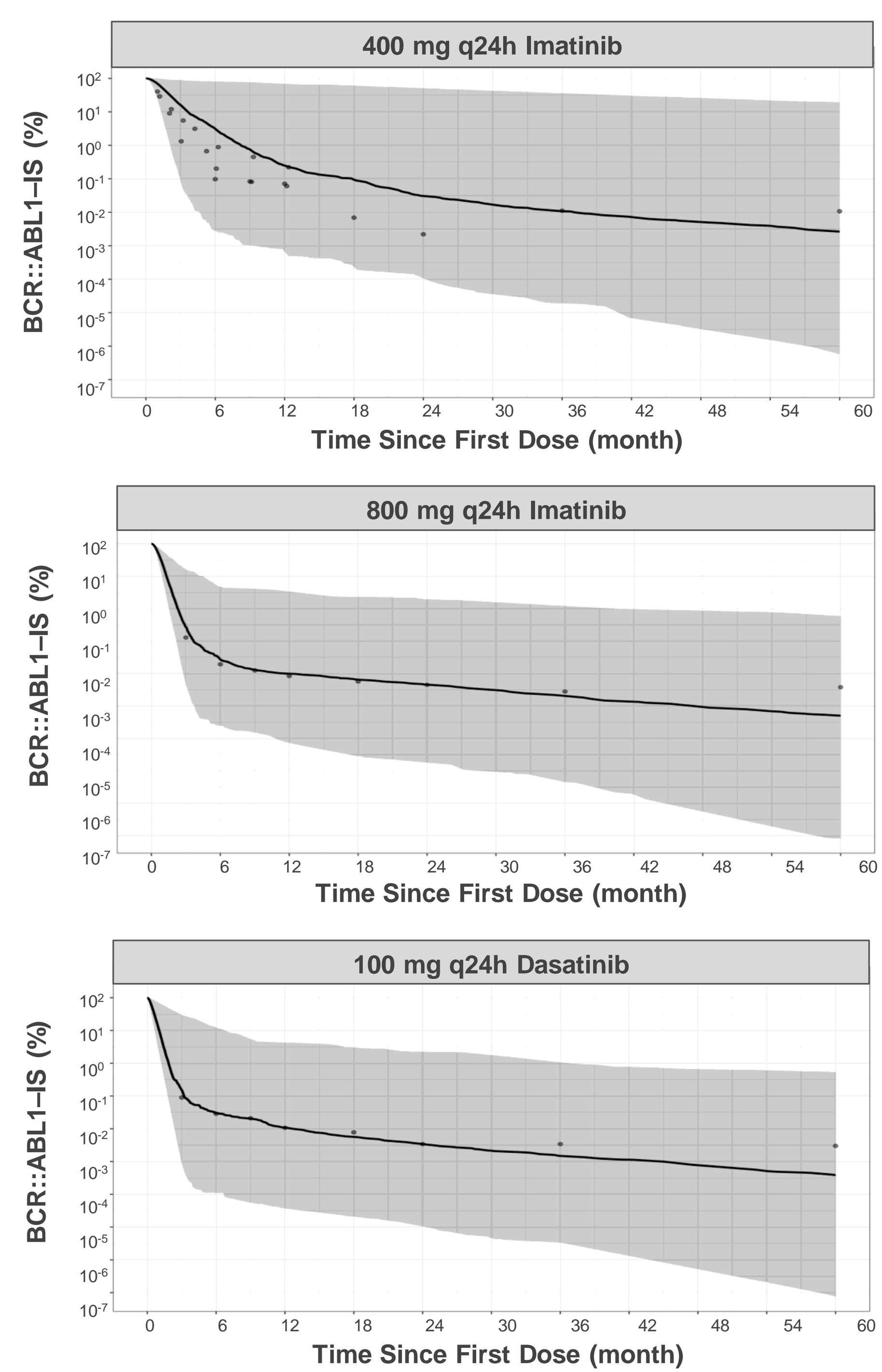
NOTE:
*Asciminib simulation was conducted with pSTAT5 IC_{90} value after correction with PPB. Simulations with the other TKIs were conducted with pCRKL IC_{50}s in the presence of 100% human serum.
There is no variability assigned to the values listed in this table.

RESULTS

Model: Predicted BCR::ABL1 vs Observed[27]

- The across TKI system parameters (I_{max} and Hill coefficient) were determined by using BCR::ABL1 data obtained from the literature for dasatinib and imatinib; while the compound specific parameters (pCRKL IC_{50}/pSTAT5 IC_{90}) were determined from *in vitro* experiments and were adjusted for PPB (pSTAT5)
- Interestingly, the I_{max} values for imatinib and dasatinib were similar, but Hill coefficients were, 1.3 and 2.5, respectively
  - The binding mode of imatinib is different from that of dasatinib, which might cause the coordinating coefficient to be different for the two drugs
- Shown in **Figure 3**, the simulated median BCR::ABL1 counts agreed well with observed data for imatinib (400 and 800mg QD) and dasatinib (100mg QD), and all observed data fell within the predicted 90% prediction interval
- After simulations were conducted with a virtual CML patient population of 200, response rates (cumulative MMR, MR4, MR4.5, and MR5.0 rate at specified time points, including 12 and 24 months) were calculated based on simulated BCR::ABL1 counts for all virtual patients

Figure 3: Model-Simulated Mean BCR::ABL1 Levels Following a 60-Month Treatment

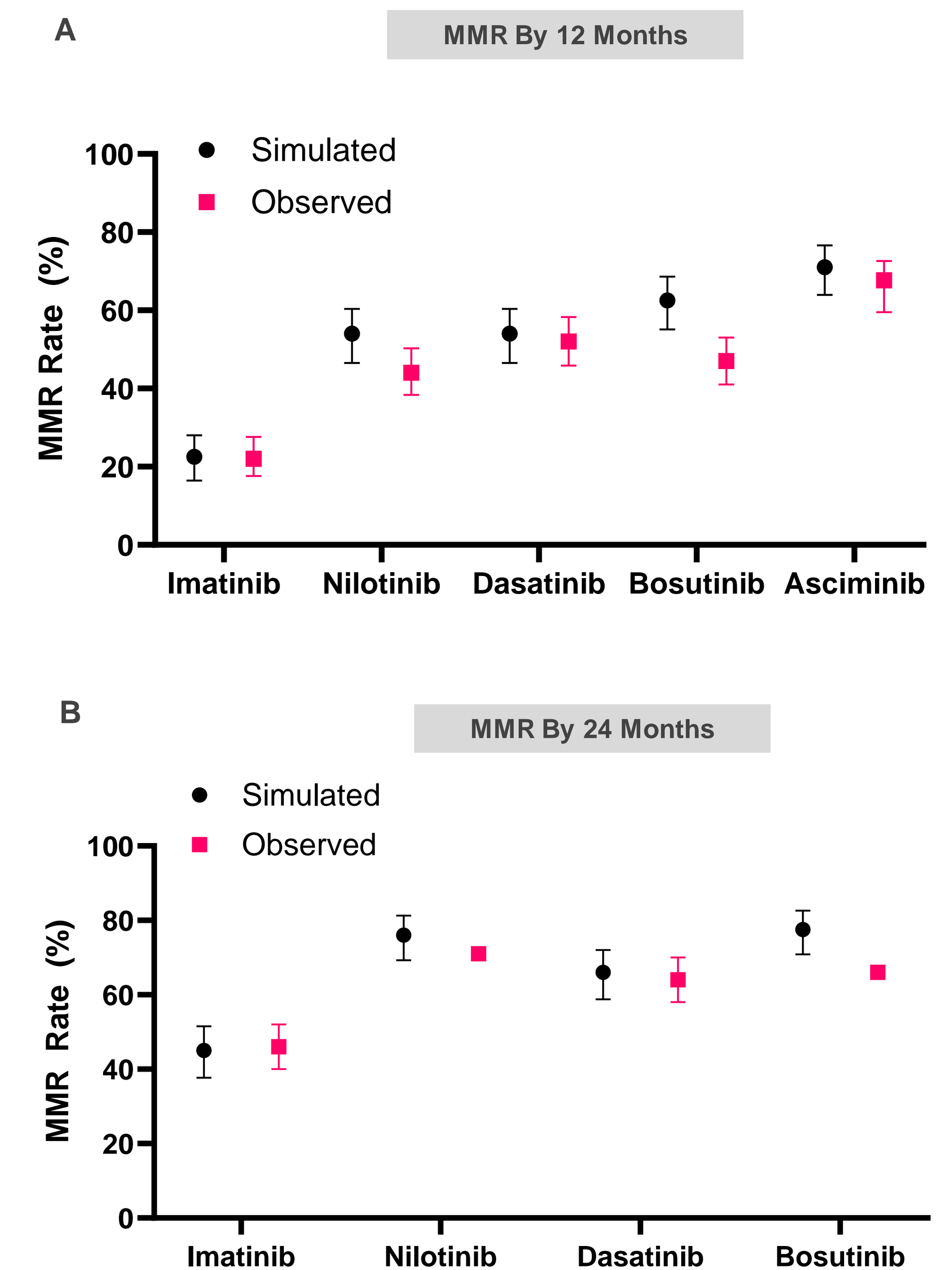


Note: Median (90% PI) shown

Population PK/PD Model Validation – Predicted vs. Observed Cumulative Molecular Response Rates of TKIs Across Studies

- Model-simulated major molecular response (MMR) rates by 12 months were comparable to literature-reported efficacy results at the labeled doses for respective TKIs, as shown in **Figure 4A**
- Model-simulated MMR rates by 24 months were also comparable to literature-reported results (**Figure 4B**)
- In addition, model-simulated MR4.0 and MR4.5 rates by 12 and 24 months were also comparable to literature-reported results (data not shown)

Figure 4: Observed vs. Model-Simulated MMR of TKIs Across Studies (A) By 12 Months; (B) By 24 Months



CONCLUSIONS

- A mechanistic popPK/PD model was developed to predict the efficacy outcome of BCR::ABL1 TKIs in newly diagnosed patients with CML
- The model-simulated results agreed with literature-reported data, with reasonable accuracy
- The model was used to predict the asciminib Phase 3 study efficacy results before it was published and it agreed with the observed MMR rate by week 48
- The model also suggests that the adherence to therapy/regimen for respective TKIs is key to achieve the desired efficacy. The overprediction of second and third generation TKIs may be related to its safety and tolerability profile rather than potency, since there was no dropout considered in the simulation.
- Overall, the system PK/PD model structure enabled estimation of drug efficacy using *in vitro* measured potency parameters (pCRKL IC_{50} or pSTAT5 IC_{90}) and simulated human PK profiles
- This model may help drug development by predicting efficacy outcomes before any significant clinical development investment

Antibody, Ab; BSA, bovine serum albumin; DMSO, dimethyl sulfoxide; ECLU, electrochemiluminescence; FBS, fetal bovine serum; HC, high control; low control, HS; human serum; IS, internal standards; LC; MSD, Meso Scale Discovery; PBS, phosphate-buffered serum; PBST, PBS + 0.05% Tween 20; pCRKL, phosphorylated CRKL; PPB, plasma protein binding

Acknowledgments: Ingrid Koo, PhD, provided editorial support for this poster.

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Presented at the 2025 American Association for Cancer Research Annual Meeting