ARTICLE

Population pharmacokinetic–pharmacodynamic modeling of PB2452, a monoclonal antibody fragment being developed as a ticagrelor reversal agent, in healthy volunteers

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Abstract

PB2452, a neutralizing monoclonal antibody fragment that binds the antiplatelet drug ticagrelor with high affinity, is being developed as a ticagrelor reversal agent. To identify a clinically useful intravenous (i.v.) reversal regimen, a semimechanistic exposure-response model was developed during the PB2452 first-in-human phase I study. From a randomized, double-blind, placebo-controlled, single-dose trial to evaluate the safety, efficacy, and pharmacokinetics (PKs) of PB2452 in 61 healthy volunteers pretreated with ticagrelor, sequential dose cohort data were used to build and refine an exposure-response model that combined population PK models for ticagrelor (TICA), ticagrelor active metabolite (TAM), and PB2452, and related their binding relationships to the PK of uncomplexed TICA and TAM which is predictive of platelet inhibition. Platelet function was assessed by multiple assays. The model was developed using Bayesian methods in NONMEM. Human PK and pharmacodynamic data from sequential dose cohorts were used to initially define and then refine model parameters. Model simulations indicated that an initial i.v. bolus of PB2452, followed by a high-rate infusion, and then a slower-rate infusion would provide immediate and sustained reversal of the antiplatelet effects of ticagrelor. Based on model predictions, a 6 g i.v. bolus followed by 6 g infused over 4 h and then 6 g over 12 h was identified and tested in study subjects and shown to provide complete reversal within 5 min of infusion onset that was sustained for 20–24 h. The model is predictive of the reversal profile of PB2452 and will inform future trials of PB2452.

Study Highlights

WHAT IS THE CURRENT KNOWLEDGE ON THE TOPIC?
Ticagrelor (TICA) is a direct acting, reversibly binding, oral $P2Y_{12}$ receptor antagonist. PB2452 (bentracimab), a neutralizing monoclonal antibody fragment that binds the antiplatelet drug TICA with high affinity, is being developed as a TICA reversal agent.

WHAT QUESTION DID THIS STUDY ADDRESS?
This study mathematically explored the pharmacokinetics (PKs) of PB2452, its interaction with ticagrelor and the active metabolite, and the ability of PB2452 to reverse the antiplatelet effects of TICA.

WHAT DOES THIS STUDY ADD TO OUR KNOWLEDGE?
A population PK/pharmacodynamic (PD) model describing the PKs of PB2452, ticagrelor, an active metabolite, and the platelet function is presented. The model was developed using data in human healthy volunteers and was used to determine potentially effective doses and schedules for administering PB2452 in future clinical trials.

HOW MIGHT THIS CHANGE DRUG DISCOVERY, DEVELOPMENT, AND/OR THERAPEUTICS?
The PK/PD model may assist with the development of PB2452 by assisting with interpretation of data and providing simulations for experimental designs. The model was developed in healthy volunteers but can be updated with patient information.

INTRODUCTION

Antiplatelet therapy is a cornerstone of secondary prevention of cardiovascular (CV) events.[1] In particular, for high-risk patients, such as those with acute coronary syndromes, with prior myocardial infarction, or undergoing stenting, the prevailing paradigm is use of dual antiplatelet therapy, that is, the combination of aspirin plus an oral $P2Y_{12}$ receptor antagonist. Ticagrelor (TICA) is a direct acting oral $P2Y_{12}$ receptor antagonist with reversible receptor binding kinetics.[2] The 180 mg loading dose followed by 90 mg twice daily, in combination with low dose aspirin, has been proven to reduce the composite of CV death, myocardial infarction (MI), or stroke in patients with acute coronary syndromes in the PLATelet inhibition and patient Outcomes (PLATO) trial.[3] More recently, the THEMIS and THEMIS-PCI trials led to the US Food and Drug Administration (FDA) approval of TICA for preventing a first MI or stroke in patients with stable coronary artery disease as well.[4–6]

A major limitation of oral $P2Y_{12}$ receptor antagonists is the increased bleeding risk during therapy that persists for several days even after drug therapy ceases. The antiplatelet effects can markedly inhibit achievement of hemostasis in patients with spontaneous or procedure-related major bleeding. If an urgent or emergency procedure is indicated, the physician performing the procedure must decide whether to proceed and accept the increased bleeding risk or whether to postpone the procedure for several days and accept the increased ischemic risk after discontinuing the antiplatelet therapy and the risk associated with delaying a medically necessary procedure. The American College of Cardiology Foundation–American Heart Association, European Society of Cardiology, and other society guidelines recommend cessation of oral $P2Y_{12}$ receptor antagonists for 3–7 days before surgery.[7,8]

PB2452, also known as bentracimab (formerly MEDI2452), is a neutralizing monoclonal antibody fragment that binds TICA and its major active circulating metabolite (TAM) with high affinity.[9] A first-in-human clinical trial was conducted to determine if PB2452 could reverse rapidly the antiplatelet effects of TICA, and in this manner reduce the risk or severity of bleeding.[10]

A model for PB2452 was developed preclinically,[11] where it was referred to as MEDI2452. The goal of the present analysis is to fit a population pharmacokinetic/pharmacodynamic (PK/PD) model to characterize the relationship between PB2452, TICA, TAM, and change in platelet aggregation and P2Y12 receptor signaling, as measured by light transmittance aggregometry (LTA) which assesses inhibition of platelet aggregation, the VerifyNow $P2Y_{12}$ assay, which assesses P2Y12 reactivity units (PRUs), and the enzyme-linked immunosorbent assay (ELISA)-based vasodilator stimulated phosphoprotein (VASP) phosphorylation assay, which assesses receptor signaling with the platelet reactivity index using clinical data with the preclinical model[11] as a starting point. This model was developed during the conduct of the first-in-human study and used to inform dosing decisions throughout the study with the aim of finding a dose and regimen that would provide complete reversal of TICA's effect on platelet aggregation within 5 min of infusion onset, that would be sustained for 20–24 h. The model was then updated with final data from the trial. This semimechanistic model was used to predict the PKs of PB2452, TICA, and TAM, as well as the PD effect of PB2452 following various dosing regimen to develop a dosing regimen to be used in subsequent studies.

MATERIALS AND METHODS

Study design

A single-center, randomized, double-blind, placebo-controlled, single-ascending-dose, phase I trial was conducted to evaluate the safety, efficacy, and PK profiles of PB2452 in healthy volunteers 18–50 years of age who were pretreated with TICA. The study had 10 cohorts of healthy volunteers. Table 1 shows the dosing regimen for each cohort.

Subjects in cohorts 1 through 3 did not receive ticagrelor. Subjects in cohorts 4–10 were pretreated with TICA for 48 h. The first dose of TICA was an oral loading dose of 180 mg followed by 90 mg administered orally twice daily for 2 days. Subjects in cohorts 4 through 6 were administered the study drug (PB2452) i.v. immediately after the fifth TICA dose, whereas subjects in cohorts 7 through 10 were administered the study drug 2 h after the fifth TICA dose at the time of peak TICA concentration. Further details on the design and conduct of the study are provided in ref. 10

Data assembly

All data used in the PK/PD analysis were obtained from subjects in the clinical trial who received TICA and/or PB2452. Subjects who received TICA alone (TICA with placebo) were included in the development of the model.

TICA concentrations, PB2452 concentrations, TAM concentrations, demographic information, and measures of platelet aggregation (PRU, LTA, and VASP) were used to build NONMEM (version 7.4; ICON Development Solutions) input data for the PK/PD analysis. The data consist of total TICA (including the PB2452-TICA complex, protein-bound TICA, and free TICA), Total TAM (including the PB2452-TAM complex, protein-bound TAM, and free TAM), uncomplexed PB2452, and total PB2452 (including uncomplexed PB2452, the PB2452-TICA complex, and the PB2452-TAM complex) along with the PD measures of platelet aggregation and activation (PRU/LTA/VASP).

Prior to modeling, PB2452, TICA, and TAM concentrations were converted to nanomolar (nM) units using the molecular weights for the analytes.

Time is defined as the time following the first administration of TICA (except for cohorts 1–3, where it is time after first administration of PB2452 because TICA was not given). Depending on the cohort, PB2452 is administered at either 48 h (cohorts 4–6) or 50 h (cohorts 7–10).

Data analysis

Population PK and PK/PD analyses were carried out using NONMEM version 7.4 (control file available as Supplementary Material S1), PDx-Pop version 5.2 and Intel Visual Fortran Compiler version 12 on Microsoft Windows 10 Professional.

The models described in the following sections are nonlinear hierarchical models that were fit using Bayesian

TABLE 1 Dosing regimen for each cohort

Cohort	Pre-PB2452 ticagrelor dosing to steady state	PB2452 dose (g)	PB2452 infusion time	N (Active:Placebo)
1		0.1	30 min	3:1
2		0.3	30 min	3:1
3		1.0	30 min	3:1
4[a]	180 mg + 90 mg b.i.d.	1.0	30 min	6:2
5[a]	180 mg + 90 mg b.i.d.	3.0	30 min	6:2
6[a]	180 mg + 90 mg b.i.d.	9.0	30 min	6:2
7[b]	180 mg + 90 mg b.i.d.	18.0	3 g 5 min + 15 g 8 h	6:2
8[b]	180 mg + 90 mg b.i.d.	18.0	6 g 15 min + 6 g 3 h + 6 g 8 h 45 min	6:2
9[b]	180 mg + 90 mg b.i.d.	18.0	6 g 15 min + 6 g 4 h + 6 g 12 h	3:1
10[b]	180 mg +90 mg b.i.d.	18.0	6 g 10 min + 6 g 3 h + 6 g 13 h	6:2

[a]The last dose of ticagrelor and PB2452 were administered simultaneously.

[b]The last dose of ticagrelor was give 2 h prior to the administration of PB2452.

Markov Chain Monte-Carlo (MCMC) techniques. The Bayesian analysis involved the estimation of the joint posterior distribution of all parameters conditional on the observed data. Generating random samples from the joint posterior distribution allows the marginal distribution of each parameter to be completely characterized. More details on Bayesian methods in general may be found elsewhere.[12] Trace plots were utilized to determine how long to run the burn-in phase and how many samples from the posterior distribution to generate. For these models, a burn-in of 5000 samples was adequate. Samples of between 10,000 and 20,000 were generated for providing posterior distribution estimates. A mix of noninformative and informative priors were utilized as discussed below.

The MU referencing technique was utilized in NONMEM.[13] In particular, MU_1 (for example) was set equal to THETA(1). Then a particular model parameter was set equal to EXP(MU_1 + ETA(1)). So, THETA represents the population value of the parameter on the log-scale. This usually helps to improve the efficiency (speed of convergence and reduced autocorrelation) of the algorithms (MCMC, but also applies to other "advanced" algorithms available) utilized.

Covariates were examined for the final PK/PD model to identify potential factors affecting the PK/PD of PB2452 and its relationship to TICA and TAM. Covariates were also examined for parameters relating uncomplexed TICA and TAM to PRU/LTA/VASP. The covariates considered include demographics (age, weight, sex, body mass index, and race), liver functions tests (aspartate aminotransferase, alanine aminotransferase, and Alk. Phos.), baseline PRU/VASP/LTA, kidney marker (estimated glomerular filtration rate [eGFR]), and hematocrit.

Development of the PK model

A diagram of the model considered is in Figure 1. The model is similar to the model developed preclinically,[11] with some adjustments made to better fit the clinical data. TICA is dosed orally and passes through two transit compartments prior to entering the central compartment. TICA is metabolized to its active metabolite TAM, and both TICA and TAM diffuse into peripheral compartments. PB2452 is dosed i.v., and directly enters the central compartment. PB2452 can also diffuse into a peripheral compartment. PB2452 binds to TICA and TAM, forming the PB2452-TICA and PB2452-TAM complexes respectively. These complexes render TICA and TAM inactive. In the model, it is assumed that the complexes are cleared at the same rate as PB2452. An important modification from the preclinical model to this clinical model was the addition of compartments

for the complex to enter where TICA and TAM return to circulation, whereas PB2452 is removed from the system are also included in the model.

PD model

The population PK/PD model relates the model predicted PK of uncomplexed TICA and TAM to the PD measures through maximum effect (E_{max}) models. The approach here is similar to models for TICA alone,[14] except that TICA and TAM were included separately in the model as

$$PRU = Base * \left(1 - \frac{Emax_1 * TICA^{\gamma}}{EC50_1^{\gamma} + TICA^{\gamma}} - \frac{Emax_2 * TAM^{\gamma}}{EC50_2^{\gamma} + TAM^{\gamma}} \right)$$

where Base refers to the baseline (prior to administration of TICA) value. E_{maxi}, $EC50_i$, and the Hill coefficient (γ) are parameters to be estimated. The same structural model was used for PRU, VASP, and LTA. The complexes PB2452-TICA and PB2452-TAM are considered to render TICA and TAM inactive, and therefore these complexes do not contribute to any PD effects.

RESULTS

A total of 61 (48 treated with PB2452 and 13 with placebo) patients who received PB2452 and/or TICA were included in the PK/PD analysis.

Initial model structure based on the preclinical model and optimization of the model

The initial model was as depicted in Figure 1, but it did not include the extra PB2452-TICA and PB-2452-TAM compartments that were added to allow TICA and TAM to return to circulation. Those two compartments were added later, as described below. The initial model was based on the premise that PB2452 formed a complex with TICA and TAM that was then presumably removed largely through the kidneys. The expected result would be an increase in PRU/LTA/VASP toward baseline (pre-TICA administration) levels. Baseline PRU/LTA/VASP were added in the PD models using the observed value from the data for each subject. An ETA term was added to allow for potential measurement error.

Due to the complexity of the model for the data collected, there are potential identifiability issues for estimating all the parameters in the model. Decisions were
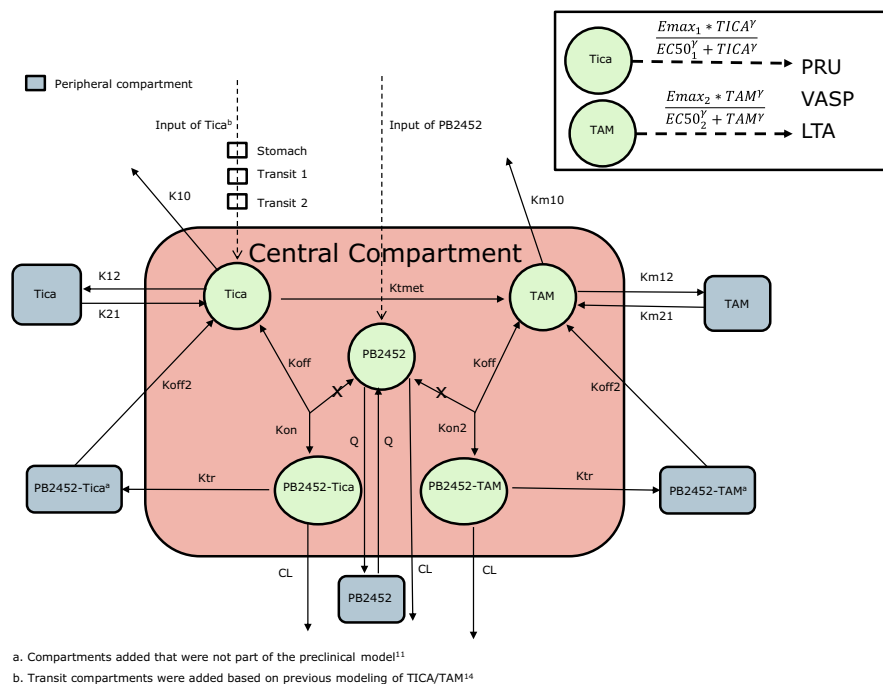
FIGURE 1 Schematic illustration of the combined ticagrelor (TICA), metabolite, and PB2452 pharmacokinetic model. Inputs into the system for ticagrelor and PB2452 are depicted with dashed lines. The model consists of two-compartment models (central and peripheral) for ticagrelor, metabolite, and PB2452. TICA is metabolized to form the active metabolite at a rate of Ktmet. PB2452 binds to TICA and the metabolite to form complexes denoted as PB2452-TICA and PB2452-TAM, respectively. These complexes dissociate to return TICA, metabolite, and PB2452 to systemic circulation. The model assumes that the clearance for the complexes and PB2452 alone are the same. Additional PB2452-TICA and PB2452-TAM compartments were added to account for the process whereby TICA and the metabolite are returned to circulation at a later timepoint. PB2452 is removed from the system. When the complexes dissociate from the original compartments, PB2452 does not return to systemic circulation in this model (represented by an X on the arrows). CL, clearance; EC50, half-maximal effective concentration; E_{max}, maximum effect; LTA, light transmittance aggregometry; PRU, P2Y12 reactivity unit; TAM, ticagrelor active metabolite; VASP, vasodilator stimulated phosphoprotein

TABLE 2 Population PK parameters of final PK model (PB2452 alone)

Parameters (units)	Final estimate	95% CI		Interindividual variability
		Lower	Upper	
CL (L/h) = EXP(THETA1)				
THETA1	0.632	0.383	0.881	37.8%
V1 (L) = EXP(THETA2)				
THETA2	1.05	0.781	1.32	40.4%
Q (L/h) = EXP(THETA3)				
THETA3	−0.770	−1.07	−0.470	42.8%
V2 (L) = EXP(THETA4)				
THETA4	1.24	0.744	1.74	62.9%
Residual variability: CV = 7.11%				

Abbreviations: CI, confidence interval; CL, clearance; CV, coefficient of variation; PK, pharmacokinetic.

made to either fix or constrain some of the parameters based on prior distributions in the Bayesian analysis. After these steps (detailed below) were taken, the trace plots with diverse starting values (Supplementary Material S2) indicate that the parameters are being estimated well.[15,16] Because TICA has been studied and modeled when administered alone, the PK parameters associated with uncomplexed TICA and TAM (and not associated directly with their relationship to PB2452) were fixed to the model-predicted values of the previous model.[14]

Because subjects from cohorts 1–3 were administered PB2452 alone (no TICA was given), data from these cohorts was used to model the PK of uncomplexed PB2452.

A two-compartment model was fit to the concentration of PB2452 data versus time. The PK parameters associated with this model are in Table 2. PB2452 has a clearance of 1.88 L/h and central volume of distribution of 2.86 L. The half-life for the distribution phase and elimination phase are 0.81 h and 6.68 h, respectively. In the PK/PD model, the parameters associated with the PK of PB2452 (that are not associated with the relationship between PB2452/TICA/TAM) were fixed to these values.

The E_{max} parameter relating TICA to PRU (as well as VASP and LTA) was fixed to 90%. The EC50 value was estimated to be large, especially relative to the concentrations expected for TICA, suggesting that the E_{max} would not be reliably estimated using the current data. It is anticipated that this parameter value would be large,[14] so it was fixed at a large value. Kd (affinity of PB2452 for TICA and TAM) was fixed to EXP(−4) = 0.018. This value was chosen based on literature.[9,11] Finally, fm was set equal to 0.30. This corresponds to about 23% metabolism for TICA to TAM (when no PB2452 is present), which is consistent with literature.[14]

For parameters that were fixed, ETAs were still added to some of them with the OMEGA set to a small value (5%–10%) to allow for some exploration of potential patterns and trends, especially related to covariates. The choice of which parameters to add an ETA to was based on literature.[14] Table 3 presents parameter estimates for the final (covariates included) model, but also indicates which parameters had an ETA added with a small, fixed OMEGA value.

For parameters that were to be estimated, most of the prior distributions were uninformative or weakly informative. The prior distribution for all THETAs in the model (natural log of model parameters) was normal with mean = 0 and variance = 25 except for the THETA corresponding to the EC50 for the effect of TICA on PRU/LTA/VASP and the THETA corresponding to the E_{max} for the effect of TAM on PRU/LTA/VASP. For the EC50 relating TICA to the PD measures, the natural log of the parameter had a normal prior with mean = 4 and variance = 100 because that parameter seemed to be large based on initial model runs. For the E_{max} relating TAM to the PD measures, the natural log of the parameter had a normal prior with mean = −0.10 and variance = 0.25 because the value was expected for E_{max} was expected to be around 90–99%.

The Hill coefficients for the E_{max} models relating the metabolism of TICA to PB2452 concentrations and relating TICA and TAM to PRU/LTA/VASP were all set equal to 2. This provided a slight improvement in model fits versus leaving the Hill coefficients set to 1. This parameter was estimated to be around 1.59 for the sum of TICA and TAM in a model where TICA and TAM were modeled alone.[14]

For interindividual variability, the exponential error structure was used. For residual variability, proportional and additive errors were utilized. Proportional was used for uncomplexed PB2452 and total TAM. Proportional plus additive was used for total PB2452 and total TICA. The additive error was used for PRU/LTA/VASP. When the proportional error alone was used for total PB2452 and total TICA, the estimate for the proportional error was excessively large, leading to simulations that were not meaningful. The problem was mitigated by adding the additive error term.

Based on the covariate plots (ETAs vs. covariates) during the initial stages of model development, it appears that the clearance of TAM has a strong relationship with weight. The clearance of TAM (the population value or THETA) was estimated instead of being fixed.

A final base model was run with the metabolism of TICA as a function of the concentration of PB2452 and with the population value (THETA) of clearance for TAM being estimated.

Final model structure – updating the model to better fit the clinical data

As the study progressed through sequential dose cohorts, it became apparent that some aspects of the PB2452 to TICA and TAM relationships were unaccounted for in the base model. Emerging data suggested that there was potentially some sequestering and recycling of TICA and/or TAM post-complexation with PB2452, resulting in these analytes returning to circulation and having an effect on PRU/LTA/VASP. This is seen in Figure 2 where the data along with simulations from the final model for cohorts 4 through 10 are presented (cohorts 1–3 were administered PB2452 without TICA). In cohorts 4, 5, and 6, it may be seen that the drug had an early effect (the PRU values were increased close to baseline values) that lasted less than 2 h before the PRU values decreased back to the levels that were seen after TICA administration and before PB2452 administration. This was anticipated for cohorts 4 and 5 but was not expected for cohort 6 (9 g PB2452 for 30 min) based on initial modeling assumptions. It was anticipated that administration of 9 g PB2452 for 30 min would be sufficient to increase PRU values to the pre-TICA baseline and sustain this increase.

Due to the rapid decrease in PRU following PB2452 administration in cohorts 4 through 6, prolonged infusion regimens of PB2452 were investigated in subsequent cohorts. For cohort 7, a bolus plus prolonged infusion was administered with the aim to provide a more sustained effect. The results from this cohort, and those that followed, revealed that a higher dose and prolonged infusion prolonged the increase in PRU. However, an unexpected loss of effect

TABLE 3 Population PK/PD parameters of final PK/PD model

Parameters (Units)	Final estimate	95% CI Lower	Upper	Inter-individual variability (CV%)
EC50 (nmol/L) = EXP(THETA1) EC50 for relating TICA to PRU				
THETA1	10.6	8.76	12.4	23.3%
E_{max} = EXP(−0.1) E_{max} for relating TICA to PRU				
Fixed in model[14]				
Kon (nmol^{-1} × h^{-1}) = EXP(THETA2)				
THETA2	−5.56	−5.73	−5.39	43.6%
Kd (nmol) = EXP(−4)				
Fixed in model[11]				
Kd2 (nmol) = EXP(THETA3)				
THETA3	2.04	1.86	2.22	25.9%
Ktr (h^{-1}) = EXP(THETA4 + THETA13*(LOG(WT)−4.35))				
THETA4	−1.22	−1.33	−1.11	25.0%
THETA13	1.46	0.841	2.08	
Kon2 (nmol^{-1} × h^{-1}) = EXP(THETA5)				
THETA5	−3.74	−3.91	−3.57	30.4%
E_{max}f = EXP(THETA6) Emax for relating metabolism of TICA to PB2452 concentrations				
THETA6	2.98	2.76	3.20	23.6%
ECf (nmol/L) = EXP(THETA7) EC50 for relating metabolism of TICA to PB2452 concentrations				
THETA7	9.36	9.10	9.62	59.8%
EC502 (nmol/L) = EXP(THETA8 + THETA12*(LOG(WT)−4.35)) EC50 for relating TAM to PRU				
THETA8	4.59	4.49	4.69	25.3%
THETA12	−0.965	−1.49	−0.442	
E_{max}2 = EXP(THETA9) Emax for relating TAM to PRU				
THETA9	0.0181	−0.0421	0.0783	20.7%
KA = EXP(2.3) Absorption parameter for TICA				
Fixed in model[14]				10% Fixed
CL/F (L/h) = EXP(2.81) clearance of TICA				
Fixed in model[14]				10% Fixed
V1/F (L) = EXP(5.04) central volume of TICA				
Fixed in model[14]				
V2/F (L) = EXP(4.02) peripheral volume of TICA				
Fixed in model[14]				
Q1/F L (h) = EXP(2.34) intercompartmental clearance of TICA				
Fixed in model[14]				10% Fixed
CLM (L/h) = EXP(THETA11 + THETA10*(LOG(WT)−4.35)) clearance of TAM				
THETA10	1.31	0.912	1.71	23.9%
THETA11	1.93	1.86	2.00	
VM1 (L) = EXP(1.95) central volume of TAM				
Fixed in model[14]				
VM2 (L) = EXP(3.74) peripheral volume of TAM				10% Fixed
Fixed in model[14]				
Q2M (L/h) = EXP(1.48) intercompartment clearance of TAM				
Fixed in model[14]				

(Continues)

TABLE 3 (Continued)

| Parameters (Units) | Final estimate | 95% CI | | Inter-individual variability (CV%) |
		Lower	Upper	
CL_ant (L/h) = EXP(0.631) clearance of PB2452				5% Fixed
Fixed in model[a]				
Q_ant (L/h) = EXP(−0.765) intercompartmental clearance of PB2452				
Fixed in model[a]				5% Fixed
V_ant (L) = EXP(1.05) central volume of PB2452				
Fixed in model[a]				5% Fixed
V_ant_perp (L) = EXP(1.28) peripheral volume of PB2452				
Fixed in model[a]				5% Fixed
Base = Log(BPRU) baseline PRU				
Fixed in model to observed baseline values				10% Fixed
fm = 0.3 Metabolism rate of TICA is fm*elimination rate of TICA. This is the value for metabolism when there is no PB2452 present.				
Fixed in model[14]				
Koff = Kon*Kd				
Koff2 = Kon*Kd2				
Residual Variability: PRU: Additive SD = 20.3 Uncomplexed PB2452: 28.2% Total PB2452: 13.7% Additive SD = 504 Total TICA: 42.4% Additive SD = 332 Total TAM: 23.1%				

Abbreviations: CI, confidence interval; CL/F, total apparent clearance; CV%, percent coefficient of variation; EC50, half-maximal effective concentration; E_{max}, maximum effect; PD, pharmacodynamic; PK, pharmacokinetic; PRU, P2Y12 reactivity unit; TICA, ticagrelor.

[a]Values were fixed based on modeling PB2452 alone in using data from the first three cohorts.

was observed post-cessation of drug infusion despite dose escalation.

To achieve improved fit to the study data, multiple adjustments were made to the model. The late loss of effect was potentially due to recycling of TICA and TAM upon elimination of PB2452, so new PB2452-TICA and PB2452-TAM compartments were added to the model, which assume the complexes move through these new compartments at a rate of Ktr and that some amount of TICA and TAM eventually return to circulation as PB2452 is removed from the system. In addition, rather than initially summing TICA and TAM as had been performed in other models,[14] these two analytes were modeled separately against PRU/LTA/VASP. These updates led to significant improvements in the fit of the model to the data. The model initially was underestimating TAM values (Supplementary Material S3). To address the apparent underestimation of TAM values, the model was further modified such that metabolism of TICA to TAM was considered a function of the concentration of PB2452. This

was an empirical addition to the model where the fraction metabolized is modeled as:

$$fm = 0.3 * \left(1 + Emax * \left(C_{PB2452}\right)^{\gamma} / \left((EC_{50})^{\gamma} + (C_{PB2452})^{\gamma}\right)\right)$$

with

Rate of metabolism from TICA to TAM = fm*(elimination rate of TICA).

where C_{PB2452} is the concentration of uncomplexed PB2452 in the full model.

Examining covariates

The final step in model development was addition of significant covariates. Based on examining the covariate plots (Supplementary Material S4), weight appeared to be a potentially strong covariate for ETA4 (corresponding to Ktr), ETA8 (corresponding to EC50 relating TAM to PRU/LTA/VASP), and ETA10 (corresponding to the
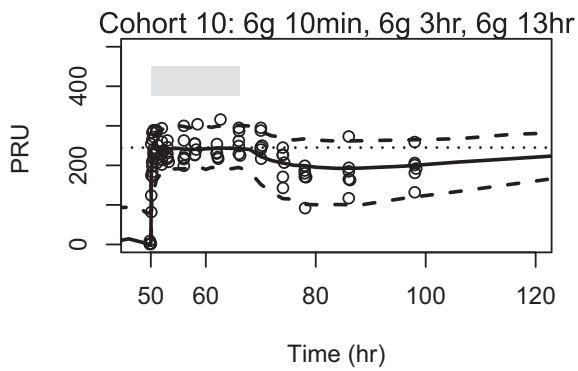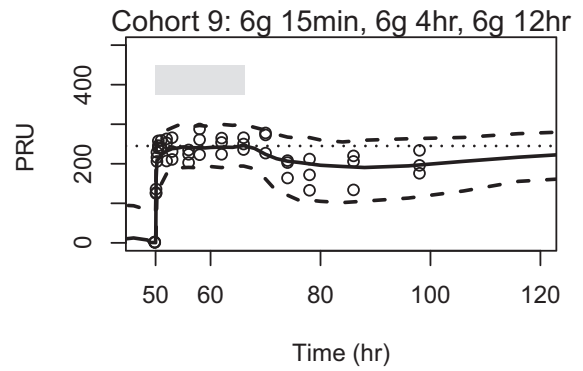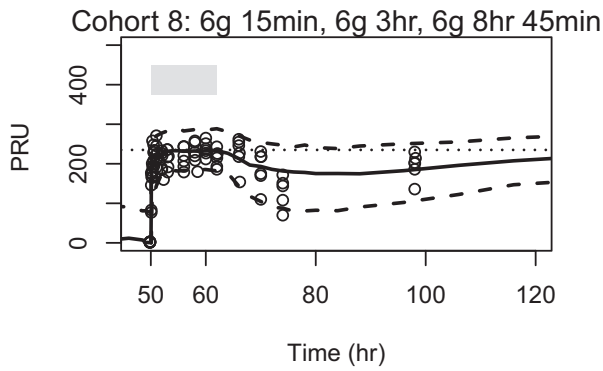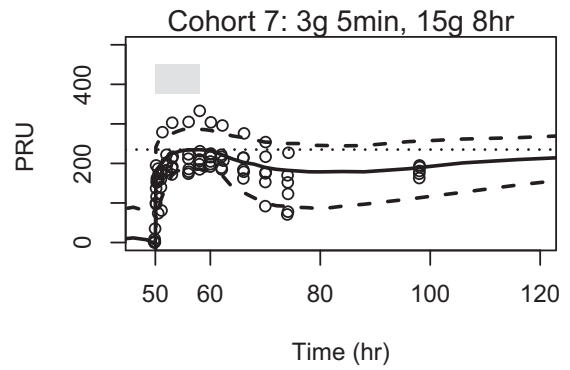
Cohort 4: 1g 30min

Cohort 5: 3g 30min

Cohort 6: 9g 30min

Cohort 7: 3g 5min, 15g 8hr

Cohort 8: 6g 15min, 6g 3hr, 6g 8hr 45min

Cohort 9: 6g 15min, 6g 4hr, 6g 12hr
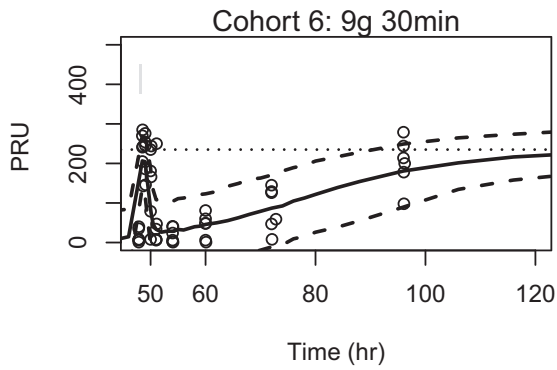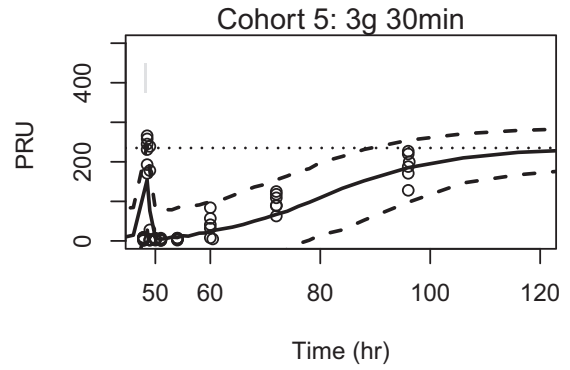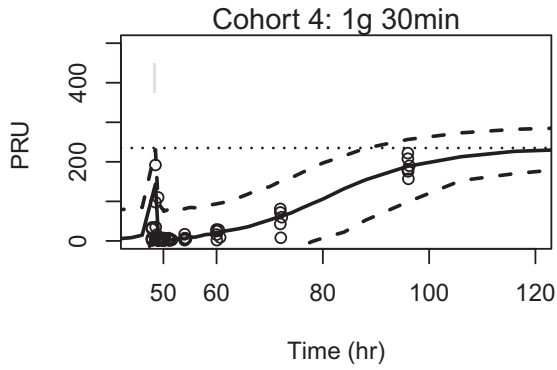
Cohort 10: 6g 10min, 6g 3hr, 6g 13hr

FIGURE 2 Simulation results for PRU with the observed data overlaid by dosing regimen of PB2452. The solid line depicts the median of the simulation while the dashed lines are the 5th and 95th percentiles. The dotted line depicts the median baseline (prior to administration of TICA) of PRU for the cohort. The gray box reflects the time from the start of the first administration of PB2452 to the end of the last administration of PB2452. PRU, P2Y12 reactivity unit; TICA, ticagrelor

clearance of TAM). Weight was added as a covariate for these terms and was found to be statistically significant for all three. Based on the covariate plots for the final model (Supplementary Material S5) with the covariates included, no other covariates were added. The impact of weight on the overall model for PRU was assessed by simulating subjects at different weights and plotting them together. The simulation results (Supplementary Material S6) suggests that the practical effect of weight is minimal.

Assessment of the final model

For the final modeling, diagnostic plots were run and appear to be reasonable for all components of the model, including PRU/LTA/VASP, total TICA, total TAM, total PB2452, and uncomplexed PB2452 (Figure 3). Conditional weighted residuals (CWRES) are displayed in (Supplementary Material S7). The model slightly overpredicted the total PB2452 and underpredicted the uncomplexed PB2452. For higher values of PRED, the CWRES are negative for TICA and total PB2452, suggesting the model is underpredicting those values. This could be due to fixing population mean values based on literature. However, the individual plots suggested that the predictions are close to the observed values. Visual predictive checks (simulations from the model for PRU with the data overlaid for the cohorts where TICA and PB2452 were both administered) were then performed and showed that he results for LTA and VASP were similar (Figure 2). Additionally, the checks indicated that the model was highly predictive of the PRU data from the current study in terms of fit and variability because the median from the simulations (solid lines) typically passed through the middle of the data, and most of the data were contained within the 5th and 95th percentiles (dashed lines). Figure 4 shows simulations from the model with data overlaid for PRU, total TICA, total TAM, total PB2452, and uncomplexed PB2452 for the cohort where PB2452 was dosed 6 g for 15 min followed by 6 g for 4 h followed by 6 g for 12 h. This figure shows that the model performed well with all of the components of the model where observed data was available.

For the final model, different sets of starting values were used for the Bayesian algorithm. Trace plots were produced to examine autocorrelation and convergence (Supplementary Material S2). For all THETAs, the model seemed to converge rapidly. THETA1 (corresponding to the EC50 for the effect of TICA on PRU) seemed to exhibit

a high degree of autocorrelation. This is likely attributed to the value being arbitrarily large and the model not being sensitive to the value of the parameter. All other THETAs seemed to have a much lower degree of autocorrelation. To minimize any impact of autocorrelation, many posterior samples were generated. A burn-in of 5000 was used per chain followed by generating a sample of 20,000 per chain.

The parameter estimates for the final population PK/PD model (PRU) are in Table 3. The parameter estimates for the population PK/PD model for LTA and VASP are similar.

DISCUSSION

A phase I trial was conducted to evaluate the safety, efficacy, and PK profiles of PB2452 in healthy volunteers 18–50 years of age who were pretreated with TICA. The data from this trial were used to develop a semimechanistic population PK/PD model. Overall, the model appears to capture the key patterns observed in the data over time and supports use of a standard three-phase infusion regimen in later phase studies. The model fits the data well based on diagnostic plots, including visual predictive checks. Simulations for the cohorts tested produced results that mirror the observed data.

Given the complex relationship between PB2452 and TICA/TAM, it was important to refine the model to be predictive of the standard 18-gram dosing regimen for PB2452 for subsequent trials. The model supports a dosing regimen initiated with an initial bolus (or a very short infusion) dose to provide immediate reversal, followed by a higher rate infusion (loading regimen) and then a slower maintenance rate infusion to maintain the values of PRU/LTA/VASP necessary for the intended patient populations. Based on model predictions, a 6 g i.v. bolus followed by 6 g infused over 4 h and then 6 g over 12 h was identified and tested in study subjects and shown to provide complete reversal within 5 min of infusion onset that was sustained for 20–24 h (Figure 4).

The model and results suggest that PB2452 may have an impact on the metabolism of TICA to TAM. This was added to the model empirically. This could potentially be due to PB2452-TICA complex being formed and some of this TICA being metabolized to TAM. The model without this component underestimated the TAM values substantially. It also shows that as PB2452 binds to TICA and TAM, more TICA and TAM may rapidly redistribute to the central
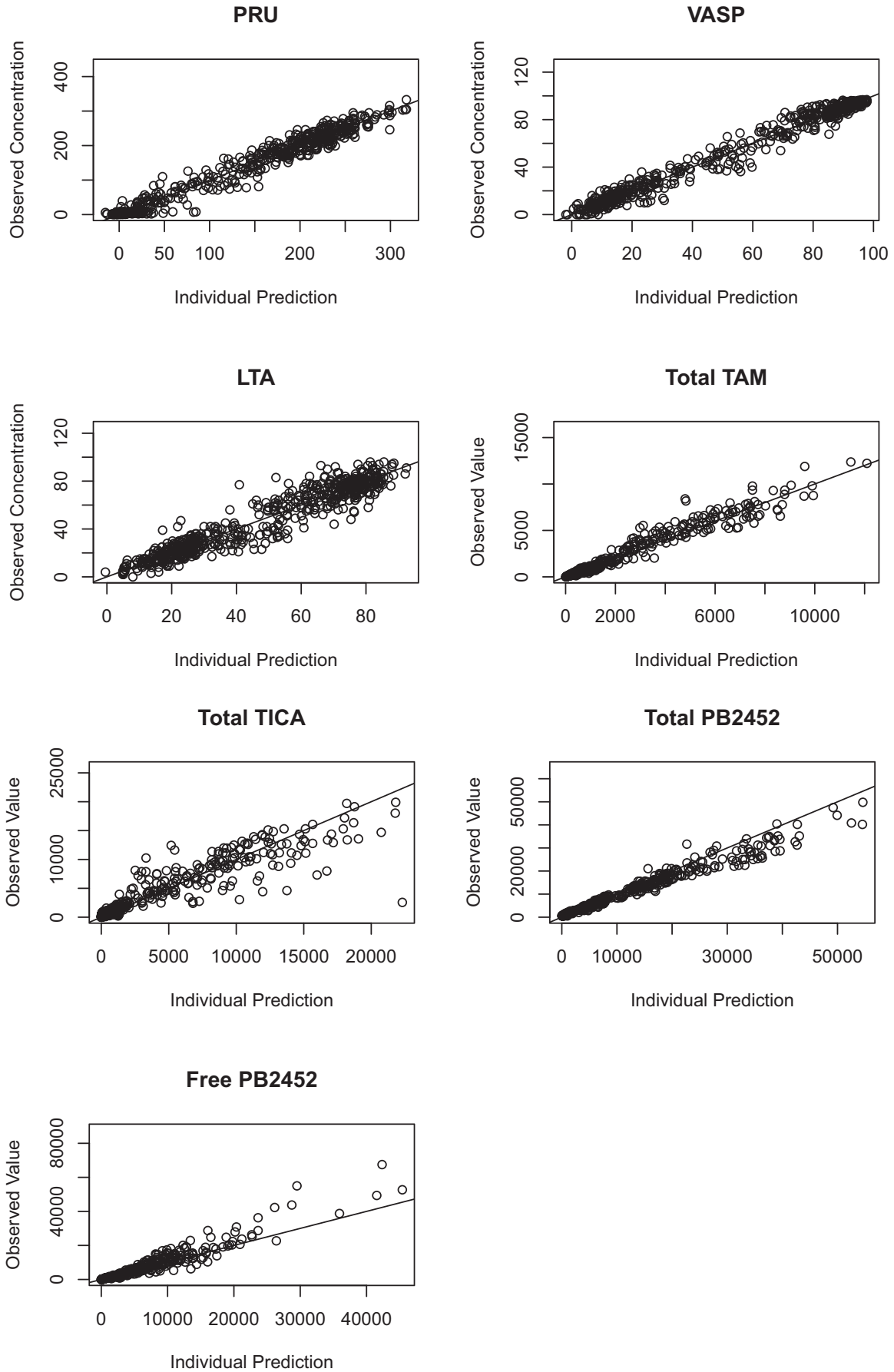
FIGURE 3 Observed versus individual prediction plots for various measured components of the model

FIGURE 4 Simulation results for various measured components of the model. The solid line depicts the median of the simulation, whereas the dashed lines are the 5th and 95th percentiles. The dosing regimen for PB2452 was 6 g for 15 min followed by 6 g for 4 h followed by 6 g for 12 h. PRU, P2Y12 reactivity unit; TAM, ticagrelor active metabolite; TICA, ticagrelor
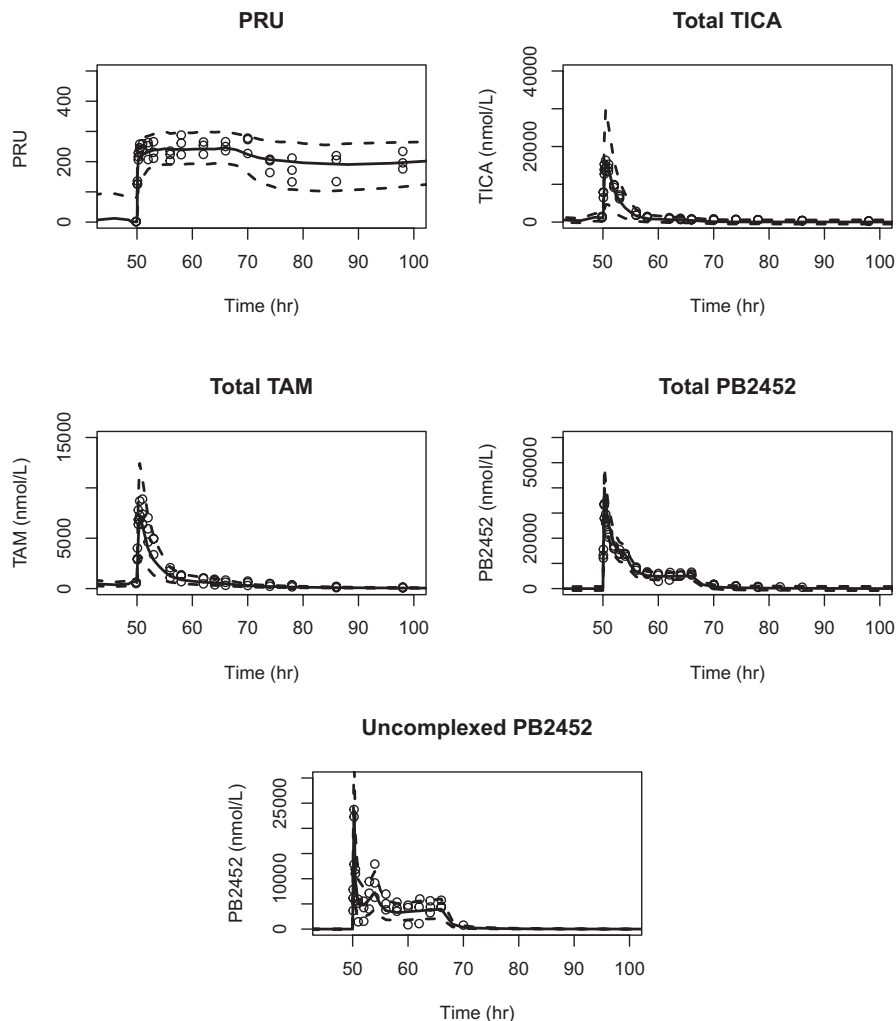
compartment from the periphery, causing a rapid return of PRU/LTA/VASP to levels observed prior to PB2452 administration. The apparent sequestering and partial recycling of TICA and TAM evident in later timepoints post-PB2452 infusion could potentially be due to post-glomerular reabsorption of PB2452-TICA and -TAM complexes into tubular cells, lysosomal degradation of PB2452, and recycling of TICA and TAM back into circulation (Supplementary Material S8). This possibility for antibody fragments is discussed in the literature.[17] The addition of extra PB2452-TICA and PB2452-TAM complex compartments allows for this to be represented in the model. They are not necessarily physiologic compartments but may depict a process of complex dissociation and TICA (or TAM) return to circulation. Some of the recirculated TICA may be metabolized to TAM could explain the observed relationship between TAM concentration and PB2452 concentrations.

The EC50 for TICA was large (greater than 20,000 nmol/L), which is well beyond the range of uncomplexed TICA expected based on the model (less than 2000 nmol/L). This suggests that the effect of TICA may be closer to a gradually increasing log linear model. The EC50 for TAM is much lower at 98.5 nmol/L. The model presented here is different from others in literature[14,18] where the relationship is either modeled as a sum of TICA and TAM or just TICA alone. The model presented here fits the placebo data (subjects who received TICA plus a placebo) well, suggesting that the model here performs as well as those used elsewhere. It is possible that, in the previous studies, the natural relationship between TICA and TAM caused it to be difficult to distinguish the contribution due to each, whereas in the present study with PB2452 added, this relationship is altered.

Because complexes of PB2452 with TICA and TAM are expected to be removed in the kidneys, either by degradation or urinary elimination, eGFR was explored as covariate. However, no effects with eGFR were observed perhaps due to the healthy study population with most eGFR values within a normal range (74.75–162.80 ml/min/1.73 m^2 calculated by the abbreviated MDRD equation). Some simulations were run to explore potential outcomes for subjects with renal impairment (Supplementary Material S9) that may be used for planning purposes.

In conclusion, we developed a semimechanistic model to explain the relationships among PB2452, TICA, TAM,

and the effects on PRU/LTA/VASP. The model explained the data well and assisted with finding a dose and administration regimen subsequent clinical trials. The model may be useful to help guide development of PB2452 as an antidote for ticagrelor in hospitalized patients. Updating the model with patient data may then help predict whether dose adjustments may be needed in different subpopulations (e.g., older patients and different ethnicities) as well.

CONFLICT OF INTEREST

Dr. Bhatt serves as Chair of REVERSE-IT, studying bentracimab, and receives funding paid to Brigham and Women's Hospital. Dr. Bhatt discloses the following relationships - Advisory Board: Cardax, CellProthera, Cereno Scientific, Elsevier Practice Update Cardiology, Janssen, Level Ex, Medscape Cardiology, MyoKardia, Novo Nordisk, PhaseBio, PLx Pharma, Regado Biosciences; Board of Directors: Boston VA Research Institute, Society of Cardiovascular Patient Care, TobeSoft; Chair: American Heart Association Quality Oversight Committee; Data Monitoring Committees: Baim Institute for Clinical Research (formerly Harvard Clinical Research Institute, for the PORTICO trial, funded by St. Jude Medical, now Abbott), Cleveland Clinic (including for the ExCEED trial, funded by Edwards), Contego Medical (Chair, PERFORMANCE 2), Duke Clinical Research Institute, Mayo Clinic, Mount Sinai School of Medicine (for the ENVISAGE trial, funded by Daiichi Sankyo), Population Health Research Institute; Honoraria: American College of Cardiology (Senior Associate Editor, Clinical Trials and News, ACC.org; Vice-Chair, ACC Accreditation Committee), Baim Institute for Clinical Research (formerly Harvard Clinical Research Institute; RE-DUAL PCI clinical trial steering committee funded by Boehringer Ingelheim; AEGIS-II executive committee funded by CSL Behring), Belvoir Publications (Editor in Chief, Harvard Heart Letter), Canadian Medical and Surgical Knowledge Translation Research Group (clinical trial steering committees), Duke Clinical Research Institute (clinical trial steering committees, including for the PRONOUNCE trial, funded by Ferring Pharmaceuticals), HMP Global (Editor in Chief, Journal of Invasive Cardiology), Journal of the American College of Cardiology (Guest Editor; Associate Editor), K2P (Co-Chair, interdisciplinary curriculum), Level Ex, Medtelligence/ReachMD (CME steering committees), MJH Life Sciences, Population Health Research Institute (for the COMPASS operations committee, publications committee, steering committee, and USA national co-leader, funded by Bayer), Slack Publications (Chief Medical Editor, Cardiology Today's Intervention), Society of Cardiovascular Patient Care (Secretary/Treasurer), WebMD (CME steering committees); Other: Clinical Cardiology (Deputy Editor), NCDR-ACTION Registry Steering Committee (Chair), VA CART Research and Publications Committee (Chair); Research Funding: Abbott, Afimmune, Amarin, Amgen, AstraZeneca, Bayer, Boehringer Ingelheim, Bristol-Myers Squibb, Cardax, Chiesi, CSL Behring, Eisai, Ethicon, Ferring Pharmaceuticals, Forest Laboratories, Fractyl, HLS Therapeutics, Idorsia, Ironwood, Ischemix, Janssen, Lexicon, Lilly, Medtronic, MyoKardia, Novo Nordisk, Owkin, Pfizer, PhaseBio, PLx Pharma, Regeneron, Roche, Sanofi, Synaptic, The Medicines Company; Royalties: Elsevier (Editor, Cardiovascular Intervention: A Companion to Braunwald's Heart Disease); Site Co-Investigator: Abbott, Biotronik, Boston Scientific, CSI, St. Jude Medical (now Abbott), Svelte; Trustee: American College of Cardiology; Unfunded Research: FlowCo, Merck, Takeda.

All other authors declared no competing interests for this work.

AUTHOR CONTRIBUTIONS
S.K. wrote the manuscript. J.L. designed the research. D.L.B., S.A., and J.L. performed the research. J.W. analyzed the data. J.L. contributed analytical tools.

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