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Subcutaneous Marzeptacog Alfa (Activated) for On-Demand Treatment of Bleeding Events in Subjects With Hemophilia A or B With Inhibitors

Alan Faraj[1], Joakim Nyberg[2], Grant E. Blouse[3], Tom Knudsen[3] and Ulrika S. H. Simonsson[1,*]

Marzeptacog alfa (MarzAA) is under development for subcutaneous treatment of episodic bleeds in patients with hemophilia A/B and was studied in a phase III trial evaluating MarzAA compared with standard-of-care (SoC) for on-demand use. The work presented here aimed to evaluate MarzAA and SoC treatment of bleeding events on a standardized four-point efficacy scale (poor, fair, good, and excellent). Two continuous-time Markov modeling approaches were explored; a four-state model analyzing all four categories of bleeding improvement and a two-state model analyzing a binarized outcome (treatment failure (poor/fair), and treatment success (good/excellent)). Different covariates impacting improvement of bleeding episodes as well as a putative relationship between MarzAA exposure and improvement of bleeding episodes were evaluated. In the final four-state model, higher baseline diastolic blood pressure and higher age (> 33 years of age) were found to negatively and positively impact improvement of bleeding condition, respectively. Bleeding events occurring in knees and ankles were found to improve faster than bleeding events at other locations. The covariate effects had most impact on early treatment success (≤ 3 hours) whereas at later timepoints (> 12 hours), treatment success was similar for all patients indicating that these covariates might be clinically relevant for early treatment response. A statistically significant relationship between MarzAA zero-order absorption and improvement of bleedings ($P < 0.05$) were identified albeit with low precision. No statistically significant difference in treatment response between MarzAA and intravenous SoC was identified, indicating the potential of MarzAA for treatment of episodic bleeding events with a favorable subcutaneous administration route.

Study Highlights

WHAT IS THE CURRENT KNOWLEDGE ON THE TOPIC?

☑ Acute bleeding events are currently treated intravenously. Subcutaneous treatment options such as marzeptacog alpha (MarzAA) activated allow for faster treatment following a bleeding event, potentially improving treatment outcomes, including quality of life and reducing bleeding-related complications.

WHAT QUESTION DID THIS STUDY ADDRESS?

☑ A model-based approach was developed for evaluating the effect of on-demand treatment of bleeding events. The model enabled evaluation of covariate and exposure effects on bleeding improvement and potential differences in treatment success between MarzAA and standard-of-care (SoC).

WHAT DOES THIS STUDY ADD TO OUR KNOWLEDGE?

☑ Subcutaneous administration of MarzAA resulted in similar treatment success as SoC, supporting the hypothesis that MarzAA can improve treatment of acute bleeding events in patients with hemophilia.

HOW MIGHT THIS CHANGE CLINICAL PHARMACOLOGY OR TRANSLATIONAL SCIENCE?

☑ This work shows a model-based method for evaluation of a four-graded treatment success scale for treatment of bleeding in hemophilia and relation to pharmacokinetics and risk factors using a Markov modeling approach.

Marzeptacog alfa (activated; MarzAA), a novel variant activated recombinant factor VII (rFVIIa) including a total of four amino acid substitutions is being developed for subcutaneous (s.c.) administration to address an unmet need in hemophilia and other bleeding disorders.[1–3] In contrast to s.c. administration, intravenous (i.v.) dosing often requires a medical professional (hemophilia

[1]Department of Pharmaceutical Biosciences, Uppsala University, Uppsala, Sweden; [2]Department of Pharmacy, Uppsala University, Uppsala, Sweden; [3]Catalyst Biosciences, South San Francisco, California, USA. *Correspondence: Ulrika S. H. Simonsson (ulrika.simonsson@uu.se)

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treatment center or home nursing) or family member to achieve venous access making home treatment cumbersome, particularly for pediatric patients.[4,5] Other challenges include patient adherence[6,7] or use of a central venous access device on a regular basis, which makes it time-consuming, painful, and negatively influences adherence.[6,7] Given the current treatment paradigm for hemophilia, there is a need for a therapy that is long-lasting, effective, and can be used for on-demand treatment of bleeds without the need for i.v. access. Compared with the i.v. route, the s.c. administration of wild-type rFVIIa extends half-life and reduces fluctuations of systemic drug concentrations, although this route is associated with reduced bioavailability.[8] Consequently, the development of rFVIIa variants with greater bioavailability and extended effect with s.c. administration promises to facilitate rapid and effective treatment of bleeding in patients with hemophilia who have breakthrough bleeding on prophylaxis or are treated on-demand for acute hemorrhage.

In a clinical prophylaxis trial, MarzAA was given as 30 μg/kg daily over 50 days and only 2 patients out of 11 experienced one bleeding event. The protocol allowed for dose escalation to 60 μg/kg daily following a bleed, after which no additional bleeding events occurred for an additional subsequent 50 days.[2] Given these results, and a pharmacokinetic modeling and simulation study[9] evaluating MarzAA exposure after different doses toward a predefined clinical target related to wt-FVIIa,[9] supported 60 μg/kg to be evaluated in the phase III Crimson-1 trial for on-demand treatment of bleeding events. The descriptive statistics of the primary efficacy end point showed that MarzAA has potential and is comparable to i.v. standard-of-care (SoC)[1] from an efficacy point of view.

A common way to analyze efficacy data in a prophylaxis setting is to apply repeated time-to-event analysis where the events are repeated bleeding events over time,[10] in which the bleed category (ordered repeated time to event analysis) can be taken into account. However, this trial was performed in an on-demand setting, and the current standard for efficacy evaluation in such a setting is improvement quantified by the four-point ordered scale (poor, fair, good, and excellent improvement) as determined by the clinical investigator. Ordered categorical data of this nature has commonly been analyzed using proportional odds models in the literature for other disease areas.[11–13] However, such a modeling approach has a limitation when testing covariate effects as a covariate effect as it impacts all scores equally.[13] For this work, we identified a continuous-time Markov model approach,[14] earlier applied in pharmacometric literature,[13,15–17] as it allowed for the possibility of evaluating covariates impacting specific scores and potential dependencies between a score and the previous score.

Although the accumulated Crimson data showed potential for s.c. MarzAA, the trial was terminated earlier for reasons unrelated to the study drug efficacy or safety, but rather to changes in business strategy. As model-based analysis has been shown to have higher statistical power compared with traditional statistical approaches,[18–20] this work aimed to compare the efficacy of MarzAA to SoC, evaluate covariate effects impacting treatment success and to evaluate the exposure-response of s.c. MarzAA using a model-based continuous-time Markov approach.

METHODS

This multicenter, global, open label, randomized, crossover phase III study was performed to evaluate the efficacy and safety of MarzAA for on-demand treatment and control of spontaneous or traumatic bleeding episodes in adolescent and adult subjects with congenital hemophilia A (HA) or hemophilia B (HB) with inhibitors compared with the SoC commercially available in each country. The primary outcome of the trial was control of bleeding episodes at 24 hours after initial dose (good/excellent improvement of the bleed). In contrast to the planned statistical approach that was not performed due to early termination of the trial, this work used the available efficacy evaluation data in a model-based analysis approach.

Study design and patients

Subjects were randomized to either sequence A or B. In sequence A, subjects were treated on-demand with 60 μg/kg s.c. MarzAA in 3-hourly intervals. Each subject could experience several bleeds during the trial and treatment response for each bleeding occasion was measured by a clinical investigator with a 4-point scale corresponding to poor, fair, good, or excellent improvement in signs of bleeding measured 1, 3, 6, 9, 12, 24, and 48 hours after initial dose. If the response was poor or fair at 3 hours after the first dose, a second dose was given. Similarly, a third dose was repeated at 6 hours after the first dose if needed. The SoC treatment period consisted of different SoC products (APCC/Feiba, Coagil VII, or NovoSeven) used dependent on clinical site country, allowing for one to three doses of i.v. administered NovoSeven, Feiba or Coagil-VII according to the dosing recommendations. In sequence B, subjects followed a reverse order in which SoC was followed by a MarzAA treatment period.

A total of 66 evaluable bleeding events from 15 patients (HA subjects) were studied in the Crimson-1 trial[1] for drug effect evaluation (32 treated with MarzAA and 33 treated with SoC). Each subject experienced five bleeds on average (90% inter-percentile range; 1–8 bleeds). The total number of efficacy evaluations were 222 and 265 for s.c. MarzAA and i.v. SoC, respectively. A total of 487 efficacy observations were included in the analysis dataset. The median (minimum-maximum) of time to cessation of bleeding, time from bleed recognition to initial dosing and number of doses per bleed was 9.0 (1.0–36.0) and 6.0 (0.8–61.0) hours, 0.75 (0.2–10.3) and 1.0 (0.0–59.0) hours, and 2.0 (1.0–5.0) and 1.0 (1.0–4.0) doses, for MarzAA and SoC, respectively (**Table S1**). One subject treated with MarzAA was found to have low-titer cross-reactive non-neutralizing anti-drug antibodies, but was transient and resolved at the end of the study 2 months after exposure to MarzAA. One subject in the MarzAA and one subject in the SoC group needed rescue therapy, defined as additional doses after 24 hours post the initial dose due to inadequate hemostasis. No injection site reactions, drug-related adverse events, or thrombotic events were reported.

Drug quantification

Functional clot activity[21] was measured in all samples using the STACLOT VIIa-rTF kits manufactured by Diagnostica Stago on the Stago ST4/Start4 coagulation analyzer and test procedure was adapted from the STACLOT kit instructions with a lower limit of quantification of 0.25 ng/mL.

Model building

Pharmacodynamic modeling. The outcome data (poor, fair, good, and excellent) were treated as an ordinal categorical variable. A continuous Markov model[14] with four-states as well as a simplified version consisting of two states were developed. In the four-state model, each state represented the probability of respective category of the response variable. The probability at the first observation (time = 0) for each bleeding event and individual was set to 1, and corresponded to poor improvement (probability for fair, good, and excellent set to 0 at

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initialization). A set of differential equations with first order transfer rates allowed transfer of the probability between the states observed during treatment of a bleed. Following an observation, the probability was reset in all compartments meanwhile the compartment representing the observed categorical response was set to 1 (100% probability). The differential equations representing the four-state model is shown in Eqs. 1–4:

$$\frac{dPr(P)}{dt} = k_{FP} \cdot Pr(F) - k_{PF} \cdot Pr(P) \quad (1)$$

$$\frac{dPr(F)}{dt} = k_{PF} \cdot Pr(P) + k_{GF} \cdot Pr(G) - k_{FP} \cdot Pr(F) - k_{FG} \cdot Pr(F) \quad (2)$$

$$\frac{dPr(G)}{dt} = k_{FG} \cdot Pr(F) + k_{EG} \cdot Pr(E) - k_{GE} \cdot Pr(G) - k_{GF} \cdot Pr(G) \quad (3)$$

$$\frac{dPr(E)}{dt} = k_{GE} \cdot \Pr(G) - k_{EG} \cdot \Pr(E) \quad (4)$$

where Pr(P), Pr(F), Pr(G), and Pr(E) describe the probability of the poor, fair, good, or excellent improvement at every timepoint. The transfer rates are represented with subscripts with two numbers in which the first number illustrate the current category and the second number stand for the direction of movement. The set of differential equation in Eqs. 1–4 served as a starting point, from which additional direct transfers were tested for (e.g., from poor to excellent without moving through other states). In addition, shared transfer rates were tested for, meaning that the same parameter could describe transfer between several states.

Although the four-state model was considered a more informative approach (no transformation of data, enabling testing for covariate effects and exposure-response relationships on specific scores), a reduced model approach was also developed where the poor and fair response corresponded to treatment failure (TF (Eq. 5)) whereas good and excellent improvement of the bleeding event was considered treatment success (TS (Eq. 6)):

$$\frac{dPr(\mathrm{TF})}{dt} = k_{TSTF} \cdot Pr(\mathrm{TS}) - k_{TFTS} \cdot Pr(TF) \quad (5)$$

$$\frac{dPr(\mathrm{TS})}{dt} = k_{TFTS} \cdot Pr(\mathrm{TF}) - k_{TSTF} \cdot Pr(\mathrm{TS}) \quad (6)$$

All bleeding events were considered to start in TF at initialization of the bleeding event and two transfer rates were estimated between the two states.

Interindividual variability (IIV) and inter-bleed variability (IBV) was evaluated on all transfer rates. Subsequently, covariates were tested using stepwise-covariate-modeling on all transfer rates for both models. The forward inclusion step was performed with a threshold P value of 0.05 followed by a stricter backward deletion step of using a P value of 0.01. Tested covariates (**Table S1**) were severity of bleed (each bleed was classified to be mild, moderate, or severe), location of bleed (knee, ankle, elbow, and other (buttock, forearm, foot, hip, hand, leg, eye, chest, ear, mouth, nose, neck, shoulder, stool, throat, vaginal, or wrist)), heart rate, systolic and baseline diastolic blood pressure (BDBP), respiratory rate, age, weight, and treatment group (MarzAA/SoC). The best models from the covariate search were taken forward to explore exposure-response relationships for MarzAA. Evaluation of variability and covariates were always performed additively on a logit scale.

Pharmacokinetic-pharmacodynamic modeling. Pharmacokinetic (PK) metrics were derived through Bayesian *post hoc* estimates[9] based on non-bleeding PK observations as bleeding PK information was too sparse. Only four observations from one individual were available. For the individuals with missing non-bleeding PK information, the typical prediction was used. Single dose area under the curve over 48 hours ($AUC_{0\text{-}48h}$), maximum concentration over 48 hours ($C_{max0\text{-}48h}$), minimum concentration over 48 hours ($C_{min0\text{-}48h}$), time until maximum concentration over 48 hours (T_{max48h}), individual clearance estimates, individual bioavailability estimates, individual absorption rate, and individual duration of zero-order release of the drug (I_{dur}) were derived using an already developed population PK model (code can be found in the supplemental material of the previous paper).[9] Each of these metrics were tested through linear and piece-wise linear functions for continuous covariates and through linear-proportional inclusion for categorical covariates (one additional parameter per category). Exponential and power relationships between exposure metrics and transfer rates were not considered as the relationships were tested on a logit scale.

Model evaluation. Discrimination between models were based on changes in objective function value (OFV) provided by NONMEM (version 7.43; Icon Development Solutions, Hanover, MD, USA).[22] Difference in OFV (ΔOFV) was considered nominally χ^2 distributed and a ΔOFV of 3.84 points was considered to correspond approximately to a P value of 0.05 for one degree of freedom for nested models. Additional discrimination metrics were visual predictive checks in which simulation-based comparisons of simulated and observed proportions of the categorical response variable were performed. Furthermore, uncertainty in parameters of the model was considered. All post-processing of results was performed in R.[23]

Covariate effects were visualized on a typical patient level for all possible combinations of covariate effects to show how the covariate effect sizes impact treatment success. For continuous covariates, a lower and higher value was visualized relative to the reference (median) in the dataset. If the covariate effect was described by a piece-wise linear function, relevant values of the covariate were studied in relation to when the slopes changed in the piece-wise function. For BDBP, 70 and 90 mmHg were used to visualize the effect of lower and higher pressures, (as compared with 80 mmHg which was considered a normal BDBP). The difference between bleeding events in elbow/ankle joints vs. knees and other joints were also studied. For the reduced two-state model, the minimum and maximum bodyweight in the dataset were used for visualization, whereas the same values were used for BDBP as for the four-state model.

Ethics

The study was conducted in accordance with the US Food and Drug Administration (FDA) regulations, the International Conference on Harmonization (ICH) E6 (R2) Guideline for Good Clinical Practice (GCP), the Declaration of Helsinki, and any applicable local health authority, and institutional review board/independent ethics committee requirements. All subjects provided written informed consent prior to participation in the study.

RESULTS

Model development

The final model was a four-state Markov model (**Figure 1** and NONMEM code S1) as this provided a good description of the data (**Figure 2**) and respected the natural order of the data without simplification, hence maximizing usage of information in the data, rather than simplified the observed data as in the two-state Markov model, although it also described the data well (**Figure S1**). No difference in treatment success was detected between s.c. MarzAA and i.v. SoC regardless of analysis approach. The final four-state model had one parameter describing worsening of the

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scores ($k_{FP\text{-}GF\text{-}EG}$), and two separate transfers describing improvement from poor/fair to fair/good ($k_{PF\text{-}FG}$) and good to excellent (k_{GE}), respectively (**Figure 1**). Typical patient parameters $k_{FP\text{-}GF\text{-}EG}$, $k_{PF\text{-}FG}$, and k_{GE} were estimated to 0.01, 0.17, and 0.2 day^{-1} (**Table 1**) respectively and IIV was estimated to 11% (coefficient of variation) and 21% for $k_{PF\text{-}FG}$ and k_{GE}, respectively. No IBV was supported for any of the model parameters. The covariate search identified a piece-wise linear relationship where patients with age above 33 years (median in the dataset) had a higher $k_{PF\text{-}FG}$ with increase in age compared with younger patients. This translates to better treatment of the bleeds in older patients compared with younger patients. Bleeding location was found to have a statistically significant impact on $k_{PF\text{-}FG}$ where bleeding events in knee and other joints were found to be associated with a higher $k_{PF\text{-}FG}$ as compared with elbow/ankle joints. Lastly, BDBP was found to be a statistically significant covariate reducing the k_{GE} rate with increasing BDBP, translating to less good treatment effect in patients with higher BDBP. A statistically significant ($P < 0.05$) exposure-response relationship was found between I_{dur} (zero-order MarzAA absorption) and $k_{FP\text{-}GF\text{-}EG}$ in the four-state Markov model but was determined with low parameter precision and was therefore removed in the final model. The *post hoc* individual PK parameters can be found in **Table S3**. In addition, none of the transfer rates were found to be statistically different between bleeding events treated with MarzAA and SoC, indicating similar efficacy between the two treatments. Alternative four-state model structures were tested where transfer was made possible without first entering an intermediate state (e.g., direct transfer from poor to good without need to enter fair first), but was not statistically significant and produced high parameter uncertainty.

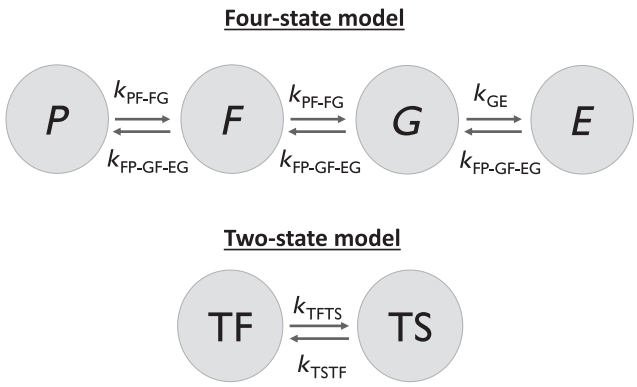

Figure 1 Illustration of the final continuous four-state Markov model and the reduced two-state model. Poor (*P*), fair (*F*), good (*G*), and excellent (*E*) illustrate the possible treatment outcomes in the final four-state model. $k_{PF\text{-}FG}$ is the first order rate describing the transfer from poor to fair and from fair to good improvement, k_{GE} is the first order rate describing the transfer from good to excellent improvement, and $k_{FP\text{-}GF\text{-}EG}$ is the first order rate describing the transfer from fair to poor score, from good to fair score, and from excellent to good improvement. In the reduced two-state model, TF and TS are the treatment failure and treatment success, respectively. k_{TFTS} is the first order rate describing transfer from TF state to TS state and k_{TSTF} is the first order rate describing the transfer from TS state to TF state.

The reduced two-state model was built on binarized data, as previously described, with two transfer rates estimated, describing transfer from treatment failure (poor/fair improvement) to treatment success (good/excellent improvement) and vice versa through k_{TFTS} and k_{TSTF}, respectively (**Figure 1**). Typical patient parameters k_{TFTS} and k_{TSTF} were estimated to 0.57 and 0.004 day^{-1}, respectively. BDBP and bodyweight were found as statistically significant ($P < 0.01$) covariates where BDBP below or equal to 80 mmHg was found to slightly increase k_{TFTS} whereas values above 80 mmHg were found to decrease k_{TFTS}, indicating that high blood pressure might negatively impact treatment success. Higher bodyweight was found to be associated with an increase in k_{TFTS}, translating to increased treatment success. In the evaluation of MarzAA PK metrics as covariate impacting the transfer rates, T_{max48h} was found as a statistically significant exposure metric decreasing k_{TFTS} ($P < 0.05$) but was determined with low parameter precision and therefore not kept in the model. Similar to the four-state modeling approach, none of the estimated transfer rates were found to be different between MarzAA and SoC. The

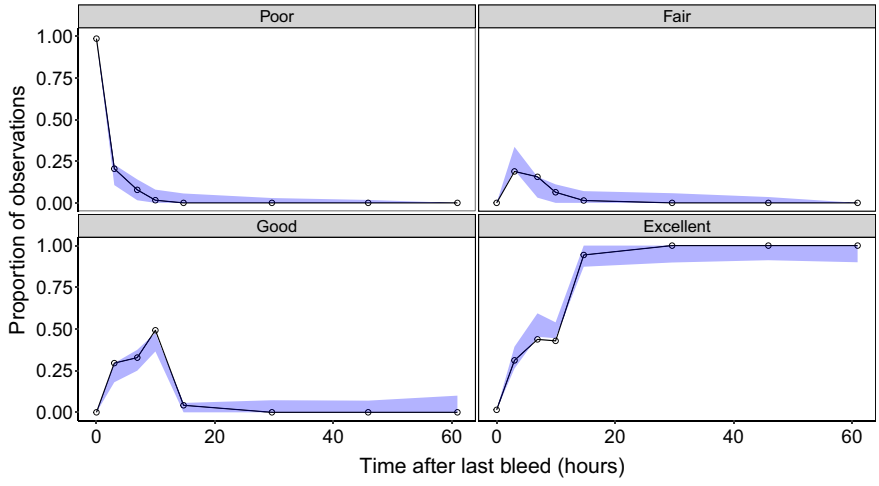

Figure 2 Prediction corrected visual predictive check of the proportion of total observations in each of the poor, fair, good and excellent treatment response states vs. time after last bleed. The circles are prediction corrected observed proportions over time and the blue shaded area is the 95% prediction interval based on 500 simulations from the final four-state Markov model.

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Table 1 Model parameter estimates of the final four-state Markov model

Typical parameter	Estimate (RSE %)	IIV % (RSE %)	Description
$k_{PF\text{-}FG}$ (day^{-1})	0.17 (7.8)	11 (65)	Shared transfer rate from poor to fair score and from fair to good score
k_{GE} (day^{-1})	0.2 (7.9)	21 (23)	Transfer rate from good to excellent score
$k_{FP\text{-}GF\text{-}EG}$ (day^{-1})	0.01 (26)	–s	Shared transfer rate from excellent to good score, from good to fair score and from fair to poor score
Covariate effects			
$Age_{k_{PF-FG}}$ [a]	1.9 (82)	–	Linear increase in $k_{PF\text{-}FG}$ above 33 years of age (no effect below 33 years of age)
$Knee_{k_{PF-FG}}$ [b]	1.2 (41)	–	Increase in $k_{PF\text{-}FG}$ for bleeding events in knee joints compared with bleeding events in elbow and ankle joints
$Other_{k_{PF-FG}}$ [c]	1.4 (35)	–	Increase in $k_{PF\text{-}FG}$ for bleeding events in other locations compared with bleeding events in elbow and ankle joints
$BDBP_{k_{GE}}$ [d]	−0.2 (45)	–	Decrease in k_{GE} with increase in baseline diastolic blood pressure

IIV, inter-individual variability expressed as coefficient of variation and in % of the parameter estimate; RSE, relative standard error.
Covariate effects, the uncertainty in the covariate effects and the uncertainty of IIV are reported on the logit scale.

[a] $k_{PF-FG} = TVk_{PF-FG} + (0 + Age_{k_{PF-FG}} * (Age - 33))$.

[b] $k_{PF-FG} = TVk_{PF-FG} + (0 + Knee_{k_{PF-FG}})$.

[c] $k_{PF-FG} = TVk_{PF-FG} + (0 + Other_{k_{PF-FG}})$.

[d] $k_{GE} = TVk_{GE} + (0 + BDBP_{k_{GE}} * (BDBP - 80))$.

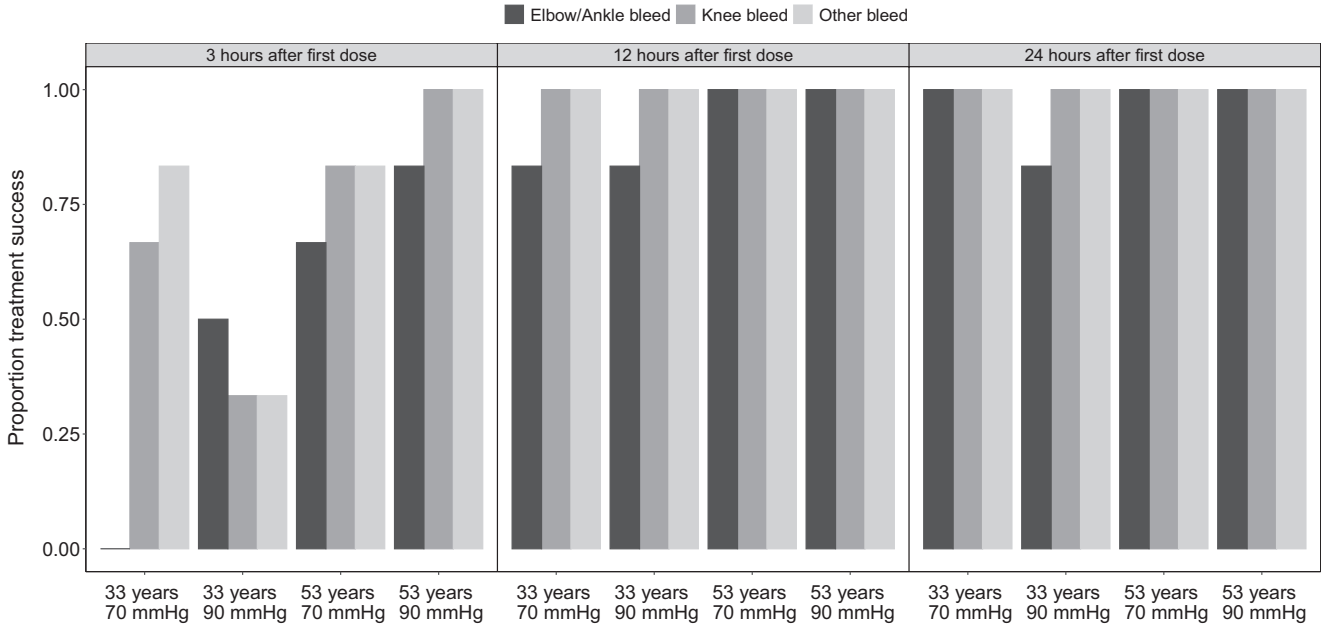

Figure 3 Prediction of proportion of successful treatment (good or excellent response) for a representative set of different subpopulations of patients for different ages, baseline diastolic blood pressure, and bleeding location at different timepoints after the first dose, based on the final four-state Markov model.

parameter estimates of the two-state model are reported in **Table S2** and the model fit is shown in **Figure S1**.

For the final four-state model, it was found that the covariate effect relationships had minor impact on treatment success (good/excellent response) at later time points (12 and 24 hours after the first dose). However, for treatment response occurring 3 hours after the first dose, a higher variation in the impact of covariate effects on the treatment response was found (**Figure 3**). A BDBP of 70 mmHg in a typical patient of 53 years of age (maximum age among the patients in the dataset) was associated with better treatment response as compared with a typical patient of 33 years of age, for bleeding events in knees or elbows/ankles (67% vs. 83% or 67% vs. 0%) as compared with bleeding events in other joints (83% for both 33 and 53 years of age). For a typical 53-year-old patient, a higher BDBP (90 compared with 70 mmHg) was associated with a better treatment response regardless of bleeding location (83%

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vs. 67%, 100% vs. 83% and 100% vs. 83% for bleeding events in elbow/ankles, knees and other joints, respectively). For a typical patient of 33 years of age, a worsened treatment response was found with a higher BDBP (90 mmHg) as compared with 70 mmHg for bleeding events in knees and other joints (33% vs. 67% and 33% vs. 83%, respectively), whereas a better treatment response was found for bleeding events in elbows/ankles (50% vs. 0%, for 90 and 70 mmHg).

Similar to the four-state model, the covariate effects impact on the treatment response in the reduced two-state model was more pronounced early (3 hours after the first dose) as compared with later at 12 and 24 hours after the first dose (**Figure S2**). A higher bodyweight was found to improve the treatment response. For a BDBP of 70 mmHg, a 104 kg typical patient was found to have proportion of treatment success of 83% whereas a 51 kg patient was found to have treatment success of 17%. A higher BDBP was found to worsen the treatment response for the same bodyweight (50% vs. 83% for 90 mmHg compared with 70 mmHg in a 104 kg typical patient). One combination of covariates (BDBP of 90 mmHg and bodyweight of 51 kg) was found to lessen the treatment success regardless of the time after the first dose (**Figure S2**).

DISCUSSION

In this work, a model-based approach for evaluation of novel treatments against episodic bleeds in patients with HA/HB was developed where a four-state Markov model was found to well describe the data (**Figure 2**) and preserve information about all scores in the data rather than categorizing the data as in the two-state model approach, which was also explored in this analysis. Further, the four-state Markov model allowed for evaluation of exposure-response on all end point scores which further supported this approach to be included in the final model. No statistically significant difference between s.c. MarzAA and SoC was found given the data in this study, independent of analysis approach. A statistically significant exposure-response relationship was identified using both analysis approaches but was too imprecise given the data, indicating that this aspect could be further studied with a larger sample size. Furthermore, patient characteristics that might be important for faster successful treatment outcome was identified in the analysis (**Figure 3**).

The World Federation of Hemophilia for patient care recommends starting treatment of episodic bleeding events and stopping bleedings as soon as possible to prevent bleeding-induced joint damages.[24] In this study, all s.c. doses of MarzAA were administered within 7 minutes from reconstitution to injection and the mean (standard deviation (SD)) of time from bleed recognition to initial dosing was 1.2 (1.8) and 3.5 (9.9) hours for s.c. MarzAA and i.v. SoC, respectively. The results from the covariate analysis in this work illustrates that some patient subgroups may have an earlier treatment response which would indicate that these patients might be easier to treat. We found that the patient subgroups that experienced a bleeding in knee joints or other bleeding locations in general had better treatment response within 3 hours after the first dose. In particular, age group of 53 years of age (maximum age in the dataset) were associated with a better response than the 33-year-old group (cutoff where the covariate effect had an impact, i.e., below 33 years, the covariate was not found to have an impact). The best treatment response was found among subgroups of 53 years of age with a BDBP of 90 mmHg and bleeding event located in knee joints or other locations as previously defined.

The final model indicated that in patients above 33 years of age, improvement of the bleeding symptoms increased with age. It is, however, hard to elucidate if age is the driver or other changes with age, such as physical activity or change in health status. In addition, bleedings occurring in knee and other joints were found to have a higher $k_{PF\text{-}FG}$ (which translates to a faster bleeding improvement) compared with elbow and ankle joints. Different joints may be differently perfused and have different capacities for clearing blood which might have an effect on hemostasis, both due to biological blood clearing mechanisms, pharmacodynamic mechanisms and due to delivery of drug to the site. Furthermore, higher BDBP was found to negatively impact k_{GE}, making it harder to move from good to excellent improvement with increasing BDBP. It is known that blood pressure is elevated among patients with hemophilia after adjusting for age and common risk factors,[25,26] potentially due to vascular changes.[26] The classification of low, normal, and high blood pressure is dependent on both systolic and diastolic pressure. However, this analysis did not find any covariate effects related to systolic blood pressure. In order to illustrate the impact of the covariate relationships on treatment success, different simulations were done to predict the proportion of treatment success in a typical patient with all possible covariate combinations (**Figure 3**, **Figure S2**). In general, the covariate relationships had minor impact on treatment success during later timepoints after the first dose (12 and 24 hours postdose) whereas during the first 3 hours after the first dose, treatment success varied more among the different covariate combinations. For instance, bleeding events in elbow/ankle joints that occurred in a 33-year-old typical patient with a BDBP of 70 mmHg resulted in almost 0% treatment success at 3 hours. However, the same combination of covariates led to desirable response at 12 and 24 hours after the first dose. In contrast, bleedings in knee joints or other locations in a 53-year-old typical patient with BDBP of 90 mmHg had almost 100% treatment success at 3 hours after the first dose. These results (**Figure 3**) suggest that some combination of covariates may lead to an increased number of needed doses (as additional dosing is guided by having poor/fair improvement at each efficacy assessment) in an on-demand setting. It is important to highlight the limited sample size in this study, which may be the reason for fairly low precision in some covariate relationships despite being statistically significant. In addition, each subgroup of characteristics in **Figure 3** was predicted based on the final model including covariate effects. As the number of individuals with a certain combination of covariates were low, there are limited data supporting each of the combinations, although each covariate was statistically significant.

In the final four-state model, MarzAA exposure (zero-order absorption (I_{dur})) was identified among the exposure metrics to impact ($k_{FP\text{-}GF\text{-}EG}$) through a piece-wise linear function ($P < 0.05$). The identified exposure-response parameters were not included in the final model due to high uncertainty (relative standard

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error > 500%). It is important to acknowledge that the exposure-response relationship was identified using PKs in patients in a non-bleeding state as it was not feasible to obtain drug concentration measurements during a bleeding event. There might be differences in PK between non-bleeding and bleeding state wherefore the true driver of episodic bleeding cessation is most likely the exposure of MarzAA at the bleeding occasion. In addition, the PKs for MarzAA varies between occasions, as shown in previous work,[9] further impacting the PKs between dosing occasions.

In summary, this work applied a four-state Markov model approach which allowed to study covariate and exposure-response relationships directly on the four-category outcome scale used in the clinical study. Furthermore, it allowed the investigation of the progression of the improvement through two different outcome scales. The work identified that age, bleeding location, and BDBP might be clinically relevant for early treatment response of MarzAA and SoC (APCC/Feiba, Coagil VII, or NovoSeven) for the treatment of bleeding in patients with HA/HB. No statistically significant difference in treatment response between s.c. MarzAA and i.v. SoC was found, indicating that s.c. MarzAA may potentially be as efficacious as current i.v. SoC for on-demand treatment of episodic bleeding events.

SUPPORTING INFORMATION

Supplementary information accompanies this paper on the *Clinical Pharmacology & Therapeutics* website (www.cpt-journal.com).

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CONFLICTS OF INTEREST

T.K. and G.E.B. were employees and shareholders of Catalyst Biosciences at the time of this work. All other authors declared no competing interests for this work.

AUTHOR CONTRIBUTIONS

All authors wrote the manuscript, designed the research, performed the research, and analyzed the data.

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1. Crimson 1: A Phase 3 study to evaluate the efficacy and safety of subcutaneous marzeptocog alfa (activated) for on-demand treatment of bleed events in subjects with hemophilia A or B, with inhibitors. *ISTH Congress Abstracts*. <https://abstracts.isth.org/abstract/crimson-1-a-phase-3-study-to-evaluate-the-efficacy-and-safety-of-subcutaneous-marzeptocog-alfa-activated-for-on-demand-treatment-of-bleed-events-in-subjects-with-hemophilia-a-or-b-with-inhibitors/>Accessed October 04, 2023.
2. Mahlangu, J. *et al*. Subcutaneous engineered factor VIIa marzeptacog alfa (activated) in hemophilia with inhibitors: phase 2 trial of pharmacokinetics, pharmacodynamics, efficacy, and safety. *Res. Pract. Thromb. Haemost.* **5**, e12576 (2021).
3. Mahlangu, J., Levy, H., Lee, M. & Del Greco, F. Efficacy and safety of subcutaneous prophylaxis with dalcinonacog alfa in adults with haemophilia B. *Haemophilia* **27**, 574–580 (2021).
4. Hartmann, J. & Croteau, S.E. 2017 clinical trials update: innovations in hemophilia therapy. *Am. J. Hematol.* **91**, 1252–1260 (2016).
5. Peyvandi, F. *et al*. A randomized trial of factor VIII and neutralizing antibodies in hemophilia A. *N. Engl. J. Med.* **374**, 2054–2064 (2016).
6. Hacker, M.R., Geraghty, S. & Manco-Johnson, M. Barriers to compliance with prophylaxis therapy in haemophilia. *Haemophilia* **7**, 392–396 (2001).
7. Stoner, K.L., Harder, H., Fallowfield, L.J. & Jenkins, V.A. Intravenous versus subcutaneous drug administration. Which do patients prefer? A systematic review. *Patient* **8**, 145–153 (2014).
8. Tiede, A. *et al*. Safety and pharmacokinetics of subcutaneously administered recombinant activated factor VII (rFVIIa). *J. Thromb. Haemost.* **9**, 1191–1199 (2011).
9. Faraj, A., Knudsen, T., Desai, S., Neuman, L., Blouse, G.E. & Simonsson, U.S.H. Phase-3 dose selection of Marzeptacog Alfa (activated) informed by population pharmacokinetic modeling: a novel hemostatic drug. *CPT Pharmacometrics Syst. Pharmacol.* **11**, 1628–1637 (2022).
10. Abrantes, J.A., Solms, A., Garmann, D., Nielsen, E.I., Jönsson, S. & Karlsson, M.O. Relationship between factor VIII activity, bleeds and individual characteristics in severe hemophilia a patients. *Haematologica* **105**, 1443–1453 (2020).
11. Kowalski, K.G., McFadyen, L., Hutmacher, M.M., Frame, B. & Miller, R. A two-part mixture model for longitudinal adverse event severity data. *J. Pharmacokinet. Pharmacodyn.* **30**, 315–336 (2003).
12. Lu, T., Yang, Y., Jin, J.Y. & Kågedal, M. Analysis of longitudinal-ordered categorical data for muscle spasm adverse event of Vismodegib: comparison between different pharmacometric models. *CPT Pharmacometrics Syst. Pharmacol.* **9**, 96–105 (2020).
13. Schindler, E. & Karlsson, M.O. A minimal continuous-time Markov Pharmacometric model. *AAPS J.* **19**, 1424–1435 (2017).
14. Markov, A.A. An example of statistical analysis of the text of Eugene Onegin Illustrating the association of trials into a chain. *Bull. Acad. imp. sci. St-Pétersbg* **7**, 153–162 (1913).
15. Bergstrand, M., Söderlind, E., Weitschies, W. & Karlsson, M.O. Mechanistic modeling of a magnetic marker monitoring study linking gastrointestinal tablet transit, in vivo drug release, and pharmacokinetics. *Clin. Pharmacol. Ther.* **86**, 77–83 (2009).
16. Lacroix, B.D., Karlsson, M.O. & Friberg, L.E. Simultaneous exposure–response modeling of ACR20, ACR50, and ACR70 improvement scores in Rheumatoid arthritis patients treated with certolizumab pegol. *CPT Pharmacometrics Syst. Pharmacol.* **3**, e143 (2014).
17. Pilla Reddy, V., Petersson, K.J., Suleiman, A.A., Vermeulen, A., Proost, J.H. & Friberg, L.E. Pharmacokinetic–pharmacodynamic modeling of severity levels of extrapyramidal side effects with Markov elements. *CPT Pharmacometrics Syst. Pharmacol.* **1**, e1 (2012).
18. Svensson, R.J., Gillespie, S.H. & Simonsson, U.S.H. Improved power for TB phase IIa trials using a model-based pharmacokinetic-pharmacodynamic approach compared with commonly used analysis methods. *J. Antimicrob. Chemother.* **72**, 2311–2319 (2017).
19. Rekić, D., Röshammar, D. & Simonsson, U.S.H. Model based design and analysis of phase II HIV-1 trials. *J. Pharmacokinet. Pharmacodyn.* **40**, 487–496 (2013).
20. Karlsson, K.E., Vong, C., Bergstrand, M., Jonsson, E.N. & Karlsson, M.O. Comparisons of analysis methods for

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proof-of-concept trials. *CPT Pharmacometrics Syst. Pharmacol.* **2**, e23 (2013).
21. Morrissey, J.H., Macik, B.G., Neuenschwander, P.F. & Comp, P.C. Quantitation of activated factor VII levels in plasma using a tissue factor mutant selectively deficient in promoting factor VII activation. *Blood* **81**, 734–744 (1993).
22. Beal, S.L., Sheiner, L.B., Boeckmann, A.J. & Bauer, R.J. *NONMEM Users Guides* (Icon Development Solutions, Ellicott City, MD, 1989-2011).
23. R: The R Project for Statistical Computing. <https://www.r-project.org/> Accessed October 04, 2023.
24. Carcao, M. & Goudemand, J. Inhibitors in hemophilia: a primer (5th edition). World Federation of Hemophilia (2018). <https://elearning.wfh.org/resource/inhibitors-in-hemophilia-a-primer/> Accessed January 14, 2024.
25. Barnes, R.F.W., Cramer, T.J., Sait, A.S., Kruse-Jarres, R., Quon, D.V.K. & von Drygalski, A. The hypertension of hemophilia is not explained by the usual cardiovascular risk factors: results of a cohort study. *Int. J. Hypertens.* **2016**, 2014201 (2016).
26. Sartori, M.T. *et al*. Endothelial dysfunction in haemophilia patients. *Haemophilia* **14**, 1055–1062 (2008).

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