Journal of Thrombosis and Haemostasis, **16**: 2184–2195 DOI: 10.1111/jth.14272

ORIGINAL ARTICLE

A randomized trial of safety, pharmacokinetics and pharmacodynamics of concizumab in people with hemophilia A

H. EICHLER,* P. ANGCHAISUKSIRI,† K. KAVAKLI,‡ P. KNOEBL,§ J. WINDYGA,¶ V. JIMÉNEZ-YUSTE,** A. HYSENI,†† U. FRIEDRICH†† and P. CHOWDARY‡‡

**Institute of Clinical Hemostaseology and Transfusion Medicine, Saarland University and University Hospital, Homburg/Saar, Germany; †Division of Hematology, Department of Medicine, Ramathibodi Hospital, Mahidol University, Bangkok, Thailand; ‡Department of Hematology, Ege University Children's Hospital, Izmir, Turkey; §Department of Medicine 1, Division of Hematology and Hemostasis, Medical University of Vienna, Vienna, Austria; ¶Department of Disorders of Hemostasis and Internal Medicine, Institute of Hematology and Transfusion Medicine, Warsaw, Poland; **Hematology Department, La Paz University Hospital, Madrid, Spain; ††Research and Development, Novo Nordisk A/S, Copenhagen, Denmark; and ‡‡Katharine Dormandy Haemophilia Centre and Thrombosis Unit, Royal Free London NHS Foundation Trust, London, UK*

To cite this article: Eichler H, Angchaisuksiri P, Kavakli K, Knoebl P, Windyga J, Jiménez-Yuste V, Hyseni A, Friedrich U, Chowdary P. A randomized trial of safety, pharmacokinetics and pharmacodynamics of concizumab in people with hemophilia A. *J Thromb Haemost* 2018; **16**: 2184–95.

Essentials

- explorer™3 was a double-blinded, multiple-dose escalation trial of subcutaneous concizumab.
- A pharmacodynamic relationship for unbound TFPI and thrombin generation was confirmed.
- No serious adverse events and no anti-drug antibodies were observed.
- explorer™3 data support further clinical development of concizumab in people with hemophilia.

Summary. *Background:* Concizumab is a humanized mAb targeting tissue factor pathway inhibitor (TFPI), leading to enhanced thrombin generation (TG) potential. explorer™3 (NCT02490787) was a phase 1b, double-blind, multiple-dose escalation trial of subcutaneous concizumab in people with severe hemophilia A without inhibitors. *Objectives:* The primary objective was to evaluate safety. Assessments of pharmacokinetics, pharmacodynamics and subcutaneous concizumab immunogenicity were secondary objectives. *Patients/Methods:* Adverse events (AEs), clinical assessments and bleeding episodes were recorded. Plasma concizumab levels and unbound TFPI levels were measured with ELISAs; residual TFPI activity was measured with a chromogenic assay. Standardized assays were used to assess TG, D-dimer and prothrombin fragment 1 + 2 (F_{1+2}) levels. explorer™3 was completed after investigation of three dose cohorts (0.25, 0.5 and 0.8 mg kg^{-1}, once every 4 days) had been completed. Twenty-four patients received 12 doses of concizumab or placebo in a 3 : 1 randomization over a 42-day period. *Results:* No serious AEs and no anti-drug antibodies were observed. Fifty-four mild and two moderate AEs were observed in 19 patients. Concizumab exposure increased with dose in a non-linear manner, confirming target-mediated drug disposition. D-dimer and F_{1+2} levels were increased mostly in the highest dose cohort, in line with previous observations. The level of unbound TFPI decreased in a dose-dependent manner, and was accompanied by a residual TFPI activity decrease and an increase in peak TG. Although the trial was not powered to evaluate efficacy, a trend towards lower bleeding rates was observed in patients in the highest dose cohort. *Conclusion:* explorer™3 data support further clinical development of concizumab for use in people with hemophilia, with or without inhibitors.

Keywords: clinical trial; factor VIII; hemophilia A; pharmacokinetics; safety.

Correspondence: Hermann Eichler, Institute of Clinical Hemostaseology and Transfusion Medicine, Saarland University and University Hospital, D-66421, Homburg/Saar, Germany
Tel: +49 684 1162 2530
E-mail: Hermann.Eichler@uks.eu

Received: 31 January 2018
Manuscript handled by: F. Peyvandi
Final decision: F. R. Rosendaal, 19 July 2018

Introduction

Congenital hemophilia is an inherited bleeding disorder arising from deficiency/absence of factor VIII (hemophilia A) or FIX (hemophilia B). People with severe hemophilia typically experience recurrent and spontaneous joint, muscle and soft tissue bleeding, which may lead to chronic

arthropathy, muscular atrophy, and other deformities. Current hemostatic treatment for hemophilia A and hemophilia B comprises replacement therapy with FVIII or FIX concentrates, respectively. In patients with severe hemophilia, prophylactic treatment is recommended to avoid the risk of life-threatening bleeds [1]. However, barriers to prophylaxis exist, such as the need for venous access, the use of central venous access devices in younger children (associated with infections), and frequent intravenous infusions with time-consuming administration [2–9]. Moreover, recent real-world data showed that an annualized bleeding rate as high as four was observed in people with severe hemophilia A receiving regular prophylaxis (although low adherence might have also played a role in this reported annualized bleeding rate value), and people with moderate disease experienced bleeds as frequently or more frequently than those with severe disease [10]. A similar trend was noted for hemophilia B patients.

Given these challenges, alternative treatment options to improve management strategies for people with hemophilia, with or without inhibitors, are of special interest, and are aimed at enhancing treatment adherence and compliance, and improving outcomes. The inhibition of tissue factor (TF) pathway inhibitor (TFPI) represents a therapeutic approach that may provide such an alternative treatment. TFPI is a potent regulator of the initiation phase during normal coagulation. Upon injury of the vessel wall, TF becomes exposed and binds to activated FVII (FVIIa). The TF–FVIIa complex activates FX to form activated FX (FXa) and FIX to form activated FIX. FXa not only leads to thrombin generation (TG) and fibrin formation to form a clot, but also enhances its own formation by activating FVII to form FVIIa. The activity of TFPI in regulating the initiation phase of coagulation is dependent on FXa binding to the TFPI Kunitz-2 domain, thus forming a TF–FVIIa–FXa–TFPI quaternary complex, in which the Kunitz-1 domain of TFPI inhibits FVIIa. TFPI simultaneously inhibits FXa and TF–FVIIa immediately following TF–FVIIa-mediated FXa generation [11,12]. Although people with hemophilia have normal initiation of coagulation, the amplification step of the coagulation process is impaired because of deficiency in FVIII or FIX, leading to impaired FXa generation [11,13]. Inhibition of TFPI results in increased FXa generation via the initiation pathway, leading to improved TG [12–14].

Concizumab is a high-affinity, humanized, mAb directed against the Kunitz-2 domain of human TFPI, designed to target and selectively block the FXa-binding site of TFPI. Concizumab abolishes TFPI inhibition of the TF pathway, resulting in increased FXa production, thus allowing sufficient TG despite FVIII or FIX deficiency in people with hemophilia A or B with or without inhibitors [15]. Non-clinical studies have shown that concizumab binds to TFPI with high affinity, and abrogates TFPI inhibition of TF–FVIIa-mediated FX activation and FXa activity *in vitro* [16]. Furthermore, concizumab has been shown to promote TF-induced TG by neutralizing TFPI in FVIII-deficient plasma [16]. Inhibition of TFPI also correlated with reduced blood loss and bleeding time in a hemophilic rabbit model [16]. As a mAb, concizumab has the advantage of being administered subcutaneously, and shows good solubility and stability, allowing administration as a liquid formulation via a ready-to-use and easy-to-use portable pen device [17].

In the first-in-human explorer™1 trial, concizumab showed a favorable safety profile, with no safety concerns being identified and no anti-drug antibodies being observed following intravenous or subcutaneous administration in healthy individuals or people with hemophilia A or B [18]. Concizumab administration was associated with a dose-dependent procoagulant effect [18]. Concizumab non-linear pharmacokinetics were observed in explorer™1, indicating dose-dependent, target-mediated clearance at low doses, which is a common characteristic of mAbs [19].

The aim of the clinical trial reported here was to investigate the safety, pharmacokinetics and pharmacodynamics of multiple doses of concizumab administered subcutaneously to hemophilia A patients without inhibitors.

Materials and methods

Trial design

explorer™3 (NCT02490787) was a multinational, multicenter, randomized, double-blind, placebo-controlled, multiple-dose, dose-escalation, phase 1b trial, conducted at 18 sites in 13 countries (Australia, Austria, Croatia, France, Germany, Israel, Poland, Spain, Thailand, Turkey, Ukraine, UK, and USA). The trial was approved according to local regulations by the appropriate health authorities and by an independent ethics committee or institutional review board, as required, and conducted in accordance with the Declaration of Helsinki and ICH Good Clinical Practice. Written informed consent was obtained from all participants prior to any trial-related activities. Investigators, participants and the sponsor were all blinded to treatment. Trial products appeared similar and were packaged blinded. All authors had access to the primary clinical trial data.

The trial was designed to investigate five escalating dose cohorts, each consisting of eight patients and divided into two blocks (Fig. 1). The trial comprised two treatment arms, concizumab and placebo. At visit 2, eligible patients were randomized 3 : 1 to receive concizumab or placebo by the use of a web-based randomization system (Interactive Web Response System). On the basis of observed changes in coagulation parameters, the trial was finalized following completion of investigation of the 0.8 mg kg^{-1} cohort (see Results and Discussion for further details). Concizumab doses were selected to reach predefined plasma levels comparable to, below and above

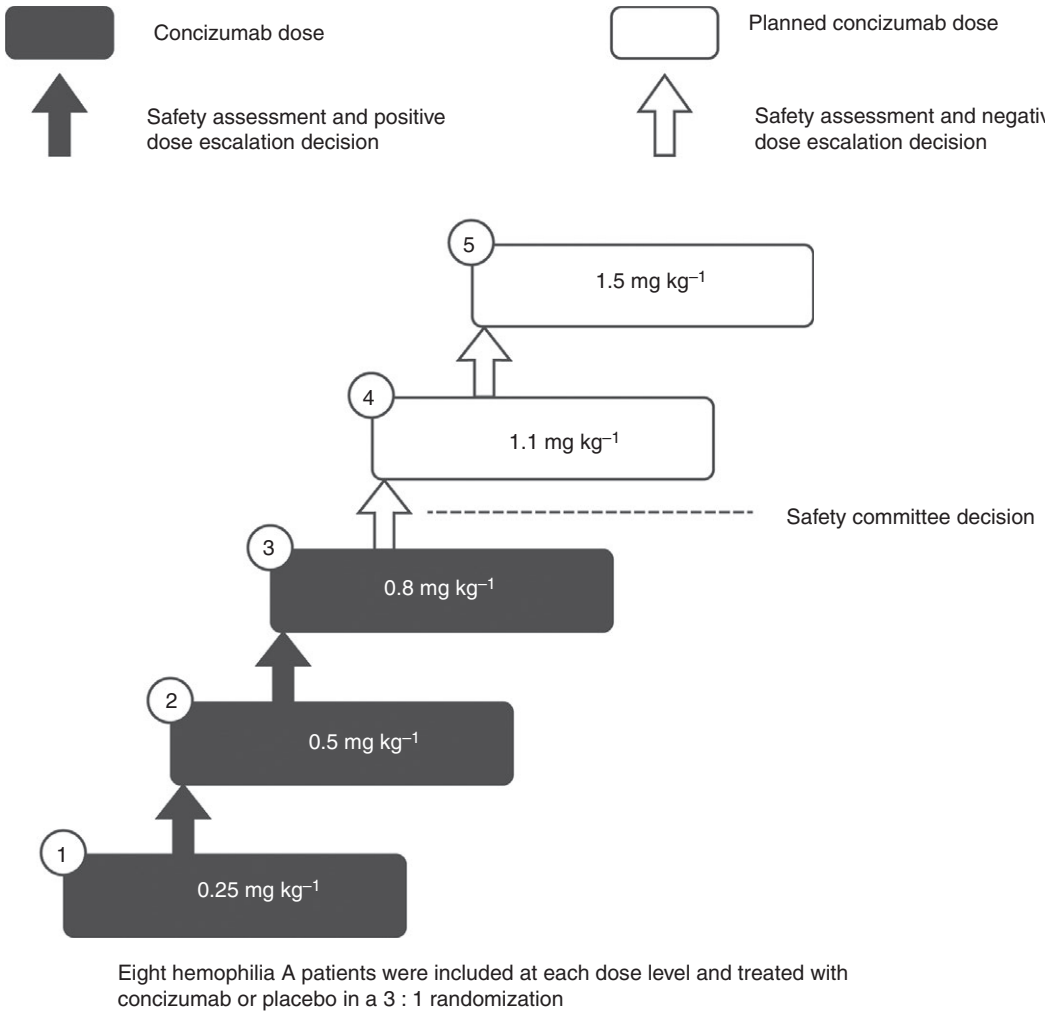

Fig. 1. explorer™3 dose escalation schedule. On the basis of human data from previous trials with concizumab, a population pharmacokinetic (PK) model providing an adequate description of the concizumab PK time course was developed. Between each dose level during the trial, the population PK model could be updated with PK data from the previous dose level(s). The dose needed to achieve the exposure level intended for the next ascending dose level was recalculated, and, if warranted, the dose could be changed as appropriate. Thus, the trial design allowed for adjustment of the dose levels based on the updated population PK model analysis of the exposure levels. Before the next dose was ascended to, an internal Novo Nordisk trial safety group reviewed blinded preliminary safety, PK and pharmacodynamic data, including D-dimers, prothrombin fragment 1 + 2, and total tissue factor pathway inhibitor (TFPI), and results for binding anti-drug antibodies, for each participant in a dose cohort and approved each dose escalation.

the exposure shown to be effective in animal studies. A population pharmacokinetic (PK) model was used to predict the doses needed to reach the target plasma levels (Fig. 1). In each dose cohort, participants were treated with 12 doses of concizumab or placebo over a period of ~ 42 days. The first two doses were administered on two consecutive days to achieve steady state at the targeted plasma level rapidly, and this was followed by subsequent dosing every fourth day. After the last dose, patients were followed up for 5 weeks.

Trial population

Patients were male hemophilia A patients without inhibitors, with documented FVIII activity levels of $\leq 2\%$, receiving on-demand treatment. Further eligibility criteria were: age, 18–64 years; body weight, 50–100 kg; and body mass index, 18–30 kg m^{-2}. Key exclusion criteria included known or suspected hypersensitivity to trial product or related products, a platelet count of $< 50 \times 10^9$ L^{-1} at screening, any clinical signs or known history of thromboembolic events, or a high risk of thromboembolic events and an increased risk of cardiovascular disease as judged by the investigator.

Patients were allowed to treat bleeding episodes with FVIII concentrate. Antifibrinolytics (e.g. tranexamic acid or aminocaproic acid), anticoagulants (e.g. heparin) and FVIII bypassing agents were not allowed during the course of the trial.

Objectives, endpoints, and assessments

The primary objective was to evaluate the safety of multiple subcutaneous doses of concizumab in hemophilia A patients, and the primary endpoint was the number of adverse events (AEs) from first trial drug administration to 11 weeks after first trial product administration. Assessment of the immunogenicity of concizumab was another safety-related secondary objective, with the frequency of anti-concizumab antibodies as a supportive secondary immunogenicity endpoint.

Safety assessments included investigation of local tolerability at the injection site, vital signs, electrocardiography, troponin T measurements, physical examination, and clinical laboratory tests, including urinalysis, hematology, biochemistry, coagulation-related parameters and anti-drug antibodies. Coagulation-related parameters included antithrombin (STA-Stachrom ATIII Kit [Diagnostica Stago, Asnières-sur-Seine, France] on the STA-R Evolution), fibrinogen (STA-Fibrinogen 5 [Diagnostica Stago] on the STA Compact), prothrombin time (Dade Innovin [Siemens Healthcare Diagnostics, Eschborn, Germany] on the BCS XP Analyzer), activated partial thromboplastin time (APTT) (Dade Actin FSL Activated PTT Reagent [Siemens Healthcare Diagnostics] on the BCS XP Analyzer), D-dimers (STA Liatest D-DI Kit [Diagnostica Stago] on the STA Compact), prothrombin fragment 1 + 2 (F_{1+2}) (Enzygnost F_{1+2} [monoclonal] [Siemens Healthcare Diagnostics] ELISA), protein C (STA-Stachrom Protein C Activity Kit [Diagnostica Stago] on the STA-R Evolution), and protein S (CRYOcheck Clot S [Precision BioLogic, Dartmouth, Canada] Destiny MAX [Tcoag]). With the exception of urinalysis and hematology (analyzed locally), all clinical laboratory analyses were performed in a central laboratory. Except for electrocardiography and anti-drug antibodies, all safety assessments were performed at each treatment visit every 4 days until the end of the 42-day treatment period and, with the exception of local injection site reactions, at each weekly visit during the 5-week follow-up period until the end of the trial. Anti-drug antibodies were assessed at the screening visit and on days 1, 18, and 42, as well as at the end of the trial. Analysis of binding anti-drug antibodies was performed with a bridging electrochemiluminescence assay. Assay quality controls and samples were pretreated with acid (glycine-HCl, pH ~ 2.5, for 15 min) to ensure that any complexes between anti-drug antibodies and the drug present in the serum samples were dissociated, while also ensuring the dissociation of any labeled concizumab from TFPI. Following acid pretreatment, all samples were neutralized with Tris buffer mixed with biotinylated and sulfo-tag-labeled concizumab. The samples were incubated at room temperature for ~ 1 h, and then transferred to a streptavidin-coated, 96-well plate. After ~ 1 h, the plate was washed, substrate was added (MSD Read Buffer; Meso Scale Diagnostics, Rockville, MD, USA), and the plate was read on an MSD SI6000 reader, with results given as relative light units.

Other secondary objectives included the assessment of the pharmacokinetics and pharmacodynamics of multiple subcutaneous doses of concizumab in hemophilia A patients. PK endpoints included the area under the plasma concentration–time curve from the penultimate dose administration (day 38) to the last dose administration (day 42) (AUC_τ), and the maximum concentration (C_{max}) and trough level (C_{trough}) of concizumab. Pharmacodynamic (PD) endpoints included residual TFPI activity, and unbound and total TFPI levels. TG potential assessment was an exploratory PD endpoint.

PK and PD assessment blood samples were collected predose at each day of dose administration, 4 h after administration on days 18 and 42, and 48 h after the last dose administration on day 42. Both PK and PD blood sample analyses were performed at the central laboratory. PK parameters were determined by quantifying plasma concizumab levels with an ELISA in which concizumab was captured by TFPI and detected with horseradish peroxidase-labeled anti-human IgG_4-specific antibodies followed by colorimetric detection of the amount of anti-TFPI with 3,3′,5,5′-tetramethylbenzidine. Unbound TFPI (plasma TFPI not bound to concizumab) was measured in ng mL^{-1} with an ELISA kit validated for determining TFPI not bound to concizumab for this study (Asserachrom TOTAL TFPI; Diagnostica Stago). Residual TPFI activity was measured with a chromogenic assay (S2222; Chromogenix, DiaPharma Group, West Chester, OH, USA) detecting FXa activity, which is reported as U mL^{-1}. The amount of TFPI present in the plasma is inversely proportional to the amount of FXa generated. Total TFPI was measured in ng mL^{-1} with an in-house ELISA using a polyclonal anti-TFPI antibody, which binds to TFPI via an epitope at some distance from the binding site of concizumab, thereby capturing both unbound and concizumab-bound TFPI. A monoclonal anti-TFPI antibody that does not bind to the concizumab epitope was used for detection. TG potential, reported as peak thrombin (nM), was measured *in vitro* with a calibrated automated thrombogram (Thrombinoscope BV, Maastricht, the Netherlands). Samples were collected in 3.2% trisodium citrate. The TG potential assay was performed at the end of each cohort. To ensure that true baseline TG potential was measured, i.e. that there was no influence of treatment of breakthrough bleeding episodes with FVIII concentrate, it was calculated from values of samples taken > 72 h following the last FVIII administration.

Bleeding episodes were reported throughout the trial, and evaluated on the basis of their cause, location and severity (mild–moderate or severe), and on whether hemostatic concomitant medication or other therapy was used.

Statistical analysis

Data were analyzed with SAS version 9.4 (TS1M2).

The sample size (eight participants per cohort; six receiving concizumab, and two receiving placebo) was chosen to enable adequate assessment of safety and PK parameters, while exposing the smallest possible number of patients to concizumab.

Safety endpoints were reported for the safety analysis set, i.e. all patients exposed to at least one dose of trial product analyzed according to the treatment received. PK and PD endpoints were reported for the full analysis set, i.e. all patients in the trial. As no assessments were invalid or excluded, the safety and full analysis sets were identical.

Safety endpoints were based on descriptive statistics. Coagulation-related parameters were presented as graphical presentations of mean values with standard error of the mean by dose cohort and time point. The AUC_τ was calculated according to the actual dosing interval, and normalized to the planned dosing interval of 4 days. All PK and PD parameters derived from concentration profiles were summarized by dose group and time point, including geometric mean and coefficient of variation. Peak thrombin was presented as individual values and medians with interquartile ranges (IQRs) by dose cohort, and graphical presentations including and excluding values obtained within 72 h of dosing with FVIII concentrate.

Results

Trial population and baseline characteristics

The trial was conducted from 10 September 2015 to 14 October 2016. A total of 35 patients were screened, and 24 eligible patients were randomized and distributed equally between the three concizumab dose groups (0.25, 0.5 and 0.8 mg kg^{-1} every fourth day). In each dose cohort, six patients received concizumab and two patients received placebo. All randomized patients completed the trial and were included in the full analysis and safety analysis sets. Baseline demographic characteristics were comparable between the three dose groups, with the exception of a decreasing trend in the mean weight of patients treated with the lowest to the highest concizumab dose (Table 1).

Safety

Each patient was exposed to 12 doses of either concizumab or placebo, with the exception of one patient in the 0.5 mg kg^{-1} dose cohort who missed a dose.

A total of 56 AEs were reported in 19 of 24 patients (Table 2). Of these, 54 AEs in 18 patients were rated as mild, and two in two patients were rated as moderate. Increasing concizumab dose was not associated with an increase in the number of AEs. There were no serious AEs (SAEs) or deaths. No anti-drug antibodies were reported, and no thromboembolic events occurred during the trial.

The most frequently reported AEs were headache (six events in four patients), nasopharyngitis (five events in four patients), and injection site erythema (three events in three patients). All remaining AEs were reported in a total of one or two patients across all treatment groups, with no apparent relationship with treatment or dose. Five local injection site reactions in four patients were reported, all of which were rated as mild. Three of the injection site reactions were recorded in two patients in the placebo group.

Table 1 Patient disposition and baseline characteristics in explorer™3

	Concizumab				
	0.25 mg kg^{-1}	0.5 mg kg^{-1}	0.8 mg kg^{-1}	Placebo	Total
No. of patients included	6	6	6	6	24
No. of patients withdrawn	0	0	0	0	0
No. of positive inhibitor tests	0	0	0	0	0
Age (years)					
Mean (SD)	39.2 (8.2)	32.3 (9.7)	27.8 (6.1)	37.0 (11.0)	34.1 (9.5)
Weight (kg)					
Mean (SD)	85.1 (9.5)	71.9 (9.7)	61.6 (7.7)	76.2 (7.6)	73.7 (11.8)
BMI (kg m^{-2})					
Mean (SD)	26.4 (2.6)	24.1 (4.1)	21.1 (2.3)	24.0 (2.1)	23.9 (3.3)
FVIII level (%)*					
Mean (SD)	0.54 (0.35)	0.36 (0.27)	0.50 (0.39)	0.50 (0.56)	0.47 (0.38)
Historical ABR					
Median (minimum; maximum)	6.0 (1.0; 60.0)	9.0 (0.0; 64.0)	11.0 (2.0; 48.0)	34.0 (0.0; 100)	11.0 (0.0; 100)
No. of patients with moderate hemophilia	0	0	1†	0	1
No. of patients with severe hemophilia	6	6	5	6	23

ABR, annualized bleeding rate; BMI, body mass index; SD, standard deviation. *Based on measurements taken at visit 2, when, according to the protocol, no treatment with FVIII was allowed to have taken place 48 h prior to the visit. †FVIII activity based on medical records: 1.2%.

Table 2 Summary of adverse events (AEs) following subcutaneous administration of concizumab in patients with hemophilia A in explorer™3 (safety analysis set)

	Concizumab *E* (*N*)	Placebo *E* (*N*)	Total *E* (*N*)
No. of patients	18	6	24
All AEs	47 (16)	9 (3)	56 (19)
AEs leading to withdrawal	0	0	0
AEs by severity			
Mild*	45 (15)	9 (3)	54 (18)
Moderate†	2 (2)	–	2 (2)
AEs probably or possibly related to trial product	8 (6)	5 (2)	13 (8)
General disorders and administration site conditions	6 (3)	3 (1)	9 (4)
Injection site erythema	2 (2)	1 (1)	3 (3)
Injection site pain	–	2 (1)	2 (1)
Fatigue	2 (2)	–	2 (2)
Inflammation	1 (1)	–	1 (1)
Pyrexia	1 (1)	–	1 (1)
Infections and infestations	8 (6)	1 (1)	9 (7)
Nasopharyngitis	4 (3)	1 (1)	5 (4)
Upper respiratory tract infection	2 (2)	–	2 (2)
Chronic tonsillitis	1 (1)	–	1 (1)
Tooth abscess	1 (1)	–	1 (1)
Nervous system disorders	6 (4)	–	6 (4)
Headache	6 (4)	–	6 (4)
Skin and subcutaneous tissue disorders	3 (2)	1 (1)	4 (3)
Subcutaneous hemorrhage	–	1 (1)	1 (1)
Maculopapular rash	2 (1)	–	2 (1)
Eczema	1 (1)	–	1 (1)
Gastrointestinal disorders	5 (3)	1 (1)	6 (4)
Flatulence	–	1 (1)	1 (1)
Abdominal pain	2 (1)	–	2 (1)
Constipation	1 (1)	–	1 (1)
Diarrhea	1 (1)	–	1 (1)
Toothache	1 (1)	–	1 (1)
Musculoskeletal/connective tissue disorders	5 (4)	–	5 (4)
Myalgia	2 (2)	–	2 (2)
Arthralgia	1 (1)	–	1 (1)
Joint swelling	1 (1)	–	1 (1)
Synovitis	1 (1)	–	1 (1)

E, number of AEs; *N*, number of patients with AEs. Two medical events of special interest (MESIs) were reported (medication errors; see the 'Safety' section for more details). A MESI was defined as an AE, serious or non-serious, fulfilling one or more of the following criteria: (i) medication errors concerning trial products (administration of wrong drug [use of wrong Data Universal Numbering system was not considered to be a medication error]; wrong route of administration, such as intramuscular instead of subcutaneous; administration of an overdose with the intention to cause harm [e.g. suicide attempt]; accidental administration of a lower or higher dose than intended [i.e. a dose lower or higher than 10% of the intended dose; however, the administered dose must have deviated from the intended dose to such an extent that clinical consequences for the trial subject were likely to have happened as judged by the investigator, although they did not necessarily occur]); (ii) project-specific and/or trial-specific AEs (anaphylactic reactions; thromboembolic events, including but not limited to, clinical signs or laboratory indications of arterial and venous thrombosis, including myocardial infarction, pulmonary embolism, stroke, deep vein thrombosis, other clinically significant thromboembolic events, and peripheral artery occlusion; disseminated intravascular coagulation; allergic reactions; and hypersensitivity type reactions). If any of the above events occurred, dosing with concizumab was to be stopped immediately and, where possible and applicable, evidence-based guidelines or best practices were to be used to guide treatment. *Headache, nasopharyngitis, and injection site erythema. †Myalgia and urinary calculus.

A total of 13 AEs in eight patients were rated by the investigators as 'possibly' or 'probably' related to trial product (Table 2). Five of these AEs occurred in two patients in the placebo group. The majority (12/13) were classified as mild, with one event being classified as moderate (myalgia in the 0.25 mg kg^{-1} concizumab cohort). All 13 events were characterized by full recovery.

Two medical events of special interest (defined in the footnote to Table 2) were reported, both medication errors resulting from the administration of 0.8 mL instead of the approximately 0.5 mL required in the placebo group and 0.8 mg kg^{-1} in the concizumab group. They were reported as an 'overdose' in the placebo group and as 'incorrect dose administered' in the 0.8 mg kg^{-1} group.

There were no clinically significant changes that were deemed to be related to concizumab administration for the following safety assessments: vital sign and physical

examination, electrocardiography, troponin T measurements, urinalysis, hematology, and biochemistry profiles, including platelets, antithrombin, prothrombin time, APTT, and protein C and protein S measurements.

Pharmacokinetics and pharmacodynamics

Successful drug uptake was demonstrated in all three dose cohorts with subcutaneous concizumab administration of 0.25, 0.5 and 0.8 mg kg^{-1} (Fig. 2A). The median (IQR) concizumab levels were 30.20 ng mL^{-1} (IQR 22.20–36.30 ng mL^{-1}), 79.10 ng mL^{-1} (IQR 60.60–91.40 ng mL^{-1}) and 162.50 ng mL^{-1} (IQR 93.20–795.00 ng mL^{-1}) in the 0.25, 0.5 and 0.8 mg kg^{-1} groups, respectively. Concizumab levels returned to baseline levels during the follow-up period after the last dose on day 42. Large differences in total exposure (AUC) across the three dose groups were observed, with high variability between patients, especially in the highest (0.8 mg kg^{-1}) dose cohort, as illustrated by the error bars for this cohort (Fig. 2A). Concizumab exposure (AUC_τ) increased with dose in a greater than linear manner, with geometric mean values increasing three-fold in the 0.5 mg kg^{-1} group and nearly 11-fold in the 0.8 mg kg^{-1} group as compared with the 0.25 mg kg^{-1} group (Table 3). Similarly, C_{max} and C_{trough} increased from the lowest to the highest concizumab dose cohort in a non-linear, but dose-dependent, manner. These observations are consistent with target-mediated drug disposition (TMDD) and similar to earlier preclinical and clinical data [18,20].

Unbound TFPI levels decreased with increasing concizumab dose, showing a tight PK–PD relationship for concizumab and unbound TFPI. The median unbound TFPI levels were 59.80 ng mL^{-1} (IQR 52.60–67.90 ng mL^{-1}),

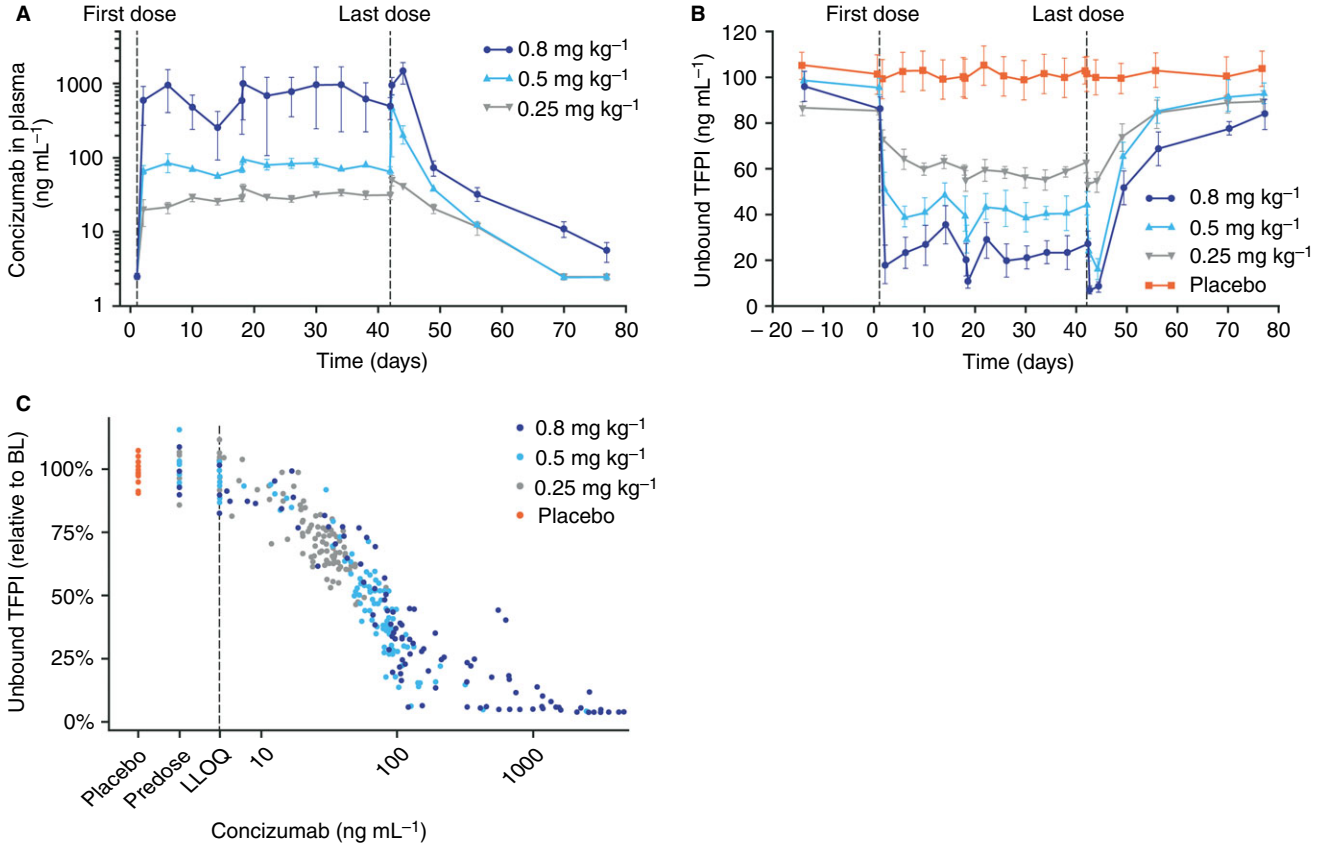

Fig. 2. Mean profiles of concizumab and unbound tissue factor pathway inhibitor (TFPI), and correlation between concizumab and unbound TFPI, following concizumab subcutaneous administration. (A, B) Mean concizumab levels (A) (log scale) evaluated with concizumab ELISA and mean unbound TFPI plasma levels (B) evaluated with TFPI ELISA in the treatment (days 1–42) and follow-up (days 44–77) periods in the placebo group and three concizumab groups. Error bars represent ± standard error of the mean. Blood samples were taken predose (trough) and 4 h after administration on days 18 and 42. Percentages show change from baseline (BL) to day 42 of mean unbound TFPI plasma levels. Blood samples for pharmacokinetic/pharmacodynamic assessments were collected predose on each day of dose administration, and at 4 h after administration on days 18 and 42. The local peaks observed on the concizumab level graph (A) at days 18 and 42 reflect the two 4-h postdose samples. The large peak at planned day 42 in the concizumab 0.50 mg kg^{-1} group was driven by an extreme value from a single patient, and was not associated with any treatment-emergent adverse events. (C) Correlation between concizumab and unbound TFPI shown as percentage relative to BL values in the placebo group and three concizumab groups. The vertical dashed line indicates the lower limit of quantification (LLOQ). [Color figure can be viewed at wileyonlinelibrary.com]

Table 3 Mean pharmacokinetic parameters of concizumab following subcutaneous administration in hemophilia A patients in explorer™3

	Concizumab 0.25 mg kg^{-1}	Concizumab 0.5 mg kg^{-1}	Concizumab 0.8 mg kg^{-1}
AUC_τ (days × ng mL^{-1}), geometric mean (minimum; maximum)	135 (113; 187)	396 (353; 441)	1470 (354; 8020)
C_{max} (ng mL^{-1}), geometric mean (minimum; maximum)	52 (42; 83)	265 (108; 2450)	1442 (154; 4630)
C_{trough} (ng mL^{-1}), geometric mean (minimum; maximum)	30 (20; 42)	67 (49; 93)	350 (90; 1180)

AUC_τ, area under the plasma concentration–time curve (calculated according to the actual dosing interval from the penultimate dose administration [day 38] to the last dose administration [day 42]); C_{max}, maximum concizumab concentration (measured 4 h after the first concizumab dose); C_{trough}, concizumab trough level (measured prior to the last dose at day 42).

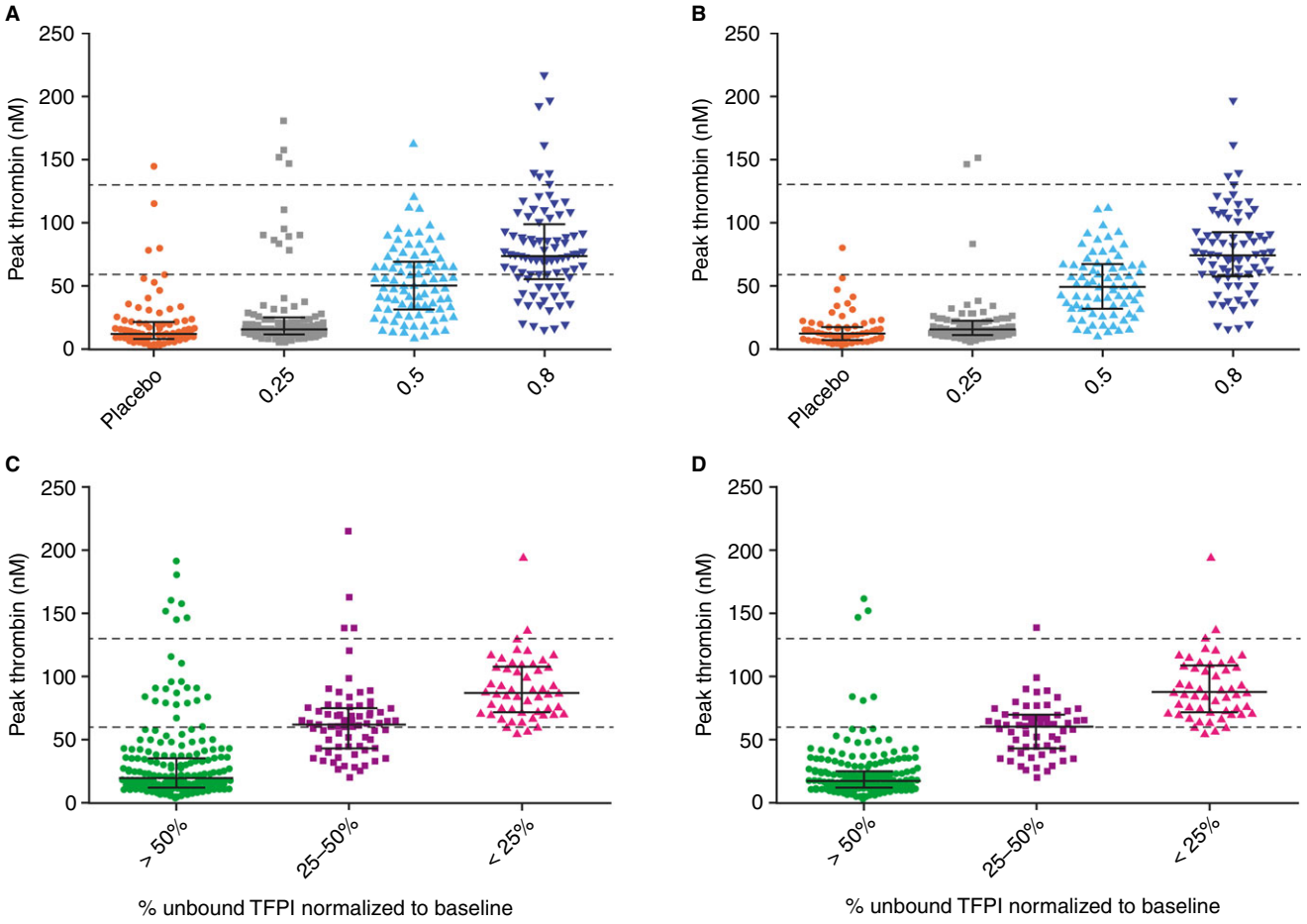

Fig. 3. Peak thrombin generation following subcutaneous concizumab and placebo administration. (A, B) Correlation between concizumab and peak thrombin generation potential by dose cohort, including (A) and excluding (B) values obtained within 72 h after dosing with FVIII concentrate. (C, D) Correlation between unbound tissue factor pathway inhibitor (TFPI) and peak thrombin generation potential, including (C) and excluding (D) values obtained within 72 h after dosing with FVIII concentrate. The numbers of postbaseline unbound TFPI measurements that were < 25% of the baseline unbound TFPI level were 0, 11 (12.64%), 42 (50.00%) and 0 in the concizumab 0.25, 0.5 and 0.8 mg kg^{-1} and placebo groups, respectively. Individual and median values with interquartile ranges of peak thrombin generation are shown. Dashed horizontal lines indicate the normal laboratory range for peak thrombin generation potential. [Color figure can be viewed at wileyonlinelibrary.com]

41.90 ng mL^{-1} (IQR 27.90–52.90 ng mL^{-1}) and 20.45 ng mL^{-1} (IQR 4.80–32.70 ng mL^{-1}) in the 0.25, 0.5 and 0.8 mg kg^{-1} groups, respectively. As compared with baseline, mean reductions in unbound TFPI levels of approximately 27%, 55% and 75% were observed in the 0.25, 0.5 and 0.8 mg kg^{-1} groups, respectively (Fig. 2B). The time needed to reach the highest level of reduction in unbound TFPI level decreased with increasing concizumab doses, with the maximal change (geometric mean [minimum; maximum]) being observed after 24 days (10; 34), 11 days

(5; 29) and 4 days (2; 22) days in the 0.25, 0.5 and 0.8 mg kg^{-1} groups, respectively. An inverse correlation was observed between concizumab and unbound TFPI; unbound TFPI values decreased to lower percentages relative to baseline with increasing concizumab levels in the three dose cohorts, showing a tight PK–PD relationship for concizumab and unbound TFPI (Fig. 2C). Mean unbound TFPI values prior to the last dose were inversely correlated with total concizumab exposure (data not shown).

Residual TFPI activity decreased in all concizumab-treated patients, with an approximately 30% reduction in the 0.25 mg kg^{-1} group. For the 0.5 mg kg^{-1} and 0.8 mg kg^{-1} groups, the residual TFPI activity assay reached saturation, showing an approximately 60–70% reduction, with no differences between the two groups (data not shown). The maximal changes (geometric mean [minimum; maximum]) in residual TFPI activity from baseline, based on trough assessments, were 0.6 U mL^{-1} (0.4; 1.0), 0.8 U mL^{-1} (0.6; 1.0) and 0.9 U mL^{-1} (0.8; 1.2) for the 0.25, 0.5 and 0.8 mg kg^{-1} groups, respectively. The time to maximum change (median [minimum; maximum]) from baseline in residual TFPI activity decreased from 29.5 days (17; 38) to 5.5 days (2; 18) in the 0.25 mg kg^{-1} and 0.5/0.8 mg kg^{-1} groups, respectively.

The TG potential increased in a concizumab dose-dependent manner, with a strong correlation between concizumab level and peak thrombin (Fig. 3A,B). The large variability observed between patients was partly influenced by treatment of bleeding episodes with FVIII concentrate. Notably, the TG potential in the 0.8 mg kg^{-1} group increased by approximately three-fold during the concizumab treatment period as compared with baseline, thus moving within the normal range (data not shown). Peak thrombin and unbound TFPI were inversely correlated, irrespective of whether bleeding episodes had been treated with FVIII concentrate $\leq$ 72 h prior to blood sampling (Fig. 3C,D).

Coagulation-related parameters

D-dimer and prothrombin F_{1+2} levels above the normal reference range were observed mostly in the highest (0.8 mg kg^{-1}) dose cohort in patients with high exposure to concizumab. This increase above the normal reference range was generally not seen in the 0.25 mg kg^{-1} and 0.5 mg kg^{-1} groups (Fig. 4A–B). Mean fibrinogen values decreased by 20% as compared with baseline in both the 0.5 mg kg^{-1} and 0.8 mg kg^{-1} groups, reaching their nadir at the end of the treatment period, although all values remained within the lower and upper limits of normal (Fig. 4C). Mean levels of soluble fibrin above the normal reference range were also observed in the 0.8 mg kg^{-1} group (Fig. 4D). These above-mentioned changes were indicative of activation of the coagulation and fibrinolytic pathways. No thromboembolic events or abnormal bleeding patterns were observed in association with the changes in these coagulation parameters. Furthermore, no significant changes in other primary coagulation-related parameters, including platelet counts, antithrombin levels, prothrombin time, APTT, protein C, and protein S, were observed.

Bleeding events

A total of 91 bleeding episodes in 21 patients were recorded during the trial. Of these, 15, 30 and 18 episodes were reported in five, six and five patients in the 0.25, 0.5 and 0.8 mg kg^{-1} groups, respectively. A total of 28 episodes were recorded in five patients in the placebo group. Ten of 15 (66.7%), 14 of 30 (46.7%) and six of 18 (33.3%) of the bleeding episodes in the 0.25, 0.5 and 0.8 mg kg^{-1} groups, respectively, required additional treatment during the treatment period. The vast majority of these bleeding episodes were treated with FVIII concentrate. The majority of bleeding episodes (67/91 [73.6%]) were spontaneous. Bleeding episodes were generally classified as mild–moderate (89/91 [97.8%]), with the exception of two severe bleeding episodes: one spontaneous hemarthrosis in the 0.5 mg kg^{-1} group, and one traumatic muscular bleeding in the placebo group. The majority of the reported bleeding episodes (52/91 [57.1%]) occurred > 2 days after the last dose.

Discussion

The results from the phase 1b explorer™3 trial confirmed the results that had been reported for concizumab in the first-in-human explorer™1 trial [18]. Furthermore, the trial generated additional data in relation to safety, TG potential and bleeding event occurrence in a setting where multiple subcutaneous concizumab doses were administered to patients for 6 weeks.

No deaths and no thromboembolic events or other SAEs were reported, and no anti-drug antibodies were detected. Only five local injection site reactions were reported. The numbers of AEs were similar in the 0.25 mg kg^{-1} and 0.5 mg kg^{-1} groups and in the 0.8 mg kg^{-1} and placebo groups, respectively, with a numerically lower number of AEs being reported in the 0.8 mg kg^{-1} and placebo groups. Therefore, no apparent relationship between concizumab dose and the number of AEs was identified. This was also the case for the 13 AEs that were rated as 'possibly' or 'probably' related to concizumab by the investigators; however, this will need to be confirmed in larger clinical trials.

Coagulation-related parameters, including D-dimer and F_{1+2}, increased above the normal reference range in the highest dose cohort (0.8 mg kg^{-1}). These changes were observed previously in explorer™1 [18], with a similar trend being observed in a first-in-human study with another anti-TFPI antibody following administration to

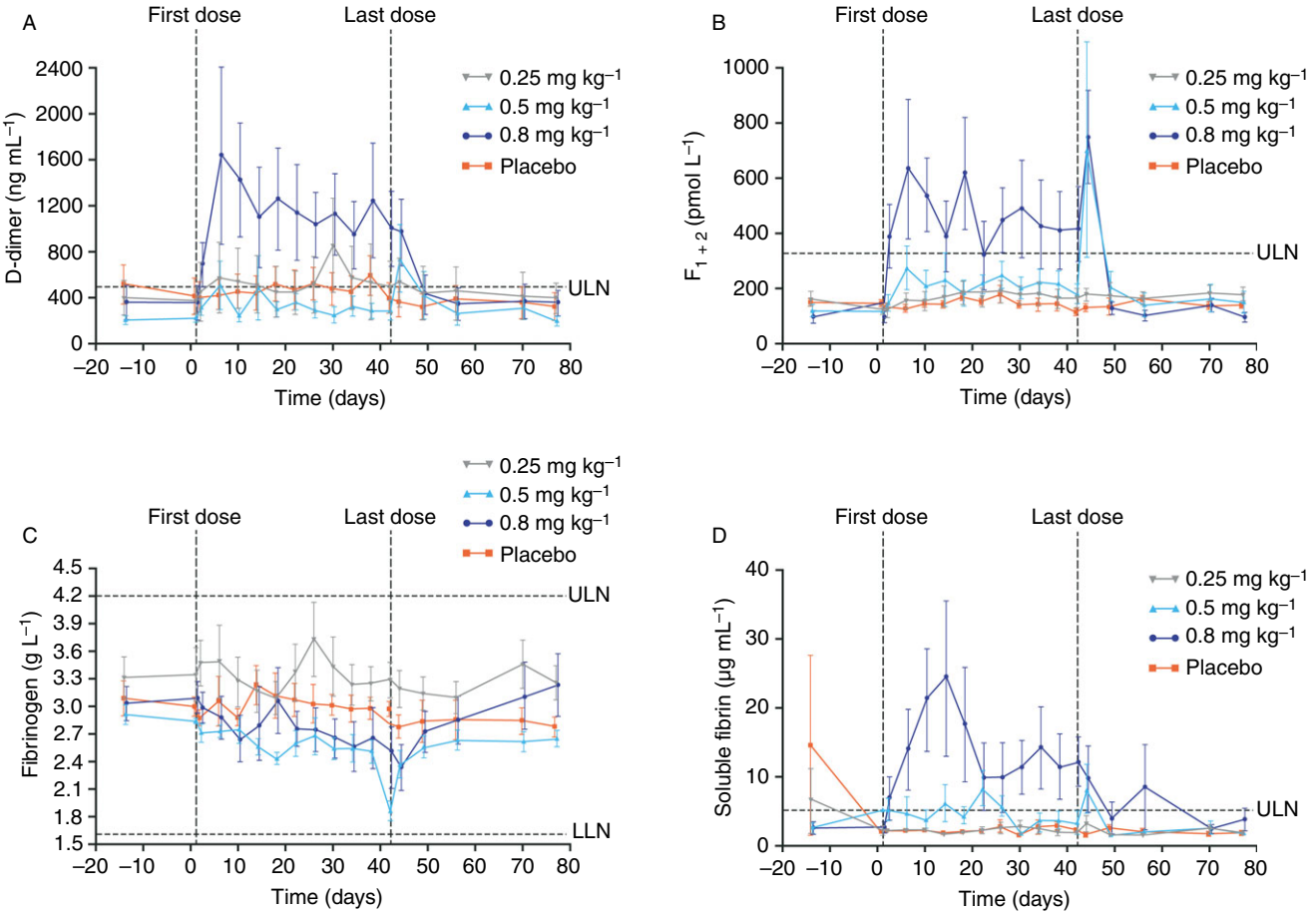

Fig. 4. Mean profiles of coagulation-related parameters following subcutaneous concizumab and placebo administration. Mean values of (A) D-dimer, (B) prothrombin fragment 1 + 2 (F_{1+2}) (C) fibrinogen levels and (D) soluble fibrin measured in the treatment (days 1–42) and follow-up (days 44–77) periods in the placebo group and the three concizumab groups are shown. Error bars represent ± standard error of the mean. Horizontal dashed lines represent references for D-dimers (fibrinogen-equivalent units), F_{1+2}, fibrinogen, and soluble fibrin. LLN, lower limit of normal range; ULN, upper limit of normal range. [Color figure can be viewed at wileyonlinelibrary.com]

healthy volunteers [21]. The increase in these markers was followed by a decrease in fibrinogen level, with values remaining within the normal range, and an increase in soluble fibrin level above the normal range, which can be partly explained by the conversion of fibrinogen to fibrin by thrombin. These changes were not followed by significant changes from baseline for platelet counts, antithrombin levels, prothrombin time, APTT, protein C, and protein S, and were not judged to be clinically significant by the investigators. Furthermore, no thromboembolic events or increases in the number of bleeding episodes followed these changes in coagulation-related parameters. Currently, there are no data to indicate that D-dimers are predictive of thrombotic risk in individuals with hemophilia. However, given the relatively short period of observation in explorer™3 (which was based on phase 1 results), future trials with longer observation periods are needed to better assess whether there is a thrombotic risk associated with increases in D-dimer and F_{1+2} levels in hemophilic patients. It would be of particular interest to assess this potential risk in individuals with hemophilia who are aged > 50 years, with a high body mass index, or with cardiovascular comorbidities.

Although it had been planned to include five concizumab dose levels in explorer™3, the trial was completed after the first three dose levels (0.25, 0.5 and 0.8 mg kg^{-1}), owing to an internal safety committee decision not to dose-escalate to the fourth concizumab dose level of 1.1 mg kg^{-1}. The decision was based on the observation of changes in coagulation and PK parameters, as well as the high interpatient variation in the PK parameters and procoagulant response to concizumab in the 0.8 mg kg^{-1} group. No clinical consequences or SAEs were observed in the completed-dose groups.

Non-linear, dose-dependent pharmacokinetics of concizumab were observed, confirming TMDD, as previously shown in both concizumab clinical trials [18] and nonclinical studies [20,22]. A clear PD response to concizumab dosing was observed, with unbound TFPI levels in plasma being inversely correlated with concizumab

levels. The tight PK–PD relationship for concizumab and unbound TFPI is consistent with previously reported clinical data [18]. This observation was supported by a decrease in residual TFPI activity, again confirming the results of previous clinical trials [18]. In addition, a dose-dependent increase in peak TG potential was observed *in vitro*, although there was large intrapatient and interpatient sample variability, which was partly influenced by the treatment of bleeding episodes with FVIII, as shown when exclusion of values from samples taken within 72 h of FVIII dosing led to a reduction in peak TG potential levels.

The treatment duration in explorer™3 (42 days) allowed a preliminary assessment of the ability of concizumab to prevent bleeding episodes. Although the study was not designed or powered to assess efficacy, a trend towards lower bleeding rates was observed in patients in the highest dose cohort (0.8 mg kg^{-1}), seen as increased on-treatment versus pretreatment and post-treatment differences in bleeding rates as compared with the lower dose cohorts.

This trial generated valuable safety, PK, PD and bleeding episode data, although the last of these needs to be considered in light of the limited cohort sizes and observation period. These results will be used to design future trials that will provide further safety and efficacy data for concizumab in hemophilic patients.

In conclusion, no safety concerns that prevent further clinical development of concizumab for use in people with hemophilia were observed in explorer™3. Concizumab pharmacokinetics were shown to be influenced by TMDD, and a PK–PD relationship for concizumab, unbound TFPI and TG potential was confirmed. Moreover, a trend towards lower bleeding rates at higher concizumab exposure levels was observed.

Addendum

A. Hyseni and U. Friedrich designed the clinical trial, and analyzed and interpreted the data. H. Eichler, P. Angchaisuksiri, K. Kavakli, P. Knoebl, J. Windyga, V. J. Yuste, and P. Chowdary recruited patients into the trial, and analyzed and interpreted the data. All authors contributed to the writing and review of the manuscript, and approved the final version.

Acknowledgements

The authors would like to thank the patients who participated in the trial and their families. The authors would like to acknowledge the contribution of all investigators (see below), co-investigators and study nurses who were involved in the explorer™3 trial and screened patients for the trial. The authors would also like to thank J. Haaning, L. Jacobsen, M. T. Kveiborg, J. Rasmussen, K. Wahlander and R. Wirz from Novo Nordisk for their review of and input to the manuscript. Medical writing support was provided by Physicians World Europe GmbH, Mannheim, Germany, and was financially supported by Novo Nordisk A/S, Denmark. The explorer™3 study was sponsored by Novo Nordisk, A/S, Denmark. The explorer™3 investigators were: P. Angchaisuksiri, P. Chowdary, H. Eichler, S. Halimeh, V. Jiménez-Yuste, K. Kavakli, R. Kazmi, G. Kenet, R. Klamroth, P. Knoebl, J. Lasky, O. Stasyshyn, M. Trossaert, J. Windyga, and S. Zupancic-Salek.

Disclosure of Conflict of Interests

H. Eichler has acted as a consultant for Bayer and Novo Nordisk; has received research funding and/or honoraria from Baxalta, Bayer, CSL Behring, Pfizer, and Sobi; and has been on the Board of Directors/advisory committees for Bayer and Novo Nordisk. K. Kavakli has received honoraria and has been on the Speakers' Bureau for Novo Nordisk. P. Knoebl has acted as a consultant for and received honoraria from Novo Nordisk, Baxalta, CSL Behring, and Octapharma. J. Windyga has received research funding and honoraria from Baxalta, Biogen Idec, Baxter Healthcare, Bayer, CSL Behring, Novo Nordisk, Octapharma, Roche, and Sanofi. V. J. Yuste has acted as a consultant and received research funding and honoraria from Novo Nordisk. A. Hyseni and U. Friedrich are employees of Novo Nordisk A/S. P. Chowdary has acted as a consultant for and received research funding/honoraria from Bayer, Novo Nordisk, Pfizer, Roche, Shire, and Sobi, and has been on the Speakers' Bureau for Novo Nordisk, Pfizer, Roche, Shire, and Sobi.

References

1 Srivastava A, Brewer AK, Mauser-Bunschoten EP, Key NS, Kitchen S, Llinas A, Ludlam CA, Mahlangu JN, Mulder K, Poon MC, Street A, Treatment Guidelines Working Group on Behalf of The World Federation Of Haemophilia. Guidelines for the management of hemophilia. *Haemophilia* 2013; **19**: e1–47.
2 Hacker MR, Geraghty S, Manco-Johnson M. Barriers to compliance with prophylaxis therapy in haemophilia. *Haemophilia* 2001; **7**: 392–6.
3 Petrini P. Identifying and overcoming barriers to prophylaxis in the management of haemophilia. *Haemophilia* 2007; **13**(Suppl. 2): 16–22.
4 Santagostino E, Mancuso ME. Venous access in haemophilic children: choice and management. *Haemophilia* 2010; **16**(Suppl. 1): 20–4.
5 Blanchette VS, Manco-Johnson MJ. Meeting unmet needs in inhibitor patients. *Haemophilia* 2010; **16**(Suppl. 3): 46–51.
6 Holstein K, Batorova A, Carvalho M, Fijnvandraat K, Holme P, Kavakli K, Lambert T, Rocino A, Jimenez-Yuste V, Astermark J, On behalf of the, European Haemophilia Therapy Strategy Board (EHTSB). Current view and outcome of ITI therapy – a change over time? *Thromb Res* 2016; **148**: 38–44.
7 Kempton CL, Meeks SL. Toward optimal therapy for inhibitors in hemophilia. *Hematology Am Soc Hematol Educ Program* 2014; **2014**: 364–71.

8 Ljung R. Aspects of prophylactic treatment of hemophilia. *Thromb J* 2016; **14**: 30.

9 Santagostino E, Morfini M, Auerswald GK, Benson GM, Salek SZ, Lambert T, Salaj P, Jimenez-Yuste V, Ljung RC. Paediatric haemophilia with inhibitors: existing management options, treatment gaps and unmet needs. *Haemophilia* 2009; **15**: 983–9.

10 Berntorp E, Dolan G, Hay C, Linari S, Santagostino E, Tosetto A, Castaman G, Alvarez-Roman MT, Parra Lopez R, Oldenburg J, Albert T, Scholz U, Holmstrom M, Schved JF, Trossaert M, Hermans C, Boban A, Ludlam C, Lethagen S. European retrospective study of real-life haemophilia treatment. *Haemophilia* 2017; **23**: 105–14.

11 Broze GJ Jr, Girard TJ. Tissue factor pathway inhibitor: structure-function. *Front Biosci (Landmark Ed)* 2012; **17**: 262–80.

12 Mast AE. Tissue factor pathway inhibitor: multiple anticoagulant activities for a single protein. *Arterioscler Thromb Vasc Biol* 2016; **36**: 9–14.

13 Hedner U, Ezban M. Tissue factor and factor VIIa as therapeutic targets in disorders of hemostasis. *Annu Rev Med* 2008; **59**: 29–41.

14 Nordfang O, Valentin S, Beck TC, Hedner U. Inhibition of extrinsic pathway inhibitor shortens the coagulation time of normal plasma and of hemophilia plasma. *Thromb Haemost* 1991; **66**: 464–7.

15 Petersen LC. Hemostatic properties of a TFPI antibody. *Thromb Res* 2012; **129**(Suppl. 2): S44–5.

16 Hilden I, Lauritzen B, Sorensen BB, Clausen JT, Jespersgaard C, Krogh BO, Bowler AN, Breinholt J, Gruhler A, Svensson LA, Petersen HH, Petersen LC, Balling KW, Hansen L, Hermit MB, Egebjerg T, Friederichsen B, Ezban M, Bjorn SE. Hemostatic effect of a monoclonal antibody mAb 2021 blocking the interaction between FXa and TFPI in a rabbit hemophilia model. *Blood* 2012; **119**: 5871–8.

17 Nielsen AM, Witt ML, Zhuang G, Friderichsen P, Norgaard LW. Switching from syringe-to-vial to a prefilled subcutaneous pen injector in haemophilia – as easy as ABC? *Haemophilia* 2017; **23**: 37–8 (abstract P015).

18 Chowdary P, Lethagen S, Friedrich U, Brand B, Hay C, Abdul Karim F, Klamroth R, Knoebl P, Laffan M, Mahlangu J, Miesbach W, Dalsgaard Nielsen J, Martin-Salces M, Angchaisuksiri P. Safety and pharmacokinetics of anti-TFPI antibody (concizumab) in healthy volunteers and patients with hemophilia: a randomized first human dose trial. *J Thromb Haemost* 2015; **13**: 743–54.

19 Wang W, Wang EQ, Balthasar JP. Monoclonal antibody pharmacokinetics and pharmacodynamics. *Clin Pharmacol Ther* 2008; **84**: 548–58.

20 Agerso H, Overgaard RV, Petersen MB, Hansen L, Hermit MB, Sorensen MH, Petersen LC, Hilden I. Pharmacokinetics of an anti-TFPI monoclonal antibody (concizumab) blocking the TFPI interaction with the active site of FXa in Cynomolgus monkeys after iv and sc administration. *Eur J Pharm Sci* 2014; **56**: 65–9.

21 Cardinal MK, Zhu T, Sun P, Pittman DD, Murphy JE, Arkin S. A first-in-human (FIH) study of the safety, tolerability, pharmacokinetics (PK) and pharmacodynamics (PD) of PF-06741086, an anti-TFPI monoclocal antibody, following administration of single subcutaneous or intravenous doses in healthy adult male volunteers. *Res Pract Thromb Haemost* 2017; **1**: 415 (PB 890).

22 Hansen L, Petersen LC, Lauritzen B, Clausen JT, Grell SN, Agerso H, Sorensen BB, Hilden I, Almholt K. Target-mediated clearance and bio-distribution of a monoclonal antibody against the Kunitz-type protease inhibitor 2 domain of tissue factor pathway inhibitor. *Thromb Res* 2014; **133**: 464–71.