Plenary Paper

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CLINICAL TRIALS AND OBSERVATIONS

Recombinant ADAMTS-13: first-in-human pharmacokinetics and safety in congenital thrombotic thrombocytopenic purpura

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Key Points

- First-in-human, phase 1 study, recombinant ADAMTS-13 was safe, nonimmunogenic, and tolerated in congenital thrombotic thrombocytopenic purpura.
- Recombinant ADAMTS-13 pharmacokinetic profile was comparable to plasma infusion studies, with evidence of pharmacodynamic activity.

Safety, tolerability, and pharmacokinetics of recombinant ADAMTS-13 (a disintegrin and metalloproteinase with a thrombospondin type 1 motif, member 13; BAX 930; SHP655) were investigated in 15 patients diagnosed with severe congenital ADAMTS-13 deficiency (plasma ADAMTS-13 activity <6%) in a prospective phase 1, first-in-human, multicenter dose escalation study. BAX 930 was well tolerated, no serious adverse events occurred, and no anti-ADAMTS-13 antibodies were observed. After single-dose administration of BAX 930 at 5, 20, or 40 U/kg body weight to adolescents and adults, there was approximate dose proportionality with respect to maximum plasma concentration (C_{max} [U/mL]) and area under the concentration–time curve (AUC [h•U/mL]). Dose-related increases of individual ADAMTS-13:Ag and activity were observed and reached a maximum within 1 hour. With escalating BAX 930 doses administered, a dose-dependent persistence of ADAMTS-13-mediated von Willebrand factor (VWF) cleavage products and reduced VWF multimeric size were observed. This study demonstrated that pharmacokinetic parameters of BAX 930 were comparable to those estimated in previous plasma infusion studies and provided evidence of pharmacodynamic activity. This study was registered at www.clinicaltrials.gov as #NCT02216084. (*Blood*. 2017;130(19):2055-2063)

Introduction

The plasma metalloprotease ADAMTS-13 (a disintegrin and metalloproteinase with a thrombospondin type 1 motif, member 13) is a constitutively active enzyme that cleaves von Willebrand factor (VWF) at the Tyr1605–Met1606 bond in the A2 domain, an otherwise cryptic site rendered susceptible, by the application of shear stress, to regulate the size of VWF multimers.[1] VWF is a multimeric glycoprotein synthesized principally by vascular endothelial cells.[2] The inability to cleave ultralarge (UL) VWF multimers into smaller forms as a result of congenital or acquired ADAMTS-13 deficiency results in thrombotic thrombocytopenic purpura (TTP), a rare potentially fatal disorder of the microcirculation caused by increased binding of platelets to UL VWF.[3] The congenital form of TTP (cTTP, previously termed hereditary TTP or Upshaw-Schulman syndrome) is an ultrarare, although likely underestimated, condition with a prevalence of approximately 1 case per million.[4,5] It exhibits an autosomal recessive mode of inheritance caused by homozygous or compound heterozygous mutations in both ADAMTS-13 alleles on chromosome 9.[6] The nature of the mutations is diverse and includes single amino acid missense substitution (approximately 60%), as well as nonsense, frame-shift, splice site mutations and deletions and insertions (collectively, approximately 40%).[1,7-9]

The principal pathophysiology arises from platelet aggregates in the microcirculation affecting critical organs, including the brain, heart, and kidneys. TTP crises are associated with cerebral vascular incidents in at least 30% of patients, with a risk for neurologic sequelae in approximately 20% of patients.[10] Acute renal failure has been reported in 11% of patients with severe cTTP,[11] and chronic, possibly progressive, renal involvement is often seen. Sudden cardiac death resulting from myocardial infarction, heart failure, and arrhythmia has also been reported.[12]

Although considered a monogenetic disorder, the clinical presentation of cTTP is variable. Symptoms develop soon after birth in some patients, whereas others remain asymptomatic until the second or third decade of life. This phenotypic variability is thought

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to be related to the causative mutations and the level of plasma ADAMTS-13 activity.[13,14] In the newborn, cTTP typically presents as neonatal jaundice and thrombocytopenia,[3] whereas in early childhood, symptomatic episodes are often associated with intercurrent infections or vaccinations. Among patients with cTTP presenting with a first TTP episode later in life, pregnancy is often the inciting event.[15] Intrauterine fetal death is common in patients with cTTP who do not receive regular plasma therapy throughout pregnancy.[16,17] Other precipitants associated with increased VWF levels, such as infection, surgery, and alcohol binge drinking, provide additional triggers for acute TTP events.[18-22] Despite the wide range of ages of the first TTP event, most patients with cTTP subsequently demonstrate a chronic, relapsing course and require prophylactic treatment to prevent long-term neurological, renal, and other sequelae.

There are no drugs currently approved for the specific treatment of cTTP. Acute TTP episodes are generally treated with infusions of fresh frozen plasma (FFP) or solvent/detergent-treated plasma, typically 10 to 20 mL/kg body weight (BW). Some intermediate-purity FVIII:VWF concentrates have been shown to contain low levels of ADAMTS-13 and have been used as an alternative treatment in select patient populations.[23] Although the half-life of infused plasma ADAMTS-13 was found to be 2 to 4 days,[24] prophylaxis with 10 to 15 mL plasma /kg BW every 2 to 3 weeks has been shown to be effective in preventing acute episodes in the majority of patients with TTP.[6,25,26]

Although the treatment of TTP via plasma infusions is generally effective, the therapy is frequently complicated by allergic and anaphylactic reactions or volume overload.[27] Plasma infusions also carry risks for infection as a result of bloodborne pathogens. In addition, plasma infusions carried out in the hospital or outpatient settings are burdensome and time-consuming, and can be stressful for younger patients. As such, the development of a recombinant ADAMTS-13 (BAX 930; SHP 655; rADAMTS-13) represents a potential new therapeutic option to improve the current standard of care.

In this phase 1, first-in-human study, we investigated the safety, including immunogenicity, tolerability, and pharmacokinetics (PKs), of rADAMTS-13 in patients diagnosed with severe congenital ADAMTS-13 deficiency to establish dosing for future studies.

Methods

Design

This was a prospective, open-label, multicenter, first-in-human dose escalation study on safety, immunogenicity, tolerability and PKs of BAX 930 in patients diagnosed with cTTP assigned to 1 of 3 dose cohorts, receiving a single dose of BAX 930 at 5, 20, or 40 U/kg BW, where 1 unit is approximately equivalent to the ADAMTS-13 activity in 1 mL plasma. The study comprised 2 dose-escalation steps. Fifteen patients were enrolled (of 16 screened; 1 was unable to participate because of a scheduling conflict) and dosed sequentially. Participants were recruited to the next higher dose level only after short-term safety had been demonstrated and reviewed by an independent Data Monitoring Committee at the preceding dose level. The study was approved by the responsible ethics committees or institutional review boards and regulatory authorities. The study was registered at www.clinicaltrials.gov as #NCT02216084. All participants gave written informed consent. Data were analyzed by biostatisticians at Shire and Quintiles; all authors had access to primary clinical trial data.

Patients

The principal inclusion criteria consisted of a documented diagnosis of severe congenital ADAMTS-13 deficiency (baseline plasma ADAMTS-13 activity $< 6\%$) confirmed by genetic testing, ages 12 to 65 years, and the absence of severe TTP symptoms at the time of screening. This included stable laboratory parameters (platelet count, $>100 \times 10^9$/L; lactate dehydrogenase [LDH] <3 times the upper limit of normal). Evidence of end organ damage such as stroke, heart, renal disease, or abnormal liver function tests were further exclusions to study enrollment. Patients with a medical history or presence of functional neutralizing ADAMTS-13 inhibitor at screening, a medical history of immunological or autoimmune disorders, or significant neurological events were excluded. A sample size of 14 was selected on the basis of feasibility.

Study drug and treatments

BAX 930 is a fully glycosylated recombinant human ADAMTS-13 protein produced in a Chinese hamster ovary (CHO) mammalian expression system in a plasma protein-free milieu. The cell culture and purification processes used in the manufacture of BAX 930 do not employ additives of human or animal origin. The purification process of BAX 930 is based on chromatography using conventionally available resins, including anion exchange, hydroxyapatite, mixed mode (hydrophobic interaction), and cation exchange. In addition, BAX 930 undergoes 2 dedicated virus reduction steps: solvent/detergent treatment and nanofiltration. The final product is lyophilized for intravenous injection. The sterile, lyophilized BAX 930 was stored in 10-mL vials, which were reconstituted with 5 mL sterile water, giving the solution a nominal strength of 300 U/mL that is stable for 3 hours at room temperature after reconstitution. BAX 930 was infused at a rate of 2 to 4 mL/minute. In patients receiving regular prophylaxis, there was at least a 10-day window between their last prophylactic treatment and their BAX 930 dose.

Safety assessments

Patients were hospitalized for at least 96 hours after investigational product infusion for safety observation and PK assessments. Follow-up visits were scheduled 12 days and 30 days (or 6, 9, and 12 days for the 2 participating adolescents) postinfusion. Safety was evaluated through clinical assessment, hematology, serum chemistry, urinalysis, coagulation tests, antibodies against ADAMTS-13 or CHO cell protein, viral serology, cardiac troponin, D-dimers, and seromarkers for brain or renal impairment. Adverse events (AEs) were recorded throughout the study, but specifically by the patient in a diary, beginning 96 hours postinfusion until the study completion visit.

ADAMTS-13 and VWF assays

ADAMTS-13 activity was calculated by analysis of fluorescence over time, using the synthetic fluorogenic VWF73 peptide substrate (FRETS-VWF73) and a commercially available, chromogenic GST-VWF73-based ADAMTS-13-activity ELISA (Technozym® ADAMTS-13 Activity ELISA).[28,29] ADAMTS-13 antigen was measured using a validated sandwich ELISA employing anti-human-ADAMTS-13 antibodies.[30-32] VWF:RCo and VWF:Ag were determined as previously described.[33,34]

Analysis of VWF multimers was performed using sodium dodecyl sulfate-agarose gel electrophoresis followed by western blotting and sensitive luminescence detection.[4,35] VWF degradation products generated by ADAMTS-13-mediated cleavage were assessed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis under reducing conditions followed by western blotting and immunostaining with a horseradish peroxidase-labeled polyclonal rabbit antihuman VWF antibody with enhanced chemiluminescence detection.[4,35,36]

Immunogenicity assessments

Total binding antibodies to ADAMTS-13 were measured by ELISA-based assays, detecting total immunoglobin (IgG, IgA, IgM). Neutralizing antibodies to ADAMTS-13 were measured using a Bethesda-like approach with the ADAMTS-13 activity assay.[37]

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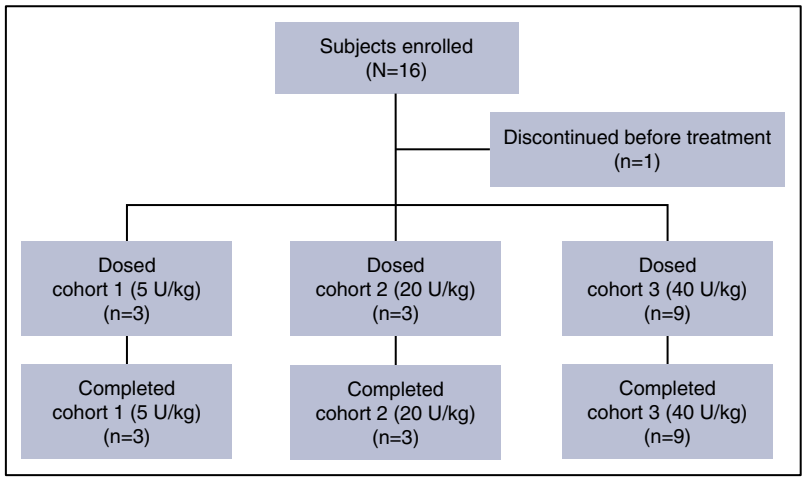
Figure 1. Study population disposition.



Pharmacokinetic assessments

PK parameters, including area under the concentration-time curve (AUC [h•U/mL]), plasma half-life ($t_{1/2}$ [hours]), clearance (Cl [mL/kg/hours]), mean residence time (MRT [hours]), volume of distribution at steady state (V_{ss} [mL/kg]), maximum plasma concentration (C_{max} [U/mL]), and incremental recovery (IR [U/mL]) were assessed for ADAMTS-13 activity and ADAMTS-13 antigen (ADAMTS-13:Ag). Concentrations were measured at standardized time points: within 60 minutes preinfusion and 15, 30, 60 minutes and 3, 6, 9, 12, 24, 48, 96, 144, 168, 192, 216, 240, 264, and 288 hours postinfusion in adults. The 2 adolescent subjects (16 and 17 years of age) had sparse sampling preinfusion and at 6, 48, 144, 216, and 288 hours postinfusion. The hematology and clinical chemistry assessments, conducted as part of the safety assessments (see earlier), were performed on EDTA anticoagulated whole blood and serum, respectively, at the screening visit, just before investigational product infusion, and at regular increments up to 288 hours postinfusion. The hematology panel included platelet counts, and the clinical chemistry panel included serum LDH, both of which were used for assessing the pharmacodynamic effects of BAX 930.

Statistical analysis

ADAMTS-13 concentration data were analyzed using a noncompartmental approach, and individual PK parameter estimates were derived. Total $AUC_{0-\infty}$ was calculated by the linear trapezoidal rule up to the last quantifiable concentration with a tail area correction. Terminal plasma half-life was determined from the terminal rate constant obtained by log-linear fitting of a regression line by the least squares deviation method to the last 5 quantifiable concentrations that were above preinfusion levels. MRT was calculated as total area under the moment curve divided by the total area under the curve corrected for the duration of the infusion. Systemic clearance (Cl) was calculated as dose per body mass (kg) divided by $AUC_{0-\infty}$. IR was calculated as C_{max} divided by dose per body mass.

To better address the variability in baseline ADAMTS-13 levels, a population PK model was also developed. A 2-compartment model with first-order elimination and constant rate infusion was selected iteratively among different structural and stochastic candidates and provided population PK parameter estimates. Individual PK parameters from a noncompartmental approach and from the population PK model were similar.

Descriptive statistics were used to assess safety in terms of product-related AEs.

Results

Fifteen patients received study drug: 3 each in the 5 and 20 U/kg dose cohorts, and 9 in the 40 U/kg dose cohort (Figure 1; Table 1); 2 patients in the 40 U/kg cohort were adolescents. All patients completed the study. Doses administered were within 0.3 U/kg of the planned doses, with the exception of 1 patient who received 47.5 U/kg instead of the planned 40 U/kg.

Table 1. Subject demographic data

Subject ID	Age (years)	Sex	Race	Age at diagnosis	Previous treatment type	Dosing cohort, U/kg	Platelet levels at screening (10^9/L)	LDH levels at screening (U/L)
1	22	F	White	15	Prophylaxis	5	106*	294*
2	28	M	White	24	On Demand	5	166	136
3	35	M	White	29	Prophylaxis	20	154	170
4	21	F	Asian	<1	Prophylaxis	40	167	151
5	24	F	Asian	17	Prophylaxis	20	220	142
6	25	M	White	2	Prophylaxis	40	190†	159
7	33	F	White	20	Prophylaxis	40	223	165
8	32	F	White	10	On Demand	40	221	146
9	40	M	White	16	Prophylaxis	40	216	125
10	41	F	White	19	On Demand	5	348	130
11	39	F	White	36	Prophylaxis	20	252	259*
12	16	M	White	14	Prophylaxis	40	214	153
13	17	F	White	<1	Prophylaxis	40	353	140
14	33	M	White	26	Prophylaxis	40	273	122†
15	30	M	Black	14	Prophylaxis	40	297	185

Table has been anonymized by permutation of the following columns: age and age at diagnosis.
*Abnormal.
†Baseline values provided; screening values not available.

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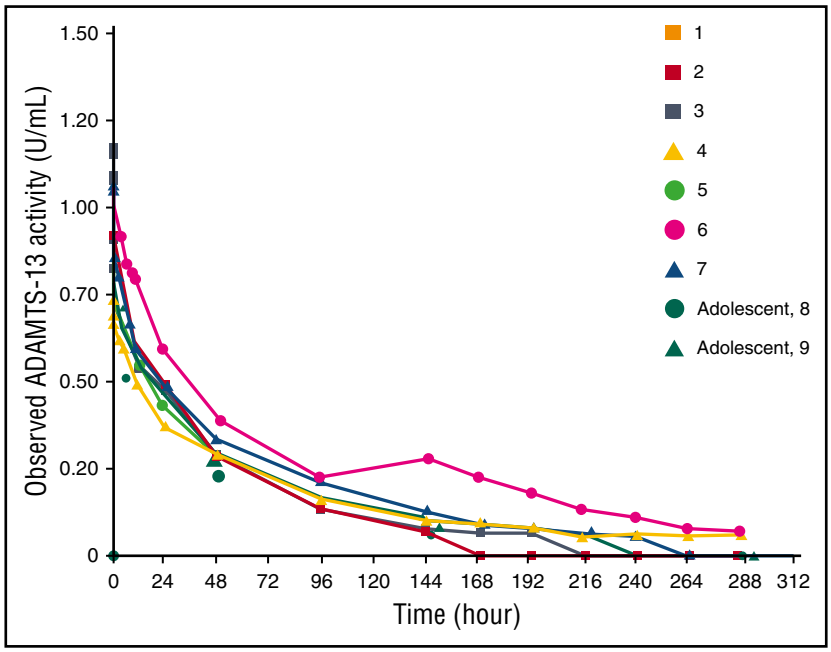

Figure 2. Observed ADAMTS-13 activity over time. ADAMTS-13 activity in plasma was measured at baseline and at times up to 288 hours, using the FRETS-VWF73 assay after a 40 U/kg administration of BAX 930.

ADAMTS-13 activity

After the single infusion of BAX 930, dose-related increases in ADAMTS-13 FRETS-VWF73 concentrations were observed (data from the 40 U/kg cohort shown in Figure 2). ADAMTS-13 activity remained quantifiable for 24 hours in the 5 U/kg cohort, for 240 hours in the 20 U/kg cohort, and for the full 288-hour study period in the 40 U/kg cohort. ADAMTS-13 activities measured by FRETS-VWF73 and chromogenic assay methods were highly comparable (Table 2).

In the 40 U/kg cohort, the geometric mean of IR was 0.0232 (U/mL•kg/U), mean terminal half-life was 59.2 hours, and initial half-life was 17.0 hours for ADAMTS-13 activity (FRETS-VWF73). ADAMTS-13 activity, C_{max}, and total exposures increased approximately in proportion to the dose escalations. The geometric mean C_{max} was 0.398 U/mL after 20 U/kg infusion and 0.948 U/mL after 40 U/kg infusion; the geometric mean $AUC_{(0\text{-}inf)}$ was 19.1 U•h/mL after 20 U/kg infusion and 53.1 U•h/mL after 40 U/kg infusion.

With the more sensitive chromogenic ELISA assay (lower limit of quantitation [LLOQ]: 0.0073 U/mL compared with 0.05 U/mL for FRETS-VWF73), low levels of ADAMTS-13 activity were measurable at the preinfusion time in all patients except 1 adult and both adolescents.

Despite the differences in the LLOQ, activity measurements by FRETS-VWF73 and chromogenic assay methods were otherwise similar (Table 2).

ADAMTS-13 antigen

ADAMTS-13 antigen (ADAMTS-13:Ag) concentrations at predose (baseline) were low, ranging from 0.003 to 0.031 U/mL, but quantifiable in all but 2 patients (1 patient each in the 5 and 40 U/kg dose cohorts). Individual postdose ADAMTS-13:Ag levels remained quantifiable in all samples up to the last collection at 288 hours postdose and were higher than baseline (predose) values.

The geometric mean of ADAMTS-13:Ag Cl was 61.5 mL/h, and of V_{ss} was 5300 mL (at 40 U/kg dose level), suggesting the protein distributed primarily within the intravascular compartment.[38] There was approximate dose proportionality with respect to C_{max} (geometric means of 0.323 μg/mL after 20 U/kg infusion and 0.672 μg/mL after 40 U/kg infusion) and $AUC_{(0\text{-}inf)}$ (geometric means of 18.3 μg•h/mL after 20 U/kg infusion and 36.0 μg•h/mL after 40 U/kg infusion).

The PK parameter estimates for ADAMTS-13:Ag were comparable to ADAMTS-13 activity. Above the 5 to 40 U/kg dose range, ADAMTS-13:Ag geometric mean C_{max} increased 5-fold between 5 and 20 U/kg, and approximately proportional to the dose between 20 and 40 U/kg (2.1-fold) (Table 2). The fold increases in geometric mean total exposures ($AUC_{(0\text{-}t)}$ and $AUC_{(0\text{-}inf)}$) were approximately proportional to the dose increase for both dose level comparisons.

ADAMTS-13 activity and ADAMTS-13 antigen in adolescents

The available concentration data for both ADAMTS-13 activity and ADAMTS-13:Ag, did not suggest any major differences between adolescents and adults. Despite the low number of adolescents in the study (n = 2) and reduced sampling per protocol, ADAMTS-13 activity, IR, $t_{1/2}$, and AUC were generally within the data ranges for adults treated at the same dose level (40 U/kg). The estimated clearances for the 2 adolescent subjects were 64.9 and 54.5 mL/h, respectively, which were within the range observed for adult subjects (n = 7; range, 44.4-115 mL/h).

Pharmacodynamic effects

In addition to the small, intermediate, and large multimeric sizes typically present in normal plasma, UL VWF multimers were observed in the samples collected before dosing, at screening or predose, in all patients, and at most points postdose. The trend for decreasing large multimers, including UL multimers, and increasing levels of the intermediate fraction was observed during the first 12 to 24 hours postinfusion in individual profiles at BAX 930 doses of 20 or 40 U/kg before slowly returning to preinfusion levels during a 288-hour period (Figure 3B).

Likewise, an apparent dose-dependent effect was seen with the detection of ADAMTS-13-mediated VWF cleavage products. In the 5 U/kg dose cohort, ADAMTS-13-mediated VWF cleavage products were present up to 3 hours postdose in all patients (100%) and up to 6 hours postdose in 1 patient (33.3%), and no longer detectable

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Table 2. Summary of key plasma ADAMTS-13 FRETS-VWF73 PK parameters in adults

	Median (range)		
Pharmacokinetic parameters	**5 U/kg (n = 3)**	**20 U/kg (n = 3)**	**40 U/kg (n = 7)**
FRETS-VWF73			
IR, U/mL*kg/U	0.0154 (0.0125-0.0187)	0.0181 (0.0153-0.0290)	0.0228 (0.0185-0.0290)
C_{max}, U/mL	0.075 (0.065-0.100)	0.361 (0.300-0.583)	0.941 (0.737-1.158)
t_{max}, h*	1.00 (0.52-1.00)	0.33 (0.25-0.53)	0.37 (0.22-0.58)
$AUC_{(0\text{-}inf)}$, U*h/mL	ND†	18.1 (15.4-24.9)	52.3 (41.3-85.4)
$AUC_{(0\text{-}t)}$, U*h/mL	0.388 (0.0528-1.68)	14.9 (11.5-21.1)	46.5 (37.2-78.8)
$t_{1/2}$, h	ND†	36.5 (29.5-69.2)	66.9 (44.1-77.8)
$MRT_{(0\text{-}inf)}$, h	ND†	48.876 (43.55-93.87)	92.468 (55.71-124.01)
Cl, mL/h	ND†	64.8 (53.3-100)	59.3 (44.4-115)
V_{ss}, mL	ND†	4900 (2820-5000)	5250 (4100-6830)
Technozym			
IR, U/mL*kg/U	0.0170 (0.0160-0.0179)	0.0157 (0.0136-0.0254)	0.0212 (0.0144-0.0261)
C_{max}, U/mL	0.087 (0.083-0.091)	0.308 (0.272-0.512)	0.844 (0.574-1.039)
t_{max}, h*	0.25 (0.25-0.52)	0.55 (0.28-0.97)	0.30 (0.25-0.58)
$AUC_{(0\text{-}inf)}$, U*h/mL	6.68 (4.32-17.4)	21.8 (18.3-25.8)	45.6 (40.6-89.1)
$AUC_{(0\text{-}t)}$, U*h/mL	6.39 (4.26-8.43)	20.6 (15.9-25.4)	43.8 (37.3-80.4)
$t_{1/2}$, h	66.2 (44.2-238)	64.4 (46.9-69.8)	62.6 (46.5-77.0)
$MRT_{(0\text{-}inf)}$, h	90.401 (69.41-383.36)	90.663 (65.38-114.38)	78.382 (63.54-124.58)
Cl, mL/h	51.8 (13.3-88.9)	54.7 (44.1-96.8)	68.4 (45.7-111)
V_{ss}, mL	5090 (4680-6170)	6260 (4000-6330)	6010 (3130-8700)
ADAMTS13 antigen			
IR, μg/mL*kg/μg	0.0176 (0.0171-0.0194)	0.0220 (0.0180-0.0354)	0.0247 (0.0205-0.0328)
C_{max}, μg/mL	0.066 (0.062-0.066)	0.296 (0.238-0.480)	0.655 (0.549-0.879)
t_{max}, h*	0.25 (0.25-0.52)	0.55 (0.53-0.97)	0.30 (0.22-1.12)
$AUC_{(0\text{-}inf)}$, μg*h/mL	4.14 (4.14-5.90)	18.1 (16.8-20.2)	33.4 (29.4-67.8)
$AUC_{(0\text{-}t)}$, μg*h/mL	4.05 (3.59-4.49)	16.4 (15.6-19.9)	30.4 (28.3-61.0)
$t_{1/2}$, h	93.9 (56.2-122)	48.0 (47.0-82.3)	70.4 (50.9-86.3)
$MRT_{(0\text{-}inf)}$, h	131.365 (75.45-192.16)	67.210 (62.37-128.74)	87.134 (68.83-125.51)
Cl, mL/h	58.3 (27.3-64.8)	40.1 (35.8-83.1)	61.8 (41.9-116)
V_{ss}, mL	5240 (4400-8510)	4610 (2690-5180)	4760 (3650-8150)

$AUC_{(0\text{-}inf)}$, area under the plasma-time concentration curve from zero to infinity; $AUC_{(0\text{-}t)}$, area under the plasma-time concentration curve from zero to the last measured point; max, maximum; min, minimum; ND, not determined; t_{max}, minimum time to reach C_{max}.

*Median (min − max).

†n = 1 for $AUC_{(0\text{-}inf)}$, $t_{1/2}$, $MRT_{(0\text{-}inf)}$, Cl, and V_{ss}.

thereafter (Figure 3A). ADAMTS-13-mediated VWF cleavage products were observed during a longer period of time at higher dose levels: for 20 U/kg, up to 24 hours postdose in all patients and up to 48 hours in 2 patients (66.7%); for 40 U/kg, up to 48 hours postdose in all patients and as long as 264 hours in 1 patient (14.3%) (Figure 3C).

In addition, the function of VWF to bind platelet GPIb, as measured by the VWF:RCo assay, was lower than mean baseline levels at most points across the 3 dose levels and decreased by approximately 30% within the first 9 hours. A modest decrease in LDH of 5% to 10% relative to baseline was also observed in the first 48 hours postinfusion. In addition, although subjects in this study were not experiencing acute manifestations of cTTP, there was an increasing trend in the platelet count up to 6 days after infusion in all dosing cohorts (Figure 4).

Safety

BAX 930 was well tolerated in all 15 patients, and no patient exhibited anaphylaxis or other allergic manifestations. No serious AEs, AEs leading to discontinuation from the study, or breakthrough TTP events were seen in the study after treatment. Overall, 12 of 15 patients (80.0%) reported at least 1 temporally emergent AE (TEAE; supplemental Table 1, available on the *Blood* Web site). Three subjects, all in the 40 U/kg BAX 930 cohort (40 U/kg: 3/9 subjects, 33.3%; overall: 3/15 subjects, 20.0%) reported a total of 5 TEAEs considered by the investigators to be temporally associated with BAX 930 infusion: decrease in VWF antigen and VWF activity at 30 minutes postinfusion, returning to normal activity levels at 1 hour postinfusion (n = 1), flatulence (n = 1), or nausea on 2 separate days (n = 1). No trends were observed over time or among the 3 dose cohorts in the laboratory parameters, vital sign assessments, electrocardiogram measurements, or physical examination.

Immunogenicity evaluation

All anti-ADAMTS-13 neutralizing antibody results were less than 0.6 BU/mL across the 3 dose cohorts and at all 3 scheduled times; that is, screening, infusion predose, and study completion, day 28 ± 3 days. None of the 15 patients who received BAX 930 exhibited anti-ADAMTS-13 binding antibodies at any time in the study. Similarly, all patients tested negative for anti-CHO protein antibodies at all times.

Discussion

Recombinant ADAMTS-13 potentially offers a novel option for the treatment of cTTP, eliminating many of the risks associated with products derived from human plasma and facilitating the introduction of more precise and individualized dosing regimens.[24,39-43] In our prospective, dose-escalating clinical trial, we investigated the safety, tolerability, and PK of BAX 930 infused for the first time in humans.

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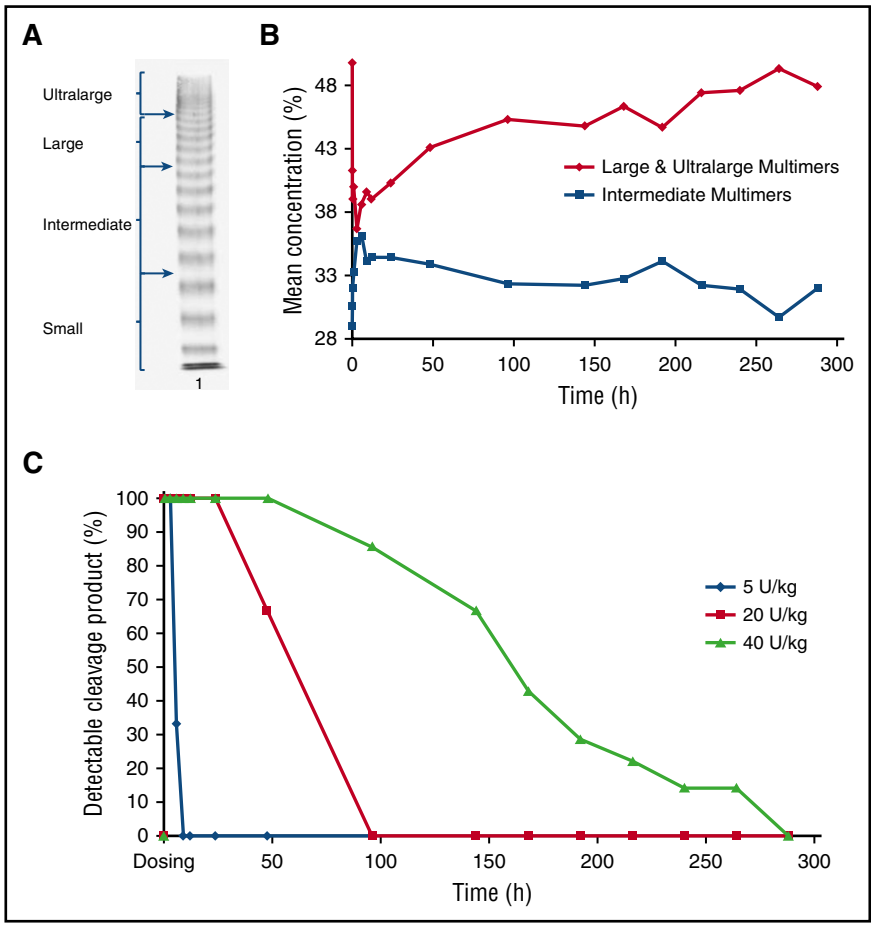

Figure 3. VWF structural analyses pre- and post-infusion of BAX 930. (A) Representative sodium dodecyl sulfate-agarose gel pattern of pretreatment TTP plasma depicting UL, large, intermediate, and small VWF multimers. (B) The proportions of intermediate and large/UL multimers of plasma VWF were estimated from sodium dodecyl sulfate-agarose gels at various times before and after infusion of 40 U/kg BAX 930. The observed concentration of large and UL multimers tended to decrease in all treated patients in the first 12 hours before gradually returning to preinfusion levels. (C) Time course of the 176-kDa ADAMTS-13 VWF cleavage product after administration of 5, 20, and 40 U/kg BAX 930. High levels of detectable VWF cleavage products are apparent just after dosing, which gradually return to preinfusion levels in a dose-related manner.

BAX 930 was expected to behave in the same way as endogenous ADAMTS-13, as demonstrated in several nonclinical in vitro and in vivo studies. In current clinical practice, infusions of 10 to 20 mL/kg FFP are recommended for treating acute episodes or prophylaxis of cTTP.[44-46] As such, a low dose of 5 U/kg, an intermediate dose of 20 U/kg, and a high dose of 40 U/kg BAX 930 were chosen for this study. The 40 U/kg dose has been calculated to correspond to doses of ADAMTS-13 typically administered during single-volume plasma exchange sessions.[47]

As would be expected in this population of patients with cTTP, predose baseline levels of ADAMTS-13:Ag and ADAMTS-13 activity (assessed by both FRETS-VWF73 and chromogenic ELISA assays) were very low or below the LLOQ. Immediately after infusion of all doses, of BAX 930 there was an increase in individual ADAMTS-13:Ag and activity observed, reaching a maximum within 1 hour postinfusion. Similar ADAMTS-13:Ag, ADAMTS-13 FRETS-VWF73, and ADAMTS-13 activity ELISA profiles suggest good correlation between protein concentrations and activity. No apparent differences were observed for ADAMTS-13 activity and ADAMTS-13:Ag between adolescents (n = 2) and adults (n = 7), treated with the same BAX 930 doses.

Overall, there was evidence suggesting dose proportionality with respect to $AUC_{(0\text{-inf})}$ and C_{max} (Table 2; Figure 5). The change in the geometric mean C_{max} for 5 to 20 U/kg was approximately 5-fold, and was approximately 2.4-fold for 20 to 40 U/kg. Greater increases were observed for geometric mean $AUC_{(0\text{-inf})}$ (47-fold for 5 to 20 U/kg and 3.1-fold for 20 to 40 U/kg), which could be attributable to concentrations falling below the LLOQ at lower doses. Available data for $AUC_{(0\text{-inf})}$ showed an approximately 2.8-fold increase in exposure for dose increases from 20 to 40 U/kg. Available data at the 20 and 40 U/kg dose levels show no major changes in Cl, V_{ss}, $t_{1/2}$, and $MRT_{(0\text{-inf})}$ with increasing dose.

PK parameters derived from the noncompartmental and population PK approaches were consistent. In the population PK model, a shorter initial half-life was observed followed by a longer terminal half-life. Whether the initial phase is a result of VWF binding, consumption of ADAMTS-13 activity, or distribution to another physiologic compartment remains to be determined.

In addition to the PK results, evidence of pharmacodynamic activity was observed. With escalating BAX 930 doses, prolonged detectable ADAMTS-13-mediated VWF cleavage products were present, in line with dose-related increases of ADAMTS-13:Ag and activity. Specifically, detectable ADAMTS-13-mediated VWF cleavage products were present in all patients (100%) and up to 48 hours after the highest dose, 40 U/kg. Patients with cTTP typically exhibit UL molecular weight forms of VWF at baseline consistent with the severe reduction in ADAMTS-13 activity. A trend for decreasing large multimers, a fraction of which also included UL multimers, and increasing levels of the intermediate fraction was observed during the first 24 hours postinfusion in individual profiles at BAX 930 doses of 20 or 40 U/kg. In addition, during the first 9 hours, the mean postdose VWF:RCo levels decreased by approximately 30%. There was also an increase in the platelet count in all dosing groups. Taken together, these findings provide evidence of in vivo ADAMTS-13 activity after BAX 930 administration.

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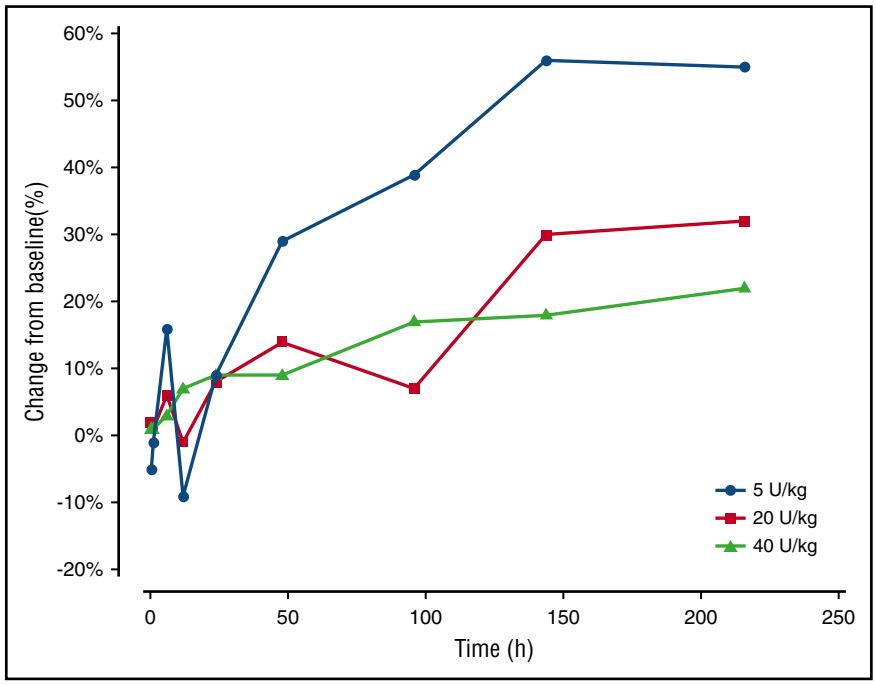
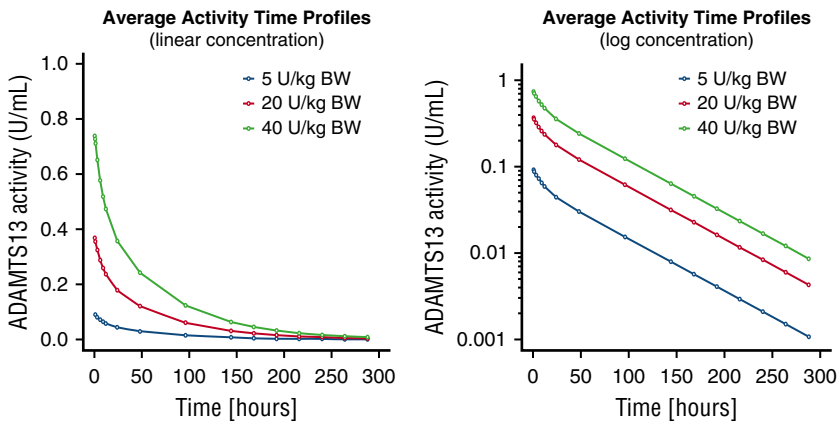


Figure 4. Changes in platelet counts after BAX 930 administration. Changes in mean platelet counts over time are depicted after administration of 5, 20, and 40 U/kg of BAX 930. An increasing trend in platelet levels was observed in all dosing cohorts.

This study demonstrated that the protein antigen and activity PK parameters of BAX 930 were comparable to those estimated from previous studies of ADAMTS-13 administered to patients with cTTP as a constituent of FFP. Furlan et al[24] reported limited PK data in patients treated with FFP and demonstrated that the recovery was nearly 100% and the half-life was 2.1 to 3.3 days. Similar results were reported by Fujimura et al.[43] The comparability of plasma-derived and recombinant ADAMTS-13 PKs suggests dosing regimens in future studies of the long-term safety and efficacy of BAX930 can be modeled on the clinical experiences with plasma-based regimens. No patients developed an inhibitor to ADAMTS-13 during this single-dose study. The lack of an apparent immune response will need to be confirmed in the upcoming pivotal study in which patients will be repeatedly exposed to BAX 930 for approximately 1 year.

In conclusion, BAX 930 appeared to be safe and well-tolerated over a dose range of 5 to 40 U/kg in patients with cTTP, and there was no evidence of an immune response to BAX 930 after a single infusion. The activity PK parameters were comparable to those estimated from FFP studies and demonstrated approximate dose proportionality with respect to C_{max} and AUC. Finally, there was evidence for BAX 930



Parameter, unit	Two compartment	NCA model
C_{max}, U/mL	0.867	0.957
IR, (U/mL)/(U/kg)	0.021	0.0234
Terminal half-life, h	57.087	60.5
MRT, h	77.593	87.656

Figure 5. Predicted PK estimates from two-compartment model. Predicted FRETS-VWF73 activity time profiles are depicted based on a 2-compartmental model according to linear and log concentrations. Both display approximate dose proportionality. The use of the 2-compartment (n = 9) and the noncompartmental (NCA; n = 7) models yield comparable results.

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activity in vivo, including effects on VWF multimers, platelet count, and serum LDH. These data provide the basis for proceeding to a phase 3 pivotal study in cTTP, using previously anticipated prophylactic and treatment dosing regimens.

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Authorship

Contribution: B.E. designed the study; C.M., J.D., C.H., L.M., and B.E. interpreted data; M.S., P.K., K.K., L.R., J.W., R.S., J.A.K.H., M.K., Y.F., and B.E. performed research; L.M. performed statistical analysis; J.D., C.H., and B.E. wrote the manuscript; and B.E. supervised the research.

Conflict-of-interest disclosure: J.D., C.H., L.M., and B.E. are employees of Shire. C.M. is an employee of Quintiles, which assisted with the conduct of this study. P.K., K.K., L.R., J.W., R.S., J.A.K.H., M.K., and Y.F. received consultancy fees for conducting the research. M.S. received honoraria for speakers' fees for Baxalta/Shire and advisory boards.

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