The von Willebrand factor A-1 domain binding aptamer BT200 elevates plasma levels of von Willebrand factor and factor VIII: a first-in-human trial

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Supplementary data

Supplementary table 1. Summary of Demographic Data in Part A

	All Placebo (N=20)	**Cohort 1 BT200 (N=6)**	**Cohort 2 BT200 (N=6)**	**Cohort 3 BT200 (N=6)**	**Cohort 4 BT200 (N=6)**	**Cohort 5 BT200 (N=6)**	**Cohort 6 BT200 (N=6)**	**Cohort 7 BT200 (N=6)**	**Cohort 8 BT200 (N=6)**	**Cohort 9 BT200 (N=6)**	**Cohort 10 BT200 (N=6)**
Gender; n (%)											
Male	18 (90.0)	5 (83.3)	6 (100)	4 (66.7)	6 (100)	6 (100)	6 (100)	6 (100)	4 (66.7)	6 (100)	5 (83.3)
Female	2 (10.0)	1 (16.7)	0	2 (33.3)	0	0	0	0	2 (33.3)	0	1 (16.7)
Ethnicity; n (%)											
Not Hispanic or Latino	20 (100)	6 (100)	6 (100)	6 (100)	6 (100)	6 (100)	6 (100)	6 (100)	6 (100)	6 (100)	6 (100)
Race; n (%)											
Asian	0	0	0	0	1 (16.7)	0	1 (16.7)	0	0	0	0
Black or African American	0	1 (16.7)	0	0	0	0	0	0	0	0	0
White	20 (100)	5 (83.3)	6 (100)	6 (100)	5 (83.3)	6 (100)	5 (83.3)	6 (100)	6 (100)	6 (100)	6 (100)
Age (years) at screening; Mean (SD)	37.6 (12.66)	44.8 (13.26)	39.8 (9.33)	47.2 (16.41)	26.2 (4.58)	28.0 (11.22)	32.5 (11.17)	31.2 (6.85)	42.8 (11.89)	40.7 (14.99)	39.5 (14.79)
Height (cm) at screening; Mean (SD)	179.8 (10.55)	176.9 (11.07)	178.0 (5.69)	175.0 (11.64)	179.8 (6.82)	174.8 (11.07)	182.3 (8.21)	189.8 (8.52)	175.7 (10.75)	179.8 (7.14)	180.7 (3.67)
Weight (kg) at screening; Mean (SD)	82.0 (16.53)	82.4 (12.35)	75.9 (14.58)	82.4 (29.22)	80.3 (7.86)	75.8 (15.92)	84.8 (11.65)	93.8 (17.49)	78.3 (6.98)	84.0 (9.65)	80.5 (10.63)

	All Placebo (N=20)	Cohort 1 BT200 (N=6)	Cohort 2 BT200 (N=6)	Cohort 3 BT200 (N=6)	Cohort 4 BT200 (N=6)	Cohort 5 BT200 (N=6)	Cohort 6 BT200 (N=6)	Cohort 7 BT200 (N=6)	Cohort 8 BT200 (N=6)	Cohort 9 BT200 (N=6)	Cohort 10 BT200 (N=6)
Weight (kg) at randomization; Mean (SD)	82.1 (16.78)	82.2 (12.17)	75.8 (14.03)	82.8 (28.81)	79.4 (8.03)	76.0 (15.54)	85.2 (11.32)	94.3 (17.28)	78.2 (7.00)	84.5 (9.69)	80.3 (10.33)
BMI (kg/m²) at screening; Mean (SD)	25.2 (3.14)	26.4 (3.90)	23.9 (4.24)	26.3 (6.05)	24.8 (2.06)	24.9 (5.34)	25.6 (3.51)	25.8 (2.66)	25.8 (5.18)	25.9 (2.07)	24.6 (3.12)

Cm=centimeter; Kg=kilogram; n=number of subjects, percentages are based on the Total; N=total number of subjects; population size;
SD=standard deviation
BT200 dose levels in cohorts 1–7 : 0.18, 0.6, 1.8, 6.0, 12.0, 18.0 and 24.0 mg, respectively (rapid SC injection)
BT200 dose levels in cohorts 8–10: 24.0, 36.0 and 48.0 mg, respectively (gradual SC infusion)

Supplementary Table 2. Summary of Demographic Data in Part D

	Placebo (N=2)	**Cohort 11 BT200 (N=6)**
Gender; n (%)		
Male	2 (100)	6 (100)
Ethnicity; n (%)		
Not Hispanic or Latino	2 (100)	6 (100)
Race; n (%)		
Asian	1 (50.0)	1 (16.7)
White	1 (50.0)	5 (83.3)
Age (years) at screening; Mean (SD)	32.5 (12.02)	31.3 (9.93)
Height (cm) at screening; Mean (SD)	188.5 (19.09)	181.3 (9.58)
Weight (kg) at screening; Mean (SD)	96.0 (41.01)	81.8 (9.97)
Weight (kg) at randomization; Mean (SD)	95.5 (41.72)	82.0 (10.20)
BMI (kg/m²) at screening; Mean (SD)	26.3 (6.19)	24.9 (2.34)

BMI=body mass index; Cm=centimeter; Kg=kilogram; n=number of subjects, percentages are based on the Total; N=total number of subjects; population size ; SD=standard deviation

Supplementary Table 3. Summary of Demographic Data in Part B

	All Placebo (N=4)	**Cohort 13 BT200 (N=6)**	**Cohort 14 BT200 (N=6)**	**Pooled BT200 (N=12)**	**All (N=16)**
Gender; n (%)					
Male	3 (75.0)	5 (83.3)	6 (100)	11 (91.7)	14 (87.5)
Female	1 (25.0)	1 (16.7)	0	1 (8.3)	2 (12.5)
Ethnicity; n (%)					
Not Hispanic or Latino	4 (100)	6 (100)	6 (100)	12 (100)	16 (100)
Race; n (%)					
Asian	0	0	1 (16.7)	1 (8.3)	1 (6.3)
White	4 (100)	6 (100)	5 (83.3)	11 (91.7)	15 (93.8)
Age (years) at screening; Mean (SD)	48.5 (10.38)	46.7 (9.05)	34.3 (14.35)	40.5 (13.13)	42.5 (12.68)
Height (cm) at screening; Mean (SD)	178.5 (11.15)	181.2 (11.62)	179.2 (10.83)	180.2 (10.76)	179.8 (10.50)
Weight (kg) at screening; Mean (SD)	80.5 (8.35)	86.3 (18.11)	91.5 (20.99)	88.9 (18.88)	86.8 (17.02)
Weight (kg) at randomization; Mean (SD)	80.3 (8.38)	86.3 (18.11)	92.0 (20.47)	89.2 (18.66)	86.9 (16.89)
BMI (kg/m^2) at screening; Mean (SD)	25.3 (1.59)	26.1 (2.94)	28.6 (6.67)	27.3 (5.08)	26.8 (4.50)

BMI=body mass index; Cm=centimeter; Kg=kilogram; n=number of subjects, percentages are based on the Total; N=total number of subjects; population size; SD=standard deviation

BT200 dose level in Cohort 13 : 12.0 mg IV infused over 2 hours plus 12.0 mg SC injection followed by four maintenance doses of 12.0 mg SC injection; BT200 dose level in Cohort 14: 24.0 mg IV infused over 2 hours plus 24.0 mg SC injection followed by four maintenance doses of 24.0 mg SC injection

Supplementary Table 4. Summary of Demographic Data in Part C

	Placebo (N=2)	**Cohort 12 BT200 (N=6)**
Gender; n (%)		
Male	2 (100)	5 (83.3)
Female	0	1 (16.7)
Ethnicity; n (%)		
Not Hispanic or Latino	2 (100)	6 (100)
Race; n (%)		
White	2 (100)	6 (100)
Age (years) at screening; Mean (SD)	31.0 (5.66)	37.5 (13.16)
Height (cm) at screening; Mean (SD)	183.0 (4.24)	177.3 (9.73)
Weight (kg) at screening; Mean (SD)	77.5 (3.54)	87.0 (18.85)
BMI (kg/m^2) at screening; Mean (SD)	23.2 (2.13)	27.5 (4.65)

BMI=body mass index; Cm=centimeter; Kg=kilogram; n=number of subjects, percentages are based on the Total; N=total number of subjects; population size ; SD=standard deviation

Supplementary Table 5a. Summary of Related Adverse Events and Bleeding Events in Part A

System Organ Class Preferred Term	Placebo (N=20) n (%) Obs	Cohort 1 BT200 (N=6) n (%) Obs	Cohort 2 BT200 (N=6) n (%) Obs	Cohort 3 BT200 (N=6) n (%) Obs	Cohort 4 BT200 (N=6) n (%) Obs	Cohort 5 BT200 (N=6) n (%) Obs	Cohort 6 BT200 (N=6) n (%) Obs	Cohort 7 BT200 (N=6) n (%) Obs	Cohort 8 BT200 (N=6) n (%) Obs	Cohort 9 BT200 (N=6) n (%) Obs	Cohort 10 BT200 (N=6) n (%) Obs
Any related AE or bleeding event	2 (10.0) 2	0	1 (16.7) 1	0	1 (16.7) 1	1 (16.7) 2	2 (33.3) 2	0	1 (16.7) 1	0	3 (50.0) 8
Respiratory, thoracic and mediastinal disorders	0	0	0	0	0	0	1 (16.7) 1	0	1 (16.7) 1	0	2 (33.3) 4
Epistaxis	0	0	0	0	0	0	0	0	1 (16.7) 1	0	2 (33.3) 4
Nasal congestion	0	0	0	0	0	0	1 (16.7) 1	0	0	0	0
Vascular disorders	0	0	0	0	1 (16.7) 1	1 (16.7) 1	0	0	0	0	1 (16.7) 2
Haematoma	0	0	0	0	1 (16.7) 1	1 (16.7) 1	0	0	0	0	1 (16.7) 2
Gastrointestinal disorders	1 (5.0) 1	0	0	0	0	0	0	0	0	0	1 (16.7) 1
Diarrhoea	1 (5.0) 1	0	0	0	0	0	0	0	0	0	0
Gingival bleeding	0	0	0	0	0	0	0	0	0	0	1 (16.7) 1
General disorders and administration site conditions	0	0	1 (16.7) 1	0	0	0	0	0	0	0	0
Vessel puncture site haematoma	0	0	1 (16.7) 1	0	0	0	0	0	0	0	0

Injury, poisoning and procedural complications	1 (5.0) 1	0	0	0	0	0	0	0	0	0	0
Subcutaneous hematoma	1 (5.0) 1	0	0	0	0	0	0	0	0	0	0
Musculoskeletal and connective tissue disorders	0	0	0	0	0	1 (16.7) 1	0	0	0	0	0
Pain in extremity	0	0	0	0	0	1 (16.7) 1	0	0	0	0	0
Nervous system disorders	0	0	0	0	0	0	0	0	0	0	1 (16.7) 1
Dizziness	0	0	0	0	0	0	0	0	0	0	1 (16.7) 1
Skin and subcutaneous tissue disorders	0	0	0	0	0	0	1 (16.7) 1	0	0	0	0
Petechie	0	0	0	0	0	0	1 (16.7) 1	0	0	0	0

AE=adverse event; IMP=investigational medicinal product; n=number of subjects, percentages are based on the Total; Obs=number of events observed

BT200 dose levels in cohorts 1–7: 0.18, 0.6, 1.8, 6.0, 12.0, 18.0, and 24.0 mg, respectively (rapid subcutaneous injection)

BT200 dose levels in cohorts 8–10: 24.0, 36.0, and 48.0 mg, respectively (gradual subcutaneous infusion)

AEs also include bleeding events. Subjects were counted only once per system organ class and preferred term (Medical Dictionary for Regulatory Activities 23.1). Related AEs are events assessed as related to IMP or possibly related to IMP.

Supplementary Table 5b. Summary of Related Adverse Events and Bleeding Events in Part B

	Placebo (N=4) n (%) Obs	**Cohort 13 BT200 (N=6) n (%) Obs**	**Cohort 14 BT200 (N=6) n (%) Obs**	**Pooled BT200 (N=12) n (%) Obs**
Any related AE or bleeding event	3 (75.0) 4	4 (66.7) 7	3 (50.0) 4	7 (58.3) 11
Vascular disorders	2 (50.0) 3	1 (16.7) 2	3 (50.0) 3	4 (33.3) 5
Haematoma	2 (50.0) 3	0	2 (33.3) 2	2 (16.7) 2
Phlebitis	0	1 (16.7) 1	0	1 (8.3) 1
Thrombosis	0	0	1 (16.7) 1	1 (8.3) 1
Venous thrombosis limb	0	1 (16.7) 1	0	1 (8.3) 1
General disorders and administration site conditions	0	1 (16.7) 2	1 (16.7) 1	2 (16.7) 3
Injection site erythema	0	1 (16.7) 2	0	1 (8.3) 2
Peripheral swelling	0	0	1 (16.7) 1	1 (8.3) 1
Skin and subcutaneous tissue disorders	0	2 (33.3) 3	0	2 (16.7) 3
Rash	0	1 (16.7) 1	0	1 (8.3) 1
Skin discolouration	0	1 (16.7) 2	0	1 (8.3) 2
Injury, poisoning and procedural complications	1 (25.0) 1	0	0	0
Contusion	1 (25.0) 1	0	0	0

AE= adverse event; IMP=investigational medicinal product; n=number of subjects, percentages are based on the Total; Obs=number of events observed

BT200 dose level in Cohort 13 : 12.0 mg IV infused over 2 hours plus 12.0 mg SC injection followed by four maintenance doses of 12.0 mg SC injection

BT200 dose level in Cohort 14: 24.0 mg IV infused over 2 hours plus 24.0 mg SC injection

AEs also include bleeding events. Subjects were counted only once per system organ class and preferred term (Medical Dictionary for Regulatory Activities 23.1). Related AEs are events assessed as related to IMP or possibly related to IMP.

Supplementary Table 5c. Summary of Related Adverse Events and Bleeding Events in Part C

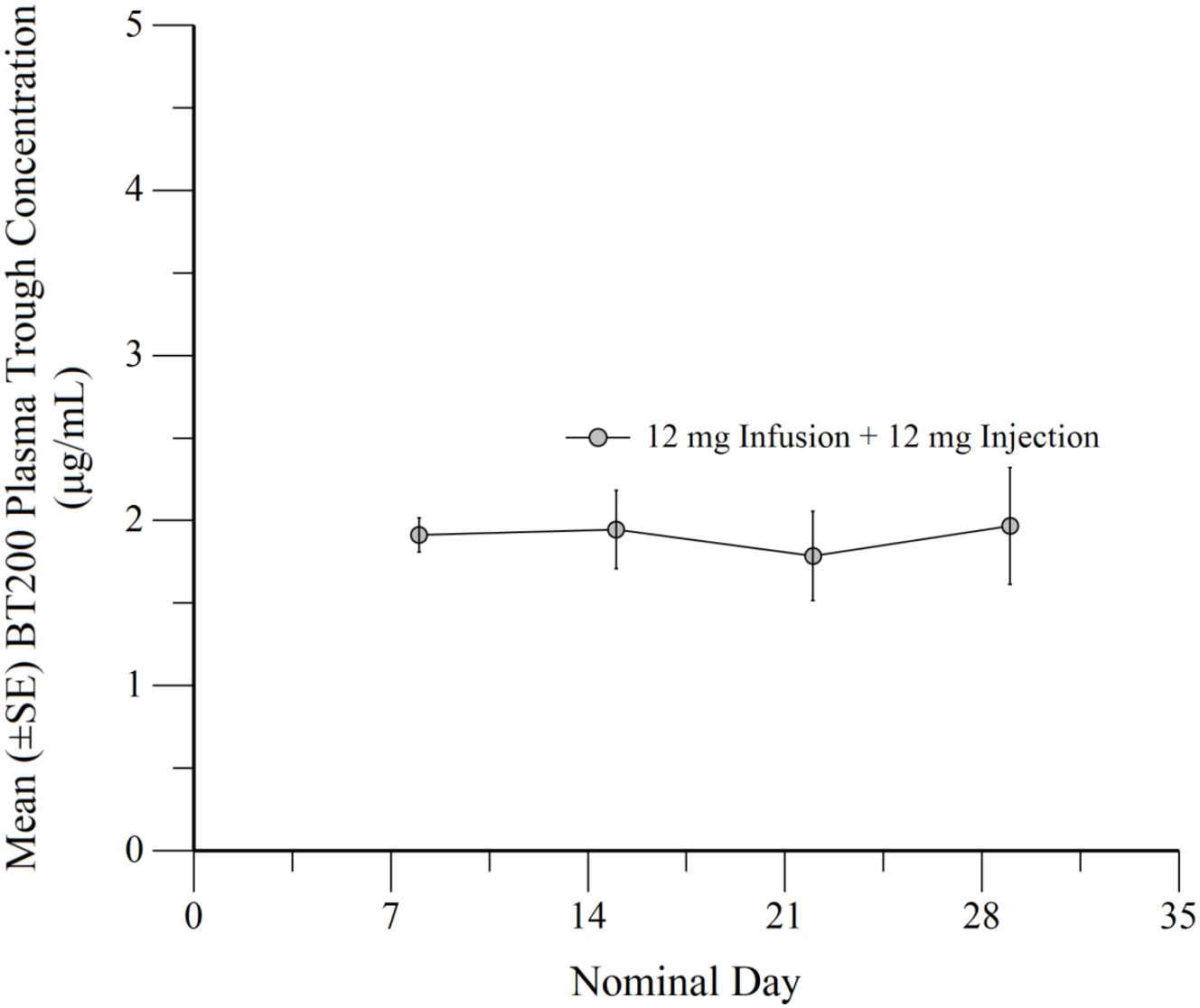
	Placebo **(N=2)** **n (%) Obs**	**Cohort 12** **BT200** **(N=6)** **n (%) Obs**
Any related AE or bleeding event	0	3 (50.0) 5
Vascular disorders	0	3 (50.0) 3
Haematoma at the blood draw site (both on right cubital vein)	0	2 (33.3) 2
Thrombosis in right cubital vein	0	1 (16.7) 1
Injury, poisoning and procedural complications	0	1 (16.7) 1
Contusion	0	1 (16.7) 1
Respiratory, thoracic and mediastinal disorders	0	1 (16.7) 1
Epistaxis	0	1 (16.7) 1

AE= adverse event; IMP=investigational medicinal product; n=number of subjects, percentages are based on the Total; Obs=number of events observed

BT200 dose level in Cohort 12: 48.0 mg single dose SC injection with an additional desmopressin 0.3 µg/kg IV infusion over 30 minutes approximately 96 hours after the BT200 SC injection

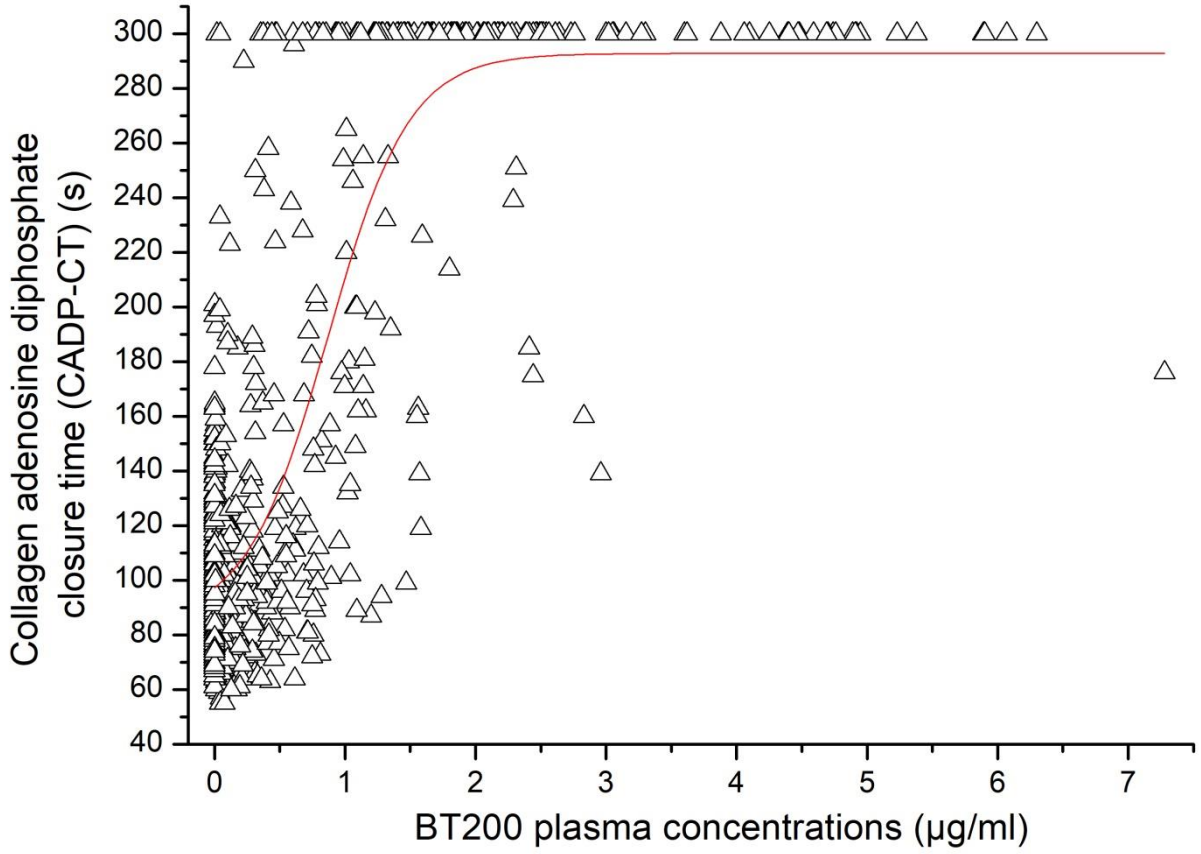
AEs also include bleeding events. Subjects were counted only once per system organ class and preferred term (Medical Dictionary for Regulatory Activities 23.1). Related AEs are events assessed as related to IMP or possibly related to IMP.

Supplementary Figure 1.



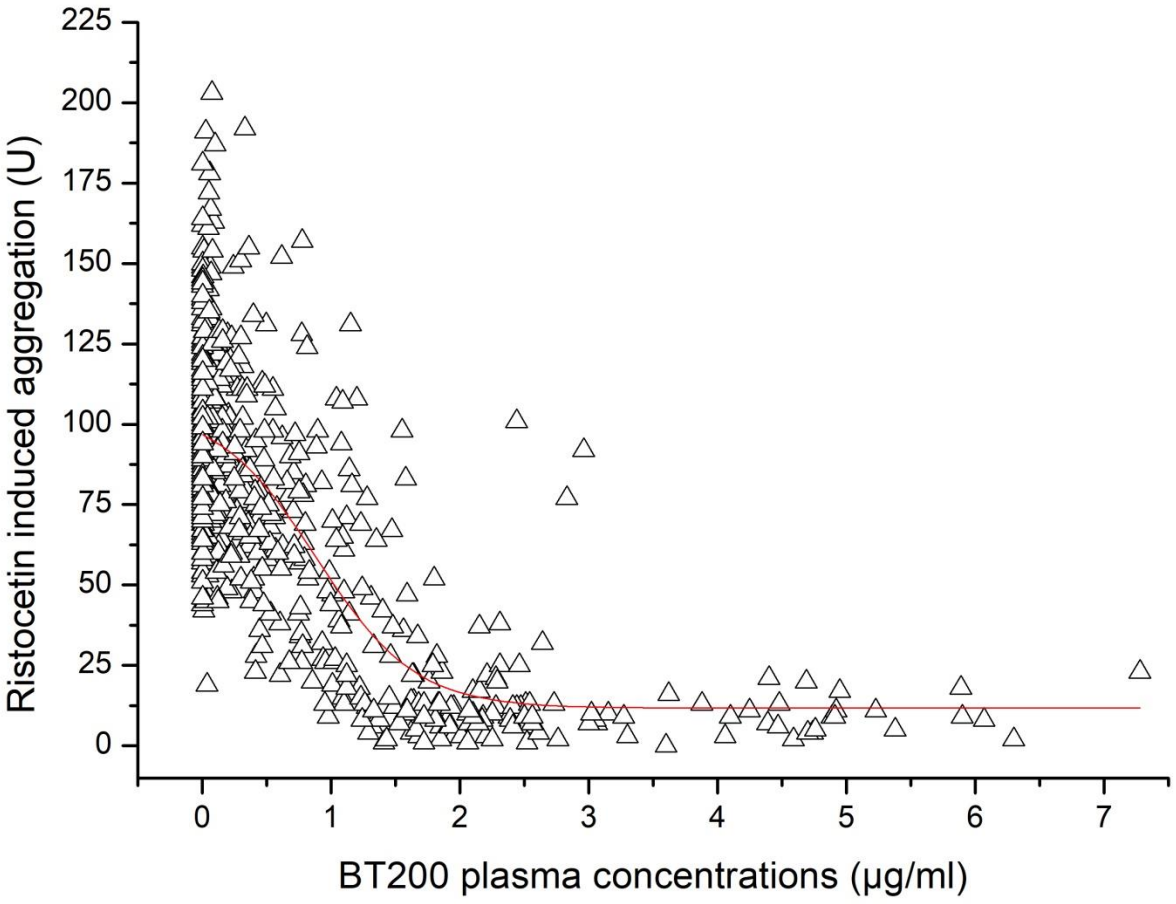
Supplementary Figure 1. Trough plasma concentrations of BT200 before each new subcutaneous dose of 12.0 mg BT200. (geometric means +/-SEM; n=6)

Supplementary Figure 2.



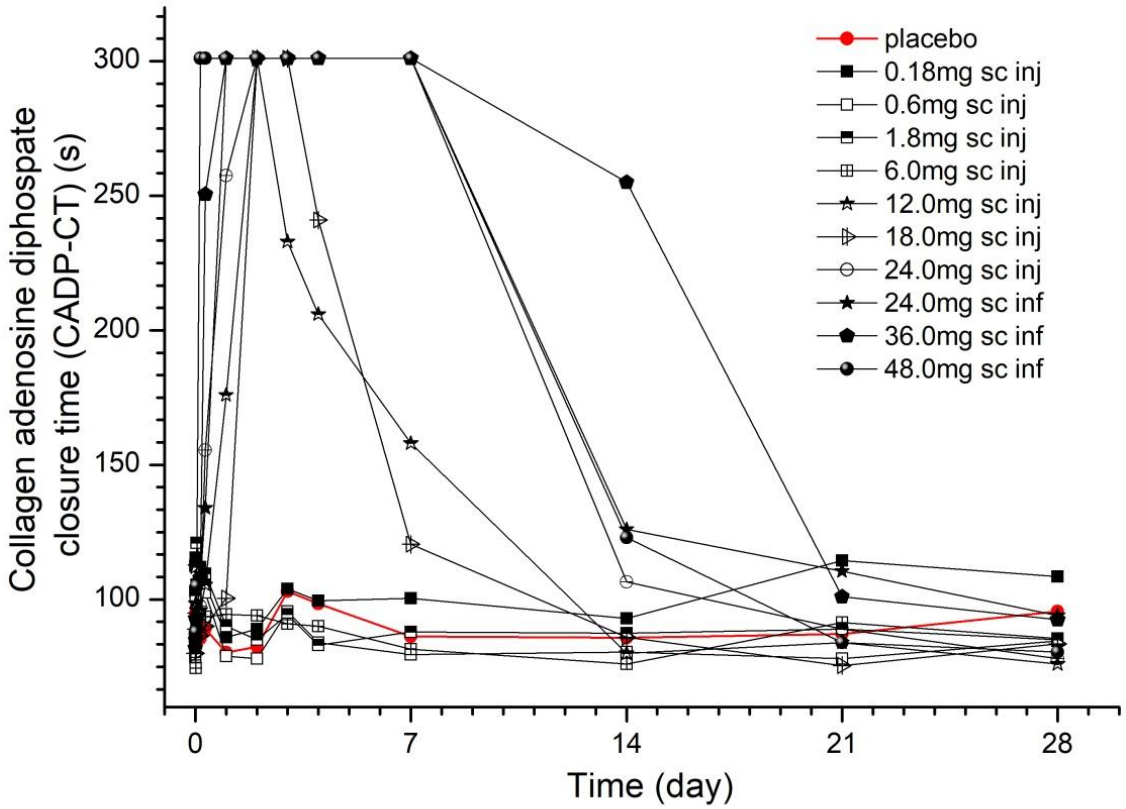
Supplementary Figure 2. Collagen adenosine diphosphate closure time (s) plotted against BT200 plasma concentrations (µg/ml) in the single ascending dose part of the trial (part A) in which BT200 was administered as subcutaneous injection or infusion.

Supplementary Figure 3.



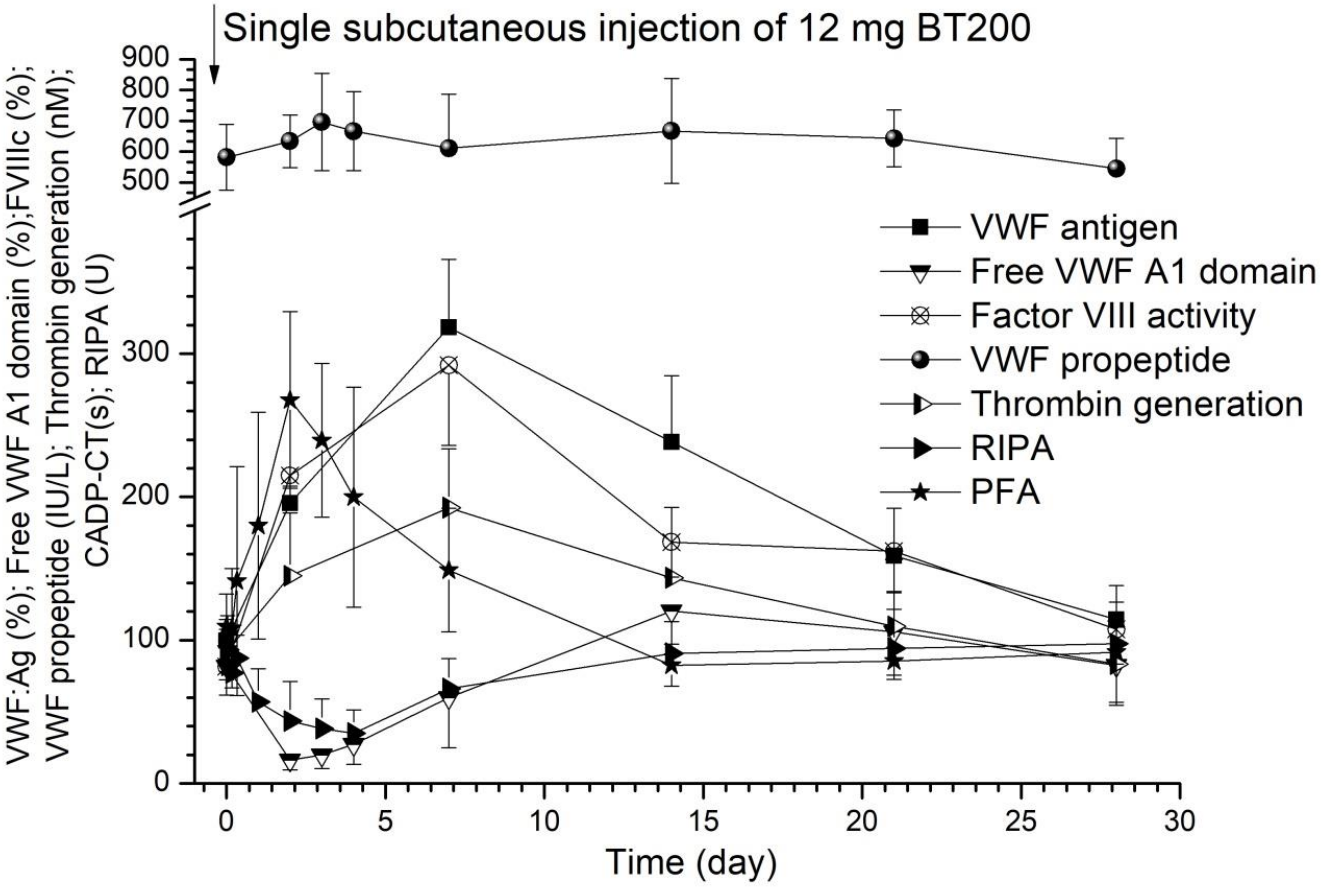
Supplementary Figure 3. Ristocetin induced platelet aggregation (U) levels plotted against BT200 plasma concentrations (µg/ml) in the single ascending dose part of the trial (part A) in which BT200 was administered as subcutaneous injection or infusion.

Supplementary Figure 4.



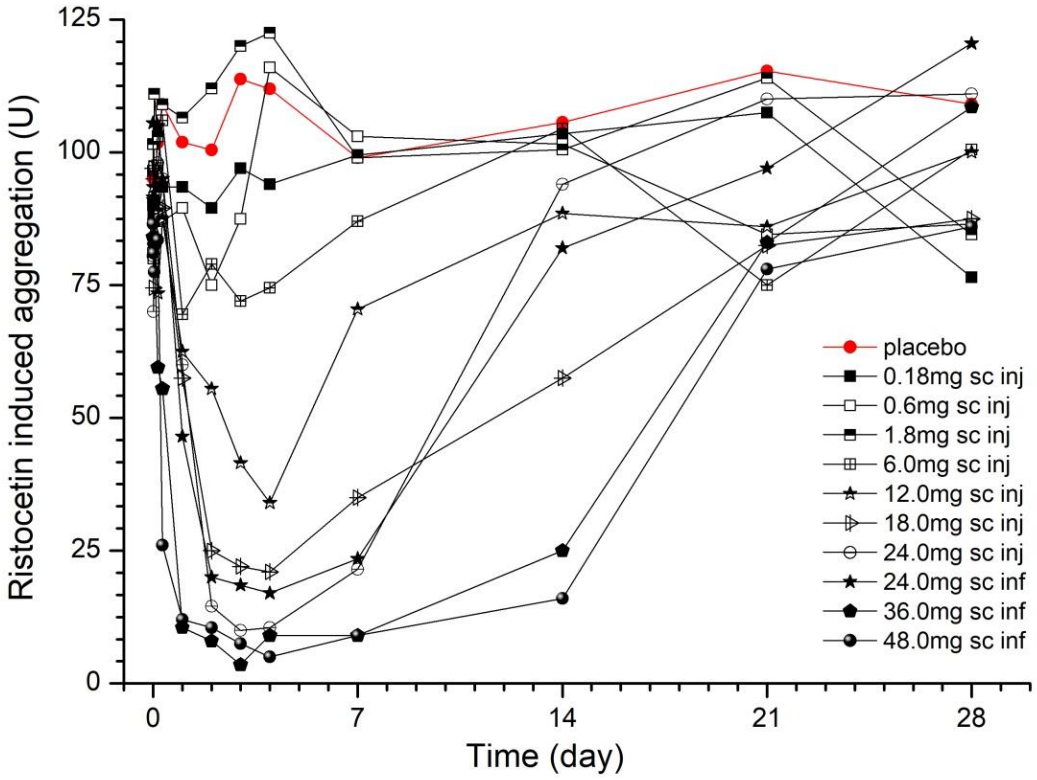
Supplementary Figure 4. Von Willebrand Factor dependent platelet function under high shear rates with the platelet function analyzer: Collagen adenosine diphosphate closure time (s) after single doses of BT200. Data are presented as mean values without error bars for better visibility (n=6 for BT200 groups, but n=20 for placebo).

Supplementary Figure 5.



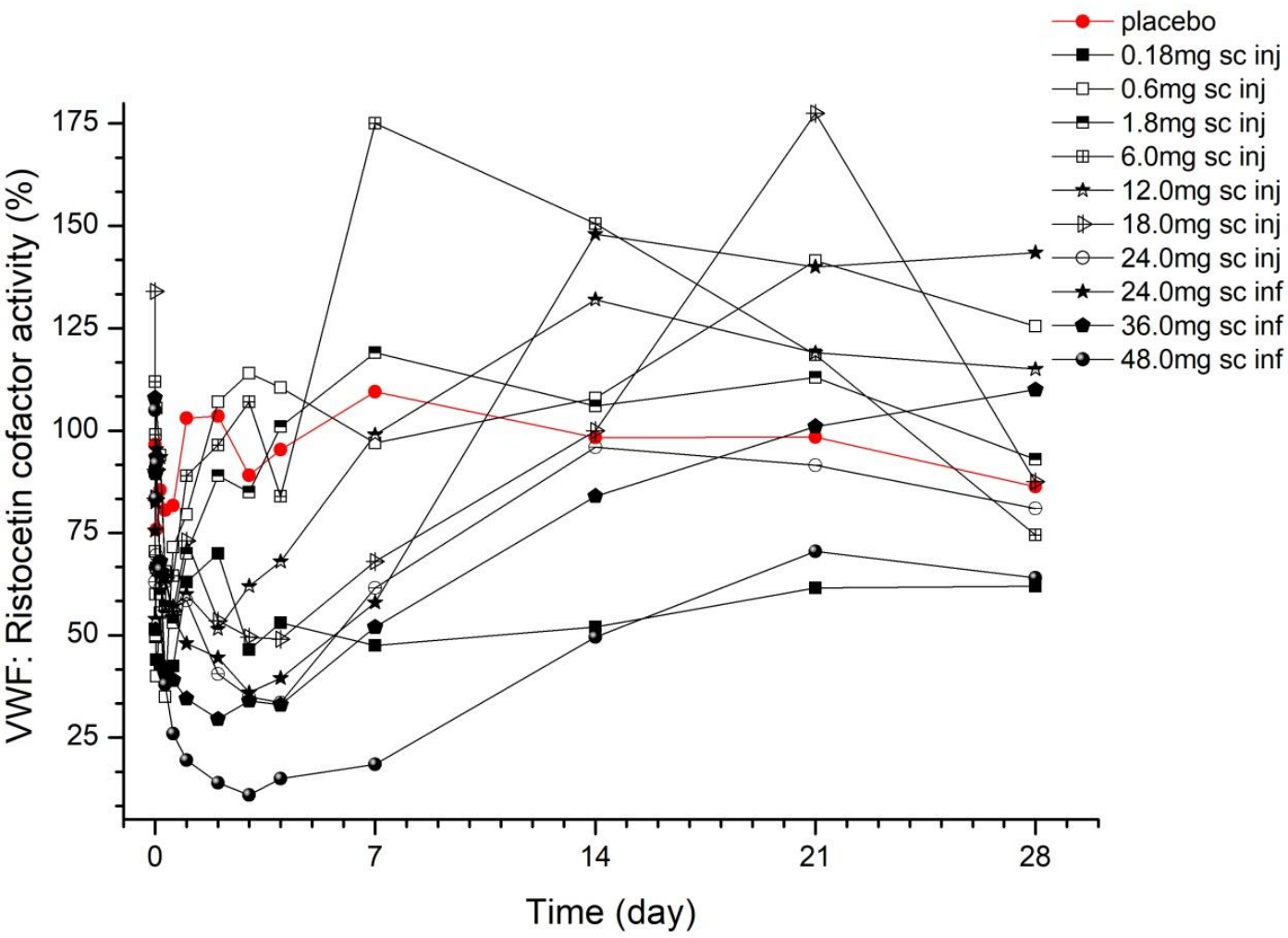
Supplementary Figure 5. Direct visual comparison of the time course of different parameters after a single subcutaneous injection of 12mg of BT200: von Willebrand factor antigen levels (%), free VWF A1 domains (%) Factor VIII activity (%), von Willebrand factor propeptide levels (IU/L), thrombin generation (nM), platelet function analyser (PFA) Collagen Adenosine Diphosphate Closure Time (s) and Ristocetin induced aggregation (RIPA)(U) Data are represented as mean values with 95% confidence interval (n=6 for BT200 group).

Supplementary Figure 6.



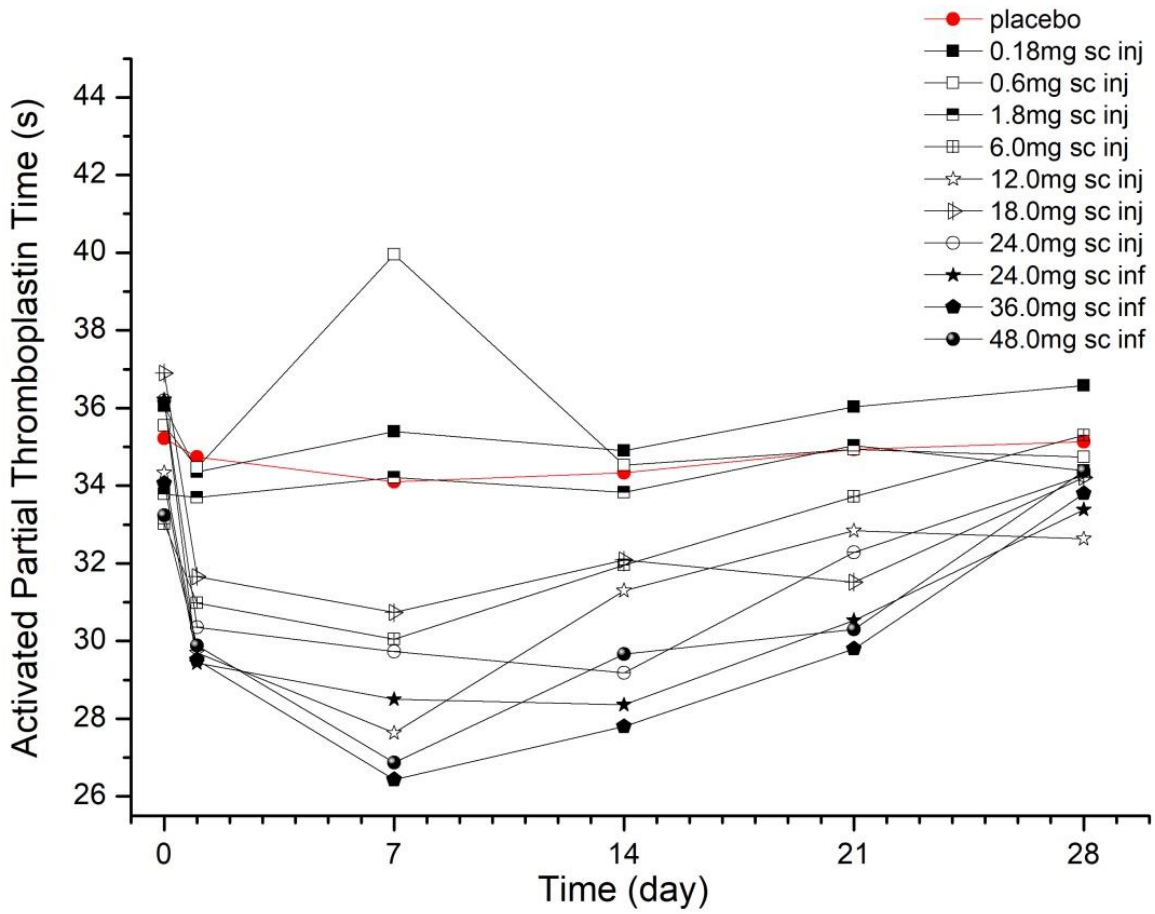
Supplementary Figure 6. Ristocetin induced platelet aggregation (U) after single doses of BT200. Data are represented as mean values without error bars for better visibility (n=6 for BT200 groups, but n=20 for placebo).

Supplementary Figure 7.



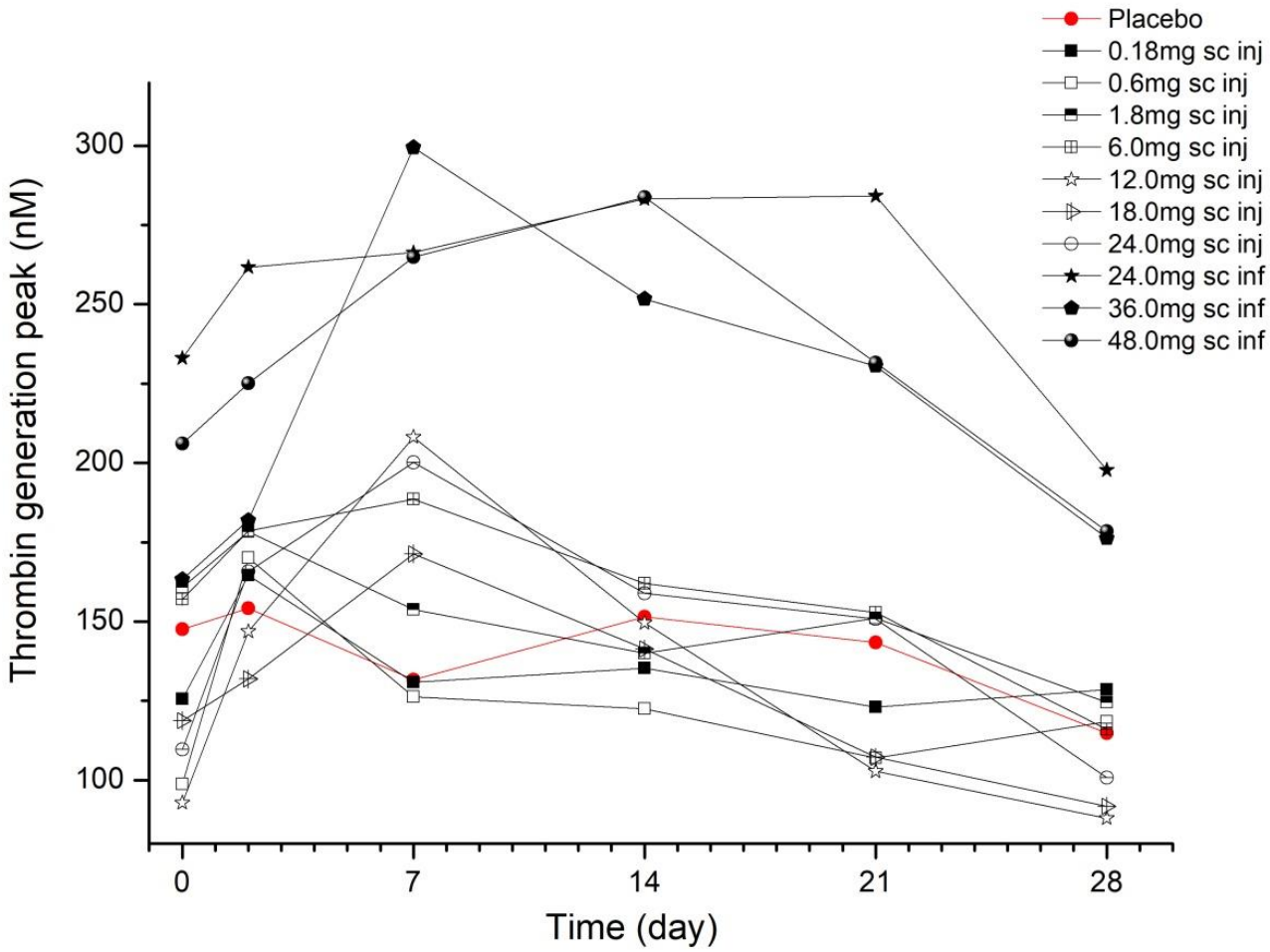
Supplementary Figure 7. Levels of von Willebrand factor ristocetin cofactor activity after single doses of BT200. Data are presented as mean values without error bars for better visibility (n=6 for BT200 groups, but n=20 for placebo).

Supplementary Figure 8.



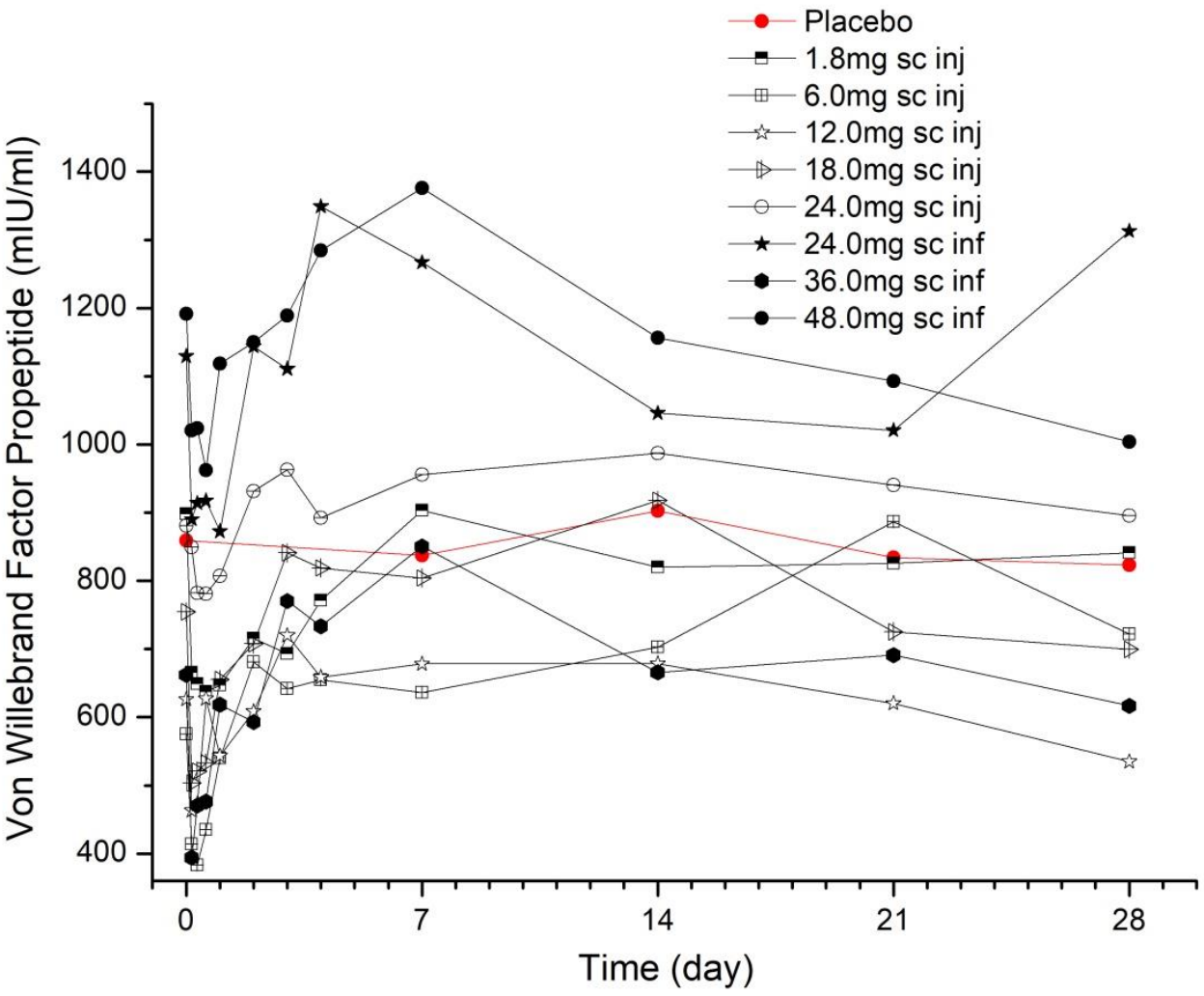
Supplementary Figure 8. Activated Partial Thromboplastin Time (aPTT FS; s) after single doses of BT200. Data are represented as mean values without error bars for better visibility (n=6 for BT200 groups, but n=20 for placebo).

Supplementary Figure 9.



Supplementary Figure 9. Peak thrombin generation (nM) after single doses of BT200. Data are mean values without error bars for better visibility (n=6 for BT200 groups, but n=20 for placebo).

Supplementary Figure 10.



Supplementary Figure 10. Von Willebrand Factor Propeptide levels (mIU/ml) after single BT200 doses. Data are represented as mean values without error bars for better visibility (n=6 for BT200 groups, but n=20 for placebo).

Methods

Analytical methods

Pharmacodynamic parameters were analyzed as previously described.[23] Factor VIIIc activity was determined by a one-stage clotting assay using elagic acid activator (Siemens Dade Actin FS reagent, FRG), to avoid putative interference of PEG with silica-based clotting assays, free unbound VWF-A1 domain (REEADS® ELISA, Corgenix, purchased from Haemochrom Diagnostic GmbH, FRG), VWF Antigen (STA LIATEST VWF:Ag; Diagnostica Stago, France), VWF Ristocetin Co-Factor (VWF:RCo; BC von Willebrand Reagent, Siemens Healthcare Diagnostics, FRG), VWF propeptide (Haemochrom Diagnostics, FRG), thrombin generation with a calibrated automated thrombogram,[24] ristocetin induced platelet aggregation with multiple electrode aggregometry (Multiplate®; Roche, Vienna, AT) and shear-induced platelet function with the platelet function analyzer (PFA200®) (Siemens Healthcare Diagnostics, FRG). The REAADS® VWF Activity ELISA Test Kit assay contains a monoclonal antibody specific for the portion of VWF, which binds platelets (A1 domain) and is coated on 96-microwell plates. Diluted plasma samples are incubated in the wells, which are washed and bound. Antigen is detected by a horseradish peroxidase conjugated anti-human VWF detection antibody. BT200 competes with antibody binding in the ELISA assay. As both BT200 and the ELISA antibody have the same target (A1 domain of VWF), in the presence of BT200 fewer A1 domains are available for the ELISA antibody, which allows for the measurement of the inhibitory effect of BT200 on VWF activity. The inhibitory effect of BT200 on VWF activity was evaluated using this ELISA kit, but with the modification that samples were assayed at 1:3 dilution, which differs from the 1:21 solution suggested by the manufacturer. This change relates to the influence of the greater dilution on the balance between free and bound aptamer by washing the aptamer off its target. Likewise, the standard curve has been adapted in accordance with the 1:3 dilution.

Plasma BT200 concentrations were measured under Good Laboratory Practice at QPS (Newark, DE) by high-performance liquid chromatography after hybridization of BT200 to a fluorescent labelled complementary oligonucleotide. The method was qualified and fully

validated according to regulatory standards with a lower limit of quantification of 1ng/mL (see online supplement for further details).

Statistical analysis

PK parameters were calculated using Phoenix® WinNonlin® v.8.3. For all analyses, SAS® (version 9.2 or higher; SAS Institute Inc., Cary, NC, USA) were used. Data were summarized by dose cohort and, where appropriate, by visit. Descriptive statistics (number of observations, mean, standard deviation, and minimum, median, maximum) were provided for continuous variables. Frequency counts and percentages were presented for categorical variables. Missing values were generally not imputed. All data exclusions, including premature terminations, were detailed and tabulated. Data listings included all enrolled subjects. Informal testing for significance was carried out by repeated measures analysis of variance. Two-sided 95% confidence intervals (CIs) were calculated for effect sizes after multiple doses. A Friedman ANOVA was applied to test the time course of changes for significance and two-sided significance levels of 5% were applied. As the different parameters are not independent variables, no correction for multiplicity was carried out. One subject randomized to receive BT200 has missing dosing information due to the needle becoming disconnected from the syringe during SC injection; results for this subject were excluded from the summary statistics.

APPENDIX

TIME POINTS FOR Pharmacokinetic Assessments

1.1. Part A: Single Ascending Dose (Cohorts 1-10)

Blood samples were collected at pre-dose, 30 min, 1, 4, 8, 14, 24, 48, 72, 96 and 168 hours post dose and on Days 14 (336 hours), 21 (504 hours), 28 (672 hours), 42 (1008 hours), and 56 (1344 hours).

Part B: Multiple Ascending Dose (Cohorts 13-14)

Blood samples were collected pre-dose, at 30 min and at1, 4, 8, 14, 24, 48, 72, 96 and 168 hours post-dose on Day 1. On Day 7, blood samples were collected pre-dose and at 168, 336, 504, 672, 840, 1008, 1175, 1510 and 1850 hours post-dose. On Day 28, blood samples were collected pre-dose and post-dose at 168, 336, 504, 672, 1008 and 1344 hours post-dose. Blood samples were also collected pre-dose on Days 7, 14, and 21.

Part C: Desmopressin Challenge Study (Cohort 12)

Blood samples were collected prior to dosing with BT200 (Day -4), and then at 30 min, 1, 2, 3, 4, 8, 14, 24, 48, 72, 96 and 168 hours post-dose. Additionally, blood was drawn on Days 14 (336 hours), 21 (504 hours), 28 (672 hours), 42 (1008 hours), and 56 (1344 hours).

Part D: Relative Bioavailability Study (Cohort 11)

Blood samples were collected pre-dose, at 30 min and at 1, 4, 8, 14, 24, 48, 72, 96 and 168 hours post-dose. Blood was also drawn on Days 14 (336 hours), 21 (504 hours), 28 (672 hours), 42 (1008 hours), and 56 (1344 hours).

The above sampling schedules differed between analytes and study parts.

1.2. Pharmacodynamic Assessments

Part A: Single Ascending Dose (Cohorts 1-10)

Blood samples were collected pre-dose, at 30 min and at 1, 4, 8, 14, 24, 48, 72, 96 and 168 hours post-dose. Blood was also drawn on Days 14 (336 hours), 21 (504 hours), 28 (672 hours).

Part B: Multiple Ascending Dose (Cohorts 13-14)

Blood samples were collected pre-dose, at 30 min and at 1, 4, 8, 14, 24, 48, 72, 96 and 168 hours post-dose on Day 1. On Day 7, blood samples were collected pre-dose and at 168, 336, 504, 672, 840, 1008 and 1176 hours post-dose. On Day 28, blood samples were collected pre-dose and post-dose at 168, 336, 504 and 672 hours. Blood samples were also collected pre-dose on Days 7, 14, 21 and 28.

Part C: Desmopressin Challenge Study (Cohort 12)

Blood samples were collected prior to administration of BT200 (Day -4), prior to desmopressin dosing and at 30 min, 1, 2, 3, 4, 8, 14, 24, 48, 72, 96 and 168 hours post desmopressin administration. Additionally, blood was drawn on days 14 (336 hours), 21 (504 hours), 28 (672 hours), 42 (1008 hours), and 56 (1344 hours).

Part D: Relative Bioavailability Study (Cohort 11)

Blood samples were collected pre-dose, at 30 min, 1, 4, 8, 14, 24, 48, 72, 96 and 168 hours post-dose and on Days 14 (336 hours), 21 (504 hours), 28 (672 hours), 42 (1008 hours), and 56 (1344 hours).

HPLC Method used for evaluation of pharmacokinetics

The plasma BT200 concentration was determined by HPLC-FL after sample preparation. The calibration range of BT200 was 10.0-2000 ng/mL using a linear regression model. BT200 reference standard came from Berkshire Sterile Manufacturing (Lee, MA), and PNA probe came from PNA Bio Inc. Company (Thousand Oaks, CA)). Instruments used were HPLC system from Shimadzu with FL detector RF-20AXS (Excitation wavelength: 436 nm; Emission wavelength: 484 nm). We used HPLC column DNAPacTM PA200, 4x250 mm from Thermo Scientific. There were two mobile phases, mobile phase A (MPA) was ACN/H2O/1M Tris-HCl, pH 8.0/0.5M EDTA, pH 8.0, and mobile phase B was 0.8M NaClO4 in MPA.

Sample preparation:

Lysis buffer was first made containing 2 mg/mL Proteinase K from Invitrogen (Carlsbad, CA), and 25% Epicenter cell and tissue lysis solution from Epicenter Biotechnology (Madison, WI) in 1x PBS buffer at pH 7.4. Twenty µL of K_2EDTA plasma samples was mixed with 20 µL of lysis buffer prior to hybridization with the PNA Probe at the final concentration of 1µM. Once combined with the PNA Probe, samples are heated at 95ºC for 15 min in a 96-well PCR plate, then snap cooled by rapid ramp-down to 4ºC in an ice bath. Once hybridized, the samples were measured by LC-fluorescence.