Pharmacology / Toxicology Review Memorandum (Final)

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To: Felice D'Agnillo Ph.D. (BLA Chairperson) and Nannette Cagungun (BLA regulatory project manager)

Subject: BLA 125606\0- Pharmacology/Toxicology review of HAEGARDA (CSL830) for routine prophylaxis to prevent Hereditary Angioedema (HAE) attacks in adolescent and adult patients

Sponsor: CSL Behring, Marburg, Germany

Recommendation: STN 125606/0 can be approved from a pharmacology and toxicology perspective.

Proposed Indication:
C1 Esterase inhibitor (HAEGARDA, CSL830) is indicated as a routine prophylaxis to prevent Hereditary Angioedema (HAE) attacks in adolescent and adult patients.

Executive summary:
HAEGARDA, CSL830 is supplied in a kit containing a lyophilized powder in a single-use vial that contains either 2000 IU or 3000 IU of C1 inhibitor with Sterile Water for Injection, USP (4 mL for reconstitution of 2000 IU or 6 mL for reconstitution of 3000 IU). Each vial of reconstituted HAEGARDA contains 500 IU/mL of C1-INH, 65 mg total protein, 10 mg glycine, 8.5 mg sodium chloride and 2.5 mg sodium citrate and reconstituted HAEGARDA is proposed a dose of 60 IU per kg body weight by subcutaneous (S.C.) injection twice weekly (every 3 or 4 days).

After twice weekly subcutaneous administration of 60 IU/kg HAEGARDA, C1-INH is slowly absorbed, with a median time to peak concentration (t_{max}) of approximately 59 hours and the mean clearance and apparent volume of distribution of C1-INH is estimated to be approximately 83 mL/hr and 4.33 L. Based on a median apparent plasma half-life of 69 hours, steady state for C1-INH is expected within 3 weeks of dosing and the steady state PK of S.C. of C1-INH was found to be independent of dose between 20-80 IU/kg in HAE subjects. Injection site reactions occurring after treatment with HAEGARDA, 95.0% were of mild intensity and 82.5% resolved within 1 day after onset.

The product demonstrates an acceptable risk to patients with hereditary angioedema at (b) (4) the proposed clinical dose as defined primarily by 14 day repeat dosing in the rat and is considered to be well tolerated after a single subcutaneous injection in Rabbit local tolerance evaluation.

The data from preclinical safety studies reveal no specific toxicity risk associated with HAEGARDA for humans based on conventional studies of safety pharmacology, single and repeat dose toxicity and local

tolerability in rats and rabbits. In vivo thrombogenicity tests in rabbits indicate that there was no prothrombotic risk associated with the I.V. or S.C. administration of HAEGARDA at the doses tested. No investigations on carcinogenicity and reproductive toxicology have been conducted. In local reaction site evaluation using rabbits by S.C. injection, HAEGARDA, Berinert® P and the placebo Berinert formulation buffer were considered to be locally well tolerated after a single subcutaneous injection.

Roster of Non-clinical Studies

Pharmacology/animal pharmacokinetics:
COM 01/04 (GLP - No) *In vitro* complement activation (Berinert® P batch (b) (4))
COM 02/10 (GLP -Yes) Influence of Berinert® P and Berinert® P/N on complement activity in human or rat plasma
57-23 (GLP - Yes) Safety pharmacology in dogs (Berinert® P batch (b) (4))
V-626 (1-3) (GLP - No) Pharmacokinetics in rats (Berinert® P batch (b) (4))
PSR (08/103) (GLP - Yes) Pharmacokinetics in rats (Berinert® P and Berinert® P/N)
PTS-4r A study on the bioavailability (F) and pharmacokinetics of subcutaneously administered Berinert P in rabbits.

Toxicology/non-clinical safety:
57-1HS - Part 1 (GLP - Yes) Acute dose toxicity in mice (Berinert® P batch (b) (4))
57-1HS - Part 2 (GLP - Yes) Acute dose toxicity in rats (Berinert® P batch (b) (4))
856351 (GLP - Yes) Repeat dose toxicity in rats (Berinert® P batch (b) (4))
57-23 (GLP - Yes) Safety pharmacology in dogs (Berinert® P batch (b) (4))
265.143.905 (GLP - Yes) Local tolerance testing of Berinert® P versus saline 0.9% in the rabbit
37872 TAL (GLP-Yes) Local tolerance study in the rabbits (CSL830 (Berinert 1500), (b) (4))

Neoantigenicity
V-211d (GLP - Yes) Neoantigenicity in rabbits and guinea pigs (Berinert® P (b) (4))
ZNA35361.001 (GLP-Yes) Neoantigenicity studies with Berinert® P and Berinert® P/N in rabbits.

Thrombogenisity
VTK 03/09 (GLP-No) Evaluation of prothrombotic effects of Berinert® P ((b) (4))
VTK 12/11 (GLP-No) Evaluation of prothrombotic effects of CSL830 ((b) (4)

Study Reviews:

COM 01/04 (GLP – No) – Influence of Berinert® P batch (b) (4) on complement activity in human or rat plasma.

Objective: To compare the effect of Berinert® P batch (b) (4) on complement activity in human and rat plasma with the end point being the IC50 for inhibition of complement components C1 r and C1 s. This study serves as a justification for the use of rats as a species which is pharmacologically similar to humans.

Methods:
Normal rat plasma and human plasma were used as the complement source. C1-INH (Berinert® P batch (b) (4)) was diluted in each plasma type over a range of concentrations from 0.0095 U/mL to 1.9 U/mL. See treatment group table below.

Materials:
Rat plasma – From (b) (4) rats (female), weight range 400-500 g from (b) (4) . Blood collected in citrate.
Human plasma – Standard human plasma, (b) (4)
Berinert® P – Batch # (b) (4)

Treatment #	Test group	Final concentration
1	Berinert® P in human plasma	1.9 U/mL
2	Berinert® P in human plasma	1.4 U/mL
3	Berinert® P in human plasma	1.19 U/mL
4	Berinert® P in human plasma	0.59 U/mL
5	Berinert® P in human plasma	0.29 U/mL
6	Berinert® P in human plasma	0.15 U/mL
7	Berinert® P in human plasma	0.095 U/mL
8	Berinert® P in human plasma	0.0095 U/mL
9	Berinert® P in rat plasma	1.9 U/mL
10	Berinert® P in rat plasma	1.4 U/mL
11	Berinert® P in rat plasma	1.19 U/mL
12	Berinert® P in rat plasma	0.59 U/mL
13	Berinert® P in rat plasma	0.29 U/mL
14	Berinert® P in rat plasma	0.15 U/mL
15	Berinert® P in rat plasma	0.095 U/mL
16	Berinert® P in rat plasma	0.0095 U/mL

Results:

	Complement activity (percent of normal)			
C1-INH concentration	Human plasma		Rat Plasma	
1.9 U/mL	11.2	12.9	9.9	10.2
1.4 U/mL	34.3	32.9	30.6	29.1

1.19 U/mL	45.4	45.0	37.8	38.5
0.59 U/mL	75.0	71.8	73.9	73.9
0.29 U/mL	84.3	94.0	88.7	85.8
0.15 U/mL	98.1	116.9	107.7	85.4
0.095 U/mL	106.6	84.3	97.0	86.8
0.00950 U/mL	103.3	116.4	93.5	93.9

Plotted averaged data (n=2 per time point) fit to a four parameter regression model indicate a C1 inhibitory concentration at 50% (IC50) = 1.0529 in human plasma and an IC50 = 1.0108 in rat plasma.

Reviewer conclusions **COM 01/04**: The data indicate a similar pharmacologic activity of Berinert® P in rat and human plasma. While, it is likely that other species would show similar C1-inhibition compared to humans, the data presented are acceptable to demonstrate that the rat is a reasonable species for demonstration of non-clinical safety.

57-23 (GLP – Yes) – Safety pharmacology study in dogs and a local intravenous tolerance study in rabbits after introduction of a (b) (4) (Berinert® P batch (b) (4)).

Objective: To evaluate the general safety pharmacology of hepatitis C virally inactivated C1-INH batches (b) (4) in beagle dogs (n=2 male and n=1 female) and local intravenous tolerance in rabbits (n=3 male and n=2 female).

Methods:
Test substance – hepatitis safe C1 inactivator HS batch (b) (4) (3,000 U/10 mL)
Vehicle control - 0.9% NaCl

Animals:
Safety pharmacology – Male and female beagle dogs from CSL's in house breeding colony (mean body weight = 10.5 kg)
Local intravenous tolerance testing – Male and female rabbits from CSL's in house breeding colony (mean body weight = 2.5 kg)

Study design:
Dosing safety pharmacology- Dogs received an escalating dose regimen of 500 U, 1000 U and 2000 U for a total of 3500 U (333 U/kg). All doses were administered into the lateral marginal ear vein.

Parameters measured:

- Arterial blood flow
- Heart, pulse and respiratory rates
- Cutaneous oxygen partial pressure
- Cardiac output
- Systemic vascular resistance
- Stroke volume
- Respiratory flow

- Central body temperature
- Blood pressure
- Pneumatogram
- dp/dt
- ECG
- Hematology (erythrocytes, leucocytes and thrombocytes)

Dosing local tolerance – Each rabbit received a single intravenous dose of 500 U (200 U/kg) (injection time 1 minute) into the lateral marginal ear vein.
Parameters measured:

- The local reaction to single injections was evaluated after administration and 24 hours post administration.

Results:
Safety Pharmacology – The cardiovascular and respiratory parameters measured at a cumulative dose of 3500U (333 U/kg) showed no abnormalities. Hematological evaluation demonstrated a slight decrease in coagulation time and thrombocyte aggregation.

Local intravenous tolerance – No abnormal histopathology was observed in tissue excised from the venous injection site area.

Reviewer's conclusions **57-23 –** At approximately 15 fold the proposed clinical replacement dose; Berinert® P does not induce any discernible adverse cardio-repiritory effects in beagle dogs. Minimal effects on thrombogenic potential could be observed at this dosing level. It is know that doses in children and neonates administered Berinert® P do exhibit thrombogenicity at 90 U/kg (4.5 fold the recommended clinical dose).

V-626 (1-3) (GLP – No) – Pharmacokinetics of C1-inactivator in rats (Berinert® P batch (b) (4) following intravenous injection. (An additional study was performed in rabbits to determine subcutaneous bioavailability).

The determination of and validation of Berinert® P in rabbit plasma is detailed in study report MEV-29r (Determination of the functional C1-Inhibitor activity in rabbit plasma)

Objective: To evaluate the pharmacokinetics of Berinert® P in the rat, one of the primary species chosen for toxicological evaluation.

Methods:
Test substance - Berinert® P lot # 00401 (50 IU/mL)

Animals:
Rat - (b) (4) CSL colony raised 200g male and female

Study design:

PK was evaluated in 1 male and 1 female animal per dosing level in anesthetized and conscious rats as follows:

Experiment 626-1 (anethetized)
Rat 1, 3 - Berinert® P 61.5 IU/kg one dose intravenous
Rat 2, 4 - Berinert® P 123 IU/kg one dose intravenous

Blood sampling (0, 5 min, 10, min, 30 min, 1 hr, 2 hr, 3 hr, 4 hr, 5 hr post injection)

Experiment 626-2 (conscious)
Rat 1, 3 - Berinert® P 61.5 IU/kg one dose intravenous
Rat 2, 4 - Berinert® P 123 IU/kg one dose intravenous

Blood sampling (0, 1 hr, 5 hr, 8 hr, 24 hr, 30 hr post injection)

Experiment 626-3 (conscious)
Rat 1, 3 - Berinert® P 61.5 IU/kg one dose intravenous
Rat 2, 4 - Berinert® P 123 IU/kg one dose intravenous

Blood sampling (0, 6 hr, 24 hr, 48 hr, 72 hr and 96 hours post injection)

Results:
In the three groups of rats the initial C1-INH levels of 24 +/- 10.8% increased approximately 8-fold within the initial hour after dosing. C1-INH activity began to decline after 24 hours and by 48 hours plasma C1-INH levels returned to baseline (approximately 25%).

V-626 (1-3) (GLP – No) – Pharmacokinetics of C1-inactivator in rats (Berinert® P batch (b) (4)) following intravenous injection. (An additional study was performed in rabbits to determine subcutaneous bioavailability).

The determination of and validation of Berinert® P in rabbit plasma is detailed in study report MEV-29r (Determination of the functional C1-Inhibitor activity in rabbit plasma)

Objective: To evaluate the pharmacokinetics of Berinert® P in the rat, one of the primary species chosen for toxicological evaluation.

Methods:
Test substance - Berinert® P lot # 00401 (50 IU/mL)

Animals:
Rat - (b) (4) CSL colony raised 200g male and female

Study design:

PK was evaluated in 1 male and 1 female animal per dosing level in anesthetized and conscious rats as follows:
Experiment 626-1 (anethetized)
Rat 1, 3 - Berinert® P 61.5 IU/kg one dose intravenous
Rat 2, 4 - Berinert® P 123 IU/kg one dose intravenous

Blood sampling (0, 5 min, 10, min, 30 min, 1 hr, 2 hr, 3 hr, 4 hr, 5 hr post injection)

Experiment 626-2 (conscious)
Rat 1, 3 - Berinert® P 61.5 IU/kg one dose intravenous
Rat 2, 4 - Berinert® P 123 IU/kg one dose intravenous

Blood sampling (0, 1 hr, 5 hr, 8 hr, 24 hr, 30 hr post injection)

Experiment 626-3 (conscious)
Rat 1, 3 - Berinert® P 61.5 IU/kg one dose intravenous
Rat 2, 4 - Berinert® P 123 IU/kg one dose intravenous

Blood sampling (0, 6 hr, 24 hr, 48 hr, 72 hr and 96 hours post injection)

Results:
In the three groups of rats the initial C1-INH levels of 24 +/- 10.8% increased approximately 8-fold within the initial hour after dosing. C1-INH activity began to decline after 24 hours and by 48 hours plasma C1-INH levels returned to baseline (approximately 25%).

COM 02/10 (GLP –Yes) – Influence of Berinert® P on complement activity in human or rat plasma
Objective: To compare the effect of Berinert® P and Berinert® P/N in human and rat plasma complement activity. The study end point being the IC50 for inhibition of complement components C1 r and C1 s. This study serves as a justification for the use of rats as a species which is pharmacologically similar to humans and comparative activity of Berinert® P and Berinert® P/N.

Methods:
Human and rat plasma was incubated with (0.074, 0.149, 0.298, 0.59, 1.19, 1.43, 1.90 U/mL) of Berinert® P and Berinert® P/N. CI-INH activity was measured using a (b) (4) Normal rat plasma and human plasma were used as the complement source.

Materials:
Rat plasma – From (b) (4) rats (female), weight range 400-500 g from (b) (4) . Blood collected in citrate.
Human plasma – Standard human plasma, (b) (4)
Berinert® P – Batch # (b) (4) and Berinert® P/N Batch# (b) (4)

Treatment #	Test group	Final concentration
1	Berinert® P in human plasma	1.9 U/mL
2	Berinert® P in human plasma	1.43 U/mL
3	Berinert® P in human plasma	1.19 U/mL
4	Berinert® P in human plasma	0.59 U/mL
5	Berinert® P in human plasma	0.298 U/mL
6	Berinert® P in human plasma	0.149 U/mL
7	Berinert® P in human plasma	0.074 U/mL
9	Berinert® P in rat plasma	1.9 U/mL
10	Berinert® P in rat plasma	1.43 U/mL

11	Berinert® P in rat plasma	1.19 U/mL
12	Berinert® P in rat plasma	0.59 U/mL
13	Berinert® P in rat plasma	0.298 U/mL
14	Berinert® P in rat plasma	0.149 U/mL
15	Berinert® P in rat plasma	0.074 U/mL
16	Berinert® P/N in human plasma	1.9 U/mL
17	Berinert® P/N in human plasma	1.43 U/mL
18	Berinert® P/N in human plasma	1.19 U/mL
19	Berinert® P/N in human plasma	0.59 U/mL
20	Berinert® P/N in human plasma	0.298 U/mL
21	Berinert® P/N in human plasma	0.149 U/mL
22	Berinert® P/N in human plasma	0.074 U/mL
23	Berinert® P/N in rat plasma	1.9 U/mL
24	Berinert® P/N in rat plasma	1.43 U/mL
25	Berinert® P/N in rat plasma	1.19 U/mL
26	Berinert® P/N in rat plasma	0.59 U/mL
27	Berinert® P/N in rat plasma	0.298 U/mL
28	Berinert® P/N in rat plasma	0.149 U/mL
29	Berinert® P/N in rat plasma	0.074 U/mL

Results:

	Complement activity (percent of normal)			
C1-INH concentration	Human plasma		Rat Plasma	
	P	P/N	P	P/N
0.074 U/mL	118	117.9	99.2	96.9
0.149 U/mL	68.4	67.3	79.5	78.5
0.298 U/mL	71.1	69.9	81.0	79.8
0.590 U/mL	61.8	63.0	73.4	73.7
1.19 U/mL	41.9	37.9	46.4	46.7
1.42 U/mL	28.4	24.2	33.5	36.2
1.42 U/mL	11.5	10.2	14.3	15.0

Plotted averaged data (n=2 per time point) fit to a four parameter regression model indicate a C1 inhibitory concentration at 50% (IC50) = 1.0529 in human plasma and an IC50 = 1.0108 in rat plasma.

Reviewer conclusions **COM 02/10**: The data indicate a similar pharmacologic activity of Berinert® P and Berinert® P/N. Differences in overall potency were approximately 6% between Berinert® P and Berinert® P/N in rat and human plasma $p<0.067$ (CI was within 80-125% of Berinert® P.

PSR (08/103) (GLP – Yes) – Pharmacokinetics of (Berinert® P and Berinert® and Berinert® P/N) following intravenous injection. (A bioequivalence study).

Objective: To evaluate the pharmacokinetics of Berinert® P and Berinert® P/N in the rat, one of the primary species chosen for toxicological evaluation.

Methods:

Test substance 1- Berinert® P lot # 31661711 (52 IU/mL, 150 U/kg)
Test substance 2- Berinert® P/N lot # 32961711 (52 IU/mL, 150 U/kg)

Animals:
Rat - (b) (4) CSL colony raised 200g male and female

Study design:

PK was evaluated in 1 male and 1 female animal per dosing level in anesthetized and conscious rats as follows:

Blood sampling (0, 4 hr, 8 hr, 24 hr post injection)

Descriptive statistics of AUC 0-24 hrs (h x mU/mL) for human C1-INH activity									
	Sex	**N**	**Mean**	**Geom. mean**	**SD**	**CV**	**Min**	**Median**	**max**
P	**M**	**10**	**30605**	**29688**	**8208**	**27**	**20496**	**28514**	**47331**
P	**F**	**10**	**31692**	**30118**	**10006**	**32**	**15548**	**33335**	**48861**
P	**All**	**20**	**31148**	**29902**	**8925**	**29**	**15548**	**30896**	**48861**
P/N	**M**	**9**	**28606**	**27772**	**6947**	**24**	**16102**	**28866**	**39332**
P/N	**F**	**10**	**25966**	**24933**	**7835**	**30**	**14127**	**25122**	**42157**
P/N	**All**	**19**	**27216**	**26240**	**7347**	**27**	**14127**	**26273**	**42157**

Reviewer conclusions **PSR (08/10)**: The data indicate a similar pharmacokinetic profile between Berinert® P and Berinert® P/N. Differences in overall PK based on AUC0-24 hr for Berinert® P and Berinert® P/N in rats was within a CI of 80-125%.

PTS-4r – A study on the bioavailability (F) and pharmacokinetics of subcutaneously administered Berinert P in rabbits.

Objective: To evaluate the bioavailability of Berinert® P after subcutaneous administration (200 U/kg) compared to the intravenous dosing (200 U/kg) route of administration. A secondary objective was to determine all PK parameters for each route of administration at the 200 U/kg dosing level.

Methods:
Test substance - Berinert® P (lot # 12161711)

Animals: (b) (4) rabbits n=10M and 10F (2.8 kg)

Study design:

Group #	Treatment	Dose/Vol./route	N (m/f)
1	Berinert® P	200 U/kg/4.0 mL/kg/intravenous	10 (5/5)
2	Berinert® P	200 U/kg/4.0 mL/kg/subcutaneous	10 (5/5)

Blood sampling: 0, 0.5 h, 1 h, 2 h, 3h, 16 h, 20 h, 1, 2, 3, 4, 14 and 21 days after a single dose.

Results:
The initial intravenous dose demonstrated a rapid decline in plasma concentration from 0 to 16 hours. Such that the end of the initial elimination phase ending at 24 hours mated the Cmax after subcutaneous dosing (2 U/mL).

Pharmacokinetic parameters PTS-4r

		Intravenous (200 U/kg)	Subcutaneous (200 U/kg)
	N	10	10
$AUC_{0\text{-tlast}}$ (d*U/mL)	Mean	6.18	4.68
	SD	0.79	0.51
$AUC_{0\text{-inf}}$ (d*U/mL)	Mean	6.29	4.80
	SD	0.82	0.51
t_{max} (days)	Mean	0.03	1.05
	SD	0.02	0.34
C_{max} (U/mL)	Mean	7.53	1.70
	SD	0.87	0.15
α elimination t1/2 (days)	Mean	0.16	---
	SD	0.03	---
β elimination t1/2 (days)	Mean	1.41	0.90
	SD	0.22	0.15
Clearance	Mean	38.9	54.1
	SD	4.3	5.6
Volume of distribution	Mean	61.0	69.8
	SD	10.3	12.7
F	***Parameter***	***Ratio***	***90% CI***
s.c./i.v.	$AUC_{0\text{-inf}}$	0.75	69.3-83.0

Reviewer conclusions **V-626(1-3) and PTS-4r** – These data suggest that comparable PK following intravenous dosing in the rabbit and human exist. The sponsor suggests that subcutaneous dosing in the rabbit shows 75% bioavailability and as a result this dosing route may be an option. The bioavailability in humans following subcutaneous dosing would require evaluation in order to accurately make this conclusion.

57-1HS – Part 1-2 (GLP – Yes) – A single-dose intravenous toxicity study of C1-inactivator HS in mice and rats

Objective: To determine the acute dose toxicity of C1-inactivator HS in mice and rats following human equivalent maximum doses (120 U and 300 U) of C1-inactivator. These doses represent the equivalent maximum clinical dose per unit body weight (20 U/kg).

Methods:
Test substance:
C1-inhibitor HS batch # (b) (4)
Control:
0.9% NaCl

Animals:
Mice - (b) (4) mice CSL breeding colony (21-25 g), 10 male and 10 female
Rats - (b) (4) (110 g – 5 weeks old), 10 male and 10 female

Dosing:
Mice – 30, 60, 120 U/animal via the tail vein
Rats – 100, 200, 300 U/animal via the tail vein

Study Design:
Mice and rats were dosed as stated above on day one, histology was performed on day 14 while clinical parameters (e.g. food/water intake, body weight and body temperature) were measured from day 1-14.

Results:
Body weight development – Normal
Clinical observations – Normal
Terminal observations (Histology) – Normal

Reviewer's conclusions – The study shows that mice and rats demonstrate a reasonable toxicity profile at the maximum clinical intended doses. However, the study does demonstrate points of weakness in its design (e.g. multitudes of the maximum clinical dose (10x) were not studied and an early evaluation group (2 days post dosing) was not included).

856351 (GLP – Yes) – 14-Day intravenous toxicity (bolus) study in the (b) (4) rat.

Objective: To assess the cumulative toxicity of Berinert® P when administered daily to rats intravenously as a bolus for 14 days. Additionally the time dependency of neutralizing antibody formation to Berinert® P or endogenous C1-inhibitor in rats was studied.

Methods:
Test substance: Berinert® P (batch # (b) (4))
Control: Sterile water for injection

Animals: (b) (4) outbred (b) (4) rats (N=60 male 135-162 g and N=60 female 112-134 g)

Study Design:

	Group 1 (0 U/kg/day)	Group 2 (20 U/kg/day)	Group 3 (60 U/kg/day)	Group3 (200 U/kg/day)
Males A	1-5	16-20	31-35	46-50
Males B	6-10	21-25	36-40	51-55
Males C	11-15	26-30	41-45	56-60

Females A	61-65	76-80	91-95	106-110
Females B	66-70	81-85	96-100	111-115
Females C	71-75	86-90	101-105	116-120
A – Sacrifice at 24 hours after 6th treatment B – Sacrifice at 24 hours after 10th treatment C – Sacrifice at 24 hours after 14th treatment Dose volume = 4 mL/kg Group 1 – control Group 2 – represents the clinical dose Group 3 – represents 3x the clinical dose Group 4 – represents 10x the clinical dose				

Observations:

Viability/mortality – Twice daily
Clinical signs – Once prior to first administration; twice daily on days 1-3; once daily thereafter during treatment period
Food consumption – Twice weekly during acclimatization and treatment
Body weight – Twice weekly during acclimatization and treatment

Ophthalmoscopic examination - During acclimatization and at the end of treatment

Clinical/laboratory investigation – days 7, 11 and 15

Hematology – days 7, 11 and 15
- erythrocyte count
- Hb
- MCV
- Red cell volume distribution width
- MCH
- MCHC
- Hemoglobin concentration distribution width
- Platelet count
- Reticulocyte count
- Reticulocyte maturity index
- Total leukocyte count
- Differential leukocyte count
- Thromboplastin time
- Activated partial thromboplastin time

Clinical Biochemistry – days 7, 11 and 15
- glucose
- urea
- creatinine
- total bilirubin
- total cholesterol
- trigycerides
- phospholipids
- AST

- ALT
- lactate dehydrogenase
- glutamate dehydrogenase
- CK
- alkaline phosphatase
- gamma-glutamyl-transferase
- sodium
- potassium
- chloride
- calcium
- phosphorous
- total protein
- albumin
- globulin

Urinalysis - days 7, 11 and 15
- volume
- specific gravity
- color
- appearance
- pH
- glucose
- ketone
- urobilinogen
- bilirubin
- erythrocytes

Antibody and C1 plasma level determination – days 7, 11, 15

Pathology - days 7, 11, 15

Results (summary):

Viability/mortality: No pre-mature mortalities

Clinical signs: No clinically relevant differences were noted in any of the animals in any group prior to administration or on any days 1-3 during the treatment period

Food consumption – No relevant changes in food intake were noted in Berinert® P treated animals at any of the dosing levels evaluated.

Body weight –No differences were found in groups treated with increasing doses of Berinert® P.

Ophthalmoscopic examination – No Berinert® P related ophthalmology changes were detected amongst groups.

Clinical/laboratory investigation – days 7, 11 and 15

Hematology – days 7, 11 and 15

Most notable was a trend toward an increase in platelets observed in female rats at the 60 and 200 U/kg dosing levels. Inconsistent changes were seen in hematological parameters (shortened PTT, elevated neutrophils, decreased MCH) in both male and female rats. However, these observations were not treatment or doses related and therefore are not likely to be attributable to Berinert® P.

Clinical Biochemistry – days 7, 11 and 15

There were no consistent treatment or dose related changes in clinical biochemistry parameters discernible at 7, 11 or 15 days. Inconsistent changes were seen in potassium, triglyceride, chloride, fatty acids and globulins. However, these observations were not treatment or doses related and therefore are not likely to be attributable to Berinert® P.

Urinalysis - days 7, 11 and 15

There were no consistent treatment or dose related changes in urinary parameters discernible at 7, 11 or 15 days. Inconsistent changes were seen in urine density and erythrocyte count. However, these observations were not treatment or doses related and therefore are not likely to be attributable to Berinert® P.

Clinical/laboratory investigation – days 7, 11 and 15

Hematology – days 7, 11 and 15

Three hematologic parameters related to coagulation were evaluated (platelets, prothrombin time (PT) and activated partial thromboplastin time (aPTT)) in male and female rats.
Male rats demonstrated an increase (not statistically significant) in platelet levels documented at the 7th daily dose. Platelet counts then declined to pre-dosing levels on day 11 and day 15. PT and aPTT and was unchanged in all male dosing groups.

Female rats demonstrated an increase (not statistically significant) in platelet levels documented at the 7th daily dose. PT remained unchanged in all female dosing groups. aPTT demonstrated a significant reduction at the 7th day of dosing. However a constistent dose and or duration of dosing dependence was not observed (se Tables 1-3 below).

Table 1: Platelets (G/l)

days of dosing (n=5/group)	Group 1 (0 U/kg/day)	Group 2 (20 U/kg/day)	Group 3 (60 U/kg/day)	Group3 (200 U/kg/day)
Males day 7	1047.6 ± 129	1204 ± 61	1294.2 ± 125	1204.6 ± 107
Males day 11	1176.4 ± 102	1081.8 ± 77	1035.0 ± 206	831 ± 149.9
Males day15	1128.2 ± 108	1142.8 ± 98.4	1067.8 ± 138	1116 ± 111
Females day 7	1179.2 ± 69	1100.0 ± 169	1208.6 ± 148	1291 ± 88
Females day 11	1050.6 ± 139	1213.6 ± 133	1114.2 ± 157	1148.8 ± 202
Females day 15	1138.2 ± 157.9	1236.2 ± 75.8	1188 ± 107	1190 ± 115
A – Sacrifice at 24 hours after 6th treatment B – Sacrifice at 24 hours after 10th treatment C – Sacrifice at 24 hours after 14th treatment Dose volume = 4 mL/kg				

Group 1 – control Group 2 – represents the clinical dose for HAE indication Group 3 – represents 3x the clinical dose for HAE indication Group 4 – represents 10x the clinical dose for HAE indication

Table 2: Prothrombin time (ratio of normal activity)

days of dosing (n=5/group)	Group 1 (0 U/kg/day)	Group 2 (20 U/kg/day)	Group 3 (60 U/kg/day)	Group3 (200 U/kg/day)
Males day 7	0.824 ± 0.081	0.820 ± 0.113	0.822 ± 0.043	0.884 ± 0.145
Males day 11	0.834 ± 0.042	0.838 ± 0.034	0.844 ± 0.088	0.876 ± 0.096
Males day15	0.882 ± 0.060	0.854 ± 0.055	0.886 ± 0.059	0.894 ± 0.086
Females day 7	0.796 ± 0.013	0.818 ± 0.038	0.814 ± 0.031	0.824 ± 0.041
Females day 11	0.830 ± 0.080	0.858 ± 0.082	0.846 ± 0.073	0.818 ± 0.043
Females day 15	0.882 ± 0.069	0.860 ± 0.023	0.872 ± 0.059	0.850 ± 0.042
A – Sacrifice at 24 hours after 6th treatment B – Sacrifice at 24 hours after 10th treatment C – Sacrifice at 24 hours after 14th treatment Dose volume = 4 mL/kg Group 1 – control Group 2 – represents the clinical dose for HAE indication Group 3 – represents 3x the clinical dose for HAE indication Group 4 – represents 10x the clinical dose for HAE indication				

Table 3: aPTT (seconds)

days of dosing (n=5/group)	Group 1 (0 U/kg/day)	Group 2 (20 U/kg/day)	Group 3 (60 U/kg/day)	Group3 (200 U/kg/day)
Males day 7	16.9 ± 2.71	18.12 ± 3.74	16.54 ± 2.13	14.56 ± 2.10
Males day 11	19.02 ± 4.56	18.10 ± 0.92	17.00 ± 3.67	15.52 ± 2.61
Males day15	14.34 ± 1.2	15.70 ± 1.91	14.00 ± 3.86	14.70 ± 3.03
Females day 7	17.72 ± 1.23	**14.18 ± 1.76**	**13.84 ± 1.98**	15.70 ± 2.52
Females day 11	16.34 ± 2.92	15.70 ± 3.46	14.76 ± 2.64	15.80 ± 3.29
Females day 15	13.26 ± 1.54	**15.92 ± 0.77**	13.64 ± 1.00	13.24 ± 1.64
A – Sacrifice at 24 hours after 6th treatment B – Sacrifice at 24 hours after 10th treatment C – Sacrifice at 24 hours after 14th treatment Dose volume = 4 mL/kg Group 1 – control Group 2 – represents the clinical dose for HAE indication Group 3 – represents 3x the clinical dose for HAE indication Group 4 – represents 10x the clinical dose for HAE indication				

C1 plasma level determination – days 7, 11, 15

	Mean C1 Esterase Inhibitor Plasma Levels (% increase over T0)					
	24 h after 6th treatment		24 h after 10th treatment		24 h after 14th treatment	
	Female	Male	Female	Male	Female	Male
Group1 (saline)	23.85	11.61	18.47	3.75	13.93	9.59
Group 2 (20 U/kg)	23.23	16.04	24.6	21.33	30.59	17.95
Group 3 (60 U/kg)	48.78	33.58	28.54	32.1	42.63	38.51
Group 4 (200 U/kg)	112.51	157.05	115.43	169.92	141.83	172.25

Antibody formation against Berinert® P

	Mean antibody against C1 Inhibitor (antibody titer – (b) (4) assay)					
	24 h after 6th treatment		24 h after 10th treatment		24 h after 14th treatment	
	Female	Male	Female	Male	Female	Male
Group1 (saline)	0.86	0.43	0.54	0.76	76.81	112.37
Group 2 (20 U/kg)	1.79	0.53	0.90	0.73	1.98	5.48
Group 3 (60 U/kg)	0.24	0.56	0.60	1.39	0.27	14.43
Group 4 (200 U/kg)	0.40	0.67	0.17	0.39	2.86	2.79

* Relative to control with the exception of the day 15 sampling in the control animals, neutralizing antibody production are not an influence on the overall toxicological findings for 14 day dosing.

Pathology - days 7, 11, 15

Organ weights - There were no consistent treatment or dose related changes in organ weights or organ/body weight ratios discernible at 7, 11 or 15 days.

Macroscopic findings - There were no consistent treatment or dose related changes in macroscopic lesions discernible at 7, 11 or 15 days in treatment groups compared to control animals.

Microscopic findings – Phlebitis, periphlebitis, thrombophlebitis and perivascular hemorrhages were observed at the injection sites of all animals (treatment and control) and are the common result of physical rather than chemically induced local trauma.

The other various microscopic findings noted do not appear to be treatment or dose related.

265.143.905 (GLP – Yes) – Local tolerance testing of Berinert® P versus saline 0.9% In the rabbit.

Objective: Determine the local tolerance of Berinert® P to physiological saline in male and female (b) (4) Rabbits following subcutaneous administration.

Methods:

Group	#/sex	Substance	Side	Route	Dose	Volume
1	3 male	Berinert® P/Saline	L/R	s.c.	25 U/kg	0.44
	3 female	Berinert® P/Saline		s.c.	25 U/kg	0.44
2	3 male	Berinert® P/Saline		s.c.	75 U/kg	1.32
	1 female	Berinert® P/Saline		s.c	75 U/kg	1.32
	2 female	Berinert® P/Saline		s.c.	75 U/kg	1.32

After s.c. injection, clinical observations were performed three times on day 0, twice daily on days 1, 2, and 3.

Results:

Erythema formation – One instance of grade 2 erythema occurred in the 25 U/kg group none in the 75 U/kg group and none in the saline treated group.

Edema formation – Edema was not observed in males or females of the control, 25 U/kg or 75 U/kg groups.

Pain reaction – One instance of a grade 1 pain reaction was noted in one male dosed with 25 U/kg. No pain reactions were noted in males or females dosed s.c. with control or 75 U/kg.

Histopathology- No product or dose related histopathology changes were noted in the tissue surrounding the s.c. injection site.

V-211d (GLP – Yes) – Testing for possible formation of antigenic components though out the modification of product processing

Through out the different phases of processing applied to CSLs C1-Inhibitor (Berinert® P) there appears to be little evidence of processes related increases in neutralizing and or non-neutralizing antibody production.

ZNA35361.001 (GLP-Yes) – Neoantigenicity studies with Berinert® P and Berinert® P/N in rabbits.

Objective: To determine if nanofiltration increases neoantigen production in Berinert® P.

Methods:
Test substance 1- Berinert® P lot # 31661711 (52 IU/mL, 150 U/kg)
Test substance 2- Berinert® P/N lot # 32961711 (52 IU/mL, 150 U/kg)

Group	#/sex	Substance	Side	Route	Dose	Volume
1	3 male	Berinert® P/Saline	L/R	s.c.	0.25 mg	1 mL
	3 female	Berinert® P/Saline		s.c.	0.25 mg	1 mL
2	3 male	Berinert® P/N		s.c.	0.25 mg	1 mL
	1 female	Berinert® P/N		s.c	0.25 mg	1 mL
	2 female	Berinert® P/Saline		s.c.	0.25 mg	1 mL

After s.c. injection, clinical observations were performed three times on day 0, twice daily on days 0, 14, and 28.

Results:

Histopathology- No product or dose related histopathology changes were noted in the tissue surrounding the s.c. injection site.

Neoantigen formation – No new antibodies were detected by (b) (4) analysis after days 0, 14 and 28 dosing when Berinert® P and Berinert® P/N was dosed in rabbits

Reviewer conclusion: There appears to be no additional risk of antigen formation with the addition of the nanofiltration step.

37872 TAL (GLP-Yes) - Local tolerance study in the rabbits following one intravenous, intra-arterial subcutaneous or intramuscular injection

Summary and reviewer's conclusion
To evaluate the local tolerance of the test item, CSL830, following one intravenous, intra-arterial, subcutaneous or intramuscular injection in rabbits. The test item, CSL830, was administered once to (b) (4) Rabbits under a dosage-volume of 3 mL/injection by intravenous, intra-arterial infusion and subcutaneous injection and under a dosage-volume of 0.5 mL/injection by intramuscular injection. The data indicate that a single intravenous or intra-arterial infusion or subcutaneous or intramuscular injection of the test item, CSL830, was clinically, locally and histologically well-tolerated in rabbits.

Materials and Methods
1. Test item
   CSL830 (Berinert 1500)

- Batch number : (b) (4)
- Protein concentration : (b) (4)
- Potency : (b) (4)
- (b) (4) : (b) (4)

Sterile (b) (4) saline solution (0.9% NaCl)
- batch Number: (b) (4)
- supplier: (b) (4)

Water for injections
- batch number: (b) (4)
- supplier: (b) (4)

2. Test system
   - Strain and species: (b) (4) rabbits
   - Number: 12 rabbits (8 males and 4 females)
   - Breeder: (b) (4)
   - Age/Weight: 3 to 4 month old, male (range: 2155 g to 2520 g), females 2458 g (range: 2385 g to 2525 g).
3. Treatment

Group:

Group	number and identity of animals	adminstraion route	dosage-volume (ml/injection)	Treatment left side	Right side
1	2 males W30141 and W30142 1 female W30158	intravenous	3	test item	control item
2	2 males W30143 and W30144 1 female W30159	intra-arterial	3	test item	control item
3	2 males W30145 and W30146 1 female W30160	Subcutanous	3	test item	control item
4	2 males W30147 and W30148 1 female W30161	intra-arterial	3	test item	control item

Administration
- intravenous infusion: marginal ear vein, 3 mL/injection
- intra-arterial infusion: central ear artery, 3 mL/injection
- subcutaneous injection: median flanks, 3 mL/injection
- intramuscular injection: dorsal muscles, 0.5 mL/injection

Duration
Following the administration, the animals were kept for an observation period of 4 days.

4. Evaluation of local reactions
   Cutaneous reactions were evaluated on day 1 before administration and 1 hour after treatment, on day 2, approximately 24 hours after the end of the administration period and then once a day on days 3 and 4, according to the following scoring scale:

   Erythema and eschar formation:
   . no erythema 0
   . very slight erythema (barely perceptible) 1
   . well-defined erythema 2
   . moderate to severe erythema 3
   . severe erythema (beet redness) to slight eschar formation (injuries in depth) 4

   Edema formation:
   . no edema …..... 0
   . very slight edema (barely perceptible) 1
   . slight edema (edges of area well-defined by definite raising) 2
   . moderate edema (raised approximately 1 millimeter) 3
   . severe edema (raised more than 1 millimeter and extending beyond area of exposure).. 4

   Other lesions including hematoma, scabs, induration were noted.

Results: There were no unscheduled deaths occurred and no recorded systemic clinical signs during the study.

1. Local reaction
   No erythema and no edema were observed at intravenous, intra-arterial and intramuscular injection sites treated with the test item. Hematoma or induration was observed at test item-treated or control sites. Erythema and edema were observed at subcutaneous injection sites treated with the test item with a slightly higher incidence and/or severity when compared to control sites. Severe edema, observed 1 hour after subcutaneous administration in 2/3 animals, was considered to be related to the injection procedure. Hematoma was also noted at the injection sites treated with the test item of the same animals.

Group	1		2		3		4	
Administration route	Intravenous		Intra-arterial		Subcutaneous		Intramuscular	
Treatment	Control item	Test item	Control item	Test item	Control item	Test item	Control item	Test item
Erythema								
- very slight	-	-	-	-	1	1	-	-
- well-defined	-	-	-	-	-	1	-	-
- LHe	1	-	3	2	-	-	-	-
Edema								
- slight	-	-	-	-	1	1	-	-
- severe	-	-	-	-	-	2	-	-
- LI	-	-	-	-	-	-	-	1
Hematoma	1	2	3	3	-	2	-	1
Induration	-	-	-	-	-	-	-	1
Total affected animals	*1/3*	*2/3*	*3/3*	*3/3*	*1/3*	*2/3*	*0/3*	*1/3*

- : not observed,
LHe : reading masked by hematoma,
LI : reading masked by induration.

2. Pathology

Macroscopic *post-mortem* examination:

No test item-related macroscopic *post-mortem* lesions were noted in any injection sites.

Microscopic examination

- Injection sites:
  At injection sites level, occasional minimal to slight test item treatment-related findings were observed in intravenous (males), subcutaneous (females) and intra-muscular (males and females) routes, while no differences were observed with intra-arterial route.
- Intravenous route:
  Minimal perivenous collagen degradation and mononuclear cell infiltrate and slight perivenous heterophil infiltrate were absorbed in the left treated site of males. There were no differences between control (right) and treated (left) sites in female. The other findings were considered to be related to the procedure administration (perivenous hemorrhage) or were considered as incidental common findings in rabbits (follicular cyst, serocellular crust or dermal subacute inflammation at the tip of ear).
- Intra-arterial route:
  No differences were observed with intra-arterial route between control (right) and treated (left) sites. Occasional needle track lesions were seen and consisted of focal subendothelial thickening or disruption of arterial wall accompanied by peri-arterial hemorrhage.
- Subcutaneous route:
  In female, slight collagen degradation was seen in the left treated site. There were no differences between control (right) and treated (left) sites in males. The other findings were considered to be related to the procedure administration (subcutaneous hemorrhage and infiltrate of mononuclear cells).
- Intramuscular route:
  Minimal to slight mononuclear cell infiltrate was seen in the left injected muscle of male and female. The other findings were considered to be related to the procedure administration (interstitial edema, degeneration/regeneration of myofibres, muscular hemorrhage, infiltrate of mononuclear cell in the fascia of muscle).

38060 TAL (GLP-Yes) – Local tolerance study in the rabbits following one subcutaneous injection

Summary and reviewer's conclusion

The objective of this study was to evaluate the local tolerance of the test item, CSL830 in direct comparison to the current marketed C1-inhibitor product Berinert® P, and its formulation buffer, following one subcutaneous injection in rabbits. The test item, CSL830 or the current marketed C1-inhibitor product Berinert® P, was administered once to (b) (4) Rabbits by subcutaneous injection. The data indicate that CSL830, Berinert® P and the placebo Berinert formulation buffer were considered to be locally well tolerated after a single subcutaneous injection.

Materials and Methods

1. Test item

   CSL830 (Berinert 1500)
   - Batch number : (b) (4)
   - Protein concentration : (b) (4)
   - Potency : (b) (4)
   - (b) (4)

   Berinert® P
   - Batch number : (b) (4)
   - Protein concentration : (b) (4)
   - Potency : (b) (4)
   - (b) (4)

   Placebo (Berinert formulation buffer: (b) (4) sodium chloride, (b) (4) glycine)
   - Batch number: (b) (4)

   Sterile (b) (4) saline solution (0.9% NaCl)
   - batch Number: (b) (4)
   - supplier: (b) (4)

   Vehicle (Water for injections)
   - batch number: (b) (4)
   - supplier: (b) (4)

2. Test system
   - Strain and species: (b) (4) rabbits
   - Number: 9 rabbits (6 males and 3 females)
   - Breeder: (b) (4)
   - Age/Weight: 3 to 4 month old, male 2479 g (range: 2350 g to 2565 g), females 2457 g (range: 2425 g to 2500 g).

3. Treatment

   Treatment group

Group	number and identity of animals	dosage-volume (ml/injection)	Treatment left side	Right side
1	2 males W30301 and W30302 1 female	3	test item No. 1	control item

	W30311			
2	2 males W30303 and W30304 1 female W30312	3	test item No. 2	control item
3	2 males W30305 and W30306 1 female W30313	3	Placebo	control item

Administration
: subcutaneous injection on the median flank, 3 mL/injection.

Duration
The dosage forms were administered once by subcutaneous injection. Following the administration, the animals were kept for a 4-day observation period.

4. Evaluation of local reactions
Cutaneous reactions were evaluated on day 1 before administration and 1 hour after treatment, on day 2, approximately 24 hours after the end of the administration period and then once a day on days 3 and 4, according to the following scoring scale:

Erythema and eschar formation:
. no erythema 0
. very slight erythema (barely perceptible) 1
. well-defined erythema 2
. moderate to severe erythema 3
. severe erythema (beet redness) to slight eschar formation (injuries in depth) 4

Edema formation:
. no edema …..... 0
. very slight edema (barely perceptible) 1
. slight edema (edges of area well-defined by definite raising) 2
. moderate edema (raised approximately 1 millimeter) 3
. severe edema (raised more than 1 millimeter and extending beyond area of exposure).. 4

Other cutaneous lesions including hematoma, scabs, induration petechiae, desquamation were noted.

Results: There were no unscheduled deaths or premature sacrifices and no clinical signs of systemic toxicity during the study.

1. Local reaction
No reactions were observed at the sites injected with NaCl 0.9%. No significant differences were observed at the sites injected with CSL830, Berinert® P or the placebo. Eythema was generally

observed after treatment and sometimes persisted until day 3. The severity of this reaction was considered as slight (the grade well-defined was only used on day 1 in one animal injected with Berinert® P). On day 4, two animals injected with one of the test items showed dryness at the injection sites. Hematoma was also noted in a single animal at the sites injected with CSL830 or the placebo after treatment and lasted until day 4. No edema was reported although this reaction could be masked 1 hour after dosing by the treatment itself.

Group	1		2		3	
Treatment	CSL830	0.9% NaCl	Berinert® P	0.9% NaCl	Berinert formulation buffer	0.9% NaCl
Erythema - very slight	1/3 (days 1 to 3)	-	2/3 (days 1 [a)] to 3 or day 2)	-	1/3 (day 1)	-
Hematoma	1/3 (days 1 to 4)	-	-	-	1/3 (days 1 to 4)	-
Dryness	1/3 (day 4)	-	1/3 (day 4)	-	-	-
Total affected animals	*2/3*	*0/3*	*2/3*	*0/3*	*2/3*	*0/3*

- : not observation.
[a)]: well-defined erythema on day 2.

2. **Pathology**

Macroscopic *post-mortem* examination:
No test item-related macroscopic *post-mortem* lesions were noted in any injection sites. A rediscoloration was observed at the injection site 2 of one male given CSL830. This correlated with minimal to slight hemorrhage in the subcutis and dermis and was considered to be related to the injection procedure.

Microscopic examination
At the injection sites, minimal acanthosis, occasionally associated with hyperkeratosis or minimal serocellular crust, was observed in most animals regardless of the injection site (0.9% NaCl, placebo or test items). A minimal or slight infiltrate of mononuclear inflammatory cells, associated with more or less heterophils, was seen in the upper dermis at sites injected with the control item, the placebo or test items, extending in the deep dermis in one animal given CSL830 and one given Berinert® P. These minimal changes were considered to be of minor importance and probably related to the administration procedure. Other changes observed occasionally (e.g. minimal infiltrate of mononuclear cell or fibroplasia in subcutis, or degeneration/regeneration of myofibers) were also considered to be related to the injection procedure.

VTK 03/09 (GLP-No) Evaluation of prothrombotic effects of Berinert® P

Summary and reviewer's conclusion
This study was conducted to investigate the thrombogenic potential of Berinert® P using an in vivo thrombogenicity test in rabbits which produces temporary venous stasis by ligating an appropriate vein and taking thrombosis incidence and thrombus dry weight as parameters for evaluation and comparison. Following i.v. administration of Berinert® P at doses of 200 to 800 U/kg, representing the 10-40 fold of the recommended clinical dose, there was no indication of thrombus formation. The data indicate that

intravenous injection of Berinert® P does not pose any thrombogenic potential at doses up to 800 U/kg under the conditions of this study

Methods

1. Study design
   The study was designed as a three-armed open trial in a total of 12 female (b) (4) rabbits. The study was performed in two parts. The second part was amended to complete the animal number to 4 per group.

   Treatment groups

Treatment	Dose (U/kg)	Volume mL/kg	Schedule	Route	N (f)
Berinert® P	200	Part 1: 3.76 mL/kg Part 2: 3.87 mL/kg	Part 1: 0.25 mL/kg*min^1 Part 2: 0.256 mL/kg*min^2 t= 0-15 min	i.v.	1 3
Berinert® P	400	Part 1: 7.52 mL/kg Part 2: 7.74 mL/kg	Part 1: 0.50 mL/kg*min^1 Part 2:0.516 mL/kg*min^2 t= 0-15 min;	i.v.	1 3
Berinert® P	800	Part 1:15.04 mL/kg Part 2: 15.47 mL/kg	Part 1: 1.00 mL/kg*min^1 Part 2: 1.032 mL/kg*min^2 t= 0-15 min;	i.v.	3 1

2. Test item
   Part 1: Berinert® P
   - Lot number : 24961711
   - Reconstitution : 53.2 U/mL
   - Dose: 200 – 800 U/kg
   - Route: I.V.
   - Volume: 3.76 – 15.04 mL/Kg

   Part 2: Berinert® P
   - Lot number : 34561711
   - Reconstitution : 51.7 U/mL
   - Dose: 200 – 800 U/kg
   - Route: I.V.
   - Volume: 3.87 – 15.47 mL/Kg

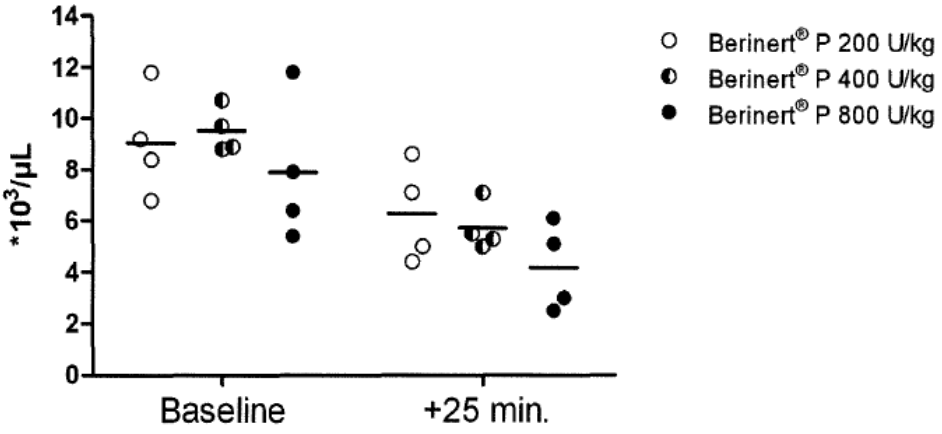
3. Test system
   - Strain and species: (b) (4) rabbits
   - Number: 12 female rabbits
   - Supply: (b) (4)
   - Age/Weight: 3 to 4 month old, range 2.6 kg to 3.3kg.

   To assess the thrombogenic potential of Berinert® P following i.v. administration, the animals were anesthetized. Once anesthetized, a jugular vein was exposed and a segment of approximately 2 cm was isolated. The test substance was infused over 15 minutes via the contra lateral ear vein. Ten minutes

after the end of the infusion, a ligature was placed around the isolated jugular vein. Blood was allowed to fill the vein segment, then a second ligature was placed approximately 1.5 cm cranial to the first one, causing a complete stasis in the isolated segment. Thirty minutes after stasis induction the vein segment was excised and dissected in a petri dish filled with sodium citrate solution. Any observed thrombi were graded according to a scoring system from 0 to 3, and their wet weight was determined.
Thrombus scores were defined as follows: 0 =no clot, 1 =one or a low number of small clots (too small to determine weights), 2 = not fully occluding clot (one or several clots of bigger size; weight can be measured), 3 =fully occluded clot (vein segment fully occluded)

Results
The results obtained using a modified (b) (4) assay to investigate the thrombogenic potential of Berinert® P following i.v. infusion showed no thrombus formation at any of the doses of Berinert® P tested. There were also no effects on hematological parameters measured with the exception of a dose-dependent reduction in WBC at 25 min post infusion compared to baseline levels.

			Venous Thrombosis Score		Thrombus wet weight (mg)	
		N	Mean (SD)	Range	Mean (SD)	Range
Berinert® P	200 U/kg	4	0.0 ± 0.0	-	n.m.	n.m.
Berinert® P	400 U/kg	4	0.0 ± 0.0	-	n.m.	n.m.
Berinert® P	800 U/kg	4	0.0 ± 0.0	-	n.m.	n.m.



VTK 12/11 (GLP-No) Evaluation of prothrombotic effects of CSL830 (C1 esterase inhibitor (human)) following I.V. adminstration

Summary and reviewer's conclusion
Berinert® and CSL830 are a (b) (4) presentation of Berinert®, comprises the (b) (4) C1-INH concentration and (b) (4) compared to Berinert®. This study was conducted to investigate the thrombogenic potential of Berinert® and CSL830 using an in vivo thrombogenicity test in rabbits. Following i.v. administration of Berinert® and CSL830, there was no

indication of thrombus formation. The data indicate that intravenous injection of Berinert® and CSL830 do not pose any thrombogenic potential at doses up to 800 U/kg under the conditions of this study.

Methods

1. Study design
   The study was designed as a three-armed open trial in a total of 10 female (b) (4) rabbits.

   Treatment groups

Treatment	Dose (U/kg)	Volume mL/kg	Schedule	Route	N
CSL830	200	0.395 mL/kg 0.371 mL/kg	0.0263 mL/kg*min. 0.0247 mL/kg*min.	i.v.	2 2
CSL830	800	1.581 mL/kg 1.484 mL/kg	0.1054 mL/kg*min. 0.099 mL/kg*min.	i.v.	2 2
Feiba NF 500E	50	2 mL/kg	0.1333 mL/kg*min.	i.v.	2

2. Test articles
   Test article 1: CSL830
   - Lot number : 00368811
   - Reconstitution : 506 U/mL
   - Dose: 200 and 800 U/kg
   - Route: I.V.
   - Volume: 0.395 – 1.581 mL/Kg

   Test article 2: CSL830
   - Lot number : 00168811
   - Reconstitution : 539 U/mL
   - Dose: 200 and 800 U/kg
   - Route: I.V.
   - Volume: 0.37 – 1.484 mL/Kg

   Test article 3: (b) (4)
   - Lot number : VNF2K039A
   - Reconstitution : 25 U/mL
   - Dose: 50 U/kg
   - Route: I.V.
   - Volume: 2 mL/Kg

3. Test system
   - Strain and species: (b) (4) rabbits
   - Number: 10 female rabbits
   - Supply: (b) (4)
   - Age/Weight: 3 to 4 month old, range 2.4 kg to 3.0kg.

   To assess the thrombogenic potential of Berinert® P following i.v. administration, the animals were anesthetized. Once anesthetized, a jugular vein was exposed and a segment of approximately 2 cm was isolated. Side branches were occluded using titanium clips. The test substance was infused over 15 minutes via the contra lateral ear vein. Ten minutes after the end of the infusion, a ligature was placed

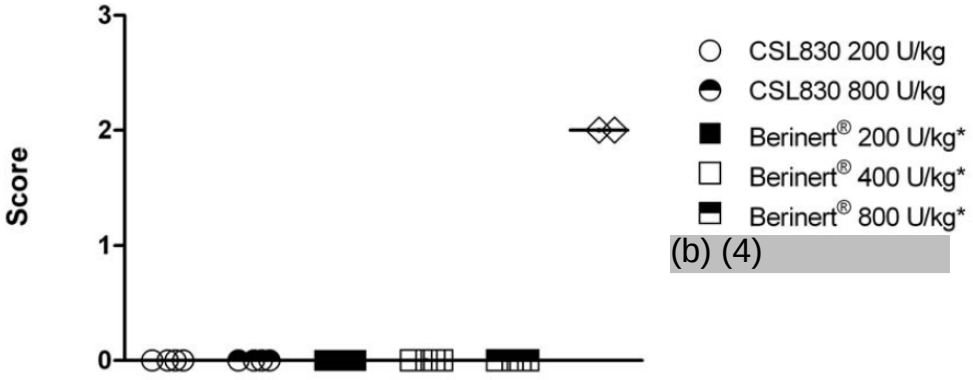
around the isolated jugular vein. Blood was allowed to fill the vein segment, then a second ligature was placed approximately 1.5 cm cranial to the first one, causing a complete stasis in the isolated segment. Thirty minutes after stasis induction the vein segment was excised and dissected in a petri dish filled with sodium citrate solution. Any observed thrombi were graded according to a scoring system from 0 to 3, and their wet weight was determined.
Thrombus scores were defined as follows: 0 =no clot, 1 =one or a low number of small clots (too small to determine weights), 2 = not fully occluding clot (one or several clots of bigger size; weight can be measured), 3 =fully occluded clot (vein segment fully occluded)

Results

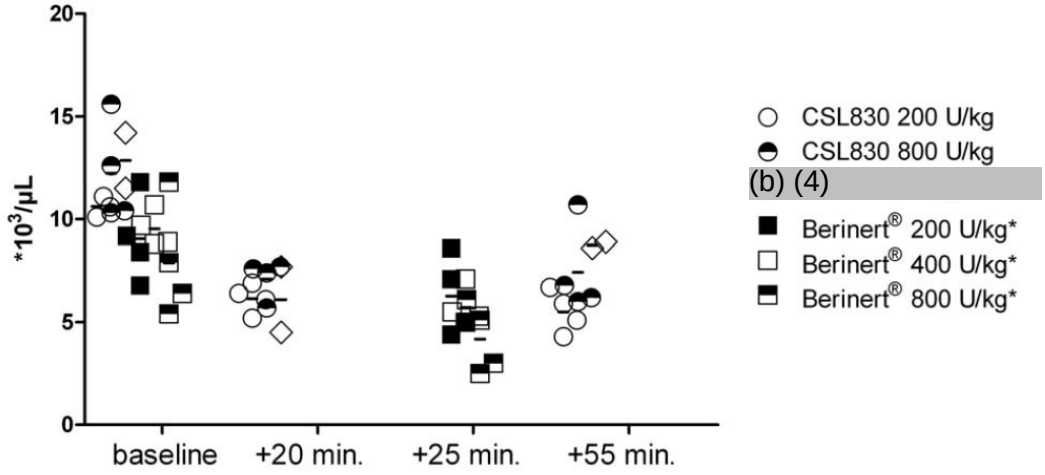
The results obtained using a modified (b) (4) assay following i.v. infusion showed that no thrombus formation can be observed at any of the doses of CSL830 or Berinert® tested.

	Dose U/kg	N	Venous Thrombosis Score Mean (SD)	Range	Thrombus wet weight (mg) Mean (SD)	Range
CSL830	200	4	0.0	-	n.m.	n.m.
CSL830	800	4	0.0	-	n.m.	n.m.
(b) (4)	50	2	2.0 ± 0.0	-	36.0 ± 21.2	21 - 51
Berinert®	200	4	0.0	-	n.m.	n.m.
Berinert®	400	4	0.0	-	n.m.	n.m.
Berinert®	800	4	0.0	-	n.m.	n.m.

Thrombus size score following i.v. infusion of CSL830 and Berinert®



There were also no effects on hematological parameters measured with the exception of a dose-dependent reduction in WBC at 25 min post infusion compared to baseline levels.


20
15
10
5
0
*10^3/µL
baseline
+20 min.
+25 min.
+55 min.
CSL830 200 U/kg
CSL830 800 U/kg
(b) (4)
Berinert® 200 U/kg*
Berinert® 400 U/kg*
Berinert® 800 U/kg*