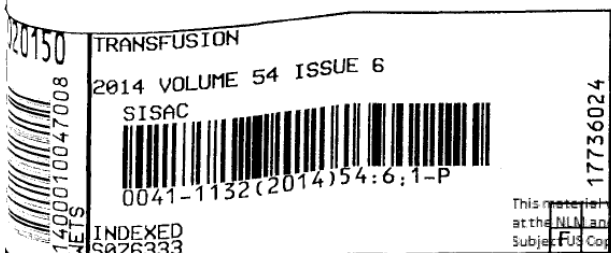
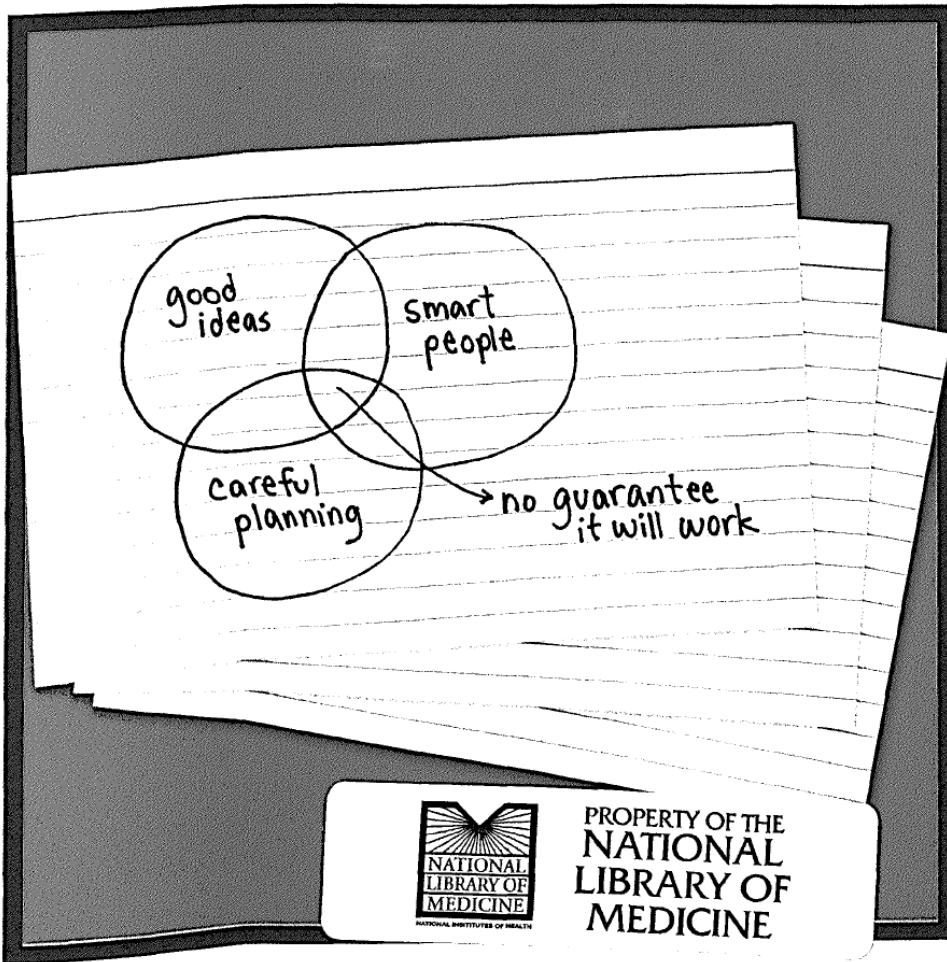


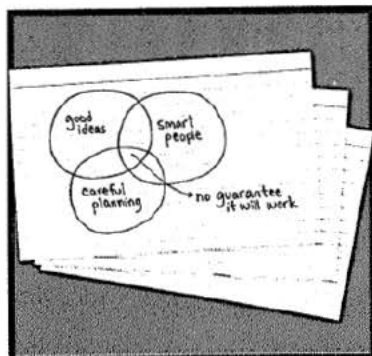
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# Table of Contents



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## EDITORIALS

- 1447 **Transfusion CME hits 50**  
*A.F. Eder and P.M. Ness*
- 1448 **"Do you catch my drift?"**  
*T. Hannon*
- 1450 **Can a comparative database study help to develop an effective risk index system?**  
*J. Dalton and E. Farag*

## HOW DO I...?

- 1452 **How do we use molecular red blood cell antigen typing to supplement pretransfusion testing?**  
*S. Sapatnekar and P.I. Figueroa*

## TRANSFUSION MEDICINE ILLUSTRATED

- 1459 **Schistocytes**  
*J.-F. Lesesve, O. Fenneteau, and G. Zini*

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## TABLE OF CONTENTS (Continued)

### BLOOD MANAGEMENT

- 1460 **Clinical predictors of postoperative hemoglobin drift**  
M.C. Grant, G.J. Whitman, W.J. Savage, P.M. Ness, and S.M. Frank
- 1469 **Predicting blood loss and transfusion requirement in patients undergoing surgery for musculoskeletal tumors**  
P.A. Thompson, D. May, P.F. Choong, M. Tacey, D. Liew, and M.F. Cole-Sinclair

### IMMUNOHEMATOLOGY

- 1478 **Expression of a single-chain human leukocyte antigen-DRA/DRB3\*01:01 molecule and differential binding of a monoclonal antibody in the presence of specifically bound human platelet antigen-1a peptide**  
E.E. Bouwmans, P.A. Smethurst, S.F. Garner, W.H. Ouwehand, and S.L. Morley
- 1486 **A new bead-based human platelet antigen antibodies detection assay versus the monoclonal antibody immobilization of platelet antigens assay**  
L. Porcelijn, E. Huiskes, I. Comijs-van Osselen, A. Chhatta, V. Rathore, M. Meyers, and M. de Haas

### TRANSPLANTATION AND CELLULAR ENGINEERING

- 1493 **Superiority of preemptive donor lymphocyte infusion based on minimal residual disease in acute leukemia patients after allogeneic hematopoietic stem cell transplantation**  
Y. Tan, K. Du, Y. Luo, J. Shi, L. Cao, Y. Zheng, G. Zheng, Y. Zhao, X. Ye, Z. Cai, and H. Huang
- 1501 **Evaluation of peripheral blood stem cell quality in products transported by traditional courier or commercial overnight shipping services**  
A. Howard, P. Chitphakdithai, E.K. Waller, W. Harris, H. Rosenthal, W. Nelson, N. Majhail, W. Navarro, S. Waldvogel, J. Lisy, J. Uecker, J. Miller, and S.R. Spellman
- 1508 **Cryopreserved stem cell products containing dimethyl sulfoxide lead to activation of the coagulation system without any impact on engraftment**  
A. Holbro, L. Graf, M. Topalidou, C. Bucher, J.R. Passweg, and D.A. Tsakiris
- 1515 **The natural killer-activating receptor, NKG2D, on CD3+CD8+ T cells plays a critical role in identifying and killing autologous myeloma cells**  
L. Talebian, D.A. Fischer, J. Wu, J.Y. Channon, C.L. Sentman, M.S. Ernstoff, and K.R. Meehan

### TRANSFUSION PRACTICE

- 1522 **Continuing Medical Education Program in Transfusion**  
A.F. Eder
- 1523 **The impact of platelet additive solution apheresis platelets on allergic transfusion reactions and corrected count increment**  
A.A.R. Tobian, A.K. Fuller, K. Uglik, D.J. Tisch, P.D. Borge, R.J. Benjamin, P.M. Ness, and K.E. King
- CME** 1530 **Perioperative and transfusion outcomes in women undergoing cesarean hysterectomy for abnormal placentation**  
K.F. Brookfield, L.T. Goodnough, D.J. Lyell, and A.J. Butwick
- 1537 **The use of an objective structured clinical examination to assess internal medicine residents' transfusion knowledge**  
E. Saldenberg and D. Pugh
- 1542 **Deferasirox improves hematologic and hepatic function with effective reduction of serum ferritin and liver iron concentration in transfusional iron overload patients with myelodysplastic syndrome or aplastic anemia**  
J.-W. Cheong, H.-J. Kim, K.-H. Lee, S.-S. Yoon, J.H. Lee, H.-S. Park, H.Y. Kim, H. Shim, C.-M. Seong, C.S. Kim, J. Chung, M.S. Hyun, D.-Y. Jo, C.W. Jung, S.K. Sohn, H.-J. Yoon, B.S. Kim, Y.-D. Joo, C.-Y. Park, and Y.H. Min for the Korean Society of Hematology Acute Myeloid Leukemia/Myelodysplastic Syndrome Working Party
- 1552 **Pharmacokinetics of plasma-derived C1-esterase inhibitor after subcutaneous versus intravenous administration in subjects with mild or moderate hereditary angioedema: the PASSION study**  
I. Martinez-Saguer, M. Cicardi, C. Suffritti, E. Rusicke, E. Aygören-Pürsün, H. Stoll, T. Rossmannith, A. Feussner, U. Kalina, and W. Kreuz

### BLOOD COMPONENTS

- 1562 **In vitro properties of platelets stored in a small container for pediatric transfusion**  
N. Tynngård, M. Trinks, and G. Berlin
- 1569 **Isoprostane and isofuran lipid mediators accumulate in stored red blood cells and influence platelet function in vitro**  
S.L. Spinelli, K.L. Lannan, A.E. Casey, A. Croasdel, T.M. Curran, K.F. Henrichs, S.J. Pollock, G.A. Milne, M.A. Refaai, C.W. Francis, R.P. Phipps, and N. Blumberg
- 1580 **Comparison of antibody titers in donor specimens and associated AS-1 leukoreduced donor units**  
E.A. Hill and B.J. Bryant
- 1585 **Quality of red blood cells washed using an automated cell processor with and without irradiation**  
A.L. Hansen, T.R. Turner, Q.-L. Yi, and J.P. Acker
- 1595 **The effects of rejuvenation during hypothermic storage on red blood cell membrane remodeling**  
J.D.R. Kurach, R. Almizraq, B. Bicalho, J.P. Acker, and J.L. Holovati

## TABLE OF CONTENTS (Continued)

- 1604 **Leukoreduced whole blood–derived platelets treated with antimicrobial peptides maintain in vitro properties during storage**  
*M. Bosch-Marcé, S. Seetharaman, J. Kurtz, K.V.K. Mohan, S.J. Wagner, and C.D. Atreya*
- 1610 **Determination of thromboelastographic responsiveness in stored single-donor platelet concentrates**  
*I.J. Bontekoe, P.F. van der Meer, and D. de Korte*

## BLOOD GROUP GENOMICS

- 1619 **HNA-3 gene frequencies in Brazilians and a new polymerase chain reaction–restriction fragment length polymorphism method for HNA-3a/3b genotyping**  
*L.B. Lopes, W. Baleotti Jr, R.B. Suzuki, A. Fabron Jr, A.K. Chiba, J.P.B. Vieira-Filho, B. de Souza Castro, A.M. Kunioshi, and J.O. Bordin*

## HEMAPHERESIS

- 1622 **Unstimulated leukapheresis in patients and donors: comparison of two apheresis systems**  
*M. Schulz, H. Bialleck, K. Thorausch, G. Bug, U. Dünzinger, E. Seifried, and H. Bönig*
- 1630 **Severe pertussis and hyperleukocytosis: is it time to change for exchange?**  
*A. Kuperman, Y. Hoffmann, D. Glikman, H. Dabbah, and Z. Zonis*

## DONOR INFECTIOUS DISEASE TESTING

- 1634 **Evaluation of a rapid colorimetric assay for detection of bacterial contamination in apheresis and pooled random-donor platelet units**  
*W.A. Heaton, C.E. Good, R. Galloway-Haskins, R.A. Yomtovian, and M.R. Jacobs*
- 1642 **A novel approach for rapid detection of bacterially contaminated platelet concentrates via sensitive measurement of microbial DNA polymerase activity**  
*D.R. Zveitzig, N.M. Riccardello, J.M. Pester, R. Jeanmonod, M.J. Kopnitsky, and S.M. O'Hara*
- 1652 **Estimating window period blood donations for human immunodeficiency virus Type 1, hepatitis C virus, and hepatitis B virus by nucleic acid amplification testing in Southern Pakistan**  
*B. Moiz, T. Moatter, U. Shaikh, S. Adil, N. Ali, F. Mahar, N. Shamsuddin, and M. Khurshid*

## BLOOD DONORS AND BLOOD COLLECTION

- 1660 **Acute hepatitis B in blood donors over a 5-year period in England and North Wales: who is getting infected?**  
*G.K. Rosenberg, S. Lattimore, S.R. Brailsford, P.E. Hewitt, K.I. Tettmar, A.D. Kitchen, S. Ijaz, and R.S. Tedder*

## TRANSFUSION COMPLICATIONS

- 1666 **Incidence of acute transfusion reactions to platelets in hospitalized pediatric patients based on the US hemovigilance reporting system**  
*N. Li, L. Williams, Z. Zhou, and Y.-Y. Wu*

## REVIEW

- 1673 **Adoptable strategic approaches to improve outcomes of allogeneic peripheral blood stem cell transplantations from unrelated donors**  
*S.K. Sohn and J.H. Moon*

## LETTERS TO THE EDITOR

- 1681 **Fresh-frozen plasma should not be given to nonbleeding premature infants with “abnormal” coagulation tests**  
*A.K. Keir and S. Stanworth*
- 1681 **In reply:**  
*C. Dani*
- 1682 **Parvovirus B19 Genotype 2 in blood donations**  
*A.M. Eis-Hübinger, U. Reber, A. Edelmann, U. Kalus, and J. Hofmann*
- 1685 **Does transfusion of salvaged blood products have a significant impact on coagulation?**  
*B. Ferro, J. Frugiuele, C. Faldini, and S. Bonarelli*

## OBITUARY

- 1686 **George Garratty, PhD, FIBMS, FRCPath, 1935-2014**  
*L.D. Petz*

## ERRATUM

1688

## Pharmacokinetics of plasma-derived C1-esterase inhibitor after subcutaneous versus intravenous administration in subjects with mild or moderate hereditary angioedema: the PASSION study

Inmaculada Martinez-Saguer,<sup>1</sup> Marco Cicardi,<sup>2</sup> Chiara Suffritti,<sup>2</sup> Eva Rusicke,<sup>3</sup>  
Emel Aygören-Pürsün,<sup>3</sup> Hildegard Stoll,<sup>4</sup> Tanja Rossmann,<sup>5</sup> Annette Feussner,<sup>6</sup> Uwe Kalina,<sup>6</sup> and  
Wolfgang Kreuz<sup>1</sup>

**BACKGROUND:** Hereditary angioedema (HAE) is a rare disease caused by C1-esterase inhibitor (C1-INH) deficiency, characterized by periodic attacks of acute edema affecting subcutaneous (SC) tissues and mucous membranes. Human C1-INH concentrate given intravenously (IV) is effective and safe, but venous access may be difficult. We compared SC and IV administration of human pasteurized C1-INH concentrate with respect to pharmacokinetics, pharmacodynamics, and safety.

**STUDY DESIGN AND METHODS:** This was a prospective, randomized, open-label, crossover study. Twenty-four subjects with mild or moderate HAE were randomly assigned during an attack-free interval to receive 1000 units of human pasteurized C1-INH concentrate IV or SC. Plasma levels of C1-INH activity and antigen, C4 antigen, cleaved high-molecular-weight kininogen (clHK), and C1-INH antibodies were measured.

**RESULTS:** The mean relative bioavailability of functional C1-INH after SC administration was 39.7%. Maximum C1-INH activity after SC administration occurred within 48 hours and persisted longer than after IV administration. C4 antigen levels increased and clHK levels decreased after IV and SC administration, indicating the pharmacodynamic action of C1-INH. The mean half-life of functional C1-INH was 62 hours after IV administration and 120 hours after SC administration ( $p = 0.0595$ ). C1-INH concentrate was safe and well tolerated when administered via both routes. As expected, SC administration resulted in a higher incidence of injection site reactions, all of which were mild.

**CONCLUSION:** With a relative bioavailability of 39.7%, SC administration of human pasteurized C1-INH yields potentially clinically relevant and sustained plasma levels of C1-INH and is safe and well tolerated.

**ABBREVIATIONS:** AE(s) = adverse event(s); AUC = area under the curve; C1-INH = C1-esterase inhibitor; CL = total clearance; clHK = cleaved high-molecular-weight kininogen;  $C_{max}$  = maximum concentration; HAE = hereditary angioedema; HK = high-molecular-weight kininogen; MRT = mean residence time; SC = subcutaneous;  $t_{1/2}$  = terminal elimination half-life;  $T_{max}$  = time of maximum concentration;  $V_z$  = volume of distribution at the terminal phase.

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**H**ereditary angioedema (HAE), caused by functional deficiency of C1-esterase inhibitor<sup>1</sup> (C1-INH), is a rare disease characterized by recurrent, spontaneous, nonallergic edema in subcutaneous (SC) tissues and mucous membranes. In case of laryngeal edema, HAE is associated with high mortality rates when there is a delay in treating the attacks.<sup>2,3</sup> HAE is a debilitating disease that can have a severe effect on quality of life.

C1-INH is a serine protease inhibitor that controls vascular permeability by acting on the initial activation phase of the complement, coagulation, contact, and fibrinolytic systems. The functional deficiency of C1-INH leads to increased activation of plasma kallikrein and Factor (F)XIIa with a subsequent release of bradykinin, which is a key mediator of vascular permeability.<sup>4</sup> Additionally, C1-INH is the main inhibitor of FXIa, which plays an important role in the generation of thrombin, a positive modulator of vasopermeability.<sup>5-8</sup>

HAE Type I results from a quantitative deficiency in functional C1-INH, whereas the less common HAE Type II, affecting 15% of patients, results from a dysfunctional form of C1-INH circulating at normal or elevated plasma concentrations.<sup>4</sup> Both defects are inherited as an autosomal dominant trait. HAE Type III is extremely rare, with mainly women being clinically affected; it is not associated with C1-INH deficiency and its pathophysiology is uncertain.<sup>9</sup> Common anti-inflammatory treatments, such as corticosteroids, antihistamines, or epinephrine, are usually inappropriate for treating acute attacks caused by HAE.<sup>10</sup> Clinical studies,<sup>11-13</sup> as well as more than 30 years of clinical use,<sup>14,15</sup> have shown that intravenous (IV) C1-INH replacement therapy with human C1-INH concentrate is an effective and safe treatment for acute edema attacks in patients with HAE. Therefore, C1-INH concentrate is recommended as first-line therapy in this indication.<sup>16</sup>

In patients with HAE requiring frequent IV treatment with C1-INH concentrate, either for acute edema attacks or for prophylaxis, venous access may become difficult over time. The SC administration of C1-INH concentrate is therefore being investigated as a potential alternative therapeutic approach, specifically for the prophylactic treatment of HAE. In support of this approach, a preclinical study with CSL Behring's human pasteurized C1-INH concentrate (Berinert, CSL Behring, Marburg, Germany) revealed a relative bioavailability of approximately 70% after SC administration in rabbits, compared with IV administration (Ingo Pragst, CSL Behring, May 2013). Building on this preclinical experience, the primary objective of our study was to compare the pharmacokinetics of the same preparation of C1-INH concentrate after IV and SC administration in subjects with mild or moderate HAE during an attack-free interval, evaluating the relative bioavailability of SC administration based on plasma levels

of C1-INH activity. In addition to assessing the safety and tolerability of C1-INH concentrate when administered via both these routes, we also assessed plasma levels of C1-INH antigen and cleaved high-molecular-weight kininogen (cHK), serum levels of C4 antigen, and the presence of C1-INH antibodies after treatment. These additional endpoints were assessed to provide insight into the pharmacokinetic and pharmacodynamic effects of the C1-INH concentrate administered.

## MATERIALS AND METHODS

### Study design and treatment

This was a single-center, prospective, randomized, open-label, crossover study in adults with mild or moderate HAE Type I or Type II. The study was conducted between September 22, 2008 (first subject first visit), and November 1, 2010 (last subject last visit). Subjects enrolled into the study had to present during an attack-free interval. In total, 24 subjects were each randomly assigned to one of two treatment sequences, AB or BA (A = IV; B = SC), at a ratio of 1:1. Blinded randomization envelopes were used. Blinding procedures were otherwise not needed because the study involved treatment with only one study drug. The sample size was calculated in accordance with the guideline on clinical investigation of recombinant or human plasma-derived FIX products (EMA/CHMP/BPWP/144552/2009).

According to the randomization sequence, human pasteurized C1-INH concentrate (Berinert, CSL Behring) was administered during an attack-free interval as either an IV infusion or an SC infusion, in each case as a single dose of 1000 U in 20 mL of solution, with a washout period of at least 7 days before study enrollment and between the two treatments. The IV infusion was administered over a time period of 3 minutes. The SC infusion was administered over a 15-minute period using two medical infusion pumps for continuous, simultaneous administration (MEDIS Infusa T1 portable syringe driver, OMT, Minden, Germany) of two doses of 500 U each at two different locations in the abdomen. A follow-up visit was performed 3 months after the second administration of C1-INH concentrate.

Plasma samples for pharmacokinetic and pharmacodynamic analyses were collected at 0, 0.25, 0.5, 0.75, 1, 2, 4, 6, 8, 12, 16, 20, 24, 36, 48, 60, 72, 120, and 168 hours after each treatment. In addition, after amending the protocol during the study, additional sampling times (at 336 ± 48 and 504 ± 48 hr after treatment) were introduced for the analysis of C1-INH activity and antigen levels, and C4 antigen levels, to investigate pharmacokinetic and pharmacodynamic trends over a longer period in six subjects. The analysis of cHK levels was also added, for which blood samples were collected at 0, 12, 36, 60, 168, and 504 hours after treatment in these six subjects.

### Study population

The study was conducted at the University Hospital Frankfurt (Frankfurt am Main, Germany), after receiving approval from the applicable ethics committee. All potential study participants provided written informed consent before being screened for eligibility. Men and women could only be enrolled if they were at least 18 years of age and presented with mild or moderate HAE Type I (C1-INH activity < 50% and C1-INH antigen < 15.4 mg/dL) or Type II (C1-INH activity < 50% and C1-INH antigen in normal or elevated concentrations) during an attack-free interval. All but two of the enrolled subjects had mild HAE. Subjects were excluded from the study if HAE was not diagnosed or if their last treatment with C1-INH concentrate (i.e., their last attack) had occurred within 7 days before enrollment. Subjects with acquired angioedema and all other types of angioedema not associated with C1-INH deficiency were also excluded. Additionally, subjects who had received any investigational drug (excluding drugs appropriate for treating acute angioedema) during the 30 days before treatment in the current study, subjects who had received any other drug appropriate for the treatment of acute angioedema within 7 days before each administration of study drug in the current study, and subjects undergoing prophylaxis with danazol, antifibrinolytics,  $\epsilon$ -aminocaproic acid, or tranexamic acid were also excluded. Further exclusion criteria included known hypersensitivity to the study drug, pregnancy (a rapid pregnancy test was required for women of childbearing potential), breast-feeding, malignant disease, immunodeficiency, and concurrent serious or acute illness or infection.

### Pharmacokinetic and pharmacodynamic assessments

The primary endpoint was the bioavailability of functional C1-INH after SC administration relative to IV administration of human pasteurized C1-INH concentrate, calculated on the basis of plasma C1-INH activity. Secondary endpoints were analyses of plasma C1-INH activity and antigen levels, serum C4 antigen levels, and plasma c1HK levels.

C1-INH activity was determined using the functional chromogenic assay (Berichrom C1-INH, commercially available from Siemens Healthcare Diagnostics, Eschborn, Germany). Standard human plasma served as the calibrator; the results are expressed as percentage of normal values. The assay was performed according to the manufacturer's instructions. The citrated plasma samples were analyzed undiluted.

Both C1-INH and C4 antigen levels were analyzed using nephelometric technology from Beckman Coulter (Brea, CA). The reagents for C1-INH antigen were obtained from Siemens Healthcare Diagnostics. The

reagents for C4 antigen analysis were obtained from Beckman Coulter. The analysis of serum for C4 levels was conducted according to the manufacturer's instructions. Citrated plasma samples were analyzed undiluted.

c1HK was assessed according to the method of Berrettini and colleagues.<sup>17</sup> Plasma samples underwent nonreducing sodium dodecyl sulfate–polyacrylamide gel electrophoresis (8% wt/vol). After electrophoretic transfer of the separated proteins from the gel onto a polyvinylidene difluoride membrane (Immobilon, Millipore, Bedford, MA), high-molecular-weight kininogen (HK) forms were identified with goat polyclonal anti-light chain-HK antibody that does not recognize the heavy chain (Nordic, Tiburg, the Netherlands) and visualized with a biotinylated rabbit anti-goat antibody (Sigma Chemical Company, St Louis, MO). The apparent molecular masses of proteins were derived by comparison with high-molecular-weight protein markers from Bio-Rad Laboratories (Richmond, CA). With this method, native HK appears as a band in the molecular range of 130 kDa and c1HK is seen as two bands in the molecular ranges of 107 and 98 kDa.

### Safety assessments

Safety endpoints included the incidence of treatment-emergent adverse events (AEs), analyses of standard laboratory safety variables, assessments of vital signs, and assessments of virus serology and the presence of C1-INH antibodies at 1 and 3 months after the last administration of C1-INH concentrate relative to baseline (before the first administration of C1-INH concentrate).

All AEs reported in this study occurred after administration of C1-INH concentrate and were considered treatment emergent. Standardized assessments were used for categorizing AEs according to intensity (mild, moderate, or severe) and causal relationship to administration of C1-INH concentrate (none, unlikely, possible, and probable). Missing values were not imputed.

Standard laboratory safety variables included hematology (hemoglobin [Hb], hematocrit, red blood cell count, total and differential white blood cell count, platelet count, mean corpuscular Hb, mean corpuscular volume), clinical chemistry (creatinine, uric acid, urea, total bilirubin, aspartate aminotransferase, alanine aminotransferase, gamma-glutamyl transferase, amylase, lipase), and coagulation (prothrombin time, activated partial thromboplastin time, fibrinogen).

Viral serology included analysis of hepatitis A virus antibodies, hepatitis B virus surface antigen, antibodies to hepatitis surface B antigen, antibodies to hepatitis B core antigen, hepatitis C virus (HCV) antibodies, human immunodeficiency virus Type 1 (HIV-1) and HIV-2 antibodies, and polymerase chain reaction (PCR) assays for HCV and HIV.

C1-INH antibodies were screened by a highly sensitive direct binding enzyme-linked immunosorbent assay (ELISA) in samples taken at the baseline, 1-month, and 3-month follow-up time points. C1-INH was coated to microplates and probed by citrated plasma samples. Antibodies were detected using a second peroxidase-conjugated antibody complex (Dako Cytomation, Glostrup, Denmark) that was not meant to be specific for single antibody isotypes. To achieve high sensitivity, the assay was designed to allow approximately 5% false-positive results. This cutoff was determined by testing of 140 samples from healthy subjects.

A result for the presence of C1-INH antibodies above cutoff was further characterized by antibody isotyping for relevant antibody isotypes, for example, immunoglobulin (Ig)M, IgG, and IgA (Sigma). In addition, C1-INH antibodies were described by obtaining relative antibody titers.

All samples with confirmed ELISA-based antibody responses above cutoff were analyzed further using a Bethesda-like assay to determine the antibody neutralization potential. For this analysis, citrated plasma samples were mixed with plasma from a normal plasma pool and incubated at 37°C for 2 hours. The residual C1-INH activity was measured using the functional chromogenic assay described above (Berichrom C1-INH).

### Statistical analyses

Two data sets were analyzed. The safety set was used for the analysis of safety assessments and included all subjects who received any part of any administration of C1-INH concentrate (24 subjects). The pharmacokinetic per-protocol set was used for the analysis of pharmacokinetic and pharmacodynamic assessments and included all subjects who fulfilled the study inclusion criteria and completed the study without any prespecified major protocol deviation (23 subjects).

The bioavailability of human pasteurized C1-INH concentrate after SC administration relative to IV administration was determined by the geometric mean quotient of individual area under the curve (AUC) values for C1-INH activity using the most conservative approach possible (i.e., not extrapolated, dose adapted, with negative values set to 0, and no interpolation of values), including 90% confidence intervals (CIs).

Assessments of C1-INH activity, C1-INH antigen levels, and C4 antigen levels were analyzed by nonlinear regression, taking into account individual endogenous concentrations of C1-INH at baseline. Individual profiles were described by a one-compartment model. Standard formulas were used to obtain individual values for variables, including AUC for the dose of 1000 U per subject, time of maximum concentration ( $T_{max}$ ), maximum concentration ( $C_{max}$ ), terminal elimination half-life ( $t_{1/2}$ ),

mean residence time (MRT), total clearance (CL), and volume of distribution at the terminal phase ( $V_z$ ).

All variables were analyzed descriptively, including 95% CIs based on log-transformed data and graphs (boxplots). No imputation of missing data was applied for any variable. An exploratory analysis of variance and graphs were used to check for the influence of the factors "treatment" and "period" on the pharmacokinetic and pharmacodynamic variables as well as for carryover effects ("treatment  $\times$  period" interaction).

The percentage of cIHK compared to total HK and the safety assessments were analyzed using summary statistics. The differences over time for laboratory safety variables (comparing values between baseline, i.e., before first treatment and 3 months after baseline) were analyzed using an analysis of covariance model with "sequence" and "treatment" as fixed effects, the baseline value as a covariate, and "subject" as a random effect. The treatment difference for vital signs was compared using a two-sided, two-sample t test. All statistical analyses were performed using computer software (SAS, Version 9.1, SAS Institute, Cary, NC).

## RESULTS

### Demographics and baseline characteristics

A total of 24 subjects with mild or moderate HAE Type I or Type II were enrolled, comprising the safety set (Table 1). One subject, in Sequence BA, was excluded from the pharmacokinetic per-protocol set because of unscheduled IV treatment with C1-INH concentrate less than 12 hours before the scheduled SC administration of C1-INH concentrate.

TABLE 1. Demographic data (safety set)\*

	Sequence AB (n = 12)	Sequence BA (n = 12)
Sex†		
Male	7 (58.33)	8 (66.67)
Female	5 (41.67)	4 (33.33)
Race†		
Caucasian	12 (100.00)	11 (91.67)
Oriental	0 (0.00)	1 (8.33)
Age (years)		
Mean (SD)	37.7 (17.45)	43.3 (18.68)
Median (range)	38.0 (18-76)	45.0 (17-71)
BMI (kg/m <sup>2</sup> )		
Mean (SD)	26.1 (5.62)	26.1 (6.55)
Median (range)	24.92 (21.1-40.8)	24.68 (18.9-41.9)

\* The sequences designate the order of administration in each treatment group: A = IV infusion; B = SC infusion.  
† Data are reported as number (%).  
BMI = body mass index.



**TABLE 2. Analysis of C1-INH activity levels after IV and SC administration of C1-INH concentrate (pharmacokinetic per-protocol set)\***

Variable	Mean (SD)		p value
	IV (n = 23)	SC (n = 23)	
AUC (hr × %)	1832.4 (927.10)	881.7 (538.10)	0.0001
AUC <sub>0-1</sub> † (hr × %)	220.5 (143.11)	104.2 (66.37)	0.0011
Bioavailability (%)‡		39.7 (27.34-57.74)	
T <sub>max</sub> (hr)§	0.5 (0-8)	48.0 (1-456)	0.0003
C <sub>max</sub> (%)	33.8 (7.513)	9.8 (4.76)	<0.0001
t <sub>1/2</sub> (hr)	62.0 (65.59)	119.6 (126.13)	0.0595
MRT (hr)	53.4 (38.25)	87.0 (51.81)	0.0149
CL [U/(hr × %)]	0.6 (0.39)	1.2 (1.50)	0.0982
V <sub>d</sub> (U/%)	36.8 (13.74)	133.3 (157.17)	0.0038

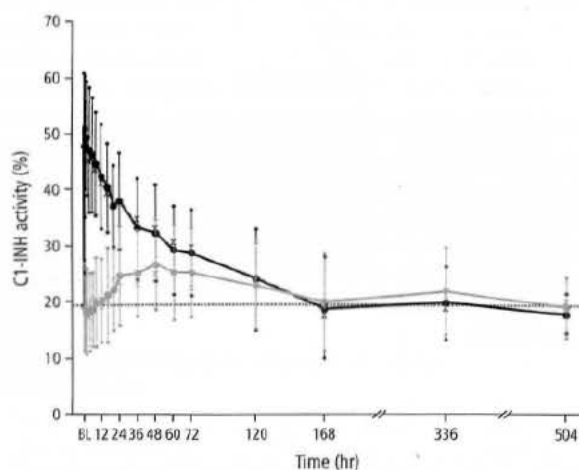
\* p values are shown for the factor of "treatment" in an analysis of variance conducted for each pharmacokinetic variable including factors of "treatment," "period," and "treatment × period." The factors of "period" and "treatment × period" had no effect on any of the pharmacokinetic variables.

† Standardized to 15 U/kg body weight.

‡ Mean bioavailability relative to IV administration based on AUC<sub>0-1</sub> (90% CI).

§ Median (range).

|| Calculation of t<sub>1/2</sub> after SC infusion was only possible for 16 of 23 subjects due to an increase in C1-INH values toward the end of the observation period in seven subjects.



**Fig. 1.** Mean C1-INH activity over time (pharmacokinetic per-protocol set, n = 23 subjects). Black and gray whiskers show SDs. Median values are indicated with "X." Horizontal lines show baseline (BL) values (before first administration of study drug). Data for mean C1-INH activity at 336 and 504 hours were obtained in only six subjects. (—●—) IV; (---■---) SC.

#### Bioavailability of C1-INH concentrate and assessments of C1-INH activity, C1-INH antigen levels, and C4 antigen levels

The mean relative bioavailability of functional C1-INH after SC administration of human pasteurized C1-INH concentrate was 39.7%, with the 90% CI ranging from 27.34% to 57.74% (Table 2). Overall, the mean plasma C1-INH activity was lower after SC administration of C1-INH concentrate than after IV administration (Fig. 1),

as also reflected in the lower AUC values after SC administration (Table 2). After SC administration, the median T<sub>max</sub> was delayed relative to IV administration, as expected. Comparing the pharmacokinetic profile of C1-INH between the two different routes of administration, a slower onset of C1-INH activity and a lower C<sub>max</sub> were observed after SC treatment (Fig. 1). The mean t<sub>1/2</sub> of C1-INH activity after IV administration was 62.0 hours. A significant effect of "treatment" was observed by analysis of variance for most variables, whereas "period" and "treatment × period" had no effect. For CL and t<sub>1/2</sub>, no factor had a significant effect.

The results for C1-INH antigen and C4 antigen levels were comparable to C1-INH activity levels in terms of the relationship between the two modes of administration. The increases in mean C1-INH antigen and C4 antigen levels were delayed and less pronounced after SC administration than after IV administration (Figs. 2 and 3). Similar to C1-INH activity, the median T<sub>max</sub> for C1-INH antigen and C4 antigen levels was markedly delayed after SC administration (Tables 3 and 4). Analysis of variance showed that only "treatment" had a significant influence (p < 0.05) on pharmacokinetic variables of C1-INH antigen and C4 antigen levels except with V<sub>d</sub> for C4 antigen levels, where "period" had a significant effect (p = 0.0048).

#### CIHK

After IV and SC administration of C1-INH concentrate, the percentage of CIHK levels decreased for up to 36 hours (Fig. 4). This was more pronounced with IV administration, where the percentage of CIHK decreased from a mean of 46.0% at baseline (0 hr) to 37.1% at 36 hours. In contrast, after SC administration, the lowest value was 42.0%, starting from a baseline value of 44.8%. After 36 hours, the CIHK levels increased again to attain approximately baseline levels.

#### Safety assessments

In total, 16 of the 24 subjects (66.7%) in the safety set experienced 46 treatment-emergent AEs (Table 5). There were no deaths during the study. One serious AE of pneumonia occurred after IV infusion of C1-INH concentrate. This event was moderate in intensity, not related to the study drug, and resolved without sequelae.

The causality of 21 AEs occurring after SC administration was categorized as "probably related." These events included application site irritation (n = 9), application site

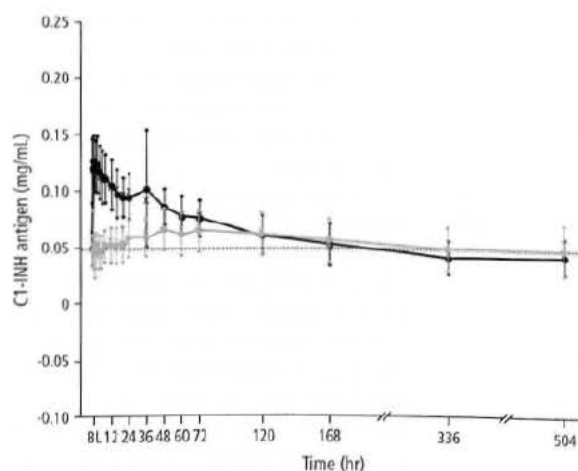


Fig. 2. Mean concentrations of C1-INH antigen over time (pharmacokinetic per-protocol set,  $n = 16$  subjects). Seven subjects with HAE Type II were excluded from the C1-INH antigen analyses. Black and gray whiskers show SDs. Median values are indicated with "X." Horizontal lines show baseline (BL) values (before first administration of study drug). Data for mean concentrations of C1-INH antigen at 336 and 504 hours were obtained in only six subjects. (—●—) IV; (---●---) SC.

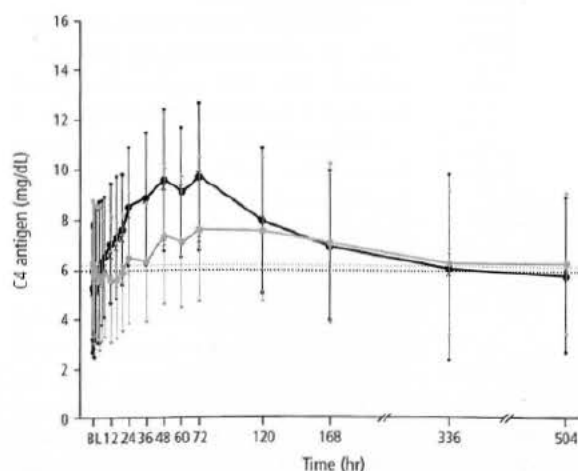


Fig. 3. Mean concentrations of C4 antigen over time (pharmacokinetic per-protocol set,  $n = 23$  subjects). Black and gray whiskers show SDs. Median values are indicated with "X." Horizontal lines show baseline (BL) values (before first administration of study drug). Data for mean concentrations of C4 antigen at 336 and 504 hours were obtained in only six subjects. (—●—) IV; (---●---) SC.

swelling ( $n = 6$ ), application site hematoma ( $n = 4$ ), application site pain ( $n = 1$ ), and back disorder ( $n = 1$ ). Two AEs were categorized as possibly related (both were application site irritation).

All AEs occurring after SC administration ( $n = 32$ ) were mild in intensity. The only moderate AEs occurred after IV administration and were one case each of rhinitis, oropharyngeal pain, sinusitis, and the serious AE of pneumonia.

No significant changes in laboratory safety variables occurred over time, and there were no significant differences in vital signs between the two routes of administration. No virus transmissions due to the administration of study drug, or positive PCR results suggestive of virus transmission, were observed.

Only one subject, after SC administration of C1-INH concentrate, exhibited a change in C1-INH antibody titer. The C1-INH antibody titer of this subject was above cutoff at screening (1:150) and further increased at 1 (1:450) and 3 months (1:450) after the last administration of study drug. No inhibitory C1-INH antibodies were detected.

## DISCUSSION

IV administration of C1-INH concentrate is a well-established and effective therapy for the acute and prophylactic treatment of HAE.<sup>18</sup> However, it can be an uncomfortable and often painful procedure for patients, especially when frequent administration is required for routine prophylaxis. SC administration may mitigate this limitation of IV therapy and thereby provide an attractive alternative therapeutic approach for HAE prophylaxis, especially for patients with limited venous access and for children.

Preclinical studies and clinical studies of SC treatment with two different preparations of C1-INH concentrate have indicated that this route of administration is safe and could achieve sustained and therapeutically potentially relevant systemic levels of C1-INH (Ingo Pragst, CSL Behring, May 2013).<sup>19,21</sup> Against this background, our study investigated the bioavailability and the pharmacokinetics, pharmacodynamics, and safety of SC administration of a marketed human pasteurized C1-INH concentrate (Berinert) that is currently approved for IV treatment of acute angioedema in patients with HAE.

Compared with IV administration, the mean relative bioavailability of functional C1-INH after SC administration of 1000 U of human pasteurized C1-INH concentrate, as assessed by C1-INH activity, was 39.7%. With the same dose of a different preparation of C1-INH concentrate, the relative bioavailability after SC administration was 62% for 1000 U and 36% for 2000 U, based on C1-INH activity; C1-INH antigen levels were 44 and 28% for 1000 and 2000 U, respectively.<sup>20</sup> The reason for this difference remains unclear. The relative bioavailability of 39.7% after SC administration of C1-INH concentrate in our study was sufficient to elicit pharmacodynamic responses, as indicated by recoveries in C4 antigen and cHK levels (increase and decrease, respectively), which was also seen after IV administration. Patients with HAE usually present with



**TABLE 3. Analysis of C1-INH antigen levels after IV and SC administration of C1-INH concentrate (pharmacokinetic per-protocol set)\***

Variable	Mean (SD)		p value
	IV (n = 16)†	SC (n = 16)†	
AUC (hr × mg/mL)	4.7 (1.07)	2.7 (1.84)	0.0008
AUC <sub>0-12</sub> (hr × mg/mL)	0.6 (0.14)	0.3 (0.24)	0.0030
T <sub>max</sub> (hr)§	0.4 (0-36)	48.1 (1-456)	0.0052
C <sub>max</sub> (mg/mL)¶	0.09 (0.044)	0.03 (0.021)	<0.0001
t <sub>1/2</sub> (hr)	53.3 (28.19)	94.4 (71.61)	0.0643
MRT (hr)	54.9 (26.55)	106.2 (58.99)	0.0041
CL [U/(hr × mg/mL)]	206.8 (64.91)	556.2 (777.85)	0.0424
V <sub>z</sub> [U/(mg/mL)]	14,842.7 (7,438.10)	49,503.6 (51,319.02)	0.0053

\* p values are shown for the factor of "treatment" in an analysis of variance conducted for each pharmacokinetic variable including factors of "treatment," "period," and "treatment × period." The factors of "period" and "treatment × period" had no effect on any of the pharmacokinetic variables.

† Seven subjects with HAE Type II were excluded from the C1-INH antigen analyses.

‡ Standardized to 15 U/kg body weight.

§ Median (range).

¶ Mean values were not rounded to one decimal place to avoid values of 0.0; decimal places of the corresponding SD were rounded accordingly.

**TABLE 4. Analysis of C4 antigen levels after IV and SC administration of C1-INH concentrate (pharmacokinetic per-protocol set)\***

Variable	Mean (SD)		p value
	IV (n = 23)	SC (n = 23)	
AUC (hr × mg/dL)	424.6 (177.07)	227.1 (206.67)	0.0013
AUC <sub>0-12</sub> (hr × mg/dL)	50.5 (23.12)	27.0 (25.01)	0.0020
T <sub>max</sub> (hr)‡	60.1 (24-168)	120.0 (4-456)	0.0121
C <sub>max</sub> (mg/dL)	4.1 (1.49)	2.0 (1.06)	<0.0001
t <sub>1/2</sub> (hr)	134.5 (216.66)	242.1 (289.30)	0.4741
MRT (hr)	83.0 (21.81)	114.3 (55.05)	0.0155
CL [U/(hr × mg/dL)]	1.9 (1.05)	2.5 (1.77)	0.3052
V <sub>z</sub> [U/(mg/dL)]	207.4 (119.53)	477.7 (293.73)	0.0034

\* p values are shown for the factor of "treatment" in an analysis of variance conducted for each pharmacokinetic variable including factors of "treatment," "period," and "treatment × period." The factors of "period" and "treatment × period" had no effect for any of the pharmacokinetic variables except V<sub>z</sub>, where "period" had a significant effect (p = 0.0048).

† Standardized to 15 U/kg body weight.

‡ Median (range).

chronic reductions in C4 plasma levels, and a recovery in C4 levels may indirectly demonstrate efficacy of the administered C1-INH concentrate.<sup>4,22</sup> In addition, cHK levels can be used to monitor bradykinin synthesis and therapeutic efficacy due to the involvement of the kinin-kallikrein system in acute HAE attacks and the presence of elevated cHK in the plasma of patients during HAE attacks.<sup>23-27</sup>

The lower activity levels of C1-INH when administered SC can likely be attributed to the gradual release of the C1-INH from the SC reservoir into the circulation. Due to its large molecular size, the C1-INH concentrate was most probably transferred from the SC reservoir to the vascular network via the lymphatic system.<sup>28</sup> The bioavailability observed in our study in humans is lower com-

pared with the relative bioavailability of approximately 70% observed previously in rabbits (Ingo Pragst, CSL Behring, May 2013). The reason for this finding is still unclear and warrants further investigations.

In our single-dose study, the mean maximum activity level of C1-INH in plasma was 33.8% after IV administration and 9.8% after SC administration. Various studies have indicated that C1-INH concentrations of at least 0.07 g/L, or approximately 30% of the functional activity level, are needed for effective long-term prophylaxis of angioedema attacks.<sup>29-33</sup> However, the data from our study cannot provide insight into whether SC administration of C1-INH concentrate yields protective levels of C1-INH in plasma because the single-dose design, while appropriate for assessing relative bioavailability, was not suited to assessing trough concentrations or prophylactic efficacy of C1-INH, which would have required a multiple-dose design.

In addition to the practical advantages of administering C1-INH concentrate SC, the apparently lower fluctuation in plasma concentrations of C1-INH associated with SC administration, relative to IV administration, may provide an additional benefit in the prophylactic treatment of HAE. Sustained concentrations of C1-INH in the circulation could potentially enhance prophylactic efficacy over time and could also mean that the frequency with which C1-INH concentrate must be administered can be reduced. However,

further research is needed to establish the extent to which these principles might apply in the light of the low relative bioavailability of C1-INH concentrate when administered SC, and the extent to which the efficacy of SC administered C1-INH can be influenced by varying the concentration and dosing regimen of the SC preparation.

No earlier data are available on the pharmacokinetics of C1-INH concentrate after SC administration that can be directly compared with the results from our study. However, with respect to IV administration, historical data are available from a number of studies with which we can compare our data. Of primary interest is that the mean t<sub>1/2</sub> observed after IV administration of human pasteurized C1-INH concentrate in our study was notably longer than mean historical values obtained with the same

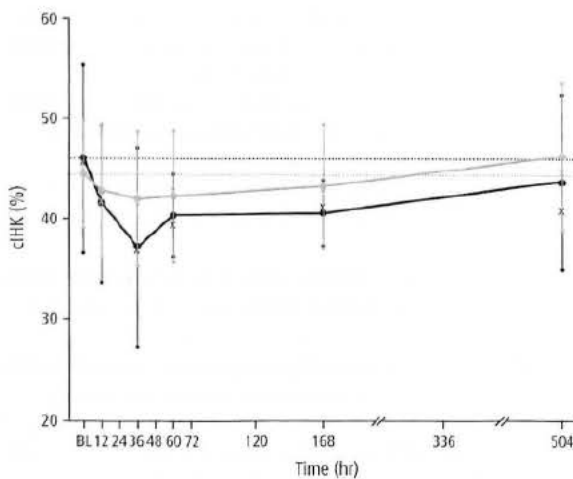


Fig. 4. Mean cHtK over time (pharmacokinetic per-protocol set,  $n = 6$  subjects). Black and gray whiskers show SDs. Median values are indicated with "X." Horizontal lines show baseline values (before first administration of study drug). (—●—) IV; (---○---) SC.

TABLE 5. Treatment-emergent AEs after IV and SC administration of C1-INH concentrate (safety set)\*

	IV ( $n = 24$ )	SC ( $n = 24$ )
Subjects with AE	7 (29.2)	15 (62.5)
Number of AEs	14	32
AE causality		
None	10 (71.4)	9 (28.1)
Unlikely	4 (28.6)	0 (0.0)
Possible	0 (0.0)	2 (6.3)
Probable	0 (0.0)	21 (65.6)
AE intensity		
Mild	10 (71.4)	32 (100.0)
Moderate	4 (28.6)	0 (0.0)
Severe	0 (0.0)	0 (0.0)

\* Data are reported as number (%).

preparation of C1-INH concentrate. The  $t_{1/2}$  of C1-INH appears to be influenced by the severity of HAE in the population analyzed and by whether the assessment was made in patients with symptomatic HAE (i.e., while experiencing an acute attack) or asymptomatic HAE (i.e., during an attack-free interval). In previous studies of the same preparation of C1-INH concentrate as used in our study, mean  $t_{1/2}$  values after IV administration were 32.7 hours (population estimate, 90% CI, 16.6–48.8 hr) in symptomatic patients,<sup>34</sup> 33.3 hours (standard deviation [SD], 19.8 hr) in asymptomatic patients with severe HAE,<sup>35</sup> and 47.8 hours (SD, 21.2 hr) in asymptomatic patients with moderate HAE,<sup>35</sup> compared with 62.0 hours (SD, 65.6 hr) in asymptomatic patients with mild or moderate HAE in our study. Similar to our result, mean  $t_{1/2}$  values of 56 (SD, 35) and 62 hours (SD, 38 hr) were reported for a

different human plasma C1-INH concentrate in patients with asymptomatic HAE,<sup>36,37</sup> and for a non-commercial C1-INH preparation the mean  $t_{1/2}$  value was 67.7 hours (SD, 4.9 hr) in patients with asymptomatic HAE and 64 hours (SD, 1.4 hr) in healthy individuals.<sup>38</sup>

Our study revealed no signals of concern regarding the tolerability and safety of SC administration of human pasteurized C1-INH concentrate. AEs were reported in 16 of the 24 subjects treated. Although more than twice as many events (32) occurred after SC administration than after IV administration (14), all of the events that occurred after SC administration were mild in intensity, and most events were associated with the injection site, such as hematoma, irritation, pain, and swelling. Relative to IV administration, such AEs are commonly associated with SC administration, as has been observed for another human plasma C1-INH concentrate<sup>20,21</sup> and for SC immunoglobulin replacement therapy.<sup>39</sup> As the C1-INH concentrate administered in our study is intended for IV administration, the volume administered was relatively high (10 mL per injection site), which may have exacerbated the injection-site reactions associated with SC administration. Increasing the concentration of a human C1-INH preparation intended for SC administration may provide a means of reducing the incidence of injection site reactions.

The development of inhibitory antibodies is often a concern with protein-based therapies, and low rates of noninhibitory antibody formation have been associated with the long-term administration of C1-INH concentrate.<sup>40</sup> Furthermore, with respect to therapeutic-based proteins, SC administration is known to be more immunogenic than IV administration.<sup>41–43</sup> In our study, only one subject exhibited an increase in C1-INH antibody titer, at 1 and 3 months after SC administration, but these antibodies were not inhibitory.

In conclusion, the relative bioavailability of functional C1-INH after SC administration of human pasteurized C1-INH concentrate was 39.7%. SC administration was safe and well tolerated. It was accompanied by pharmacodynamic responses expected of C1-INH therapy and indications that SC administration of C1-INH concentrate may achieve prolonged and less fluctuating systemic exposure to human pasteurized C1-INH concentrate than with IV administration. These observations, together with the additional convenience of SC administration, suggest that further investigation of the SC administration of C1-INH concentrate for prophylactic treatment of HAE may be warranted.

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### CONFLICT OF INTEREST

IMS has received honoraria for lectures and funding for travel expenses from CSL Behring. She is a consultant for the following companies: Baxter, Bayer, CSL Behring, Octapharma, Shire, and Sobi. MC has been a speaker for Dyax/Sigma Tau, CSL Behring, ViroPharma, Pharming/SOBI, and Shire. He has attended advisory boards of the companies mentioned above, as well as of BioChrist, and he has been a consultant for Dyax/Sigma Tau, CSL Behring, and Pharming/SOBI. EAP has attended advisory boards, has received honoraria for lectures, and has participated in clinical trials, all sponsored by CSL Behring. She has also received sponsorship for educational purposes and has provided consultancy services or has participated in clinical trials sponsored by CSL Behring, Jerini AG/Shire, Sobi, and ViroPharma. HS is an employee of Siemens Healthcare Diagnostics, Germany. AF and UK are employees of CSL Behring, Germany. WK is a consultant for and has received honoraria for lectures and funding for travel expenses from the following companies: Baxter, Bayer, CSL Behring, Biotest, Grifols, Intersero, Novo Nordisk, Octapharma, Shire, Sobi, and ViroPharma. CS, ER, and TR declare no conflicts of interest relevant to this article.

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