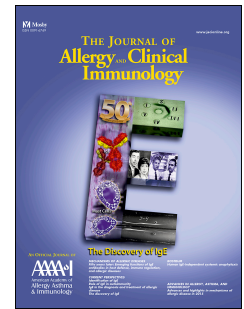


Accepted Manuscript

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PII: S0091-6749(18)30901-1

DOI: [10.1016/j.jaci.2018.06.014](https://doi.org/10.1016/j.jaci.2018.06.014)

Reference: YMAI 13492

To appear in: *Journal of Allergy and Clinical Immunology*

Received Date: 26 July 2017

Revised Date: 11 May 2018

Accepted Date: 12 June 2018

Please cite this article as: Cao H, Biondo M, Lioe H, Busfield S, Rayzman V, Nieswandt B, Bork K, Harrison LC, Auyeung P, Farkas H, Csuka D, Pelzing M, Dower S, Wilson MJ, Nash A, Nolte MW, Panousis C, Antibody-mediated inhibition of FXIIa blocks downstream bradykinin generation, *Journal of Allergy and Clinical Immunology* (2018), doi: 10.1016/j.jaci.2018.06.014.

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Antibody-mediated inhibition of FXIIa blocks downstream bradykinin generation

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This work was supported by CSL Limited

Capsule Summary

HAE results in swelling due to KKS dysregulation and bradykinin production. FXII initiates the KKS and we have developed a human antibody that potently inhibits FXII protease activity as a novel prophylactic therapy for HAE.

Key words

Factor XII; angioedema; bradykinin; kallikrein-kinin system; C1 esterase inhibitor; monoclonal antibody

Disclosure of potential conflict of interest

H. Farkas received honoraria and travel grants from CSL Behring, Shire, Swedish Orphan Biovitrum and Pharming and/or served as a consultant for these companies and has participated in clinical trials/registries for BioCryst, CSL Behring, Pharming and Shire. K. Bork has been a speaker for CSL and Shire. L. Harrison has been a consultant for CSL Limited in 2015 and 2016. H. Cao, M. Biondo, H. Lioe, V. Rayzman, M. Pelzing, S. Dower, M. Wilson, A. Nash and C. Panousis are employees of CSL Limited. M. Nolte is an employee of CSL Behring. The rest of the authors declare that they have no relevant conflicts of interest.

To the Editor:

Hereditary angioedema (HAE) is a rare genetic disorder with four known disease types that are all clinically characterized by local swellings of the skin (i.e., edema of the extremities, genitals and face), abdominal pain attacks, and, occasionally, life-threatening attacks of laryngeal edema.¹

In the event of an HAE attack, therapeutic options include the intravenous (i.v.) administration of C1-inhibitor (C1-INH) concentrate¹⁻², kallikrein inhibitors (e.g., Ecallantide) and a bradykinin (BK) receptor antagonist (e.g., Icatibant). Despite a number of acute treatment options for HAE attacks, the prophylactic treatment of HAE remains an area of unmet medical need. Current prophylactic treatments include i.v. administered C1-INH (CINRYZE[®]), and the recently approved subcutaneous (s.c.) C1-INH product HAEGARDA[®].

Factor XII (FXII) is the principal initiator of the plasma contact system and autoactivates to FXIIa upon contact with a negatively-charged surface. Activation of FXII leads to the production of the proinflammatory mediator BK through the kallikrein-kinin system (KKS). The binding of BK to BK type 2 receptors activates various intracellular signaling pathways that dilate vessels, induce chemotaxis of neutrophils and increase vascular permeability and fluid efflux.³ BK production is increased during HAE attacks and is thought to be the primary mediator of swelling in HAE.^{4,5} Like BK, plasma levels of FXIIa have also been shown to increase in patients during HAE attacks.^{6,7} However, although FXII is key to the initiation of the plasma contact system, it has not been explored as a target for the treatment of HAE. We previously reported the generation of a fully human recombinant antibody (3F7) that specifically and potently inhibited FXIIa and prevented contact-mediated thrombosis without impairing hemostasis in mouse and rabbit models.⁸ In this study we investigate the potential

for 3F7, and its affinity matured variant CSL312, to modulate KKS-mediated BK production and subsequent edema formation in a number of *in vitro* assays and *in vivo* models.

We first examined the effect of 3F7 on mast cell heparin-initiated activation of the KKS using a murine passive cutaneous anaphylaxis (PCA) model.⁹ 3F7 administered i.p. 4 hours prior to antigen challenge dose-dependently inhibited ear skin edema, with ~90% inhibition observed at 25 mg/kg of 3F7 (Fig 1, A and B). Benchmarking studies were performed to compare the efficacy of 3F7 with the HAE drugs, Icatibant (a BK receptor 2 antagonist) and Ecallantide (a plasma kallikrein inhibitor). When administered 10 min prior to antigen challenge, Icatibant and Ecallantide treatment inhibited edema by approximately 40% and 65%, respectively, compared to ~90% inhibition with 3F7 (Fig 1, C). Whilst 3F7 maintained marked inhibition of the PCA response when administered 4 hours prior to antigen injection, the other inhibitors administered at the same time frame displayed reduced efficacy (Fig 1, D), likely due to faster serum clearance. These results highlight the potential of anti-FXIIa antibodies to provide prolonged protection from edema, with significance for prophylactic management of HAE.

The effect of 3F7 was also investigated in a C1-INH^{-/-} murine model of angiotensin converting enzyme (ACE) inhibitor-induced angioedema. Captopril treatment increases vascular permeability which can be quantitated by extraction of co-administered Evans blue dye from the colons of treated mice, and was greater in C1-INH^{-/-} than in their wild type littermates (Fig 1, E). 3F7 administered prior to captopril treatment ablated vascular permeability increase at doses of 2.5 mg/kg or greater (Fig 1, F).

To enhance the potency of 3F7, affinity maturation was undertaken and a lead antibody clone (CSL312) was selected for further development. Compared to the parental 3F7 antibody,⁸ CSL312 displays a 44-fold increase in affinity to activated β FXIIa ($K_D = 140$ pM) and improved potency in an aPTT assay with human plasma (see Fig E1 in this articles Online Repository at www.jacionline.org). Whilst displaying picomolar affinity to activated FXII, CSL312 binding to zymogen FXII is a weaker interaction with rapid dissociation (see Fig E2 in this articles Online Repository at www.jacionline.org). CSL312 specificity for human FXII was tested against a panel of relevant human serine proteases (FVIIa, FIXa, FXa, FXIa, kallikrein, tPA, APC and uPA) using in vitro chromogenic activity assays. At enzyme to antibody ratios of up to 1:500, no IC₅₀ concentration was reached for any protease tested (data not shown). Prothrombin time (PT) was unaltered at any CSL312 concentration tested (data not shown).

CSL312 potently inhibits FXIIa chromogenic activity following dextran sulfate (DXS) activation of diluted plasma from normal, HAE types I/II, HAE with normal C1-INH (nC1-INH-HAE), and acquired angioedema with C1-INH deficiency (C1-INH-AAE) donors (see Fig E3 in this articles Online Repository at www.jacionline.org), with maximal FXIIa inhibition observed at 10 μ g/mL for all plasma samples tested. A small but statistically significant increase in FXII levels was observed in patients with C1-INH-HAE Type I and nC1-INH-HAE when compared to normal donors.

A liquid chromatography – tandem mass spectrometry (LC-MS/MS) assay was developed to measure the concentration of BK in undiluted plasma. Using DXS-activated pooled healthy human plasma, CSL312 completely inhibited BK generation at concentrations of >133 nM (Fig 2, A). When compared against clinically approved HAE therapeutics (C1-INH and

Ecaltantide), CSL312 displayed the most potent inhibition (Fig 2, A). This assay was also utilized to investigate the efficacy of CSL312 with 48 HAE plasma samples and 4 cynomolgus plasma samples (Fig 2, B). Here it can be seen that all HAE and cynomolgus samples were completely protected against DXS-mediated activation, with most HAE samples requiring less than 200 nM (30 µg/mL) of CSL312 for complete inhibition and no observed difference in the efficacy of CSL312 between HAE types. Cynomolgus plasma samples require a slightly higher CSL312 concentration (267 nM) for complete protection.

The pharmacokinetic (PK) and pharmacodynamic (PD) profile of CSL312 following i.v. infusion to cynomolgus monkeys was investigated. The pharmacokinetics of CSL312 in cynomolgus monkeys were typical for a monoclonal antibody (Fig 2, C). The beta phase constituted approximately 40% of total clearance, and the terminal half-life was approximately 9.1 days, with neither parameter showing any significant non-linearity over the dose range (3, 9, 27 mg/kg), as determined by ANOVA. CSL312 administration was well tolerated and a dose-dependent and prolonged increase in aPTT was observed in animals treated with CSL312 (Fig 2, D). There was no increase in PT at any doses tested (data not shown).

In this study we show that specific antibody-mediated inhibition of FXII protease activity effectively reduced edema formation in animal models and prevented BK formation following contact activation of normal, HAE (C1-INH-HAE Type I, II and HAE with normal C1-INH), and acquired angioedema plasma samples. CSL312 displayed a long plasma half-life in cynomolgous monkeys of 9.1 days and prolonged inhibition of FXIIa activity following a single dose over the course of a 36 day study. Coupled with the ability to formulate at high concentrations (>100 mg/ml) it is well suited to s.c. prophylactic application in HAE with potential dosing regimens of once every 2 weeks, or less

frequently. We are currently undertaking a Phase I clinical study with CSL312 in normal human volunteers and aim to investigate the efficacy of CSL312 in HAE patients in the near future.

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This work was supported by CSL Limited

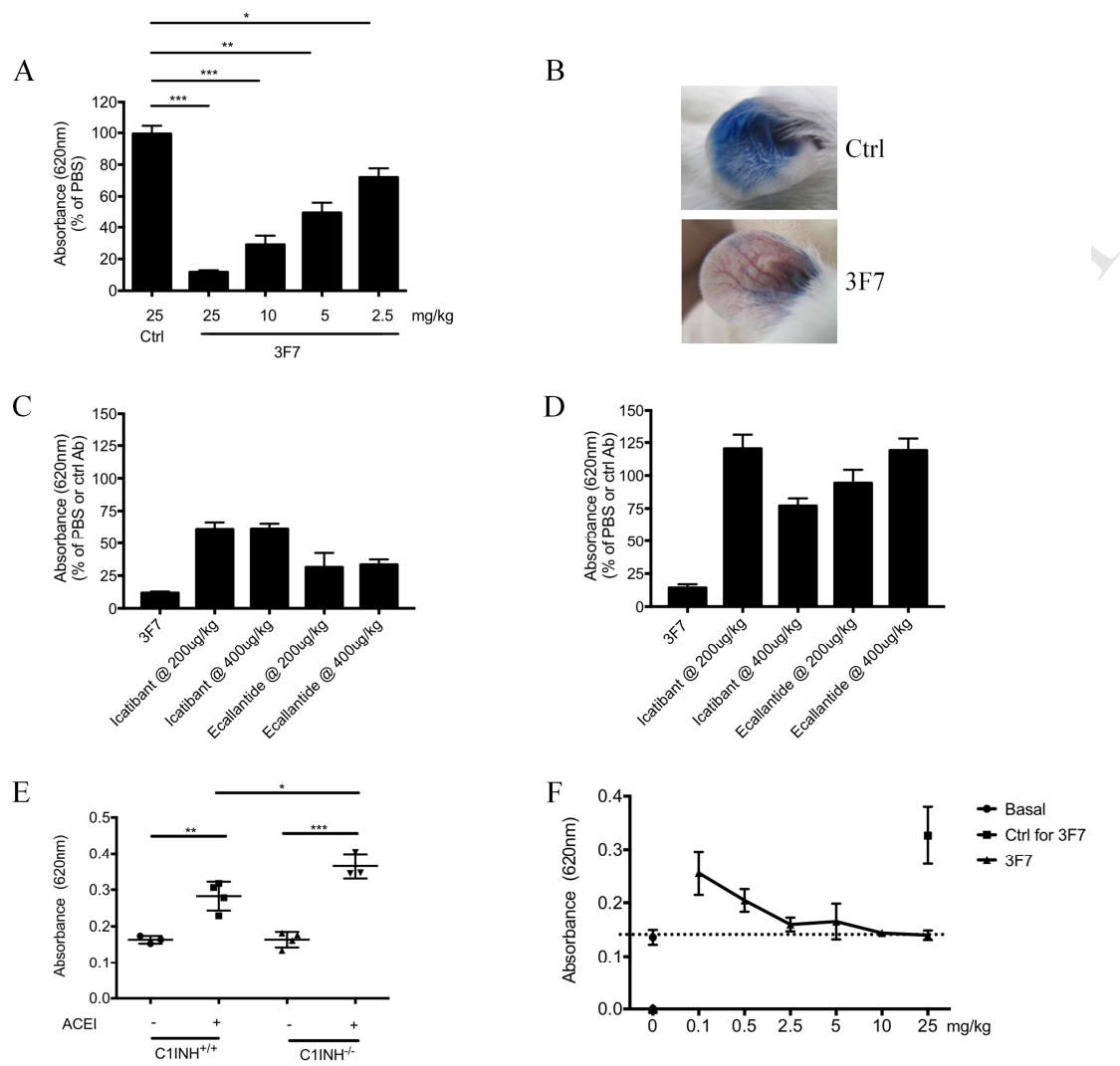
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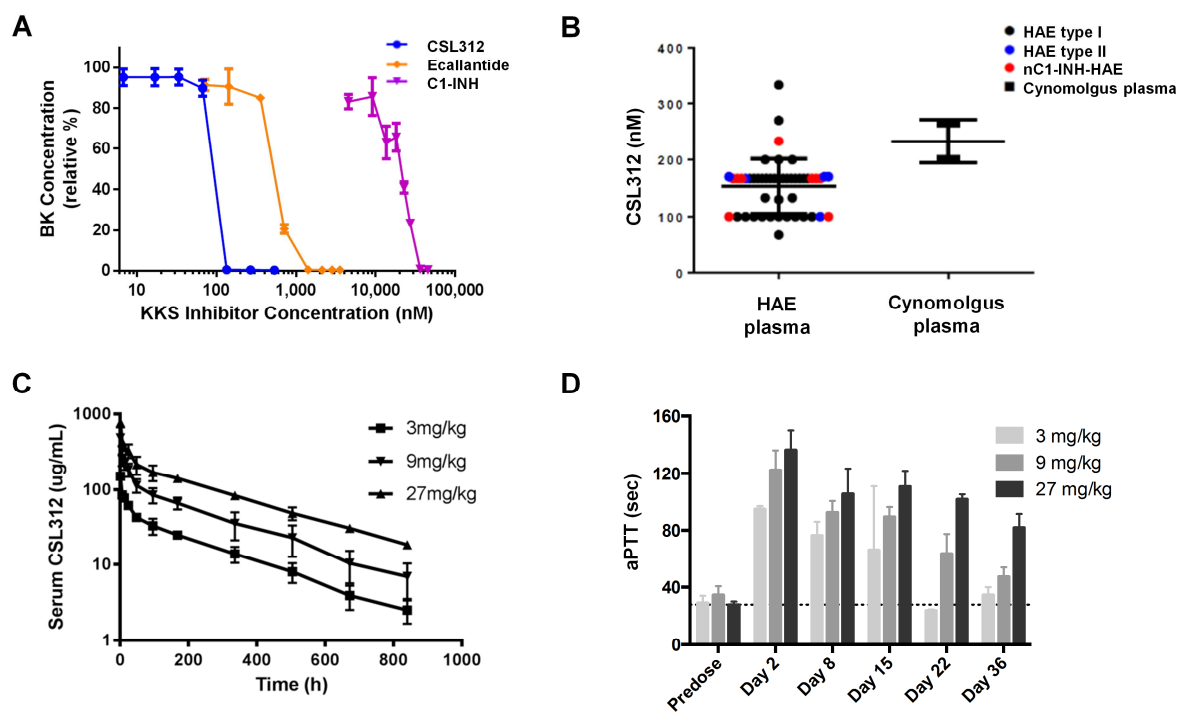
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FIGURE LEGENDS

FIG 1. Effect of anti-FXIIa mAb 3F7 on edema inhibition in mouse models of PCA (A-D) and ACEI-induced edema (E-F). In PCA, anti-DNP IgE was i.d. injected into mouse ears. After 24 hr, the mice were i.v. challenged with DNP-HSA and Evans blue. 30 min after the challenge, blue dye in the ear was extracted and the absorbance at 620 nm was measured. 3F7 and the control mAb (25 mg/kg i.p.); and, Ecallantide and Icatibant (i.v.) were injected 10 min (C) or 4 hr (D) prior to the antigen challenge. In ACEI-induced edema, mice were i.v. administered with captopril and Evans blue. Colons were removed 30 min later, and dye was extracted and the absorbance was measured at 620 nm. ACEI-induced colon edema was higher in C1INH deficient mice than in their wild type littermates (E). ACEI-induced vascular permeability increase was completely abolished by 3F7 treatment (F). Means $\pm$ SDs (n=3-5 per group) are shown. Student *t* test; *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$.

FIG 2. (A) Plot of BK concentrations (in relative %) generated by DXS-mediated activation of pooled healthy human plasma samples titrated with various KKS inhibitors. CSL312 completely inhibited BK generation at concentrations >133 nM. (B) Scatter plot of CSL312 concentration required for complete inhibition of DXS-generated BK from 48 HAE and 4 cynomolgus plasma samples. The majority of HAE samples require <200 nM of CSL312 for complete inhibition. (C) PK profile of CSL312 following a single i.v. dose at 3, 9 and 27 mg/kg in cynomolgus monkeys. (D) aPTT evaluation of cynomolgus plasma samples taken during course of CSL312 PK study.





ONLINE REPOSITORY

METHODS

In vitro FXIIa and β FXIIa amidolytic activity assay

CSL312 inhibitory activity against human FXIIa and β FXIIa (Enzyme Research Laboratories) was assessed using an *in vitro* protease assay. Briefly, 62.5 nM FXIIa or β FXIIa (20 μ L) were incubated in the presence of varying concentrations of CSL312 in a volume of 160 μ L for 5 min at 37°C. Forty μ L of 4 mM chromogenic substrate S-2302 (Haemochrom Diagnostica) was added and the plate was further incubated for 15 min at 37°C. The reaction was stopped with the addition of 40 μ L of 20% acetic acid and the absorbance measured spectrophotometrically at 405 nm.

Activated partial thromboplastin time (aPTT)

The aPTT was determined on a Blood Coagulation System (BCS) by adding 50 μ L of human plasma samples to 50 μ L Pathromtin SL (Siemens Healthcare Diagnostic Products GmbH), followed by incubation for 120 seconds at 37°C. Subsequently, 50 μ L of a calcium chloride solution was added to start the reaction.

Qualitative Biosensor analysis of CSL312 binding to activated human FXIIa and FXII zymogen

A Biacore® T-200 instrument was used for all biosensor analysis. Anti huIgG was chemically immobilized to a CM-5 sensor chip. CSL312 antibody was then captured on flowcell 1, leaving flowcell 2 as a reference cell. Equimolar concentrations of plasma-derived FXIIa and recombinant huFXII zymogen were injected in duplicate for 300 seconds. Responses for binding to the reference flow cell were subtracted from those of the other flow cells. Responses from blank injections were then subtracted from those of all other samples to produce double-referenced sensorgrams.

Murine model of mast cell heparin-induced edema: Passive cutaneous (PCA) anaphylaxis

BALB/c mice originally purchased from Jackson Laboratory (Bar Harbor, ME) were bred and maintained under specific pathogen-free conditions in the animal facility of Bio21 Institute, University of Melbourne. Studies were approved by the Institutional Animal Care and Use Committee and performed on mice of 8 to 10 weeks of age.

To induce local cutaneous anaphylaxis (PCA), BALB/c mice were anesthetized with an i.p. administration of ketamine (80 mg/kg) and xylazine (16 mg/kg), and then 20 ng of anti-DNP IgE (Sigma-Aldrich) in 10 μ L of saline was intradermally (i.d.) injected into ear skin. The PCA reaction was induced 24 hr later by an intravenous (tail vein, i.v.) administration of 100 μ g DNP-HSA (Sigma-Aldrich) in 200 μ L of 0.5% Evans blue. After 30 min the mice were sacrificed and increase in vascular permeability was assessed by dye extraction of the ear skin, which was conducted in 0.5 mL dimethylformamide at 55°C overnight. The absorbance of the extracted dye was measured with a spectrophotometer at 620 nm.

In some experiments, mice were intraperitoneally (i.p.) administered with the anti-FXIIa antibody 3F7 or an isotype control antibody (murine IgG1) at the indicated doses 4 hr before the injection of DNP-HSA. In other experiments, mice were i.v. injected with antagonists or inhibitors of the plasma KKS prior to antigen challenge. The bradykinin B2 receptor antagonist Icatibant (Shire), kallikrein inhibitor Ecallantide (Dyax Corp.) or saline were injected 10 min or 4 hr prior to the induction of the anaphylactic reaction.

Murine model of angiotensin converting enzyme (ACE) inhibitor-induced edema

Administration of the ACE inhibitor captopril (ACEI) causes a widespread extravasation of Evans blue dye in the airway, gut and footpads in mice.^{E1-E2} Heterozygous C1-INH-deficient (C1-INH^{+/-}) mice were purchased from Taconic Biosciences (Hudson, NY) and homozygous (C1-INH^{-/-}) mice were generated by crossing the heterozygous animals. C1-INH deficiency was confirmed by Western blot analysis of C1-INH protein expression (data not shown). Animal studies were approved by the Institutional Animal Care and Use Committee and performed on mice of 8 to 10 weeks of age. C1-INH^{-/-} mice and wild type littermates (C1-INH^{+/+}) were treated i.v. with 2.5 mg/kg of captopril (Sigma-Aldrich). Five minutes later, Evans blue dye (30 mg/kg) in saline was injected into mice via the tail vein. After 30 min, colons were removed and Evans blue extracted with 0.5 mL dimethylformamide at 55°C overnight and quantitated spectrophotometrically at 620 nm. 3F7 or the isotype control antibody (Ctrl) were i.p. administered 4 hr prior to captopril injection.

Affinity maturation of 3F7

The affinity maturation of 3F7 was undertaken using methods described previously^{E3}. Briefly, Fab-phage mutagenesis libraries targeting 6 amino acids at a time were designed within the heavy chain variable region CDRs 1, 2 and 3; and the light chain variable region CDRs 1 and 3 of 3F7. Each amino acid position within the 6-mer libraries was randomized for 19 different amino acids (except cysteine) and generated libraries with a diversity of 4.7×10^7 . Libraries were cycled through five rounds of selection with decreasing concentration of biotinylated β FXIIa. The target concentration was reduced 10-fold with each round, from 40 nM in Round 1 to 4 pM in Round 5. At the completion of panning, a number of phage clones were selected from each enriched library and sequenced. Unique clones from each library were then selected based on sequence and reformatted into fully human IgG4/lambda antibodies for binding analysis.

FXIIa Chromogenic Activity in Activated Plasma from Normal Human Donors and Patients with HAE Types I/II/III, and Acquired Angioedema (C1-INH-AAE)

Inhibition of FXIIa activity with CSL312 was measured with a chromogenic-based assay utilising substrate S-2302 (Chromogenix). Each plasma sample was assayed in duplicate, with a maximum of four samples per assay plate. Being a three component based assay, all solutions were prepared at three-fold strength to account for dilution once aliquoted into wells. CSL312 was prepared at 90 μ g/mL in TRIS buffer (200 mM TRIS, pH 7.8) and then serially diluted three fold in TRIS buffer to cover an effective titration range. Fifty microliters of prepared CSL312 was aliquoted into a flat bottom 96 well plate. An isotype control antibody was also prepared at 90 μ g/mL and aliquoted into the appropriate wells. HAE and

normal human plasma was thawed on ice and diluted 1/3 in TRIS buffer. Fifty microliters of diluted plasma was then aliquoted into all wells. Finally, a 3 µg/mL solution of Dextran Sulfate (Calbiochem) was prepared in TRIS buffer and 50 µL added to appropriate wells. Fifty microliters of TRIS buffer was added to control wells lacking dextran sulfate. With the addition of Dextran Sulphate to activate plasma, the 96 well plate was placed at 37 degrees for 20 min. After incubation, 40 µL of S-2302 was added to all wells and incubated for 15 min. After incubation, 20 µL of 20% Acetic Acid was added to all wells and plate analyzed at 405 nm.

FXII levels in normal and patient plasma was determined using the Human Factor XII ELISA Kit (Abcam Catalog# ab108835) according to the manufacturer's instructions.

All HAE plasma samples used in this study were collected during the symptom-free period between attacks. All subjects gave written informed consent following approval by the local ethics committee.

LC-MS/MS bradykinin assay

A LC-MS/MS analytical method was developed for bradykinin quantitation (manuscript in preparation). The assay is capable of detecting and quantifying bradykinin (BK) and hydroxyproline bradykinin (HypBK), and their breakdown products BK₁₋₅ and HypBK₁₋₅, BK₁₋₇ and HypBK₁₋₇, BK₁₋₈ and HypBK₁₋₈, and BK₂₋₉ and HypBK₂₋₉. Four internal standards were used in the assay: (i) [²H₅-D-Phe⁵]Bradykinin (BK*) as quantitative internal standard for full length BK and HypBK (ii) [²H₅-D-Phe⁵, ²H₅-D-Phe⁷]Bradykinin (BK**) as recovery internal standard, (iii) [²H₅-D-Phe⁵]Bradykinin (1-5) (BK₁₋₅*) as quantitative internal standard for bradykinin fragments and their hydroxylated variants, and (iv) Sar-[D-Phe⁸]-des-Arg⁹-Bradykinin (Sar-BK) as internal standard to check instrument and assay performance.

For each KKS inhibitor experiment, 100 µL of protease inhibitor mixture that consists of 50 µM (final concentration) of Captopril, Diprotin A, Plummer's Inhibitor and Bestatin was added to 1.0 mL of frozen plasma aliquot and the mixture was allowed to thaw slowly. Once thawed, the plasma-inhibitor mixture was gently vortexed and centrifuged at 3,000 rpm for 10 min at 4 °C. The supernatant was carefully divided into ten 100 µL aliquots for spiking with different amount of KKS inhibitor and individual dextran sulfate activation. Each of nine aliquots was added with 10 µL of CSL312 stock solution (or C1-Esterase inhibitor solution or Ecallantide solution) of varying concentration.

The plasma and KKS inhibitor mixture were stirred, gently centrifuged and allowed to stand for at least 10 min at room temperature before proceeding. Then, 10 µL of Internal Standard Stock solution 1 (consisting of BK*, BK₁₋₅* and Sar-BK) was added to the mixture and immediately followed by 10 µL of dextran sulfate (final concentration = 100 µg/mL) and the mixture stirred, gently centrifuged and allowed to stand at room temperature for 3 minutes to effect contact activation. The reaction was stopped by addition of 390 µL of ice-cold methanol to precipitate proteins and extract BK and related species. The mixture was stirred and left in ice for 1 h to ensure complete precipitation and centrifuged at 13,000 rpm for 10

min (RT). A small amount of the supernatant (30 μ L) was diluted and mixed with 100 μ L of 0.1% acetic acid in water and 20 μ L of Internal Standard Stock solution 2 (consisting of BK**) and the mixture analyzed by LC-MS/MS for quantitation.

The remaining plasma-inhibitor aliquot was mixed with 10 μ L of PBS, 10 μ L of Internal Standard 1, and 10 μ L of MilliQ water and used as negative control for measurement in non-activated plasma. The mixture was stirred, gently centrifuged, allowed to stand for 3 minutes at room temperature, added with 390 μ L of ice-cold methanol to precipitate proteins and extract BK. The mixture was left in ice for 1 h and centrifuged at 13,000 rpm for 10 minutes (RT). A small amount of the supernatant (30 μ L) was diluted and mixed with 100 μ L of 0.1% acetic acid in water and 20 μ L of Internal Standard Stock solution 2 and the mixture analyzed by LC-MS/MS for quantitation.

All LC-MS/MS experiments were performed using Sciex QTRAP 6500 triple quadrupole mass spectrometer combined with Agilent 1290 HPLC. For the HPLC conditions, mobile phase A used was 0.1% Acetic acid in water and mobile phase B used was 0.1% Acetic Acid in 98% Methanol: 2% water. Phenomenex Aeris Widepore XB-C18 2.1 x 100 mm 3.6 μ m was used as the analytical column and Phenomenex Widepore C18 4 x 2 mm (ID) Security Guard Cartridges was used as the guard column. The LC-MS/MS run time for each sample is 15 minutes with the following gradient:

Time (minutes)	Flowrate (μ L/min)	B(%)
0.0	300	5
7.0	300	40
7.1	300	100
10.0	300	100
10.1	300	5
15.0	300	5

A total of 14 multiple reaction monitoring (MRM) transitions were simultaneously measured during each LC-MS/MS run:

Q1 mass (Da)	Q3 mass (Da)	MRM ID
289.7	408.1	BK[1-5]*2+.b4
287.2	408.1	BK[1-5]2+. b4
379.1	642.4	BK[1-7]2+. b6
452.7	642.3	BK[1-8]2+. b6
452.7	807.4	BK[2-9]2+. y7
354.1	419.1	BK3+. y3
357.6	424.3	Di-labeled BK3+. y3
295.2	424.1	Hyp3-BK1-5. b4
387.1	658.4	Hyp3-BK1-7. b6
460.7	658.4	Hyp3-BK1-8. b6
460.7	823.4	Hyp3-BK2-9. y7
359.4	419.1	Hyp3-BK3+.2
355.9	419.1	Mono-labeled BK3+. y3
488.3	713.3	Sar-BK2+. 2

A Single-dose pharmacokinetic and pharmacodynamic study of CSL312 administered intravenously to cynomolgus monkeys

Three naive male cynomolgus monkeys per group were selected for use on the study. The animals received a single dose of CSL312 at 3, 9 and 27 mg/kg via i.v. infusion over 30 min at a dose volume of 6 mL/kg. All animals were observed closely for any reactions to treatment during the study. Body weights were measured and recorded prior to dosing and at the end of the study. Food consumption was measured on a daily basis. Blood samples for analysis of clinical chemistry, hematology, coagulation (aPTT and prothrombin time (PT)) and PK characteristics were collected at multiple time points up to 36 days.

CSL312 plasma levels were quantified using a Mesoscale Diagnostics (MSD) platform. MSD high bind plates were coated with recombinant FXIIa overnight at 4°C, 2 µg/mL in D-PBS. 25 µL of coating reagent was added per well and plate placed on the plate shaker overnight at 4°C. Contents of the plate was decanted and washed 5 times by hand with 280 µL of D-PBS per well. 200 µL of blocking buffer was added per well to block the plate. Plate was incubated for 2 hours at room temperature. While the plate was blocking, the standards (9000 ng/mL to 0.412 ng/mL), internal plate control and diluted test samples were prepared in 0.5% serum matrix. Contents of the plate was decanted and washed 5 times by hand with 280 µL of D-PBS per well. 25 µL of standard, blank, internal plate controls and diluted test samples were added to the appropriate wells. Plate was incubated for 1h on the plate shaker at RT. Contents of the plate was decanted and washed 5 times by hand with 280 µL of D-PBS per well. The Sulfo-tag detection antibody (MDS, 1 µg/mL) was prepared in blocking buffer and 25 µL added to the appropriate wells. Plate was incubated for 1h at RT on the plate shaker, protecting the plate from light by covering with foil. Contents of the plate was decanted and washed 5 times by hand with 280 µL of D-PBS per well. 2X Read buffer was prepared following manufacturers protocol. 150 µL of 2X read buffer was added to the appropriate wells. Care was taken to avoid addition of air bubbles. Plate was read within 30 min on the MSD® Sector Imager 2400.

REFERENCES

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FIGURE LEGENDS

FIG E1. *In vitro* potency testing of anti-FXIIa antibodies 3F7 and CSL312. Dose-dependent prolongation of aPTT clotting time in normal human plasma with CSL312 and the parental 3F7.

FIG E2. Biosensor analysis of CSL312 binding to human FXIIa (blue) and zymogen FXII (red).

FIG E3. FXII plasma concentrations and FXIIa CSL312-mediated inhibition in normal human donors and HAE subtypes. **A)** FXII plasma concentration was quantified for all plasma samples and median FXII levels are shown by the horizontal line. Statistically significant differences were evaluated for each patient group compared to normal donors using a one-way ANOVA. Groups displaying a significant difference are indicated. **B-F)** CSL312-mediated inhibition of FXIIa chromogenic activity in DXS-activated plasma samples from normal human donors (B) and patients with HAE types I, II, III (nC1-INH-HAE) (B-E); and acquired angioedema with C1-INH deficiency (C1-INH-AAE) (F). For the HAE Type III samples (E), patients with a mutation in FXII at amino acid position 309 (T309K/R) are indicated in red.

