Quantitative Systems Biology Modeling Estimates Extent of Excessive Kallikrein Generation in Hereditary Angioedema Patients

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Poster 003

Introduction

- Hereditary angioedema (HAE) is characterized by C1 esterase inhibitor (C1INH) deficiency, leading to uninhibited kallikrein-kinin system (KKS) activation and bradykinin production resulting in angioedema attacks[1]
- One potential approach to treat HAE is to target the kallikrein pathway to rebalance the KKS
  - Currently approved therapies that inhibit kallikrein or reduce the production of prekallikrein have an acceptable benefit/risk profile with no known long-term safety implications[2]
  - Furthermore, hereditary prekallikrein deficiency, also known as Fletcher factor deficiency, is generally considered not to be associated with long-term safety concerns, although case reports are limited[3]
- Lonvoguran ziclumeran (lonvo-z) is an investigational *in vivo* CRISPR-based therapy designed to permanently inactivate the *KLKB1* gene to reduce prekallikrein production to treat HAE[4,5]
  - To date, in preclinical models and ongoing clinical studies, there have been no identified safety concerns with inactivating the *KLKB1* gene with a one-time treatment[4,5]; an ongoing Phase 3 study will establish the benefit-risk profile of lonvo-z and long-term follow-up is ongoing[6,7]
- Here, we evaluated a mathematical model to predict the effect on the KKS after permanent *KLKB1* inactivation with lonvo-z

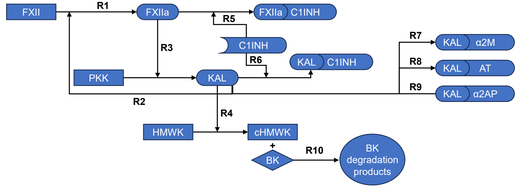
Objective

- Describe KKS activation kinetics and estimate bradykinin production capacity under varying physiological conditions to better understand the potential impact of lonvo-z 50 mg relative to healthy individuals, those with C1INH deficiency (ie, HAE), or those with prekallikrein deficiency (ie, Fletcher factor deficiency)

Methods

- We developed a quantitative systems pharmacology model of KKS activation incorporating 14 molecular species and 10 biochemical interactions, including the activated factor XII (FXIIa)-kallikrein amplification loop (**Figure 1**; **Tables 1** and **2**)
- The model approximates conditions in an *in vitro* assay, sampling a snapshot of the KKS pathway
- The simulated reactions were uniformly initiated with a small amount of FXIIa
- Biochemical reactions were formulated mathematically using Python to simulate clinical conditions
- Reactant concentrations were tracked kinetically for 1 hour post initiation
- Starting concentrations of key reactants were varied according to typical values reflecting real-world normal and pathophysiological C1INH (26-2600 nM) and prekallikrein (0-485 nM) levels
- HAE was assumed to have a range of 5%-50% normal C1INH and a simulated treatment with lonvo-z 50 mg was assumed to result in an 85% reduction in prekallikrein in the background of a C1INH deficiency based on clinical data[4,5]
- Please scan the QR code for Supplementary Methods

Figure 1. Model diagram



α2AP, alpha-2-antiplasmin; α2M, alpha2-macroglobulin; AT, antithrombin; BK, bradykinin; C1INH, C1 esterase inhibitor; cHMWK, cleaved HMWK; FXII, factor XII; FXIIa, activated factor XII; HWMK, high-molecular weight kininogen; KAL, kallikrein; PKK, prekallikrein.

Methods (continued)

Table 1. Reaction rate equations and parameters

ID	Reaction	Reactants	Rate equation	Kinetic parameters
R1	FXII autoactivation	FXII	$R1 = k_1[FXII]$	k_1 = 1.6×10^{-4} s^{-1}
R2	FXII activation by kallikrein	FXII	$R2 = \frac{k_{cat2}[KAL][FXII]}{K_{m2} + [FXII]}$	k_{cat2} = 0.01 s^{-1} K_{m2} = 11000 nM
R3	Prekallikrein activation	PKK	$R3 = \frac{k_{cat3}[PKK][FXIIa]}{K_{m3} + [PKK]}$	k_{cat3} = 0.7 s^{-1} K_{m3} = 291 nM
R4	HMWK cleavage	HMWK	$R4 = \frac{k_{cat4}[KAL][HMWK]}{K_{m4} + [HMWK]}$	k_{cat4} = 0.63 s^{-1} K_{m4} = 440 nM
R5	FXIIa inhibition by C1INH	FXIIa + C1INH	$R5 = k_5[FXIIa][C1INH]$	k_5 = 3.6×10^3 M^{-1}s^{-1}
R6	Kallikrein inhibition by C1INH	KAL + C1INH	$R6 = k_6[KAL][C1INH]$	k_6 = 1.7×10^4 M^{-1}s^{-1}
R7	Kallikrein inhibition by α2-macroglobulin	KAL + α2M	$R7 = k_7[KAL][\alpha 2M]$	k_7 = 5.8×10^3 M^{-1}s^{-1}
R8	Kallikrein inhibition by antithrombin	KAL + AT	$R8 = k_8[KAL][AT]$	k_8 = 240 M^{-1}s^{-1}
R9	Kallikrein inhibition by α2-antiplasmin	KAL + α2AP	$R9 = k_9[KAL][\alpha 2AP]$	k_9 = 180 M^{-1}s^{-1}
R10	BK degradation	BK	$R10 = k_{10}[BK]$	k_{10} = 0.046 s^{-1}

α2AP, alpha-2-antiplasmin; α2M, alpha2-macroglobulin; AT, antithrombin; BK, bradykinin; C1INH, C1 esterase inhibitor; FXII, factor XII; FXIIa, activated factor XII; HWMK, high-molecular weight kininogen; KAL, kallikrein; PKK, prekallikrein.

Table 2. Molecular species and initial concentrations

Species	Initial concentration (nM)	Role
FXII	375.0	Zymogen
FXIIa	0.0375	Active enzyme
PKK	485.0 (averaged normal)	Zymogen
KAL	0.0	Active enzyme
HMWK	670.0	Substrate
cHMWK	3.77	Cleaved product
BK	0.0	Peptide mediator
C1INH	2600.0 (averaged normal)	Inhibitor
α2M	1670.0	Inhibitor
AT	3500.0 (average)	Inhibitor
α2AP	1000.0	Inhibitor

α2AP, alpha-2-antiplasmin; α2M, alpha2-macroglobulin; AT, antithrombin III; BK, bradykinin; C1INH, C1 esterase inhibitor; cHMWK, cleaved HMWK; FXII, factor XII; FXIIa, activated factor XII; HWMK, high-molecular weight kininogen; KAL, kallikrein; PKK, prekallikrein.

Results

- Calculated peak concentrations of kallikrein and bradykinin in a model of HAE treated with lonvo-z 50 mg were consistent with those of a healthy individual (**Table 3, Figure 2**)
  - Healthy individuals (100% C1INH, 100% prekallikrein) generated mean peak concentrations of 36.5 nM and 132.1 nM for free kallikrein and bradykinin, respectively
  - Simulated patients with HAE with low C1INH (5%-15% of normal) showed approximately 4.9× normal peak kallikrein levels (mean±SD, 177.3±12.8 nM) and 1.5× elevated bradykinin potential (mean±SD, 207.4±3.0 nM)
  - An 85% reduction in prekallikrein concentration (representing typical reductions in patients with HAE treated with lonvo-z 50 mg) was shown to reduce mean peak free kallikrein and bradykinin to near normal ranges (22.5 nM free kallikrein and 112.0 nM bradykinin)
- Modeling reduction of prekallikrein following treatment with lonvo-z 50 mg suggested that patients can produce free kallikrein and bradykinin to levels within the normal range across a range of C1INH concentrations without reaching the lower levels seen in prekallikrein deficiency (**Figure 3**)
- The model suggested that the kinetics of free kallikrein levels in patients with HAE following lonvo-z 50 mg were similar to that of healthy individuals who have typical C1INH levels available to quell excessive kallikrein production (**Figure 4**)
  - A simulated deficiency in C1INH (HAE) resulted in a rapid increase and decrease in active kallikrein; however, following a simulated treatment with lonvo-z, kallikrein peaks were more consistent with healthy individuals
  - In contrast, in conditions of severe prekallikrein deficiency, there was minimal active kallikrein
- Simulations showed the capacity to produce bradykinin was retained in those with HAE, including in patients treated with lonvo-z 50 mg (**Figure 5**)
  - In prekallikrein deficiency, cleavage of HMWK was markedly slowed, aligning with the reduced bradykinin

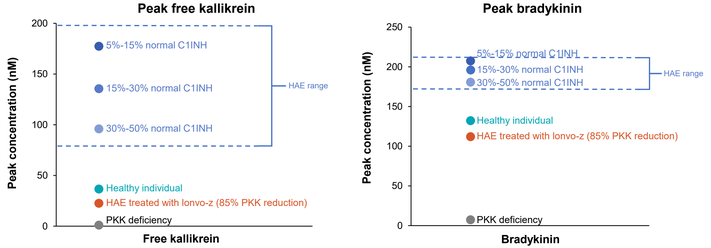
Table 3. Simulation of healthy and C1INH-deficient patient biomarkers

	Modeled clinical condition	Average C1INH, nM (%)	Prekallikrein, nM (%)	Maximum free kallikrein, nM (mean)	Maximum bradykinin, nM (mean)
Normal C1INH	Healthy individual	2600 (100)	485.0 (100)	36.5	132.1
85% reduction in prekallikrein with C1INH deficiency	HAE treated with lonvo-z	390 (15)	72.8 (15)	22.5	112.0
30%-50% of normal C1INH	HAE	1040 (40)	485.0 (100)	95.9 Range: 79.0-115.4	180.7 Range: 172.0-189.3
15%-30% of normal C1INH	HAE	585 (23)	485.0 (100)	135.5 Range: 115.4-158.2	196.1 Range: 189.2-202.5
5%-15% of normal C1INH	HAE	260 (10)	485.0 (100)	177.3 Range: 158.2-198.1	207.4 Range: 202.5-212.0
Prekallikrein deficiency	Fletcher factor deficiency	2600 (100)	9.7 (2)	1.0	7.3
Prekallikrein deficiency with 15% normal C1INH	HAE with prekallikrein deficiency	390 (15)	9.7 (2)	3.0	22.4

C1INH, C1 esterase inhibitor; HAE, hereditary angioedema.

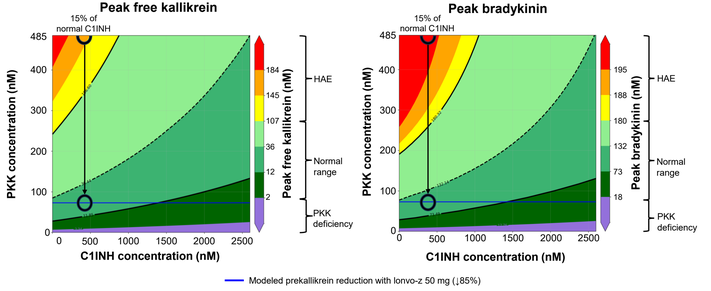
Results (continued)

Figure 2. Simulation of peak free kallikrein and bradykinin concentration



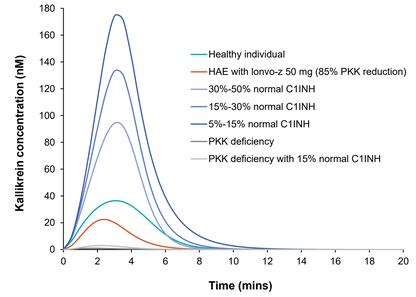
C1INH, C1 esterase inhibitor; HAE, hereditary angioedema; PKK, prekallikrein.

Figure 3. Impact of prekallikrein reduction on free, active kallikrein and bradykinin levels in the presence of reduced C1INH



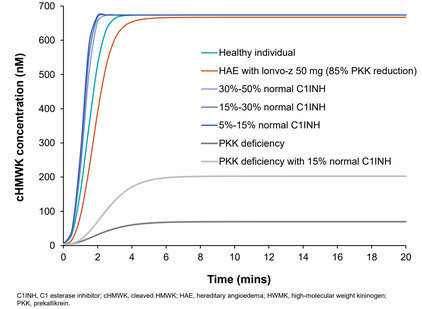
C1INH, C1 esterase inhibitor; HAE, hereditary angioedema; PKK, prekallikrein.

Figure 4. Kinetic estimation of free, active kallikrein



C1INH, C1 esterase inhibitor; HAE, hereditary angioedema; PKK, prekallikrein.

Figure 5. Kinetic estimation of cleaved HMWK



C1INH, C1 esterase inhibitor; cHMWK, cleaved HMWK; HAE, hereditary angioedema; HWMK, high-molecular weight kininogen; PKK, prekallikrein.

Conclusions

- This model suggests that patients with HAE with significant C1INH deficiency generate up to 5.4× excess free plasma kallikrein compared with healthy individuals, directly correlating with bradykinin increases
- In this model, an 85% reduction in prekallikrein, representing typical reductions seen with lonvo-z 50 mg, appeared to reset the KKS consistent with that of a healthy individual, suggesting lonvo-z may be an effective treatment for HAE
- The kinetics and capacity to cleave high-molecular weight kininogen (HMWK) were consistent between modeled healthy and HAE populations with a simulated 85% reduction in prekallikrein, suggesting the KKS is biochemically functional
  - Prekallikrein deficiency was associated with slower kinetics and a diminished capacity to fully cleave HMWK relative to healthy individuals
- Furthermore, modeled treatment with lonvo-z did not appear to yield a Fletcher factor deficiency phenotype. Any potential safety implications of having a prekallikrein deficiency are therefore expected to be avoided; however, the clinical impact of prekallikrein deficiency remains inconclusive
- This model may provide quantitative benchmarks for evaluating HAE therapeutics and the impact within the KKS

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This presentation includes data for an investigational product not yet approved by regulatory authorities.

Disclosures: APK has nothing to disclose. **CM**, **MYS**, **DM**, **JSB**, and **JAP** are employees of and hold equity in Intellia Therapeutics.

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References: 1. Zuraw BL. *N Engl J Med*. 2008;359(10):1027-1036. 2. Uminski K, et al. *Adv Ther*. 2025;42(12):5879-5895. 3. Barco S, et al. *J Thromb Haemost*. 2020;18(7):1598-1617. 4. Longhurst HJ, et al. *N Engl J Med*. 2024;390(5):432-441. 5. Cohn DM, et al. *N Engl J Med*. 2025;392(5):458-467. 6. ClinicalTrials.gov. Accessed Feb 17, 2026. https://clinicaltrials.gov/study/NCT06634420. 7. ClinicalTrials.gov. Accessed Feb 23, 2026. https://clinicaltrials.gov/study/NCT06262399.

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Supplementary Methods

- We developed a quantitative systems pharmacology model of kallikrein-kinin system (KKS) activation incorporating 14 molecular species and 10 biochemical interactions, including the activated factor XII (FXIIa)-kallikrein amplification loop (**Figure 1**; **Tables 1** and **2**)
- The model approximates conditions in an *in vitro* assay, sampling a snapshot of the KKS pathway
- The simulated reactions were uniformly initiated with a small amount of FXIIa
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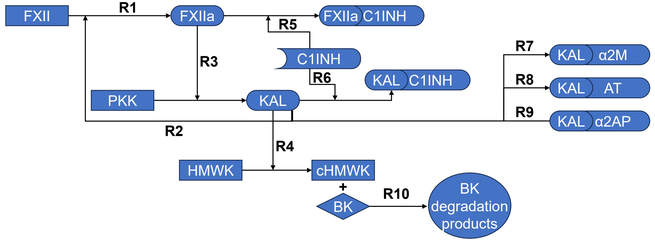
- Reactant concentrations were tracked kinetically for 1 hour post initiation
- Starting concentrations of key reactants were varied according to typical values reflecting real-world normal and pathophysiological C1 esterase inhibitor (C1INH; 26-2600 nM) and prekallikrein (0-485 nM) levels
- Hereditary angioedema was assumed to have a range of 5% to 50% normal C1INH and treatment with lonvo-z was assumed to result in an 85% reduction in prekallikrein

Figure 1. Model diagram



α2AP, alpha-2-antiplasmin; α2M, alpha2-macroglobulin; AT, antithrombin; BK, bradykinin; C1INH, C1 esterase inhibitor; cHMWK, cleaved HMWK; FXII, factor XII; FXIIa, activated factor XII; HWMK, high-molecular weight kininogen; KAL, kallikrein; PKK, prekallikrein.

Table 1. Reaction rate equations and parameters

ID	Reaction	Reactants	Enzyme	Products	Rate equation	Kinetic parameters	Equation type	Reference
R1	FXII autoactivation	FXII	-	FXIIa	$R1 = k_1[FXII]$	k_1 = 1.6×10^{-4} s^{-1}	First order	Chatterjee MS, et al. *PLoS Comput Biol*. 2010;6(9):e1000950. Loiseau C, et al. *Eur J Biochem*. 1996;239(3):692-701.
R2	FXII activation by kallikrein	FXII	KAL	FXIIa	$R2 = \frac{k_{cat2}[KAL][FXII]}{K_{m2} + [FXII]}$	k_{cat2} = 0.01 s^{-1} K_{m2} = 11000 nM	Michaelis-Menten	Tankersley DL, et al. *Biochemistry*. 1984;23(2):273-279.
R3	Prekallikrein activation	PKK	FXIIa	KAL	$R3 = \frac{k_{cat3}[PKK][FXIIa]}{K_{m3} + [PKK]}$	k_{cat3} = 0.7 s^{-1} K_{m3} = 291 nM	Michaelis-Menten	Tankersley DL, et al. *Biochemistry*. 1984;23(2):273-279. Shamanaev A, et al. *J Thromb Haemost*. 2021;19(2):330-341.
R4	HMWK cleavage	HMWK	KAL	cHMWK + BK	$R4 = \frac{k_{cat4}[KAL][HMWK]}{K_{m4} + [HMWK]}$	k_{cat4} = 0.63 s^{-1} K_{m4} = 440 nM	Michaelis-Menten	Weidmann H, et al. *Biochim Biophys Acta Mol Cell Res*. 2017;1864(11 Pt B):2118-2127. Tayeh MA, et al. *J Biol Chem*. 1994;269(23):16318-16325.
R5	FXIIa inhibition by C1INH	FXIIa + C1INH	-	FXIIa:C1INH complex	$R5 = k_5[FXIIa][C1INH]$	k_5 = 3.6×10^3 M^{-1}s^{-1}	Bimolecular (irreversible)	Pixley RA, et al. *J Biol Chem*. 1985;260(3):1723-1729.
R6	Kallikrein inhibition by C1INH	KAL + C1INH	-	KAL:C1INH complex	$R6 = k_6[KAL][C1INH]$	k_6 = 1.7×10^4 M^{-1}s^{-1}	Bimolecular (irreversible)	Chatterjee MS, et al. *PLoS Comput Biol*. 2010;6(9):e1000950. Silverberg M, et al. *J Biol Chem*. 1986;261(32):14965-14968.
R7	Kallikrein inhibition by α2-macroglobulin	KAL + α2M	-	KAL:α2M complex	$R7 = k_7[KAL][\alpha 2M]$	k_7 = 5.8×10^3 M^{-1}s^{-1}	Bimolecular (irreversible)	Cugno M, et al. *Blood*. 1997;89(9):3213-3218. van der Graaf F, et al. *Biochemistry*. 1984;23(8):1760-1766.
R8	Kallikrein inhibition by antithrombin	KAL + AT	-	KAL:AT complex	$R8 = k_8[KAL][AT]$	k_8 = 240 M^{-1}s^{-1}	Bimolecular (irreversible)	Gozzo AJ, et al. *Biol Chem*. 2006;387(8):1129-1138.
R9	Kallikrein inhibition by α2-antiplasmin	KAL + α2AP	-	KAL:α2AP complex	$R9 = k_9[KAL][\alpha 2AP]$	k_9 = 180 M^{-1}s^{-1}	Bimolecular (irreversible)	Pixley RA, et al. *J Biol Chem*. 1985;260(3):1723-1729.
R10	BK degradation	BK	-	BK degradation products	$R10 = k_{10}[BK]$	k_{10} = 0.046 s^{-1}	First order	Maurer M, et al. *Allergy*. 2011;66(11):1397-1406.

α2AP, alpha-2-antiplasmin; α2M, alpha2-macroglobulin; AT, antithrombin; BK, bradykinin; C1INH, C1 esterase inhibitor; FXII, factor XII; FXIIa, activated factor XII; HWMK, high-molecular weight kininogen; KAL, kallikrein; PKK, prekallikrein.

Table 2. Molecular species and initial concentrations

Species	Initial Concentration (nM)	Role	References
FXII	375.0	Zymogen	Weidmann H, et al. *Biochim Biophys Acta Mol Cell Res*. 2017;1864(11 Pt B):2118-2127.; Rezvani-Sharif A, et al. *PLoS Comput Biol*. 2024;20(11):e1012552.; Sexton D, et al. *J Pharmacokinet Pharmacodyn*. 2024;51(6):721-734.
FXIIa	0.0375	Active enzyme	Pixley RA, et al. *J Biol Chem*. 1985;260(3):1723-1729.
PKK	485.0 (averaged normal)	Zymogen	Weidmann H, et al. *Biochim Biophys Acta Mol Cell Res*. 2017;1864(11 Pt B):2118-2127.; Rezvani-Sharif A, et al. *PLoS Comput Biol*. 2024;20(11):e1012552.; Sexton D, et al. *J Pharmacokinet Pharmacodyn*. 2024;51(6):721-734.
KAL	0.0	Active enzyme	Røjkjaer R, et al. *Eur J Biochem*. 1997;243(1-2):160-166; Konings J, et al. *PLoS One*. 2013;8(8):e74043.
HMWK	670.0	Substrate	Sexton D, et al. *J Pharmacokinet Pharmacodyn*. 2024;51(6):721-734.; Weidmann H, et al. *Biochim Biophys Acta Mol Cell Res*. 2017;1864(11 Pt B):2118-2127.; Colman RW, et al. *Blood*. 1997;90(10):3819-3843.
cHMWK	3.77	Cleaved product	Rezvani-Sharif A, et al. *PLoS Comput Biol*. 2024;20(11):e1012552.; Zhang G, et al. *Bioanalysis*. 2017;9(19):1477-1491.
BK	0.0	Peptide mediator	Nussberger J, et al. *Lancet*. 1998;351(9117):1693-1697.
C1INH	2600.0 (averaged normal)	Inhibitor	Weidmann H, et al. *Biochim Biophys Acta Mol Cell Res*. 2017;1864(11 Pt B):2118-2127.; Pixley RA, et al. *J Biol Chem*. 1985;260(3):1723-1729.; Sexton D, et al. *J Pharmacokinet Pharmacodyn*. 2024;51(6):721-734.
α2M	1670.0	Inhibitor	Weidmann H, et al. *Biochim Biophys Acta Mol Cell Res*. 2017;1864(11 Pt B):2118-2127.; Coan MH, et al. *Biol Chem Hoppe Seyler*. 1989;370(7):673-676.; Pixley RA, et al. *J Biol Chem*. 1985;260(3):1723-1729.
AT	3500.0 (average)	Inhibitor	Weidmann H, et al. *Biochim Biophys Acta Mol Cell Res*. 2017;1864(11 Pt B):2118-2127.; Pixley RA, et al. *J Biol Chem*. 1985;260(3):1723-1729.
α2AP	1000.0	Inhibitor	Weidmann H, et al. *Biochim Biophys Acta Mol Cell Res*. 2017;1864(11 Pt B):2118-2127.; Pixley RA, et al. *J Biol Chem*. 1985;260(3):1723-1729.

α2AP, alpha-2-antiplasmin; α2M, alpha2-macroglobulin; AT, antithrombin III; BK, bradykinin; C1INH, C1 esterase inhibitor; cHMWK, cleaved HMWK; FXII, factor XII; FXIIa, activated factor XII; HWMK, high-molecular weight kininogen; KAL, kallikrein; PKK, prekallikrein.

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