Discovery and characterization of a novel engineered Fc-fused IgE cleaving enzyme for treatment of IgE mediated diseases

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ABSTRACT

Rationale: The dysregulation of humoral immune mechanisms results in pathogenic IgE production that contributes to a range of allergic and atopic diseases such as food allergies, acute anaphylaxis, allergic asthma, allergic rhinitis and chronic spontaneous urticaria. We present a novel Fc-fused bacterially-derived IgE protease that was engineered using a proprietary machine learning enabled platform to reduce immunogenicity and improve manufacturability while maintaining potency. The protease selectively cleaves IgE, eliminating it from circulation, the cell-surface and immune-complexes, and provides a novel therapeutic opportunity to treat IgE-mediated inflammation.

Methods: The engineered Fc-fused IgE protease was identified using our proprietary IMPACT platform and was characterized in vitro to determine its ability to cleave IgE using MSD and flow cytometry-based cleavage assays. Immunogenicity was assessed using T cell proliferation assays. Pharmacokinetics, pharmacodynamics and in vivo efficacy were tested using relevant preclinical models.

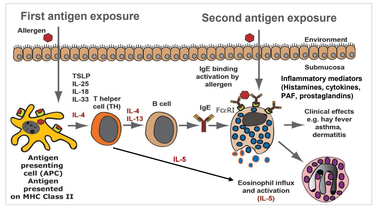
Results: The engineered Fc-protease selectively cleaves IgE, while improving stability and demonstrating low immunogenicity. It cleaves soluble IgE in human plasma with high potency, IgE$^+$ BCR and IgE bound to CD23/FcεRII in B cell lines. Remarkably, this protease shows extended pharmacokinetics and efficacy in preclinical models of local and systemic acute anaphylaxis, suggesting an impact on IgE-mediated effector functions.

Conclusions: Given its ability to simultaneously address multiple aspects of IgE pathogenesis and its efficacy in preclinical models of anaphylaxis, the engineered Fc-protease offers a new approach to targeted therapy for allergic and atopic diseases where IgE is a key driver.

INTRODUCTION

Allergic Immune Response

Dysregulation of humoral immune mechanisms results in production of IgE autoantibodies which are directly pathogenic in a wide range of allergic and atopic diseases.



Schematic overview of an allergic immune response starting with the allergen's first contact when it enters through the skin, respiratory, or gastrointestinal mucosa
(Adapted from Hellman et al; Frontiers in Immunology, vol. 8, 2017)

IMPACT Platform

A naturally occurring IgE protease derived from bacteria was fused to a human IgG Fc domain to prolong its half-life and engineered with Seismic Therapeutic's propriety machine learning IMPACT platform. The resulting molecule, selectively cleaves soluble, membrane-bound, BCR IgE and downregulated FcεRI on effector cells, without impacting other immunoglobulin (Ig) isotypes.

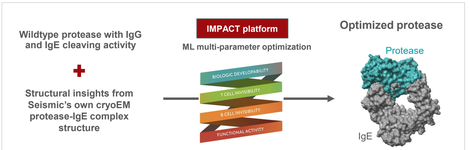


FIGURE 1: Seismic Therapeutic's proprietary machine learning enabled IMPACT platform and insights from protease-IgE complex structure, were used to engineer IgE protease and optimize multiple properties in parallel, including enzyme stability, activity, selectivity, reduction of immunogenicity, and manufacturability.

METHODS

***In vitro* assays for developability profile measurements:** Human IgE and IgG cleavage activity of the protease was measured using a CE-SDS assay. Aggregation or degradation was analyzed by analytical size exclusion chromatography (aSEC). Polyreactivity of the molecule was tested against a panel of 4 well established targets in a binding ELISA method. Thermostability and viscosity was measured using nanoDSF and viscometer respectively.

***In vitro* immunogenicity assay:** ProImmune's ProMap® Immunogenicity System T cell proliferation assay was used to enable *in vitro* assessment of potential antigenicity of molecules tested. PBMCs from HLA-typed healthy human donors were CFSE-labeled and cultured with test articles. Cells were stained with anti-CD4 antibody and proliferation was determined by measuring CFSE intensity using flow cytometric analysis. Reference antigens comprising known MHC class II epitopes were used in this study. Percentage antigenicity is expressed as the frequency of positive donor cell responses.

***In vitro* IgE and IgG cleavage:** Plasma from healthy donors was treated overnight with varying doses of IgE protease at 37°C. A Meso Scale Discovery (MSD) plate was coated with anti-human IgE or anti-human light chain antibody overnight. Next, the MSD plate was blocked and human plasma was added. Biotinylated anti-human light chain or anti-human IgG Fc antibody was used for detection of IgE and IgG respectively. Intact and fully cleaved human IgE and IgG were detected by JESS western blot system.

***In vitro* BCR and CD23 bound IgE cleavage:** U266 B1 human myeloma cells were treated with varying doses of IgE protease overnight at 37°C. Cells were stained with anti-CD19, anti-IgE and anti-lambda antibodies for flow cytometry analysis. RPMI8866 cells were pre-incubated with human IgE, followed by treatment with protease as indicated above. Cells were then stained with anti-CD19, anti-CD23, anti-IgE and anti-kappa antibodies for flow cytometry analysis.

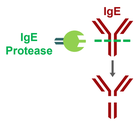
***In vivo* human IgE cleavage and FcεRI expression:** Humanized IgE/FcεRI mice (purchased from Genoway) received human IgE and IgE protease via intravenous administration. Blood was collected at different timepoints to quantify protease levels and IgE reduction following the MSD protocol described above. Human FcεRI expression was determined by staining mouse whole blood basophils with anti-CD49b and anti-FcεRI antibodies and analyzing by flow cytometry. Bone marrow cells from humanized mice were differentiated into mast cells and treated with varying concentrations of IgE protease. Cells were stained with anti-CD117 and anti-FcεRI antibodies for flow cytometry analysis.

***In vivo* passive systemic and cutaneous anaphylaxis (PSA and PCA) studies:** Humanized IgE/FcεRI mice were sensitized with anti-NIP IgE intraperitoneally (PSA) or intradermally in ear (PCA) and injected with IgE protease or vehicle by intravenous (IV) administration. Next day, mice were challenged with NIP-BSA via IV or IP administration. Evan's blue dye extravasation assay in the ear was performed (PCA), while changes in body core temperature were measured every 10 minutes until 120 minutes (PSA).

***In vivo* rat allergic asthma model:** Rats were sensitized with house dust mite (HDM) allergen intraperitoneally and then administered with a single dose of IgE protease or vehicle via intravenous or subcutaneous route. Dexamethasone was administered intraperitoneally three times during the course of the study. Rats were challenged with HDM via intra-tracheal instillation. Blood was collected at various timepoints for detecting IgE protease and rat IgE levels using MSD assay. Eosinophil counts were determined in the cells isolated from the broncho-alveolar lavage from the lungs.

RESULTS

IgE protease offers a multi-mechanistic approach for allergic and atopic diseases



Targets multiple orthogonal pathogenic mechanisms in autoantibody-mediated disease	IgE Protease	Approved anti-IgE
Rapid reduction of soluble IgE	✓	✓
Systemic and target tissue activity	✓	✓
Cleavage of IgE BCR	✓	✗
Reduction of FcεRI expression	✓	✓

FIGURE 2: IgE protease selectively cleaves soluble, membrane-bound and BCR IgE, splitting the Fc portion from the Fab arms, thereby eliminating IgE binding to high affinity FcεRI receptor on effector cells and preventing degranulation in hypersensitivity reactions.

Engineered IgE protease demonstrates improved IgE activity and no IgG cleavage compared to parental molecule

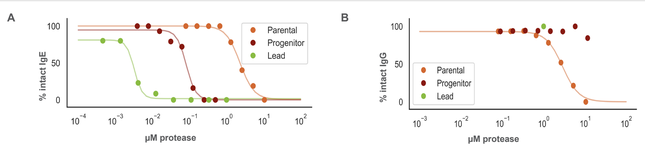


FIGURE 3: A parental protease discovered from an evolutionary screen of cysteine proteases was re-engineered to improve potency and specificity using machine learning combined with structure-based and data-driven rational design. Through a statistical model trained on an alignment of related sequences, mutations were introduced at positions with potential implications for activity and specificity, identified by analysis of the protein structure in complex with substrate A) and B) Parental protease cleaves all IgG subclasses and showed minimal IgE cleavage; progenitor molecule greatly improved potency with no IgG cleavage and the engineered lead protease molecule showed significantly greater potency with no IgG cleavage.

IgE protease shows favorable developability profile

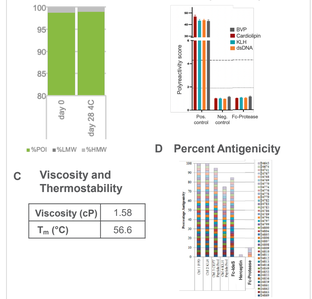


FIGURE 4: IgE protease is A) highly stable and does not aggregate at 4°C until 28 days B) is not polyreactive predictive of favorable PK and C) has low viscosity measurement suitable for subcutaneous injection and has favorable thermostability D) has low total percent antigenicity split by donor

IgE protease selectively cleaves human IgE in plasma of healthy donors

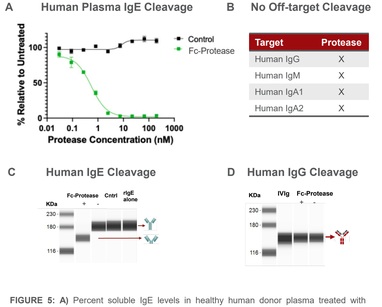


FIGURE 5: A) Percent soluble IgE levels in healthy human donor plasma treated with increasing protease concentrations overnight and quantified by MSD B) Recombinant human IgG, IgM and IgA1 and IgA2 cleavage analyzed by CE-SDS assay C) Intact and fully cleaved human IgE and D) Intact human IgG cleavage species were detected by JESS western blot system.

IgE protease cleaves IgE BCR and CD23-bound IgE on B cell lines *in vitro*

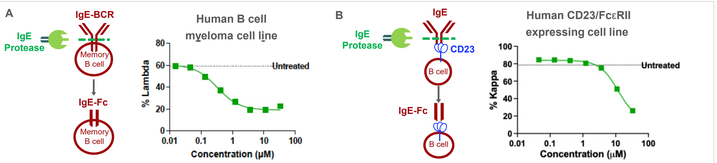


FIGURE 6: A) Percentage of lambda IgE+ B cells in human myeloma B cell line treated with increasing protease concentrations overnight resulting in protease-mediated BCR cleavage in vitro and B) CD23/FcεRII-bound IgE cleavage depicted as percent kappa reduction in CD23-expressing RPMI8866 cells.

IgE protease exhibits favorable PK and rapid reduction of human IgE and FcεRI levels in vivo

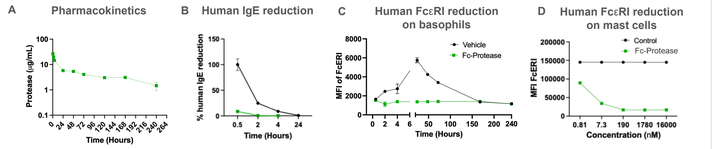


FIGURE 7: A) Pharmacokinetics B) Human IgE reduction and C) Human FcεRI reduction, after single dose of IgE protease in humanized IgE/FcεRI mice. Blood was collected at different timepoints to quantify IgE reduction using MSD method D) Rapid reduction of human FcεRI on bone-marrow-derived mast cells from humanized IgE/FcεRI mice after protease treatment ex-vivo.

IgE protease demonstrates comparable efficacy to approved anti-IgE therapy in animal models of anaphylaxis and allergy

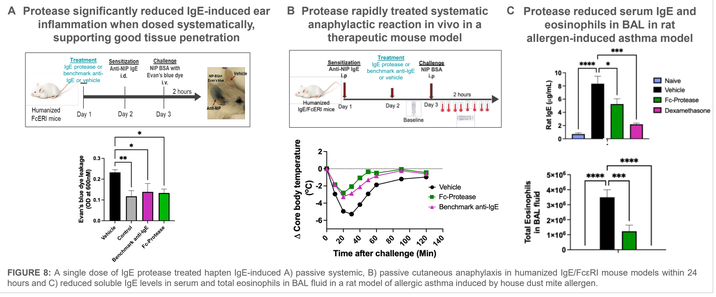


FIGURE 8: A single dose of IgE protease treated hapten IgE-induced A) passive systemic, B) passive cutaneous anaphylaxis in humanized IgE/FcεRI mouse models within 24 hours and C) reduced soluble IgE levels in serum and total eosinophils in BAL fluid in a rat model of allergic asthma induced by house dust mite allergen.

CONCLUSIONS

- IgE protease is a novel engineered first-in-class IgE degrading enzyme that demonstrates favorable manufacturability profile and low antigenicity.
- PK and PD studies show rapid reduction of soluble human IgE and FcεRI expression on effector cells at low doses, favorable for convenient and infrequent subcutaneous administration for patients.
- It cleaves circulating, membrane-bound and BCR IgE thereby removing the antigen-presenting portion of the IgE-BCR that will prevent memory B cell activation as well as antigen internalization/presentation to memory T cells.
- IgE protease is efficacious at low therapeutic doses in pre-clinical mouse models of systemic and cutaneous anaphylaxis comparable to benchmark anti-IgE, as well as reduces soluble IgE levels and eosinophils in allergen-induced rat asthma model.

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