Preclinical pharmacology of S-1117, a novel engineered Fc-fused IgG cleaving enzyme, for chronic treatment of autoantibody-mediated diseases including myasthenia gravis

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ABSTRACT

Objective: To assess the PK, PD and preclinical efficacy of an engineered pan-IgG protease.

Introduction: Pathogenic autoantibodies are key effectors of inflammation, promoting immune cell responses that cause tissue damage in autoantibody-mediated diseases such as myasthenia gravis (MG). Antibody degradation using an IgG protease represents a new therapeutic opportunity.

S-1117, a novel Fc-fused pan-IgG protease, was engineered for chronic administration using a proprietary machine learning enabled platform to augment manufacturability while maintaining potency and reduce immunogenicity. S-1117 addresses multiple mechanisms of autoimmunity by cleaving plasma IgG and B cell receptor (BCR) on memory B cells while reducing IgG effector functions and immune complex (IC)-mediated cell activation.

Methods: Plasma IgG, BCR, and IC cleavage assays were performed *in vitro*. A murine PK/PD model evaluated S-1117 function *in vivo*.

Results: S-1117 cleaves soluble IgG and BCR on memory B cells derived from healthy individuals and MG patients with comparable potency. S-1117 directly eliminates IgG effector functions including ADCC, CDC, and IC-mediated immune cell activation *in vitro*.

A single dose of S-1117 induced a rapid (<24 hours), deep (>90%), and sustained reduction of human IgG administered to mice. Human PK/PD QSP modeling indicates that infrequent chronic low doses will achieve titratable IgG reductions of 90% or greater as clinically indicated.

Summary: S-1117 is a novel engineered pan-IgG protease that demonstrates rapid and sustained reduction of human IgG levels and effector function. Advantages of enzymatic degradation and sustained PK enable a convenient treatment regimen. Since S-1117 addresses multiple pathogenic mechanisms as a single drug, it has potential to achieve superior clinical outcomes in MG.

INTRODUCTION

Mechanisms of humoral immunity can result in the production of immunoglobulins (Igs) which are directly pathogenic in a wide range of autoantibody-mediated diseases, including MG, where the predominant pathogenic autoantibody isotype is IgG. A naturally occurring pan-IgG 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 (ML) IMPACT platform. The IMPACT platform was used to enhance drug-like properties, reduce T and B cell epitopes to lower immunogenicity, and remove chemical and manufacturing liabilities, while maintaining enzymatic activity (Fig 1). The resulting molecule, S-1117, selectively cleaves soluble, immune-complexed, membrane-bound, and BCR IgG without impacting other Ig isotypes.

IMPACT platform

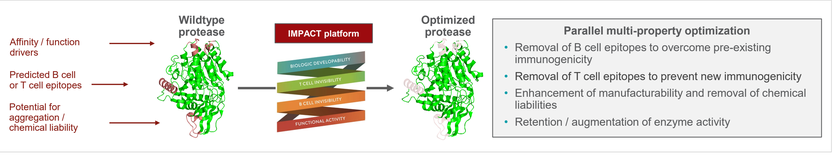

FIGURE 1: Seismic Therapeutic's proprietary machine learning enabled IMPACT platform was used to engineer S-1117 and optimize multiple properties in parallel, including enzyme stability, reduction of immunogenicity, and manufacturability, while maintaining enzyme activity.

METHODS

***In vitro* IgG cleavage:** Plasma from healthy or myasthenia gravis donors was treated overnight with varying doses of S-1117 at 37°C. A Meso Scale Discovery (MSD) plate was coated with anti-human light chain antibody overnight. The following day, the MSD plate was blocked and human plasma was added. Biotinylated anti-human IgG Fc antibody was used for detection.

Immune complex (IC) activation of peripheral blood mononuclear cells (PBMC): Pre-formed ICs were incubated with S-1117 for 2 hrs. S-1117 treated or untreated ICs, antigen (RNP), or antibody (anti-RNP) were added to human PBMC overnight. Supernatants were collected to determine IFN-α levels by ELISA. Statistical analyses were performed with one-way ANOVA followed by Tukey's post hoc test.

***In vitro* BCR cleavage:** Human whole blood was treated with varying doses of S-1117 for 20 hrs at 37°C. Cells were stained with anti-IgG, anti-CD20, anti-IgD, anti-IgM, anti-lambda and anti-kappa antibodies after red blood cell (RBC) lysis. Flow cytometry was performed on stained cells.

***In vivo* BCR cleavage:** New Zealand rabbits received S-1117 intravenously (IV) or subcutaneously (SC). Blood was collected and PBMC isolated at different timepoints. Cells were stained with anti-CD14, anti-IgG, anti-IgM, and anti-light chain. Flow cytometry was performed on stained cells.

***In vivo* IgG cleavage:** C57BL6 mice received 9 mg of IVIG intraperitoneally (IP). The day after, S-1117 or benchmark FcRN inhibitor (FcRNi) were injected. At study day 9, mice received a second injection of 4.5 mg IVIG. Blood was collected at different timepoints to quantify IVIG reduction following MSD protocol described above.

Fc-mediated effector function assays: Performed by a contract research organization (CRO). Complement-dependent cytotoxicity (CDC): Ramos cells were incubated with rituximab or control antibody for 15 min. Then, S-1117 or benchmark molecules were added in a dose response manner for 2 hrs prior to the addition of 1% normal human serum. Viability was assessed by luciferase activity. Antibody-dependent cellular cytotoxicity (ADCC): Stained SKBR3 cells were opsonized with trastuzumab or control antibody and incubated with S-1117 or benchmark molecules in a dose response manner. PBMC were added and lysis quantified by flow cytometry.

Human dose projection modeling: Performed by Applied BioMath (ABM) and Jiyun Sunny Chen. Quantitative systems pharmacology (QSP) modeling was performed using available *in vitro* and *in vivo* data to estimate human pharmacokinetics (PK) and pharmacodynamics (PD) at different IV and SC doses as well as single or chronic dose administration of S-1117.

RESULTS

S-1117 offers a multi-mechanistic approach for myasthenia gravis

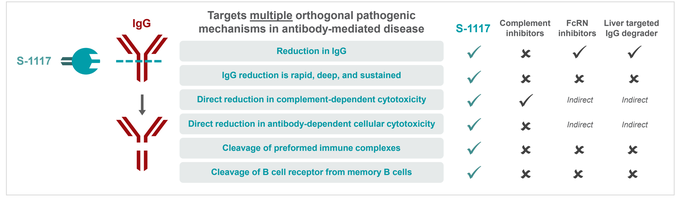

FIGURE 2: S-1117 selectively cleaves soluble, immune-complexed, membrane-bound, and BCR IgG, splitting the Fc portion from the Fab arms, thereby also eliminating IgG effector function.

S-1117 cleaves soluble IgG in plasma of healthy volunteers and MG patients

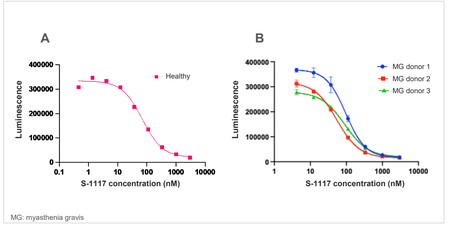

FIGURE 3: A) Total IgG levels in healthy human plasma at increasing S-1117 concentrations as quantified by MSD. B) Total IgG levels in plasma from MG patients at increasing S-1117 concentrations as quantified by MSD.

S-1117 cleaves preformed immune complexes

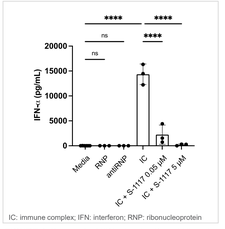

FIGURE 4: Supernatant IFN-α levels after addition of S-1117 treated or untreated ICs (RNP + anti-RNP), RNP, or anti-RNP to PBMC. **** p<0.0001

S-1117 cleaves IgG BCR on memory B cells *in vitro* and *in vivo*

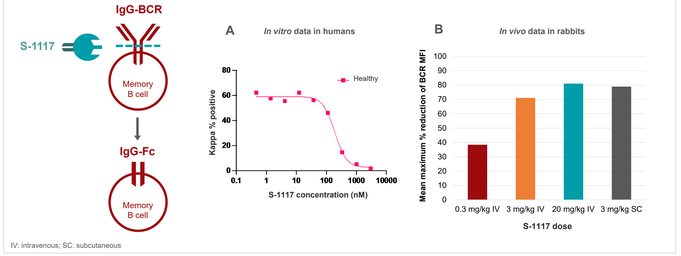

FIGURE 5: A) Percentage of kappa positive IgG+ B cells in healthy human whole blood at increasing S-1117 concentrations resulting from S-1117-mediated BCR cleavage of human B cells *in vitro*. B) Peak IgG-BCR cleavage *in vivo* expressed as mean maximum percent reduction of BCR at three IV dose levels and one SC dose level.

S-1117 shows faster, deeper, more sustained reduction of human IVIG compared to FcRN inhibitor

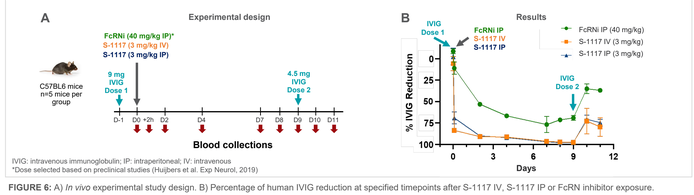

FIGURE 6: A) *In vivo* experimental study design. B) Percentage of human IVIG reduction at specified timepoints after S-1117 IV, S-1117 IP or FcRN inhibitor exposure.

S-1117 directly reduces IgG effector function, including CDC and ADCC

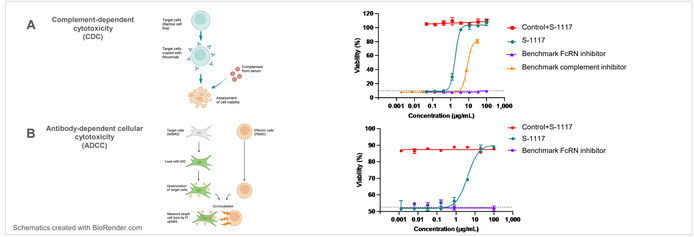

FIGURE 7: A) CDC from rituximab exposure on Ramos cells after S-1117, FcRN inhibitor or complement inhibitor incubation in a dose response manner. B) Target cell lysis after trastuzumab-induced cytotoxicity after S-1117 or FcRN inhibitor incubation in a dose response manner.

S-1117 human PK/PD dose projections predict a titratable, convenient treatment regimen

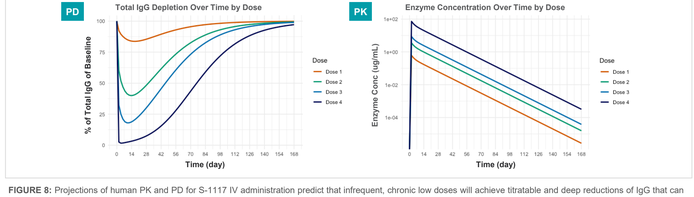

FIGURE 8: Projections of human PK and PD for S-1117 IV administration predict that infrequent, chronic low doses will achieve titratable and deep reductions of IgG that can be used in chronic and acute situations. Modeling based on *in vitro* and *in vivo* studies.

CONCLUSIONS

- S-1117 is a novel engineered pan-IgG protease that demonstrates rapid and sustained reduction of human IgG (all subtypes)
- It addresses multiple, clinically-validated, orthogonal pathogenic mechanisms in autoimmunity within a single molecule, many of which are important in myasthenia gravis
- It directly cleaves circulating, immune-complexed, membrane-bound, and BCR IgG without affecting other Ig isotypes
- S-1117 is expected to achieve dosing regimens that can be adjusted for chronic and acute treatment and used in a "treat-to-target" approach which may be tailored to the patient's disease activity
- It is being developed for subcutaneous self-administration every 4-6 weeks for patient convenience
- S-1117 will enter the clinic in the first half of 2025

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