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

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ABSTRACT

Background/Purpose: Pathogenic autoantibodies are key effectors of inflammation, promoting immune cell responses that cause tissue damage in autoantibody-mediated diseases such as idiopathic inflammatory myopathies (IIMs), lupus nephritis (LN), Sjogren's syndrome (SjS), antiphospholipid syndrome (APS), and ANCA-associated vasculitis (AAV). Antibody degradation using an IgG protease represents a new therapeutic opportunity.

Methods: Plasma IgG, IgG+ B cell receptor (BCR), immune complex (IC) cleavage, and antibody mediated effector function assays were performed *in vitro*. S-1117 function was tested pre-clinically in rabbits, cynomolgus monkeys, and mice *in vivo*.

Results: S-1117 potently cleaves all IgG subclasses in plasma from healthy individuals. It directly eliminates IgG effector function, including antibody-dependent and complement-dependent cytotoxicity, and IC-mediated immune cell activation *in vitro*. Moreover, it cleaves the IgG+ BCR on memory B cells in humans *in vitro* and rabbits *in vivo*.

In vivo, a single dose of S-1117 induces rapid (< 24 hours), deep (>90%), and sustained (10 or more days) reduction of endogenous IgG in rabbits and of human IgG administered in cynomolgus monkeys. Chronic administration of S-1117 in mice is well tolerated and demonstrates maintenance of drug exposure and activity. Human pharmacokinetic (PK) / pharmacodynamic (PD) quantitative systems pharmacology modeling predicts that infrequent chronic low doses of S-1117 can achieve a range of IgG reductions up to 90% or greater, titrated to the clinical needs of each patient.

Conclusion: S-1117 is a novel engineered pan-IgG protease that demonstrates rapid, deep, and sustained reduction of IgG levels and IgG effector function, as well as cleavage of the IgG+ BCR on memory B cells. Advantages of enzymatic degradation, sustained PK, and titratable PD are expected to enable a convenient patient-tailored treatment regimen. Since S-1117 addresses multiple pathogenic mechanisms as a single drug, it has the potential to provide superior clinical outcomes in autoantibody-mediated diseases with complex pathology.

INTRODUCTION

Mechanisms of humoral immunity can result in the production of IgG autoantibodies which are directly pathogenic in a wide range of autoantibody-mediated diseases, such as IIMs, LN, SjS, APS, and AAV. 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 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 immunoglobulin (Ig) isotypes.

IMPACT platform

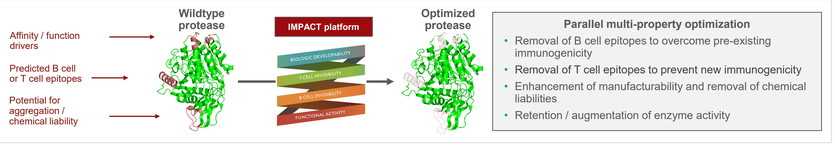


FIGURE 1: Seismic Therapeutic's proprietary machine learning enabled IMPACT platform was used to engineer S-1117 and optimize multiple properties in parallel, including enzyme stability, reduction of immunogenicity, and manufacturability, while maintaining enzyme activity.

METHODS

***In vitro* IgG cleavage:** Plasma from healthy 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. Next, the MSD plate was blocked and human plasma was added. Biotinylated anti-human IgG Fc antibody was used for detection.

***In vitro* 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* human IgG cleavage in mouse:** C57BL6 mice received 9 mg of IVIG intraperitoneally (IP). The following day, S-1117 or benchmark FcRN inhibitor (FcRNi) were injected. At study day 9, mice received a second injection of 4.5 mg intravenous immunoglobulin (IVIG). Blood was collected at different timepoints to quantify IVIG reduction following the 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.

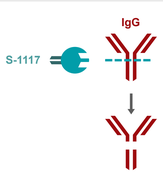
Rabbit PK/PD and BCR cleavage: New Zealand rabbits received S-1117 or vehicle by intravenous (IV) or subcutaneous (SC) administration. Blood was collected and serum and PBMC isolated at different timepoints. Serum rabbit IgG levels were quantified by MSD. PBMC were stained with anti-CD14, anti-IgG, anti-IgM, and anti-light chain. Flow cytometry was performed on stained cells.

Cynomolgus monkey PK/PD: Macaca fascicularis cynomolgus macaques received human IgG1 as a tracer antibody since S-1117 does not cleave all non-human primate IgG subclasses. The next day, animals received S-1117 or vehicle by IV or SC administration. Blood was collected at different timepoints to quantify human IgG1 reduction by MSD.

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 PK and 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 autoimmune diseases



Targets multiple orthogonal pathogenic mechanisms in autoantibody-mediated disease	S-1117	Complement inhibitors	FcRN inhibitors	Liver targeted IgG degrader
Reduction in IgG	✓	✗	✓	✓
IgG reduction is rapid, deep, and sustained	✓	✗	✗	✗
Direct reduction in complement-dependent cytotoxicity	✓	✓	*Indirect*	*Indirect*
Direct reduction in antibody-dependent cellular cytotoxicity	✓	✗	*Indirect*	*Indirect*
Cleavage of preformed immune complexes	✓	✗	✗	✗
Cleavage of B cell receptor from memory B cells	✓	✗	✗	✗

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 donors

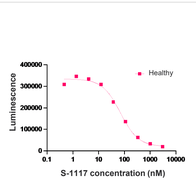


FIGURE 3: Total IgG levels in healthy human plasma at increasing S-1117 concentrations as quantified by MSD.

S-1117 cleaves preformed immune complexes

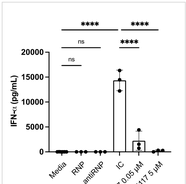


FIGURE 4: Supernatant IFN-α levels after addition of S-1117 treated or untreated ICs (RNP + anti-RNP), RNP, or anti-RNP to PBMC. **** p<0.0001

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

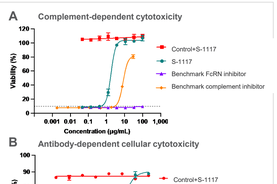


FIGURE 5: A) CDC from rituximab exposure on Ramos cells after S-1117, FcRN inhibitor, or complement inhibitor incubation in a dose response manner. B) Target cell lysis from trastuzumab-induced cytotoxicity after S-1117 or FcRN inhibitor incubation in a dose response manner.

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

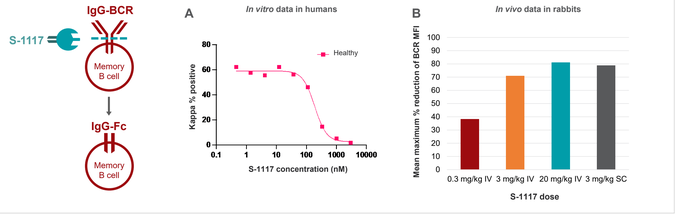
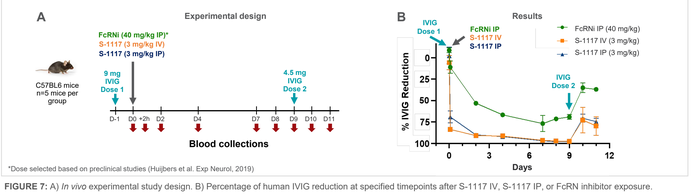


FIGURE 6: A) Percentage of kappa positive IgG+ B cells in healthy human whole blood at increasing S-1117 concentrations resulting from S-1117-mediated BCR cleavage *in vitro*. B) Peak rabbit IgG-BCR cleavage expressed as mean maximum percent reduction of BCR at three IV dose levels and one SC dose level *in vivo*.

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



*Dose selected based on preclinical studies (Huijbers et al. Exp Neurol, 2019)

FIGURE 7: 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 achieves rapid and deep reduction of IgG *in vivo* in rabbits and cynomolgus monkeys

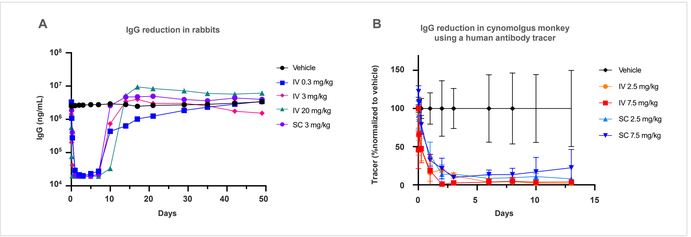


FIGURE 8: A) A single dose of S-1117 reduced rabbit IgG to undetectable levels within 24 hrs at all dose levels after both IV and SC administration. B) Using a human antibody tracer, a single dose of S-1117 demonstrated rapid and deep reduction of IgG.

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

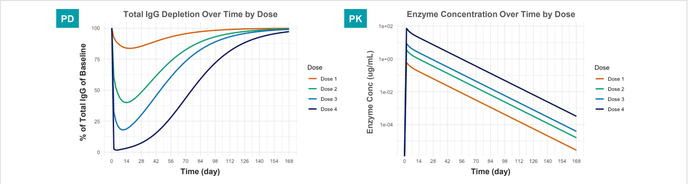


FIGURE 9: Projections of human PK and PD for S-1117 IV administration predict that infrequent, chronic low doses will achieve titratable and deep reductions of IgG that can be used in chronic and acute situations. Modeling based on *in vitro* and *in vivo* studies.

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 autoantibody-mediated diseases.
- 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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