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

Julia Manasson, Liliana Sanmarco, Alex Pellerin, Jordan Anderson, Nathan Rollins, Tobias Green, Agustin Plasencia, Andita Newton, Ryan Peckner, Yi Xing, Heather Vital, Nathan Higginson-Scott, John S. Sundy, Kevin L. Otipoby, Ivan Mascanfroni

Seismic Therapeutic, Cambridge, MA USA

ABSTRACT

Introduction: Pathogenic autoantibodies are key effectors of inflammation, promoting immune cell responses that cause tissue damage in autoantibody-mediated diseases such as immune thrombocytopenia (ITP), warm autoimmune hemolytic anemia, and Evans syndrome. Antibody degradation using an IgG protease represents a new therapeutic opportunity. S-1117 is a novel pan-IgG protease fused to an effector function silent human IgG1 Fc domain and engineered for chronic subcutaneous administration using a proprietary machine learning enabled platform to reduce immunogenicity and augment manufacturability while maintaining activity and selectivity. S-1117 cleaves and reduces soluble IgG, eliminates IgG effector function, degrades IgG immune complexes (IC), and cleaves the membrane-bound IgG B cell receptor (BCR) on memory B cells. These features allow S-1117 to simultaneously address multiple mechanisms of autoimmunity.

Methods: Plasma IgG, IgG BCR, and IC cleavage assays, as well as antibody-mediated effector function assays were performed *in vitro*. *In vivo*, human intravenous immunoglobulin (IVIG) was injected into mice and IVIG reduction was compared between S-1117 and a benchmark FcRn inhibitor. Prophylactic and therapeutic efficacy of S-1117 were tested in murine ITP models, where disease was induced with rabbit anti-mouse platelet serum (RAMS). Quantitative systems pharmacology (QSP) modeling was used to estimate human pharmacokinetics (PK) and pharmacodynamics (PD).

Results: S-1117 cleaves all IgG subclasses in human plasma. It directly eliminates IgG effector function, reducing antibody-dependent and complement-dependent cytotoxicity, antibody-dependent cellular phagocytosis, and IC-mediated immune cell activation *in vitro*. Moreover, S-1117 cleaves the IgG BCR on human memory B cells *in vitro*. *In vivo*, a single dose of S-1117 provides greater (>90%), faster (<24 hours), and more prolonged (>10 days) IgG reduction compared to a benchmark FcRn inhibitor (maximal IgG reduction of ~70% at 7 days). This correlates with superior efficacy against RAMS-induced platelet depletion in prophylactic and therapeutic murine models of ITP. Human PK/PD QSP 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 demonstrate superiority in human IgG cleavage compared to a benchmark FcRn inhibitor and are expected to enable a convenient patient-tailored treatment regimen. Given its ability to addresses multiple pathogenic mechanisms as a single drug, S-1117 has the potential to provide improved clinical outcomes in autoantibody-mediated diseases with complex pathology, such as ITP and other cytopenias, as demonstrated in the prophylactic and therapeutic ITP murine models.

INTRODUCTION

Mechanisms of humoral immunity can result in the production of IgG autoantibodies which are directly pathogenic in autoantibody-mediated diseases such as ITP. 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.

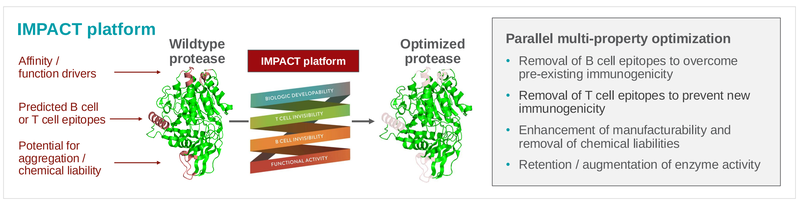


FIGURE 1: Seismic Therapeutic's proprietary machine learning enabled IMPACT platform was used to engineer S-1117 and optimize multiple properties in parallel, including enzyme stability, reduction of immunogenicity, and manufacturability, while maintaining enzyme activity.

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 reduction in mouse:** C57BL/6 mice received 9 mg of IVIG intraperitoneally (IP). The following day, S-1117 or benchmark FcRN inhibitor (FcRNi) were injected. Mice received a second injection of 4.5 mg IVIG on day 9. Blood was collected at several 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 intravenously (IV) or subcutaneously (SC). Blood was collected and serum and PBMC isolated at several 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.

***In vivo* ITP models**: A single dose of 3 mg/kg S-1117 (IV or IP), 40 mg/kg benchmark FcRNi (IP), or vehicle was injected into C57BL/6 mice the day prior to ITP induction (prophylactic) or 4 hrs post ITP induction (therapeutic). ITP was induced by rabbit anti-mouse platelet serum (RAMS) injections on days 0 and 2 for prophylactic or days 0 and 3 for therapeutic models, respectively. Blood was collected at several timepoints to quantify platelets via the Hemavet instrument.

RESULTS

S-1117 offers a multi-mechanistic approach for autoimmune diseases

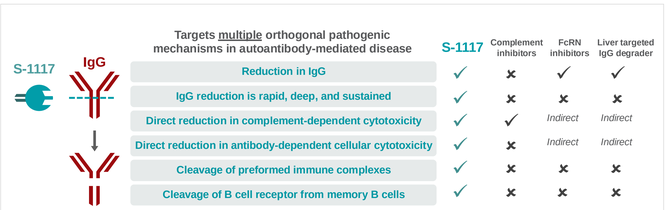


FIGURE 2: S-1117 selectively cleaves soluble, immune-complexed, membrane-bound, and BCR IgG, splitting the Fc portion from the Fab arms, thereby also eliminating IgG effector function.

S-1117 cleaves soluble IgG in plasma of healthy donors

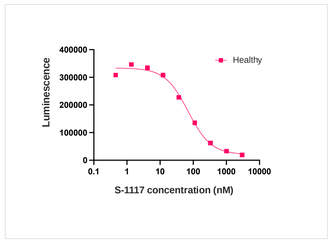


FIGURE 3: Total IgG levels in healthy human plasma at increasing S-1117 concentrations as quantified by MSD.

S-1117 cleaves preformed immune complexes

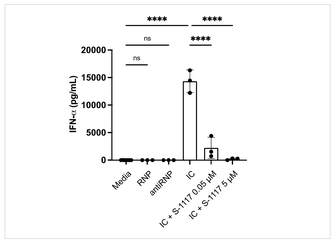


FIGURE 4: Supernatant IFN-α levels after addition of S-1117 treated or untreated ICs (RNP + anti-RNP), RNP, or anti-RNP to PBMC. **** p<0.0001

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

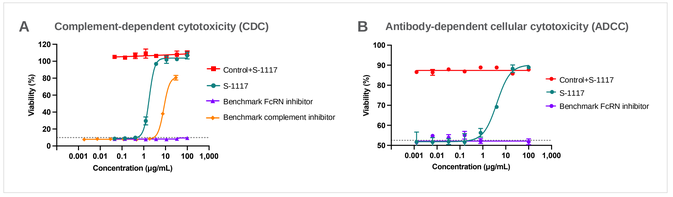


FIGURE 5: A) CDC from rituximab exposure on Ramos cells after S-1117, FcRN inhibitor, or complement inhibitor incubation in a dose response manner. B) Target cell lysis from trastuzumab-induced cytotoxicity after S-1117 or FcRN inhibitor incubation in a dose response manner.

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

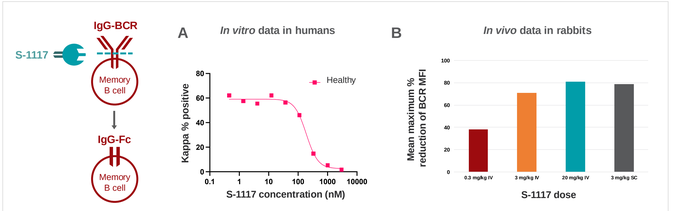
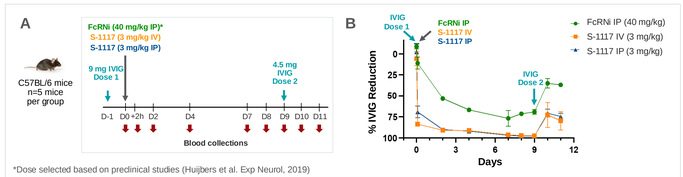


FIGURE 6: A) Percentage of kappa positive IgG+ B cells in healthy human whole blood at increasing S-1117 concentrations resulting from S-1117-mediated BCR cleavage *in vitro*. B) Peak rabbit IgG-BCR cleavage expressed as mean maximum percent reduction of BCR at three IV dose levels and one SC dose level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

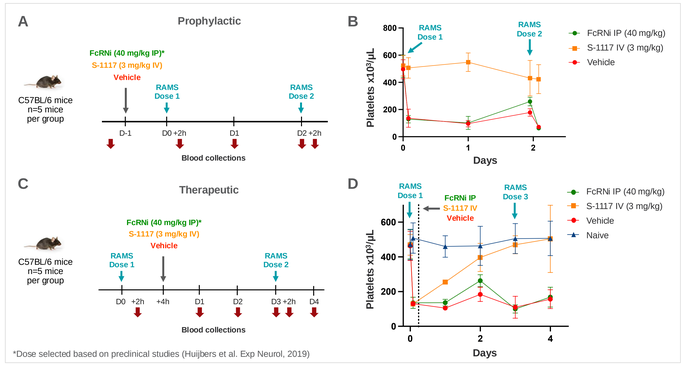
S-1117 shows faster, deeper, more sustained reduction of human IVIG compared to FcRN inhibitor



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

FIGURE 7: A) Experimental design. B) Percentage of human IVIG reduction at specified timepoints after S-1117 IV, S-1117 IP, or FcRN inhibitor exposure.

S-1117 demonstrates superior efficacy in prophylactic and therapeutic ITP models compared to FcRN inhibitor



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

FIGURE 8: A,C) Experimental design of prophylactic and therapeutic ITP models, respectively. B,D) Platelet counts at specified timepoints after S-1117 IV, FcRN inhibitor IP, or vehicle exposure.

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

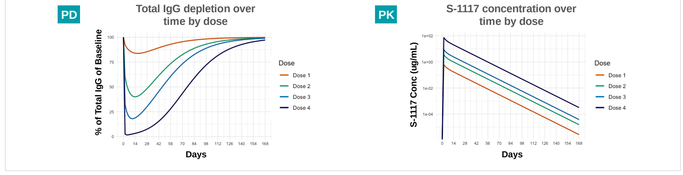


FIGURE 9: Projections of human PK and PD for S-1117 IV administration predict that infrequent, chronic low doses will achieve titratable and deep reductions of IgG that can be used in chronic and acute situations. Modeling based on *in vitro* and *in vivo* studies.

CONCLUSIONS

- S-1117 is a novel engineered pan-IgG protease that demonstrates rapid and sustained reduction of all human IgG subclasses.
- It addresses multiple, clinically-validated, orthogonal pathogenic mechanisms in autoimmunity within a single molecule, many of which are important in ITP.
- It directly cleaves circulating, immune-complexed, membrane-bound, and BCR IgG without affecting other Ig isotypes.
- It demonstrates superior efficacy in both prophylactic and therapeutic models of ITP compared to a benchmark FcRn inhibitor.
- S-1117 is being developed for both chronic and acute treatment of IgG autoantibody driven diseases.
- Titratable dosing of S-1117 is expected to enable a "treat-to-target" approach tailored to the patient's disease activity; it is formulated for convenient subcutaneous self-administration potentially every 4-6 weeks
- S-1117 is entering Phase 1 studies in the first half of 2025.

Contact: Julia Manasson, julia.manasson@seismictx.com, Seismic Therapeutic, Inc