Characterization of S-1117, a novel pan-IgG protease engineered for reduced immunogenicity using the IMPACT platform

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ABSTRACT

Background/Purpose: Pathogenic autoantibodies are key effectors of inflammation, promoting tissue damage in autoantibody-mediated diseases such as inflammatory myopathies, lupus nephritis, Sjogren's syndrome, antiphospholipid syndrome, and ANCA-associated vasculitis. Antibody degradation using an IgG protease represents a new therapeutic opportunity.

We present S-1117, a novel pan-IgG protease fused to an effectorless 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 potency. S-1117 cleaves and reduces soluble IgG, disrupts IgG effector function, degrades IgG immune complexes (IC), and cleaves the IgG+ B cell receptor (BCR) on the surface of memory B cells, reducing IgG+ BCR mediated activation. These features allow S-1117 to simultaneously address multiple mechanisms of autoimmunity.

Methods: Immunogenicity of S-1117 was assessed *in vitro* by major histocompatibility complex (MHC)-associated peptide proteomics (MAPPs) and validated by CD4 T cell proliferation assays. *In vivo*, IgG antidrug antibody (ADA) was quantified in C57BL/6 mice in response to S-1117 protease domain administration vs. the parental protease. The polypharmacology of S-1117 was determined through *in vitro* plasma IgG, IgG+ BCR, and IC cleavage, as well as antibody mediated effector function assays. S-1117 function was tested pre-clinically *in vivo* in rabbits and mice.

Results: *In vitro*, S-1117 demonstrates reduced epitope display compared to the wildtype protease by MAPPs. Furthermore, the wildtype protease elicits CD4 T cell proliferation responses from 65% of healthy donors with a response index of 2.60 (magnitude of response over background signal). In comparison, S-1117 demonstrates a reduction in the number of responders to 20% and a 10-fold lower response index. *In vivo*, a single dose of S-1117 induces rapid (< 24 hours), deep (>90%), and sustained (over 10 days) reduction of endogenous IgG in rabbits. In mice, repeat dosing of parental protease induced immediate hypersensitivity reactions, causing mortality in 60% of mice and high ADA response. In contrast, all mice treated with the protease domain of S-1117 survived multiple doses without anaphylactic reactions or induction of ADA.

Functionally, S-1117 cleaves all IgG subclasses in human plasma. It directly eliminates IgG effector function and IC-mediated immune cell activation. Moreover, it cleaves the IgG+ BCR on memory B cells in humans *in vitro*, reducing IgG+ BCR driven B cell activation.

Conclusion: S-1117 is a novel pan-IgG protease engineered with our machine learning enabled platform to reduce immunogenicity. It demonstrates rapid, deep, and sustained reduction of IgG levels, IgG effector function, and cleavage of the IgG+ BCR on memory B cells, reducing BCR mediated B cell activation. 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

Many natural and *de novo* proteins have therapeutic potential, but are limited by their immunogenicity, presenting a significant challenge in the field of drug development. One such example is IdeS, a bacterial enzyme derived from *Streptococcus pyogenes*, that selectively degrades IgG and offers a powerful approach for treating IgG antibody-mediated diseases.[1,2] However, IdeS cannot be administered chronically due to its high rate of anti-drug antibody (ADA) formation.

We previously introduced S-1117, a novel pan-IgG protease derived from IdeS,[3] fused to an effectorless human IgG1 Fc domain to extend its half-life. S-1117 selectively degrades all IgG subclasses without impacting other immunoglobulins, directly eliminates IgG effector function (e.g., complement-dependent cytotoxicity [CDC], antibody-dependent cellular cytotoxicity [ADCC], antibody-dependent cellular phagocytosis [ADCP]) and immune-complex mediated immune cell activation, and cleaves the IgG+ (BCR) on memory B cells, reducing IgG+ BCR driven B cell activation.

We applied Seismic Therapeutic's proprietary IMPACT platform to reduce immunogenicity by addressing both pre-existing ADA (B cell mediated) and the formation of new ADA via T cell activation (T cell mediated) and tested the engineered protease across multiple *in vitro* and *in vivo* assays.

[1] von Pawel-Rammingen U, et al. "IdeS, a novel streptococcal cysteine proteinase with unique specificity for immunoglobulin G." *EMBO J* 2002. 21(7): 1607-1615.
[2] Huang E, et al. "Imlifidase for the treatment of anti-HLA antibody-mediated processes in kidney transplantation." *Am J Transplant*. 2022. 22:691–697.
[3] Manasson J, et al. Preclinical Polypharmacology of S-1117, a Novel Engineered Fc-fused IgG Degrading Enzyme, for Chronic Treatment of Autoantibody-mediated Diseases [abstract]. Arthritis Rheumatol. 2024; 76 (suppl 9). https://acrabstracts.org/abstract/preclinical-polypharmacology-of-s-1117-a-novel-engineered-fc-fused-igg-degrading-enzyme-for-chronic-treatment-of-autoantibody-mediated-diseases. Accessed October 21, 2025.

S-1117, derived from a bacterial protease, offers a multi-mechanistic approach for treatment of autoimmune diseases

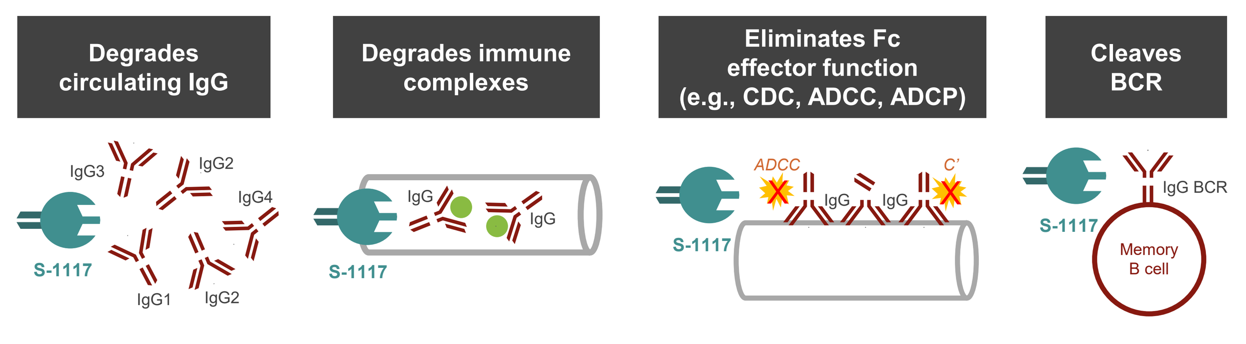


FIGURE 1: S-1117 selectively degrades circulating (soluble) and immune-complexed IgG, directly eliminates IgG Fc effector function, and cleaves the IgG+ BCR on memory B cells without affecting other Ig isotypes. Supportive *in vitro* and *in vivo* data previously presented at ACR Convergence 2024.[3]

METHODS

<u>Binding of pre-existing antibodies</u>: S-1117 and Fc-IdeS were captured on Meso Scale Discovery (MSD) plates using anti-Fc antibody. Plates were incubated at room temperature for 1 hour and blocked with isolated cleaved Fc. Plasma samples from 48 donors were diluted 1:100 and added to the plates following blocking. Detection of bound anti-drug antibody (ADA) was performed using anti-human Ig light chain antibody.

<u>MHC-associated protein proteomics (MAPPs)</u>: Monocytes were isolated from peripheral blood mononuclear cells (PBMC) of 12-15 human donors and matured to dendritic cells in the presence of S-1117 of Fc-IdeS. On Day 8, cells were lysed and HLA/peptide complexes purified using magnetic beads. Peptides bound to HLA were eluted and purified by solid phase extraction. Peptides were analyzed by high resolution sequencing mass spectrometry. HLA-DR, DP, and DQ epitope display was performed by a contract research organization (CRO).

<u>T cell proliferation</u>: PBMC from 40 human donors were labeled with CFSE and incubated with S-1117 or IdeS at 0.34 µM for 7 days. Unstimulated controls were included as background. After incubation, cells were stained with anti-CD4 antibody. Proliferation (CFSE dilution) was analyzed by flow cytometry. Assay was performed by a CRO.

<u>*In vivo* mouse immunogenicity assessment</u>: <u>Study 1</u>: C57BL/6 mice received 3 biweekly 1 mg/kg IV doses of S-1117 protease domain (n=5) or 2 biweekly 1 mg/kg IV doses of IdeS (n=5). Number of surviving animals was noted after each dose. <u>Study 2</u>: C57BL/6 mice received a 3 mg/kg IV dose of S-1117 protease domain (n=10) or IdeS (n=10). After 60 days, 5 IdeS-treated mice received a second dose of IdeS and 5 IdeS-treated mice received S-1117 protease domain. Similarly, after 60 days, S-1117 protease domain-dosed mice received a second dose of IdeS or S-1117 protease domain. Plasma was collected for ADA assessment by MSD.

<u>*In vivo* mouse ADA assessment by MSD</u>: MSD plate was coated with human anti-His Tag antibody overnight. The plate was blocked, and inactivated proteases (S-1117 protease domain or IdeS) were added. Plasma was diluted in sample buffer and added to the protease-coated MSD plate. Goat anti-mouse IgG biotinylated antibody was used for detection.

RESULTS

Seismic IMPACT platform was applied to reduce B cell and T cell mediated immunogenicity

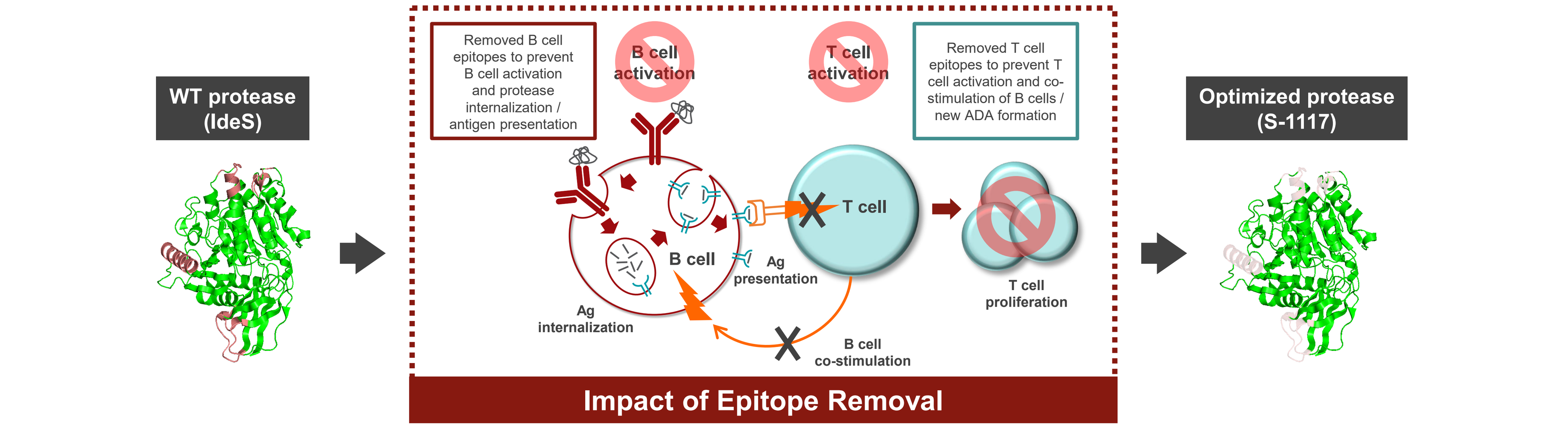


FIGURE 2: S-1117 was optimized from the wildtype bacterial protease, IdeS, by applying Seismic Therapeutic's machine learning IMPACT platform to reduce immunogenicity. B cell epitopes were removed to prevent B cell activation, protease internalization, and antigen presentation. T cell epitopes were removed to prevent T cell activation and new ADA formation.

Removal of B cell epitopes reduces pre-existing antibody binding to S-1117 *in vitro*

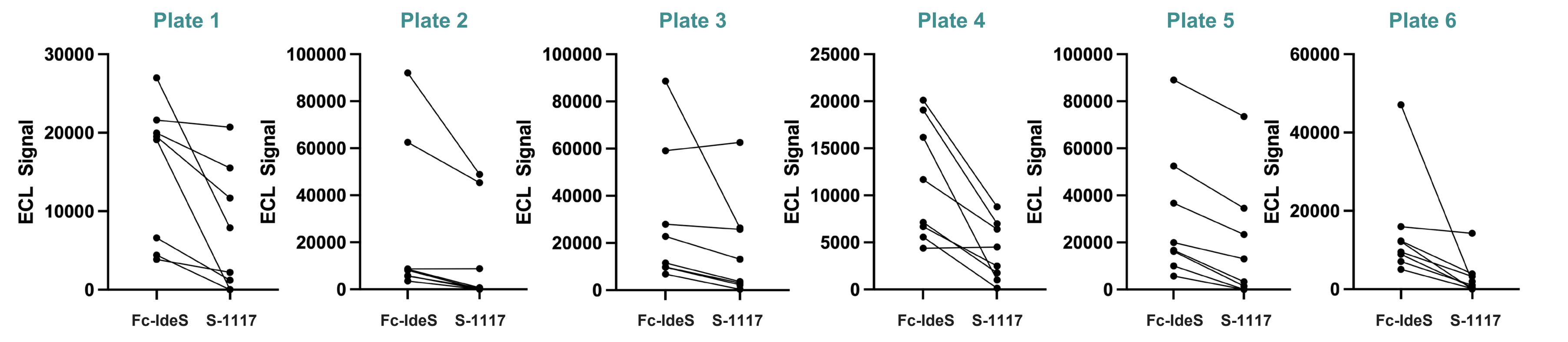


FIGURE 3: Plasma from 45 of 48 human donors demonstrated reduced levels of pre-existing antibody binding to S-1117 compared to Fc-IdeS. Each line represents a single donor.

T cell epitope removal reduces antigen presentation and T cell proliferative responses to S-1117 *in vitro*

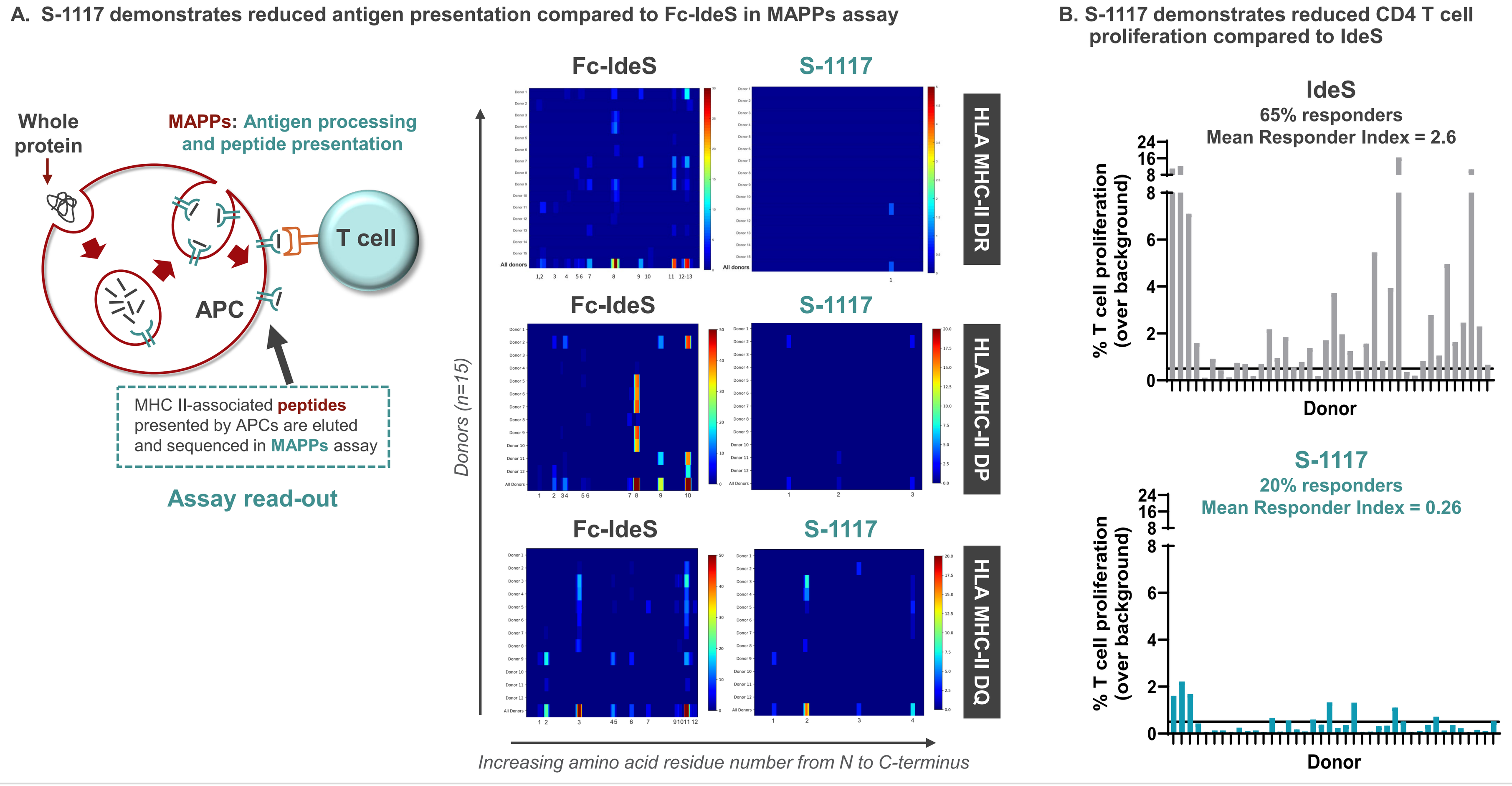
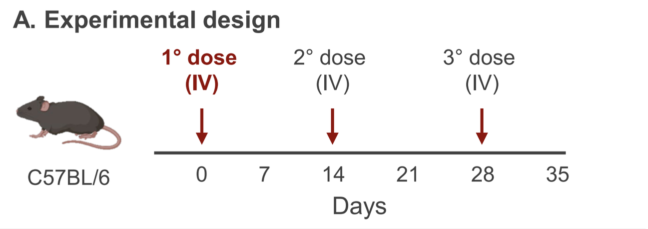


FIGURE 4: A) MAPPs shows significantly fewer peptides presented by HLA-DR, DP and DQ in antigen presenting cells (APCs) from 12-15 healthy donors in response to S-1117 (right) compared to Fc-IdeS (left). Bottom row of each heatmap shows composite results from all donors. Heatmap scale indicates the number of peptides identified for each individual sequence region (red = strong cluster, dark blue = weak cluster or single peptides) and is different for each heatmap. **B)** Percentage of CD4 T cell proliferation was lower in response to S-1117 (bottom) compared to IdeS (top) across 40 donors. Each bar represents the mean proliferative response of a donor in sextuplicate. Solid line represents assay cut-off. Responders were donors with 2 of 6 replicates above the background and Responder Index was calculated based on the percentage of responders as well as the strength of their response.

After T cell epitope removal, single dose of S-1117 does not induce ADA in murine model compared to IdeS



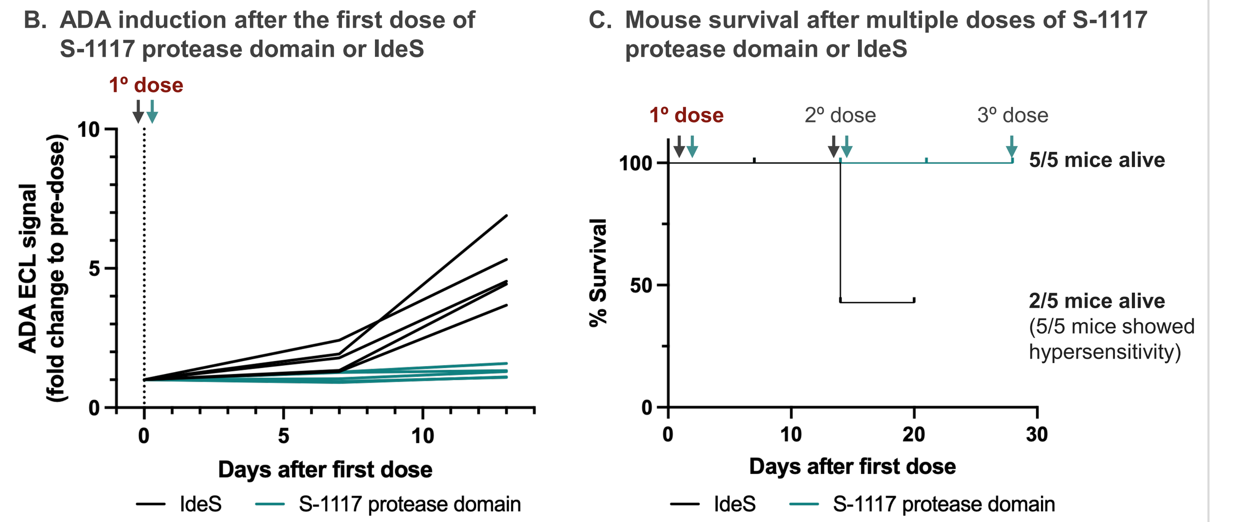


FIGURE 5: *In silico* prediction demonstrated removal of all IdeS-specific T cell epitopes from the S-1117 protease domain in C57BL/6 (H-2 I-Ab) mice. To test ADA induction *in vivo*, **A)** C57BL/6 mice received 3 biweekly 1 mg/kg IV doses of S-1117 protease domain or 2 biweekly 1 mg/kg IV doses of IdeS. **B)** Unlike IdeS, S-1117 protease domain did not demonstrate ADA induction 14 days after the first dose. **C)** Mouse survival was not impacted after the second dose of S-1117 protease domain, but 5/5 (100%) mice showed hypersensitivity and 3/5 (60%) mice died after a second dose of IdeS, consistent with ADA induction.

Multiple doses of S-1117 do not induce ADA in murine model compared to IdeS

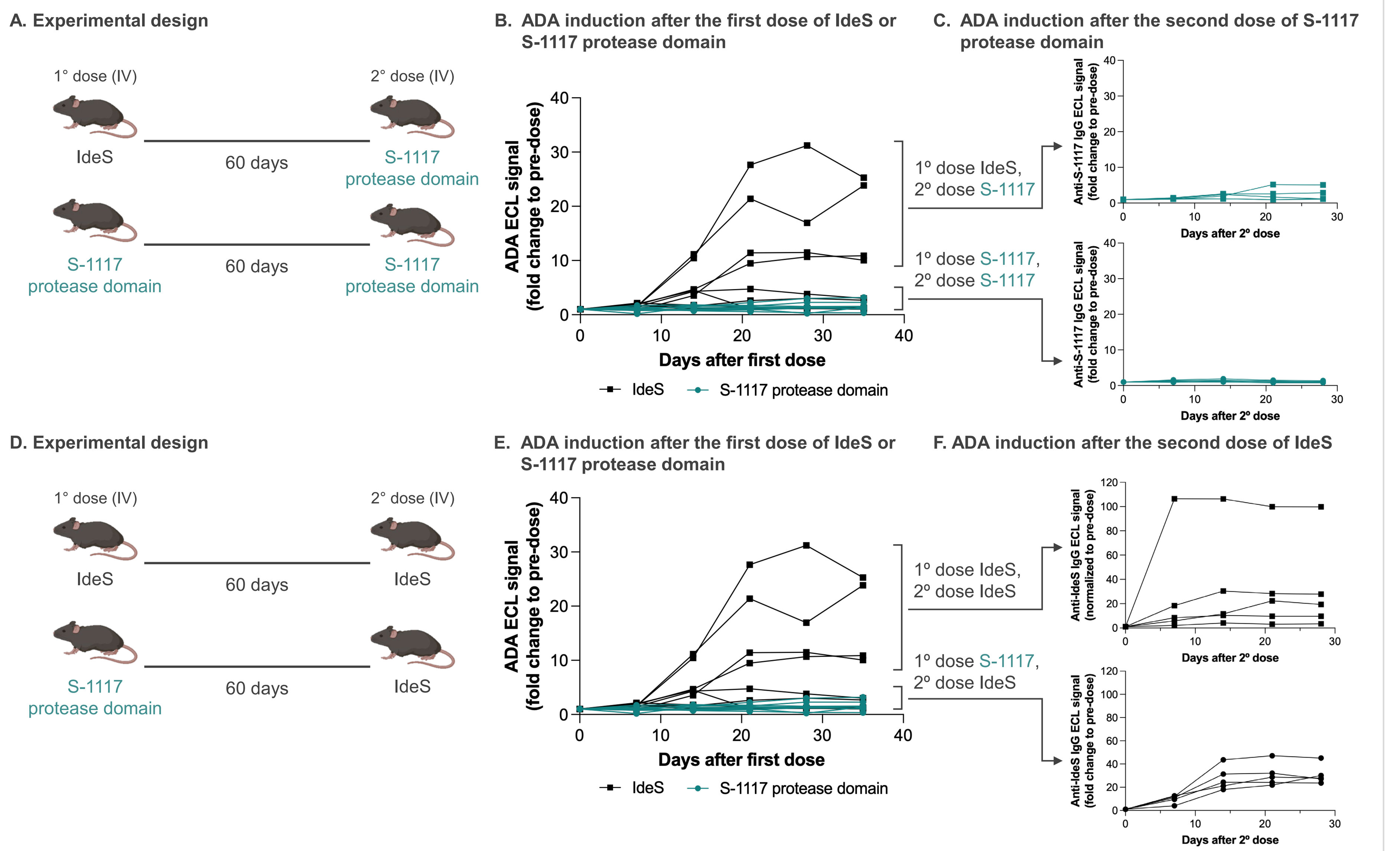


FIGURE 6: To test ADA induction after multiple doses in an *in vivo* setting, **A)** C57BL/6 mice received an IV dose of S-1117 protease domain or IdeS, which was followed by a second IV dose of S-1117 protease domain or IdeS 60 days later. **B,E)** S-1117 protease domain did not generate robust ADA induction after the first dose, unlike IdeS **C,F)** Similarly, S-1117 protease domain demonstrated very limited ADA induction after the second dose, unlike IdeS.

CONCLUSIONS

- Immunogenicity poses a significant challenge in the field of drug development.
- We applied Seismic Therapeutic's machine learning IMPACT platform to reduce immunogenicity and engineer one of our novel pan-IgG proteases, S-1117, by removing B cell epitopes to address pre-existing ADA and T cell epitopes to avoid generation of novel ADA by T cell activation.
- *In vitro*, removal of B cell epitopes demonstrated reduction of pre-existing antibody binding to S-1117 compared to the parental protease, IdeS, while removal of T cell epitopes demonstrated reduced antigen presentation by MAPPs and CD4 T cell proliferation compared to IdeS.
- *In vivo*, removal of T cell epitopes blunted ADA induction in C57BL/6 mice after a single dose and multiple doses of S-1117 compared to IdeS.
- The preclinical results are currently being validated in an ongoing Ph 1 clinical trial.

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