

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

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ABSTRACT

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

Background: Pathogenic autoantibodies are key effectors of inflammation, promoting complement activation and immune cell responses that cause tissue damage in autoantibody-mediated diseases such as myasthenia gravis (MG) and chronic inflammatory demyelinating polyneuropathy (CIDP). IgG degrading proteases represent a new therapeutic opportunity.

S-1117, a novel Fc-fused pan-IgG protease, was engineered using a proprietary machine learning enabled platform to reduce immunogenicity and augment manufacturability and stability while maintaining enzyme activity. S-1117 addresses multiple mechanisms of autoimmunity by lowering IgG and immune complex (IC) levels, reducing IgG effector functions and cleaving the antigen B cell receptor (BCR) on memory B cells.

Design/methods: Soluble IgG in plasma, BCR and IC cleavage assays, as well as Fc-mediated effector function assays were performed *in vitro*. A murine PK/PD model and a murine nephritis model evaluated S-1117 function *in vivo*.

Results: S-1117 cleaves soluble IgG from healthy volunteers and MG patients, and the BCR on memory B cells with comparable potency. S-1117 significantly reduces antibody-dependent cytotoxicity (ADCC), complement dependent cytotoxicity (CDC) and IC-mediated peripheral blood mononuclear cell (PBMC) activation *in vitro*.

When tested *in vivo*, a deep reduction of human IgG (>90%) occurred within 30 minutes of dosing. A PK/PD model developed for human dose projections predicts rapid, deep and sustained reduction of IgG levels. In an anti-glomerular basement membrane (GBM) model, a prototype Fc-fused pan-IgG protease reduced complement, IC deposition, proteinuria (protein score: 3.3 disease vs. 1.9 treated), BUN (49 mg/dl disease vs. 1.9 mg/dl treated) and renal pathology (crescent score: 3.6 disease vs. 0.6 treated).

Conclusions: S-1117 is a novel engineered pan-IgG protease that demonstrates rapid and sustained reduction of human IgG levels in a murine PK/PD model and ameliorates several pathologic manifestations in a murine anti-GBM nephritis model. Given its ability to address multiple pathogenic mechanisms as a single drug, it has the potential to achieve improved clinical outcomes in autoantibody-mediated diseases.

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 and CIDP, where the predominant pathogenic autoantibody isotype is IgG. A naturally occurring pan-IgG protease was fused to an 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 and membrane-bound IgG without impacting other Ig isotypes (Fig 2).

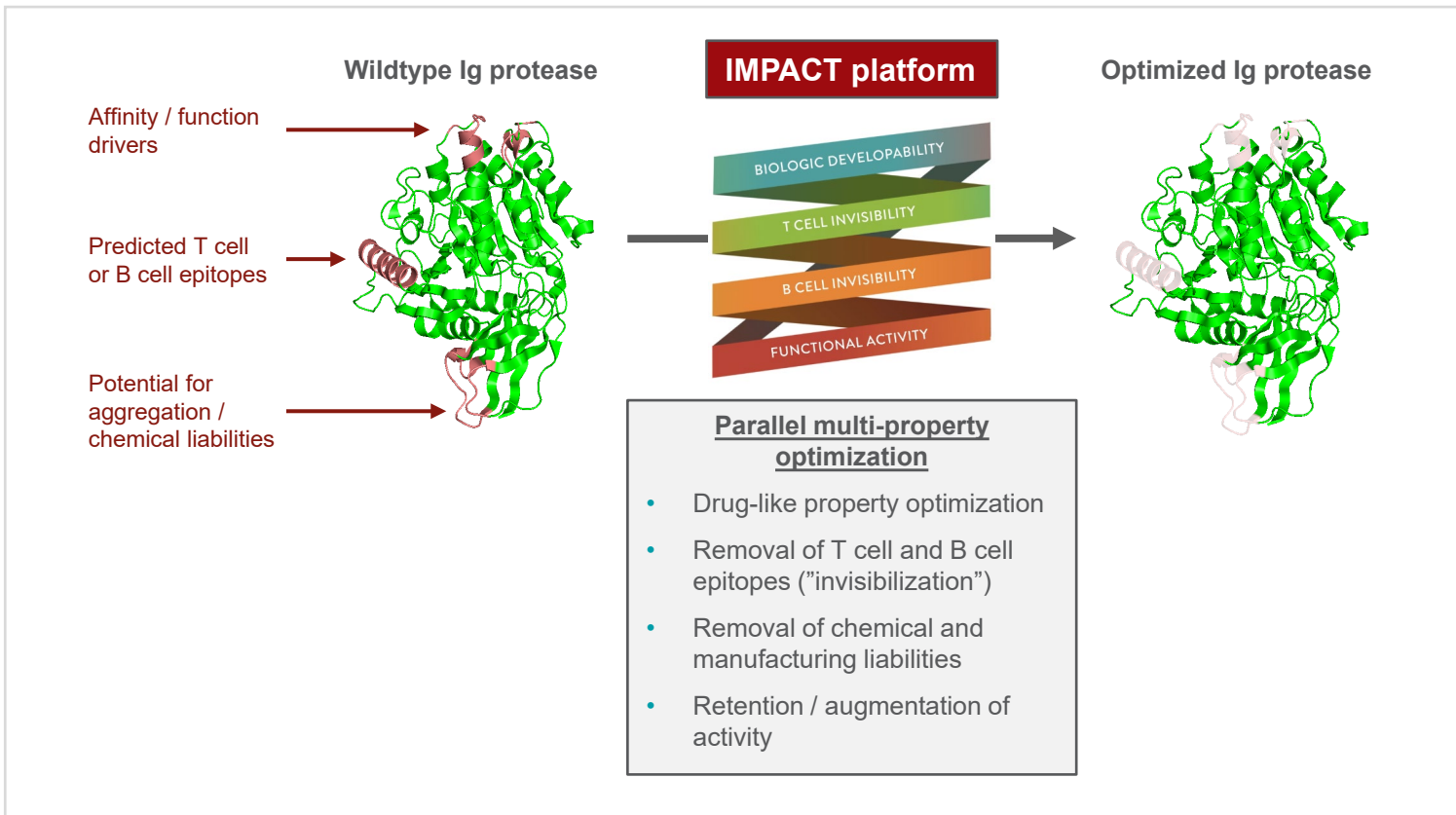


FIGURE 1: Seismic Therapeutic's ML enabled IMPACT platform was used to engineer S-1117 and optimize multiple properties in parallel, including enzyme stability, reduction of immunogenicity, and manufacturability, while maintaining enzyme activity.

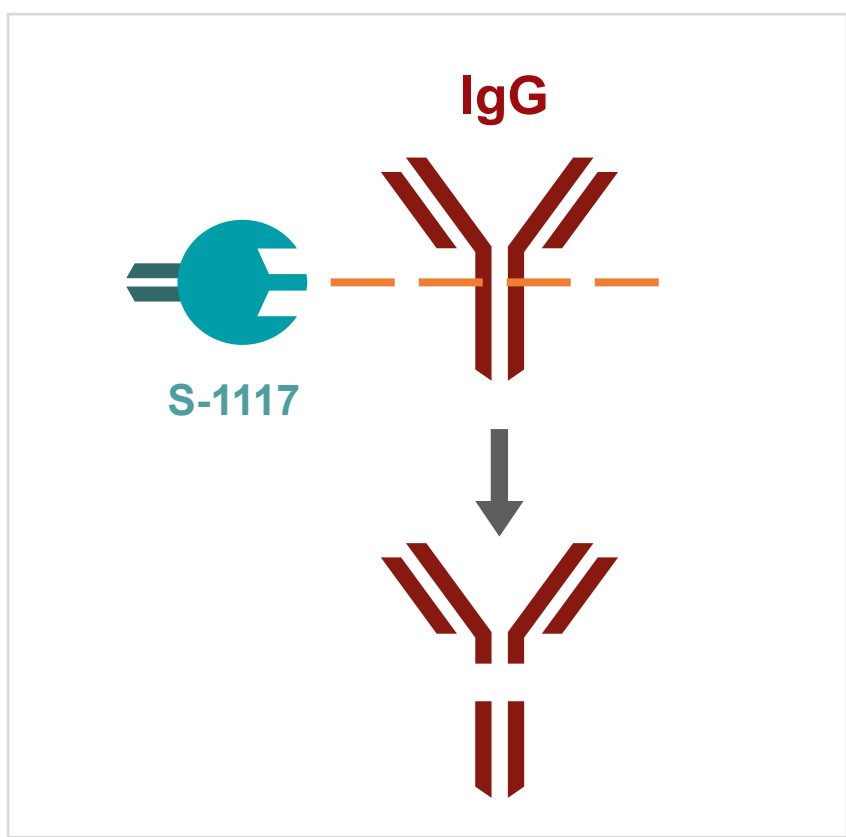


FIGURE 2: S-1117 selectively cleaves soluble and membrane-bound IgG, splitting the Fc portion from the Fab arms, thereby eliminating effector function.

METHODS

- IgG detection by MSD:** Meso Scale Discovery (MSD) plate was coated with anti-human light chain antibody overnight. The following day, the MSD plate was blocked and plasma or IVIG standard curve was added. Biotinylated anti-human IgG Fc antibody was used for detection.
- BCR cleavage:** Human whole blood was treated with S-1117 in a dose response manner for 20 hrs at 37°C. Plasma was subsequently collected for MSD and cells stained with anti-IgG, anti-CD20, anti-IgD, anti-IgM, anti-lambda and anti-kappa after RBC lysis. Flow cytometry was performed on stained cells.
- Fc-mediated effector function assays:** Performed by a contract research organization (CRO). **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 (NHS). Viability was assessed by luciferase activity. **ADCC:** Stained SKBR3 cells were opsonized with trastuzumab or control antibody and incubated with S-1117 or benchmark molecules in a dose response manner. Peripheral blood mononuclear cells (PBMC) were added and lysis quantified by flow cytometry.
- IC activation of PBMCs:** 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 PBMCs 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 vivo PK/PD:** C57BL6 mice received S-1117 or vehicle (PBS) IV and human IVIG was injected retro-orbitally (RO) at different timepoints. Each timepoint was tracked by a separate murine cohort (6 animals: 3 S-1117, 3 vehicle). Plasma was collected 15 min before or 2 hrs after IVIG injection. Mice were euthanized 2 hrs after IVIG injection. Measurements of IVIG in plasma (PD) were normalized to vehicles in each timepoint cohort.
- Human dose projection modeling:** Performed by Applied BioMath (ABM). Quantitative systems pharmacology (QSP) modeling was performed using available *in vitro* and *in vivo* data to estimate human PK and PD at different IV doses as well as single or chronic dose administration of S-1117.
- Anti-GBM model:** Performed by a CRO. On day -5, mice were immunized intraperitoneally (IP) with sheep IgG/CFA. On day -1, animals were treated with parental Fc-pan-IgG protease IV or controls (no treatment, vehicle (PBS) or dexamethasone). On day 0, plasma samples were collected for Fc-protease PK and disease was induced by IV injection of sheep anti-rat GBM serum. Mice were euthanized on day 10, blood was collected for BUN, and kidneys fixed in formalin for histopathology.
- Parental Fc-pan-IgG protease detection by MSD:** MSD plate was coated with anti-protease antibody overnight. The following day, plasma samples or parental Fc-pan-IgG protease were added to the plate. Biotinylated anti-human IgG Fc antibody was used for detection.

RESULTS

S-1117 treatment cleaves soluble IgG and the BCR with similar potency

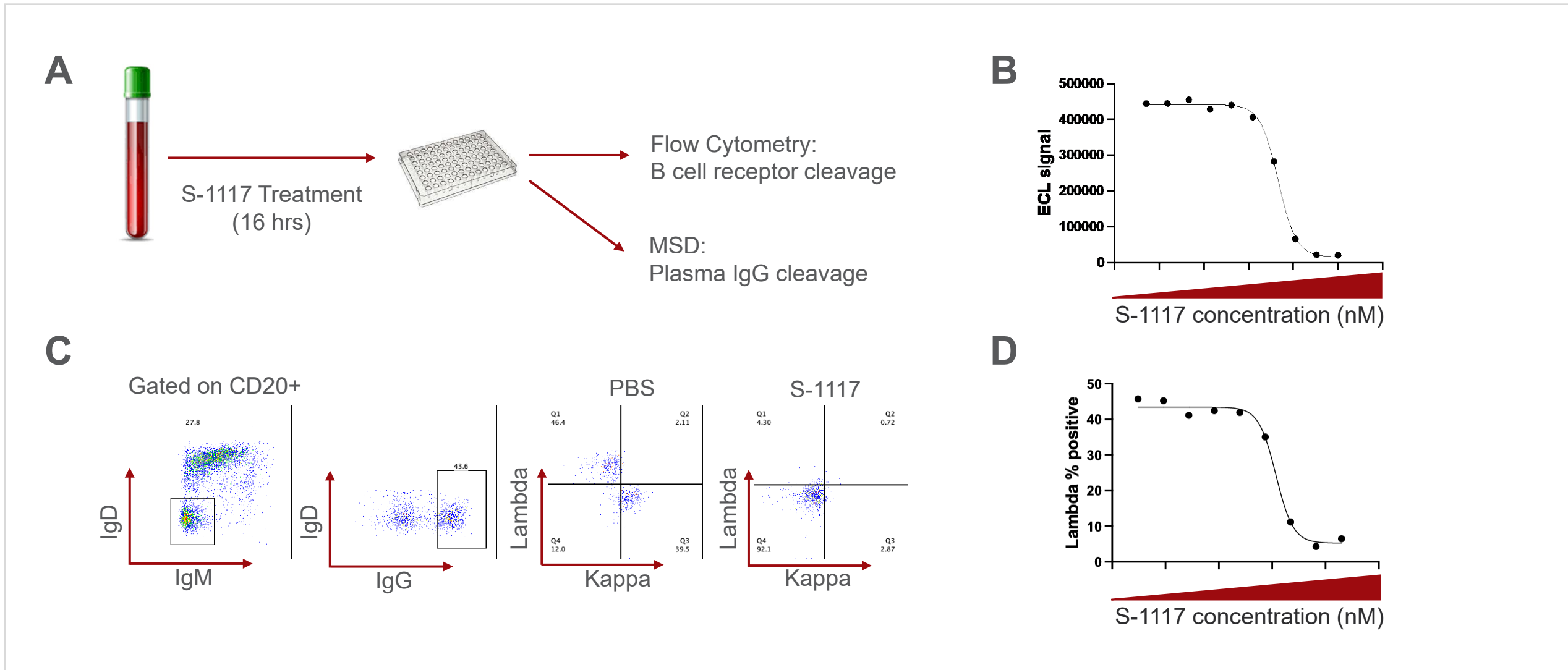


FIGURE 3: A) Study design to test S-1117 cleavage activity *in vitro*. B) IgG levels in human plasma at increasing S-1117 concentrations as quantified by MSD. C) Representative flow cytometry dot plots and D) percentage of lambda positive B cells C-D) resulting from S-1117-mediated BCR cleavage of human B cells *in vitro*.

Magnitude of soluble IgG cleavage by S-1117 in MG patients is similar to healthy volunteers

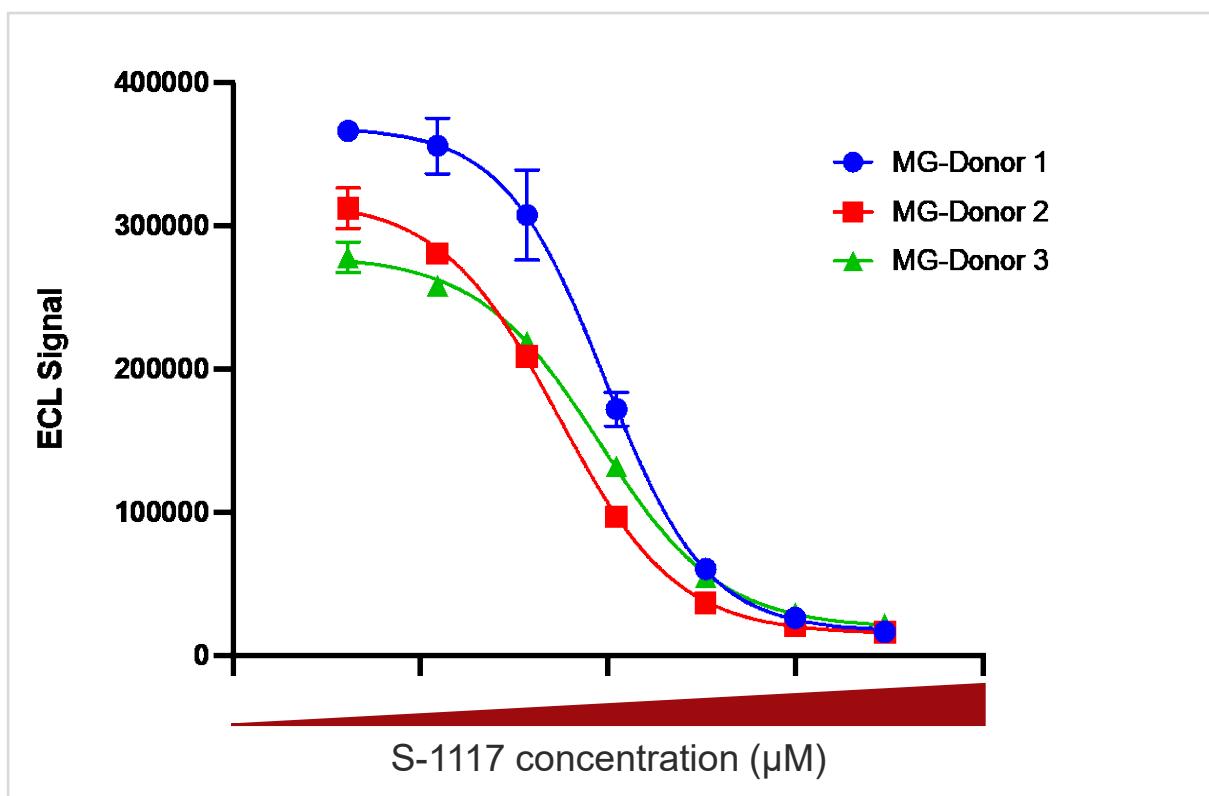


FIGURE 4: IgG levels in human plasma from MG patients at various S-1117 concentrations as quantified by MSD.

S-1117 reduces ADCC, CDC and IC-mediated PBMC activation effector function in vitro

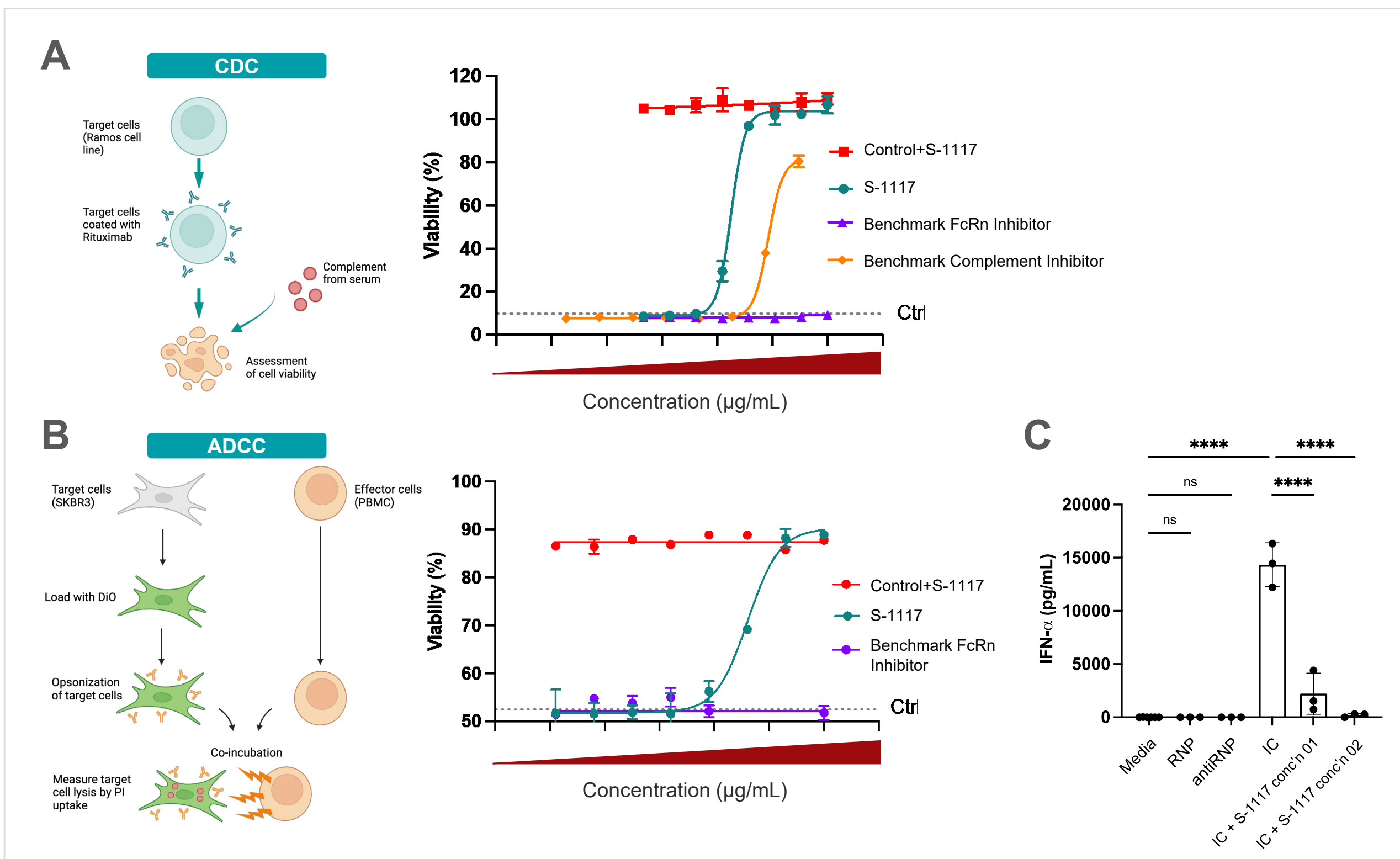


FIGURE 5: A) CDC from rituximab exposure on Ramos cells after S-1117, FcRn inhibitor or complement inhibitor incubation in a dose response manner. B) Target cell lysis after trastuzumab-induced cytotoxicity at various doses of S-1117 or efgartigimod. C) Supernatant IFN- α levels after addition of S-1117 treated or untreated ICs, RNP, or anti-RNP to PBMCs. **** p<0.0001

S-1117 induces a deep and sustained reduction of human IgG in an *in vivo* murine model

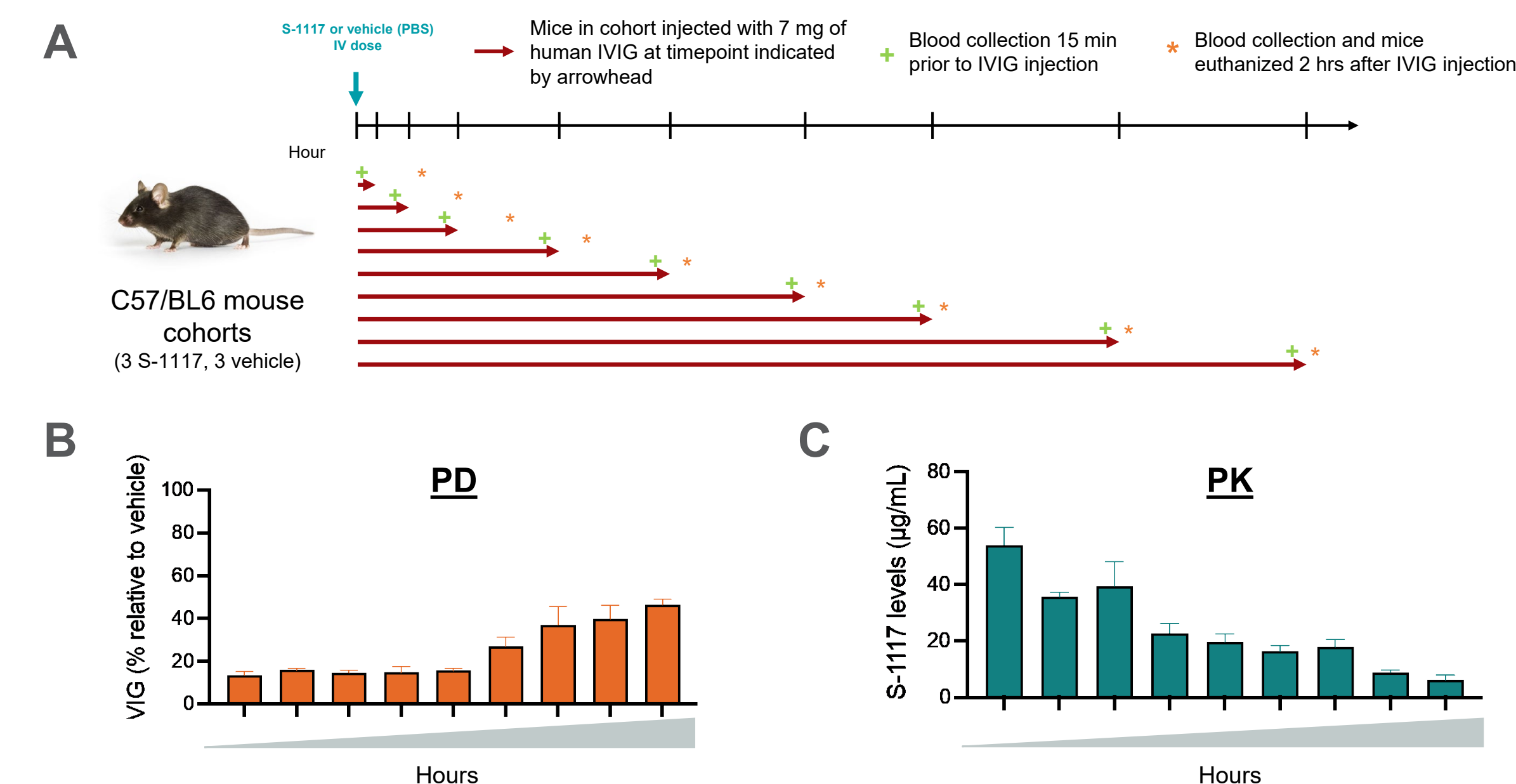


FIGURE 6: A) *In vivo* PK/PD study design. B) Percentage of IVIG relative to vehicle (PD) and C) S-1117 levels (PK) in plasma at specified timepoints after S-1117 injection as measured by MSD. Each timepoint is represented by a different cohort of mice, including vehicles, which received PBS in lieu of S-1117.

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

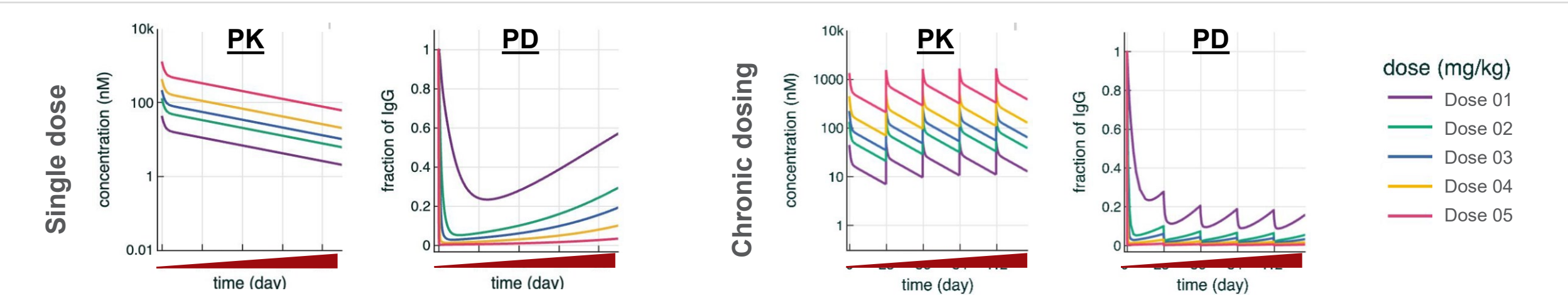


FIGURE 7: Projections of human PK and PD for S-1117 IV administration indicate that infrequent chronic low doses will achieve titratable IgG reductions of 90% or greater. Modeling based on *in vivo* murine studies.

Parental Fc-pan-IgG protease reduces tissue damage in a murine anti-GBM kidney model

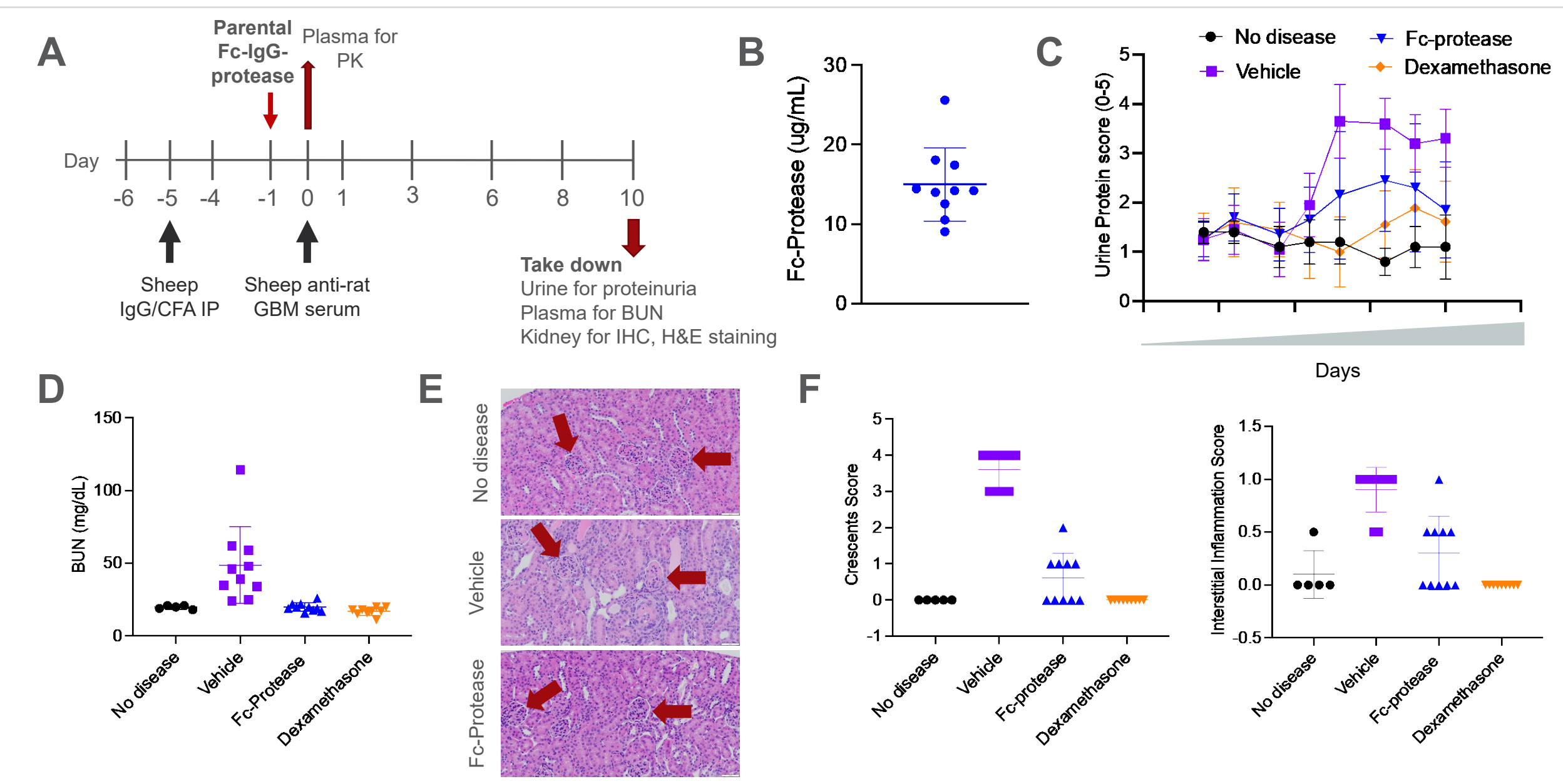


FIGURE 8: A) Study design. B) Parental Fc-pan-IgG protease levels in plasma 1 day following IV injection. C) Urine protein score measured by urine dipstick. D-F) Effect of parental Fc-pan-IgG protease, dexamethasone, or vehicle on D) BUN in plasma, E) renal histology (representative H&E stains), and F) crescent and interstitial inflammation scores.

CONCLUSIONS

S-1117 is a novel pan-IgG protease that cleaves IgG and the IgG B cell receptor. It also reduces IgG-mediated effector function *in vitro*, addressing multiple pathogenic mechanisms. A parental Fc-pan-IgG-protease mitigates renal injury in an *in vivo* IgG IC-mediated kidney disease model. Human dose projections through QSP modeling predict a rapid, deep and sustained reduction of IgG levels that would enable small volume IV or SC administration and infrequent dosing in patients.