S-4321, a novel dual cell bifunctional PD-1:FcγRIIb selective agonist antibody for autoimmune disease, maintains expression of PD-1 on target cells and enhances inhibitory receptor expression on T cells *in vivo*

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ABSTRACT

Background/Purpose: S-4321 is a novel antibody that agonizes the inhibitory PD-1 checkpoint receptor on T cells without competing for binding with its natural ligand, PD-L1, while selectively engaging and signaling through the inhibitory FcγRIIb on B cells/antigen presenting cells (APCs). S-4321 is expected to restore immune homeostasis in diseases such as RA, SLE, GCA, and IBD.

Methods: PD-1 expression was measured on activated T cells after culturing with S-4321 or benchmark PD-1 agonists. FcγRIIb selectivity of the S-4321 Fc domain was demonstrated via binding assays. The lack of proinflammatory activity of the S-4321 Fc domain was assessed using TNFα production from activated monocyte-derived dendritic cells or antibody-dependent cellular cytotoxicity (ADCC) of target Tregs by human NK cells. S-4321 was tested in *in vitro* Treg differentiation assays. S-4321 was assessed *in vivo* using the B6D2F1 model of GvHD and naïve mice and in cynomolgus monkeys.

Results: Treatment with S-4321 *in vivo* does not result in loss of PD-1 target expression, unlike first-generation PD-1 agonists. S-4321 enhances induced Treg (iTreg) differentiation (p<0.05) *in vitro*. *In vivo*, S-4321 reduces T cell expansion and proinflammatory cytokine production (p<0.05) in a murine model of GvHD and induces TIGIT expression on the surface of PD-1+ T cells in naïve mice. S-4321 demonstrates dose-proportional exposure and ~70% bioavailability with subcutaneous dosing in cynomolgus monkeys.

Conclusion: In contrast to first-generation PD-1 agonists, treatment with S-4321 does not result in loss of PD-1 expression on T cells, induction of proinflammatory cytokines, or depletion of PD-1+ Tregs. S-4321 enhances TIGIT expression on PD-1+ T cells. S-4321 has the potential to restore immune homeostasis in cell-mediated autoimmune diseases, and its high bioavailability will allow for convenient drug administration.

INTRODUCTION

Autoimmunity results from dysregulated interactions between T cells and B cells/APCs in response to self-antigens.[1,2] Inhibitory receptors (IRs) upregulated on activated lymphocytes play a critical role in restoring and maintaining immune homeostasis to control autoimmunity. Accordingly, IR deficiency is associated with autoimmunity in mice and humans,[3,4] and pharmacological blockade of IRs by checkpoint inhibitors causes immune-related adverse events.[5] We and others have hypothesized that agonism of IR pathways may restore normal immune homeostasis in patients with autoimmune disease.[6]

PD-1 is a well-characterized IR expressed on activated effector and regulatory T cells. Several PD-1 targeted therapeutic antibodies have been investigated in patients with autoimmune disease with mixed results clinically.[7,8] These first-generation antibodies have now been shown to primarily deplete PD-1+ T cells with little to no demonstrated PD-1 agonism in humans *in vivo*.

S-4321 is a next generation PD-1 agonist designed to activate multiple IRs at the T cell: B cell/APC synapse in a single bifunctional antibody. The anti-human-PD-1 $F(ab')_2$ domain of S-4321 has low affinity for PD-1 (similar to the natural ligand) and achieves PD-1 agonism without causing PD-1 loss on T cells or IL-2 production.[9] The Fc domain of S-4321 selectively agonizes the inhibitory FcγRIIb and avoids proinflammatory cytokine production or PD-1+ Treg depletion. S-4321 presents a novel therapeutic approach for multiple cell-mediated autoimmune diseases.

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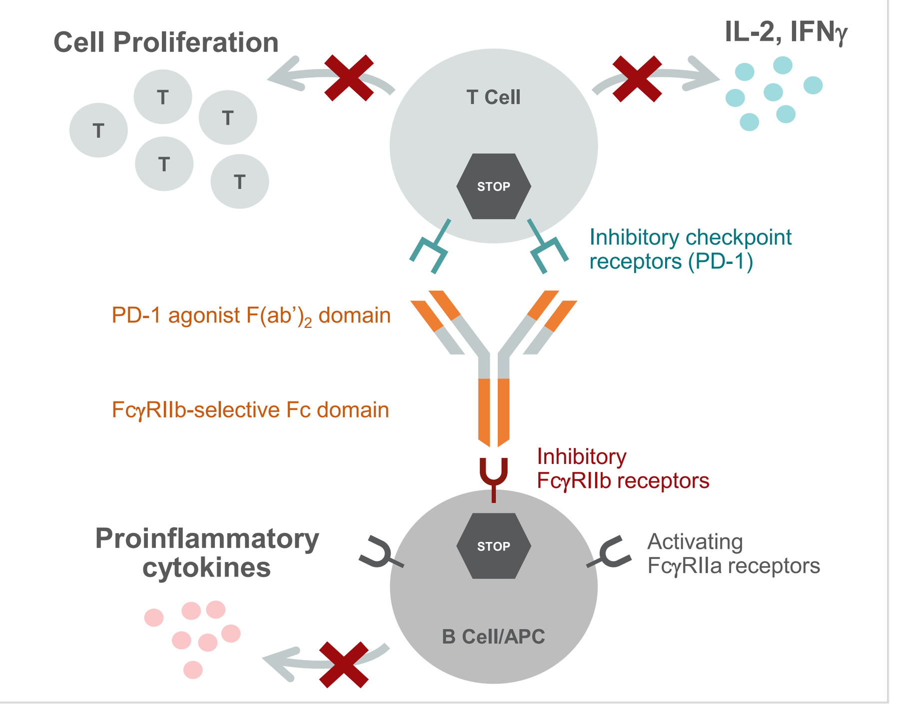

FIGURE 1: Seismic Therapeutic's IMPACT platform was used to engineer S-4321, a dual-cell bidirectional (DcB) PD-1:FcγRIIb antibody that agonizes the inhibitory PD-1 receptor on T cells ($F(ab')_2$ domain) and selectively agonizes the inhibitory FcγRIIb on APCs (Fc domain), eliciting an inhibitory signal at the immune cell synapse to re-establish immune homeostasis. By selectively binding FcγRIIb, S-4321 mitigates the unwanted production of inflammatory cytokines triggered by the engagement of activating FcγRIIa.

METHODS

SHP2 recruitment assay: Jurkat PD-1-SHP2 reporter cell lines were co-cultured with cell lines expressing FcγRIIb in the presence of S-4321 or PD-L1-Fc and luminescence measured after 24 hours.

SEB activation of PBMC: Peripheral blood mononuclear cells (PBMC) isolated from 3 healthy human donors were activated with Staphylococcal enterotoxin B (SEB) in the presence of S-4321 or benchmark high affinity PD-1 depleters/non-depleting agonists (benchmark PD-1 engagers) for 4 days to allow for PD-1 upregulation on T cells. On Day 4, activated PBMC were immunophenotyped to measure PD-1 expression on the surface of T cells and supernatant collected to quantify IL-2 production by Meso Scale Discovery (MSD) assay.

SHIP-1 recruitment assay: PBMC were isolated and activated, as described above, in the presence of S-4321 or benchmark PD-1 engagers. On Day 4, activated PBMC were co-cultured with SHIP-1 293 reporter cell lines expressing FcγRIIb. Luminescence was measured after 30 minutes of co-culture.

Cytokine release assay: Human monocyte derived dendritic cells (MoDCs) were activated with a TLR2 agonist and assessed for their capability to produce TNFα when cultured for 24 hours in plates coated with wildtype (WT) IgG1 or FcγRIIb selective binders (S-4321, P238D IgG1).

Treg ADCC assay: Human Tregs were cell-trace labeled and incubated with allogenic human NK cells for 24 hours at a 1:5 ratio in the presence of S-4321 or benchmark PD-1 engagers. Cells were labeled with a live/dead stain and % ADCC was calculated via flow cytometry.

Immunophenotyping in naïve human PD-1 knock-in mice: Human PD-1 (huPD-1) KI mice received a single dose of PBS or 1 mg/kg S-4321. Spleens were collected on Day 7 post-dosing. Fraction of Tregs as well as TIGIT, LAG3 and CD39 expression on CD4+ T cells were measured by flow cytometry.

GvHD murine model: GvHD was generated by transferring T cells isolated from a human PD-1 knock-in (KI) mouse into a B6D2F1/J model and treated with S-4321 after cell transfer. Expansion of engrafted cells was measured by flow cytometry and inflammatory cytokine production quantified by ELISPOT.

NULISA: Plasma proinflammatory mediators (GvHD model) and soluble PD-1 (cynomolgus monkey) were measured by NULISA assay (Alamar Biosciences).

Cynomolgus monkey pharmacokinetics (PK): Cynomolgus monkeys received S-4321 intravenously (IV) or subcutaneously (SC). PK was collected at several timepoints. S-4321 was captured on a plate using anti-idiotype antibody to the variable region of S-4321. Bound S-4321 was detected using an anti-human Fc biotinylated antibody followed by streptavidin SULFO-TAG. The standard was prepared in a drug naïve serum.

RESULTS

S-4321 offers a novel approach over first generation PD-1 engagers

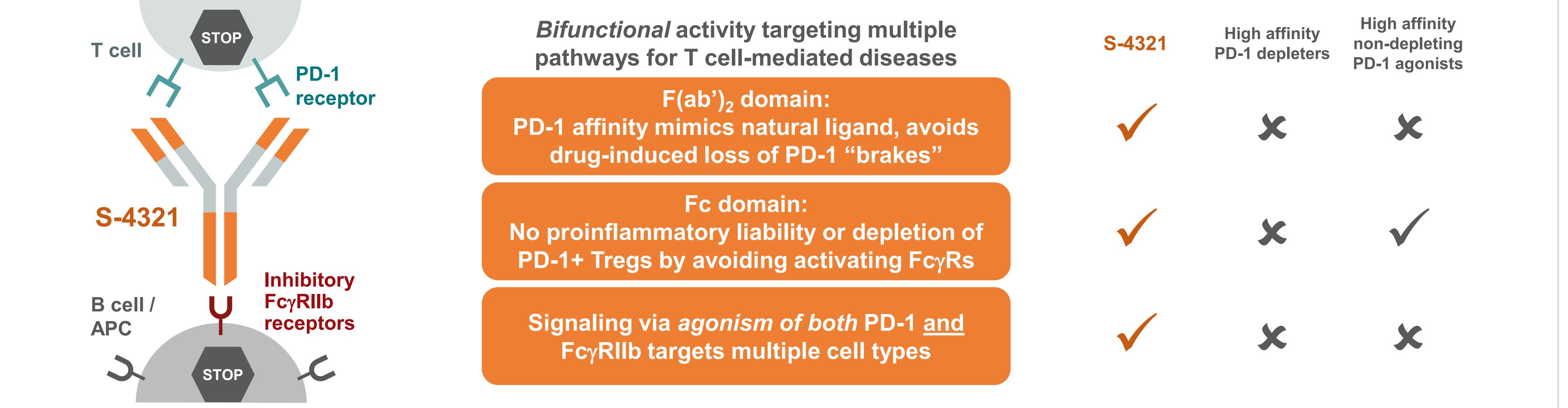

FIGURE 2: S-4321 offers a novel differentiated approach over benchmark PD-1 engagers, with bifunctional activity allowing the potential for greater clinical efficacy.

S-4321 agonizes PD-1 and FcγRIIb inhibitory receptors

A. S-4321 agonizes PD-1 similarly to the natural ligand PD-L1 in SHP2 recruitment assay *in vitro*

B. S-4321 agonizes FcγRIIb in SHIP-1 recruitment assay *in vitro*

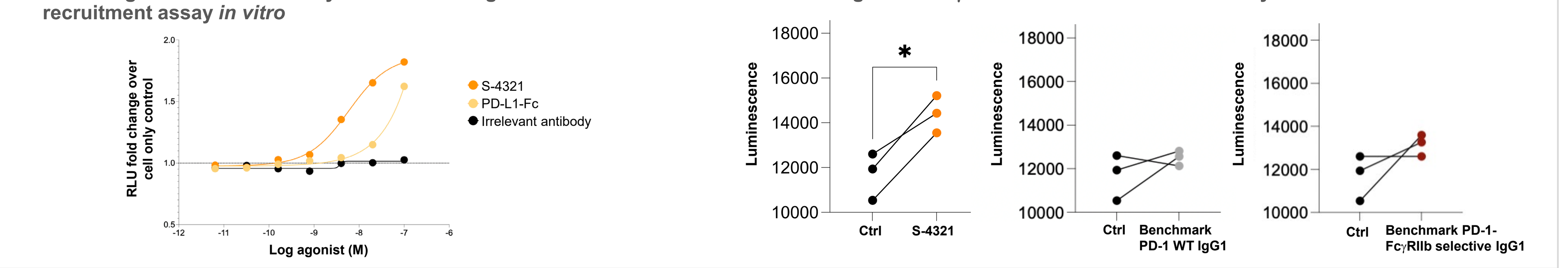

FIGURE 3: A) Treatment with S-4321 and PD-L1 induces SHP2 recruitment, a proxy of PD-1 agonism, in a Jurkat PD-1 reporter line cocultured with a FcγRIIb expressing cell line. **B)** Treatment with S-4321 induces SHIP-1 recruitment, a proxy for FcγRIIb agonism. In contrast, benchmark high-affinity depleter (WT IgG1 Fc) and benchmark high-affinity non-depleting PD-1 agonist (FcγRIIb-selective Fc) do not significantly induce SHIP-1 recruitment, likely due to loss of PD-1 surface expression and lack of an efficient cell synapse to support FcγRIIb agonism.

Low affinity $F(ab')_2$ domain of S-4321 preserves PD-1 expression

A. Low affinity for PD-1 correlates with maintenance of PD-1 expression

B. Maintenance of PD-1 expression correlates with control of IL-2 production

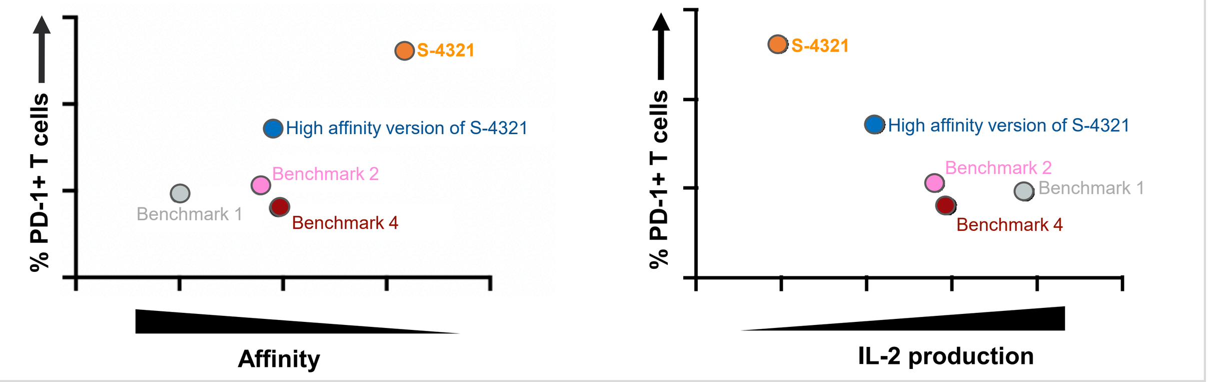

FIGURE 4: Frequency of PD-1 expression on SEB-activated T cells in the presence of S-4321 or benchmark PD-1 engagers versus **A)** their binding affinity to the PD-1 receptor and **B)** supernatant IL-2 levels produced by SEB-activated PBMC in the presence of S-4321 or the same benchmark PD-1 engagers. S-4321 has low affinity for PD-1, which results in maintenance of PD-1 expression and avoids IL-2 production. Loss of PD-1 expression with a benchmark PD-1 engager, but not with S-4321, has also been confirmed *in vivo* in naïve mice (data not shown).

FcγRIIb selective Fc domain of S-4321 avoids proinflammatory cytokine induction

S-4321 does not elicit TNFα production in MoDCs

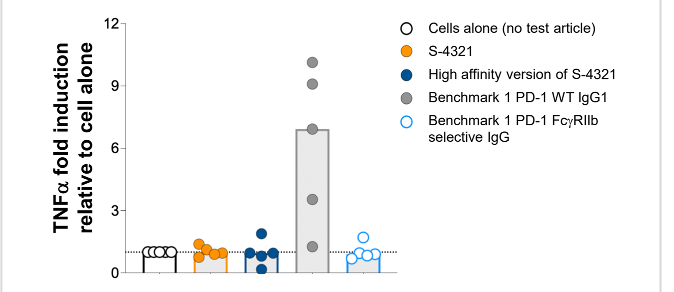

FIGURE 5: Unlike the benchmark PD-1 engager with a WT IgG1 Fc, S-4321 and a high affinity version of S-4321 do not elicit TNFα production. When the benchmark PD-1 engager Fc is replaced with an FcγRIIb selective IgG, TNFα production is reduced.

In vivo studies confirm functional differentiation of S-4321

A. S-4321 does not deplete PD-1+ Tregs

B. S-4321 agonism drives Treg expansion *in vivo*

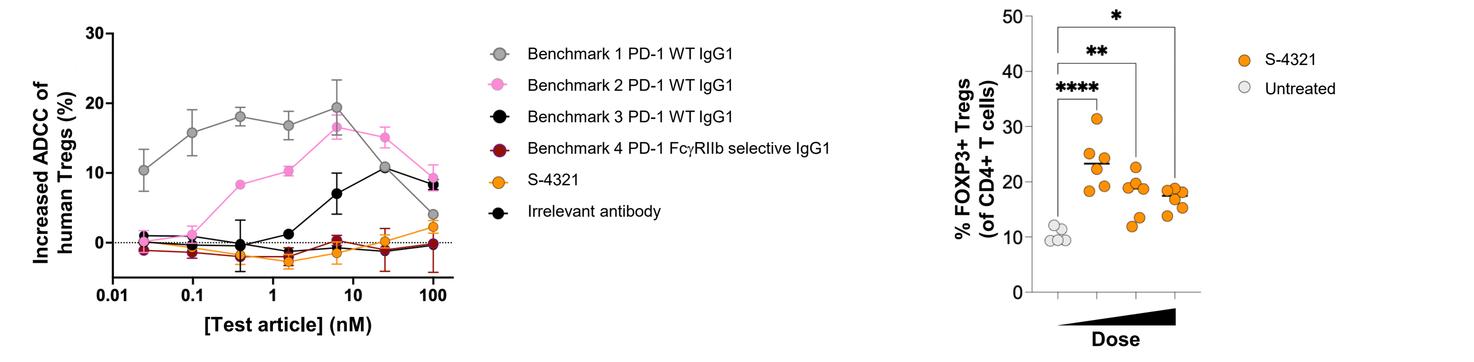

FIGURE 6: A) ADCC activity of human NK cells incubated with human Tregs in the presence of S-4321 or benchmark PD-1 engagers. ADCC requires a WT Fc; it is abrogated when the PD-1 agonist or PD-1 engager carries an FcγRIIb-selective Fc. **B)** Frequency of FOXP3+ Tregs increases in the spleen of huPD-1 KI mice on Day 7 following a single dose of S-4321 at several drug concentrations.

S-4321 treatment inhibits GvHD and downregulates secreted proteins involved in T cell activation

A. S-4321 abrogates GvHD phenotype and proinflammatory mediators in a murine model

B. Downmodulated mediators associated with T cell activation pathways

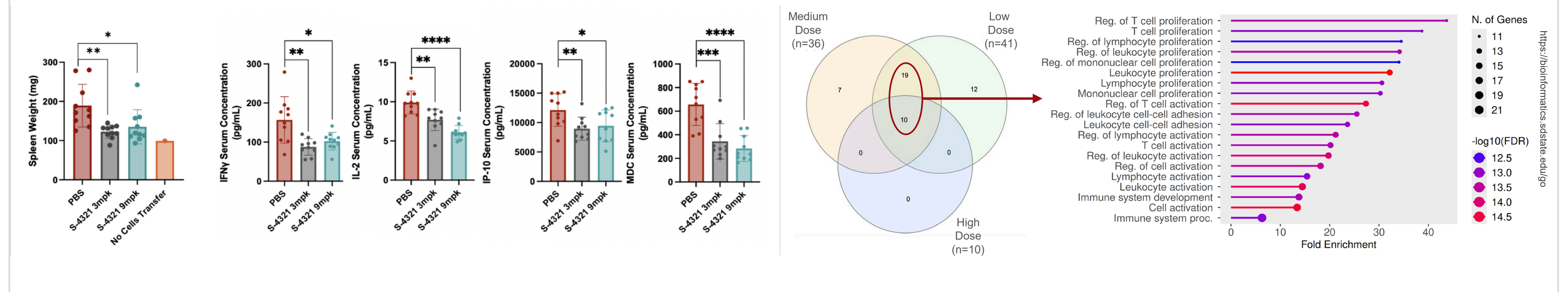

FIGURE 7: A) S-4321 treatment in the acute B6D2F1/J murine model of GvHD leads to significant reduction of splenomegaly and proinflammatory cytokine production. Changes observed in IL-2 and myeloid derived cells (MDC; CCL22) suggest that S-4321 acts at the synapse between T cells and APCs. * p<0.05, ** p<0.01, *** p<0.001. mpk = mg/kg. **B)** Proinflammatory mediators were measured by NULISA following S-4321 treatment at three dose levels. Downmodulated mediators were interrogated in a gene ontology (GO) enrichment analysis to identify pathways modulated by S-4321.

S-4321 increases expression of inhibitory receptors and CD39 in human PD-1 knock-in mice

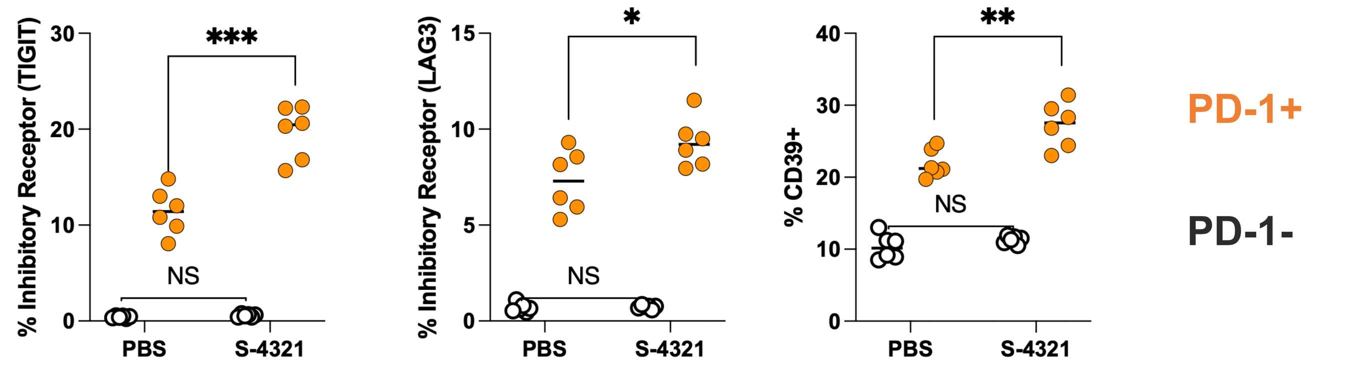

FIGURE 8: A single dose of S-4321 significantly increases expression of TIGIT, LAG3, and CD39 on PD-1+ CD4+ T cells in the spleens of huPD-1 KI mice. No increase is observed on PD-1− cells.

S-4321 demonstrates dose responsive PK and increased soluble PD-1 in non-human primates (NHP)

A. PK in cynomolgus monkeys

B. Soluble PD-1 in cynomolgus monkeys

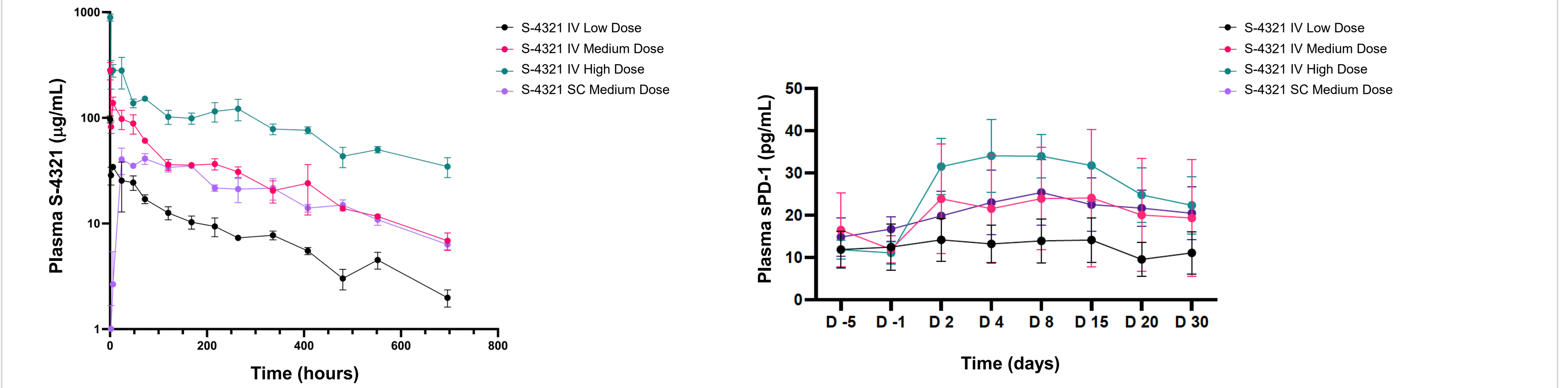

FIGURE 9: A) S-4321 PK at various doses and following both IV and SC administration. **B)** Plasma levels of soluble PD-1 (sPD-1) evaluated by NULISA pre- and post-administration of S-4321. Error bars represent standard deviation (SD) of 3 animals in each group.

CONCLUSIONS

- Unlike established benchmark PD-1 engagers, S-4321 is a novel PD-1:FcγRIIb antibody that agonizes the inhibitory PD-1 receptor on T cells and selectively agonizes the inhibitory FcγRIIb on APCs, which has potential for greater clinical benefit in diseases where pathogenesis is caused by the dysregulation of cell-mediated immunity.
- The $F(ab')_2$ domain of S-4321 demonstrates low affinity for PD-1 and achieves PD-1 agonism without causing target/cell depletion or production of proinflammatory cytokines.
- S-4321 is effective in a murine model of acute GvHD across multiple parameters. In naïve animals, it promotes Treg induction and enhances expression of immunosuppressive molecules on CD4+ T cells, including TIGIT, LAG3, and CD39.
- NHP studies show dose-proportional exposure and approximately 70% bioavailability of S-4321 with SC dosing. The increase in sPD-1 is considered an extension of its half-life upon binding with S-4321.
- S-4321 Phase 1 clinical trial in healthy participants is ongoing.

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