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S-4321, a novel dual-cell bidirectional PD-1:FcγRIIb selective agonist antibody for the treatment of autoimmune disease

Julia Manasson, Marisella Panduro, Michael Cianci, Minasri Borah, Stephanie Grebinoski, Joshua Vitlip, Stephen Lutz, Ishan Sharma, Elliott Wittenberg, Allison Colthart, Samuel Perry, Maria Cecilia Ramello, Chelsea R. Parker Harp, Jyothsna Visweswaraiah, Ryan Peckner, Alex Pellerin, Heather Vital, John S. Sundy, Nathan Higginson-Scott, Kevin L. Otipoby, Daniela Cipolletta

Seismic Therapeutic, Cambridge, MA USA

ABSTRACT

Background/Purpose: The dysregulation of immune checkpoint receptors on T cells and antigen presenting cells (APCs) drives autoimmunity while receptor agonism is expected to restore immune homeostasis in diseases such as rheumatoid arthritis, systemic lupus erythematosus, Sjogren's syndrome, and giant cell arteritis. PD-1 is a checkpoint receptor expressed on T cells, including Tfh and Treg subtypes. Strong PD-1 agonism requires super-clustering, which can be achieved by therapeutic antibodies binding to Fc gamma receptors (FcγR) on APCs. S-4321 is a novel dual-cell bidirectional (DcB) PD-1:FcγRIIb antibody that agonizes the inhibitory PD-1 receptor on T cells (Fab domain) and selectively engages the inhibitory FcγRIIb on APCs (Fc domain), eliciting an inhibitory signal at the immune cell synapse to re-establish homeostasis.

Methods: Staphylococcal enterotoxin B (SEB) activated human peripheral blood mononuclear cells (PBMC) were cultured with S-4321 and benchmark PD-1 agonist antibodies. T cell PD-1 expression was measured by flow cytometry and IL-2 production by Meso Scale Discovery (MSD) assay. Induced Tregs (iTregs) were differentiated *in vitro* by culturing human CD4 naïve T cells with TGFβ and IL-2 in the presence of S-4321. A graft versus host disease (GvHD) model was generated by transferring T cells isolated from a human PD-1 knock-in mouse into a B6D2F1/J model. Immunophenotyping was performed by flow cytometry and cytokine production measured by ELISPOT and Luminex assays. S-4321 function was tested pre-clinically in cynomolgus monkeys.

Results: S-4321 achieves prolonged agonism by binding to PD-1 with low affinity (similar to PD-L1) thus allowing for the retention of PD-1 expression on the T cell surface. This ensures continued presence of target and maintains a regulatory check on effector cell activity. In contrast, higher affinity PD-1 agonists induce a marked decrease in surface PD-1, associated with increased IL-2 production in SEB activated cultures. S-4321 also selectively engages FcγRIIb, avoiding the induction of proinflammatory cytokines and undesirable depletion of PD-1 expressing T cells, such as Tregs, by antibody-dependent cellular cytotoxicity (ADCC) through engagement of activating FcγR.

S-4321 promotes iTregs in the presence of TGFβ and IL-2 ($p<0.05$) *in vitro*. Using the B6D2F1/J model of murine GvHD, prophylactic treatment with S-4321 reduces engrafted T cell expansion and proinflammatory cytokine production ($p<0.05$). Cynomolgus monkey data demonstrate dose-proportional exposure and ~70% bioavailability of S-4321 with subcutaneous dosing.

Conclusion: S-4321 is a novel DcB PD-1:FcγRIIb agonist that engages inhibitory receptors on both sides of the T cell-APC synapse. It agonizes PD-1 without causing target or cell depletion and its high bioavailability allows for convenient administration. *In vivo*, S-4321 decreases T cell activation that contributes to the pathogenesis of autoimmune diseases and avoids the depletion of Tregs, important for restoring peripheral tolerance.

INTRODUCTION

The dysregulation of the immune response against self-antigens results in the development of autoimmunity. Antigen activation induces lymphocytes to upregulate inhibitory receptors (IRs) as a feedback mechanism for limiting effector activity triggered by the antigens. Not surprisingly, IR deficiency such as PD-1, is associated with autoimmunity in mice and humans.[1,2] Since the loss of IR pathways gives rise to inflammation, agonism of these pathways is expected to restore normal immune homeostasis.

PD-1 is a well characterized IR expressed on activated T cells. Strong PD-1 agonism requires clustering of multiple PD-1 molecules, which can be achieved through therapeutic antibody binding to FcγR on APCs.[3] Seismic Therapeutic's proprietary machine learning IMPACT platform enabled the identification of S-4321, a PD-1 agonist antibody that is also selective for FcγRIIb, the only inhibitory human FcγR. S-4321 aims to restore immune homeostasis by agonizing multiple IRs with a single DcB antibody at the T cell:B cell/APC synapse.

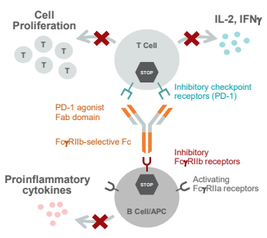


FIGURE 1: Seismic Therapeutic's IMPACT platform was used to engineer S-4321, a DcB PD-1:FcγRIIb antibody that agonizes the inhibitory PD-1 receptor on T cells (Fab domain) and selectively engages the inhibitory FcγRIIb on APCs (Fc domain), eliciting an inhibitory signal at the immune cell synapse to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

[1] Wang, Han et al. "Establishment of NOD-Pdcd1-/- mice as an efficient animal model of type I diabetes." *Proceedings of the National Academy of Sciences of the United States of America* vol. 102,33 (2005): 11823-8. doi:10.1073/pnas.0505497102

[2] Ogishi, Masaaki et al. "Inherited PD-1 deficiency underlies tuberculosis and autoimmunity in a child." *Nature medicine* vol. 27,9 (2021): 1646-1654. doi:10.1038/s41591-021-01386-8

[3] Suzuki, Kensuke et al. "Anti-PD-1 antibodies recognizing the membrane-proximal region are PD-1 agonists that can down-regulate inflammatory diseases." *Science immunology* vol. 5,79 (2020): eaaw9977. doi:10.1126/sciimmunol.aaw9977

METHODS

SEB activation of PBMC: PBMC were isolated from whole blood of healthy donors and incubated with SEB in the presence of a PD-1 antagonist, an isotype control (anti-RSV:FcγRIIb selective), benchmark high affinity PD-1 agonist antibodies, or Seismic Therapeutic PD-1 agonists at several concentrations. After 4 days in culture, PBMC were collected and immunophenotyped to measure PD-1 expression on the cell surface of T cells and supernatant collected to quantify IL-2 production by MSD assay.

Binding measurements to FcγR: Binding measurements to human FcγRIIa, FcγRIIb, FcγRIII, and FcRn were performed via surface plasmon resonance (SPR, Carterra). Confirmatory cell binding measurements were performed using CHO lines engineered to express FcγRIIb, FcγRIIa H131, or FcγRIIa R131.

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).

ADCC assay: Human natural killer (NK) cells were incubated overnight with Jurkat cells expressing human PD-1 at a ratio of 1:5 in the presence of S-4321 at different concentrations or benchmark PD-1 agonist antibodies. ADCC activity was measured by quantifying cellular viability via flow cytometry.

iTreg differentiation assay: iTregs were differentiated *in vitro* by culturing human CD4 naïve T cells with TGFβ and IL-2 in the presence of S-4321 for 3 days. Induction of Tregs were measured by quantification of FOXP3 and CD25 expression via flow cytometry.

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

Cynomolgus monkey pharmacokinetics (PK): Cynomolgus monkeys received S-4321 intravenously (IV) or subcutaneously (SC). Blood was collected at different timepoints to quantify PK. 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 drug naïve serum.

RESULTS

S-4321 offers a novel differentiated approach over clinical stage PD-1 agonists

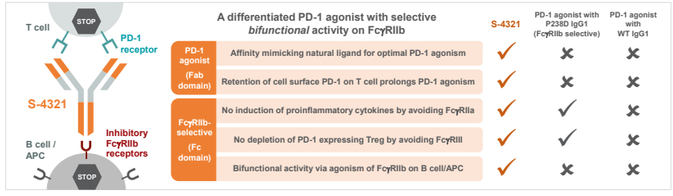


FIGURE 2: S-4321 offers a novel differentiated approach over clinical stage PD-1 agonists, with bifunctional activity allowing the potential for greater clinical efficacy.

S-4321 low affinity Fab domain preserves PD-1 expression on T cells and maintains control of IL-2 production by activated cells

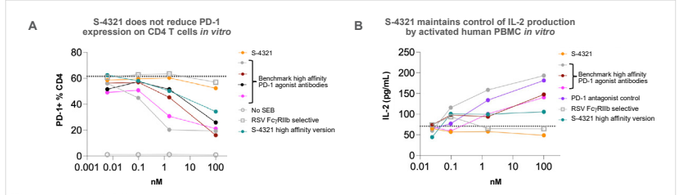


FIGURE 3: A) Frequency of PD-1 expression on SEB-activated CD4 T cells in the presence of increasing concentrations of test article. B) Supernatant IL-2 levels produced by SEB-activated PBMC with increasing concentrations of test article.

S-4321 Fc domain selectively binds the inhibitory FcγRIIb receptor

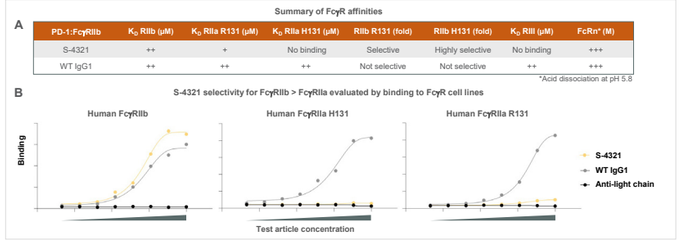


FIGURE 4: A) Binding measurement to human FcγRIIa, FcγRIIb, FcγRIII, and FcRn via surface plasmon resonance (Carterra). B) Cell binding measurements in CHO lines engineered to express FcγRIIb, FcγRIIa H131, or FcγRIIa R131.

S-4321 FcγRIIb-selective Fc domain does not elicit the production of proinflammatory cytokines by APCs or deplete PD-1+ cells

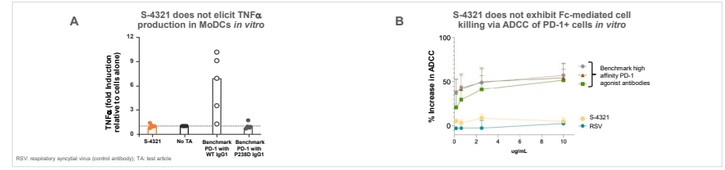


FIGURE 5: A) TNFα production by MoDCs cultured with S-4321, Benchmark PD-1 with IgG1 WT, or Benchmark PD-1 with P238D IgG1 (FcγRIIb selective binder). B) ADCC activity of human NK cells incubated with Jurkat PD-1 cells in the presence S-4321 or benchmark high affinity PD-1 agonist antibodies.

S-4321 promotes the induction of Tregs *in vitro*

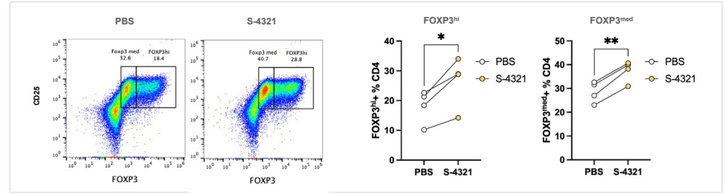
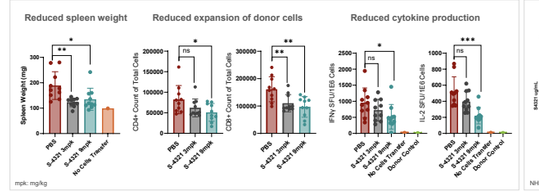


FIGURE 6: Induced differentiation of Tregs (FOXP3+CD4+) *in vitro* in the presence of IL-2, TGFβ, and S-4321. * $p<0.05$, ** $p<0.01$

Treatment with S-4321 inhibits GvHD in a murine model



S-4321 exhibits dose-linear PK and high bioavailability in NHP

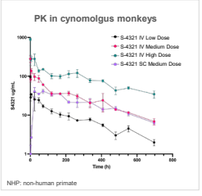


FIGURE 7: S-4321 treatment in the acute B6D2F1/J model of murine GvHD leads to significant reduction of splenomegaly, engraftment of CD4+ and CD8+ donor cells, and pro-inflammatory cytokine production. ns: not significant, * $p<0.05$, ** $p<0.01$, *** $p<0.001$

FIGURE 8: S-4321 PK at various doses and following both IV and SC administration.

CONCLUSIONS

- The dysregulation of IRs, such as PD-1, drives autoimmunity, while checkpoint receptor agonism is expected to restore normal immune homeostasis.
- Unlike established PD-1 agonists, S-4321 is a novel PD-1:FcγRIIb antibody that agonizes the inhibitory PD-1 receptor on T cells and selectively engages 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 Fab domain of S-4321 demonstrates low affinity for PD-1 and achieves PD-1 agonism without causing target depletion or IL-2 production.
- The Fc domain of S-4321 selectively engages the inhibitory FcγRIIb receptor, preventing the depletion of PD-1 expressing T cells, such as Tregs, and avoiding the production of proinflammatory cytokines.
- Furthermore, S-4321 promotes the induction of Tregs and prevents GvHD progression in a murine model.
- Non-human primate studies demonstrate dose-proportional exposure and ~70% bioavailability of S-4321 with SC dosing.
- S-4321 will enter the clinic in the first half of 2025.

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