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ALPN-101, a First-in-Class Dual ICOS/CD28 Antagonist, Suppresses Key Effector Mechanisms Associated with Sjögren's Syndrome and Systemic Lupus Erythematosus

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Abstract

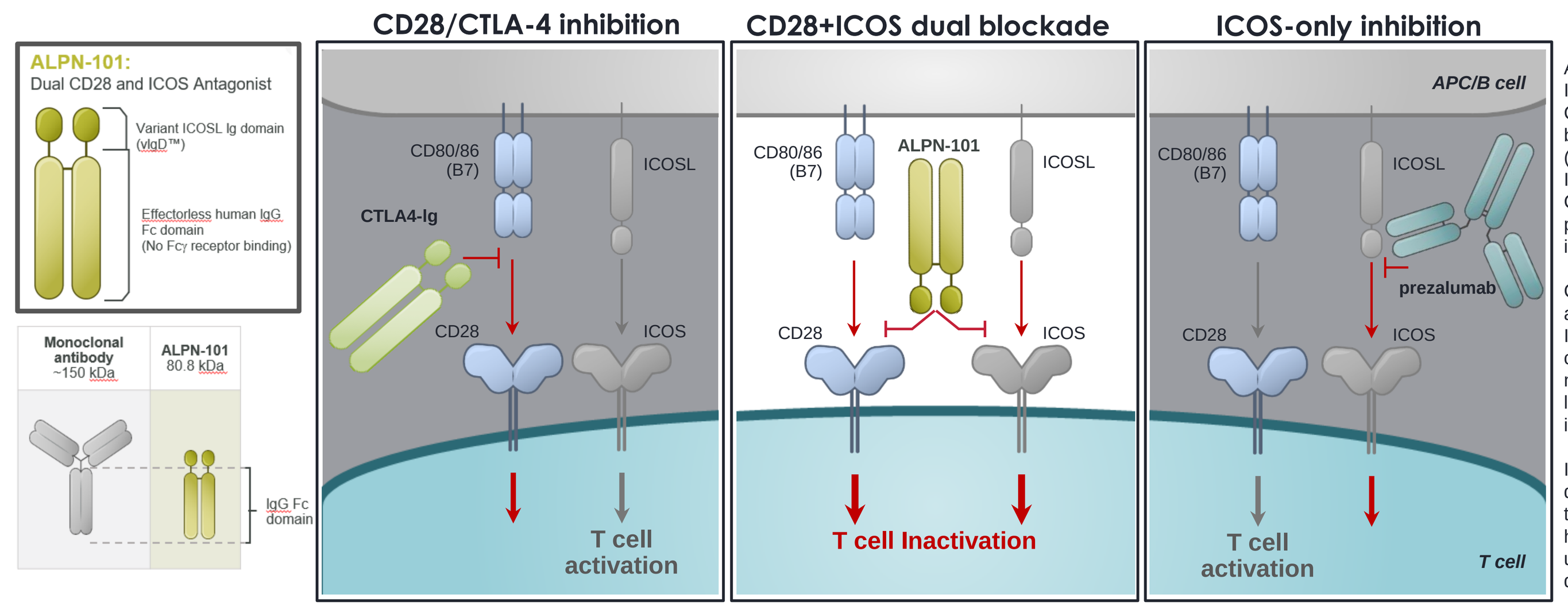
INTRODUCTION: ALPN-101 is an Fc fusion protein of a human inducible T cell costimulator ligand (ICOSL) variant immunoglobulin domain (vIgD™) designed to inhibit simultaneously the CD28 and ICOS costimulatory pathways. CD28 and ICOS each play a role in T cell activation and adaptive immunity which can contribute to autoimmune disease when dysregulated. ALPN-101 has previously been shown to have potent immunosuppressive activity in various *in vitro* and *in vivo* models of disease, including acute graft versus host disease, inflammatory arthritis, and multiple sclerosis. We report here *in vitro* assays using peripheral blood mononuclear cells (PBMC) from healthy donors vs. patients to analyze human T cell and B cell activation and suppression of antibodies and inflammatory mediators thought to contribute to the pathogenesis of Sjögren's syndrome (SjS) and other connective tissue diseases. Additionally, the efficacy of ALPN-101 was confirmed *in vivo* in mouse immunization models, and in mouse models of SjS and systemic lupus erythematosus (SLE).

METHODS: Primary cell assays were performed with healthy donor and SjS patient PBMC stimulated with K562 cells expressing CD80, CD86, ICOSL, and anti-CD3 (OKT3) to evaluate the potency of ALPN-101 to suppress cytokine production and alter gene expression. The activity of dual pathway inhibition by ALPN-101 was compared to the CD28-only inhibitor abatacept (CTLA4-Ig; Bristol-Myers Squibb, via Catalent) and to the ICOS pathway inhibitor prezalumab (AMG-557/anti-ICOSL, Creative Biolabs), or a combination of the two. ALPN-101 was compared to abatacept *in vivo* in standard mouse immunization models (KLH, sheep RBC), in a model of SjS involving anti PD-L1 antibody-mediated acceleration of sialadenitis in non-obese diabetic (NOD) mice, and in the bm12 inducible model of lupus.

RESULTS: Compared to abatacept, prezalumab, or combination abatacept + prezalumab, ALPN-101 demonstrated superior suppression of pro-inflammatory cytokine (i.e. TNFα, IFNγ, IL-1β, IL-2, IL-6, IL-17A, GM-CSF, etc.) release from stimulated healthy SjS, or SLE patient PBMCs (Fig. 3). ALPN-101 treatment reduced germinal center (GC) B cells and follicular helper T cells (T_{FH}) and inhibited antibody production *in vivo* in mouse immunization models (not shown) and in the bm12 model (Fig. 9), and suppressed proliferation and antibody production in human B cell/T_{FH} cell co-cultures (Fig. 5). In anti PD-L1-treated NOD mice, ALPN-101 suppressed sialadenitis, insulitis, blood glucose levels, and autoantibodies with activity often superior to that of abatacept (Fig. 6-8, and data not shown).

CONCLUSION: The efficacy of dual CD28/ICOS antagonist ALPN-101 is superior to CD28 or ICOS costimulatory pathway inhibitors, administered individually or in combination, in human *in vitro* and/or mouse *in vivo* translational studies. A Phase 1 clinical trial with ALPN-101 in healthy volunteers is ongoing (NCT03748836), and trials in inflammatory diseases are planned.

Figure 1: ALPN-101 (ICOSL vIgD-Fc), Generated Using the vIgD™ Directed Evolution Strategy, Blocks Both CD28 and ICOS T Cell Costimulation Pathways

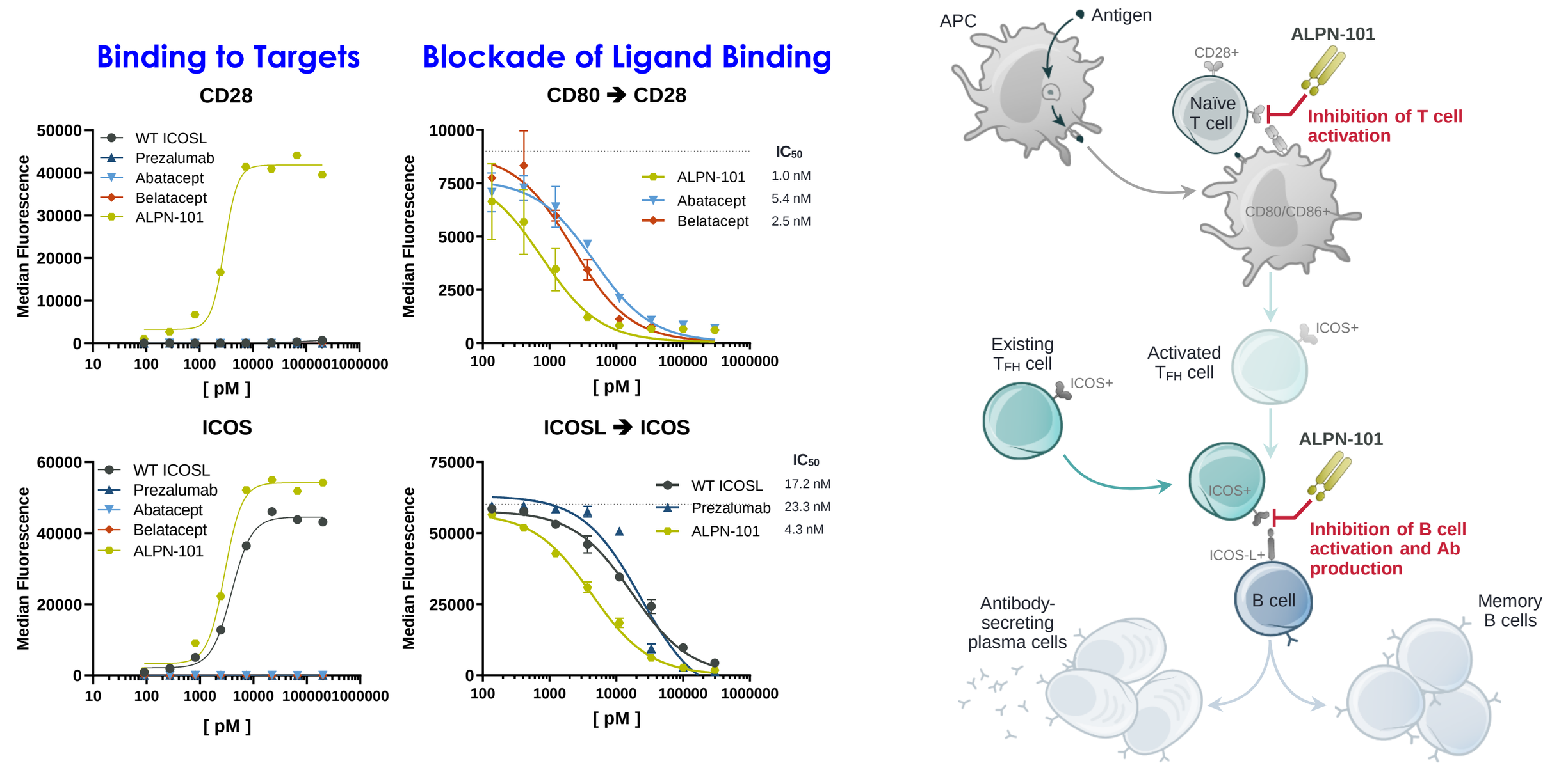


ALPN-101 inhibits both CD28 and ICOS-mediated costimulation. Comparators such as abatacept / belatacept (CTLA4-Ig, BMS) or FR104 (anti CD28 Fab, OSE Immunotherapeutics) inhibit only the CD28/CTLA-4 pathway, while prezalumab (anti ICOSL mAb, Amgen) inhibits only ICOS costimulation.

CD28 costimulation is critical for naïve and memory $CD4^+$ T cell activation. ICOS signaling augments CD28 costimulation but also plays non-redundant roles; ICOS deficiency leads to defective humoral responses in both mice and humans.[1]

ICOS signaling is crucial for the development of human T_H17 cells[2] and the function & maintenance of follicular helper T cells.[3] ALPN-101 may thus be uniquely suited to treat diseases driven by these pathogenic cell types.

Figure 2: ALPN-101 Binds CD28 and ICOS and Blocks Binding to Their Ligands, Impacting B Cell/T Cell Collaboration During Antibody Responses



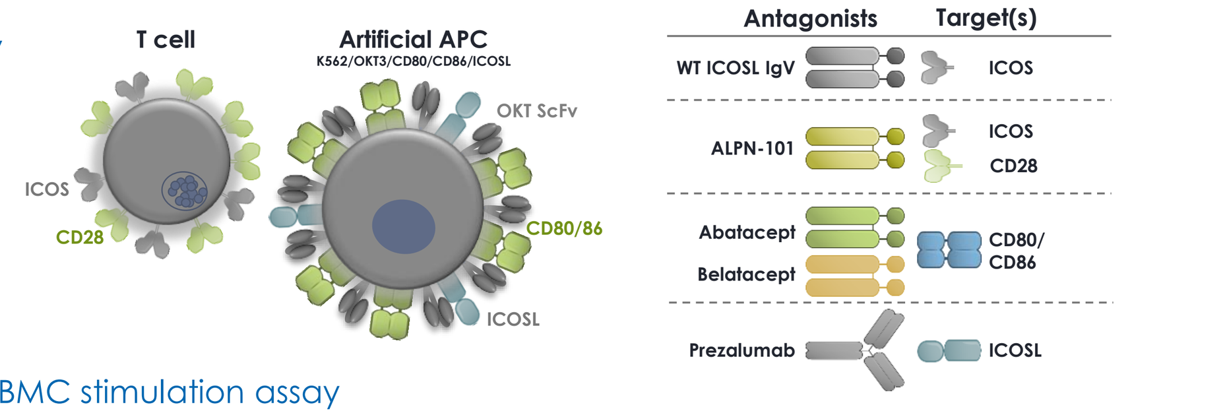
- Primary immune responses depend heavily upon the CD28 pathway
- Secondary immune responses (e.g. memory, effector) can be CD28-independent.
- ICOS is upregulated upon T-cell activation and is the most closely related protein to CD28, yet plays a non-redundant function in many immune responses, such as follicular helper T cell (T_{FH}) differentiation and function.[1,3]
- ICOS+ T cells escape treatment with drugs only blocking the CD28/CTLA-4 pathway, like abatacept.

Our Thesis: Blockade of both CD28 and ICOS pathways by ALPN-101 should provide superior efficacy to therapeutics which only interfere with a single pathway

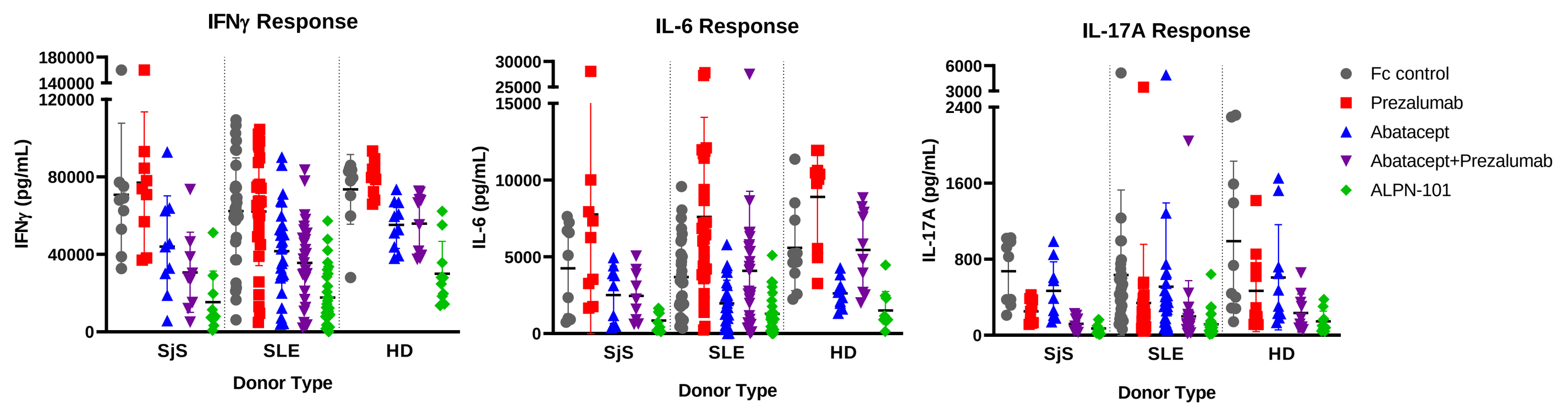
Figure 3: ALPN-101 Inhibits Cytokine Production from Human Sjögren's and SLE Patient PBMC *In Vitro* More Potently Than Single CD28 or ICOS Pathway Inhibitors

(A) Diagram of the PBMC stimulation assay

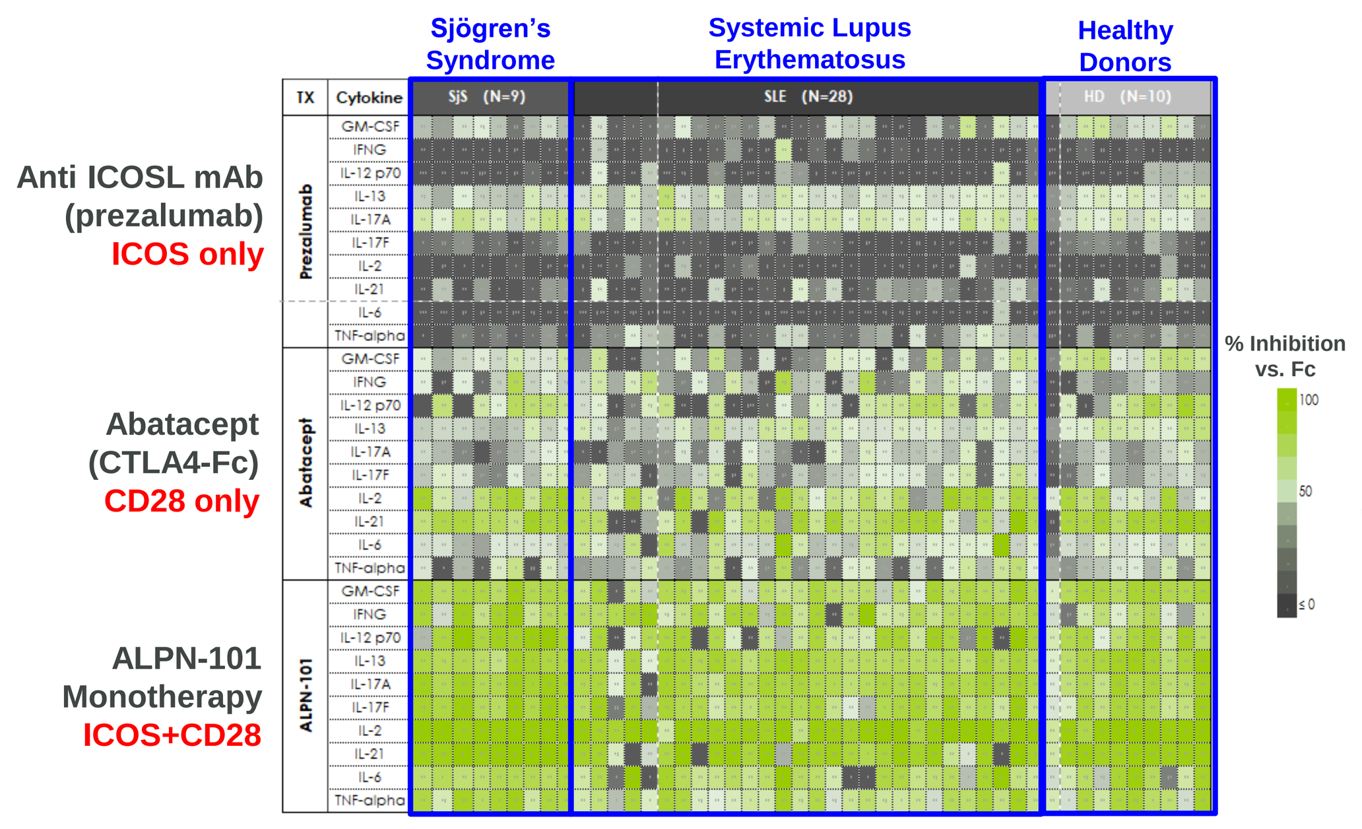
Patient or healthy donor peripheral blood mononuclear cells (PBMC) were stimulated with fixed artificial APCs (shown at right) for 48 hrs in the presence of ALPN-101 (100nM) or comparators:
- Prezalumab (anti ICOSL mAb)
- Abatacept alone (CTLA4-Ig)
- Abatacept + prezalumab



(B) Examples of cytokine responses in the PBMC stimulation assay



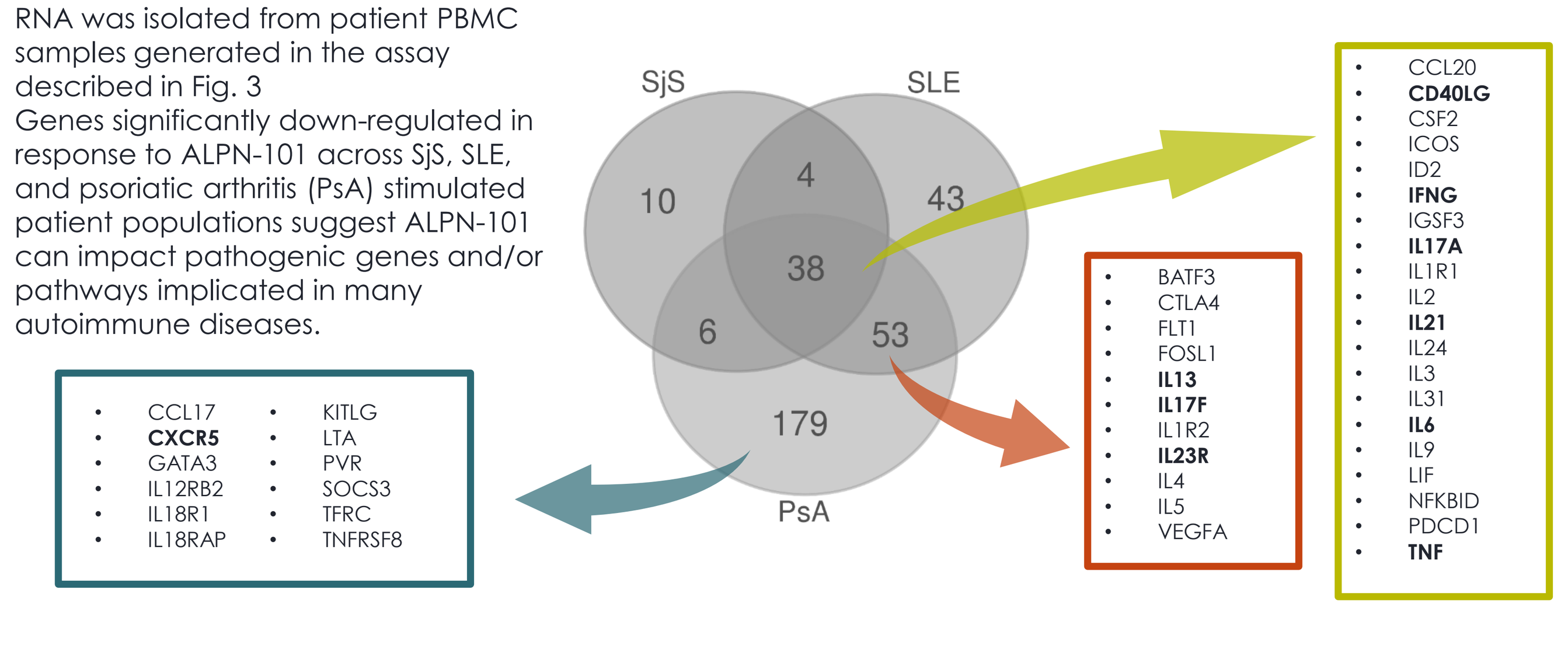
(C) Summary of cytokine responses in the PBMC stimulation assay



(A) *In vitro* stimulation of healthy donor, Sjögren's syndrome (SjS), and SLE patient PBMC with artificial APC [fixed K562 expressing cell surface OKT3 (anti CD3)/CD80/ CD86/ICOSL)] at a 20:1 ratio. Test articles were added at 100 nM and supernatants were collected and assayed for cytokine concentrations after 48 hr incubation. **(B)** IFNγ, IL-6, and IL-17A levels are shown as examples, though similar trends were observed for all cytokines measured, as summarized in C. **(C)** ALPN-101 demonstrated statistically significant superiority to prezalumab, abatacept, or a combination of prezalumab+ abatacept for the majority of donors and analytes tested.

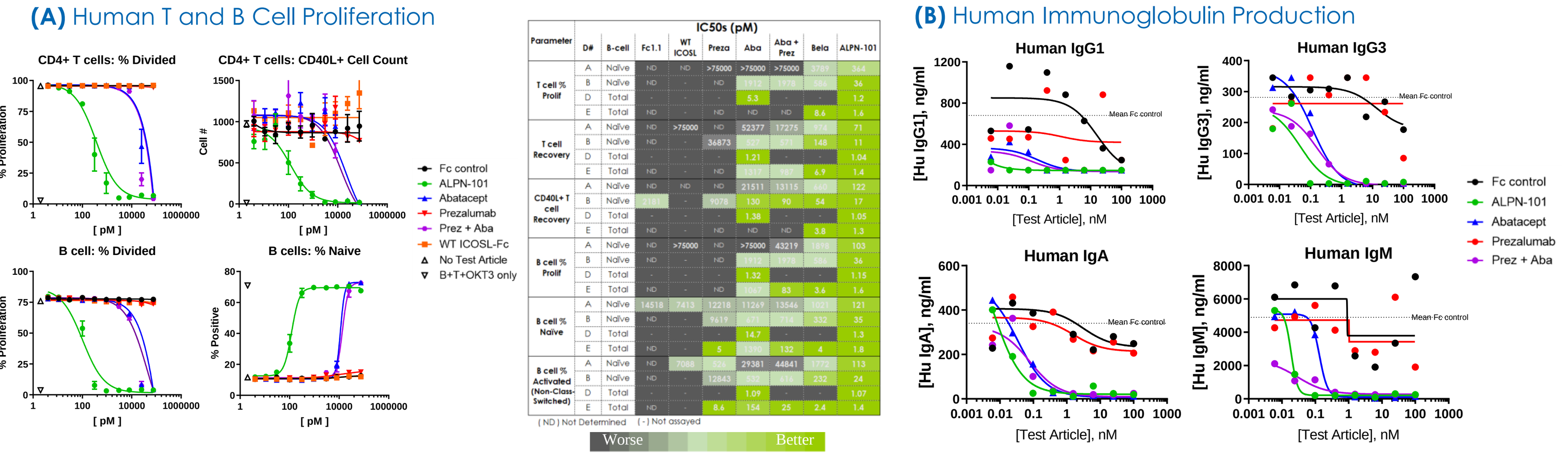
Figure 4: ALPN-101 Suppresses Expression of Many Genes Associated with SjS/SLE Mechanisms and Phenotypes

- RNA was isolated from patient PBMC samples generated in the assay described in Fig. 3
- Genes significantly down-regulated in response to ALPN-101 across SjS, SLE, and psoriatic arthritis (PsA) stimulated patient populations suggest ALPN-101 can impact pathogenic genes and/or pathways implicated in many autoimmune diseases.


SjS
SLE
PsA
10
4
43
38
6
53
179
CCL17
CXCR5
GATA3
IL12RB2
IL18R1
IL18RAP
KITLG
LTA
PVR
SOCS3
TFRC
TNFRSF8
BATF3
CTLA4
FLT1
FOSL1
IL13
IL17F
IL1R2
IL23R
IL4
IL5
VEGFA
CCL20
CD40LG
CSF2
ICOS
ID2
IFNG
IGSF3
IL17A
IL1R1
IL2
IL21
IL24
IL3
IL31
IL6
IL9
LIF
NFKBID
PDCD1
TNF

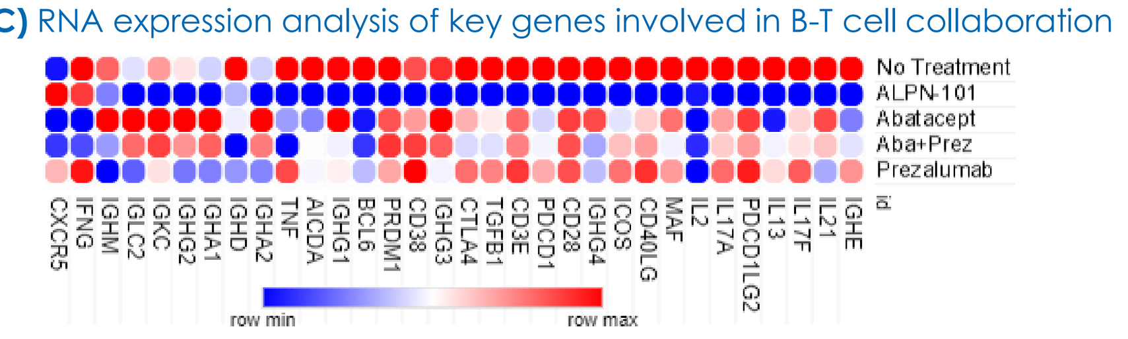
See also: Poster #1531. Evans et al. (2019) **ALPN-101, a First-in-Class Dual ICOS/CD28 Antagonist, Suppresses Key Effector Mechanisms Underlying Rheumatoid and Psoriatic Arthritis.** ACR Annual Meeting; November 11, 2019.[4]

Figure 5: ALPN-101 Suppresses Proliferation and Antibody Responses in Human B-T_{FH} Cell Co-Cultures and Affects Expression of Genes Involved in B-T Cell Collaboration



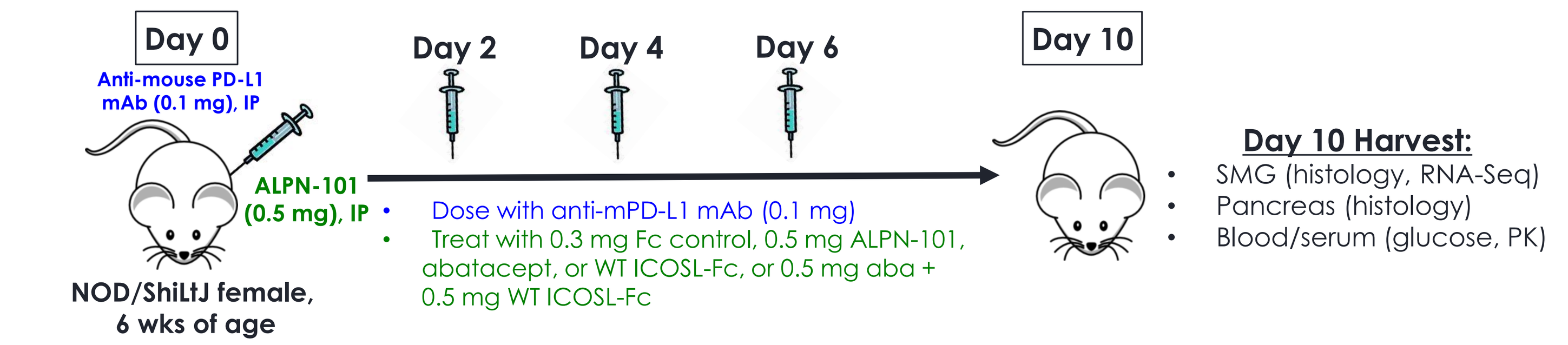
(A) Autologous circulating human B and T_{FH} cells isolated from PBMC from healthy donors were mixed with artificial APCs (K562/CD80) and soluble OKT3, and titrations of the test articles indicated. Cultures were assessed on Day 7 for cellular activation/proliferation and IC_{50} values are summarized in the table.
(B) In a separate study using a similar protocol, supernatants were collected on Day 8, diluted 1:10, and assayed for human immunoglobulin (Ig) secretion. Data in (B) are representative of results from 8 donors.

(C) RNA expression analysis of key genes involved in B-T cell collaboration


No Treatment
ALPN-101
Abatacept
Aba+Prez
Prezalumab
CXCR5
IFNG
IGHM
IGLC2
IGKC
IGHG2
IGHA1
IGHD
IGHA2
TNF
AICDA
IGHG1
BCL6
PRDM1
CD38
IGHG3
CTLA4
TGFB1
CD3E
PDCD1
CD28
IGHG4
ICOS
CD40LG
MAF
IL2
IL17A
PDCD1LG2
IL13
IL17F
IL21
IGHE
row min
row max

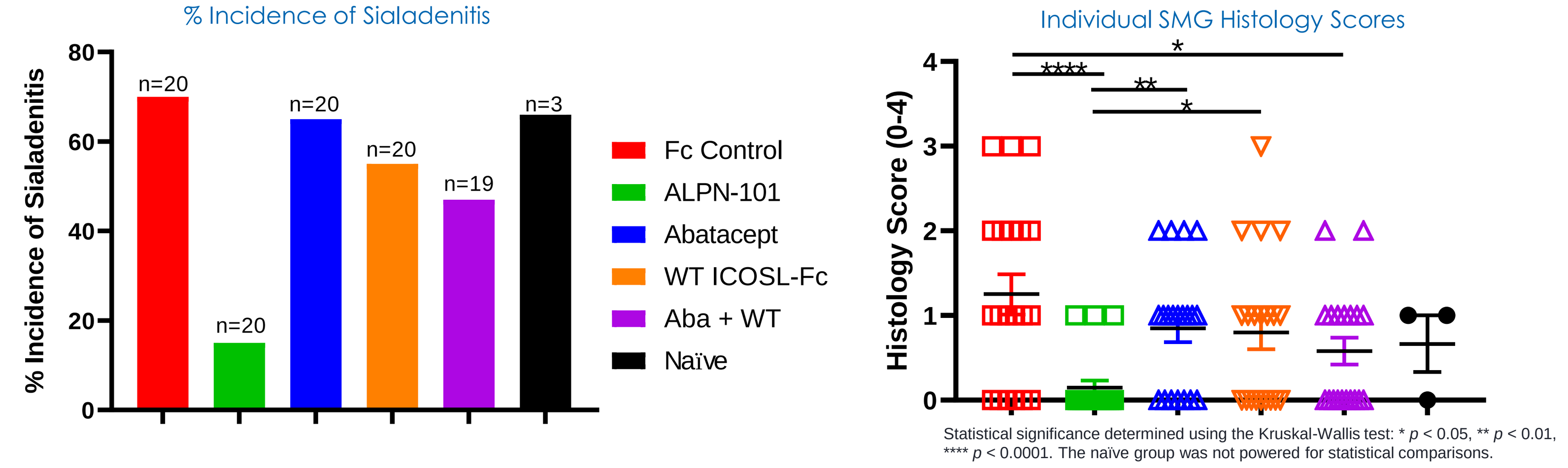
(C) RNA isolated from a B/T_{FH} co-culture similar to the one described in **A** was analyzed for relative gene expression levels by next generation sequencing. Expression values in RPKM are plotted on a relative scale from 0 to 1 for each gene between treatment groups, with degree of red reflecting higher expression and degree of blue representing lower values for each gene. The list of genes shown was sorted by level of inhibition by ALPN-101 (least to most inhibited, from L→R).

Figure 6: The Anti-PD-L1 mAb-Induced NOD Mouse Model of Sjögren's Syndrome


Day 0
Anti-mouse PD-L1 mAb (0.1 mg), IP
ALPN-101 (0.5 mg), IP
NOD/ShiLtJ female, 6 wks of age
Day 2
Day 4
Day 6
Dose with anti-mPD-L1 mAb (0.1 mg)
Treat with 0.3 mg Fc control, 0.5 mg ALPN-101, abatacept, or WT ICOSL-Fc, or 0.5 mg aba + 0.5 mg WT ICOSL-Fc
Day 10
Day 10 Harvest:
SMG (histology, RNA-Seq)
Pancreas (histology)
Blood/serum (glucose, PK)

A mouse model of Sjögren's syndrome induced in female diabetes-prone NOD/ShiLtJ mice using repeat dosing (on Days 0, 2, 4, and 6) with anti-mouse PD-L1 antibody (based on a modified version of a protocol published by Zhou et al., 2016[5]) was used to evaluate the impact of ALPN-101 and comparator treatments on the development of sialadenitis and insulitis, the levels of blood glucose, serum autoantibodies, and gene expression in the submandibular gland (SMG).

Figure 7: ALPN-101 Reduces the Incidence and Severity of Sialadenitis in NOD Mice Enrolled in the Anti PD-L1 mAb-Induced Model of Sjögren's Syndrome



Statistical significance determined by the Kruskal-Wallis test: * p < 0.05, ** p < 0.01, **** p < 0.0001. The naïve group was not powered for statistical comparisons.

Representative H&E Images of SMG

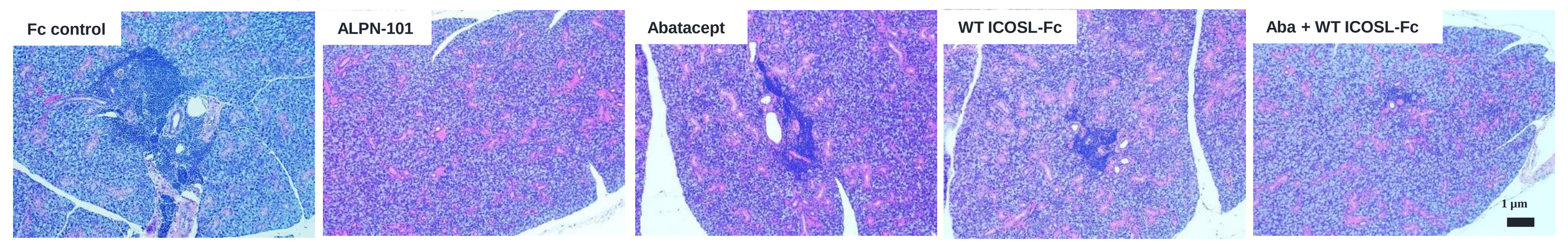
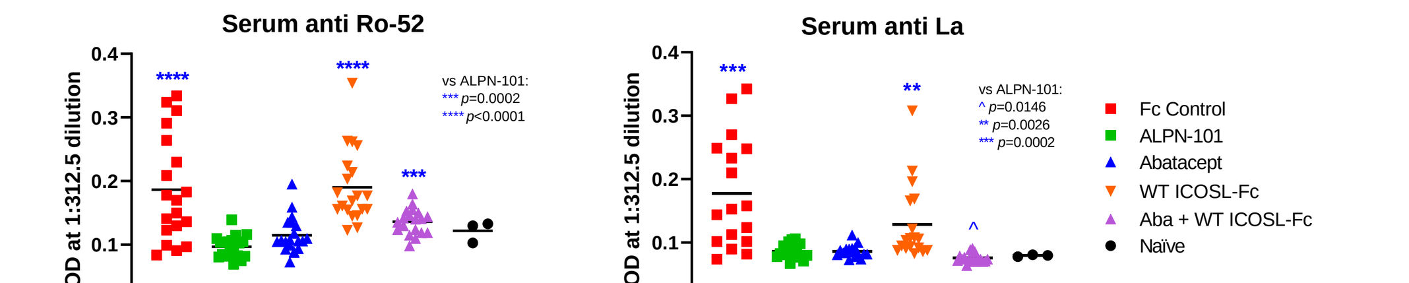


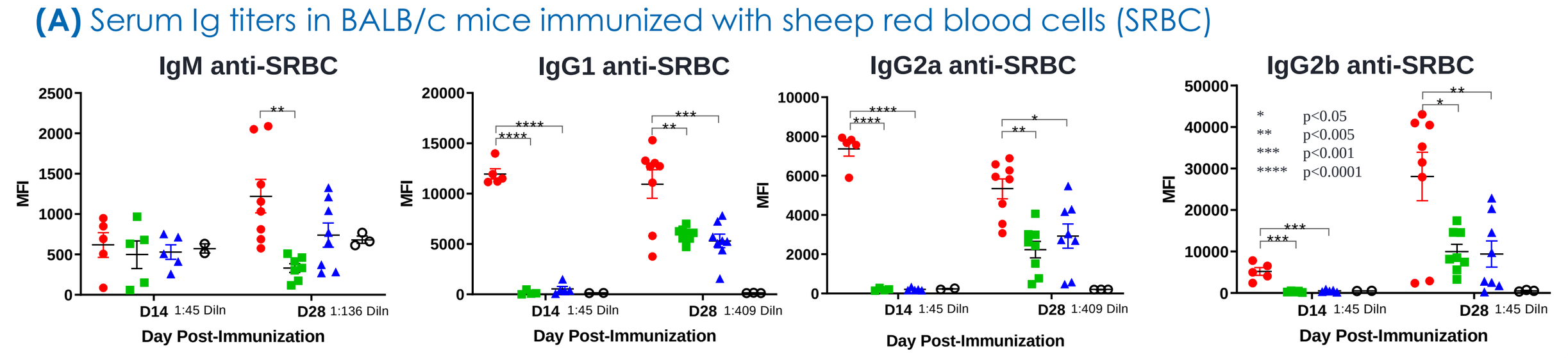
Figure 8: ALPN-101 Reduces Serum Autoantibody Titers in the Anti PD-L1 mAb-Induced NOD Mouse Model of Sjögren's



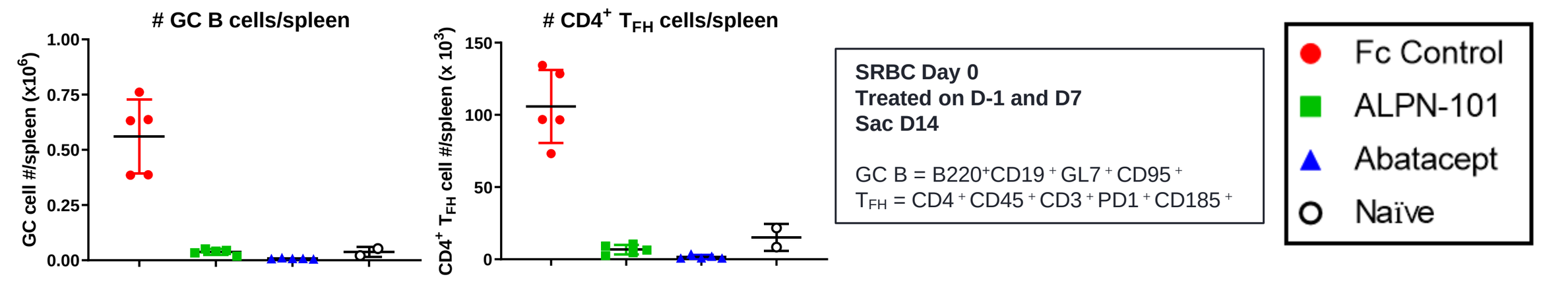
Anti Ro-52 and anti La Abs were detected by ELISA (Signosis, Santa Clara, CA) in diluted serum collected on Day 10 from mice in the SjS study described in Figs 6-7. Statistical differences noted between the ALPN-101 group and comparator treatments were determined using an uncorrected non-parametric Dunn's test.

Figure 9: ALPN-101 Reduces GC B and T_{FH} Cells and Suppresses Antibody Responses in Normal Mice and in the bm12 Inducible Mouse Model of SLE

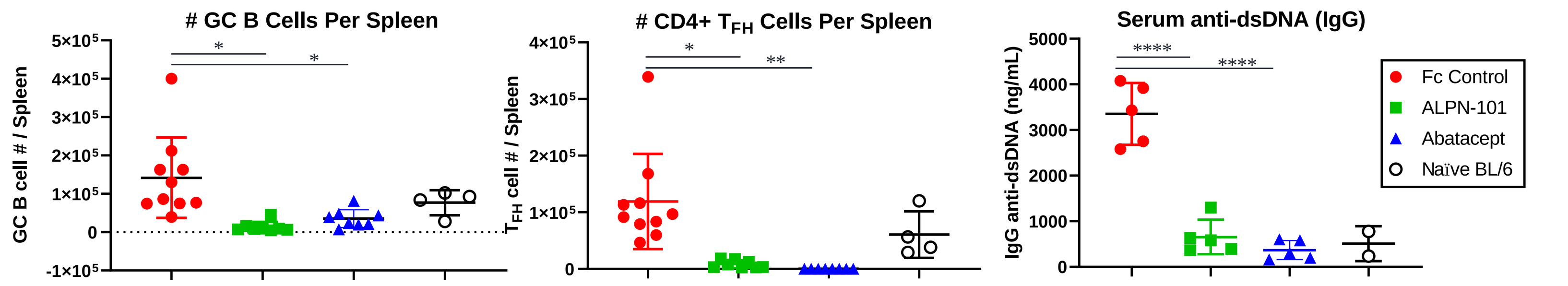
(A) Serum Ig titers in BALB/c mice immunized with sheep red blood cells (SRBC)



(B) Numbers of splenic GC B and T_{FH} in BALB/c mice 14 days after immunization with SRBC



(C) Numbers of splenic GC B and T_{FH} and serum anti-dsDNA titers in the bm12 inducible model of SLE



Statistical significance was determined for GC B and T_{FH} using an unpaired t-test, and for anti dsDNA concentrations using an uncorrected Fisher's least significant differences test; *p<0.05, **p<0.01, and ****p<0.0001.

(A) BALB/c mice were immunized with SRBC on Day 0 (D0). Half the mice were dosed on D-1 & D7, and half on D14 & D21 with 1.5 mg ALPN-101 or abatacept, or a molar-matched amount of Fc control. The second group was boosted with SRBC on D15 and all mice were euthanized 1 wk after their 2nd dose (on D14 and D28, respectively). Serum anti-SRBC antibodies on D14 and D28 were measured via flow cytometry by detecting binding to SRBC using isotype-specific anti-mouse Ig Abs. p values were determined using a ordinary one-way ANOVA with Tukey's multiple comparisons test.
(B) Spleens were harvested on D14 from the first cohort of mice (treated on D-1 & D7) and analyzed by flow cytometry.
(C) Per a published protocol[6], splenocyte suspensions from female I-A^{bm12}B6(C)-*H2*-$Ab1^{bm12}$/KhEgJ ('bm12') mice were injected IP into an equivalent number (1:1) of 9 wk old female C57BL/6NJ recipient mice on Day 0. H2-$Ab1^{bm12}$ differs from H2-$Ab1^{b}$ by 3 amino acids in the β-chain of the I-A molecule. Alloactivation of donor CD4+ T cells by recipient APC leads to a cGvHD disease with symptoms resembling SLE. Starting on Day 0, recipient mice were treated 2x/wk for 11 wks with 400 μg ALPN-101 or abatacept, or a molar-matched amount (250 μg) of Fc control protein. At study termination on Day 82, GC B cells and T_{FH} cells were enumerated by flow cytometry. Anti-double stranded (ds) DNA IgG titers were measured on D14 using the Mouse Anti-dsDNA IgG Antibody Assay kit from Chondrex (Redmond WA).

Summary and Conclusions

- ALPN-101 (ICOSL vIgD-Fc) is a dual CD28 and ICOS T cell co-stimulation pathway inhibitor targeting both naïve and activated pathogenic T cells, including ICOS+ cells that escape currently available CD28 pathway inhibitors.[7]
- ALPN-101 inhibits cytokine production *in vitro* from human Sjögren's and SLE patient PBMC, more potently than single CD28 or ICOS pathway inhibitors.
- ALPN-101 affects genes involved in B cell differentiation and suppresses proliferation and antibody responses *in vitro* in human B cell-T_{FH} cell co-cultures.
- ALPN-101 suppresses anti-SRBC (and anti-KLH, not shown) antibody responses *in vivo* in normal mice, and reduces autoantibody titers in mouse models of Sjögren's syndrome and SLE.
- ALPN-101 reduces the incidence and severity of sialadenitis and insulitis, and reduces blood glucose levels (Fig. 7 and data not shown), in NOD mice enrolled in the anti PD-L1-induced model of Sjögren's syndrome.
- ALPN-101 is a novel therapeutic candidate for Sjögren's syndrome, SLE, and potentially other connective tissue and/or serious autoimmune diseases. A phase 1 clinical trial with ALPN-101 in healthy volunteers is ongoing (NCT03748836), and trials in inflammatory diseases are planned.

References

1. Panneton et al. (2019) **Inducible T cell Costimulator: Signaling Mechanisms in T Follicular Helper Cells and Beyond**. Immunol Rev. 291:91-103
2. Paulos et al. (2010) **The Inducible Costimulator (ICOS) is Critical for the Development of Human TH17 Cells**. Sci Transl Med. 2(55):55ra78.
3. Weber et al. (2015) **ICOS Maintains the T Follicular Helper Cell Phenotype by Down-Regulating Krüppel-Like Factor 2.** J Exp Med. 212(2):217-33.
4. Evans et al. (2019) **ALPN-101, a First-in-Class Dual ICOS/CD28 Antagonist, Suppresses Key Effector Mechanisms Underlying Rheumatoid and Psoriatic Arthritis.** Poster #1531, American College of Rheumatology Annual Meeting 2019; Atlanta, GA.
5. Zhou et al. (2016) **Endogenous Programmed Death Ligand-1 Restrains the Development of Sjögren's Syndrome in Non-Obese Diabetic Mice.** Sci Rep. 6: 39105.
6. Klarquist & Janssen (2015) **The bm12 Inducible Model of Systemic Lupus Erythematosus (SLE) in C57BL/6 Mice.** J Vis Exp. (105): e53319.
7. Dillon et al. (2019) **ALPN-101, a Dual ICOS/CD28 Antagonist, Demonstrates Potent and Dose-Dependent Suppression of Graft vs. Host Disease (GvHD) in a Human/NSG™ Mouse Xenograft Model, with Activity Superior to CD28 or ICOS Single Pathway Antagonists.** Poster #426, Transplantation & Cellular Therapy Meeting of ASBMT and CIBMTR, February 2019, Houston, TX.

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