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ALPN-101, a First-in-Class Dual ICOS/CD28 Antagonist, Suppresses Key Effector Mechanisms Underlying Rheumatoid and Psoriatic Arthritis

Lawrence S. Evans, Stacey R. Dillon, Katherine E. Lewis, Susan Bort, Erika Rickel, Jing Yang, Martin F. Wolfson, Sherri Mudri, Kayla Susmilch, Steven D. Levin, Sean MacNeil, Mark W. Rixon, Jan L. Hillson, Stanford L. Peng, and Kristine M. Swiderek.
Alpine Immune Sciences, Inc. Seattle, WA

Abstract

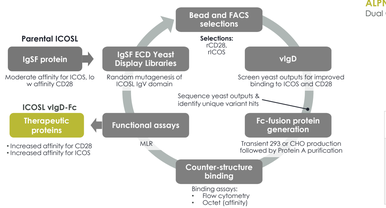
BACKGROUND / PURPOSE: ALPN-101 is an Fc fusion protein of a human inducible T cell costimulator ligand (ICOSL) variant immunoglobulin domain (vIgD™) designed to simultaneously inhibit the CD28 and ICOS costimulatory pathways. CD28 and ICOS each play a role in T cell activation and adaptive immunity which 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 and multiple sclerosis. We report here *in vitro* analyses using PBMC from rheumatoid arthritis (RA) and psoriatic arthritis (PsA) patients and from healthy donors. ALPN-101 demonstrated superior suppression of human T cell activation and potent reduction of inflammatory mediators known to contribute to the pathogenesis of RA, PsA, and juvenile idiopathic arthritis (JIA). Additionally, the efficacy of ALPN-101 was confirmed *in vivo* in a mouse model of collagen-induced arthritis (CIA).

METHODS: Healthy donor, RA, and PsA patient PBMC or Th17-skewed T cell cultures were stimulated with K562 cells expressing CD80, CD86, ICOSL, and anti-CD3 (OKT3) to evaluate the potency of ALPN-101 to suppress pro-inflammatory cytokine production. The activity of dual pathway inhibition by ALPN-101 was compared to the CD28-only inhibitor abatacept (CTLA-4-Fc, Bristol-Myers Squibb via Catalent) and to the ICOS pathway inhibitor prezalumab (AMG-557; anti-ICOSL, Creative Biolabs). ALPN-101 was tested *in vivo* against abatacept in a CIA model in which male DBA/1 mice were immunized with bovine collagen in Freund's adjuvant on Days 0 and 18.

RESULTS: Compared to abatacept, prezalumab, or combination abatacept + prezalumab, ALPN-101 demonstrated superior suppression of pro-inflammatory cytokine (i.e. TNF-α, IFN-γ, IL-2, IL-6, IL-17A, GM-CSF, etc.) release from stimulated healthy and patient PBMCs, and suppressed T cell proliferation in Th17-skewed cultures. The administration of ALPN-101 resulted in significant disease reduction in the mouse CIA model (including decreased paw inflammation, serum cytokines, and anti-collagen antibodies), matching or exceeding the activity of abatacept.

CONCLUSIONS: 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. The data suggest that ALPN-101 may significantly improve upon the clinical efficacy of currently approved therapeutics like abatacept for treatment of inflammatory diseases, including rheumatoid, psoriatic, and juvenile idiopathic arthritis. 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 is an ICOSL variant immunoglobulin domain (vIgD™) engineered for enhanced affinity for CD28 and ICOS



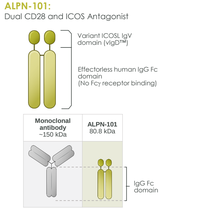
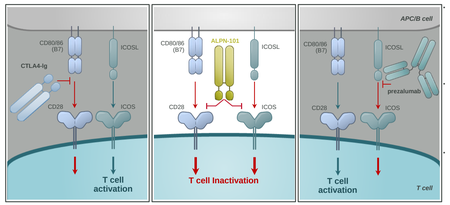


Figure 2: ALPN-101 Blocks Both CD28 and ICOS Pathways



- 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 (Panneton 2019 imm Revs).
- ICOS signaling is crucial for the development of human Th17 cells (Paulos 2010 Sci Transl Med) and function and maintenance of follicular helper T-cells (Tfh, Weber 2015 JEM). ALPN-101 may thus be uniquely suited to treat diseases driven by these pathogenic cell types.

Figure 3: Rationale for ALPN-101 in RA, PsA or TFH Autoimmune Mediated Diseases

Indication	Pathogenic Cell Type	Clinical Effect of CD28 Inhibition	ALPN-101 In Vivo Model Efficacy	Potential Role for ICOS in Disease Pathogenesis
RA	T & B-cells	Abatacept approved (2005)	Yes	↑ PD-1+ ICOS+CXCR5+ CD4+ T cell population in joints and blood that provide B-cell help and correlate with seropositivity (rheumatoid factor or anti-citrullinated peptide) in RA patients (Rao 2017 Nature)
PsA	T_H17	Abatacept approved (2017)	Yes	ICOS – ICOSL critical for the development of T_H17 cells, increased ICOS expression on T_H17 vs T_H1 or T_H2 cells (Paulos 2010 Sci Transl Med)
Sjogren's (SjS)	T & B-cells	Abatacept failed to meet primary endpoints (NCT02915159)	Yes	↑ numbers of circulating ICOS+ T_{FH} cells correlating with ↑ plasmablasts, plasma cells, and autoantibody levels (Brokstad 2018 Scand J Immunol); ↑ numbers of circulating ICOS+ T_{FH} cells correlates with anti-SSA/Ro 60, anti-SSA/Ro 50 ab levels and ESSDAI scores (Fonseca 2018 Arthritis Rheumatol)
Systemic Lupus Erythematosus (SLE)	T & B-cells	Abatacept failed to prevent disease flare (NCT00119678)	Yes	↑ numbers of ICOS+ T_{FH} cells correlating with ↑ circulating plasmablasts, levels of serum anti-dsDNA and anti-nucleosar antibodies (ANA)(Zhang 2015 Lupus); ↑ ICOS+ T_{FH} cells correlate with SLEDAI, circulating plasmablasts, and anti-dsDNA positivity, reflecting active disease (Choi 2015 Arthritis Rheumatol)

Please see also: **Dillon et. al. ALPN-101, a First-In-Class Dual ICOS/CD28 Antagonist, Suppresses Key Effector Mechanisms Associated with Sjögren's Syndrome and Systemic Lupus Erythematosus (Poster #2414; Sjögren's Syndrome – Basic & Clinical Science Poster I, Tuesday, November 12, 2019, 9-11 AM)**

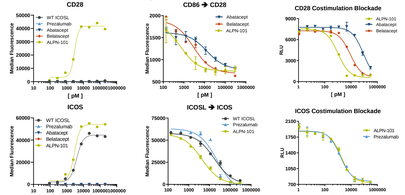
Figure 4: ALPN-101 Binds CD28 and ICOS and Prevents Ligand Binding

A) ALPN-101 was titrated and incubated with CHO cells expressing human CD28 or ICOS. Bound protein was detected with anti-human IgG-PE and measured by flow cytometry.

B) ALPN-101 or comparators were titrated and incubated with mixed fixed amounts of CD86 or ICOSL labeled with AlexaFluor-647. Proteins were added to CHO cells expressing human CD28 or ICOS. After incubation, residual protein washed away and bound Alexa-647 levels measured by flow cytometric analysis. IC50 levels determined using Graphpad Prism and shown below.

Ligand Binding IC50s (nM)

Cell Surface Expressed Target		CD28		ICOS
	Ligand	CD80	CD86	ICOSL
Multiplier	WT ICOSL	-	-	17.2
	Prezalumab	-	-	23.3
	Abatacept	5.4	12.9	-
	Belatacept	2.5	2.8	-
	ALPN-101	1.0	0.7	4.3

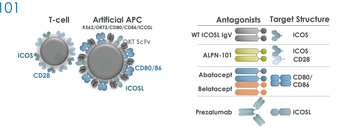


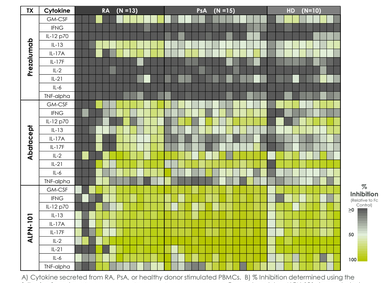
C) CD28 costimulation blockade demonstrated by inhibition of artificial APC (aAPC) expressing OKT3 + human CD86 from stimulating via endogenous CD28 on Jurkat/IL-2 cells expressing luciferase driven from an IL-2 promoter (Promega). ICOS blockade demonstrated by inhibition of aAPC with OKT3 + human ICOSL from stimulating ICOS on Jurkat/IL-2 cells transduced with extracellular domain of human ICOS and intracellular domain of CD28.

Figure 5: Superior Inhibition of Cytokine Secretion from Stimulated Patient or Healthy Donor PBMCs with ALPN-101

Patient or healthy donor peripheral blood mononuclear cells (PBMC) were stimulated with fixed artificial APCs (shown at right) for 48 hours in the presence of ALPN-101 (100nM) or comparators:
- Prezalumab (AMG-557; anti-ICOSL, Creative BioLabs)
- Abatacept (CTLA4-Ig)
- Abatacept + prezalumab

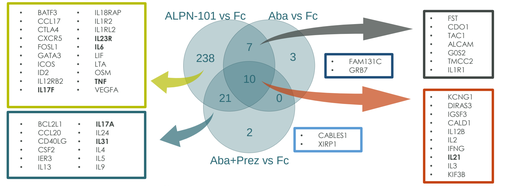
Assay Read-outs:
- Cytokine secretion measured by ELISA or multi-plex analysis (EMD Millipore)
- RNAseq analysis





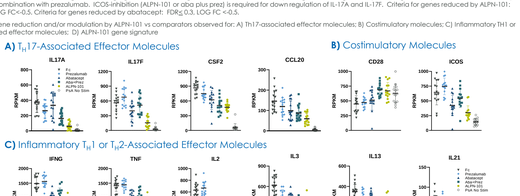
A) Cytokine secreted from RA, PsA, or healthy donor stimulated PBMCs. B) % Inhibition determined using the following formula: ((Fc control value – Exp value)/Fc control value))*100. For most analytes, ALPN-101 demonstrated greater cytokine inhibition than observed with abatacept or prezalumab alone or combined (i.e. IL-17A).

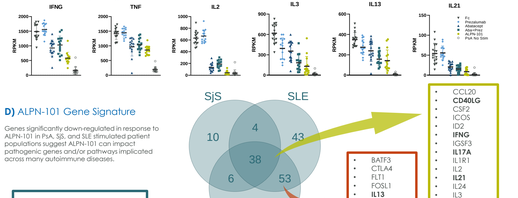
Figure 6: Significant Increase in Downregulated Genes Associated with PsA Disease Pathogenesis by ALPN-101 Relative to Comparators



Key genes related to disease pathogenesis are highlighted in bold. More genes associated with disease pathogenesis are significantly downregulated by ALPN-101 than abatacept alone or in combination with prezalumab. ICOS-inhibition (ALPN-101 or aba plus prez) is required for down-regulation of IL-17A and IL-17F. Criteria for genes reduced by ALPN-101: FDR≤0.05, LOG FC<-0.5. Criteria for genes reduced by abatacept: FDR≤ 0.3, LOG FC <-0.3.

Significant gene reduction and/or modulation by ALPN-101 vs comparators observed for: A) Th17-associated effector molecules; B) Costimulatory molecules; C) Inflammatory TH1 or TH2-associated effector molecules; D) ALPN-101 gene signature

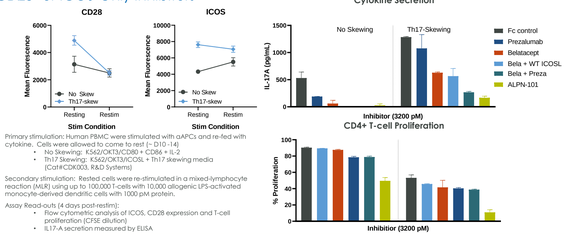




D) ALPN-101 Gene Signature

Genes significantly down-regulated in response to ALPN-101 in PsA, SjS, and SLE stimulated patient population suggest ALPN-101 can impact pathogenic genes and/or pathways implicated across many autoimmune diseases.

Figure 7: ALPN-101 Demonstrates Greater Suppression of Th17-Skewed Cells vs CD28- or ICOS-Only Inhibitors



Primary stimulation: Human PBMC were stimulated with aAPCs and re-fed with cytokine. Cells were allowed to come to rest (~ D10-14):
- No Skewing: K562/OKT3/CD80 + CD86 + IL-2
- Th17 Skewing: K562/OKT3/ICOSL + Th17 skewing media (CytRICDK003, R&D Systems)

Secondary stimulation: Rested cells were re-stimulated in a mixed-lymphocyte reaction (MLR) using up to 100,000 T-cells with 10,000 allogenic LPS-activated monocyte-derived dendritic cells with 1000 pM protein.

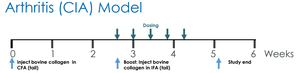
Assay Read-outs (4 days post-restim):
- Flow cytometric analysis of ICOS, CD28 expression and T-cell proliferation (CFSE dilution)
- IL17-A secretion measured by ELISA

Figure 8: ALPN-101 is More Effective than Abatacept in a Semi-Therapeutic Dosing Regimen in a Mouse Collagen-Induced Arthritis (CIA) Model

(A) Study Design

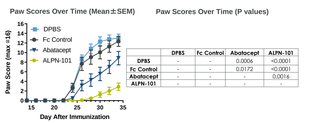
Male DBA/1 mice were immunized with bovine collagen in complete Freund's adjuvant (CFA) on Day 0 and boosted with bovine collagen/incomplete Freund's adjuvant (IFA) on Day 18 (Hooke Laboratories, Lawrence, MA)

Mice were treated intraperitoneally (IP) every 3 days for a total of 5 doses (Q3Dx5) with vehicle (Dulbecco's phosphate buffered saline/DPBS), Fc control, abatacept (Orencia®), or ALPN-101 in a semi-therapeutic regimen, starting before administration of the collagen boost on Day 18.

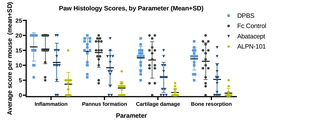


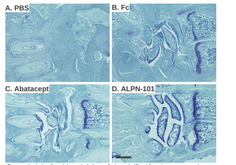
The study was terminated on Day 35. Endpoints include: Disease scores; flow cytometric analysis of popliteal lymph nodes; serum cytokine analysis; anti-collagen IgG analysis

(B) ALPN-101 Significantly Reduces Inflammation and Structural Damage as Measured by Paw Swelling and Histological Analysis



	DPBS	Fc Control	Abatacept	ALPN-101
DPBS	-	-	0.0006	<0.0001
Fc Control	-	-	0.0172	<0.0001
Abatacept	-	-	-	0.00016
ALPN-101	-	-	-	-

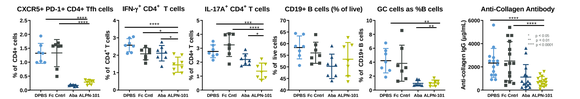




Example toluidine blue staining of decalcified front paws are shown:

- A) Severe diffuse inflammatory infiltrate with severe edema with total or near total destruction of joint architecture, with multifocal chondrocyte loss and full thickness defects in cortical bone with destruction of joint architecture in a PBS-treated animal.
- B) Severe diffuse inflammatory infiltrate with severe edema with extensive destruction of joint architecture, with marked chondrocyte loss and full thickness defects in cortical bone with distortion of the cortical surface in a Fc-treated animal.
- C) Moderate to severe inflammatory infiltrate with marked destruction of joint architecture, minimal to mild disruption of structure and some areas of bone-resorption in an abatacept-treated animal.
- D) Absence of inflammation, pannus, cartilage damage or bone resorption in an ALPN-101-treated animal. Bar, 500 µm.

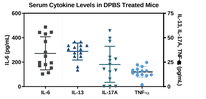
(C) Flow Cytometric Analysis of Popliteal LNs



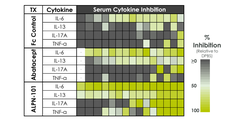
C) Popliteal LNs were harvested and processed into single cell suspensions, surface stained for various markers: T_{FH} cells (CD3, CD4, CXCR5, and PD-1); germinal center (GC) B-cells (CD19, GL-7, CD95). For intracellular cytokine staining, cells were stimulated for 5 hours with PMA/Ionomycin in the presence of GolgiStop and GolgiPlug. Cells were then surface stained for CD3, CD4, fixed, permeabilized and stained for IFN-gamma or IL-17A. D) The concentration of total anti-mouse Type II collagen IgG antibody in the sera of immunized mice was determined by ELISA (Chondrex, Cat#20341).

(D) Anti-Collagen Ab Response

(E) Greater Inhibition of Cytokine in Sera of Mice Treated with ALPN-101



E) Average serum cytokine levels were determined for DPBS-treated mice. % Inhibition of cytokine was determined for individual mice from the Fc control, Abatacept, or ALPN-101-treated mice relative to DPBS mice using the following formula: ((Ave DPBS – Exp Animal)/Ave DPBS))*100



Summary and Conclusions

- ALPN-101 binds both CD28 and ICOS and demonstrates immunosuppressive efficacy superior to CD28 or ICOS costimulatory pathway inhibitors, administered individually or in combination, in human in vitro and/or mouse in vivo translational studies.
- The data suggest that ALPN-101 could significantly and uniquely improve upon the clinical efficacy of currently approved therapeutics like abatacept or IL-17 inhibitors for treatment of inflammatory diseases, including rheumatoid, psoriatic, juvenile idiopathic and other arthritis conditions.
- A Phase 1 clinical trial with ALPN-101 in healthy volunteers is ongoing (NCT03748836), and trials in inflammatory diseases are planned.

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