ALPN-101, A FIRST-IN-CLASS DUAL ICOS/CD28 ANTAGONIST, DEMONSTRATES EFFICACY IN PATIENT-DERIVED PBMC *IN VITRO* AND IN AN *IN VIVO* T CELL TRANSFER MODEL OF CHRONIC INFLAMMATORY BOWEL DISEASE (IBD)

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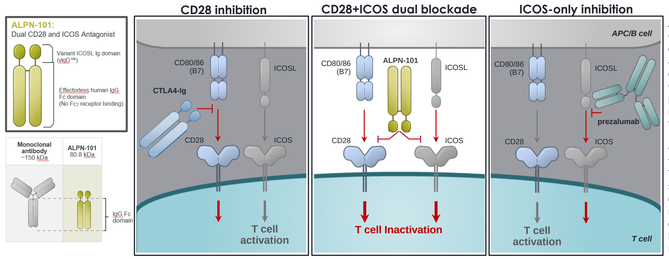
Introduction

- T cell costimulation is strongly implicated in the pathogenesis of IBD, yet CD28 costimulatory pathway inhibitors (e.g. abatacept) have not proven clinically efficacious, implicating an alternative costimulatory pathway.
- CD28 predominates in naïve T cells and is less critical in activated, effector T cells. In contrast, costimulatory receptor ICOS (Inducible T cell Co-Stimulator) is upregulated and mediates costimulation in post-activation T cells - suggesting ICOS may be more relevant in active disease.
- ALPN-101 (ICOSL vIgD-Fc) is an Fc fusion protein of a human inducible T cell costimulator ligand (ICOSL) variant immunoglobulin domain (vIgD™) engineered to inhibit both CD28 and ICOS.
- ALPN-101 has potent *in vitro* immunosuppressive activity and *in vivo* efficacy in models of disease for which both CD28 and ICOS have been implicated (aGvHD, RA, Sjögren's, Lupus, MS).
- Here, we demonstrate potent activity of ALPN-101: (1) *in vitro* using PBMC from Crohn's and ulcerative colitis patients, demonstrating superior suppression of T cell activation and cytokine release, and (2) *in vivo* in a mouse T cell transfer model of chronic colitis, showing its efficacy to both prevent and treat disease.

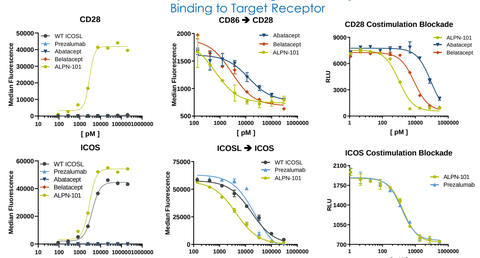
Figure 1: ALPN-101 Blocks Both CD28 & ICOS T Cell Costimulation Pathways



ALPN-101 inhibits both CD28- and ICOS-mediated costimulation. In contrast, abatacept (BMS) inhibits only the CD28/CTLA-4 - CD80/CD86 axis, while prezalumab (anti ICOSL mAb, Amgen) inhibits only the ICOS pathway.

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.

Figure 2: ALPN-101 Binds CD28 and ICOS and Prevents Ligand Binding



D) Ligand Binding IC_{50} (nM)

Cell Surface Expressed Target		CD28		ICOS
	Ligand	CD80	CD86	ICOSL
Inhibitor	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

IC_{50} values calculated using GraphPad Prism

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

B. ALPN-101 or comparators titrated and incubated with fixed amounts of labeled CD86 or ICOSL and added to CHO cells expressing human CD28 or ICOS; binding measured by flow cytometry.

C. CD28 blockade demonstrated by inhibition of artificial APC expressing OKT3 and human CD86 stimulating CD28⁺ Jurkat/IL-2 cells (IL-2 promotor driven luciferase expression; Promega). ICOS blockade demonstrated by inhibition of aAPC expressing OKT3 and human ICOSL stimulating ICOS⁺ Jurkat/IL-2 cells (transduced with a chimeric molecule consisting of the extracellular domain of human ICOS and the intracellular domain of CD28).

Figure 3: ICOS/CD28 Role in Inflammatory Bowel Disease

Indication	Pathogenic Cell Type	Clinical Effect of CD28 Pathway Inhibition	Potential Role for ICOS in Disease Pathogenesis
Crohn's Disease (CD)	T cells	Not efficacious in moderate to severe CD [1]	ICOS expression & CD4⁺ ICOS⁺ lamina propria mononuclear cells (LPMC) elevated in inflamed mucosa compared to non-inflamed or PBMCs [2] ICOS expression on CD4⁺ LPMC directly correlates with endoscopic disease activity scores (CDAI > 150) Blocking ICOS on CD28-deficient T cells in a CD45RB^{hi} adoptive transfer model prevented colitis [3]
Ulcerative Colitis (UC)	T cells	Not efficacious in moderate to severe UC [1]	ICOS expression & CD4⁺ ICOS⁺ LPMC elevated in inflamed mucosa compared to non-inflamed or PBMCs [2] ICOS expression on CD4⁺ LPMC directly correlates with endoscopic disease activity scores (Matts grade >3)
Immune-Related Adverse Event Colitis Checkpoint Inhibitor-Mediated	T cells	*Not Tested*	CD4⁺ ICOS⁺ T cells elevated in lamina propria in checkpoint inhibitor-mediated colitis from patients treated with anti-CTLA-4 and anti-PD-1 [4]
Celiac Disease (CeD)	T cells	*Not Tested*	Several studies identified genetic linkage & association of CeD with the 2q33 locus, a region harboring the genes CD28, CTLA-4, and ICOS (CELIAC3), three important regulators of T cell activity [5,6,7]

[1] Sandborn 2012: Gastroenterology 143; [2] Sato 2004: Gastroenterology 126; [3] de Jong 2004: Int Immunol 16; [4] Coutzac 2017: J Crohns Colitis 10;
[5] Amudsun 2004: Tissue Antigens 64; [6] Gough 2005: Immunological Reviews 204; [7] Haimila 2009: Genes&Immunity 10

ICOS⁺ T cells escape inhibition with CD28 –CD80/CD86 pathway blockers (e. g. abatacept)

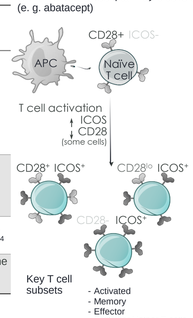
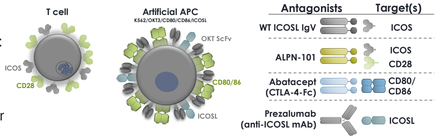


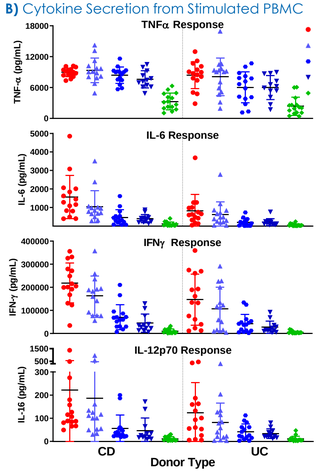
Figure 4: Superior Inhibition of Cytokine Secretion from Stimulated Patient PBMCs with ALPN-101

A) *In Vitro* PBMC stimulation assay

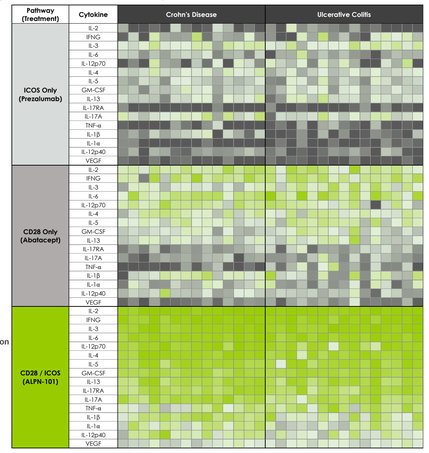
- Crohn's Disease (CD) or Ulcerative Colitis (UC) patient PBMC were stimulated at a 20:1 ratio with artificial APC [fixed K562 expressing cell surface OKT3 (anti-CD3), CD80, CD86, ICOSL]
- Antagonists were added at 100 nM
- After 48 h, supernatants were collected and assayed for cytokine concentrations



B) Cytokine Secretion from Stimulated PBMC



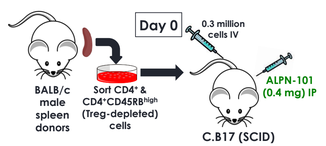
C) Percent Inhibition of Cytokine Secretion Relative to Fc Control

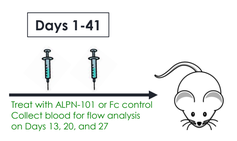


B. Cytokines secreted from stimulated PBMC from Crohn's Disease (CD) or Ulcerative Colitis (UC) patients were analyzed by ELISA or Milliplex® (EMD Millipore)

C. % 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 5: ALPN-101 Treatment in the CD4⁺CD45RB^{high} T Cell-Induced Mouse Model of Colitis





Days 42-43: Study Termination

- Colon weight (mg)/length (mm)
- Colon & small intestine (histology)
- Distal 1/3 of colon cultured to assess cytokine secretion *ex vivo*
- Blood, mesenteric LN for flow analysis
- Serum (PK, cytokines)

Treatment Groups

Cohort	n	Name	Cells injected		Dosing Regimen (IP, 0.1 ml))		
1	12	Vehicle (no colitis) Day 0-41	CD4+	Vehicle	-	2x/week: Day 0 – Day 41
2	16	Fc1.1 Day 0-41		Fc control	260 ug	2x/week: Day 0 – Day 41
3	12	ALPN-101 Day 0-41		ALPN-101	400 ug	2x/week: Day 0 – Day 41
4	12	ALPN-101 Day 0	CD4⁺CD45RB^{high}	ALPN-101	400 ug	1x on Day 0*
5	12	ALPN-101 Day 14		ALPN-101	400 ug	1x on Day 14*
6	12	ALPN-101 Day 14-41		ALPN-101	400 ug	2x/week: Day 14 – Day 41*

Colon Histology Scoring

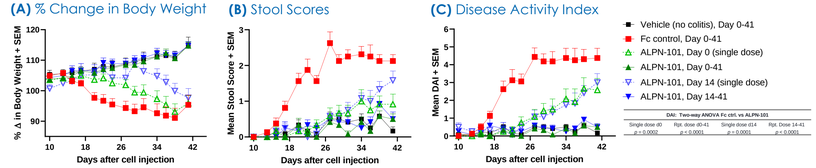
Histology Score		Criteria
Mucosa[1]	0	Normal
	1	3–10 IEL/HPF[2], Focal damage[3]
	2	>10 IEL/HPF, Rare crypt abscesses
	3	>10 IEL/HPF, Multiple crypt abscesses, erosion/ulceration
Submucosa	0	Normal, widely scattered leukocytes
	1	Focal aggregates of leukocytes
	2	Leukocyte infiltration with expansion of submucosa
	3	Diffuse leukocyte infiltration
Muscularis	0	Normal, widely scattered leukocytes
	1	Widely scattered leukocyte aggregates between muscle layers
	2	Leukocyte infiltration with focal effacement of the muscularis
	3	Extensive leukocyte infiltration with transmural effacement of muscularis

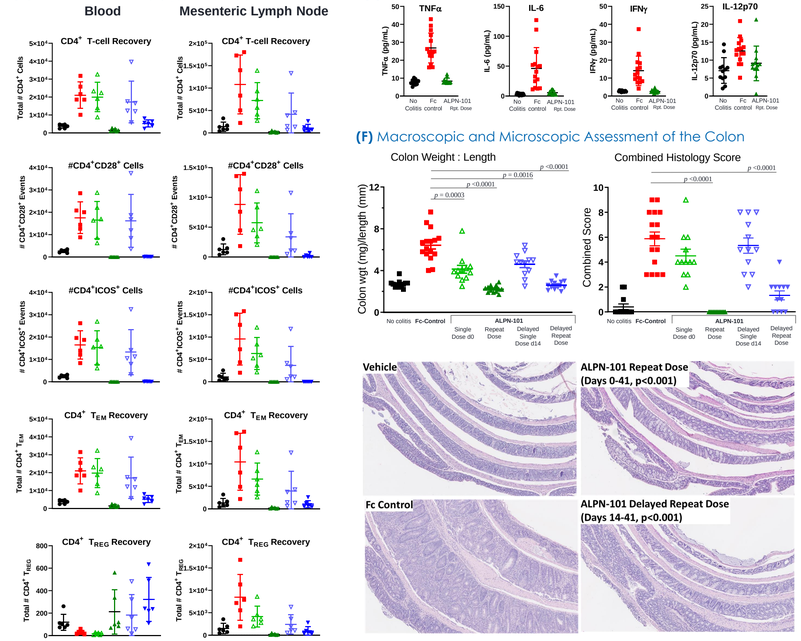
[1] Epithelium and lamina propria
[2] IEL, intraepithelial lymphocytes; HPF, high power field
[3] Immature enterocytes, increased mitotic figures

Scoring System: Disease Activity Index (DAI): Body Weight Score + Stool Score

Score	Clinical observations: Stool	Body Weight / Day 0 weight
0	Stool fairly compact with no signs of mucus. When pressed, keeps shape, does not fall apart	≥ 99%
1	Stool soft, no signs of mucus. When pressed, compacts easily, then breaks apart; OR No stool, but a clear liquid can be seen	[95-99%)
2	Very soft stool, not quite diarrhea; OR Wet pellet is formed, falls apart when pressure applied; OR No stool, but colored mucus visible	[90-95%)
3	Mucus and some diarrhea present. Stool does not hold shape when touched; OR No stool, but some signs of diarrhea	[85-90%)
4	Diarrhea is present; OR No stool, but excessive signs of diarrhea	< 85%

Figure 6: ALPN-101 Significantly Reduces Disease in the CD4⁺CD45RB^{high} T Cell-Induced Mouse Model of Colitis





Efficacy of ALPN-101 in a murine T cell transfer model of colitis (Fig. 5), using various dosing regimens, was evaluated based on the improvement of the disease activity index (A-C), suppression of T cells in blood and mesenteric lymph nodes (D), suppression of pro-inflammatory cytokines in serum (E), and macroscopic and microscopic assessment of the colon post mortem (F).

Summary and Conclusions

- ALPN-101 (ICOSL vIgD-Fc), a novel therapeutic candidate for inflammatory disease, is a dual CD28 and ICOS T cell co-stimulation pathway inhibitor that targets both naïve and activated pathogenic T cells, including ICOS⁺ cells that may escape inhibitors that target only the CD28 pathway
- ALPN-101 inhibits cytokine production *in vitro* from human colitis patient PBMC more potently than single CD28 or ICOS pathway inhibitors
- ALPN-101 demonstrates effector memory T cell and cytokine suppression in mouse *in vivo* translational models of inflammatory bowel disease, and appears to completely prevent development of colitis even with delayed repeat dose administration. Single dose administration at day 0 or day 14 still resulted in milder colitis compared to Fc control.
- A Ph1 healthy volunteer study to evaluate safety and pharmacodynamic activity of single and multiple intravenous and subcutaneous escalating doses of ALPN-101 has recently been completed (NCT03748836). Therapeutic studies in inflammatory diseases, including acute graft-versus-host disease (NCT04227938, BALANCE; Yang 2019), are in preparation.

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