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

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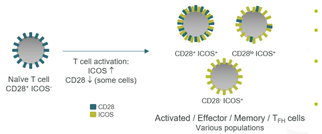
Abstract

BACKGROUND: ALPN-101 (ICOSL vIgD-Fc) is an Fc fusion protein of a human ICOSL variant immunoglobulin domain (vIgD™) designed to simultaneously inhibit the ICOS and CD28 costimulatory pathways. ICOS and CD28 bind ICOSL and CD80/CD86, respectively, and play critical roles in T cell activation and adaptive immunity. ALPN-101 has demonstrated preliminary efficacy in a model of graft versus host disease (GvHD) (2018 BMT abstract #244). Here we examined its efficacy, exposure, and pharmacodynamics in preparation for upcoming clinical studies.

RESULTS: ALPN-101 inhibited T cell activation in the GvHD model with all dose regimens tested (Fig 1). Significantly enhanced survival and reduced disease scores were observed in mice treated with ALPN-101 compared to mice treated with belatacept (i.e. 100% vs. 40% survival at Day 42, respectively; $p < 0.01$ by Mantel-Cox log rank test). Notably, single dose (100 µg) administration of ALPN-101 resulted in similar protection from disease as repeat dosing of 100 µg belatacept. The observed serum exposure of ALPN-101 in the GvHD model was 45% lower than that of normal mice. The trough concentrations of ALPN-101 were 60-80% lower than those of belatacept at the same dose level. Flow cytometric analysis of blood and serum cytokine analyses at end of study demonstrated ALPN-101 suppressed activation and expansion of transferred human T cells and cytokine production, and no acute cytokine release was observed at any point in the study. In contrast, serum inflammatory cytokines and activated human T cells expressing ICOS were readily detectable in the belatacept-treated mice. While most of the transferred human T cells initially expressed CD28 and just ~15% were ICOS+, the activated T cells remaining in the saline- or belatacept-treated mice at termination/end of study were >80% ICOS+ (Fig 2).

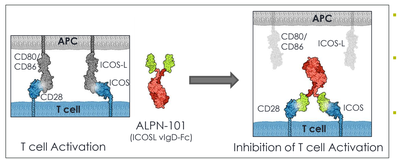
CONCLUSION: ALPN-101 is a potent dual ICOS/CD28 T cell antagonist capable–even with a single dose–of inhibiting lethal inflammatory processes with superior efficacy vs continuous CD28 or ICOS single pathway inhibition despite lower PK exposure, likely attributable to superior control of ICOS+ T cells which otherwise escape single pathway blockade. ALPN-101 is thus a promising novel therapeutic candidate for GvHD, and upcoming clinical trials will explore its therapeutic potential in GvHD and other inflammatory diseases.

Figure 1: Biological Rationale for Coinhibition of CD28 and ICOS



- Naïve T cells generally express CD28 but little to no ICOS
- Following antigen encounter, most activated T cells become CD28+ ICOS+
- A subset of activated T cells downregulate CD28 and become CD28^{low} ICOS^{+} or CD28^{-} ICOS^{+}, accounting for up to half the total circulating T cell population in the elderly
- CD28^{-} ICOS^{+} cells are resistant to CTLA-4-Fc therapy and appear to play important roles in inflammatory and autoimmune diseases[1]

Figure 2: ALPN-101, a Dual ICOS/CD28 Antagonist



- Extracellular subdomain of native human ICOS ligand (ICOSL) was evolved with vIgD platform yielding ICOSL vIgD
- ICOSL vIgD binds both ICOS and CD28 with high affinity, and blocks their interaction with ICOSL and CD80/CD86, respectively
- The ICOSL vIgD (*green*) was fused to an effector function negative Fc (*red*) to create the homodimeric Fc-fusion protein therapeutic candidate ALPN-101

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2. Sato et al. **ICOS up-regulation on activated T cells in chronic graft-versus-host disease after dog leukocyte antigen-nonidentical hematopoietic cell transplantation: a potential therapeutic target**. Transplantation. 2013 Jul 15;96(1):34-41.
3. Adom et al. **ICOSL+ Plasmacytoid Dendritic Cells as Biomarker and Inducer of GvHD.** Oral presentation #355, Session 722; American Society of Hematology (ASH) Conference; 2018; San Diego, CA.
4. Dillon et al. **A Dual ICOS/CD28 Antagonist ICOSL Variant Ig Domain (vIgD™) Potently Suppresses Human Mixed Lymphocyte Reactions and Human/NSG™ Mouse Xenograft Graft vs. Host Disease (GvHD).** Poster, BMT/Tandem; 2018; Salt Lake City, UT.

Acknowledgements

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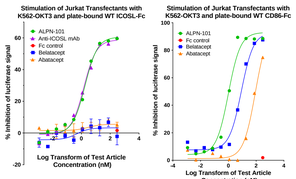
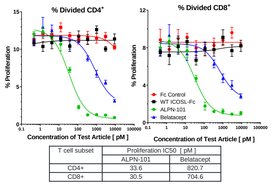
Figure 3: ALPN-101 Inhibits Both CD28 and ICOS Pathways *In Vitro*

A. Jurkat cells transfected with chimeric ICOS/CD28



B. Human Mixed Lymphocyte Reaction (MLR)



T cell subset	Proliferation IC50 [pM]	
	ALPN-101	Belatacept
CD4+	33.6	820.7
CD8+	30.5	704.6

A) Human Jurkat T cells expressing endogenous CD28, an IL-2-luciferase reporter gene, and a transfected chimeric ICOS/CD28 molecule (i.e. the ICOS extracellular domain fused to the intracellular tail of CD28) were stimulated with plate-bound WT ICOSL-Fc or WT CD86-Fc in the presence or absence of titrated amounts (4-fold dilutions from 0.001-300 nM) of ALPN-101, belatacept/abatacept, anti-ICOSL mAb, or Fc control. The % inhibition of the luciferase signal in the presence of various concentrations of the test articles [defined as (1 – experimental value/mean value of Fc control wells) x 100] is plotted vs. the log transform of test article concentration (nM). B) Proliferation in the human MLR was determined by quantitating the percentage of CFSE-labeled cells diluting CFSE over time. Effect of ALPN-101 and controls on the % proliferation of CD4+ vs. CD8+ T cells is shown.

Figure 4: ALPN-101 Potently Protects Mice from Disease in the Human PBMC-NSG™ GvHD Model

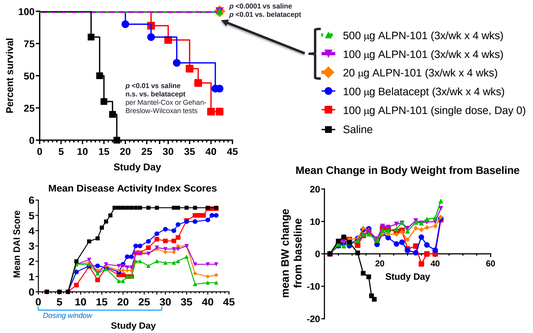
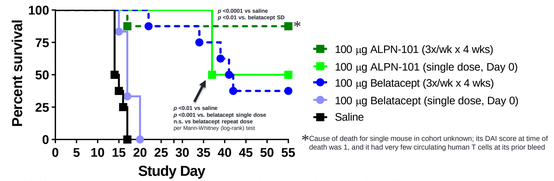


Figure 5: Single Dose ALPN-101 Protects Mice from GvHD as Effectively as Repeat Dose Belatacept, While Single Dose Belatacept Provides No Protection

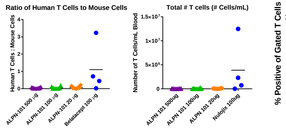


*Cause of death for single mouse in cohort unknown; its DAI score at time of death was 1, and it had very few circulating human T cells at its prior bleed

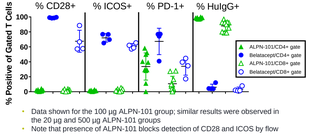
Human PBMC→NSG™ GvHD Model: On Day –1, female NOD.Cg-Prkdc<scid> Il2rg<tm1Wjl>/SzJ (NSG™) mice (6-10/group) were irradiated (100 rad) & administered 10 mg human gamma globulin SC. On Day 0, mice received 10x10^6 human PBMC, IV. For the repeat dose groups, IP dosing began on Day 0 and continued 3x weekly (M, W, F) through Day 28. The studies were terminated on Day 42 (**Figure 4**) or Day 55 (**Figure 5**), blood was collected from the surviving mice in the indicated groups, and engrafted human CD45+ cells were characterized by flow cytometry.

Figure 6: ALPN-101 Suppresses Activated T Cell Expansion & Inflammatory Cytokine Production in the Human PBMC-NSG™ GvHD Model (Fig. 4 study)

A. T Cells in Terminal Blood (Flow Cytometry)

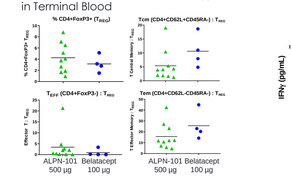


B. Activated and Drug-Bound T Cells in Terminal Blood



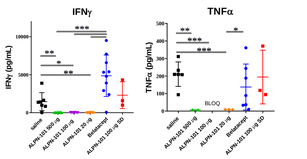
- Data shown for the 100 µg ALPN-101 group; similar results were observed in the 20 µg and 500 µg ALPN-101 groups
- Note that presence of ALPN-101 blocks detection of CD28 and ICOS by flow

C. Effector (T_{EFF}) and Regulatory T (T_{REG}) Cells in Terminal Blood



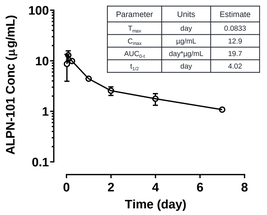
- Similar ratios were observed in the 20 µg and 100 µg ALPN-101 groups

D. Serum Cytokines (Terminal Bleeds)

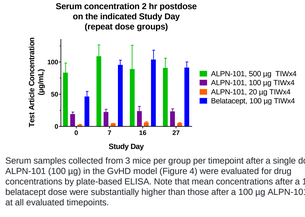


- Other cytokines similarly inhibited by ALPN-101 treatment included: IL-5, IL-10, IL-12p70, IL-13, & IL-17A.
- BLOQ = below lower limit of quantitation. SD = single dose.
- * p<0.05; ** p<0.01; ***p<0.001 by unpaired t-test with Gaussian distribution.

Figure 7: Pharmacokinetics of ALPN-101 in the HuPBMC-NSG™ Model (Fig. 4 study)



Parameter	Units	Estimate
T_{max}	day	0.0833
C_{max}	µg/mL	12.9
AUC_{0-t}	day*µg/mL	19.7
$t_{1/2}$	day	4.02



Serum samples collected from 3 mice per group per timepoint after a single dose of ALPN-101 (100 µg) in the GvHD model (Figure 4) were evaluated for drug concentrations by plate-based ELISA. Note that mean concentrations after a 100 µg belatacept dose were substantially higher than those after a 100 µg ALPN-101 dose at all evaluated timepoints.

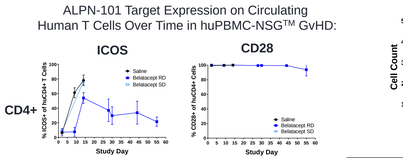
Summary and Conclusions

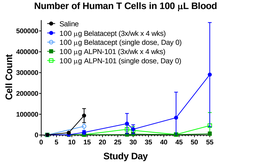
Both CD28 and ICOS costimulatory pathways appear to promote GvHD[2,3]. Currently approved biologic therapies are limited to CD28-only inhibition, permitting the escape of activated ICOS+ T cells. ALPN-101 (ICOSL vIgD-Fc) is a dual CD28 and ICOS T cell costimulation pathway inhibitor and thus can target ICOS+ cells that escape CD28-only blockade.

- ALPN-101 consistently demonstrates superior inhibition of T cell proliferation and cytokine production[4] in MLRs compared to single pathway blockade.
- Repeat dosing with ALPN-101 can completely protect mice from disease in a humanized GvHD model and, impressively, a single equimolar dose of ALPN-101 provides similar protection as repeat doses of belatacept, while a single dose of belatacept confers no protection from disease.
- Following a single 100 µg IP dose of ALPN-101 in the GvHD model, Tmax was observed at 2 hr post dose, and the serum half-life was ~4 days. Mean concentrations after a 100 µg belatacept dose were substantially higher than after a 100 µg ALPN-101 dose at all evaluated timepoints, consistent with superior potency and pharmacodynamic effects of ALPN-101.
- ALPN-101 potently inhibits inflammatory cytokine secretion and effector T cell expansion in the GvHD model.
- The huPBMC-NSG GvHD model reproduces the increase of ICOS expression on activated human T cells in GvHD patients over time, and the increase of circulating ICOS+ T cells in the model correlates with disease activity, while there is no correlation with CD28+ or PD-1+ expression. Thus, one mechanism of action of ALPN-101 may be to inhibit the emergence of activated ICOS+ pathogenic T cells that escape CD28 single pathway blockade.

Dual antagonism of ICOS and CD28 via ALPN-101 may be a particularly effective therapeutic and/or preventative treatment for GvHD and other acute and chronic T cell-mediated inflammatory diseases. A clinical trial with ALPN-101 in GvHD is being planned.

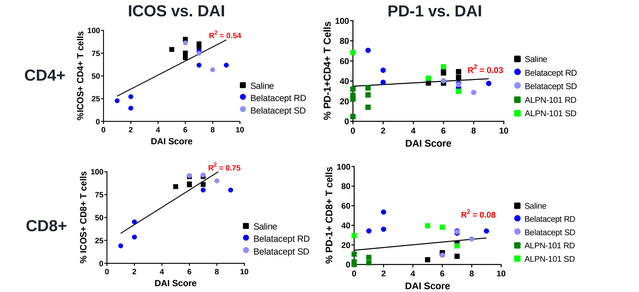
Figure 8: Upregulation of ICOS, but not PD-1, on Activated CD4+ and CD8+ T Cells Escaping Suppression by Belatacept Correlates with Disease Severity (Fig. 5 study)





Marker	T Cell Subset	Correlation Coefficient (R^2 Value) for % Marker Expression vs. Each Endpoint: DAI	% Change in BW	Survival Day
ICOS	CD4+	0.54	-0.68	-0.83
	CD8+	0.75	-0.78	-0.76
CD28	CD4+	0.38	-0.39	-0.29
	CD8+	0.34	-0.50	-0.74
PD-1	CD4+	0.03	-0.05	-0.01
	CD8+	0.08	-0.03	0.02

Negative R^2 values denote an inverse correlation



Terminal blood from each mouse treated as indicated with saline or single dose (SD) or repeat dose (RD) belatacept or ALPN-101 (from GvHD study shown in **Figure 5**) was collected and evaluated by flow cytometry for ICOS, CD28, and PD-1 expression on CD4+ and CD8+ human T cells. Linear regression curves are shown for %ICOS+ or PD-1+ of CD4+ or CD8+ T cells vs. terminal DAI score. Correlation coefficients (R^2) are indicated in red font for each dataset. Additional correlation analyses for % marker expression vs DAI, % change in body weight, and survival day are summarized in the table above the graphs. Note that ALPN-101 blocks the binding of anti-ICOS and anti-CD28 flow mAbs, so data from the ALPN-101 groups were omitted from the ICOS and CD28 correlation analyses.

Disclosures: All authors are employees or contractors at Alpine Immune Sciences, Inc.

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