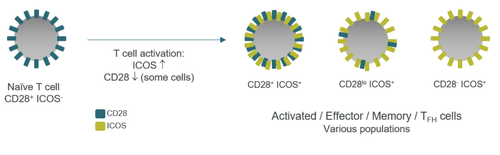
# Therapeutic Candidate ALPN-101, a Dual ICOS/CD28 Antagonist, Potently Suppresses Human/NSG Mouse Xenograft Graft vs. Host Disease (GvHD) in a Dose Ranging Study and Reduces Disease Activity in a Mouse Model of Hemophagocytic Lymphohistiocytosis (HLH)

Stacey R. Dillon[1], Katherine E. Lewis[1], Katherine Verbist[2], Paige Tedrick[2], Sabrin Albeituni[2], Ryan Swanson[1], Lawrence S. Evans[1], Steven D. Levin[1], Rebecca Wu[1], Sherri Mudri[1], Erika Rickel[1], Mark W. Rixon[1], Stanford L. Peng[1], Kim E. Nichols[2], and Kristine M. Swiderek[1]
[1]Alpine Immune Sciences, Inc. Seattle, WA and [2]St. Jude Children's Research Hospital, Department of Oncology, Memphis, Tennessee, USA

## Abstract

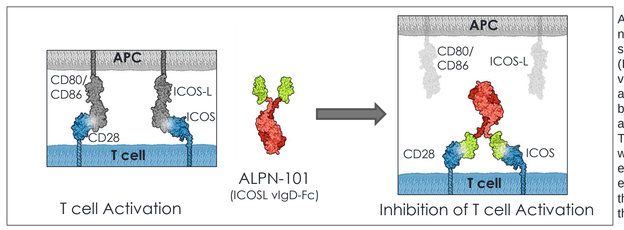
**Background/Purpose:** ALPN-101 is a potent dual inhibitor of the ICOS and CD28 T cell costimulatory pathways designed for therapeutic application in inflammatory diseases. CD28 and ICOS bind CD80/CD86 and ICOS ligand (ICOSL), respectively, and play critical roles in T cell activation and adaptive immunity. ALPN-101 has previously been demonstrated to have potent efficacy – superior to wild type ICOSL-Fc – in models of graft versus host disease (GvHD), a disease reflecting immune-mediated attack of recipient tissue by donor T cells. Here, we examined the efficacy of a single dose of ALPN-101 or repeat dosing with different dose levels in GvHD. We also explored the potential therapeutic benefit of ALPN-101 in another T cell-driven inflammatory disease, hemophagocytic lymphohistiocytosis (HLH), a spectrum of disorders of the immune system characterized by the excessive production of cytokines by activated T cells and macrophages accumulating in organs such as the liver, spleen, bone marrow, and brain, which mediate significant tissue damage.
**Results:** ALPN-101 significantly attenuated T cell activation in the human PBMC-NSG GvHD model at a single 100ug dose and at all multiple doses tested, protecting mice from the effects of xenogeneic T cell activation *in vivo*. Treated animals exhibited enhanced survival and reduced disease scores compared to control mice treated with saline or belatacept. Flow cytometric analyses of blood collected at 1-2 weeks post cell transfer demonstrated ALPN-101 reduced both the number and activation state of the transferred human CD4+ and CD8+ T cells. In the HLH model, ALPN-101 lessened several of the clinical and laboratory manifestations of HLH, including organomegaly, anemia, CD8+ T cell expansion, and liver inflammation.
**Conclusion:** ALPN-101 is a potent T cell inhibitor capable, even with a single dose, of preventing T cell activation, such as that observed in the huPBMC-NSG™ GvHD and the LCMV-induced HLH models, and thus is a promising novel therapeutic candidate for GvHD and other inflammatory diseases. Preclinical development is underway to support clinical studies of this potentially first-in-class dual ICOS and CD28 inhibitor.

## 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 various diseases, e.g. cancer, autoimmunity, and atherosclerosis[1]

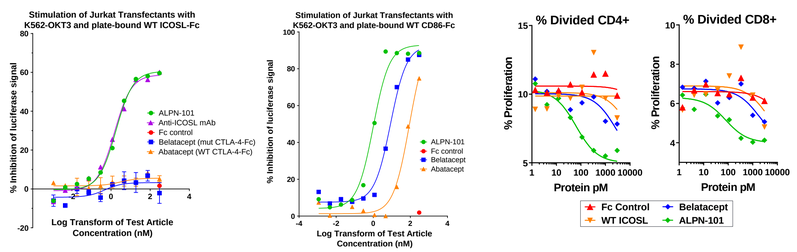
## Figure 2: ALPN-101 (ICOSL vIgD-Fc), a Dual ICOS/CD28 Antagonist




An extracellular subdomain of the naturally occurring human Ig superfamily member ICOS ligand (ICOSL) was evolved on the vIgD™ platform to bind both ICOS and CD28 with high affinity, and block their interaction with ICOSL and CD80/CD86, respectively. The variant ICOSL vIgD (*green*) was fused to an Fc (*red*) engineered to reduce Fc-mediated effector function activity to create the homodimeric Fc-fusion protein therapeutic candidate ALPN-101.

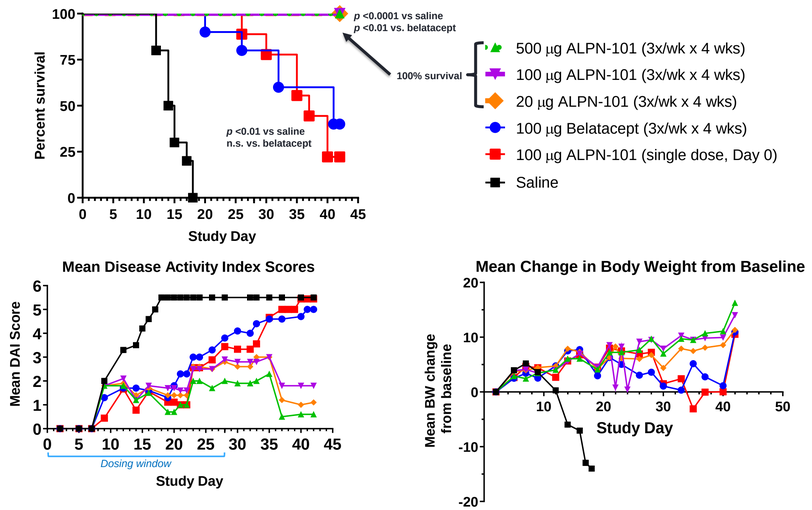
## Figure 3: ALPN-101 Inhibits Both CD28 and ICOS Pathways *In Vitro*

A. Jurkat cells transfected with chimeric ICOS/CD28 B. Human Mixed Lymphocyte Reaction (MLR)



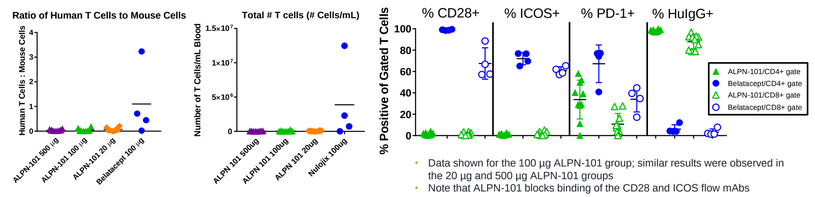

A) Human Jurkat T cells expressing endogenous CD28, an IL-2-luciferase reporter gene, and a transfected chimeric ICOS/CD28 molecule (i.e. the ICOS extracellular domain fused to the intracellular tail of CD28) were stimulated with plate-bound WT ICOSL-Fc or WT CD86-Fc in the presence or absence of titrated 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, Even After a Single Dose



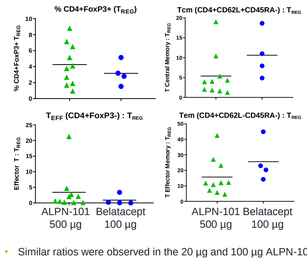

## Figure 5: ALPN-101 Suppresses Activated T cell Expansion & Inflammatory Cytokine Production in the Human PBMC-NSG™ GvHD Model

A. T Cells in Terminal Blood (Flow Cytometry) B. Activated and Drug-Bound T Cells in Terminal Blood



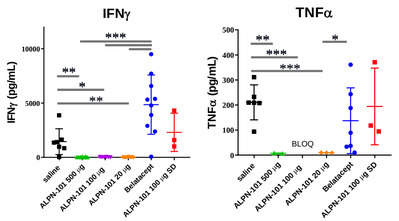

- Data shown for the 100 μg ALPN-101 group; similar results were observed in the 20 μg and 500 μg ALPN-101 groups
- Note that ALPN-101 blocks binding of the CD28 and ICOS flow mAbs

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)

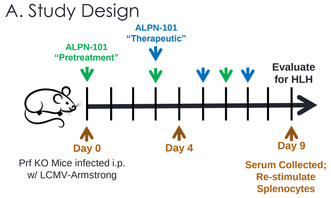



- Other cytokines similarly inhibited by ALPN-101 treatment included: IL-5, IL-10, IL-12p70, IL-13, & IL-17A.
- BLQ = below lower limit of quantitation. SD = single dose.
- * p<0.05; ** p<0.01; ***p<0.001 by unpaired t-test with Gaussian distribution.

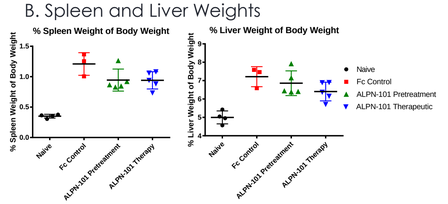
**Human PBMC→NSG™ GvHD Model:** On Day –1, female NOD.Cg-Prkdc<scid> Il2rg<tm1Wjl>/SzJ (NSG™) mice (10/group) were irradiated (100 rad) & administered 10 mg human gamma globulin SC. On Day 0, mice received 10x10$^6$ human PBMC, IV. For the repeat dose groups, IP dosing began on Day 0 and continued 3x weekly (M, W, F) through Day 28 with saline, or with 20, 100, or 500 μg ALPN-101 or 100 μg of belatacept (molar equivalent to 100 μg ALPN-101). The study was terminated on Day 42, blood was collected from the surviving mice in the repeat dose groups, and engrafted human CD45+ cells were detected by flow cytometry. ALPN-101 significantly protected the mice from death, per the log-rank (Mantel-Cox) or Gehan-Breslow-Wilcoxon tests for significance, as indicated in Figure 4.

## Figure 6: ALPN-101 Reduces CD4+ T Cell Activation and Liver Inflammation in the Perforin (Prf) Knockout (KO)/LCMV Mouse Model of Hemophagocytic Lymphohistiocytosis (HLH)

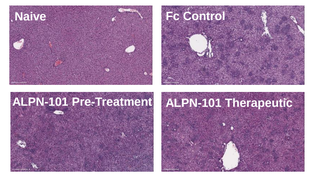
A. Study Design



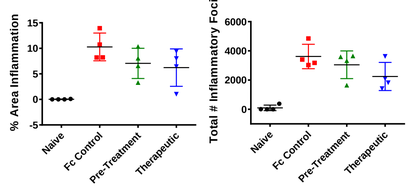

B. Spleen and Liver Weights



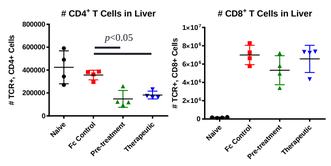

C. Liver Histology (H&E)



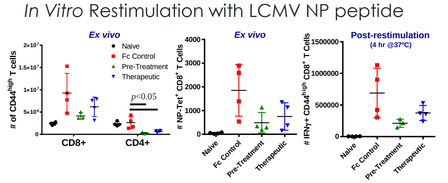

D. Liver Inflammation




E. Number of T Cells in Liver




F. Activated T Cells in Spleen *Ex Vivo* & After *In Vitro* Restimulation with LCMV NP peptide




## Summary and Conclusions

- ALPN-101 (ICOSL vIgD-Fc) potently inhibits both the CD28 and ICOS T cell costimulation pathways *in vitro*, consistently demonstrating superior inhibition of T cell proliferation and cytokine production[2] in MLRs.
- Repeat dosing with ALPN-101 completely protects mice from disease in a humanized GvHD model and a single dose provides similar protection to repeat doses of belatacept, at least in part by preventing the emergence of activated (ICOS+/PD-1+) T cells that escape CD28 single pathway inhibition.
- ALPN-101 inhibits the expansion of both effector and regulatory T cells, while not significantly perturbing the $T_{EFF}$:$T_{REG}$ balance, and potently inhibits inflammatory cytokine production.
- ALPN-101 reduces CD4+ T cell activation and liver inflammation in a mouse model of HLH primarily driven by CD8+ T cells. In this model, treatment with ALPN-101 inhibits the generation of CD44$^{high}$ activated T cells without significantly affecting the LCMV-specific CD8+ T cell response.
- Dual antagonism of ICOS and CD28 may be an effective therapeutic approach in T cell-mediated inflammatory diseases. Clinical trials with ALPN-101 are expected to begin in early 2019.

## References

1. Betjes, M.G.H. **Clinical Consequences of Circulating CD28-Negative T Cells for Solid Organ Transplantation.** Transplant International 29:274-84 (2016).
2. Dillon, Lewis, Swanson, Evans, 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 presented at BMT/Tandem; 2018 Feb 21-25; Salt Lake City, UT.
3. Adom, Ramadan, Kushekhar, Lewis, Peng, Dillon, and Paczesny. **ICOSL+ Plasmacytoid Dendritic Cells as Biomarker and Inducer of GvHD.** Oral presentation #355, Session 722; **ASH 2018, Sunday, Dec 2 (9:30 am)**; San Diego, CA.

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