# ALPN-101, a Dual ICOS/CD28 Antagonist, Potently Suppresses Disease in Multiple Mouse Models of Autoimmunity

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## Abstract

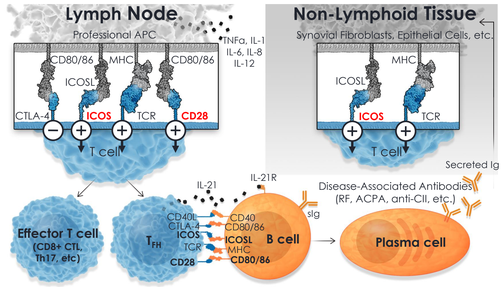
**BACKGROUND:** CD28 and Inducible T-cell Costimulator (ICOS) are two related costimulatory molecules within the immunoglobulin superfamily (IgSF) expressed on T cells and interacting with CD80/CD86 and ICOS ligand (ICOSL), respectively. Both play critical roles in T cell activation and adaptive immunity, but when dysregulated can contribute to autoimmunity. We used our proprietary platform to create a variant Ig domain (vIgD™), an engineered version of ICOSL capable of binding both ICOS and CD28 and blocking the interaction of these costimulatory molecules with their respective receptors. This ICOSL vIgD was fused to a human Fc lacking effector function (i.e. FcR binding and complement fixation) to create the therapeutic candidate ALPN-101, which has previously been shown to have potent immunosuppressive activity *in vitro*. We report here *in vivo* activity data in mouse models of autoimmune disease supporting the potent immunosuppressive activity of ALPN-101.

**METHODS:** ALPN-101 was evaluated for immunosuppressive activity in multiple mouse models, including the collagen-induced arthritis (CIA) model with either prophylactic or therapeutic dosing. ALPN-101 was dosed a maximum of 4 times either prior to or just after disease onset. Comparator molecules were administered at molar equivalent doses in regimens matching ALPN-101.

**RESULTS:** ALPN-101, when given either prophylactically or therapeutically, significantly attenuated disease activity in the collagen-induced arthritis model. ALPN-101 mediated significant disease reduction in CIA, matching or exceeding CD28-only or ICOS-only inhibitors. Similar effects were observed in additional disease models.

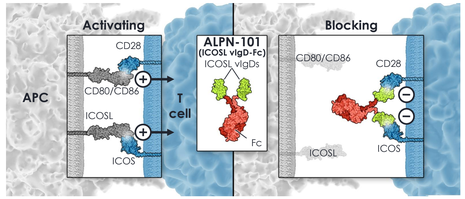
**CONCLUSION:** Efficacy *in vivo* of ALPN-101 is superior to wild-type ICOSL domains or CD28-only inhibitors. The increased efficacy of ALPN-101 was made possible by engineering the wild-type ICOSL IgSF to create a vIgD with altered affinity between ICOSL and ICOS and through specifically-directed alterations in ICOSL/CD28 binding. Preclinical development of ALPN-101 is underway to support clinical studies of this potentially first-in-class dual ICOS and CD28 inhibitor.

## Figure 1: CD28 and ICOS Mediate T Cell Costimulation

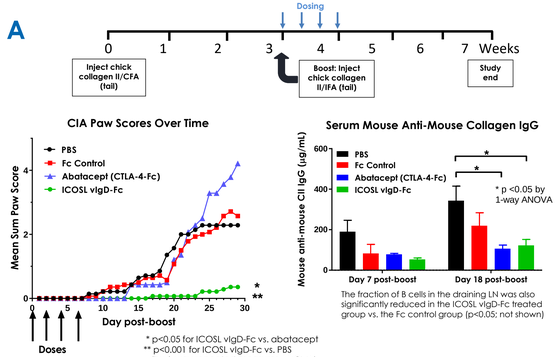



T cells express the costimulatory molecules CD28 and ICOS, which interact with CD80/CD86 and ICOS-L respectively, on antigen presenting cells (APC). In lymphoid organs, professional APC (i.e. dendritic cells, macrophages, and B cells) express CD80, CD86, and ICOSL and engage CD28+/ICOS+ T cells. Activated T cells can then differentiate into effector cells such as CD8+ cytotoxic T cells (CTL), IL-17A/F-secreting CD4+ Th17 cells, or CD4+ follicular helper ($T_{FH}$) cells. $T_{FH}$ expressing CD40L engage B cells in lymphoid follicles and release cytokines (e.g. IL-21) inducing differentiation of B cells to antibody (Ab)-secreting plasma cells. Plasma cells can produce tissue-damaging Abs like rheumatoid factor (RF) and anti–citrullinated peptide antibodies (ACPA) in humans, and anti-collagen (CII) Abs in mice, forming immune complexes which can be deposited in the joints and other tissues. ICOSL can also be expressed on non-professional APCs, leading to T cell activation in non-lymphoid tissues and further damage to the tissues and joints.

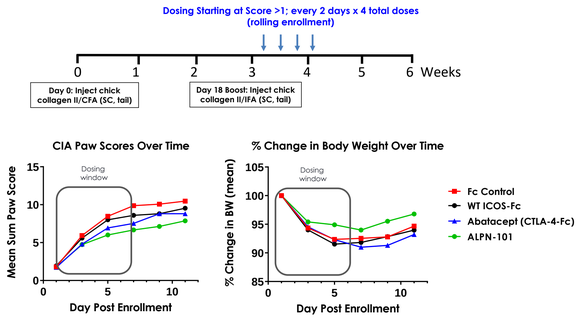
## Figure 2: ALPN-101 (ICOSL vIgD-Fc), a Dual ICOS/CD28 Antagonist



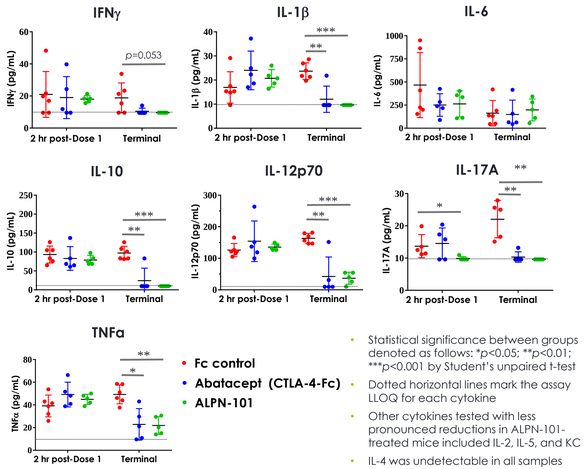

## Figure 3: ICOSL vIgD-Fc is More Effective than Abatacept in the CIA Mouse Model of RA When Dosed Prophylactically




## Figure 4: Delayed Dosing with ALPN-101 Suppresses Disease in a Therapeutic CIA Model

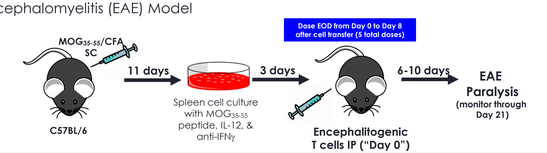



## Figure 5: ALPN-101 Suppresses Serum Cytokine Production in a Therapeutic CIA Model

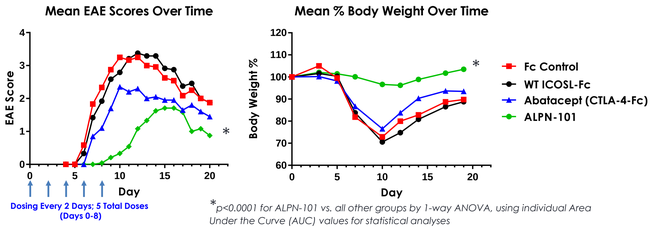



- Statistical significance between groups denoted as follows: *p<0.05; **p<0.01; ***p<0.001 by Student's unpaired t-test
- Dotted horizontal lines mark the assay LLOQ for each cytokine
- Other cytokines tested with less pronounced reductions in ALPN-101-treated mice included IL-2, IL-5, and KC
- IL-4 was undetectable in all samples

## Figure 6: ALPN-101 Inhibits Disease in an Adoptive Transfer Experimental Autoimmune Encephalomyelitis (EAE) Model




| Score | Clinical observations |
|---|---|
| 0 | No obvious changes in motor functions |
| 1 | Limp tail |
| 2 | Limp tail and weakness of hind legs |
| 3 | Limp tail and complete paralysis of hind legs. OR Limp tail with paralysis of one front and one hind leg, OR ALL of: 1) Severe head tilting, 2) Walking only along the edges of the cage, 3) Pushing against the cage wall, 4) Spinning when picked up by the tail. |
| 4 | Limp tail, complete hind leg and partial front leg paralysis |
| 5 | Complete hind and complete front leg paralysis, no movement; OR Mouse is spontaneously rolling in the cage; OR Mouse found dead due to paralysis. |




*p<0.0001 for ALPN-101 vs. all other groups by 1-way ANOVA, using individual Area Under the Curve (AUC) values for statistical analyses

## Summary and Conclusions

- ALPN-101 is a dual ICOS/CD28 antagonist engineered to inhibit the CD28 and ICOS T cell costimulatory pathways and comprised of a variant immunoglobulin domain (vIgD) of the human inducible T cell costimulator ligand (ICOSL) formatted as an Fc fusion protein.
- ALPN-101 potently inhibits human and mouse T cells responses *in vitro*, and demonstrates superior efficacy to CD28- or ICOS-only pathway blockade *in vivo* across multiple acute and chronic inflammatory disease models, including delayed type hypersensitivity (ref. 1), humanized GvHD (ref. 1), collagen-induced arthritis (Figs 3-5), EAE (Fig 6, ref. 2), and others.
- ALPN-101 inhibits multiple indicators of disease in the collagen-induced arthritis mouse model, including reducing paw swelling and inflammatory cell infiltrates, lowering serum levels of inflammatory cytokines and anti-collagen autoantibodies, and decreasing $T_{FH}$ cells, B cells, & activated T cells in the paw-draining lymph nodes.
- Our data corroborate previous evidence that the CD28 and ICOS pathways play important roles in inflammatory arthritis, but suggest that their redundancy may explain the only partial benefit of single pathway blockade (ref. 3).
- Dual antagonism of ICOS and CD28 may therefore be an effective therapeutic approach in inflammatory disease, including multiple forms of arthritis.
- Clinical trials with ALPN-101 are expected to begin shortly.

## References

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(2) Dillon SR, Lewis KE, Swanson R, Evans LS, Komacker MG, Levin SD, Wolfson MF, Rickel E, Bort SJ, Mudri S, Moss AM, Seaberg MA, Bhandari J, MacNeil S., Hoover J, Rixon MW, and SL Peng. **Therapeutic Candidate ALPN-101, a Dual ICOS/CD28 Antagonist, Demonstrates *In Vivo* Efficacy in an Experimental Autoimmune Encephalomyelitis (EAE) Model.** Poster presented at American Neurological Association Annual Meeting: 2018 Oct 21-23; Atlanta, GA.

(3) Panneton V, Bagherzadeh Yazdchi S, Witalis M, Chang J, and Suh WK. (2018). **ICOS Signaling Controls Induction and Maintenance of Collagen-Induced Arthritis.** *J Immunol.* 200(9): 3067-3076.

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