# Therapeutic Candidate ALPN-101, a Dual ICOS/CD28 Antagonist, Demonstrates *In Vivo* Efficacy in an Experimental Autoimmune Encephalomyelitis (EAE) Model

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## Abstract

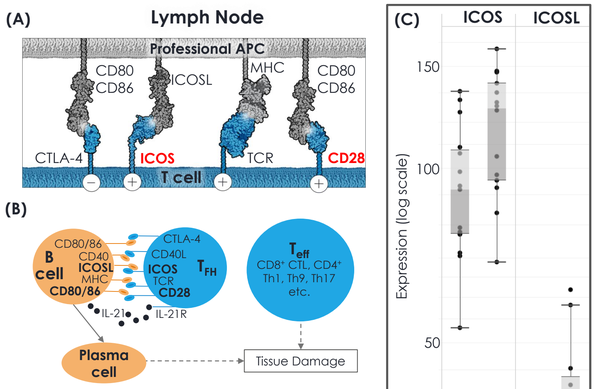
**Background/Purpose:** ALPN-101 is a dual ICOS/CD28 antagonist ICOS Ligand variant Ig domain (vIgD$^{TM}$) fused to an Fc lacking effector function. ALPN-101 was created using a proprietary technology platform enabling the discovery of novel proteins with tailored specificity and affinity through directed evolution of immunoglobulin superfamily (IgSF) proteins. CD28 and Inducible T-cell Costimulator (ICOS) are two related costimulatory molecules expressed on T cells that interact with CD80/CD86 and ICOSL, respectively. Both play critical roles in T cell activation and adaptive immunity. ALPN-101 is capable of binding both ICOS and CD28 and blocking the interaction of these costimulatory molecules with their respective counterstructures. Based on demonstrated *in vitro* and *in vivo* potency, ALPN-101 is being developed for the treatment of autoimmune and inflammatory diseases.

**Methods:** We used our proprietary technology platform to create a tailored ICOSL vIgD-Fc fusion protein (ALPN-101) capable of binding both ICOS and CD28 with high affinity and inhibiting their T cell costimulatory pathways. In an allogeneic mixed lymphocyte reaction (MLR), co-culturing negatively selected human pan T cells with activated human monocyte-derived dendritic cells, ALPN-101 demonstrates potent *in vitro* functional activity. ALPN-101 has been further evaluated in multiple *in vivo* mouse disease models, including experimental autoimmune encephalomyelitis (EAE).

**Results:** ALPN-101 significantly attenuates T cell activation *in vitro* as assessed by suppressed proliferation and cytokine production in MLR. ALPN-101 mediates significant disease reduction in the EAE model, as compared to control comparators, assessed by body weights and clinical scores.

**Conclusion:** ALPN-101 is capable of delivering dual inhibitory signals to T cell co-stimulators CD28 and ICOS, which may therapeutically translate to diminishing the severity of autoimmune and inflammatory diseases. ALPN-101 has demonstrated activity in the EAE model, a commonly used experimental model for multiple sclerosis. The efficacy of ALPN-101 appears superior to wild-type ICOSL-Fc domains in this model, presumably due to its ability to bind the cognate ligand ICOS with enhanced affinity and to bind the additional counter-structure CD28. IND-enabling studies have been initiated to support planned clinical studies.

## Figure 1: CD28 and ICOS Mediated T Cell Costimulation Contributes to Pro-Inflammatory State in Multiple Sclerosis (MS)







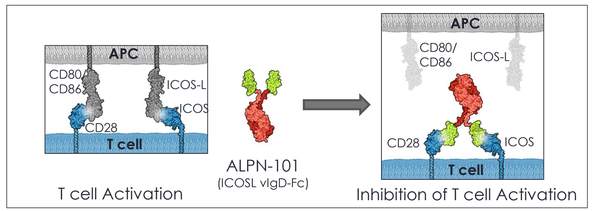
**(A)** Costimulatory receptors CD28 and ICOS are expressed on T cells, counter-structures CD80/CD86 and ICOS-L, respectively, are expressed on antigen presenting cells (APC)

**(B)** Professional APC in lymph nodes (dendritic cells, macrophages, B cells) engage CD28$^+$/ICOS$^+$ T cells, activated T cells differentiate into effector cells

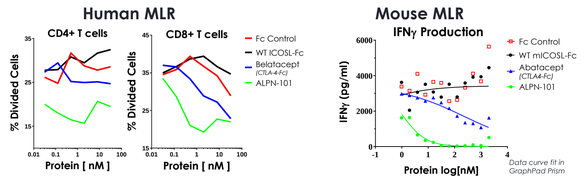
- CD4$^+$ Th1-, Th9- and Th17-cells, implicated as key contributors to MS by increasing inflammation within the CNS in both MS and experimental autoimmune encephalomyelitis (1)
- CD4+ICOS+CXCR5+ T follicular helper cells are increased in PBMC in relapsing-remitting and correlate with disease progression in secondary progressive MS. In both, a significantly increased ICOS gene expression in cerebrospinal fluid cells, in secondary progressive MS, an increased percentage of total monocytes and monocytes expressing ICOSL is observed (2)
- ICOSL also expressed on non-professional APCs, leading to T cell activation in non-lymphoid tissues and further tissue damage

**(C)** Expression studies corroborate upregulation of ICOS and ICOSL in MS (3)

## Figure 2: ALPN-101 a Dual ICOS/CD28 Antagonist Designed to Block T Cell Activation



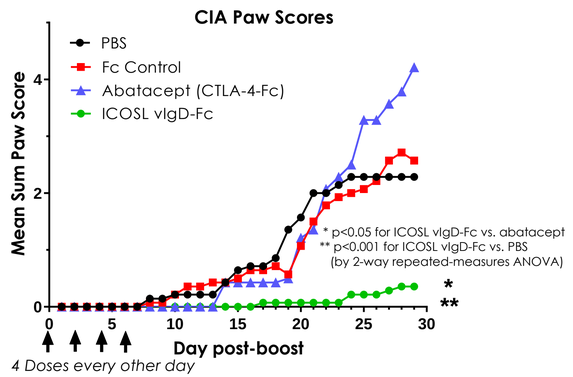

## Figure 3: ALPN-101 Potently Inhibits T Cells in Mixed Lymphocyte Reactions (MLR)




- ALPN-101 blocks proliferation of CD8$^+$ **and** CD4$^+$ T cells more potently than belatacept and controls
  - ALPN-101 blocks $T_{HF}$, TH17, TH9 cells implicated in MS disease progression (1)
- ALPN-101 significantly reduces cytokines (e. g. IFNγ, IL-21, not shown)
- T cell proliferation was determined by quantitating dilution of % CFSE-labeled cells over time

- ALPN-101 inhibits IFNγ production
- Test system:
  C57BL/6 anti-BALB/c MLR
  i.e. splenic T cell responders and bone marrow-derived dendritic cell stimulators
  - Inhibition of T cells determined by quantitating IFNγ production

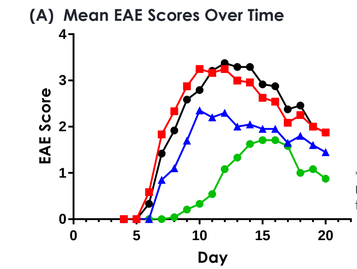
## Figure 4: ICOSL vIgD-Fc is More Effective than Abatacept *In Vivo* in a Collagen-Induced Mouse Model of Rheumatoid Arthritis



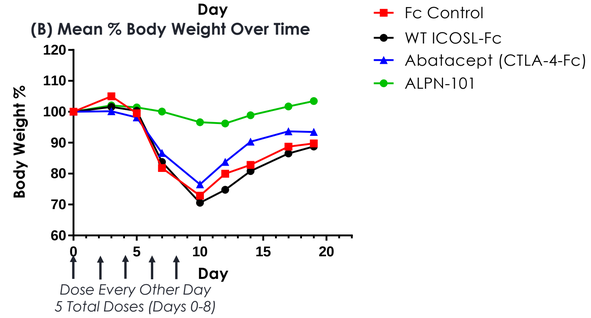

*4 Doses every other day*

- Male DBA/1J mice (n=15/group) immunized with chick collagen in Complete Freund's Adjuvant on Day -21; boosted with chick collagen in Incomplete Freund's Adjuvant on Day 0
- Dosing started on day of boost:
  100 µg/dose ICOSL vIgD-Fc or molar equivalents of controls
- Paws scored daily for disease:
  max score per paw = 4; max sum paw score per mouse = 16

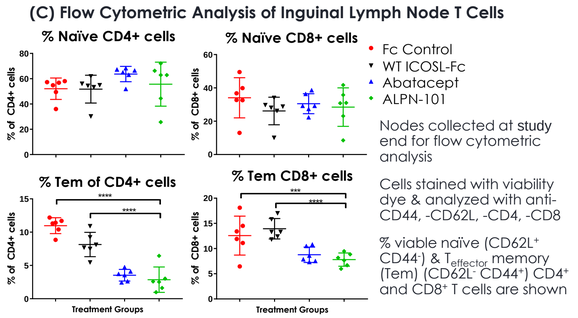
## Figure 5: ALPN-101 Inhibits Disease in an Adoptive Transfer Experimental Autoimmune Encephalomyelitis (EAE) Model




*p<0.0001 by 1-way ANOVA Area Under the Curve (AUC): ALPN-101 to controls




*Dose Every Other Day*
*5 Total Doses (Days 0-8)*



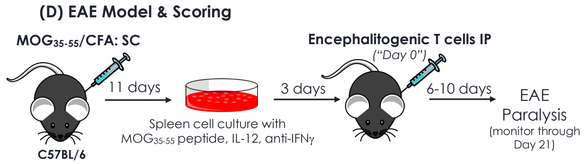

Nodes collected at study end for flow cytometric analysis

Cells stained with viability dye & analyzed with anti-CD44, -CD62L, -CD4, -CD8

% viable naïve (CD62L$^+$ CD44$^-$) & $T_{effector}$ memory (Tem) (CD62L$^-$ CD44$^+$) CD4$^+$ and CD8$^+$ T cells are shown

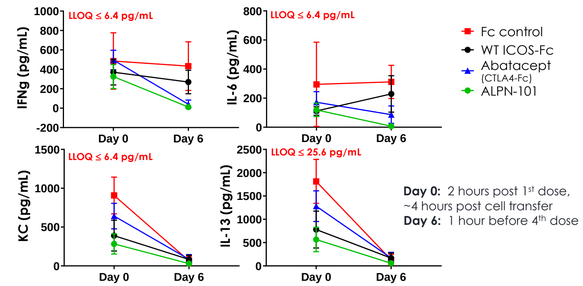
**** p<0.0001; *** p<0.001 by 1-way ANOVA

- CD4$^+$ and CD8$^+$ Tem cells reduced with ALPN-101 treatment




| Score | Clinical observations |
|---|---|
| 0 | No obvious changes in motor functions |
| 1 | Limp tail |
| 2 | Limp tail and weakness of hind legs |
| 3 | Limp tail & 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. |

## Figure 6: ALPN-101 Treatment Reduces Serum Cytokine Levels in an Adoptive Transfer EAE Model




**Day 0:** 2 hours post 1$^{st}$ dose, ~4 hours post cell transfer
**Day 6:** 1 hour before 4$^{th}$ dose

- ALPN-101 causes reduction of pro-inflammatory cytokines in serum on Day 0, including IL-5, IL-10, IL-12p70, TNFα *(data not shown)*
- Potent suppression of serum IFNγ and IL-6 production by ALPN-101 on Day 6, while IL-6 and IFNγ remain detectable in control groups
  - IL-6 and IFNγ known contributors to pathology in EAE and MS
  - ALPN-101 inhibits initial activation of transferred T cells
- Serum levels of IL-1β, IL-2, IL-4, and IL-17A below limit of detection
- Additional preclinical studies to evaluate cytokine levels and immune cells in CNS planned

## 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 inhibits T cell activation and disease in the adoptive transfer EAE mouse model superior to abatacept. Our data corroborate ICOS plays an important role in multiple sclerosis (6)
- Dual antagonism of ICOS and CD28 may therefore be an effective therapeutic approach in inflammatory disease including in one or more forms of multiple sclerosis
- ALPN-101 potently inhibits T cell response in vitro and demonstrates superior efficacy to CD28- or ICOS-only pathway blockade across multiple acute and chronic inflammatory disease models, including delayed type hypersensitivity, GvHD, collagen-induced arthritis and others (4, 5)
- Clinical trials with ALPN-101 are expected to begin shortly

## References

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