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Dual Blockade of ICOS and CD28 with Acazicolcept (ALPN-101) Reveals Non-Redundant Roles of T Cell Costimulation Pathways in Systemic Lupus Erythematosus

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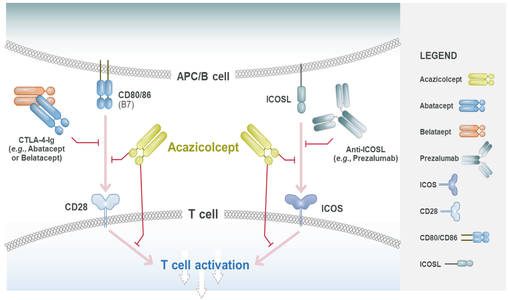
BACKGROUND:

CD28 and inducible T cell costimulator (ICOS) play nonredundant roles in T cell activation and inhibiting these pathways can ameliorate an (auto)immune response. Systemic lupus erythematosus (SLE) is characterized by the dysregulation of T and B cell activation. Transcriptional analyses have revealed upregulation of CD28 and ICOS ligand/receptor genes in SLE[1], but single pathway inhibition has not proven clinically effective in SLE and related diseases.[2,3] Acazicolcept (ALPN-101) is an Fc fusion protein of a human variant ICOS-ligand (ICOSL) domain designed to block both CD28 and ICOS. We conducted *in vitro* assays with healthy donor (HD) and SLE patient PBMCs to analyze acazicolcept or comparators to suppress inflammatory mediators that promote disease pathogenesis. Additionally, acazicolcept was evaluated in the bm12 induced mouse model[4] of SLE.

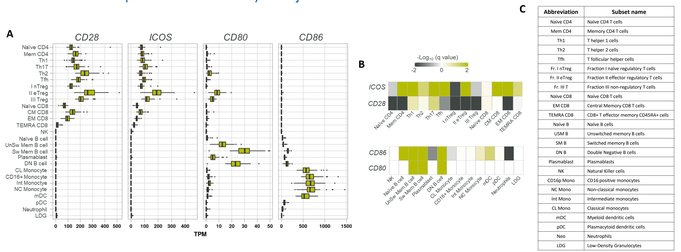
Figure 1: Acazicolcept Inhibits Both CD28- and ICOS-Mediated Costimulatory Pathways



Acazicolcept (ALPN-101), an ICOSL variant Immunoglobulin (Ig) domain (vIgD™) fused to an "effectorless" IgG Fc, is a first-in-class dual inhibitor of the CD28 and ICOS T cell costimulatory pathways.

- Dysregulation of T cell activation is a significant contributor to autoimmune diseases, including SLE[5].
- CD28 and ICOS are both Ig superfamily proteins with structural similarities, but distinct downstream signaling pathways[6]. CD28 stimulation is crucial for activation of naïve and memory CD4+ T cells, augmenting and prolonging the production of IL-2, and promoting T cell survival. ICOS stimulation triggers clonal expansion of T cells and T cell-dependent antibody production and can also initiate germinal center formation and isotype switching of B cells.
- Acazicolcept is an Fc fusion protein of a human variant ICOSL domain designed to inhibit CD28 and ICOS co-stimulation and has demonstrated efficacy in preclinical models of multiple sclerosis[7], inflammatory bowel disease[8], inflammatory arthritis[9], graft-versus-host disease[10], systemic sclerosis[11], and uveitis[12].

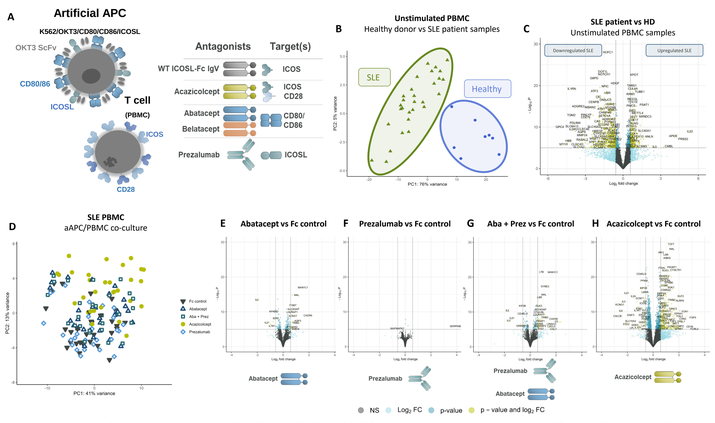
Figure 2: Acazicolcept Target-Related Genes are Upregulated in the T and B cells of SLE Patients Compared to Healthy Subjects



Abbreviation	Subset name
Naive CD4	Naive CD4 T cells
Mem CD4	Memory CD4 T cells
Th1	T helper 1 cells
Th2	T helper 2 cells
Tfh	T follicular helper cells
Fr. I nTreg	Fraction I naive regulatory T cells
Fr. II nTreg	Fraction II effector regulatory T cells
Fr. III T	Fraction III non-regulatory T cells
Naive CD8	Naive CD8 T cells
EM CD8	Central Memory CD8 T cells
TEMRA CD8	CD8+ T effector memory CD45RA+ cells
Naive B	Naive B cells
USM B	Unswitched memory B cells
SM B	Switched memory B cells
DN B	Double Negative B cells
Plasmablast	Plasmablasts
NK	Natural Killer cells
CD16p Mono	CD16 positive monocytes
NC Mono	Non-classical monocytes
Int Mono	Intermediate monocytes
CL Mono	Classical monocytes
mDC	Myeloid dendritic cells
pDC	Plasmacytoid dendritic cells
Neo	Neutrophils
LDG	Low-Density Granulocytes

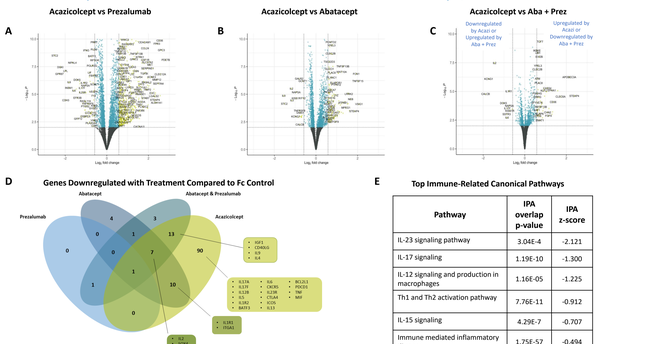
RNA-Seq expression of acazicolcept target-related genes in sorted peripheral mononuclear cells (PBMC) subsets reported by Ota et al.[1] **A** Transcripts per million (TPM) expression values for healthy sample immune cell subsets. **B** TPM values were $\log_2$ transformed and converted to z-scores for SLE vs. healthy donor (HD) comparison. Heatmap colors represent negative $\log_{10}$ q-values from Wilcoxon tests between 62 SLE and 79 HDs with positive (*olive green*) values representing higher SLE patient expression and negative (*black*) values representing lower SLE patient expression. White heatmap values indicate either non-significance (q-values ≈1) or low expression (subject median expression ≤10). TPM values displayed were upper quartile normalized. **C** Summary of PBMC immune cell subset classification abbreviations used in A and B.

Figure 3: Acazicolcept Potently Suppresses Genes Associated with T Cell Activation in SLE PBMCs Stimulated with Artificial APCs



A Visual schematic of experimental design: PBMC from 8 HD or 30 SLE patients were stimulated with artificial antigen presenting cells (aAPCs) expressing anti-CD3 (OKT3), CD80, CD86, and ICOSL for 48h with 100 nm of Fc control protein, acazicolcept, or comparators directed against the CD28 (abatacept; [CTLA-4-Ig]) or ICOS (prezalumab; [anti-ICOS-L monoclonal antibody]) pathways or combined (abatacept + prezalumab). **B** PBMCs that were not exposed to artificial aAPCs exhibit distinct clustering of healthy donor and SLE patient samples by variance stabilized transformed (VST) gene counts plotted using principal component analysis (PCA) (PC1 = 76%; PC2 = 5%). **C** Volcano plots (total genes = 11868) highlight differentially expressed genes between HD and SLE samples, with 516 downregulated and 841 upregulated in the SLE patient population. Green dots represent differentially expressed genes that pass significance threshold with a p-value < 10E-06 and a $\log_2$ fold change cutoff with an absolute value greater than 0.58. Light blue dots pass the p-value cutoff but not the fold change threshold, dark blue dots pass a fold change cutoff but not the p-value threshold, and gray dots are not significant. **D** PCA was performed on transformed gene expression data from each SLE PBMC sample co-cultured with aAPCs in the presence of differing treatments (abatacept, prezalumab, abatacept + prezalumab, Fc control and acazicolcept). PCA identified acazicolcept clustering away from Fc control. **E-H** Volcano plots displaying differentially expressed genes comparing each treatment (**E** abatacept, **F** prezalumab, **G** abatacept + prezalumab (aba + prez), **H** acazicolcept) versus Fc control for SLE patient samples.

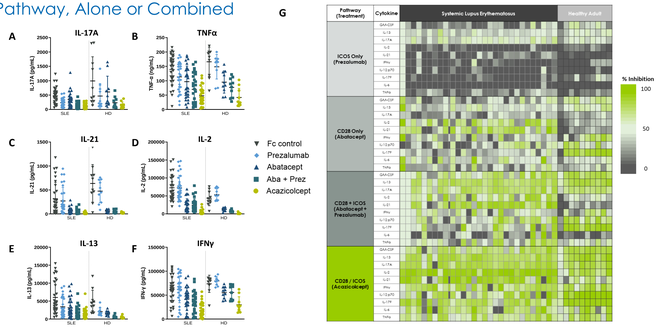
Figure 4: Acazicolcept Downregulates Genes Implicated in the Pathogenesis of SLE More Potently than Inhibition of the CD28 or ICOS Pathways, Alone or Combined



Pathway	IPA overlap p-value	IPA z-score
IL-23 signaling pathway	3.04E-4	-2.121
IL-17 signaling	1.19E-10	-1.300
IL-12 signaling and production in macrophages	1.16E-05	-1.225
Th1 and Th2 activation pathway	7.76E-11	-0.912
IL-15 signaling	4.29E-7	-0.707
Immune mediated inflammatory disease	1.75E-57	-0.494

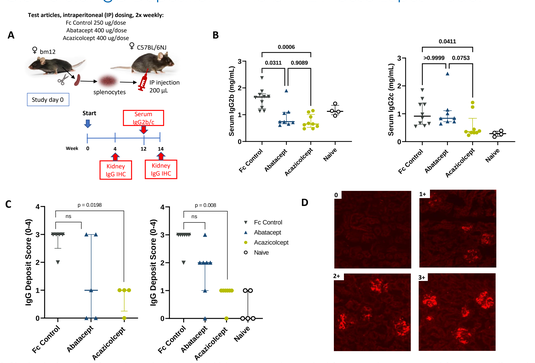
Acazicolcept strongly modulates genes associated with T cell activation as compared to **A** prezalumab, **B** abatacept, and the **C** combination of abatacept and prezalumab. Acazicolcept more potently suppresses transcription of genes related to T cell activation and immune inflammation including IL-2, IL-17F, CTLA4, ICOS and IL-6. Volcano plots highlight differentially expressed genes between HD and SLE samples with 516 downregulated and 841 upregulated in SLE patient population. Green dots represent differentially expressed genes that pass significance threshold with a p-value <0.001 and a $\log_2$ fold change cutoff with an absolute value greater than 0.58. Light blue dots pass the p-value cutoff but not the fold change threshold, dark blue dots pass a fold change cutoff but not the p-value threshold, and gray dots are not significant. Additionally, using Ingenuity Pathway Analysis (IPA, v94302991, QIAGEN) indicates that acazicolcept significantly downregulates genes associated with systemic autoimmune syndromes (IPA z-score = -1.357, IPA p-value = 5.95E-15) and helper T lymphocyte differentiation (IPA z-score = -1.374, IPA p-value = 6.77E-6) pathways compared to abatacept + prezalumab highlighting the advantage of dual inhibition over independent pathway blockade. **D** Venn diagram analysis of downregulated differentially expressed genes from **Fig 3E-H** (that pass significance threshold with a p-value <10E-06 and a $\log_2$ fold change cutoff less than 0.58) comparing individual treatment conditions in SLE patient samples to Fc control highlighting the downregulation of key pro-inflammatory regulators of interest. **E** Differentially expressed genes from **Fig 3H** were analyzed using IPA to identify top immune-related canonical pathways that were downregulated with acazicolcept. Multiple pathways implicated in the pathogenesis of SLE were downregulated including IL-17 signaling, IL-23 signaling, and Th1/Th2 activation.

Figure 5: Acazicolcept Suppresses Pro-Inflammatory Cytokine Production in PBMC/aAPC Co-Cultures More Potently than Inhibition of the CD28 or ICOS Pathway, Alone or Combined



Acazicolcept demonstrates superior cytokine inhibition as compared to Fc control, abatacept, or prezalumab. **A-F** Individual cytokine levels (pg/mL) for IL-17A, TNFα, IL-21, IL-2, IL-13, and IFNγ from supernatants harvested from PBMC/aAPC co-cultures as described in **Fig. 3A**. Acazicolcept suppresses key cytokines produced by activated T cells and myeloid cells that have been implicated in SLE pathogenesis. **G** Inhibition of cytokines for abatacept, prezalumab, aba + prez, and acazicolcept were normalized to Fc control to demonstrate enhanced suppression, indicating inhibition of both CD28 and ICOS pathways yield decreased inflammatory cytokines compared to single-agent molecules. Statistically significantly ($p<0.5$) enhanced suppression of IFNγ, IL-2, and IL-6 by acazicolcept vs. combined aba + prez was observed in SLE PBMC cultures.

Figure 6: Acazicolcept Reduces Pathogenic Hyper-IgG and Glomerular IgG Deposition in the bm12 Mouse Lupus Model



A Study design showing at weeks 4 and 14, kidneys were harvested from each mouse and frozen, sectioned, and stained with anti-mouse IgG antibody, and serum was collected at week 12 for total levels of pathogenic IgG2b and 2c antibodies. **B** Serum levels of IgG2b and 2c (week 12) are plotted. **C** The extent of mouse IgG staining was semi-quantitatively scored: the glomerular IgG deposit scores for each kidney collected for each treatment group at week 4 (*left*) and week 14 (*right*) are shown. **D** Representative sections (original magnification, 10X) for each score are shown. Data shown in **B** and **C** are from individual mice; horizontal bars and vertical error bars represent the group median ± IQR, respectively. Statistically significant differences between treatment groups were assessed via Kruskal-Wallis test with uncorrected Dunn's test; *p*- values <0.05 were considered statistically significant. Naïve group was not included in statistical analyses.

Summary and Conclusions

- Acazicolcept (ALPN-101; ICOSL vIgD-Fc) is a dual CD28 and ICOS T cell costimulation pathway inhibitor targeting both naïve and activated pathogenic T cells.
- Acazicolcept target-related genes (*ICOS, CD80, CD86*) are upregulated in the T and B cells of SLE patients compared to healthy subjects.
- Acazicolcept suppresses pro-inflammatory cytokine production and expression of genes implicated in the pathogenesis of SLE more potently than inhibitors of the CD28 or ICOS pathways alone (or often when combined), including numerous genes associated with T cell activation.
- Acazicolcept reduces pathogenic hypergammaglobulinemia and glomerular IgG deposition in the bm12 mouse model of lupus.
- These findings indicate that simultaneously inhibiting ICOS and CD28 pathways with acazicolcept may significantly improve disease activity in lupus, with activity superior to agents targeting only one of these pathways, alone or combined.
- Clinical investigation of acazicolcept for the treatment of SLE remains strongly supported. A global, randomized, placebo-controlled, double-blind, phase 2 trial of acazicolcept in SLE (NCT04835441/Synergy) is currently enrolling.

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