ACR CONVERGENCE 2023 | NOVEMBER 10-15, 2023 | SAN DIEGO, CA, USA

Upregulation of Both APRIL and BAFF in Systemic Lupus Erythematosus Suggests Non-Redundant Roles, Further Revealed by Dual Inhibition with Povetacicept (ALPN-303)

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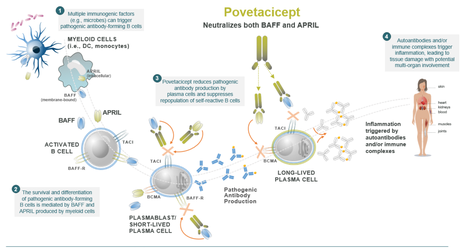
Background:

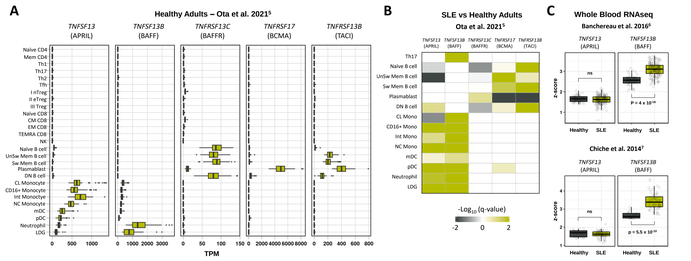
B cell activating factor (BAFF) and a proliferation-inducing ligand (APRIL) are cytokines that signal through transmembrane activator and CAML interactor (TACI), B cell maturation antigen (BCMA), and/or BAFF-Receptor (BAFF-R); these cytokines play important roles in the activation, differentiation and/or survival of B cells, particularly antibody-secreting cells, as well as T cells and innate immune cells. Therapeutic agents targeting BAFF and/or APRIL have demonstrated promising clinical potential in systemic lupus erythematosus (SLE), lupus nephritis, and other B cell-related diseases, but have generally targeted only BAFF, only APRIL, or predominantly BAFF. Due to the overlapping and non-redundant roles of BAFF and APRIL, more potent and/or complete inhibition of both cytokines is likely required for optimal efficacy. Povetacicept (ALPN-303) is an Fc fusion protein of a variant TACI domain engineered to have enhanced affinity for BAFF and APRIL, providing more potent dual inhibition of both BAFF and APRIL than wild type (WT) TACI-Fc or BAFF- or APRIL-specific antibodies. In a first-in-human study with healthy volunteers (NCT05034484), povetacicept was well tolerated, exhibited dose-dependent pharmacokinetics (PK), and exhibited expected pharmacodynamic (PD) effects on circulating immunoglobulin (Ig) and B cell populations.[1] In multiple disease models, povetacicept suppresses autoantibody production better than other B cell modulating therapies, including WT TACI-Fc, anti-CD20, and neonatal Fc receptor (FcRn) inhibition.[2-4]

Figure 1: Povetacicept is an Enhanced APRIL + BAFF Antagonist that Potently Modulates B Cells and Pathogenic Autoantibody Development



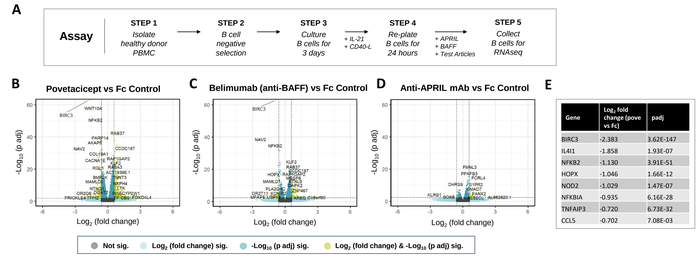
Povetacicept (ALPN-303) is a modified TACI-Fc fusion protein, engineered for high affinity to both BAFF and APRIL, which mediates enhanced inhibition of both cytokines compared to WT TACI-Fc

Figure 2: BAFF, APRIL, BAFF-R, BCMA, and TACI Gene Expression is Increased in Circulating Myeloid/B Cell Populations in SLE Patients as Compared to Healthy Adults



Publicly available transcriptional datasets were used to evaluate the expression of **(A)** BAFF- and APRIL-related genes in immune cell subsets sorted for RNA sequencing (RNASeq) from healthy adults[5]. Samples from SLE patients were compared to healthy adults using **(B)** transcript per million (TPM) values that were log_2 transformed and converted to z-scores. Heatmap colors represent -log_{10} q-values from Wilcoxon tests between SLE and healthy adults. Positive (*olive green*) values represent higher SLE patient expression, and negative (*black*) values represent lower SLE patient expression. White values indicate either non-significance (q-values =1) or low expression (healthy adult median expression ≤10). TPM values were upper quartile normalized. Upregulation of BAFF and APRIL receptor genes *TNFRSF13C* (BAFF-R), *TNFRSF17* (BCMA), *TNFRSF13B* (TACI) was predominantly observed in B cell subsets from SLE patients as compared to healthy controls. **(C)** *TNFSF13* (APRIL) and *TNFSF13B* (BAFF) expression were compared between SLE vs. healthy adults from whole blood RNASeq analyses using datasets published by Banchereau et al., 2016[6] (SLE N=158) and Chiche et al., 2014[7] (SLE N=62). Analysis of these publicly available immune cell subset-specific RNASeq and whole blood RNASeq datasets reveals a trend towards elevated *TNFSF13B* (BAFF) expression in SLE patients as compared to healthy adults; *TNFSF13* (APRIL) expression is more variable across all datasets but is elevated in sorted myeloid cells from SLE patients[5]. These observations suggest a potential role for the BAFF/APRIL pathways in SLE disease pathogenesis. Immune cell subset abbreviation definitions: (Un)Sw Mem=(un)switched memory, DN=double negative (IgD-CD27-), CL Mono=classical monocytes, Int=intermediate, NC=non-classical, mDC=myeloid dendritic cells, pDC=plasmacytoid DC, LDG=low-density granulocytes.

Figure 3: Dual Pathway Inhibition of BAFF + APRIL Signaling with Povetacicept, as Compared to Single Pathway Inhibitors, More Potently Inhibits Expression of Genes Associated with B Cell Activation



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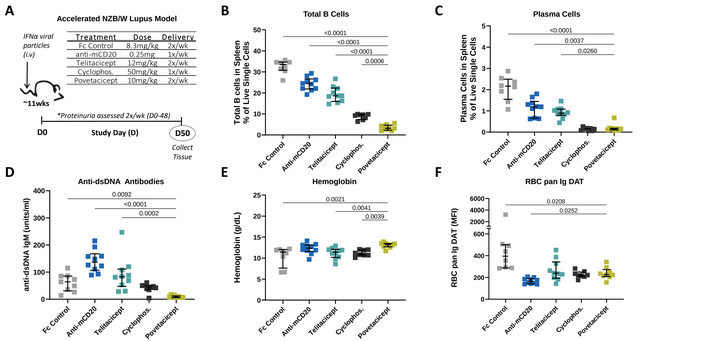
Gene	Log_2 fold change (povo vs Fc)	padj
BIRC3	-2.383	3.62E-147
IL4I1	-1.858	1.93E-07
NFKB2	-1.130	3.91E-51
HOPX	-1.046	1.66E-12
NOD2	-1.029	1.47E-07
NFKBIA	-0.935	6.16E-28
TNFAIP3	-0.720	6.73E-32
CCL5	-0.702	7.08E-03

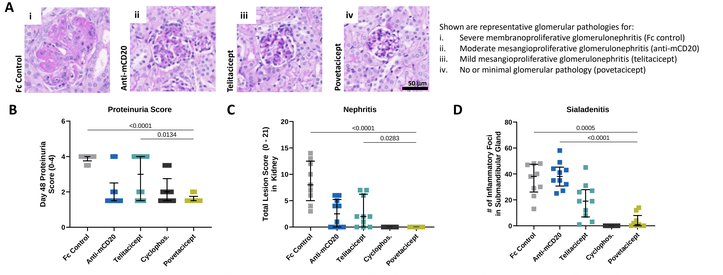
Purified human B cells from healthy donors were cultured as outlined in **(A)** and treated with 100 nM of the following test articles: Fc control, povetacicept, belimumab (anti-BAFF) monoclonal antibody (mAb) or anti-APRIL mAb (produced at Alpine based on sibeprenlimab sequence, per WHO drug information Volume 34 number 4 2020). BAFF, APRIL, and test articles were co-incubated with shaking (80 rpm) at room temp for 15 min prior to addition to cells. Following treatment, B cells were processed for RNASeq and differential expression was performed between various groups. Volcano plots highlight differentially expressed genes from B cells cultured in the presence of BAFF (10 nM) + APRIL (5 nM) and treated with either **(B)** povetacicept, **(C)** belimumab, or **(D)** anti-APRIL mAb versus (vs) Fc control. Green dots represent differentially expressed genes that pass significance (sig.) threshold with an adjusted p-value (padj) >0.005 and log_2 fold cutoff value with an absolute value greater than 0.58. Genes presented as light blue dots pass the p-value cutoff but do not pass the log_2 fold change threshold, dark blue dots represent genes that pass fold change cutoff, but not significantly, and genes shown as gray dots pass neither log_2 fold change nor adjusted p-value cutoffs. **(E)** List of genes associated with B cell activation that are significantly decreased with povetacicept as compared to Fc control from volcano plot in **Fig 3B**.

Figure 4: Povetacicept Reduces B Cells, Plasma Cells, and Autoantibodies, and Suppresses Autoimmune Anemia in the IFNα-Accelerated NZB/W Mouse Lupus Model More Potently than Anti-CD20 or WT TACI-Fc



The interferon alpha (IFNα)-accelerated NZB/W mouse lupus model described in **(A)** was used to evaluate the impact of povetacicept and comparator molecules on disease development and severity. On Day 50 (D50), spleens were collected for immunophenotyping. Flow cytometric analysis of spleens revealed that povetacicept reduced the frequency of **(B)** total B cells (CD19+B220+CD11b-) and **(C)** plasma cells (TACI+CD138+) as compared to Fc control, anti-mCD20, or telitacicept (wild-type TACI-Fc). **(D)** Lower anti-double stranded (ds) DNA levels were detected in serum of povetacicept-treated mice compared to Fc control, anti-mCD20, or telitacicept groups. **(E)** Higher hemoglobin levels were observed in povetacicept-treated mice as compared to Fc control and telitacicept groups. **(F)** Reduced red blood cell (RBC) direct anti-globulin test (DAT) pan Ig was observed in mice treated with povetacicept compared to Fc control or anti-mCD20. Data from individual mice are plotted. Horizontal bars and vertical error bars represent the mean ± standard deviation for data shown in **B**, or the group median ± interquartile range (IQR) for **C.** Statistically significant differences between treatment groups were assessed via 1-way ANOVA with uncorrected Fisher's LSD test in **B**. In **C-F**, Kruskal-Wallis test with an uncorrected Dunn's test was used to determine significance. P-values < 0.05 were considered significant. For simplicity, statistically significant p-values are only shown for comparisons of povetacicept versus each of the other treatment groups.

Figure 5: Treatment with Povetacicept Significantly Reduces Nephritis, Proteinuria, and Sialadenitis in the IFNα-Accelerated NZB/W Mouse Lupus Model



Mice enrolled in the accelerated preclinical NZB/W lupus study outlined in **Fig. 4A** were assessed for proteinuria on Day 48 (D48) and tissues were collected for histological analysis on D50. **(A)** Representative images of paraffin embedded periodic acid-Schiff (PAS)-stained kidneys. Histopathology of cyclophosphamide-treated kidneys (not shown) was similar to that of the povetacicept treatment group. **(B)** Proteinuria scores on D48 were reduced in mice treated with povetacicept as compared to Fc control or telitacicept. **(C)** Total lesion scores (glomerular + tubular/interstitial lesions) were lower in the povetacicept group compared to kidneys from mice treated with Fc control or telitacicept. **(D)** Lower numbers of inflammatory foci were observed in H&E-stained submandibular gland of mice treated with povetacicept compared to Fc control or anti-mCD20 groups. Each symbol represents data from individual mice. Horizontal bars and vertical error bars represent the group median ± IQR, respectively. Statistical analysis performed as described in **Fig 4 (C-F)**. Scale bar: 50 µm.

Summary and Conclusions

- BAFF- and APRIL-related genes (i.e., *BAFF*, *APRIL*, *TACI*, and *BCMA*) are increased in myeloid lineage cells and B cells in SLE patients compared to healthy adults.
- Povetacicept, as compared to single BAFF or APRIL pathway inhibitors, more potently downregulates genes associated with activation in B cells.
- Povetacicept significantly reduces multiple disease parameters in the IFNα-accelerated NZB/W mouse model of lupus, more effectively than WT TACI-Fc or conventional B cell depletion.
- Dual, potent inhibition of both BAFF and APRIL may be required to achieve optimal suppression of pathogenic pathways in SLE and related diseases.
- These results strongly support the clinical evaluation of povetacicept in SLE, as well as in other B cell- and/or autoantibody-related diseases.
- A clinical study in SLE is in preparation; clinical trials in autoimmune glomerulonephritis, including lupus nephritis (NCT05732402), and autoimmune cytopenias (NCT05757570) are ongoing.

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Acknowledgements

We thank our colleagues at Hooke Laboratories (Lawrence, MA) for conducting the IFNa-accelerated NZB/W mouse model study, Jennifer Austin (Biocraft Studio) for illustrations, Julie Crider for medical writing support, and the rest of our team at Alpine Immune Sciences for their contributions to the development of povetacicept.

Financial Disclosures

All authors are current or former employees of Alpine Immune Sciences.