The Enhanced Dual BAFF/APRIL Inhibitor Povetacicept (TACI vTD-Fc; ALPN-303) More Potently Reduces Disease Activity in Murine Lupus Compared to CD20 Depletion and WT TACI-Ig, Associated with Greater End-Organ Distribution

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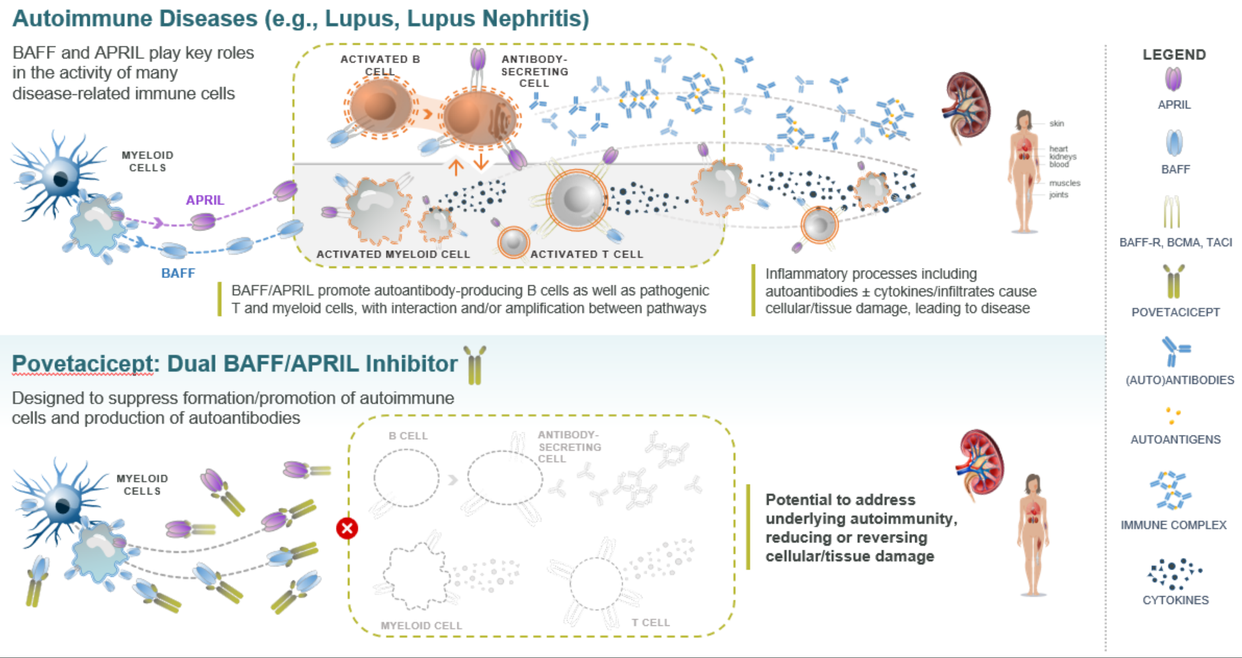
INTRODUCTION

- BAFF and APRIL signal through TACI, BCMA, and/or BAFF-R, and play important roles in the activation, differentiation, and/or survival of B cells, including antibody-secreting cells, as well as T cells and innate immune cells.[1]
- Therapeutic agents targeting BAFF and/or APRIL have demonstrated promising clinical potential in systemic lupus erythematosus (SLE), lupus nephritis (LN), and other B cell-related diseases; however, these inhibit only BAFF, only APRIL, or predominantly BAFF, and more potent inhibition of both cytokines is likely required for optimal efficacy.
- Povetacicept (TACI vTD-Fc; ALPN-303) is a potent, enhanced dual BAFF/APRIL antagonist.[2] In previous pre-clinical studies and disease models, povetacicept demonstrated greater potency in reducing autoantibodies and B and T cell populations that play a key role in antibody responses than treatment with wild-type (WT) TACI-Ig, while also exhibiting greater serum exposure and pharmacodynamic effects.[2]
- The objectives of these studies were to ascertain the expression of BAFF and APRIL in SLE patients using transcriptional datasets; and to compare povetacicept to WT TACI-Ig in tissue distribution and lupus efficacy using mouse models.

Povetacicept: a Dual BAFF/APRIL Inhibitor for Multiple Autoimmune Diseases

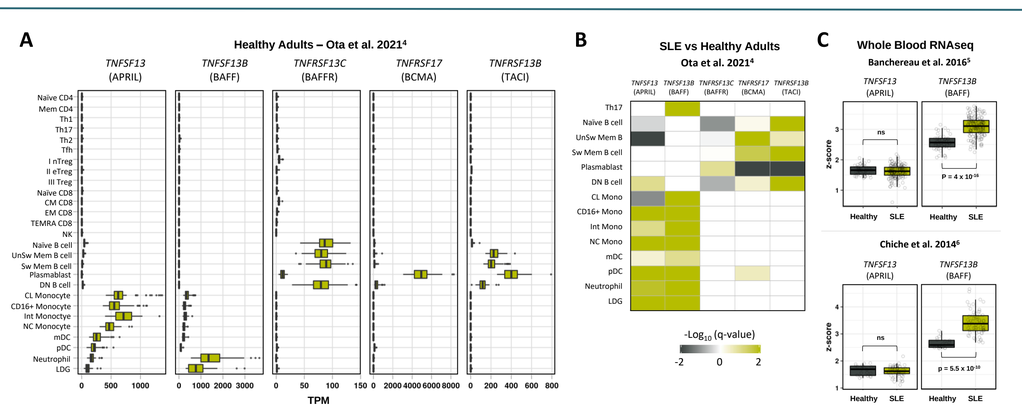


METHODS

- APRIL and BAFF gene expression was assessed in published transcriptional datasets from healthy donors and SLE patients.
- To evaluate *in vivo* biodistribution, povetacicept (10 mg/kg) or a molar-matched dose of WT TACI-Ig (telitacicept; sourced from Clinigen) was administered IV to healthy C57BL/6 mice. Lupus-related tissues were collected ~18 hours later, following perfusion, and evaluated by quantitative immunohistochemistry for human Fc staining.
- Affinity determination by surface plasmon resonance (SPR) was conducted on a Biacore 3000 optical biosensor equipped with a CM5 sensor chip (GE) prepared with goat anti-human IgG capture antibody (Jackson ImmunoResearch). Additional details are provided in Evans et al.[2]
- Povetacicept was also evaluated in an IFNα-accelerated NZB/W mouse model of SLE[3], in which 11-week-old female NZB/W mice were given an IFNα-expressing adenovirus IV on Day 0.
  - Mice were administered twice weekly intraperitoneal injections of povetacicept (10 mg/kg), a molar-matched dose of an Fc control or telitacicept, or weekly cyclophosphamide (50 mg/kg) or a depleting anti-mouse CD20 mAb (0.25 mg).
  - Mice were analyzed 7 weeks later for disease endpoints, including levels of double-stranded DNA in serum, anemia, proteinuria, nephritis, glomerular immune complex deposition, and sialadenitis.

RESULTS

Figure 1: 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[4]. 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[5] (SLE N=158) and Chiche et al., 2014[6] (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: APRIL = a proliferation-inducing ligand; BAFF = B cell activating factor; (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 2: Povetacicept Exhibits Greater Distribution to Lupus-Related Organs than WT TACI-Ig (Telitacicept)

Differences in Size, Target Affinity, and/or Isoelectric Point (pI) of Povetacicept vs. WT TACI-Ig May Drive Enhanced Tissue Penetrance

Tissue	% Human Fc-Positive (Mean ± SEM)		Fold Increase (Povetacicept vs. Telitacicept)
	Povetacicept	Telitacicept	
Kidney	1.9 ± 0.2	0.1 ± 0.0	**19.0**
SMG	1.03 ± 0.25	0.12 ± 0.02	**8.6**
Lung	0.49 ± 0.13	0.13 ± 0.05	**3.8**
Skin (abdomen)	2.7 ± 0.4	0.8 ± 0.2	**3.4**
Brain	0.1 ± 0.03	0.03 ± 0.01	**3.3**
Spleen	1.13 ± 0.21	0.87 ± 0.2	**1.3**
Liver	0.4 ± 0.2	2.5 ± 0.4	**0.2**

Povetacicept (12 mg/kg) or a molar-matched dose of telitacicept was administered IV to healthy C57BL/6 mice. Tissues were collected ~18 hours later and evaluated by quantitative immunohistochemistry for human Fc staining. SEM = standard error of the mean; SMG = submandibular gland.

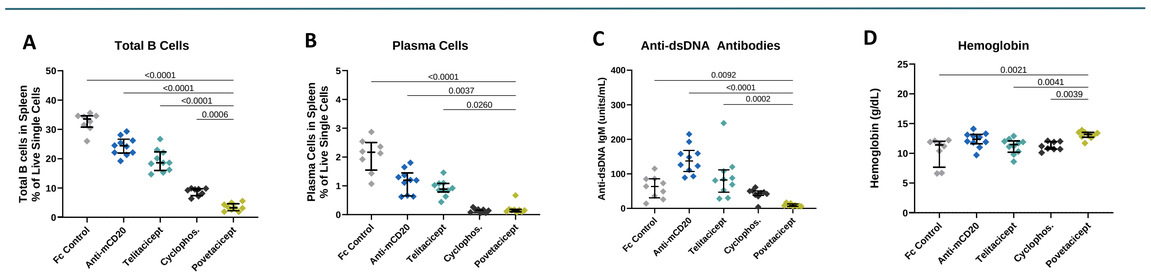
Parameter	Povetacicept	Telitacicept (Tai'ai®)
MW (kDa)	62.6	75.3 – 75.4[1]
K_D, BAFF (SPR)	59 pM	491 pM
K_D, APRIL (SPR)	~1 pM	CNBD *(multiple/variable off rates)*
pI [2]	6.5 – 7.0	7.1 – 8.4

[1] Theoretical values shown; MW reported as 80.24 kDa in *J Clin Pharmacol* 56:948 (2016)

[2] Theoretical, calculated by Expasy (web.expasy.org/compute_pi/), Prot pI (Protpi.ch/Calculator/Protein Tool), or at Alpine based on *Anal. Biochem* 179:319 (1989)

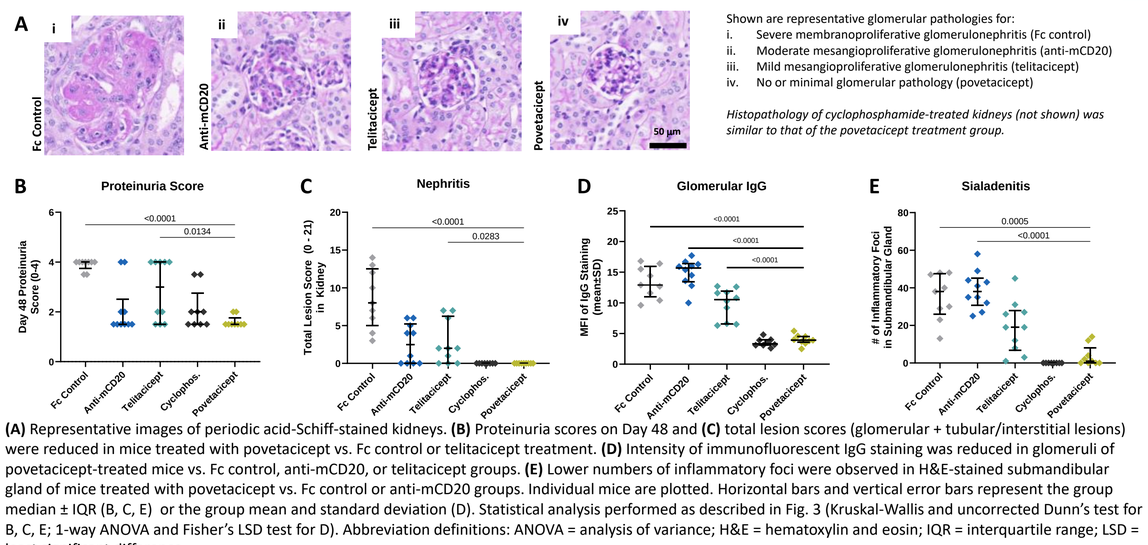
IV = intravenous; MW = molecular weight; kDa = kilodaltons; ; KD = dissociation constant; SPR = surface plasmon resonance; CNBD = could not be determined; pI = isoelectric point (pH at which the net charge of a protein is zero). Telitacicept was sourced from Clinigen.

Figure 3: Povetacicept Reduces B Cells, Plasma Cells, and Autoantibodies, and Suppresses Autoimmune Anemia in the IFNα-Accelerated NZB/W Mouse Lupus Model[3] More Potently than Anti-CD20 or WT TACI-Ig



Flow cytometric analysis of spleens collected at the end of the study (Day 50) revealed that povetacicept reduced the frequency of **(A)** total B cells (CD19+B220+CD11b-) and **(B)** plasma cells (live TACI+CD138+ cells) as compared to Fc control, anti-mCD20, or telitacicept (wild-type TACI-Ig). **(C)** Lower anti-double stranded (ds) DNA levels were detected in serum of povetacicept-treated mice compared to Fc control, anti-mCD20, or telitacicept groups. **(D)** Higher hemoglobin levels were observed in povetacicept-treated mice as compared to Fc control, telitacicept, or cyclophosphamide groups. Data from individual mice are plotted. Horizontal bars and vertical error bars represent the mean ± standard deviation for data shown in A, or the group median ± interquartile range (IQR) for B-D. *P* values for statistically significant differences between povetacicept and other treatment groups are shown, calculated using 1-way ANOVA with uncorrected Fisher's Least Significant Difference (LSD) test (A), or Kruskal-Wallis test with uncorrected Dunn's test (B – D). *P* values < 0.05 considered statistically significant. Abbreviation definitions: ANOVA = analysis of variance; TACI = TACI, transmembrane activator and CAML interactor.

Figure 4: Treatment with Povetacicept Significantly Reduces Proteinuria, Nephritis, Glomerular IgG Deposition, and Sialadenitis in the IFNα-Accelerated NZB/W Mouse Lupus Model[3]



(A) Representative images of periodic acid-Schiff-stained kidneys. **(B)** Proteinuria scores on Day 48 and **(C)** total lesion scores (glomerular + tubular/interstitial lesions) were reduced in mice treated with povetacicept vs. Fc control or telitacicept treatment. **(D)** Intensity of immunofluorescent IgG staining was reduced in glomeruli of povetacicept-treated mice vs. Fc control, anti-mCD20, or telitacicept groups. **(E)** Lower numbers of inflammatory foci were observed in H&E-stained submandibular gland of mice treated with povetacicept vs. Fc control or anti-mCD20 groups. Individual mice are plotted. Horizontal bars and vertical error bars represent the group median ± IQR (B, C, E) or the group mean and standard deviation (D). Statistical analysis performed as described in Fig. 3 (Kruskal-Wallis and uncorrected Dunn's test for B, C, E; 1-way ANOVA and Fisher's LSD test for D). Abbreviation definitions: ANOVA = analysis of variance; H&E = hematoxylin and eosin; IQR = interquartile range; LSD = least significant difference.

SUMMARY/CONCLUSIONS

- BAFF- and APRIL-related genes (i.e., *TNFSF13B*/BAFF, *TNFSF13*/APRIL, *TNFRSF13C*/BAFF-R, and *TNFRSF17*/BCMA) are increased in myeloid lineage cells and B cells in SLE patients compared to healthy adults.
- Povetacicept significantly reduces multiple disease parameters in the IFNα-accelerated NZB/W mouse model of lupus, more effectively than WT TACI-Ig or conventional B cell depletion.
- Povetacicept exhibits greater distribution to lupus-related tissues in normal mice than WT TACI-Fc (telitacicept), which may reflect its smaller size, higher target affinity, and/or lower isoelectric point, possibly contributing to its greater potency in the NZB/W disease model [9].
- 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 phase 2 study in SLE (DENALI) is in preparation; clinical trials in autoimmune glomerulonephritis, including lupus nephritis (NCT05732402), and autoimmune cytopenias (NCT05757570) are ongoing.

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ABBREVIATIONS

APRIL, a proliferation-inducing ligand; BAFF, B cell activating factor; BCMA, B-cell maturation antigen; Ig, immunoglobulin; IV, intravenous; kDa, kilodaltons; KD, dissociation constant; LN, lupus nephritis; TACI, transmembrane activator and CAML interactor; vTD, variable TNF/R domain; WT, wild-type.

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