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ALPN-303, an Engineered Dual BAFF/APRIL Antagonist, Potently Inhibits Pathogenic Autoantibodies in Preclinical Models, with Corresponding Pharmacodynamic Activity in Humans

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Background

- B-cell activating factor of the TNF family (BAFF) and a proliferation-inducing ligand (APRIL) are TNF superfamily members that bind transmembrane activator and calcium-modulator and cyclophilin ligand (CAML) interactor (TACI), B-cell maturation antigen (BCMA), and/or BAFF receptor (BAFF-R) on B cells and together support B cell development, differentiation, and survival.
- ALPN-303 is an Fc fusion protein of a human TACI variant TNFRSF domain (vTD) engineered by directed evolution to have enhanced affinity for BAFF and APRIL.
- In preclinical studies, ALPN-303 mediates significantly improved inhibition of both BAFF and APRIL in vitro and enhanced pharmacokinetic (PK) and immunomodulatory properties in vivo (e.g., by reducing germinal center B cells, follicular helper T cells, and plasma cells), as compared to wild-type (WT) TACI-Fc molecules.
- B-cell-targeting therapies like the WT TACI-Fc fusions atacicept and telitacicept, as well as belimumab (anti-BAFF), BION-1301 and sibeprenlimab (anti-APRIL), and ianalumab (anti-BAFF-R), have demonstrated promising clinical potential in B cell / antibody-related diseases like systemic lupus erythematosus (SLE), IgA nephropathy (IgAN), and Sjögren's syndrome.
- While WT TACI-Fc binds BAFF better than APRIL, ALPN-303 has enhanced inhibitory activity against BAFF and particularly improved APRIL binding vs WT TACI-Fc, and thus may further improve clinical outcomes in these serious antibody-related diseases.

Figure 1: ALPN-303 is a Modified TACI-Fc Fusion Protein Generated Via Directed Evolution

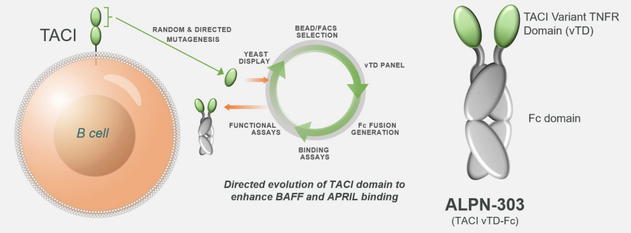
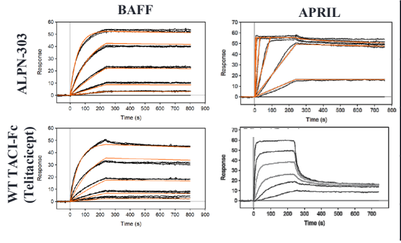


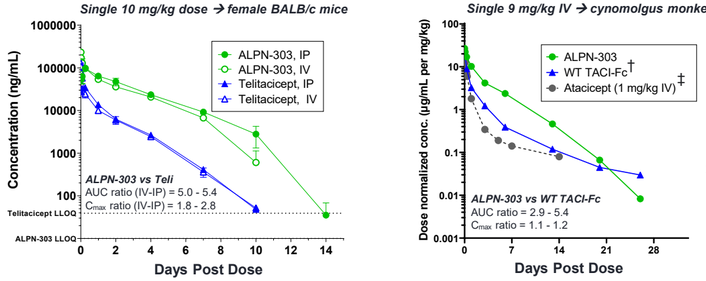
Figure 2: ALPN-303 has Significantly Enhanced Affinity for BAFF and APRIL as Compared to WT TACI-Fc



	BAFF		APRIL	
	ALPN-303	WT TACI-Fc*	ALPN-303	WT TACI-Fc*
k_a 1x10^5 ($M^{-1}s^{-1}$)	6.19 ± 0.01	1.75 ± 0.01	7.0 ± 7	CNBD
k_d 1x10^{-5} (s^{-1})	3.67 ± 0.02	8.63 ± 0.02	7.0 ± 6	CNBD
R_{max} (RU)	52.8 ± 0.3	47.6 ± 0.4	55.5 ± 0.1	CNBD
K_D (pM)	**59.3** ± 0.1	**491** ± 1	**~1.00** ± 0.04	**CNBD**

Affinity measurements of ALPN-303 and WT TACI-Fc binding to recombinant human BAFF and APRIL as determined by surface plasmon resonance (SPR). SPR sensorgrams are shown in black lines and results from non-linear least squares regression analysis of the data in orange lines. CNBD, could not be determined; the WT TACI-Fc/APRIL interaction displayed multiple on- and off-rates, preventing an accurate data fit using a 1:1 model. *Telitacicept commercial drug product (obtained from Clinigen, Burton upon Trent, UK).

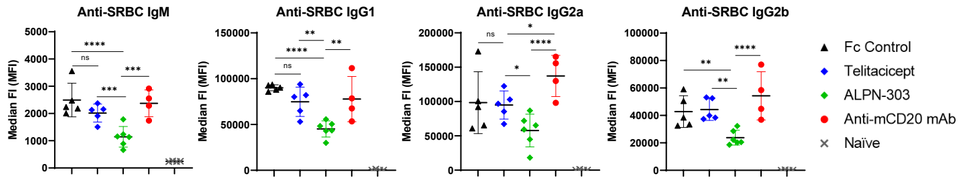
Figure 3: ALPN-303 Exhibits Higher Serum Exposure than WT TACI-Fc in Mice and Cynomolgus Monkeys



†Generated at AIS based on the sequence included in the telitacicept WHO INN submission, WHO Drug Information, Vol. 32, No. 4, 2018.

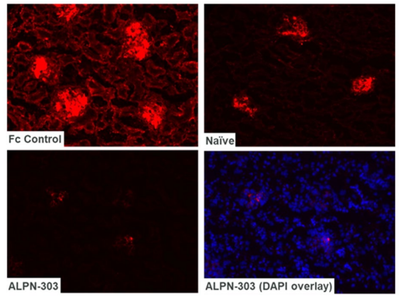
‡Atacicept PK data extracted from Carbonatto et al. (2008) *Toxicol Sci* 105: 200–210

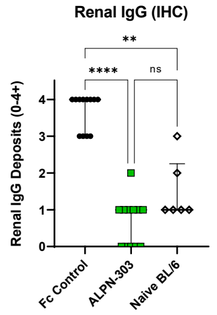
Figure 4: ALPN-303 Reduces T Cell-Dependent Antibody Responses in a Mouse SRBC Immunization Model More Potently than WT TACI-Fc or Anti-CD20 mAb



Following intraperitoneal (IP) SRBC immunization on Day 0, female BALB/c mice were dosed IP twice, on Days 1 and 6, with 0.2 mg ALPN-303 or molar-matched amounts of Fc control or telitacicept (WT TACI-Fc); a depleting anti-mouse CD20 mAb was administered once on Day 1. Anti-SRBC Ig concentrations in serum were measured on Day 15. Statistically significant differences between the SRBC immunized groups were determined by one-way ANOVA and Fisher's LSD multiple comparisons test (ns = not significant; *p<0.05, **p<0.01, ***p<0.001, ***p<0.0001).

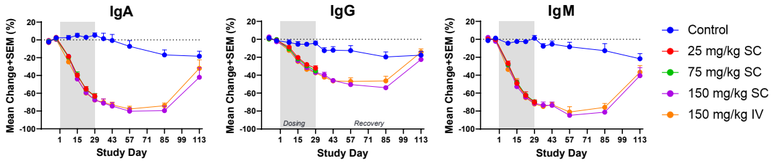
Figure 5: ALPN-303 Significantly Reduces Glomerular IgG Deposition in the bm12 Inducible Mouse Model of Lupus





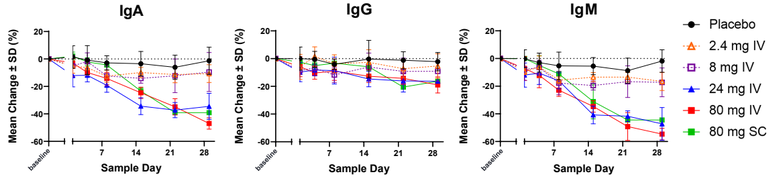
C57BL/6NJ mice were injected IP with bm12 splenocytes (~1 donor spleen per recipient) on Day 0, and dosed 2x/wk from Day 5 until Day 88 (6d prior to termination), with 0.2 mg ALPN-303 or with a molar-matched amount (0.16 mg) of Fc control protein. Kidneys collected on Day 95 were frozen in OCT blocks, sectioned, stained with AF594-conjugated goat anti-mouse IgG, followed by staining with 4',6-diamidino-2-phenylindole (DAPI) diluted 1:5000 in PBS. Glomerular IgG deposition was scored as per Kelkka et al. (2014) Antioxid Redox Signal. 21:2231-45. Statistical significance between treatment groups was determined by the Kruskal-Wallis test, followed by uncorrected Dunn's test for multiple comparisons (ns = not significant; **p<0.01, **** p<0.0001).

Figure 6: ALPN-303 Potently and Reversibly Reduces Serum Ig in a 1-Month Toxicology Study in Non-Human Primates (NHP)



Male and female cynomolgus monkeys (n = 6/group for dosing period; n = 4/group for recovery) were dosed weekly for up to 5 doses at 25, 75, or 150 mg/kg ALPN-303 via SC administration, or at 150 mg/kg via 30 min IV infusion. No test article-related toxicities were observed; the no-observed-adverse-effect level (NOAEL) was 150 mg/kg, the highest dose administered.

Figure 7: Single Dose ALPN-303 Preliminarily Reduces Serum Ig in a Dose-Dependent Manner in Adult Healthy Volunteers



In an ongoing Phase 1 trial in adult healthy volunteers (NCT05034484), subjects have been administered a single dose of 2.4, 8, 24, or 80 mg ALPN-303 (100 mg/mL) via 30 min IV infusion, or 80 mg via SC administration (each ALPN-303 group n = 4; placebo n = 10). To date, there have been no treatment-related serious adverse events, no infusion-related or injection site reactions, and no adverse trends in safety laboratories observed. Dose escalation beyond 80 mg is still in progress.

Conclusions

To address a key limitation of WT TACI-Fc, which binds to APRIL relatively weakly, ALPN-303 was engineered to have significantly enhanced affinity for APRIL (and BAFF) and is thus a potentially best-in-class BAFF/APRIL antagonist.

ALPN-303 is well-tolerated in rodents and NHP and demonstrates encouraging immunomodulatory activity and efficacy, including significant reversible reductions in circulating Ig.

ALPN-303 exhibits higher serum exposures and more potent immunomodulatory activities than WT TACI-Fc in preclinical models.

In an ongoing study in adult healthy volunteers (NCT05034484), ALPN-303 has been well tolerated and exhibits correspondingly encouraging pharmacodynamic activity, which may translate to lower and/or less frequent doses, and/or improved efficacy vs WT TACI-Fc-based therapies and potentially other BAFF/APRIL-targeted therapies.

ALPN-303 is an attractive development candidate for the treatment of multiple autoimmune and inflammatory diseases.

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