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A Randomized, Placebo-Controlled, Phase 1 Study in Healthy Adult Volunteers of the Safety, Tolerability, Pharmacokinetics, and Pharmacodynamics of ALPN-303, a Potent Dual BAFF/APRIL Antagonist for the Treatment of Autoimmune Cytopenias

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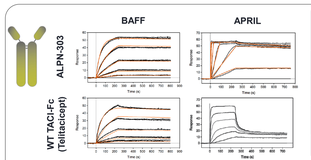
#3763

Introduction

B cell activating factor (BAFF) and a proliferation-inducing ligand (APRIL) are tumor necrosis factor superfamily (TNFSF) members that bind transmembrane activator and calcium-modulating cyclophilin ligand interactor (TACI), B-cell maturation antigen (BCMA), and/or BAFF receptor (BAFF-R) on B cells and together support B-cell development, survival, and differentiation into antibody-secreting cells (ASC). In preclinical studies, co-neutralization of BAFF and APRIL can directly suppress ASC and reduce circulating immunoglobulins. Autoimmune hemolytic anemia (AIHA) and immune thrombocytopenic purpura (ITP) are diseases characterized by autoantibodies directed against red blood cells and platelets, respectively. Significantly higher levels of both BAFF and APRIL have been observed in the serum of patients with AIHA and ITP compared to healthy subjects, and polymorphisms in BAFF and TACI have been associated with ITP.[1-4] The B cell-targeting agents currently used to treat AIHA or ITP, rituximab (anti-CD20) and belimumab (anti-BAFF), cannot directly deplete antibody-secreting plasma cells. To address these shortcomings, we have developed ALPN-303, an Fc fusion of an engineered TACI variant TNFRSF domain, which substantially improves upon the ligand affinity liabilities of wild type (WT) TACI, resulting in highly potent dual BAFF/APRIL inhibition superior to WT TACI-Fc, or to BAFF- or APRIL-specific monoclonal Abs (mAb).

Figure 1: ALPN-303 is an Engineered TACI-Fc Fusion with Enhanced Affinity for BAFF and APRIL



	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 2: ALPN-303 Inhibits Autoantibody Production to Disrupt Antibody-Driven Platelet Destruction in ITP

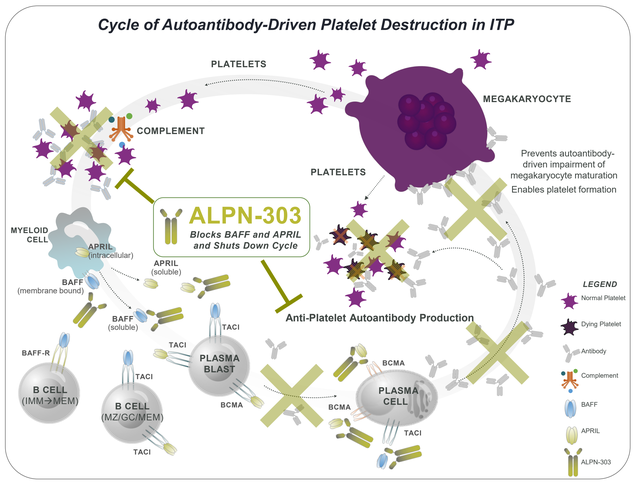
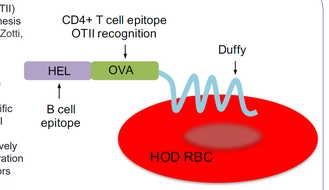
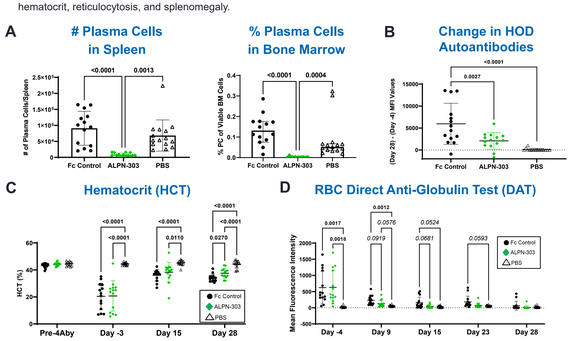


Figure 3: ALPN-303 Treatment Provides Benefit in the HOD Mouse Model of AIHA

- There are few mouse models of AIHA, though 'HOD' mice cross-bred with OTII transgenic mice (HODxOTII) represent a model system that reflects the pathogenesis observed in human patients (Desmarets, 2009; Dei Zotti, 2021).
- HOD mice express an RBC-restricted triple fusion protein of hen egg lysozyme (HEL), a portion of ovalbumin (OVA), and human blood group molecule Duffy (HEL-OVA-Duffy, aka 'HOD').
- OTII mice express a transgenic T cell receptor specific for OVA presented in the context of the MHC class II molecule I-A^b.
- AIHA develops in aged HODxOTII mice, or alternatively the disease onset can be accelerated with administration of monoclonal antibodies against checkpoint inhibitors and IL-10R to reverse tolerance.
- Disease in this model is accompanied by high titer RBC autoantibodies, increased activated B cells, decreased hematocrit, reticulocytosis, and splenomegaly.





METHODS

- Young HODxOTII mice (1.5 to 3.5 months old) were intraperitoneally (IP) administered antibodies against CTLA-4, IL-10R, LAG-3, and PD-1 for 2.5 weeks to accelerate disease onset, then randomized to treatment groups (N=15) based on HOD autoantibody titers and treated IP with 10 mg/kg ALPN-303 or a molar-matched dose of Fc control twice weekly for 3.5 weeks. A PBS control group that did not receive the 4-antibody induction regimen was also included for comparison. Assessments included measurement of circulating RBC autoantibodies and autoantigens, hematocrit and reticulocytes over time, and immuno-phenotyping of B and T cell subsets in spleen and bone marrow (BM) at the end of the study (Day 29). Detection of autoantibodies bound to peripheral RBCs was evaluated by staining RBCs with goat anti-mouse Ig and measuring signal by flow cytometry. 1-way ANOVA was used for both A and B; 2-way ANOVA was used for C and D; *p* values <0.05 were considered statistically significant.

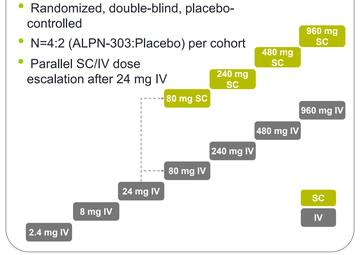
RESULTS:

- As compared to Fc control, ALPN-303 treatment significantly reduced numbers of plasma cells in spleen and frequency of PC in bone marrow (**A**), significantly suppressed the generation of antigen (HOD)-specific autoantibodies in serum (**B**), significantly increased whole blood hematocrit (**C**), and tended to lower anti-erythrocyte autoantibodies (**D**).

Figure 4: RUBY-1 Study Design

Study Design

- RUBY-1 (NCT05054484) is a Phase 1 trial evaluating single ascending doses of 2.4 to 960 mg ALPN-303 administered IV or SC to healthy volunteers
- Randomized, double-blind, placebo-controlled
- N=4:2 (ALPN-303:Placebo) per cohort
- Parallel SC/IV dose escalation after 24 mg IV



Demographics and Disposition

Attribute	Subjects (N=66)
Age (year)[1]	32 (18, 64)
Female	46 (70%)
Race	
White	40 (61%)
Asian	13 (20%)
Other	13 (20%)
BMI (kg/m^2)[1]	25 (18, 31)
eGFR[2]	
≥90	53 (80%)
60–89	13 (20%)
Completed[3]	39 (59%)

Data Extract: 29JUL2022

[1] Median (min, max)
[2] mL/min/1.73 m^2
[3] As of 29JUL2022
BMI = body mass index
eGFR = estimated glomerular filtration rate

Table 1: ALPN-303 is Well-Tolerated Overall

- ALPN-303 has been well-tolerated overall; most common adverse events (AEs) include mild (G1) headache, dizziness, low Ig (an expected pharmacodynamic [PD] effect), or back pain. Incidence of infection has not significantly differed from placebo.
- No G4-5 AEs, serious AEs, serious or severe infection, severe hypogammaglobulinemia, or cytokine release (no significant changes in: GM-CSF, IFNγ, IL-3, IL-5, IL-6, IL-7, IL-8, IL-10, IL-18, MIP-1α, MIP-1β, MCP-1, TNFα, or TNFβ).

Treatment-Emergent Adverse Event (TEAE)	All Placebo (N=22)	All ALPN-303 (N=44)
Any TEAE[1]	**12 (55%)**	**27 (61%)**
Grade 1	7 (32%)	20 (45%)
Grade 2	4 (18%)[2]	6 (14%)[3]
Grade 3	1 (5%)[4]	1 (2%)[4]
Any Adverse Event of Interest	**1 (5%)**	**1 (2%)**
Administration-Related Reaction[5]	1 (5%)	1 (2%)
Injection Site Pain (Grade 1)	1 (5%)	1 (2%)
Serious or Severe Infection	0	0
Severe Hypogammaglobulinemia	0	0
Cytokine Release Syndrome	0	0

[1] Follow-up through Day 29 post-dose.
[2] Upper respiratory tract infection, hyperlipasemia, dyspepsia, fatigue and iron deficiency anemia (n=1 each).
[3] Urinary tract infection, headache, migraine, nausea, and back pain (n=1 each); lipase increased (n=2).
[4] Blood creatine phosphokinase increased, attributed to strenuous exercise.
[5] Infusion-related reaction, injection-related reaction, injection site pain, or injection site reaction.

Data Extract: 29JUL2022

Most Common[1] TEAEs

Preferred Term (Any Grade)	All Placebo (N=22)	All ALPN-303 (N=44)
Headache or Migraine	**4 (18%)**	**11 (25%)**
Grade 1	4 (18%)	9 (20%)
Grade 2	0	2 (5%)
Infections[2]	**4 (18%)**	**6 (14%)**
Grade 1	3 (14%)	5 (11%)
Grade 2	1 (5%)	1 (2%)
Dizziness[3]	**1 (5%)**	**5 (11%)**
Grade 1	1 (5%)	4 (9%)
Grade 2	0	1 (2%)
Hypogammaglobulinemia[4]	**0**	**4 (9%)**
Grade 1	0	4 (9%)

[1] Experienced by more than 5% of subjects treated with ALPN-303.
[2] Events coded in system organ class of Infections and Infestations including COVID-19, nasopharyngitis, viral URTI (all G1); URTI (G2) in subjects in the placebo group and COVID-19 (n=2), URTI (n=2), furuncle (all G1); urinary tract infection (G2) in subjects treated with ALPN-303.
[3] 'Dizziness,' 'dizziness postural' or 'presyncope.'
[4] 'Blood immunoglobulin M decreased' or 'hypogammaglobulinemia.'
G1=Grade 1; G2=Grade 2; URTI=upper respiratory tract infection.

Data Extract: 29JUL2022

Figure 5: ALPN-303 Provides Dose-Dependent Pharmacokinetics and Target Coverage

- Dose-dependent PK was observed by both IV and SC routes with good bioavailability (60 to >100% at 80 to 960 mg).
- Estimated half-life ($t_{1/2}$) at 80 to 240 mg is 3.1 to 7.5 days.
- Through Day 28 post-dose, >95% coverage of APRIL achieved by both IV and SC 240 mg doses.

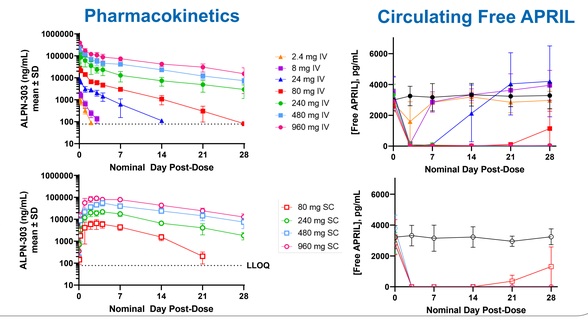
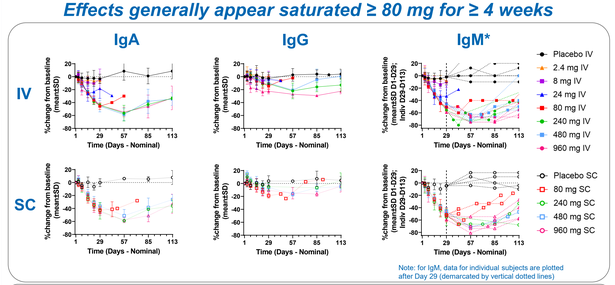


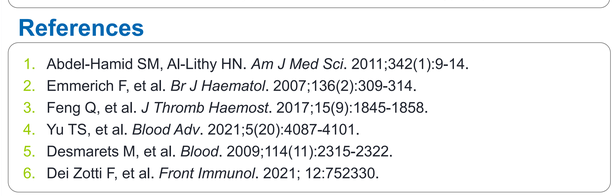
Figure 6: ALPN-303 Dose-Dependently Reduces Circulating Immunoglobulins and Antibody-Secreting Cells (ASC)

Effects generally appear saturated ≥ 80 mg for ≥ 4 weeks



Antibody-Secreting Cells

CD38^{hi} plasmablasts & plasma cells, of gated CD19+CD27+IgD- memory B cells



References

1. Abdel-Hamid SM, Al-Lithy HN. *Am J Med Sci*. 2011;342(1):9-14.
2. Emmerich F, et al. *Br J Haematol*. 2007;136(2):309-314.
3. Feng Q, et al. *J Thromb Haemost*. 2017;15(9):1845-1858.
4. Yu TS, et al. *Blood Adv*. 2021;5(20):4087-4101.
5. Desmarets M, et al. *Blood*. 2009;114(11):2315-2322.
6. Dei Zotti F, et al. *Front Immunol*. 2021; 12:752330.

Summary and Conclusions

- ALPN-303 is an engineered variant TACI-Fc fusion with enhanced affinity as compared to WT TACI-Fc for both BAFF and APRIL.
- ALPN-303 is efficacious in a mouse model of AIHA, significantly increasing hematocrit while reducing pathogenic anti-RBC autoantibodies.
- In this first-in-human study, ALPN-303 has been well-tolerated as single IV or SC doses of up to 960 mg in adult healthy volunteers. The most frequent adverse event has been mild headache. No severe infections or hypogammaglobulinemia, or cytokine release of any grade, have been observed.
- ALPN-303 demonstrates dose-dependent PK/PD. Coverage of free APRIL is maintained for ≥ 4 wk with 240 mg, corresponding to reductions in serum Ig and antibody-secreting cells. These data support a dose regimen of up to 240 mg SC every 4 weeks in future studies.
- Further clinical development of ALPN-303 in autoimmune cytopenias and other autoantibody-related diseases is strongly supported. Clinical trials in autoimmune cytopenias, including warm AIHA, cold agglutinin disease (CAD), and ITP, as well as in other B cell-associated disorders (e.g., lupus, IgA nephropathy, lupus nephritis, and membranous nephropathy), are in preparation.

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