OP0039

ALPN-303, AN ENHANCED, POTENT DUAL BAFF/APRIL ANTAGONIST ENGINEERED BY DIRECTED EVOLUTION FOR THE TREATMENT OF SYSTEMIC LUPUS ERYTHEMATOSUS (SLE) AND OTHER B CELL-RELATED AUTOIMMUNE DISEASES

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Background: BAFF and APRIL are TNF superfamily members that form homo- and heteromultimers that bind TACI and BCMA on B cells; BAFF also binds BAFF-R. BAFF and APRIL support B cell development, differentiation, and survival, particularly for plasmablasts and plasma cells, and play critical roles in the pathogenesis of B cell-related autoimmune diseases. In nonclinical models, inhibition of either BAFF or APRIL alone mediates relatively modest effects, whereas their co-neutralization dramatically reduces B cell function, including antibody production. Fc fusions of wild-type (WT) TACI (e.g. atacicept and telitacicept) target both BAFF and APRIL and have demonstrated promising clinical potential in e.g. systemic lupus erythematosus (SLE) and IgA nephropathy but have not yet clearly exhibited long-term and/or complete disease remissions.
Objectives: To generate a dual BAFF/APRIL antagonist with inhibitory activity superior to WT TACI and BCMA and with the potential to improve clinical outcomes in B cell-mediated diseases.
Methods: Our directed evolution platform was used to identify a potent variant TNFR domain (vTD) of TACI that exhibits significantly enhanced affinity for BAFF and APRIL as compared to WT TACI; this TACI vTD domain was fused to a human IgG Fc to generate the therapeutic candidate ALPN-303. ALPN-303 was evaluated for functional activity in: 1) human lymphocyte assays, 2) the NOD.*Aec1Aec2* spontaneous model of Sjogren's syndrome (SjS), 3) the bm12-induced mouse model of lupus, 4) the (NZB/NZW)F_1 spontaneous model of lupus, and 5) preclinical rodent and cynomolgus monkey pharmacokinetic/pharmacodynamic studies.
Results: ALPN-303 inhibited BAFF- and APRIL-mediated signaling *in vitro* in human lymphocyte assays, with significantly lower IC_{50} values than WT TACI-Fc and belimumab comparators. In all mouse models evaluated, administration of ALPN-303 rapidly and significantly reduced key lymphocyte subsets including plasma cells, germinal center B cells, and follicular T helper cells. ALPN-303 significantly reduced autoantibodies and sialadenitis in the spontaneous SjS model, inhibited glomerular IgG deposition in the bm12-induced model of lupus, and potently suppressed anti-dsDNA autoAbs, blood urea nitrogen levels, proteinuria, sialadenitis, kidney lesions, and renal immune complex deposition in the NZB/W lupus model. As compared to WT TACI-Fc, ALPN-303 exhibited higher serum exposure and significantly and persistently decreased titers of serum IgM, IgG, and IgA antibodies in mice and cynomolgus monkeys (Figure 1).

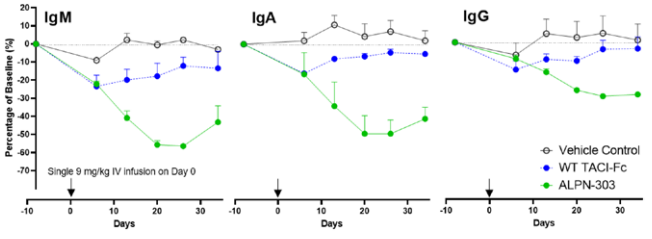

Figure 1. ALPN-303 induces more potent suppression, as compared to WT TACI-Fc, of serum immunoglobulins following a single 9 mg/kg IV infusion (on Day 0; arrows) in female cynomolgus monkeys.

Conclusion: ALPN-303 is a potent BAFF/APRIL antagonist derived from our directed evolution platform that consistently demonstrates encouraging immunomodulatory activity and efficacy *in vitro* and *in vivo*, superior in preclinical studies to anti-BAFF antibody and WT TACI-Fc. This novel Fc fusion molecule demonstrates favorable preliminary developability characteristics, including higher serum exposures and more potent immunosuppressive activities, which may enable lower clinical doses and/or longer dosing intervals than WT TACI-Fc therapeutics. ALPN-303 may thus be an attractive development candidate for the treatment of multiple autoimmune and inflammatory diseases, particularly B cell-related diseases such as SLE, SjS, and other connective tissue diseases. Preclinical development is underway to enable the initiation of clinical trials later this year.

Disclosure of Interests: Stacey R. Dillon Shareholder of: Alpine Immune Sciences, Bristol Myers Squibb, Employee of: Alpine Immune Sciences, Bristol Myers Squibb, Lawrence S. Evans Shareholder of: Alpine Immune Sciences, Employee of: Alpine Immune Sciences, Katherine E. Lewis Shareholder of: Alpine Immune Sciences, Employee of: Alpine Immune Sciences, Jing Yang Shareholder of: Alpine Immune Sciences, Employee of: Alpine Immune Sciences, Mark W. Rixon Shareholder of: Alpine Immune Sciences, Employee of: Alpine Immune Sciences, Joe Kuijper Shareholder of: Alpine Immune Sciences, Employee of: Alpine Immune Sciences, Dan Demonte Shareholder of: Alpine Immune Sciences, Employee of: Alpine Immune Sciences, Janhavi Bhandari Shareholder of: Alpine Immune Sciences, Employee of: Alpine Immune Sciences, Steve Levin Shareholder of: Alpine Immune Sciences, Employee of: Alpine Immune Sciences, Kayla Kleist Shareholder of: Alpine Immune Sciences, Employee of: Alpine Immune Sciences, Sherri Mudri Shareholder of: Alpine Immune Sciences, Employee of: Alpine Immune Sciences, Susan Bort Shareholder of: Alpine Immune Sciences, Employee of: Alpine Immune Sciences, Daniel Ardourel Shareholder of: Alpine Immune Sciences, Employee of: Alpine Immune Sciences, Michelle A. Seaberg Shareholder of: Alpine Immune Sciences, Employee of: Alpine Immune Sciences, Rachel Wang Shareholder of: Alpine Immune Sciences, Employee of: Alpine Immune Sciences, Chelsea Gudgeon Shareholder of: Alpine Immune Sciences, Employee of: Alpine Immune Sciences, Russell Sanderson Shareholder of: Alpine Immune Sciences, Employee of: Alpine Immune Sciences, Martin F. Wolfson Shareholder of: Alpine Immune Sciences, Employee of: Alpine Immune Sciences, Jan Hillson Shareholder of: Alpine Immune Sciences, Employee of: Alpine Immune Sciences, Stanford L. Peng Shareholder of: Alpine Immune Sciences, Employee of: Alpine Immune Sciences
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OP0040

HIPPOCAMPAL IMMUNE CELL TRAFFICKING AND A MYELOID PREDOMINANT INFLAMMATORY RESPONSE WITH ENHANCED ANTIGEN PRESENTATION AND DECREASED LEVELS OF NEUROTRANSMITTERS UNDERLY THE NEUROPSYCHIATRIC PHENOTYPE OF THE NZW/NZB MURINE LUPUS MODEL

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Background: Neuropsychiatric events are common in patients with systemic lupus erythematosus (SLE), yet the underlying pathogenesis remains ill-defined, as the access to brain tissue is limited. We have previously shown that NZW/NZB F1 murine lupus model recapitulates the neuropsychiatric lupus phenotype including depressive-like behavior, increased rates of anxiety, cognitive dysfunction and motor disturbances, both at pre-nephritic and nephritic stages of the disease.
Objectives: To dissect specific regions in the brain, which account for this phenotype and elucidate inflammatory and non-inflammatory mechanisms involved.
Methods: Four distinct brain regions (hippocampus, amygdala, striatum and pre-frontal cortex) were dissected from brains of female C57BL/6 (WT) and NZW/NZB F1 mice at the age of 3 months (pre-nephritic) and 6 months (nephritic stage) (n=5-8/condition/experiment). Since most of the behavioral phenotype corresponds to the hippocampus, we first examined in depth the hippocampal pathology by bulk RNA sequencing, measurements of neurotransmitters levels via high-performance liquid chromatography (HPLC) and by immunophenotyping via flow cytometry analyses. For comparisons, statistical significance was indicated as a two-sided P<0.05.
Results: Transcriptomic analysis revealed aberrant immune mediated response in the hippocampus of 6 month-old lupus mice compared to WT. Specifically, inflammatory pathways including both innate and adaptive immune responses, increased cytokine production, increased antigen presentation and immune cell trafficking, along with increased apoptosis and decreased cell proliferation suggest that immune aberrancies may lead to neuronal damage. These aberrancies were present in mice at 3 month-old, yet were progressed with time being more prominent at 6 month of age in lupus hippocampus. The RNA sequencing date were validated by immunophenotyping on lupus hippocampus demonstrating increased reactive GFAP+ astrocytes both at 3 and 6-month old mice. Activated IBA1+ microglia and CD11b+CD45hi CNS myeloid cells were increased only at 6 months of age. Furthermore, increased immune cell infiltration from the periphery including lymphocytes (CD45+CD11b-) mainly T cells (CD4+/CD8+) and monocytes (CD45+CD11b+Ly6G-Ly6C+), was evident only in 6 month-old lupus hippocampus compared to WT. Importantly, microglia cells in lupus hippocampus at 6 but not at 3 month of age, exhibited increased expression of antigen presenting markers including CD80, CD86 and MHC-II

indicating that microglia cells may carry out the antigen presentation process seen in transcriptomic data. Low levels of serotonin and noradrenaline were observed at both 3 and 6 months of age in lupus mice; these aberrancies were mainly attributed to decreased serotonin synthesis as evidenced by intact serotonin metabolism (no differences were observed at its metabolite: 5-hydroxyindoleacetic acid). Analysis of the remaining regions of the brain combined with studies of metabolic activities of various brain regions by PET-CT scanning is in progress.

Conclusion: Immune cell trafficking from the periphery combined with marked inflammatory response in the hippocampus underlie the neuropsychiatric phenotype in NZW/B murine lupus. Our data indicate increased expression of activated myeloid cells -including microglia- in the hippocampus of lupus mice culminating in increased antigen presentation and decreased neurotransmitter levels.

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OP0041 SALIVA AND SERUM LEVELS OF CXCL13: ASSOCIATION WITH THE SEVERITY OF SALIVARY GLAND LESIONS AND LYMPHOMA IN PATIENTS WITH SJÖGREN'S SYNDROME (SS)

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Background: CXCL13 has been implicated in the formation of ectopic germinal centers (GC) in minor salivary gland (MSG) inflammatory lesions of SS patients. Recent studies suggest that serum CXCL13 levels associate with disease severity and risk for non-Hodgkin's lymphoma (NHL) development.

Objectives: To validate the clinical utility of CXCL13 by investigating potential associations of saliva and serum CXCL13 levels with various histopathologic (including severity of MSG autoimmune infiltrates and GC formation), serologic and clinical features of the disease, as well as NHL.

Methods: CXCL13 levels were measured by a commercially available ELISA (sensitivity: 1 pg/ml; Abcam, Cambridge, UK) in paired serum and saliva specimens from 25 SS patients (9 with NHL; SSL), 9 sicca controls (SC; sicca-complaining individuals with no infiltrates in diagnostic MSG biopsy and negative autoantibody profile) and 6 healthy controls (HC). From the 16 SS patients without evidence of NHL, 5 had mild, 6 intermediate and 5 severe lesions at MSGs, as arbitrarily defined by focus (FS) and Tarpley (TS) biopsy scores (mild: FS:1-1.7, TS:1, intermediate: FS:1.8-2.95, TS:2 and severe: FS: 3.0-11, TS: 3-4). Furthermore, the organization of the MSG infiltrates to GCs has been evaluated in 23 patients revealing 10 with GCs.

Results: Kruskal-Wallis analysis revealed that serum CXCL13 levels were significantly increased in SS patients without or with NHL (median: 94.83 pg/ml and 96.70 pg/ml, respectively), compared to SC and HC (35.44 and 40.92 pg/ml respectively; p<0.05), whereas saliva levels were only marginally increased (76.47, 84.10, 55.98 and 65.30 pg/ml in SS, SSL, SC and HC, respectively, p=0.051). Among SS patients with distinct MSG lesion severity, only those with severe lesions were found to express significantly higher serum CXCL13 levels (149.3 pg/ml) from SC and HC (p: 0,0051 and 0.0166, respectively). Spearman's Rank correlation analysis showed that both serum and saliva levels correlated with SG biopsy focus score (r: 0.6889, p=0.0001 and r: 0.4222, p=0.01, respectively). Mann-Whitney test revealed that serum CXCL13 levels were significantly elevated in patients with GCs at MSG lesions (156.1 *vs* 69.64 pg/ml, p:0.0015), rheumatoid factor (105.0 *vs* 53.72 pg/ml, p: 0.015) and marginally with anti-Ro/La antibodies (121.8 *vs* 65.05 pg/ml, p: 0.06) compared to those without. Furthermore, CXCL13 levels were significantly increased in SS patients at high risk to develop NHL compared to low risk (149.3 *vs* 71.54 pg/ml, respectively, p: 0.0275). Saliva levels were not found to associate with the studied features.

Conclusion: Serum and to a lesser extend saliva CXCL13 levels are increased in SS and SSL patients and associate with the degree of MSG infiltration, as assessed by focus score. Serum, but not saliva, CXCL13 associates with various disease features, including GC formation, and may have a clinical utility in identifying SS patients at high risk to develop lymphoma.

Disclosure of Interests: None declared

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OP0042 BLOCKING OF CD103+ TISSUE RESIDENT MEMORY T CELLS (TRM) AS A THERAPEUTIC STRATEGY IN SJOGREN'S SYNDROME

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Background: Tissue-resident memory T cells (TRM), are a recently identified T cells population featuring tissue localization and expression of markers of tissue homing, CD69 and CD103. Recently, the expansion of CD8+ TRMs and their involvement in the sialadenitis was described in a murine model of SS. However, CD4+ and CD8+ TRM's functional relevance in pSS is still not fully understood, and the TRM therapeutic targeting unexplored.

Objectives: The study aimed to address the role of $CD4^+$ and $CD8^+$ TRMs in the pathogenesis of pSS and to explore the therapeutic targeting of the tissue residency marker of TRM CD103.

Methods: An animal model of experimental (ESS) obtained by immunization of female C57BL/6 mice (n=10) with salivary glands (SG) protein extract and Freund's complete adjuvant used to investigate the dynamic of infiltration of SG by $CD4^+$ and $CD8^+$ TRMs, their frequency, and the impact of CD103 blockade. For the therapeutic intervention, at 10-weeks post-immunization, the salivary gland was cannulated via Wharton's duct, and an anti-CD103 neutralizing antibody or vehicle-injected. The mice's saliva flow rate was assessed, and SGs were analyzed by Flow-cytometry and immunohistochemistry (IHC).

The frequency and localization of TRMs was analyzed in minor SG of sicca syndrome (nSS) and pSS patients (n=39) by flow cytometry and IHC. The expression of genes involved in the tissue retention of TRMs was assessed in SG by RT-PCR.

Results: Upon the ESS progression, a significant progressive increase in $CD45^+CD103+$ cells frequency was observed from 5wk to 20wk post-immunization (p<0.001), where the $CD8^+$ were the most abundant, followed by $CD4^+$. Consistently, $CD103^+CD8^+$ T cells were detected within the lymphocytic infiltration of SG from ESS mice. Sorted purified SG $CD10^+CD3^+CD8^+$ T cells showed higher Granzyme B, TNF-alpha expression compared to $CD103^-CD3^+CD8^+$ at both mRNA and protein levels. Notably, ESS mice treated with anti-CD103 showed improvement in salivary function (p<0.05) and reduced lymphocytic infiltrations measured as focus score (FS) (p<0.01) and area-fraction (p<0.01). Consistently, anti-CD103 treatment consistently reduced $CD103^+$ cells and IFN-gamma$^+$, Granzyme B^+, and $TNFa^+$ $CD8^+$ cells. We next performed phenotypic analysis of $CD45^+CD103^+$ immune cells in the SG of pSS patients observing an increase in both with $CD8^+CD103^+CD69^+$ and $CD4^+CD103^+CD69^+$ (p<0.05). Finally, IHC showed that the expansion of TRMs in pSS salivary glands was accompanied by a down-regulation of E-cadherin glandular expression and their migration outside the epithelium in the context of inflammatory infiltrates. SG of patients with pSS showed a significant up-regulation of BLIMP1, KFL-2, and S1PR1 and down-regulation of ITGB2. CXCL9 and CXCL10, and IL-15 involved in the tissue recruitment and long-term survival of TRMs were significantly modulated in pSS salivary glands.

Conclusion: TRM are expanded and activated in the SG of pSS and ESS, participating in the organization of tissue inflammation. Although the mechanisms behind this expansion are still not fully understood, CD103 could be a valuable novel therapeutic target to prevent lymphocytic infiltrations and glandular destruction in Sjogren syndrome.

Disclosure of Interests: None declared

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OP0043 INCREASED RISK OF SEVERE INFECTIONS AND MORTALITY IN PATIENTS WITH NEWLY DIAGNOSED SYSTEMIC LUPUS ERYTHEMATOSUS: A POPULATION-BASED STUDY

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Background: Systemic lupus erythematosus (SLE) is a chronic disease with a broad spectrum of autoantibodies and clinical manifestations. As much as 45%