**ABSTRACT**

BTLA is a co-inhibitory checkpoint receptor that regulates activation of T cells, B cells and dendritic cells. The interaction of BTLA with its ligand HVEM induces inhibitory signals that regulate immune cell function. ANB032 is a novel BTLA agonist (IgG4 antibody that does not compete with the binding of BTLA to HVEM). Upon binding to BTLA, and simultaneous Fc receptor engagement to an opposing cell, ANB032 induced inhibitory signaling, reduced T cell proliferation and reduced inflammatory cytokine secretion. In vitro, ANB032 reduced Th1, Th2, Th17 and Th22 inflammatory cytokine secretion from atopic dermatitis patient derived PBMCs. To determine the efficacy and the immune regulatory effects of ANB032, we ran an experiment comparing it to reference BTLA agonist antibodies in a human xenograft GvHD mouse model. Compared to the reference antibodies, ANB032 demonstrated superior in vivo efficacy on key endpoints, including improved survival, body weight maintenance, reduced human T cell expansion and reduced plasma inflammatory cytokines. ANB032 demonstrated, both in vitro and in vivo, the potential therapeutic benefit that BTLA agonism may provide in the treatment of autoimmune or inflammatory diseases and is being evaluated in an ongoing Phase 2 study in atopic dermatitis (NCT05935085).

**BACKGROUND AND OBJECTIVE**

- Co-inhibitory immune checkpoint receptors, such as B and T Lymphocyte Attenuator (BTLA), regulate the immune system in part through their expression pattern on specific immune cells, including effector T cells, and attenuate an overactive immune response when agonized
- BTLA is expressed preferentially on activated T cells, B cells, and dendritic cells (DCs), which are key contributors to inflammatory diseases
- ANB032 is an investigational BTLA agonist antibody that has been shown to reduce activated T cell proliferation, reduce inflammatory cytokine secretion (Th1, Th2, Th17, Th22), and modulate DC function while inducing Treg
- Atopic dermatitis (AD) is a systemic, heterogenous inflammatory disease with pathogenesis driven by Th1, Th2, Th17, Th22, and DCs, both in the skin and in the peripheral blood
- In vivo efficacy and immune regulatory effects of ANB032 were tested in a humanized mouse model of GvHD, where human peripheral blood mononuclear cells (PBMCs) were adoptively transferred into irradiated NSG mice. Body weight, human T cell expansion and survival were assessed
- ANB032 and two reference BTLA agonists were evaluated to determine the effect of potency as well as the blocking of the natural HVEM/BTLA binding interaction.
- ANB032 is non-blocking of HVEM (HVEM sparing) and secreted IgG4 Ref1 is a BTLA agonist that spares HVEM binding to BTLA and Ref2 blocks HVEM binding to BTLA: both Ref1 and Ref2 lack Fc receptor engagement due to mutations made in the Fc domain

**METHODS**

**Humanized Mouse Model of Xenogeneic Acute GvHD**
- NOD-skid IL-2/γnull mice were engrafted with human PBMCs one day prior to 4-week BiW dosing with BTLA agonist antibodies or isotype control (Figure 1)
- Blood was collected for fluorescence-activated cell sorting (FACS) analysis at study day 18 midpoint (determined when isotype control animals exhibited GvHD manifestations) (Figure 2)
- Clinical observations of survival, body weight and DAi (fur, skin, posture and activity) were collected (Figure 3)
- Inflammatory cytokine levels were measured from plasma collected at midpoint (SD18) (Figure 4)

**AD Patient-Derived PBMCs**
- PBMCs from AD donors were treated with anti-CD3 and anti-CD28 in vitro for 72h to stimulate T cell proliferation, in the presence of ANB032 or isotype control mAb. Supernatants were collected for analysis by MSD to measure inflammatory cytokine secretion (Figure 5).

**CONCLUSIONS**

- In a human xenograft GvHD mouse model, ANB032:
  - Reduced T cell expansion
  - Reduced inflammatory cytokines in plasma
- Demonstrated superior in vivo efficacy on key endpoints, including prolonged survival, maintained body weight and an overall reduced disease activity index (DAI), compared to reference BTLA agonist antibodies
- Illustrated the importance of not blocking HVEM, the natural ligand, resulting in enhanced potency when compared to a leading BTLA agonist reference
- ANB032 reduced Th1, Th2, Th17 and Th22 inflammatory cytokine secretion from atopic dermatitis patient-derived PBMCs
- ANB032 is currently being evaluated in an ongoing Phase 2 study in moderate-to-severe AD (NCT05935085)

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**REFERENCES**