ANB032, a BTLA Checkpoint Agonist Monoclonal Antibody, Reduced T Cell Proliferation, Inflammatory Cytokine Secretion and Prevented Graft versus Host Disease (GvHD) in a Mouse Model

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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).

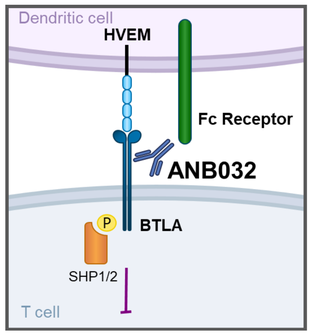
BACKGROUND AND OBJECTIVE

- 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 Tregs[1]
- 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 periphery; ANB032 may potentially play a role in reducing inflammation and restoring immune homeostasis
- 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 to 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 WT IgG4. Ref1 is a BTLA agonist that spares HVEM binding to BTLA and Ref2 blocks HVEM binding to BTLA; both Ref1 and Ref2 lack FcR engagement due to mutations made in the Fc domain

Antibody characteristics of ANB032, Ref1 & Ref2

	ANB032	Ref1	Ref2
HVEM Sparing	✓	✓	✘
FcR Engagement	✓	✘	✘

Proposed Mechanism of Action for ANB032



Schematic of proposed MOA

BTLA is key node of immune regulation
- BTLA is a potent co-inhibitory checkpoint receptor
- Expressed only on immune cells and preferentially on activated immune cells
- Dysregulation of BTLA pathway accelerates onset and exacerbates disease

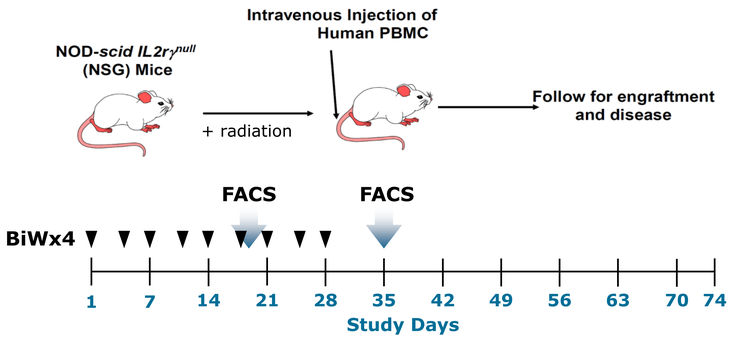
ANB032: IgG4 antibody (non-depleting)
- Binds BTLA proximal to immune cell
- Fc receptor binding contributes to differentiated potency
- Non-blocking of HVEM engagement

METHODS

Humanized Mouse Model of Xenogeneic Acute GvHD
- NOD-skid IL-2rγnull 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 TiW **(Figure 3)**
- Inflammatory cytokines 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)**.

Figure 1. Humanized Mouse Model of GvHD



Group	N	Test Agent	Dose
1	6	Isotype Control	3 mg/kg
2	15	ANB032	3 mg/kg
3	15	BTLA Agonist Ref1	3 mg/kg
4	5	BTLA Agonist Ref2	10 mg/kg

Terminal Endpoints:
- Weight loss
- Death
- GvHD disease activity index (DAI) scores (Fur, skin, posture, activity)

Figure 1. Schematic of humanized mouse model of GvHD with study details

Figure 2. ANB032 Reduced Human T Cell Expansion

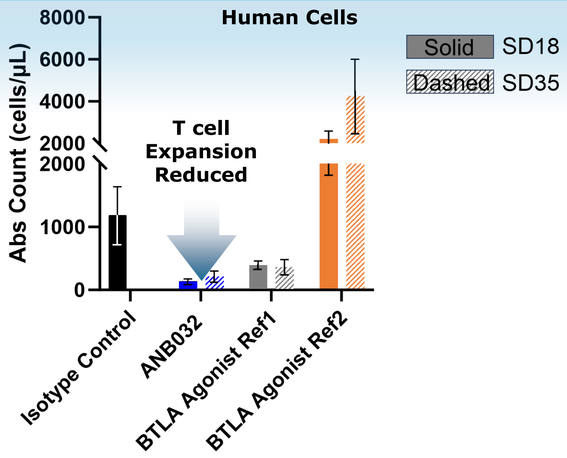


Figure 2. Quantification of circulating human CD45+ cells in whole blood of mice surviving at Study Day 18 (SD18 solid fill) and 35 (SD35 dashed fill)

Figure 3. ANB032 Resulted in Prolonged Survival and Reduced Disease Activity Index

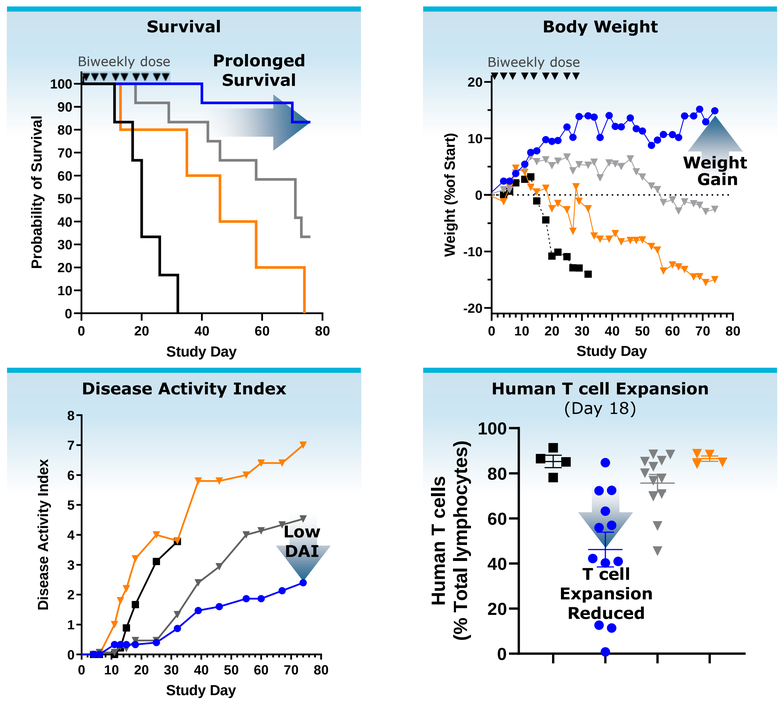


Figure 3. Evaluation of clinical observations (survival, body weight, and disease activity index) and human T cell expansion

Figure 4. ANB032 Reduced Inflammatory Cytokines

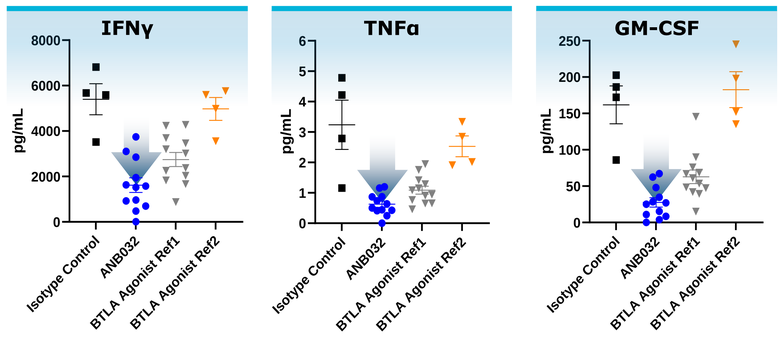


Figure 4. Plasma inflammatory cytokines were evaluated at Study Day 18 in the GvHD model

Figure 5. ANB032 Reduced Th1, Th2, Th17 and Th22 Cytokine Secretion in AD patient-derived PBMCs in vitro

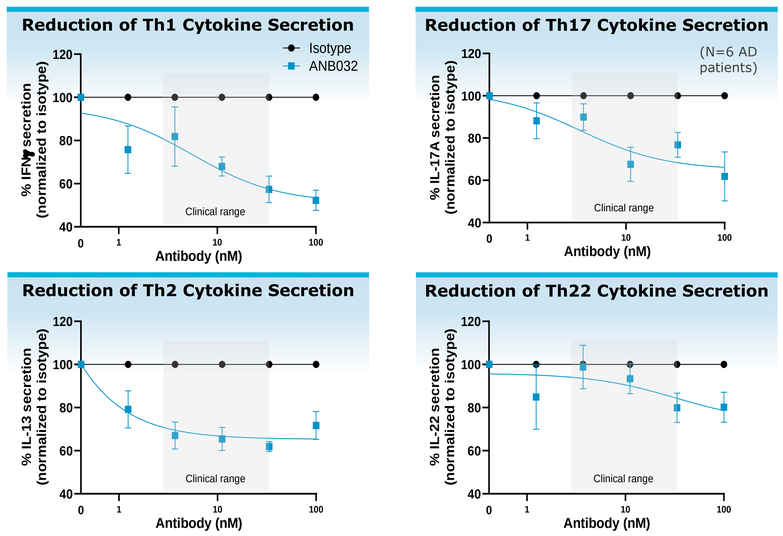


Figure 5. In vitro evaluation of IFN-γ, IL-17A, IL-13, and IL-22 secretion in AD patient-derived PBMCs

- ANB032 broadly inhibited T cell inflammatory cytokine secretion in AD patient-derived PBMCs

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)

ACKNOWLEDGEMENTS

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2. Author disclosures: All authors are employees of Anaptys

REFERENCES

1. Muench, et al. Presented at AAD 2024.