POSTER# TU131

ANB033, a Novel CD122 Antagonist in Development for the Treatment of Inflammatory and Autoimmune Diseases, Inhibited IL-15 and IL-2 Signaling in CD8+ and CD4+ T Cells In Vitro and in a Murine Model of GvHD

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ABSTRACT

CD122 is the beta subunit of the receptors for both IL-15 and IL-2. It regulates cytotoxic CD8+ T, memory CD4+ T, and natural killer (NK) cell survival, proliferation, and differentiation. In inflammatory or autoimmune diseases, IL-15 and IL-2 overexpression and signaling via CD122 can lead to and the persistence of pathogenic CD8+ T, CD4+ T, and NK cells; therefore, CD122 represents a promising therapeutic target. ANB033 is a novel CD122 antagonist monoclonal antibody engineered to bind to an optimal epitope on CD122 with high affinity, resulting in potent inhibition of IL-15 and IL-2 signaling. In vitro, ANB033 inhibited IL-15- and IL-2-mediated STAT5 signaling in cytotoxic CD8+ T cells, memory CD4+ T cells, and NK cells, leading to downstream inhibition of proliferation and inflammatory cytokine production. ANB033 was evaluated in a xenogeneic graft-versus-host disease (X-GvHD) model, which reflects pathogenic mechanisms driven by activated CD8+ and CD4+ T cells. Mice treated with ANB033 demonstrated potent reduction of human CD8+ and CD4+ T cell numbers, reduced body weight loss, and a greater survival benefit compared to isotype control or belatacept (CTLA-4 Ig). Additionally, mice treated with ANB033 exhibited prolonged survival beyond the treatment period. ANB033 significantly decreased GvHD-related pathology in the murine model and may provide therapeutic value in T cell mediated inflammatory and autoimmune diseases where IL-15 and IL-2 are pathogenic drivers of disease. These data support the rationale for evaluating ANB033 in an ongoing Phase 1b study in celiac disease.

BACKGROUND & OBJECTIVE

CD122

- Shared beta subunit (IL2Rβ) of IL-15 and IL-2 receptors
  - Forms intermediate affinity dimeric receptor for IL-2 and IL-15 when paired with CD132 (Fig.1)
  - Forms high affinity trimeric IL-2 receptor or IL-15 receptor when CD132 is paired with CD25 or CD215, respectively
- IL-15 and IL-2 are central cytokines in pathogenic inflammation:
  - Signaling drives proliferation and survival of cytotoxic CD8+ and CD4+ T cell subsets, and NK cells
  - Sustaining T cell activation and downstream Th1 and Th2 cytokine production
- CD122 is expressed on subsets of CD8+ and CD4+ T cells, and NK cells
- In inflammatory or autoimmune diseases, including celiac disease and EoE, CD122 is also overexpressed on intraepithelial lymphocytes (IELs), including CD8+ T cells and NK cells

ANB033 (CD122 antagonist mAb)

Proposed mechanism of action and impact on pathogenic immune cells

- Inhibition of both IL-15 and IL-2 signaling in subsets of CD122-expressing CD8+ T cells, CD4+ T cells, and NK cells resulting in:
  - Reduced proliferation
  - Reduced survival
  - Reduced inflammatory cytokine secretion

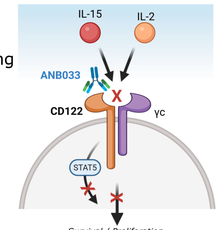

Figure 1. Proposed MOA of ANB033

Objective

To evaluate impact of ANB033 on:

- Inhibition of IL-15- and IL-2 STAT5 signaling, proliferation and cytokine production in CD8+ T cells, CD4+ T cells, and NK cells in vitro
- Pathogenic mechanisms driven by activated CD8+ and CD4+ T cells in a murine model of GvHD

METHODS

In vitro Pharmacology

Phospho-STAT5 (pSTAT5) was analyzed via flow cytometric analysis of PBMCs from 6 healthy donors that were stimulated with a combination of IL-2 and IL-15 for 20 minutes to assess the effect of inhibition of short-term immediate CD122 signaling. Background MFI was subtracted before normalizing the MFI at each concentration with the MFI observed following treatment with an equivalent concentration of isotype control. To assess longer-term impacts of CD122 inhibition, cytokine-induced cell proliferation was evaluated via flow cytometric analysis of Ki67 expression in PBMCs from 6 healthy donors that were stimulated with a combination of IL-2 and IL-15 for 6 days. The percent of proliferating cells (Ki67+) were normalized at each concentration to that of an equivalent concentration of isotype control. To assess the impact of ANB033 on TCR-induced cytokine production, PBMCs from 4 healthy donors were stimulated with anti-CD3/CD28 for 3 days, followed by MSD analysis. Cytokine concentrations were normalized to an isotype control and statistical analysis was performed using a one-way ANOVA with a Dunnett's Test for 2 simultaneous comparisons (*p<0.05, **p<0.01, ***p<.001, ****p<0.0001).

Murine Model of GvHD

A xenogeneic graft versus host disease (X-GvHD) model, in which human PBMCs were transferred into immunodeficient mice that constitutively expressed human IL-15, was run for 60 days (Fig. 2). Mice were dosed 3mg/kg intraperitoneally twice weekly with isotype control, a reference anti-CD122 antibody, or ANB033 for 28 days. Belatacept (CTLA-4 Ig), a reference standard of care (SOC), was dosed 3 times/week at 75 µg for 28 days as a positive control. Efficacy was evaluated through measurements of survival, body weight loss, plasma cytokines, bulk RNAseq, flow cytometry and IHC at study day 17 (SD17), defined as the timepoint of disease onset for isotype control animals.

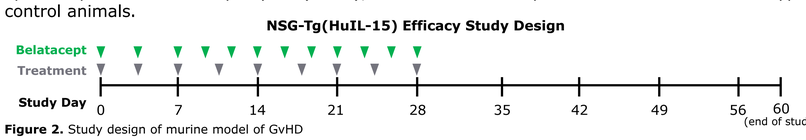

Figure 2. Study design of murine model of GvHD

RESULTS

ANB033 reduced pSTAT5, proliferation, and cytokine release from stimulated human PBMCs

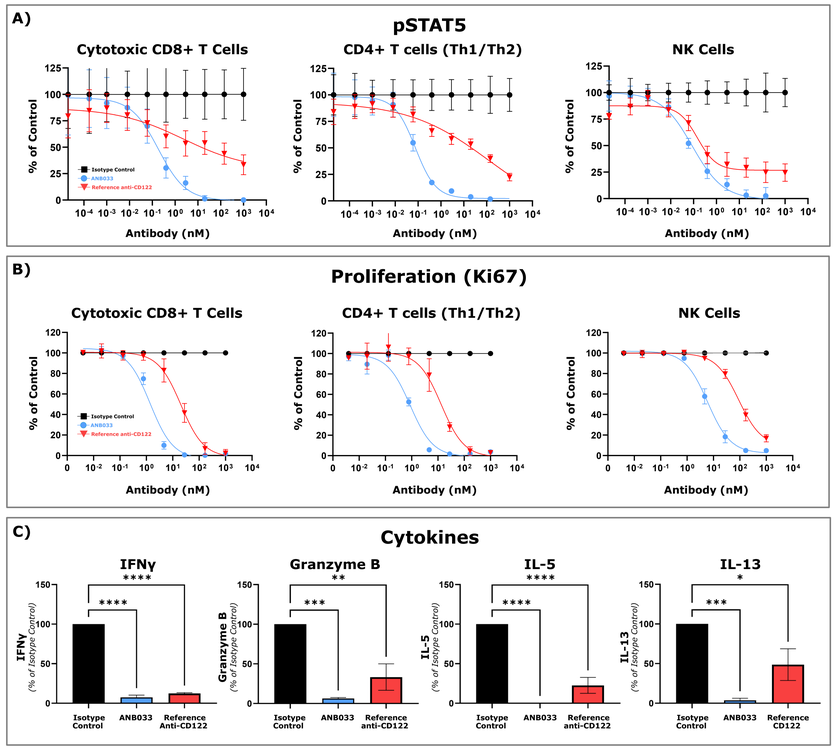

Figure 3. Evaluation of cytokine-induced pSTAT5 (A), cytokine-induced proliferation (B), and anti-CD3/CD28-induced cytokine release in PBMCs from healthy donors (C)

In human PBMCs, compared to isotype control and a reference anti-CD122 mAb:

- ANB033 reduced pSTAT5 signaling and proliferation of CD8+, CD4+ T cells and NK cells
- ANB033 reduced cytokine production (IFNγ, granzyme B, IL-5, and IL-13)

ANB033 showed greater survival benefit and reduced body weight loss compared to standard of care and isotype control in a murine model of GvHD

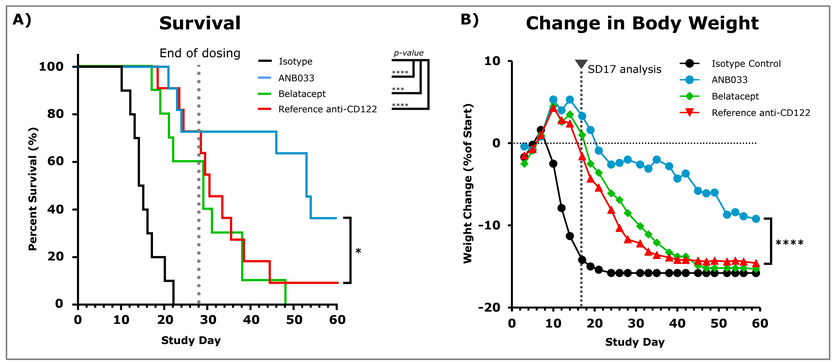

Figure 4. Overall survival (A) and cumulative percentage change from baseline body weight curves (B) of mice in a X-GvHD model following treatment with isotype control antibody, belatacept, a reference anti-CD122 mAb, or ANB033

In a murine model of GvHD, mice treated with ANB033 demonstrated:

- Significantly improved survival benefit over isotype control, belatacept, and reference anti-CD122 (p<0.0001, 0.003 and 0.03 respectively, log-rank Mantel-Cox test)
- Prolonged survival beyond the 28-day treatment period through Day 60
  - 38.5, 23 and 22 days greater median survival over isotype control, belatacept and a reference anti-CD122 mAb, respectively
- Significantly less body weight loss over the duration of the study compared to isotype control (p<0.001), belatacept (p=0.0016), and a reference anti-CD122 mAb (p=0.0003); unpaired Student's t-test

RESULTS

ANB033 reduced plasma cytokines, cytolytic gene expression, splenic CD8+ and CD4+ T cells, and intestinal T cell infiltration

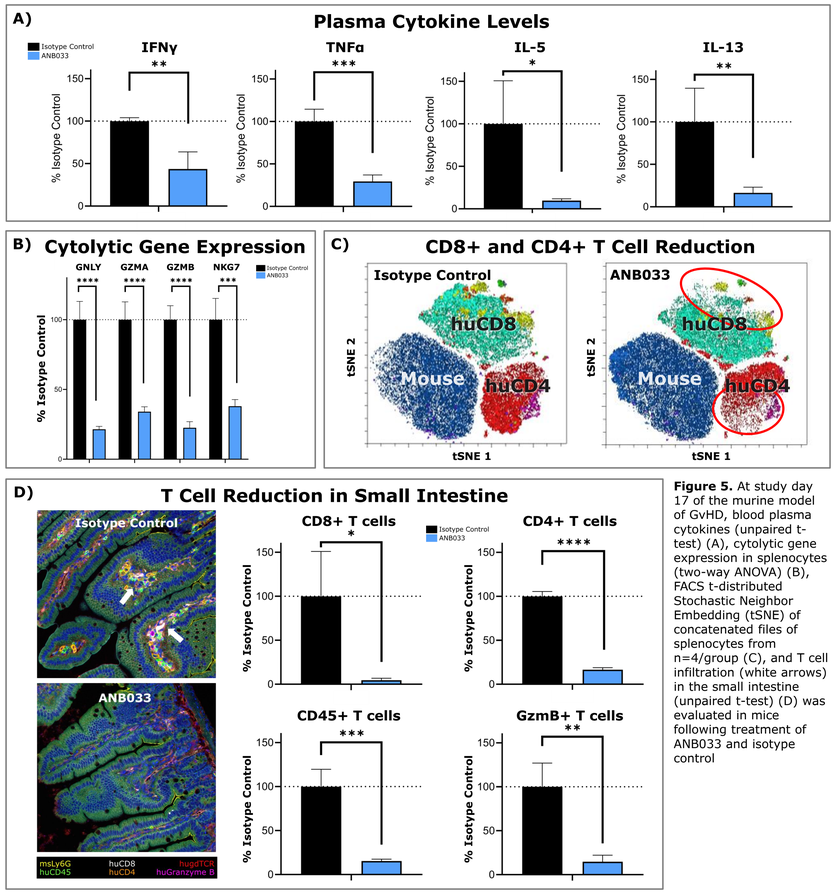

Figure 5. At study day 17 of the murine model of GvHD, blood plasma cytokines (unpaired t-test) (A), cytolytic gene expression in splenocytes (two-way ANOVA) (B), FACS t-distributed Stochastic Neighbor Embedding (tSNE) of concatenated files of splenocytes from n=4/group (C), and T cell infiltration (white arrows) in the small intestine (unpaired t-test) (D) was evaluated in mice following treatment of ANB033 and isotype control

In a murine model of GvHD, compared to isotype control:

- ANB033 reduced plasma cytokines (IFNγ, TNFα, IL-5, and IL-13)
- ANB033 reduced cytolytic gene expression of GNLY, GZMA, GZMB, and NKG7
- ANB033 reduced CD8+ and CD4+ T cells in splenocytes (circled, Fig. 5C)
- ANB033 reduced small intestinal infiltration of CD8+, CD4+, CD45+, and GzmB+ T cells

CONCLUSION

In stimulated human PBMCs, compared to isotype control and a reference anti-CD122 antibody, ANB033 demonstrated:

- Greater reduction of pSTAT5 and proliferation induction by IL-15 and IL-2
- Greater reduction of inflammatory cytokines (IFNγ, IL-5, IL-13) and cytotoxicity mediators (Granzyme B) induced by anti-CD3/CD28 stimulation

In a murine model of GvHD, ANB033 demonstrated:

Compared to isotype control, reference anti-CD122 mAb, and belatacept:
- Reduced body weight loss over the duration of the study
- Improved survival benefit and prolonged survival >30 days beyond the treatment period

Compared to isotype control:
- Inhibited human PBMC engraftment
- Reduced plasma cytokine levels, corroborating RNAseq DEG results
- Reduced CD8+ and CD4+ T cells and cytolytic gene expression in splenocytes
- Reduced small intestinal inflammation and T cell infiltration into the gut

ACKNOWLEDGEMENTS

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