

ANB032, a Novel BTLA/HVEM Therapeutic Antibody, Inhibited T cells Derived from Atopic Dermatitis Patients *In-vitro*



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Abstract

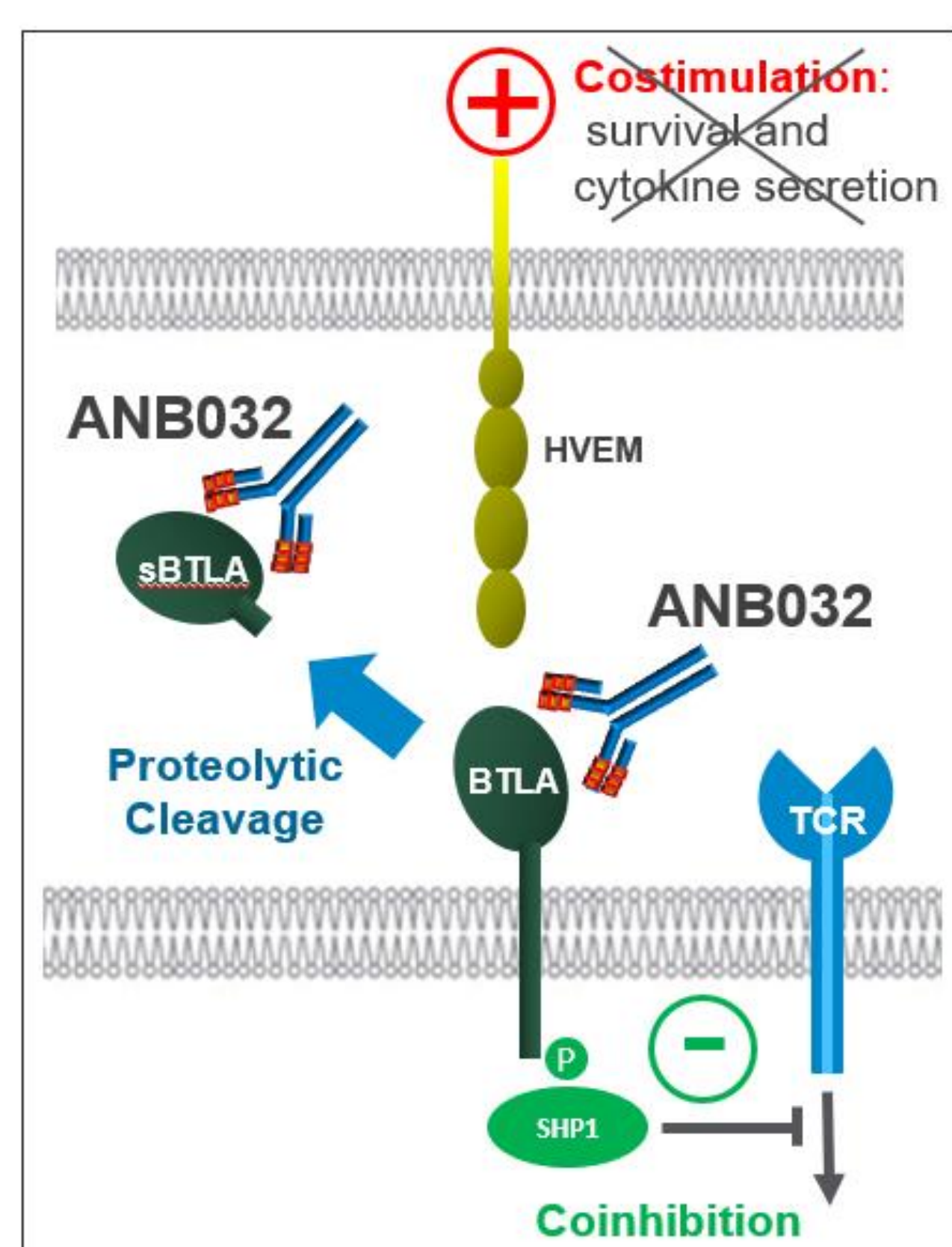
B and T lymphocyte attenuator (BTLA) is an immune checkpoint molecule that contributes to the regulation of T cell function. The role of BTLA as a regulator of inflammatory disease and cancer has been demonstrated via genetics and animal models. Recently, several studies have described soluble checkpoint molecules, including soluble BTLA (sBTLA), in the serum or in tumors of diseased patients that correlates negatively with disease outcome, suggesting that sBTLA is a biomarker and/or a direct mediator of immune suppression.

ANB032 is a novel anti-BTLA monoclonal antibody that modulates BTLA signaling *in-vivo*. In an acute humanized murine GvHD disease model ANB032 significantly prolonged animal survival, reduced pathogenic human T cell activation and expansion, and reduced BTLA expression in a dose-dependent manner. Furthermore, ANB032 induced human sBTLA release from human T cells in the GvHD model. ANB032 also induced sBTLA release in cynomolgus monkeys treated with ANB032, in a dose-dependent manner. Together, the data demonstrates that ANB032 inhibits T cell-mediated inflammation *in-vivo* and ANB032-mediated release of sBTLA is a biomarker and/or direct mediator of the immunosuppressive mechanism of ANB032 *in vivo*.

We investigated the levels of sBTLA and LIGHT in atopic dermatitis patient-derived serum samples. We observed that sBTLA was significantly decreased, while LIGHT, an inflammatory ligand of HVEM, was significantly increased compared to healthy donors, suggesting dysregulation of a normal immune suppressive pathway in the setting of inflammatory disease.

Using *in-vitro* human primary patient-derived PBMC stimulation assays, we demonstrated that ANA032 reduced T cell proliferation, reduced secretion of IFN γ , and reduced T cell BTLA expression from both atopic dermatitis-derived and healthy donor-derived PBMC. We propose that ANB032, by directly inhibiting T cell activity and inducing sBTLA release, has the potential to restore immune suppression in inflammatory diseases where dysregulated T cells-mediate pathology, such as in atopic dermatitis.

Introduction: ANB032 inhibits T cell proliferation and induces the release of soluble BTLA *in-vivo*



BTLA, which is closely related to PD-1 and CTLA-4, was first identified as a negative immunological regulator on T and B cells. BTLA expression levels vary among lymphoid and myeloid subtypes, with highest expression on B, but also significant expression on T cells as well as on mature dendritic cells, macrophages and natural killer cells (Han et al., 2004; Hurchla et al., 2005). Herpes-Virus entry mediator (HVEM) is the ligand of BTLA. HVEM has appeared as a major and complex co-stimulatory signaling molecule in the past decade.

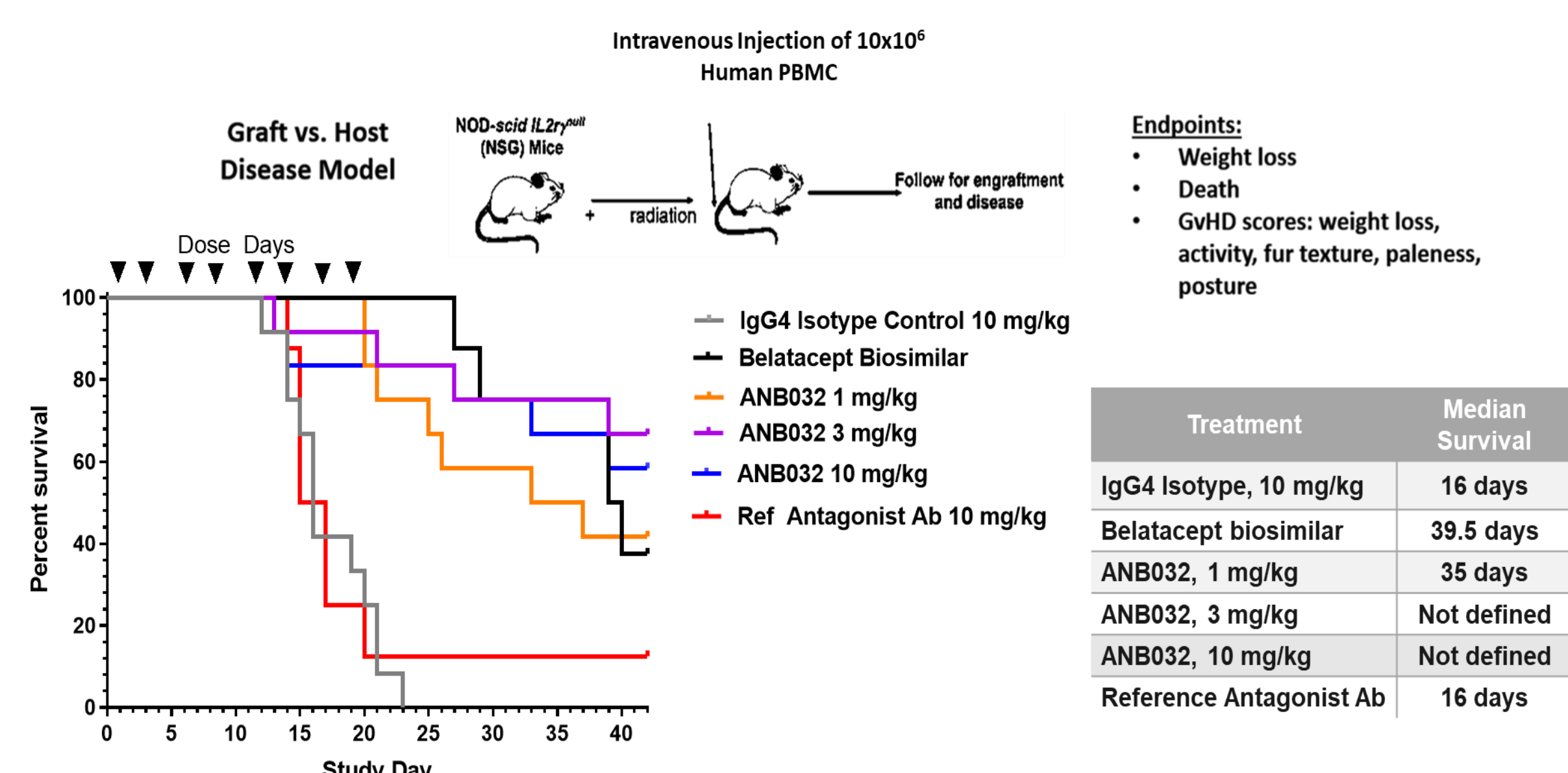
When BTLA and HVEM are engaged in trans, bi-directional signaling occurs with co-inhibition on the BTLA side and co-stimulation on the HVEM side. We've previously shown that ANB032 binds to BTLA on an epitope that doesn't disrupt trans HVEM interaction, triggering BTLA agonism that recruits SHP1 and causes co-inhibition on the BTLA side, while disrupting HVEM signaling in trans and preventing co-stimulation.

Although the interactions between BTLA and HVEM have been studied, new evidence has shown that soluble checkpoint molecules, such as soluble BTLA (sBTLA), are present in a variety of cancer contexts where immunosuppression is occurring.

Atopic dermatitis (AD), a common chronic inflammatory skin disorder, is driven by T-cell response and has a complex pathogenesis that involves dysfunctional T-cell co-inhibition signaling. Co-inhibitors, such as BTLA, are associated with mediating peripheral self-tolerance and limit the immune response. BTLA-deficient T cells in mice show increased proliferation and susceptibility to spontaneous development of autoimmune diseases, including dermatitis, demonstrating that BTLA negatively regulates T cell activation and proliferation (Nakagami et al. 2013 and Bekiaris et al. 2013). Furthermore, BTLA agonistic antibodies in murine dermatitis models reduce T cell proliferation, IFN γ secretion, tissue homing and disease severity.

In this study, we show the ability of ANB032 to inhibit the T cell mediated pathology of atopic dermatitis by reducing T cell proliferation, secretion of IFN γ , and T cell BTLA expression in human primary atopic dermatitis patient-derived PBMC stimulation assays.

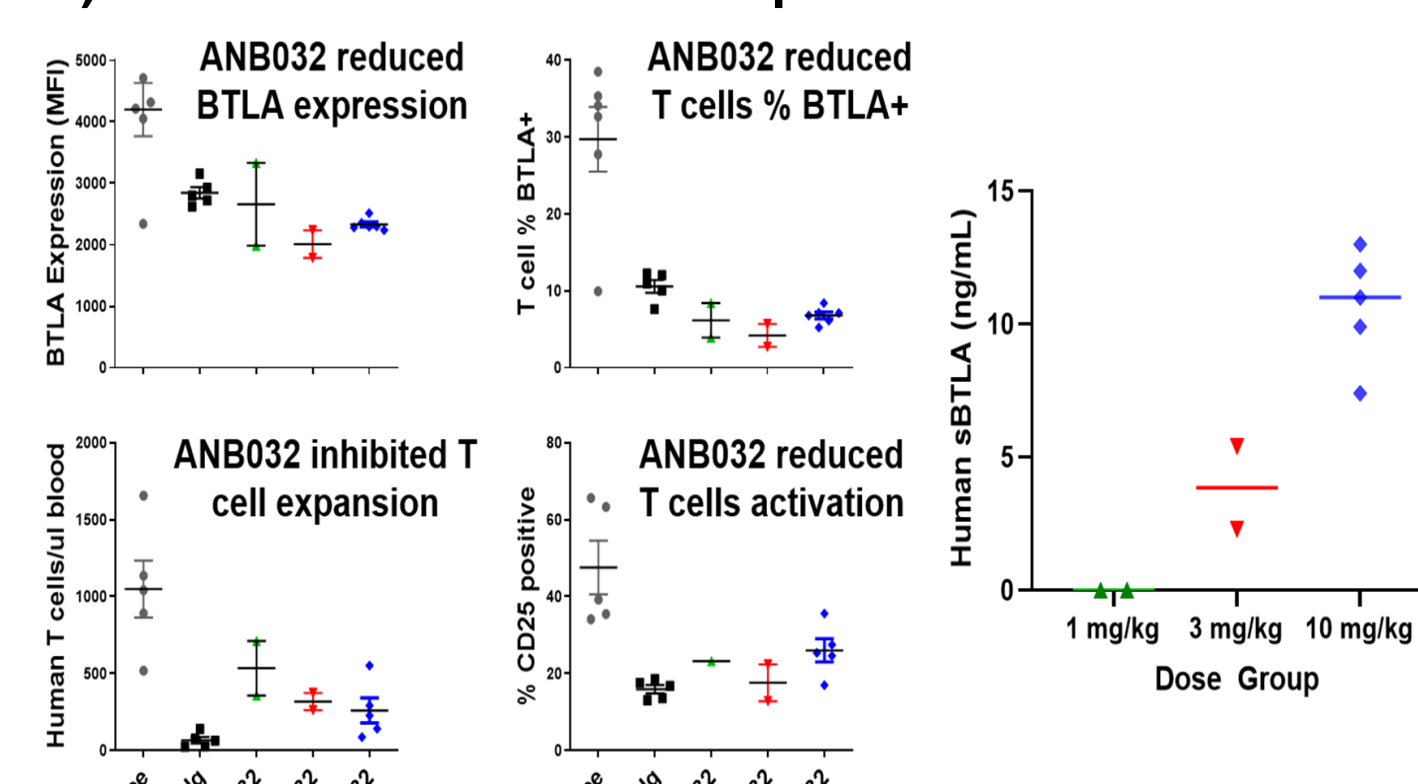
Figure 1: ANB032 is highly efficacious in a humanized murine GvHD model



The *in-vivo* efficacy of ANB032 was evaluated in an acute humanized murine GvHD disease model. This model is a T cell-mediated disease induced by engraftment of human PBMCs in sub-lethally irradiated NSG mice. At the end of the study on Day 42, animals treated with ANB032 at all doses had statistically significant higher overall survival than animals treated with isotype control antibody (10 mg/kg ANB032 dose group versus isotype, $p=0.0019$; 3 mg/kg ANB032 versus isotype, $p=0.0002$; 1 mg/kg ANB032 versus isotype, $p=0.0001$). The 10 mg/kg and 3 mg/kg ANB032 dose groups did not have a significant difference in survival, suggesting that efficacy in the GvHD model may be maximum at doses of 3 mg/kg and higher. No survival benefit was observed in animals treated with the reference antagonist antibody compared to isotype control treated animals.

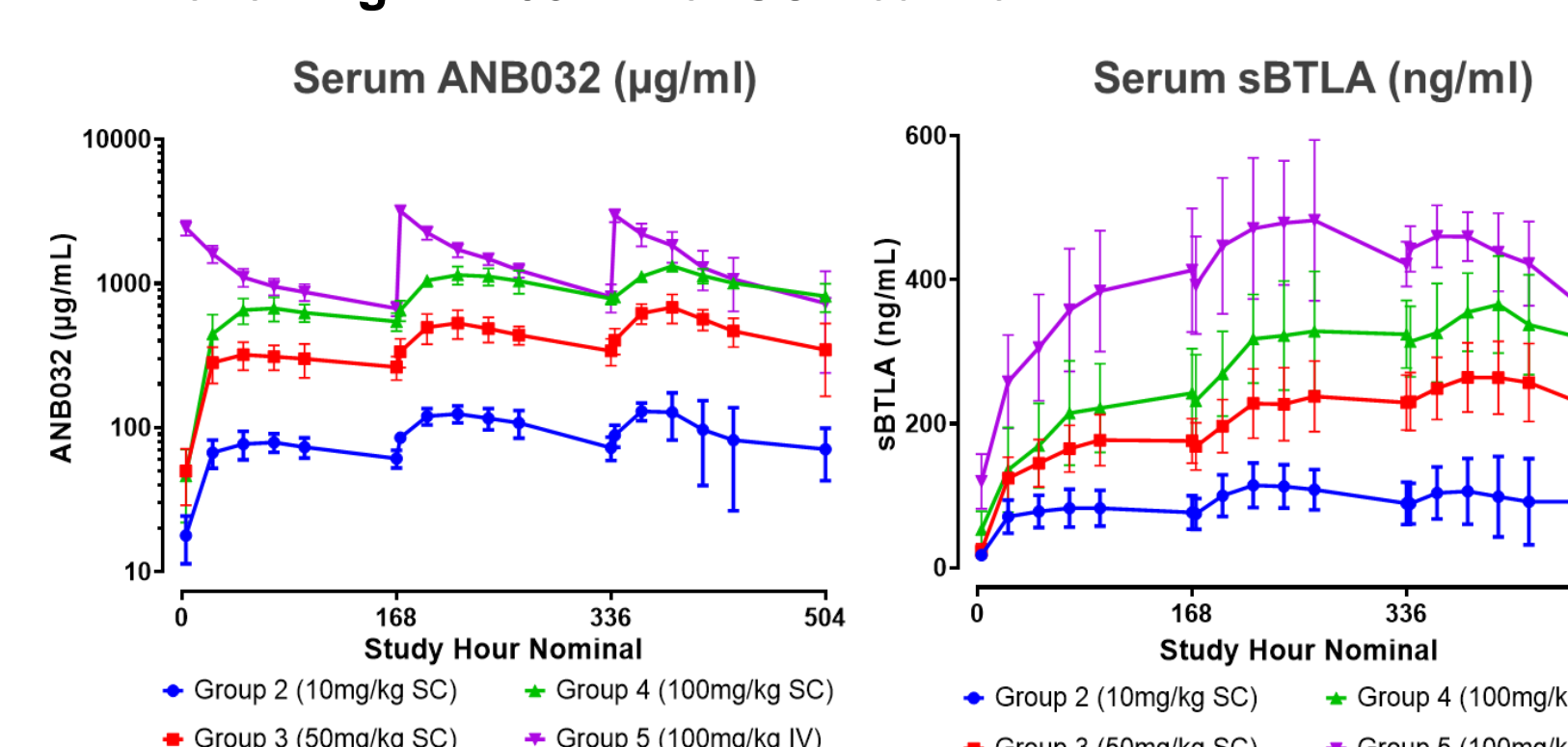
Figure 2: ANB032 inhibited human T cells in an *in-vivo* model of GvHD and induced sBTLA release in two *in-vivo* settings

A) ANB032 reduced BTLA expression and inhibited T cell expansion



On day fourteen of the GvHD model, circulating human T cells were analyzed by flow cytometry to evaluate BTLA expression, enumerate human T cell numbers, and the activation marker CD25. ANB032 reduced BTLA expression on human T cells, inhibited T cell expansion in a dose-dependent manner and reduced expression of the activation marker CD25 at all doses. Plasma concentrations of human sBTLA were measured by ELISA and shown to increase in a dose-dependent manner.

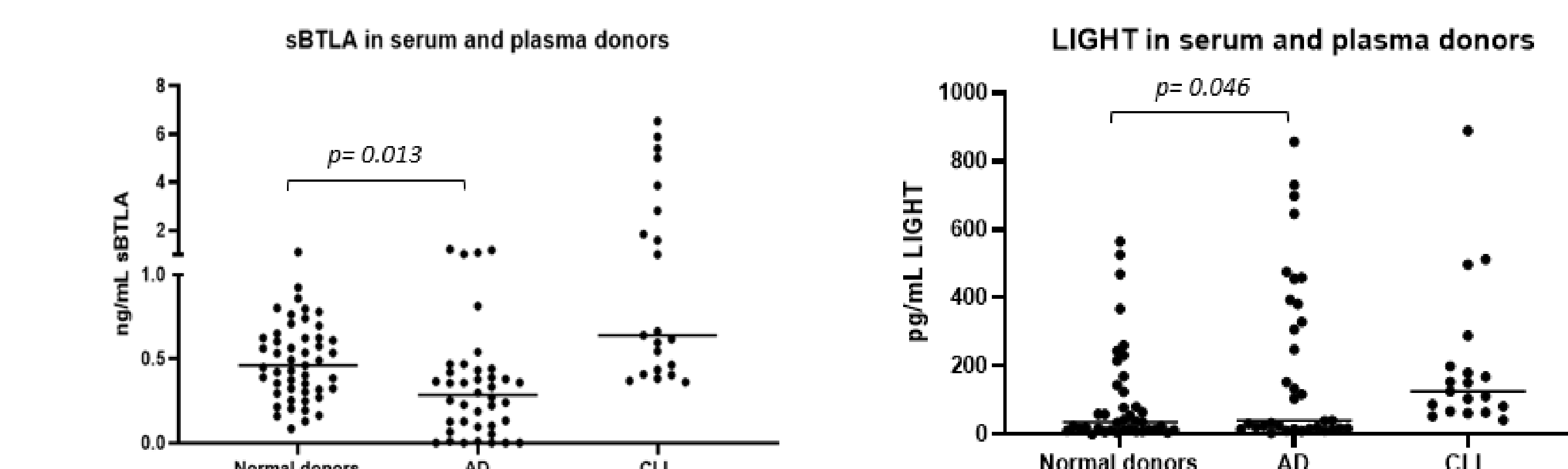
B) The level of shed BTLA increased in cynomolgus monkey following ANB032 IV or SC treatment



An electrochemiluminescence (ECL) sandwich assay was designed to quantify sBTLA concentrations utilizing AnaptysBio antibody clone 10D8, which binds to different sBTLA epitope than ANB032, and a commercial human anti-BTLA polyclonal antibody (PA5-95592) tagged with biotin as a detection antibody.

No measurable levels of sBTLA were detected in serum samples from cynomolgus monkeys at Day 1, pre-dose or vehicle control treated samples. Shed sBTLA was found in serum samples from all cynomolgus monkeys treated with ANB032, confirming that sBTLA is a pharmacodynamic marker of ANB032 activity *in vivo*.

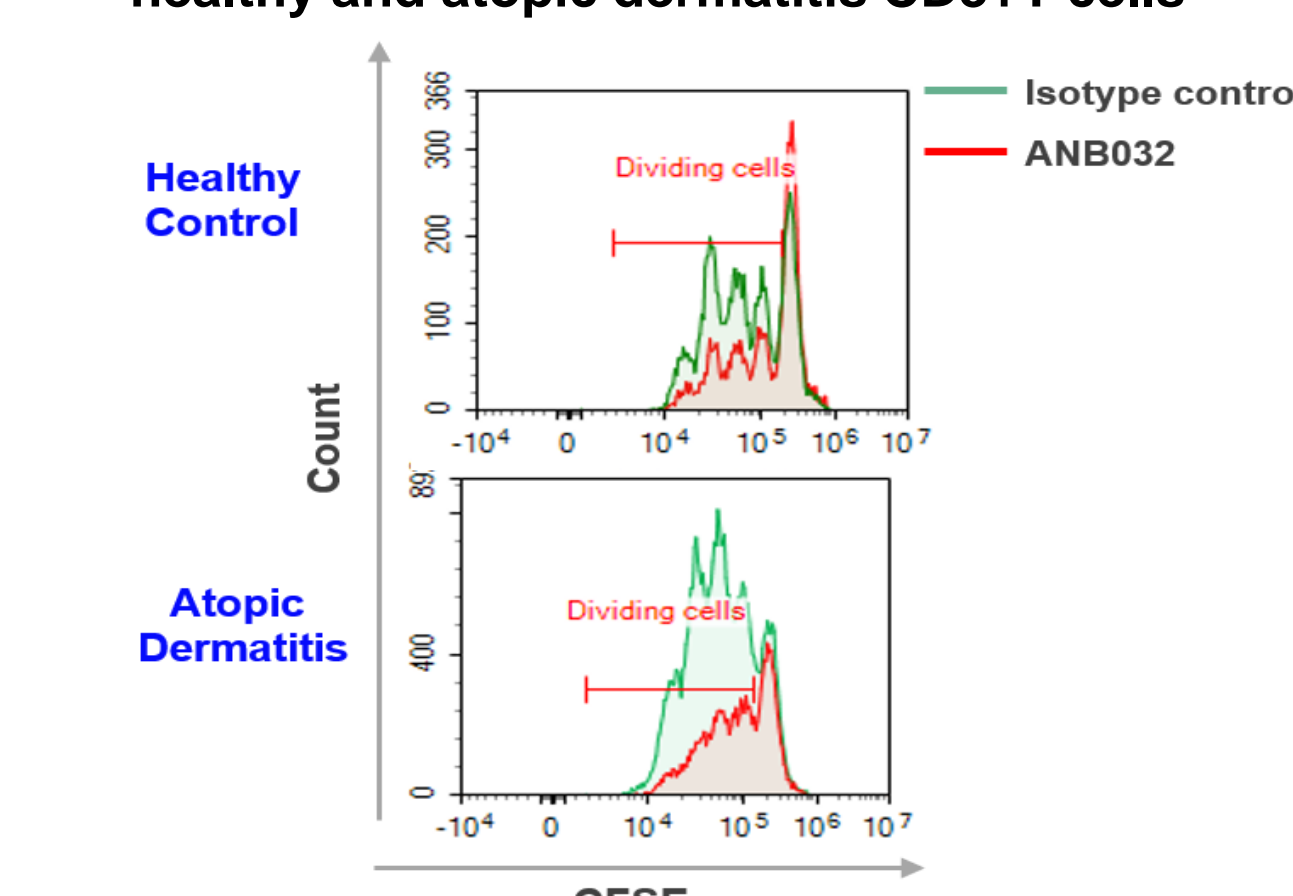
Figure 3: Reduced BTLA and increased LIGHT serum/plasma levels were detected in patients with atopic dermatitis



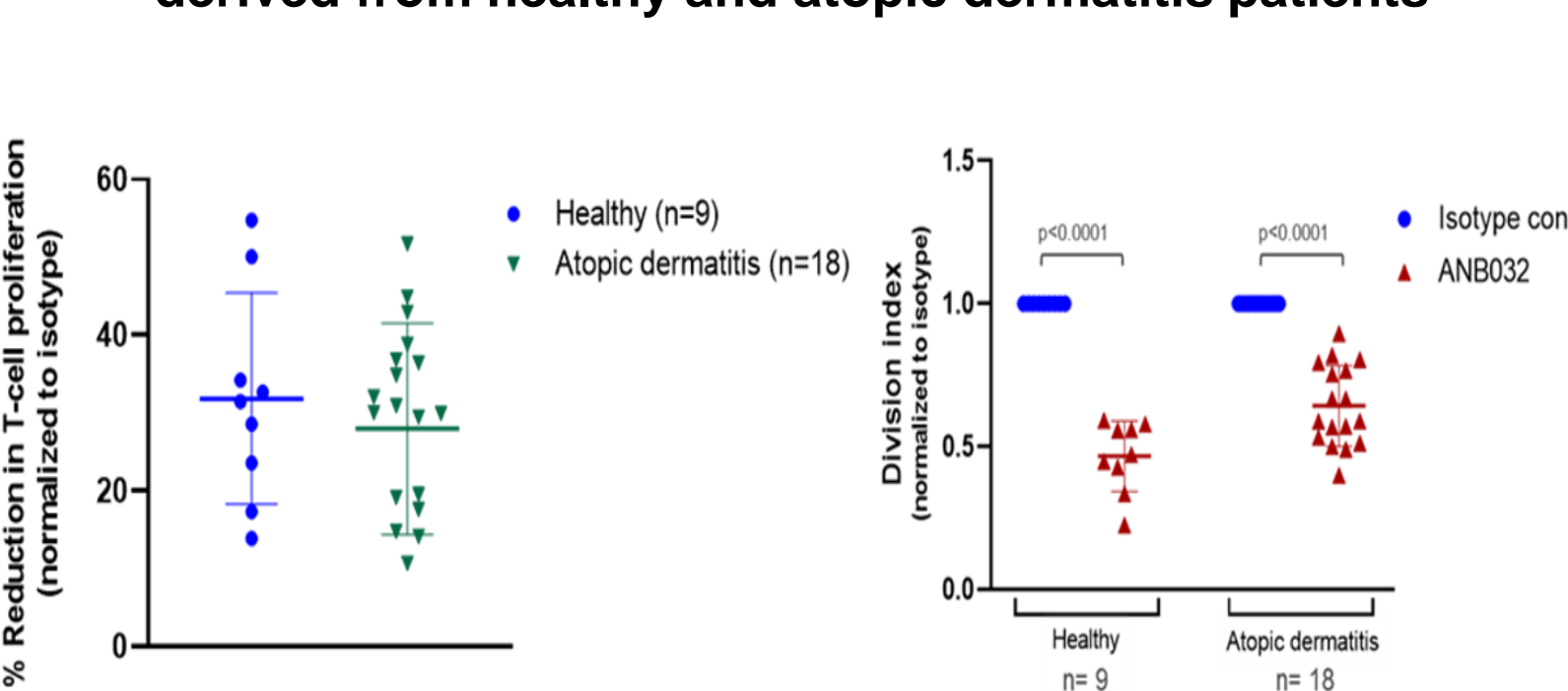
Literature has described sBTLA is a potential biomarker of immune suppressive activity in CLL. In order to identify inflammatory indications that may have dysregulation or reduced immune suppression, we analyzed circulating levels of sBTLA and LIGHT in plasma/serum by multiplex immunoassay from healthy donors, AD patients and CLL patients (Abbreviations; AD (Atopic Dermatitis) and CLL (Chronic Lymphocytic Leukemia)). Reduced sBTLA and elevated LIGHT in AD patients, compared to healthy donors suggests a reduction of BTLA immune suppressive activity.

Figure 4: ANB032 inhibited T proliferation and cytokine secretion from atopic dermatitis patient-derived PBMC *in-vitro*

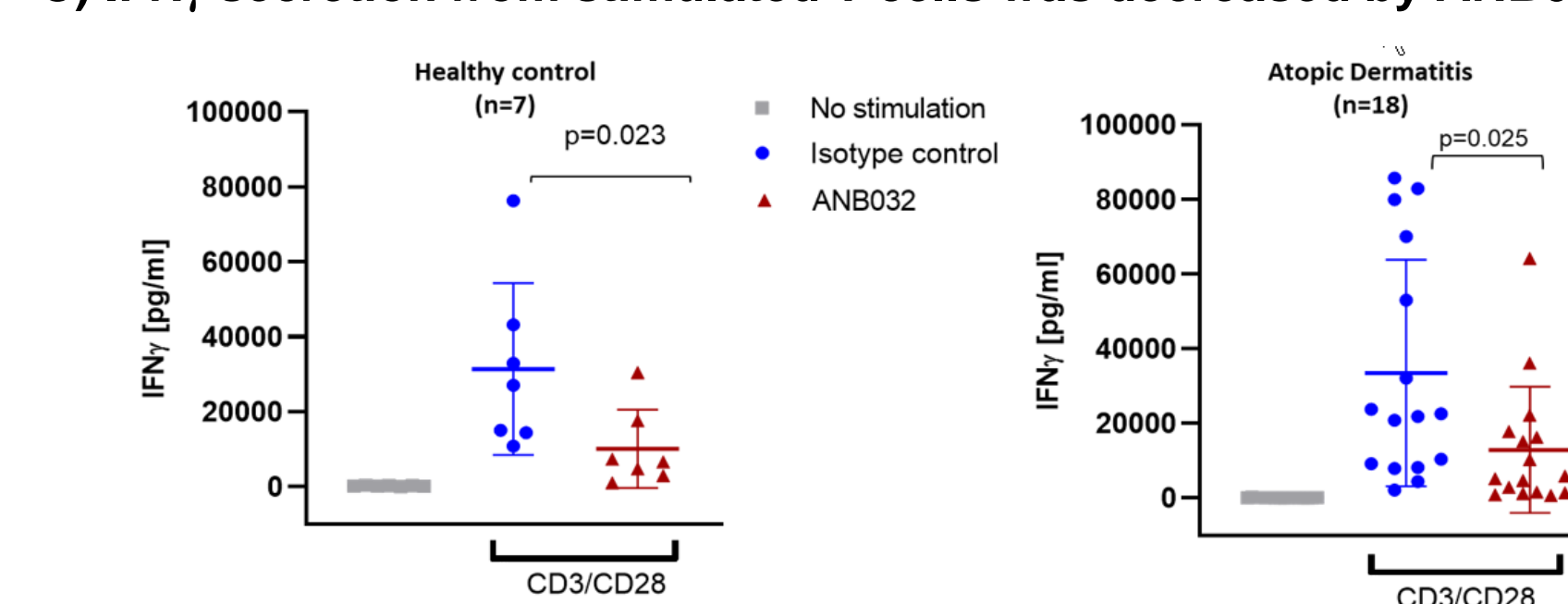
A) Flow cytometry profile of proliferating healthy and atopic dermatitis CD3+T cells



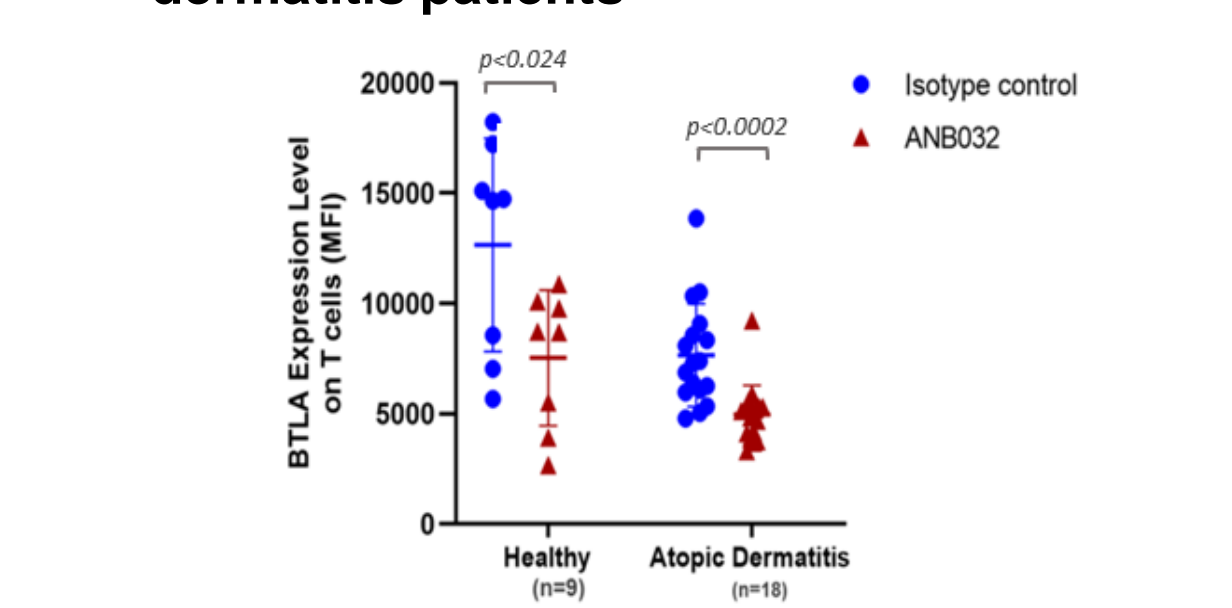
B) ANB032 inhibited T proliferation in stimulated PBMC derived from healthy and atopic dermatitis patients



C) IFN γ secretion from stimulated T cells was decreased by ANB032



D) ANB032 reduced BTLA expression on stimulated T cells from healthy and atopic dermatitis patients



T cell activity was assessed *in vitro* in the presence of ANB032 (100nM) or isotype control after three days of anti-CD3/anti-CD28 stimulated PBMCs from healthy and atopic dermatitis subjects (100nM HyHel-IgG4). (A) CFSE dilution was used to measure the proliferated fraction, and representative histogram overlays showed the flow cytometry profile of proliferating T cells. (B) Suppression of T cell division was measured as the percent reduction in proliferation or the reduction in division index. ANB032 significantly reduced T cell proliferation in both healthy and atopic dermatitis T cells. (C) Consistent with the suppression of T cell proliferation, ANB032 reduced IFN γ secretion from stimulated healthy and atopic dermatitis-donor derived PBMCs (D) ANB032 reduced BTLA expression on both healthy and atopic dermatitis T cells.

Conclusions

- We investigated the ability of ANB032, a novel BTLA/HVEM modulating therapeutic antibody, to inhibit T cell function *in-vivo* in a humanized model of GvHD and in *in-vitro* assays of T cell function using PBMC derived from atopic dermatitis patients.
- ANB032 was efficacious in a humanized model of GvHD, reducing T cell activation, expansion and BTLA expression. ANB032 also induced the release of human sBTLA into the serum in the GvHD model. Additionally, sBTLA was released into the serum in cynomolgus monkeys treated with ANB032, and BTLA expression was reduced on T and B cells in a dose dependent manner (data not shown). We hypothesize that BTLA is shed from the surface of T cells and that sBTLA is a pharmacodynamic marker of ANB032 activity *in vivo*.
- Using sBTLA as a biomarker of immune suppression, we identified that atopic dermatitis patients had lower serum sBTLA and higher LIGHT levels compared to healthy normal control individuals, suggesting dysregulation of BTLA activity *in-vivo*.
- Using *in-vitro* human primary patient-derived PBMC stimulation assays, we demonstrated that ANA032 reduced T cell proliferation, reduced secretion of IFN γ , and reduced T cell BTLA expression from both atopic dermatitis-derived and healthy donor-derived PBMC.
- We propose that ANB032, by directly inhibiting T cell activity, has the potential to restore immune suppression in inflammatory diseases where dysregulated T cells mediate pathology, such as in atopic dermatitis.