ANB032, an Investigational BTLA Checkpoint Agonist Antibody, Attenuates Dendritic Cell Maturation and Function: A Novel Mechanism Addressing Atopic Dermatitis Pathophysiology

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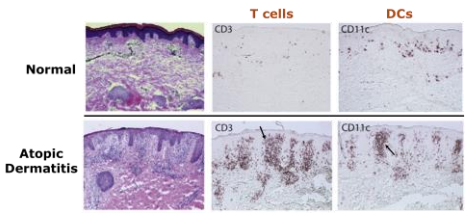
ABSTRACT

AD is characterized by heterogeneous immunologic drivers including broad T cell and DC activity. Modulating coinhibitory checkpoint receptors such as B and T Cell Lymphocyte Attenuator (BTLA) is a promising strategy to regulate immune cells for treatment of systemic, inflammatory diseases like AD. ANB032 has been shown to reduce T cell proliferation and secretion of inflammatory cytokines (Th1, Th2, Th17, Th22) in AD patient-derived peripheral blood mononuclear cells (PBMCs). Since DCs are critical in initiating adaptive immune responses and maintaining immune tolerance, the role of ANB032 in modulating DC activation and maturation was investigated. Purified monocytes from healthy PBMCs were differentiated to DCs and lipopolysaccharide (LPS)-mediated assays were used to assess BTLA expression on DCs and the effect of ANB032 on DC maturation, and expression of MHC II and costimulatory molecules. ANB032-treated DCs were co-cultured with allogeneic naïve T cells in a mixed lymphocyte reaction (MLR) to evaluate the frequency of differentiated FOXP3+ Tregs and secretion of inflammatory cytokines. LPS induced rapid DC maturation and high BTLA expression on mature DCs. ANB032 reduced the absolute number of mature DCs by 53%, and reduced HLA-DR expression and costimulatory molecule expression of CD80, CD86, CD40, and OX40L. ANB032-treated DCs increased the frequency of FOXP3+ Tregs and reduced the secretion of Th1 and Th2 cytokines in the MLR. These data demonstrate the induction of BTLA expression on mature DCs by a stimuli relevant in AD and provides insight on the effect of BTLA agonism on DC maturation and function. ANB032 has the potential to restore immune balance in the treatment of AD by impacting a broad range of pathogenic immune cells, including T cells and DCs, while inducing Tregs. These data, additional in vitro data, and results from a Phase 1 healthy volunteer study support the ongoing double-blind, placebo-controlled, global Phase 2b study of ANB032 in moderate-to-severe AD (NCT05935085).

BACKGROUND & OBJECTIVE



Guttman-Yassky et al, JACI, 2011

- Atopic dermatitis (AD) is a systemic, heterogenous inflammatory disease with pathogenesis driven by Th1, Th2, Th17, Th22, and DCs both in skin and the periphery
- There are significantly more dendritic cells (DCs) in the skin of AD patients, with up to 10-fold increase in the epidermis and up to 3.5-fold increase in the dermis[1]
- BTLA is a co-inhibitory checkpoint receptor expressed preferentially on activated T cells, B cells, and DCs, key contributors to inflammatory diseases, and preclinical studies have shown that BTLA dysregulation is linked to the development of autoimmune diseases, including dermatitis[2,3]
- Although the expression of BTLA on subsets of DCs has been reported, BTLA's role in modulating DC maturation and function has not been thoroughly investigated
- ANB032 is an investigational non-depleting BTLA agonist that does not compete with the binding of BTLA to herpesvirus entry mediator (HVEM), its ligand (**Figure 1**)

Proposed Mechanism of Action for ANB032

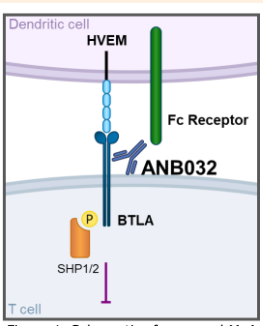


Figure 1. Schematic of proposed MoA

ANB032: IgG4 antibody (non-depleting)

- Binds to BTLA on membrane proximal epitope
- Fc receptor binding profile contributes to differentiated potency
- Non-blocking of HVEM engagement

ANB032's agonist signal modulates immune cells

- Inhibits activated T cell proliferation
- Reduces inflammatory cytokine secretion
- Modulates DC function, including inducing Tregs

Objective: Investigate the role of BTLA and effect of ANB032 on DC maturation and activation in a preclinical model

METHODS

BTLA expression on DCs

- Purified monocytes from healthy PBMCs were differentiated to DCs
- Differentiated DCs were either stimulated with lipopolysaccharide (LPS) or rested in fresh medium, then stained for MHC II and CD11c, and the BTLA expression was evaluated on immature and mature DCs

Effect of ANB032 on DCs

- Differentiated DCs were treated with either ANB032 or isotype control, then stimulated with LPS and evaluated by flow cytometry to assess the maturation state of DCs, absolute number of mature DCs, expression of MHC II and costimulatory molecules

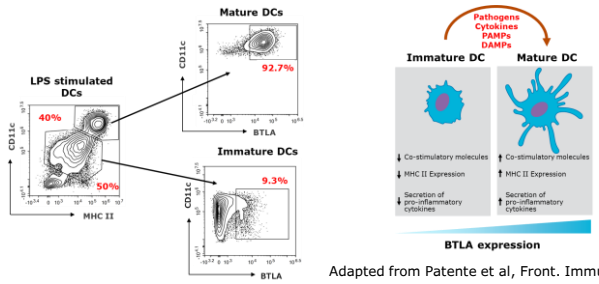
Effect of ANB032 on Tregs and inflammatory cytokines

- Differentiated DCs were treated with either ANB032 or isotype control
- ANB032-treated DCs were washed and then co-cultured with allogenic naïve CD4 T cells, then T cells were evaluated for CD4, CD25, and intracellular Foxp3 expression to identify inducible regulatory T cells (iTreg)
- The frequency of differentiated iTregs and secretion of inflammatory cytokines were evaluated by FACS and MSD, respectively

RESULTS

BTLA was Highly Expressed on Mature DCs with LPS Stimulation

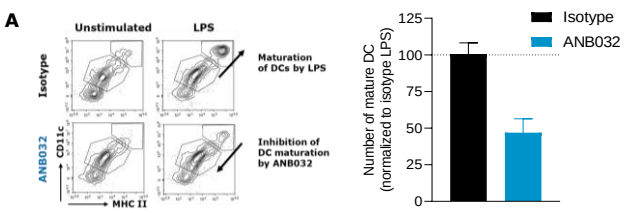
- LPS-stimulated DCs resulted in 40% mature and 50% immature DCs
- BTLA expression was seen in 92.7% of mature DCs versus 9.3% of immature DCs (**Figure 2**)



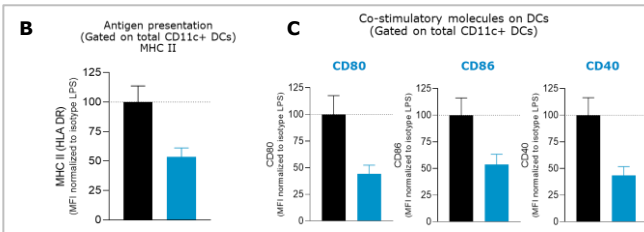
Adapted from Patente et al, Front. Immunol., 2019

Figure 2. BTLA expression on DCs

ANB032 Inhibits DC Maturation and Reduces Antigen Presentation & Co-Stimulatory Molecules



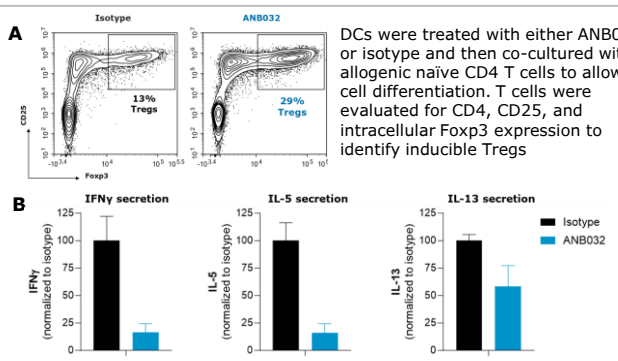
Maturation of LPS-stimulated DCs was inhibited by ANB032 resulting in reduced absolute numbers of mature DCs compared to isotype control treatment



ANB032-treated LPS-stimulated DCs had lower expression of MHC II and reduced expression of co-stimulatory molecules, CD80, CD86, and CD40, compared to the isotype control

Figure 3. Effect of ANB032 on DC maturation (A), MHC II presentation (B), and co-stimulatory molecule expression (C)

ANB032-treated DCs Induce Functional Tregs



DCs were treated with either ANB032 or isotype and then co-cultured with allogenic naïve CD4 T cells to allow T cell differentiation. T cells were evaluated for CD4, CD25, and intracellular Foxp3 expression to identify inducible Tregs

ANB032-treated DCs induced a higher frequency of Tregs and reduced inflammatory cytokines compared to isotype control-treated DCs

Figure 4. Effect of ANB032-treated DCs on functional Tregs (A) and cytokine secretion (B)

CONCLUSIONS

- BTLA is highly expressed on mature DCs
- Preclinical evaluation of ANB032 demonstrated:
  - Inhibition of DC maturation and reduction of antigen presentation and co-stimulatory molecule expression
  - Modulation of DC function that results in increased Foxp3+ Tregs that contribute to reduced inflammatory cytokine production
  - Inhibition of a broad range of T cell subsets and DCs, while inducing Tregs, supports a potential for restoring immune balance
- Based on these findings, ANB032 may provide therapeutic value in the treatment of autoimmune and inflammatory diseases, including AD
- ANB032 is being evaluated in an ongoing Phase 2 study in moderate-to-severe AD (NCT05935085)

REFERENCES

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2. Nakagomi et al. J Invest Dermatol 2013;133:702-11.
3. Bekiaris et al. Immunity 2013;39:1082-94.

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