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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Disclosures

- All authors are employees of AnaptysBio, Inc., San Diego, CA, USA

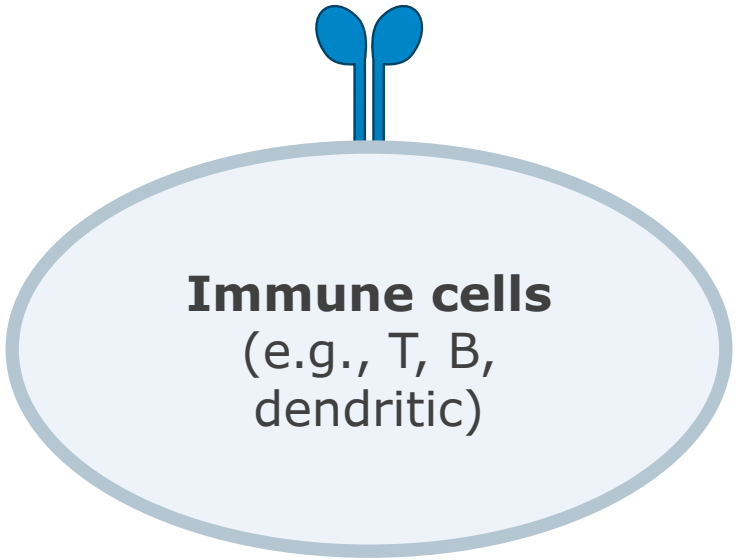
Checkpoint Receptors Modulate Immune Cells

Checkpoint antagonists:
"release the brakes"

Treat cancers:

Unleash immune response

Checkpoint receptors (e.g., PD-1, BTLA)

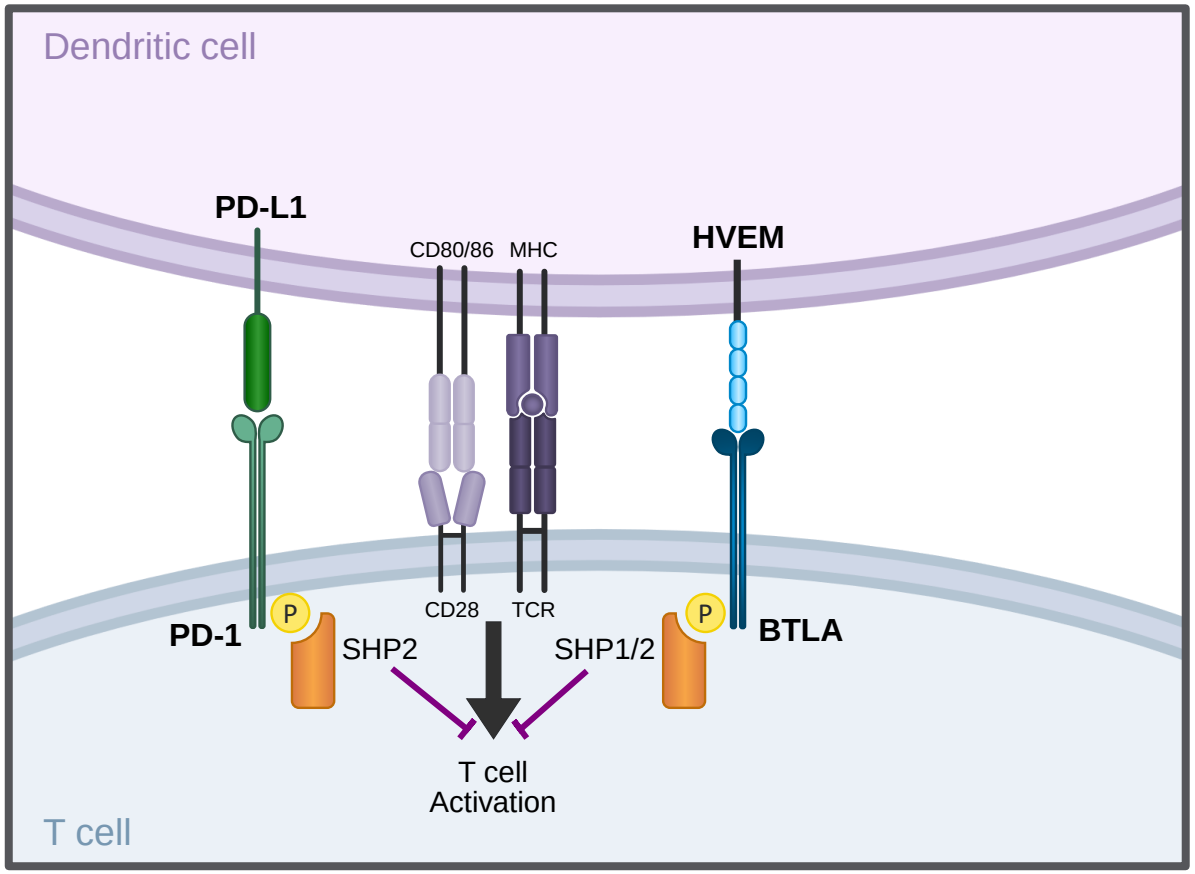


Checkpoint agonists:
"tap the brakes"

Treat inflammation:

Attenuate overactive/persistent immune response

BTLA is a Key Node Of Immune Regulation



- B and T lymphocyte attenuator (BTLA) is a potent modulator of T cells, B cells and dendritic cells (DC)
- Expressed only on immune cells and preferentially on activated immune cells
- Dysregulation of BTLA pathway accelerates onset and exacerbates disease

Adapted from Xu et al, J. Cell Biol. 2020 Vol. 219 No. 6

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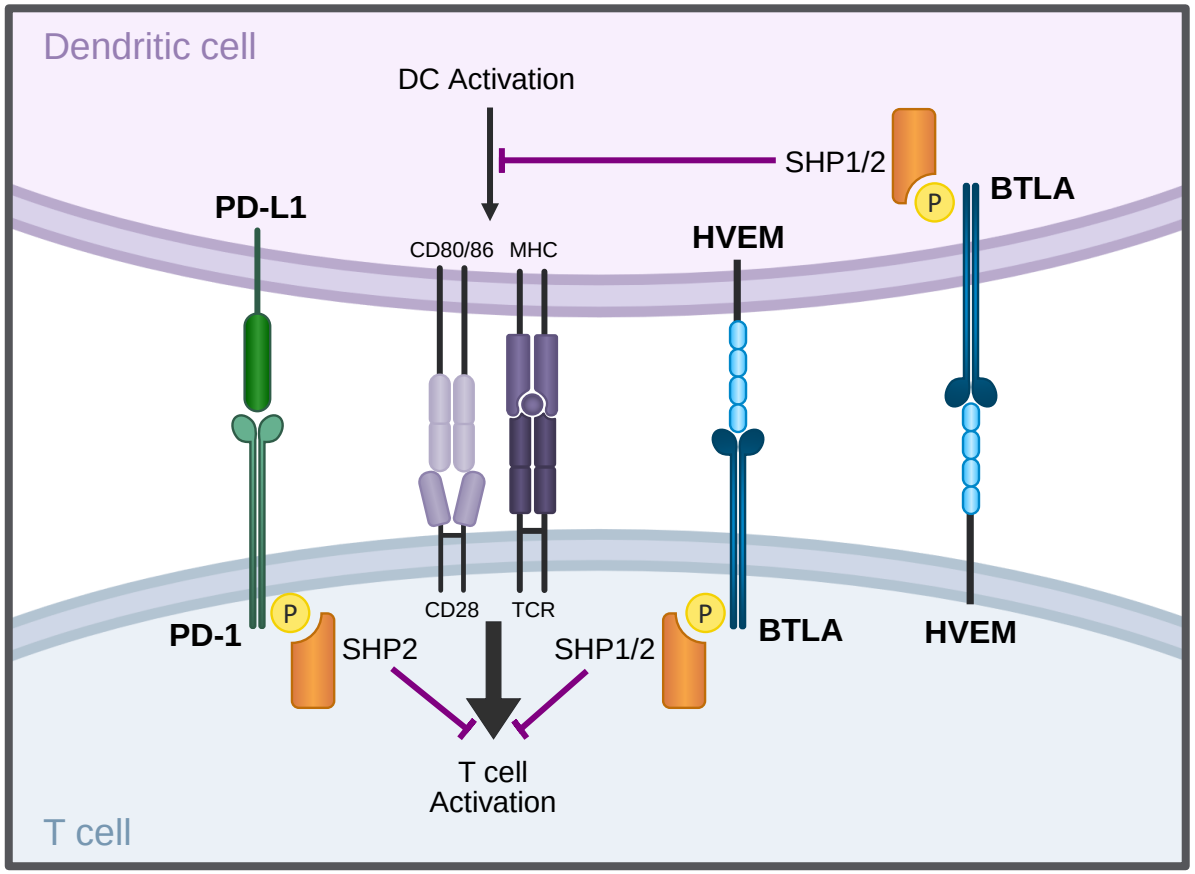

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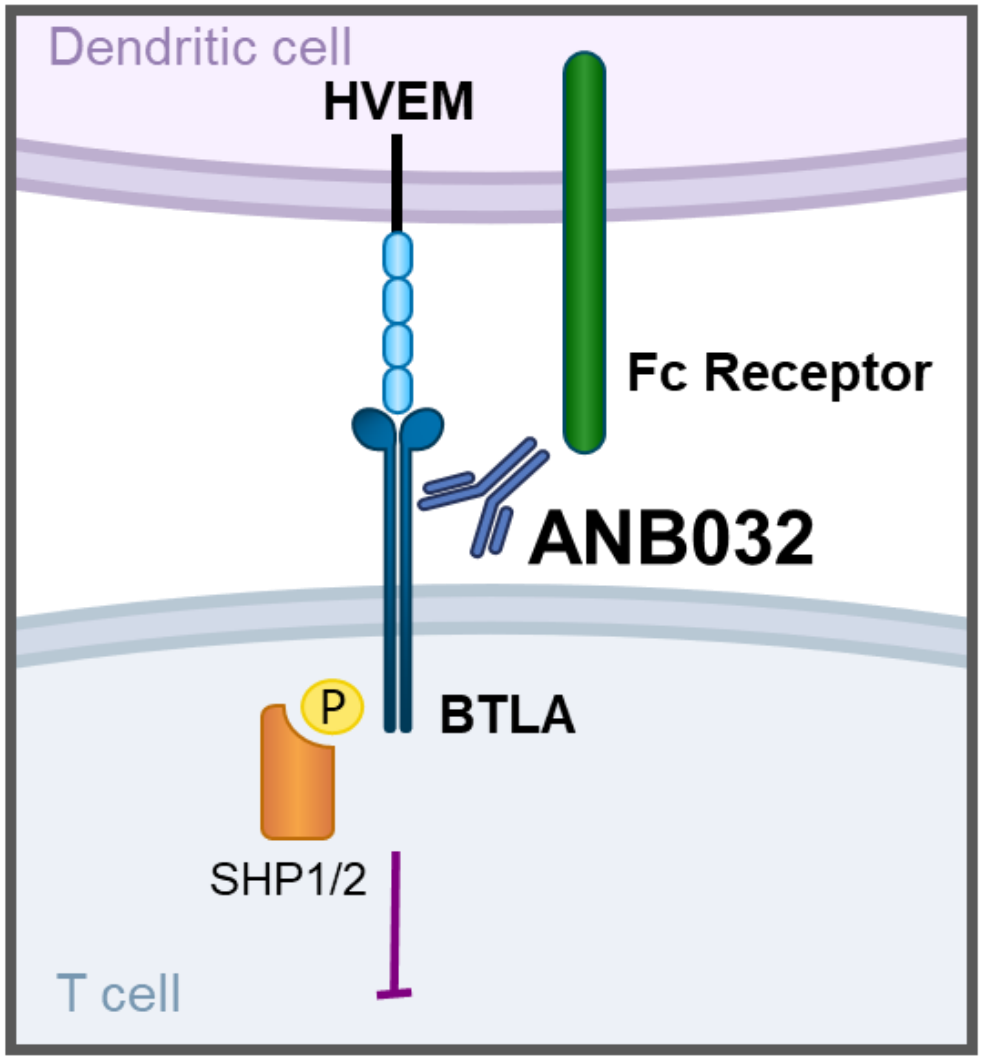
Proposed Mechanism of Action for ANB032



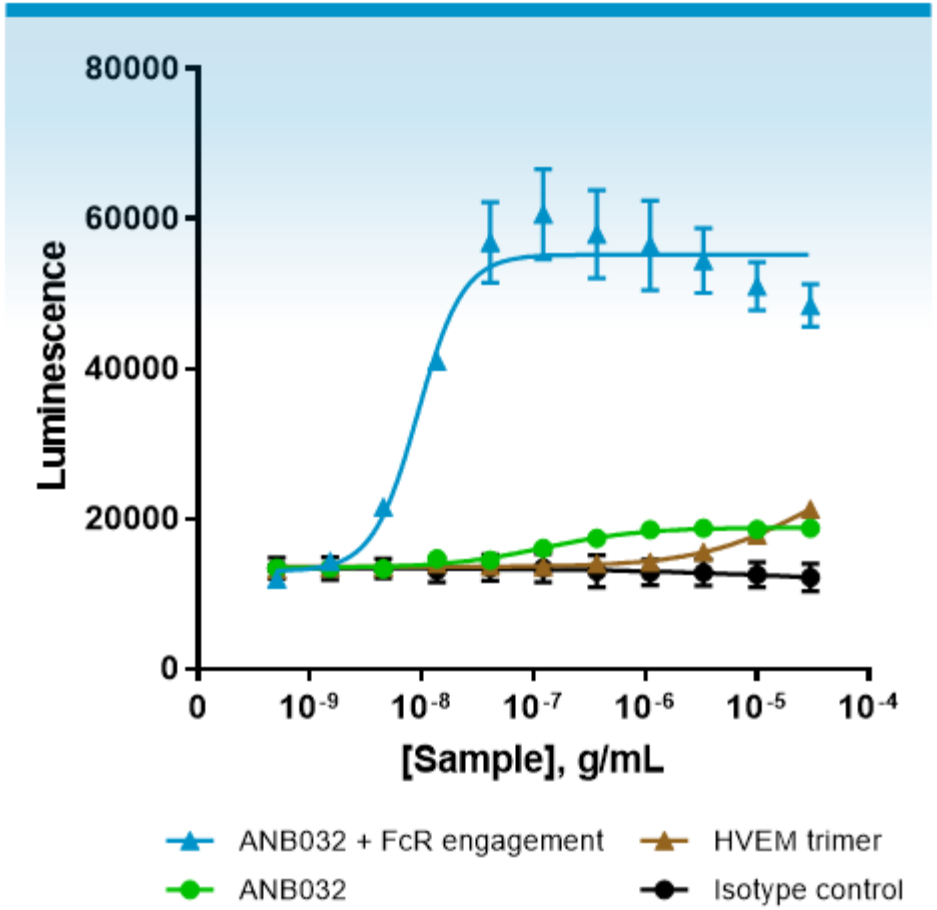
ANB032: IgG4 antibody (non-depleting)

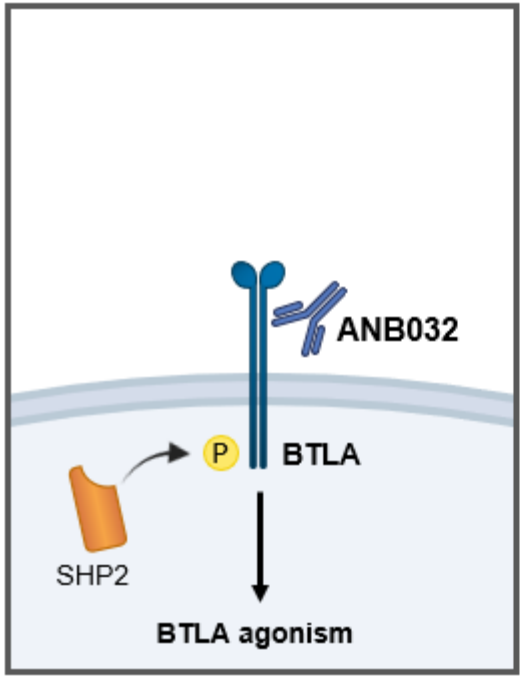
- Binds to BTLA on epitope proximal to immune cell
- Fc receptor binding profile contributes to differentiated potency
- Non-blocking of HVEM engagement with optimized antigen binding affinity

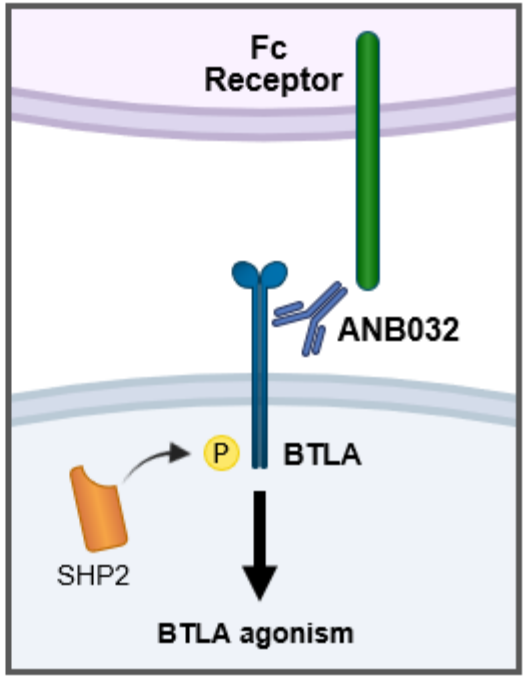
ANB032's agonist signal modulates immune cells

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

Fc Receptor Engagement by ANB032 Enhances BTLA Agonism, Measured by SHP2 Recruitment Assay



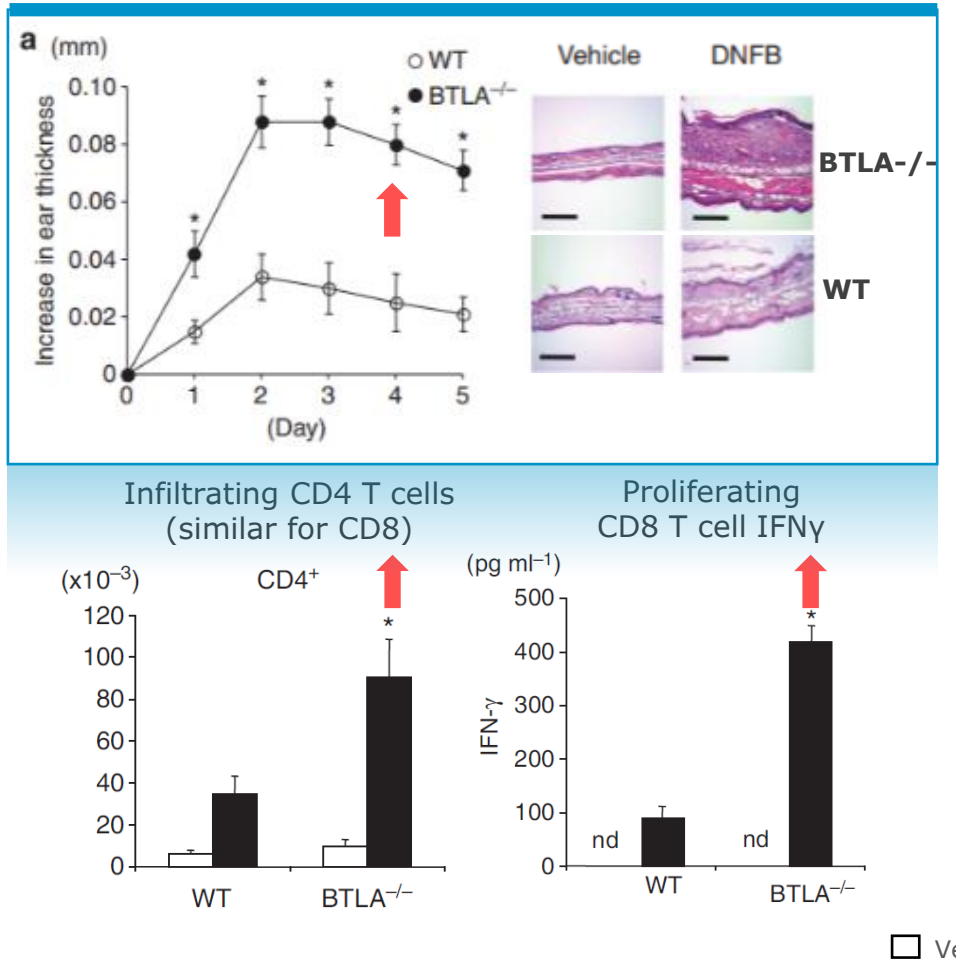




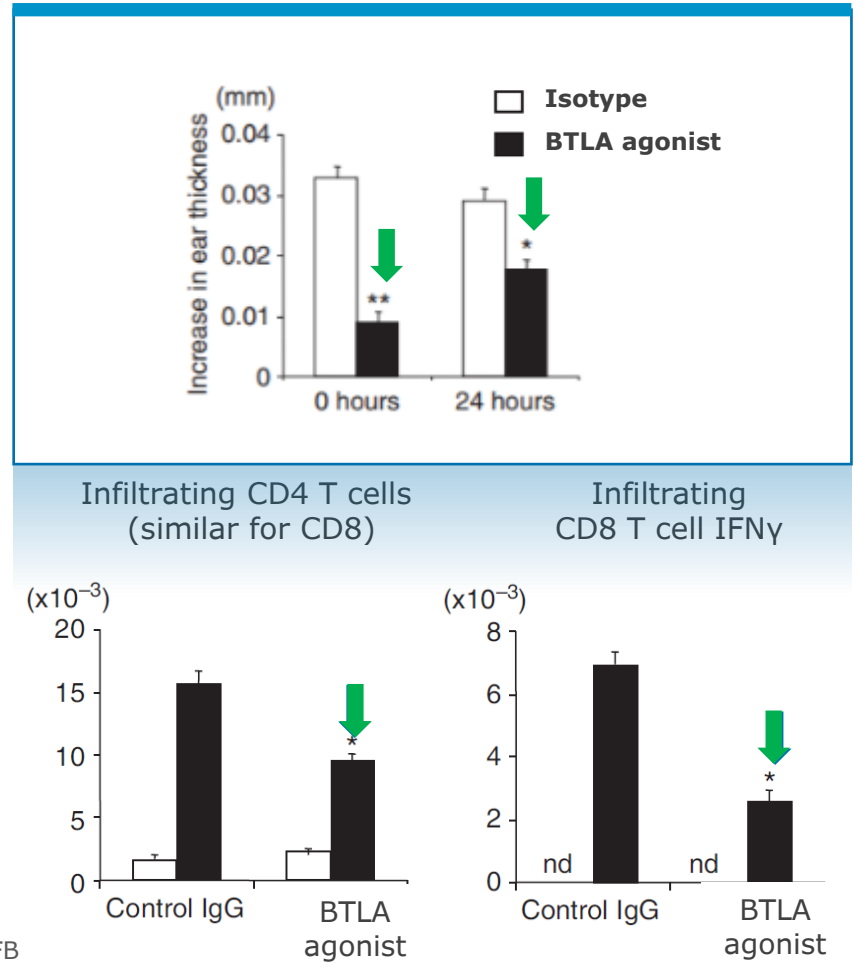
Jurkat BTLA SHP2 Recruitment Assay methodology: BTLA and SHP2 are fused with complementary enzyme fragments, when SHP2 is recruited to activated phosphorylated BTLA, the enzyme donor and enzyme acceptor form active β-gal that is detected by chemiluminescence

Agonism of BTLA in a Preclinical Mouse Dermatitis Model

BTLA knock-out mice have exacerbated T cell-mediated skin disease

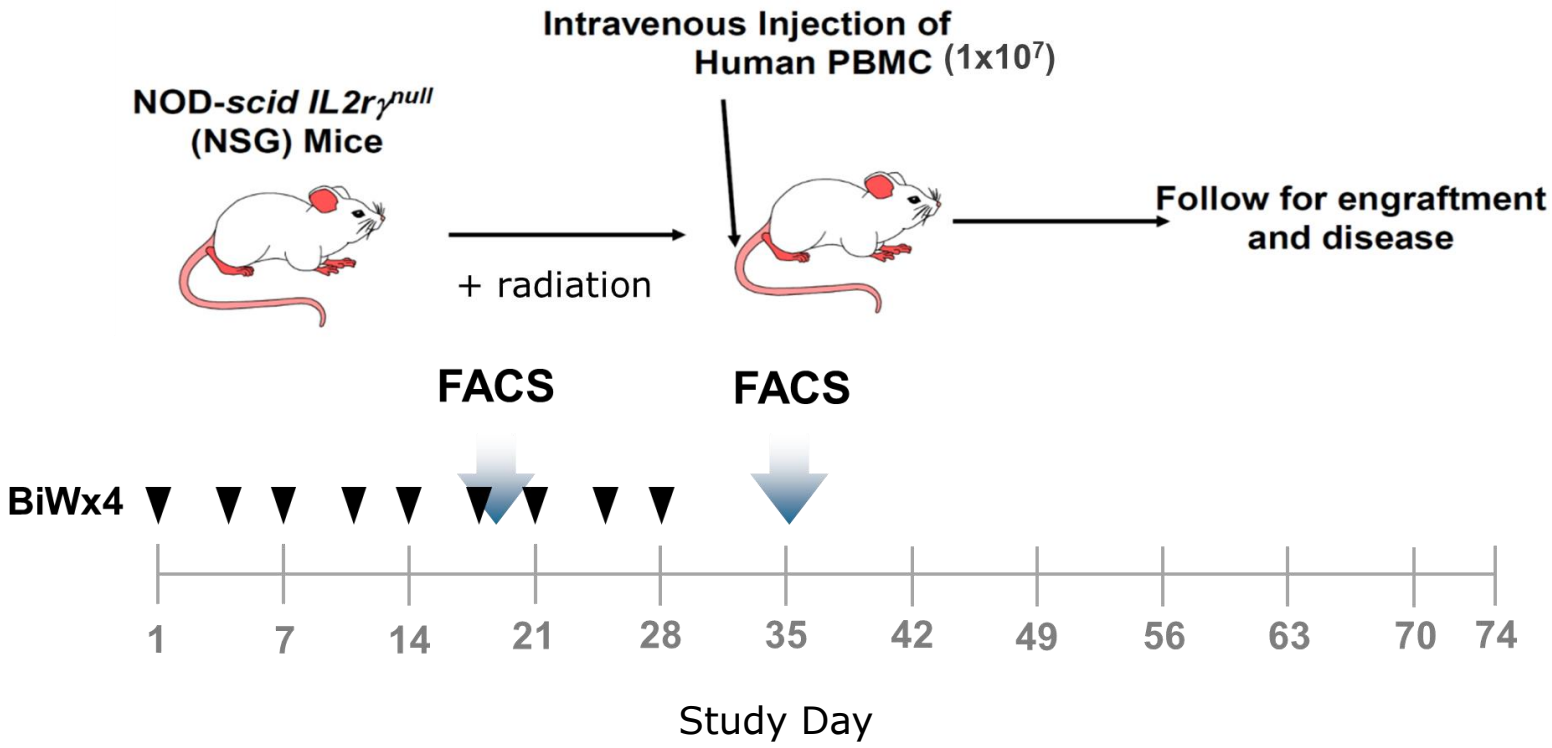


BTLA agonist-treated WT mice have reduced T cell-mediated skin disease



Nakagomi et al, Journal of Investigative Dermatology, 2013; Surrogate Murine BTLA agonist third-party data

Determining the Efficacy and Immune Regulatory Effects of ANB032 in a Humanized Mouse Model of Graft-versus-host Disease (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

Endpoints:

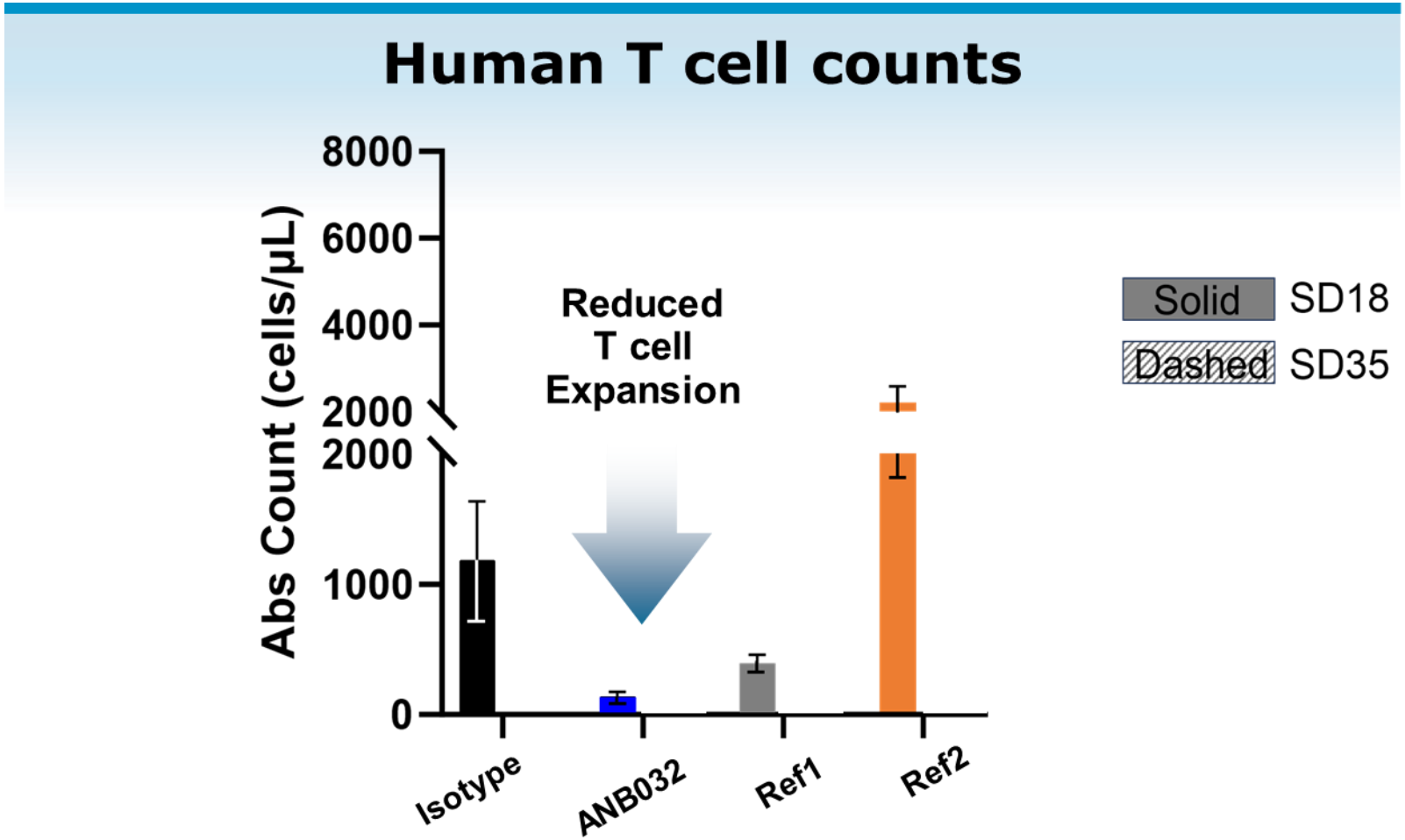
- Weight loss
- Death
- GvHD disease activity index (DAI) scores (Fur, skin, posture, activity)

Evaluating the Contribution of Both Epitope Binding and FcR Engagement to Efficacy

Antibody characteristics

	ANB032 IgG4	Ref1 Mutated IgG4	Ref2 Mutated IgG4
Binding epitope is HVEM sparing	✓	✓	✗
FcR engagement	✓	✗	✗

ANB032 Reduced Human T Cell Expansion



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

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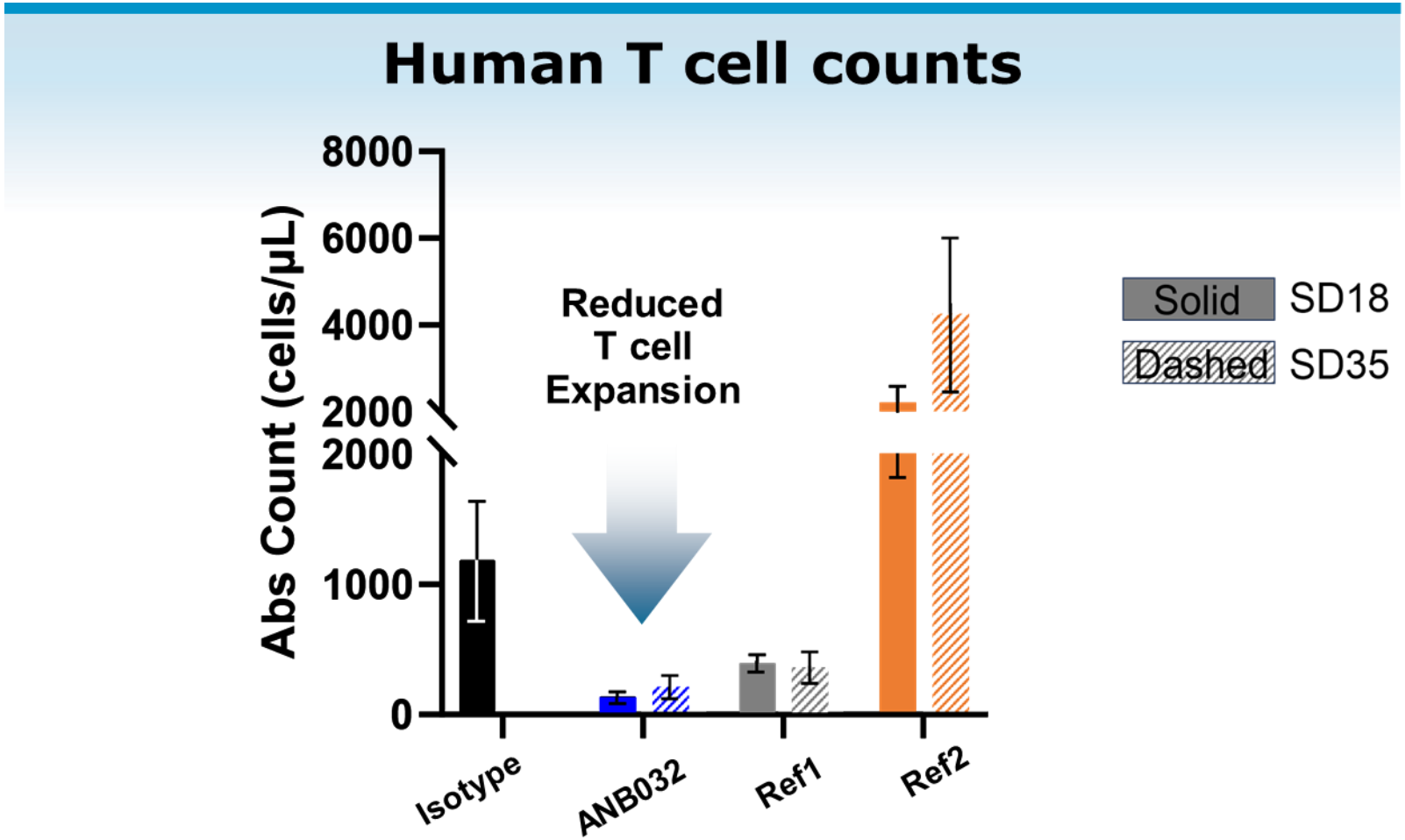

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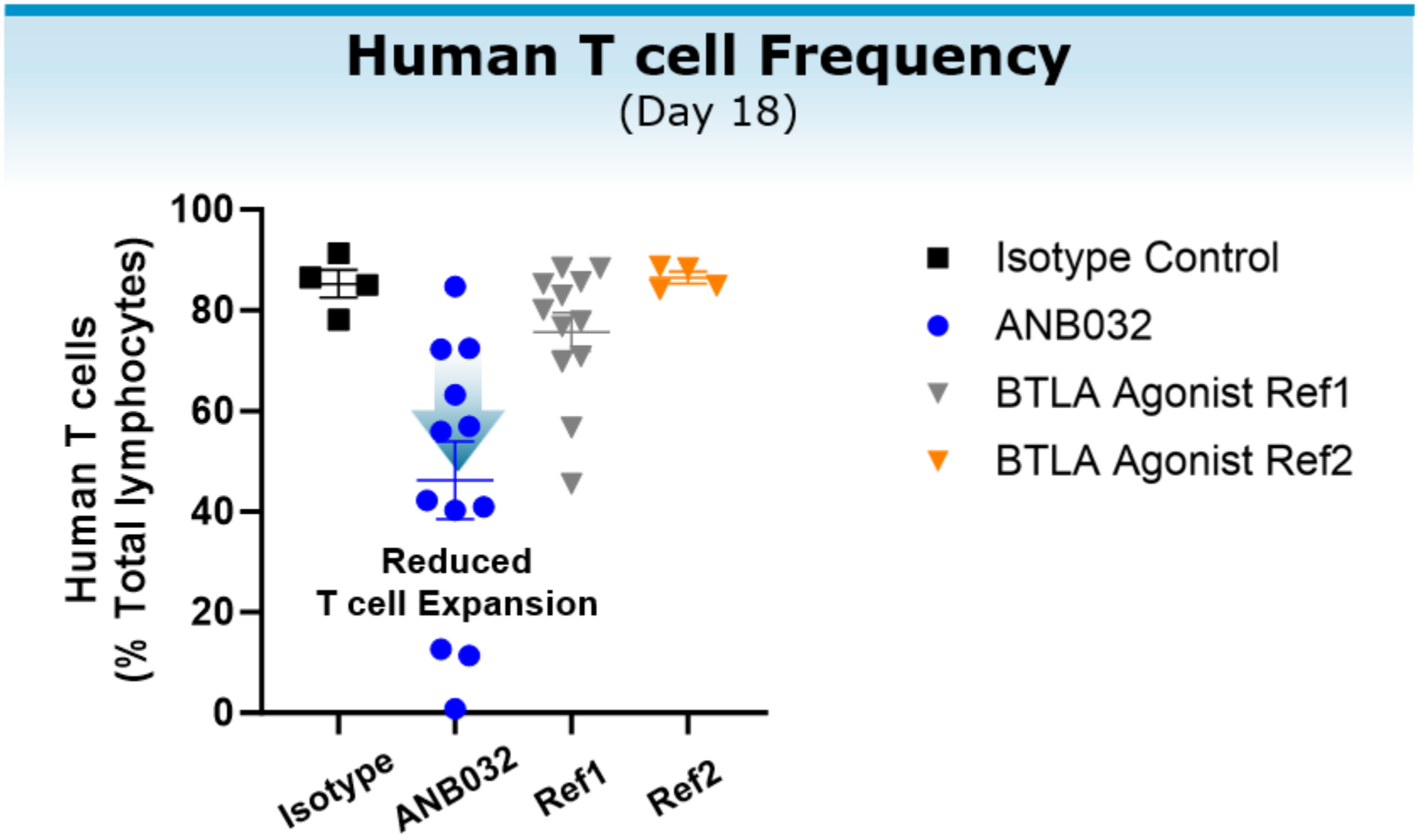
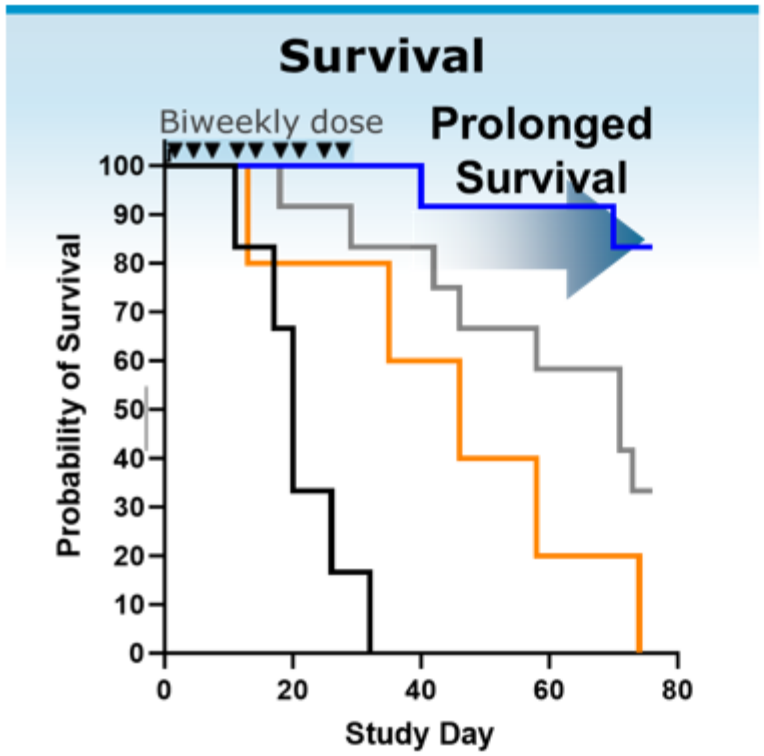
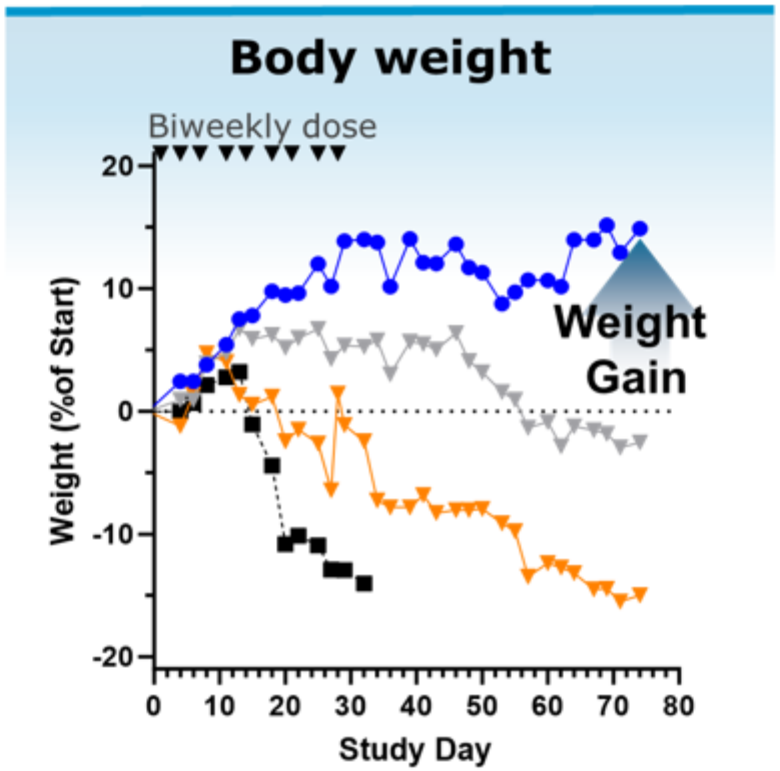
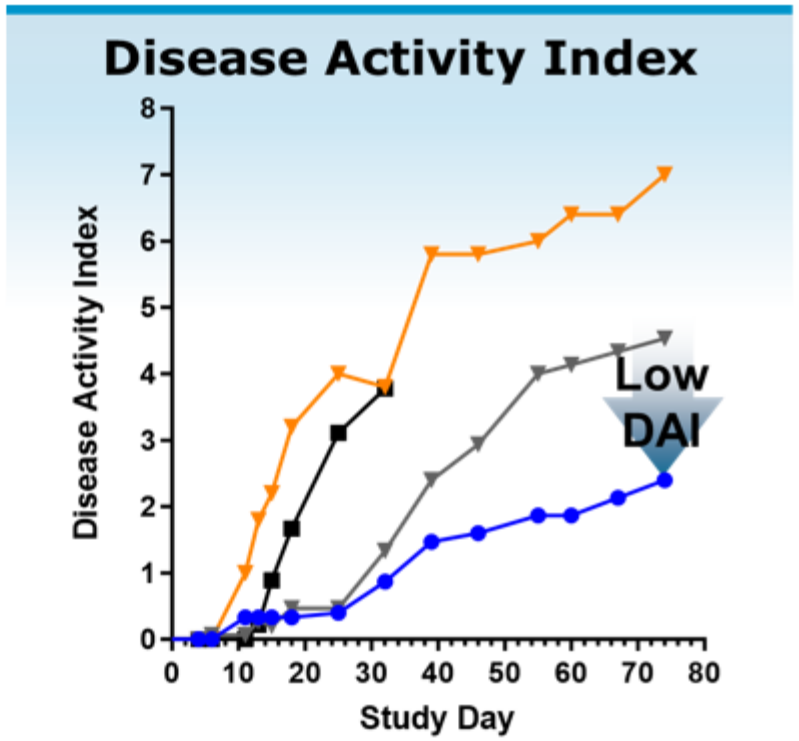


ANB032 Treatment Resulted in Prolonged Survival and Reduced Disease Activity Index

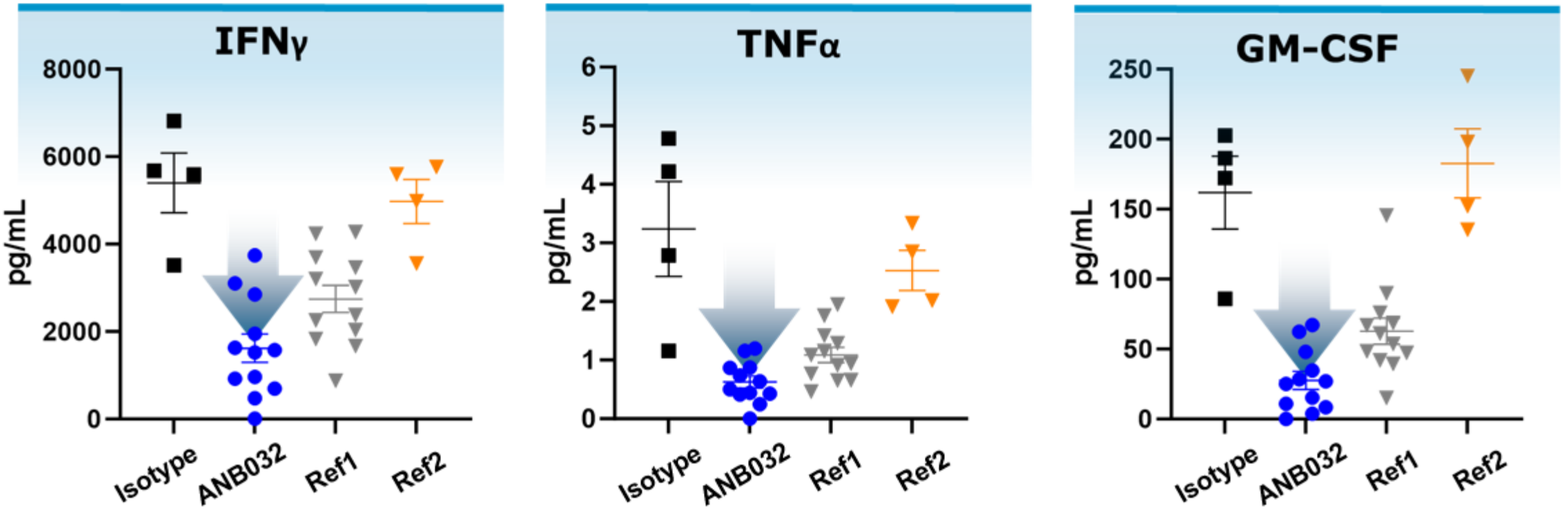




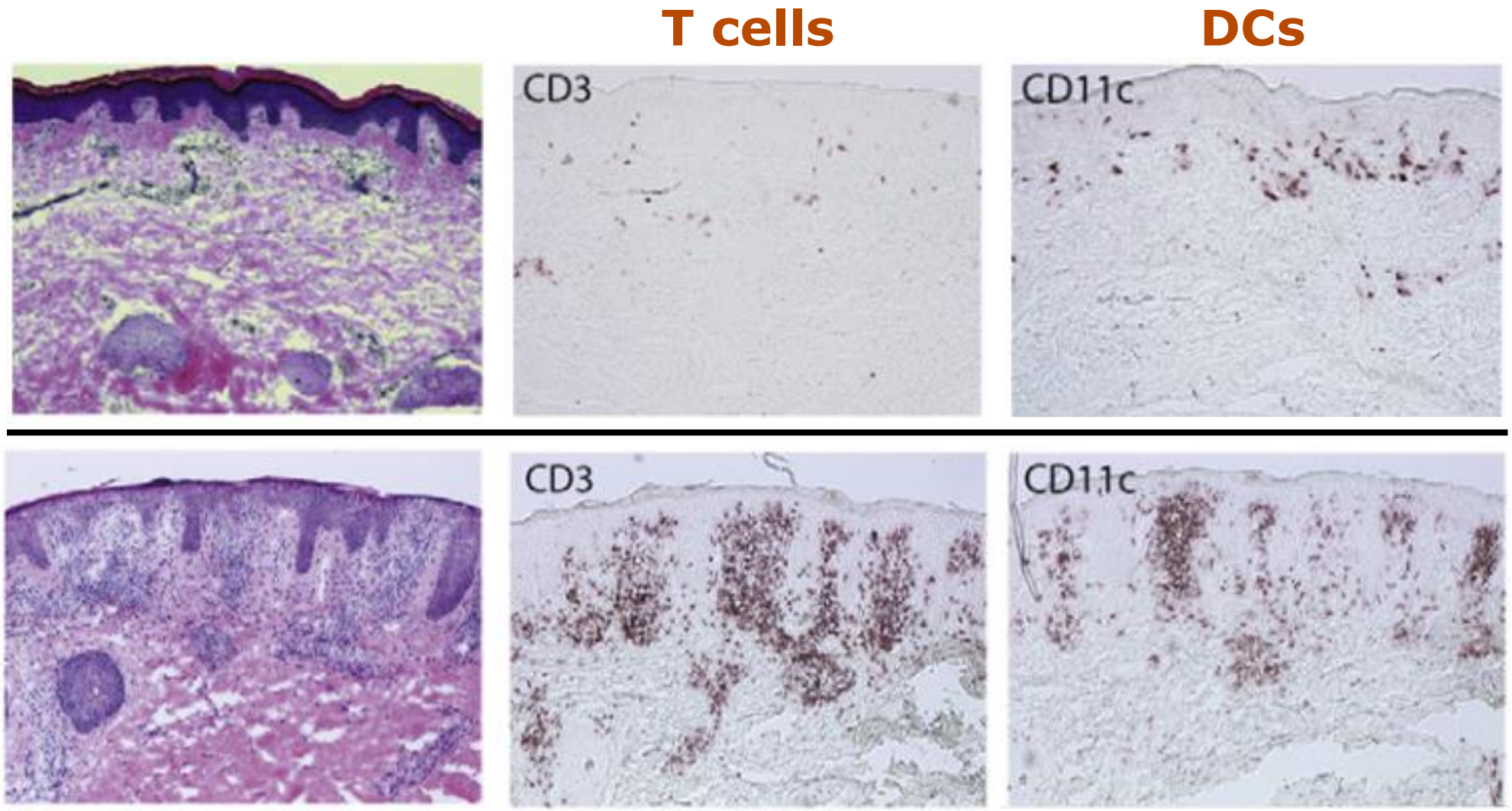


ANB032 Reduced Serum Inflammatory Cytokines

Study Day 18

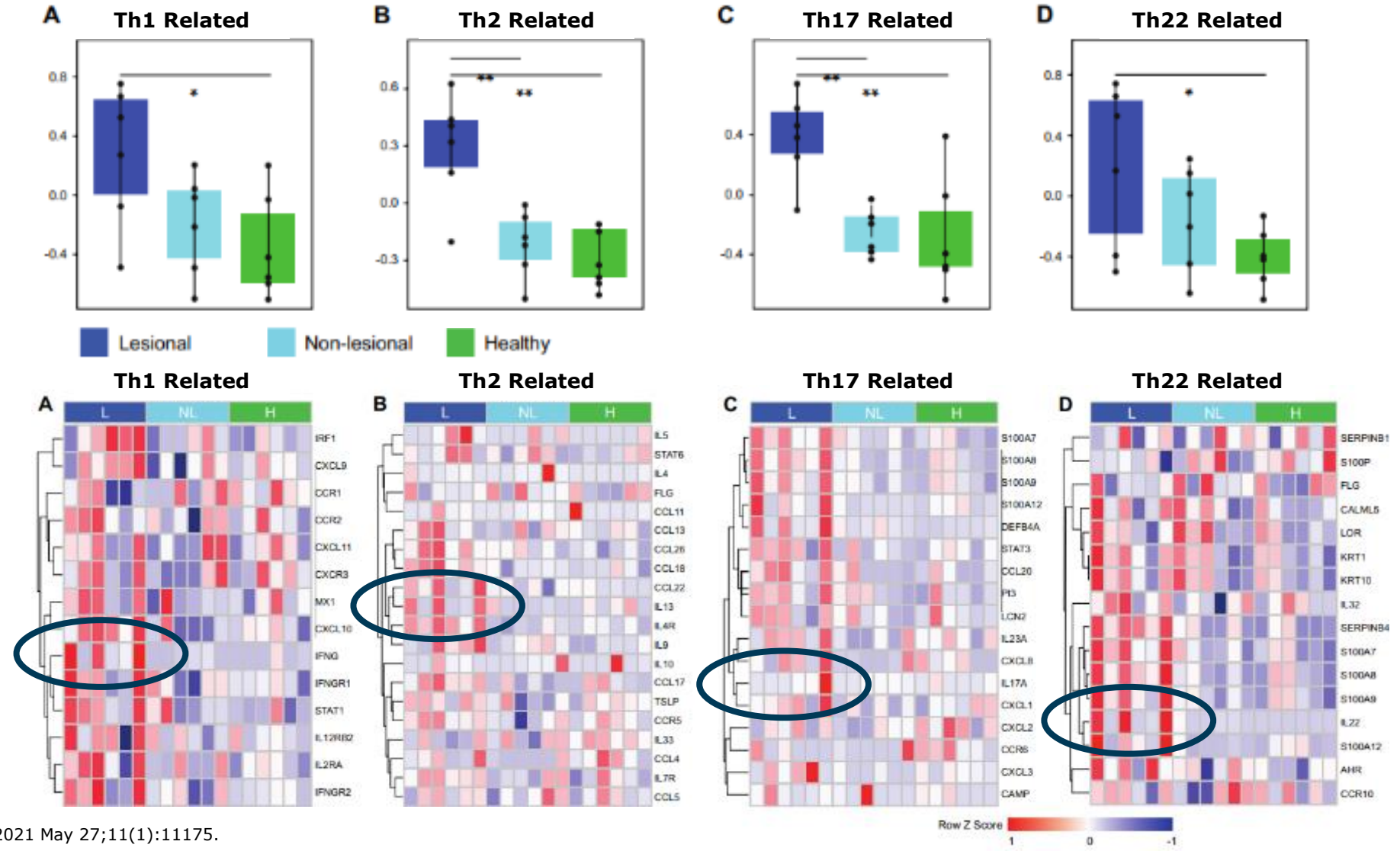


Elevated T Cells and Dendritic Cells are Hallmarks of Atopic Dermatitis (AD) Skin



Guttman-Yassky et al, JACI, 2011.

Broad T Cell (Th1, Th2, Th17 and Th22) Signatures Characterize AD



Wongvibulsin et al, Sci Rep. 2021 May 27;11(1):11175.

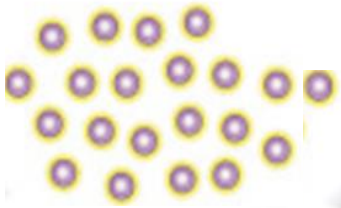
PBMC Assay for Assessment of Pharmacology in Patient-Derived Samples

PBMC isolation and CFSE labeling

PBMCs from Healthy or Disease Donors

CFSE labeling

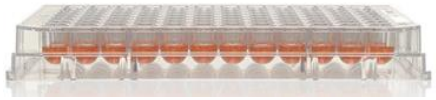
T cell activation and proliferation



CFSE labeled
PBMCs (200K/well)

ANB032 vs.
comparators,
Isotype control

sCD3/CD28



3 days

Analysis

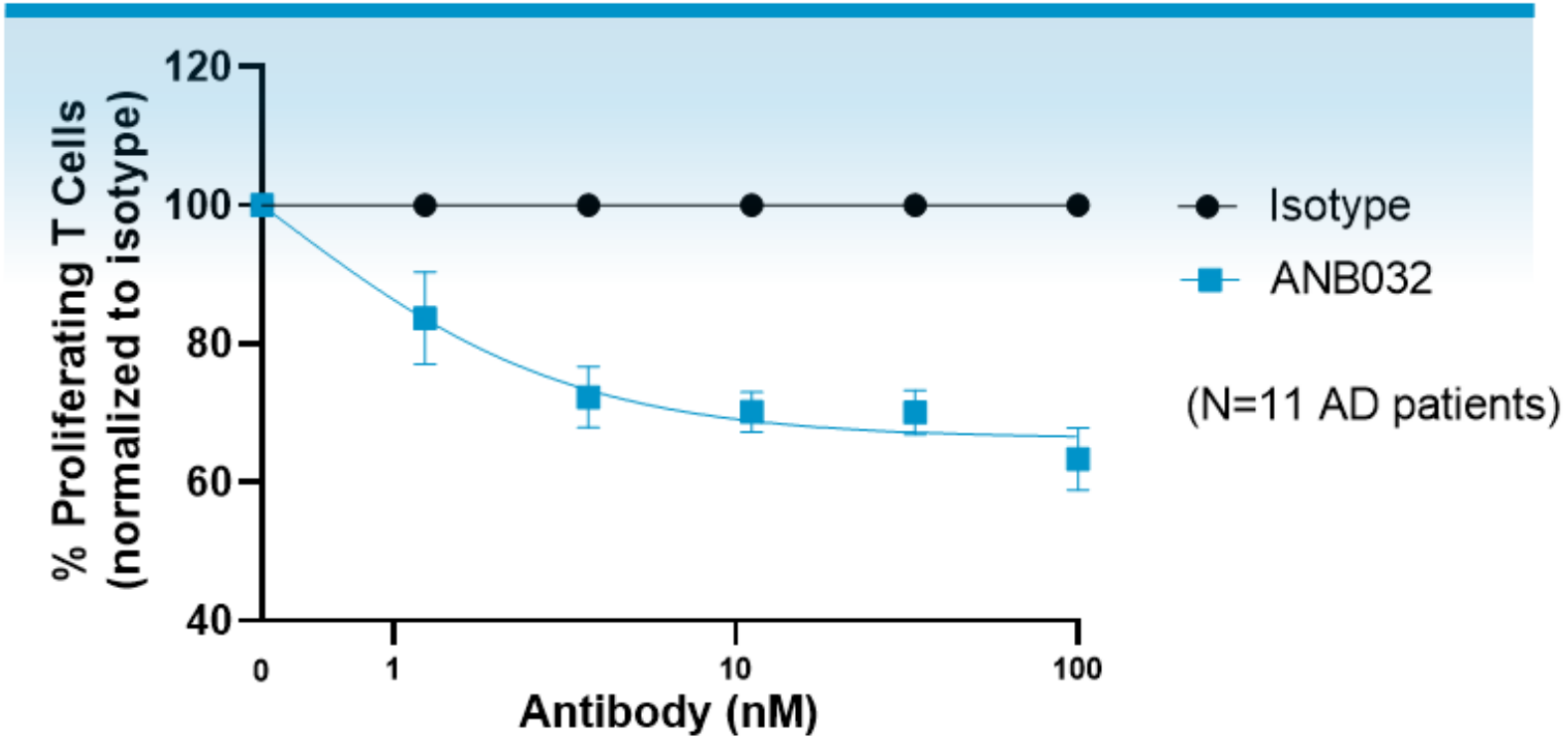
MSD
IFN-γ, IL-17A,
IL-13, IL-22, etc.

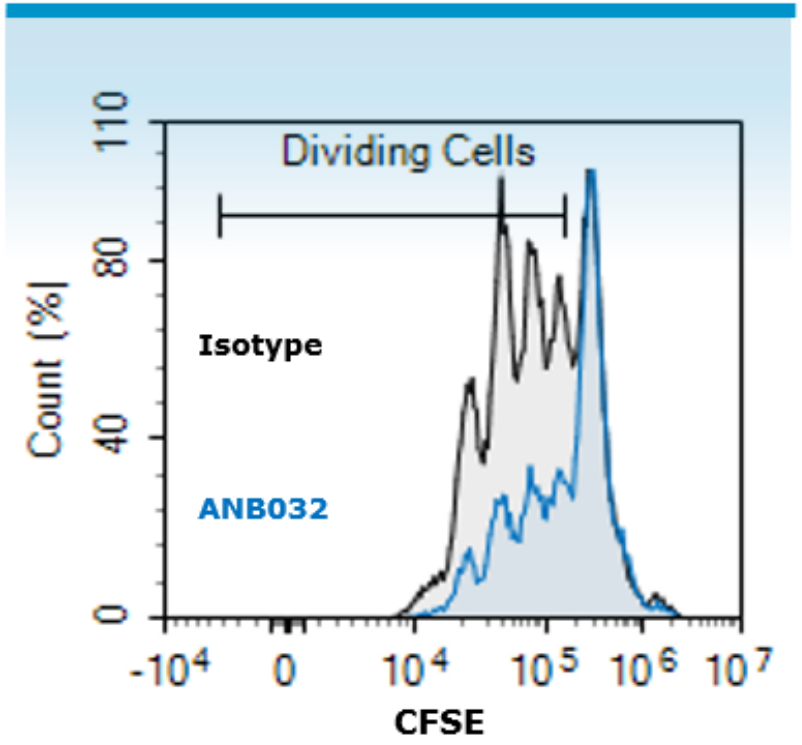
sup

cells

17 color FACS

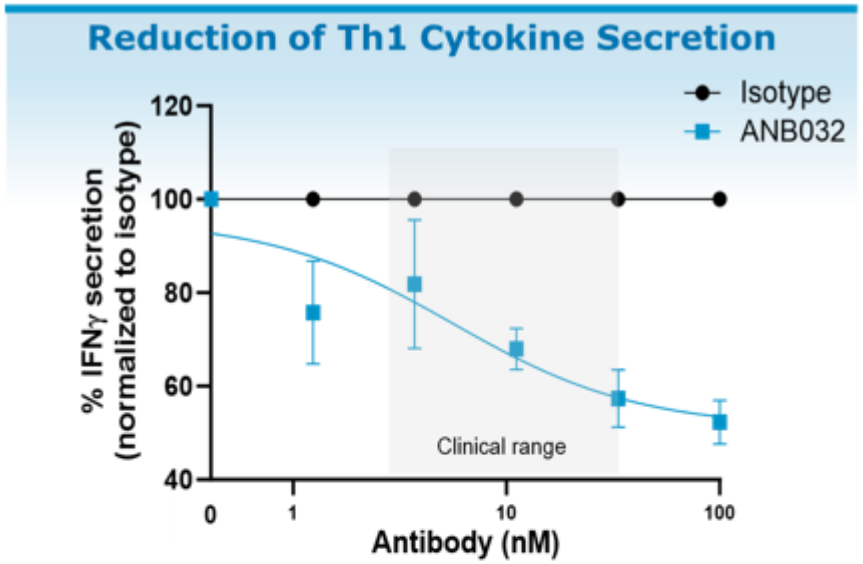
ANB032 Reduced T Cell Proliferation in AD Patient-Derived PBMCs

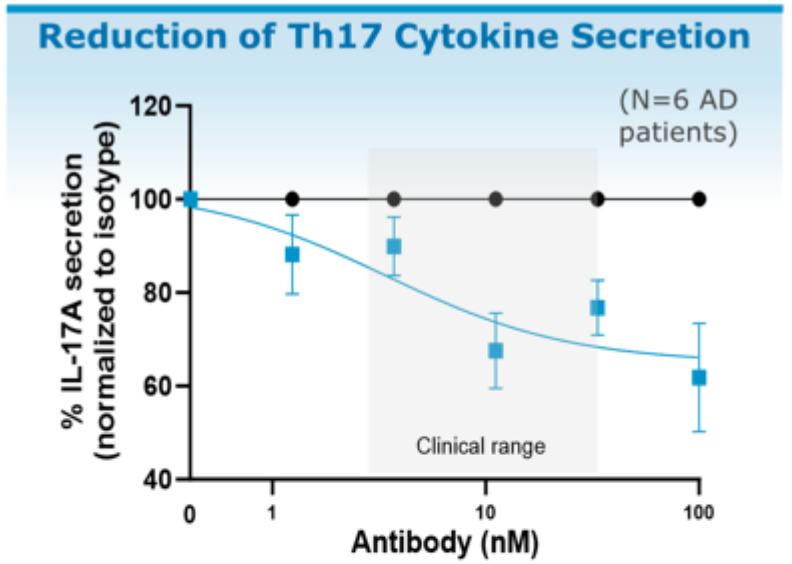


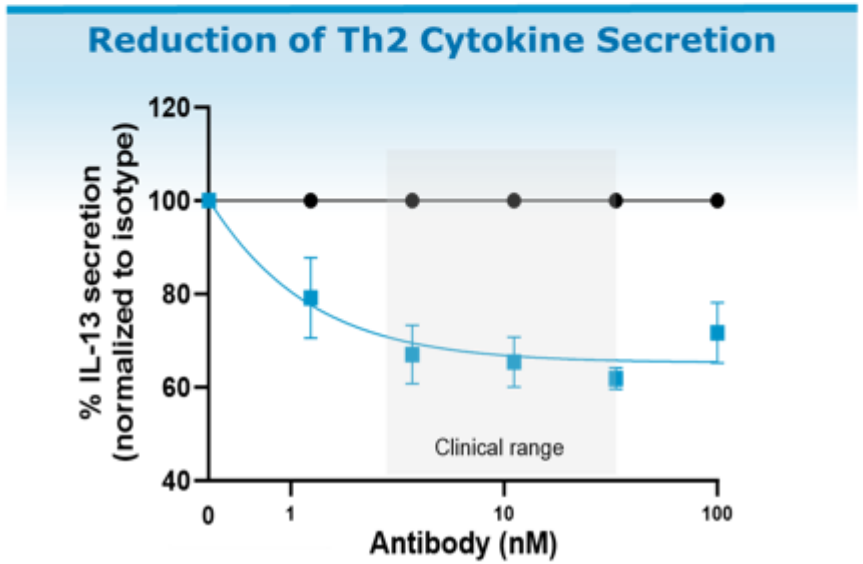


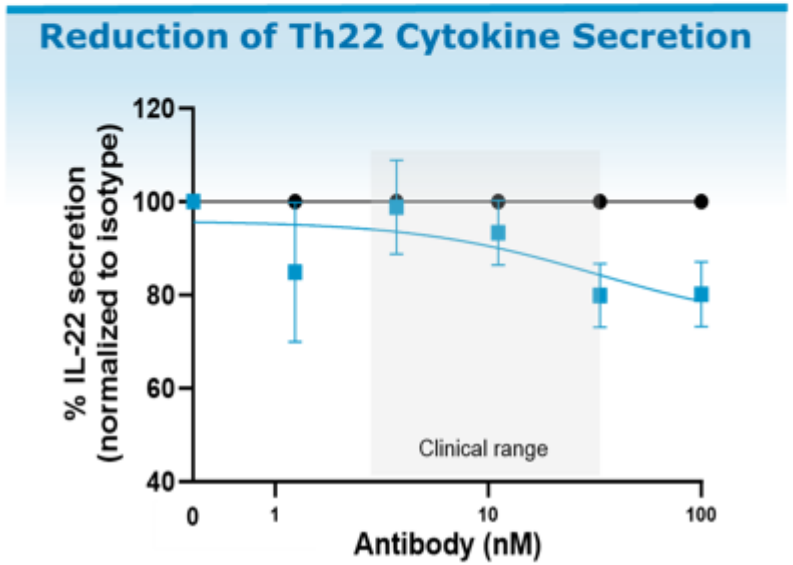
Hsu et al. Presented at the Antibody Engineering & Therapeutics Conference US, San Diego, Dec 12-16, 2021

ANB032 Reduced Th1, Th2, Th17 and Th22 Cytokine Secretion in AD Patient-Derived PBMCs In Vitro

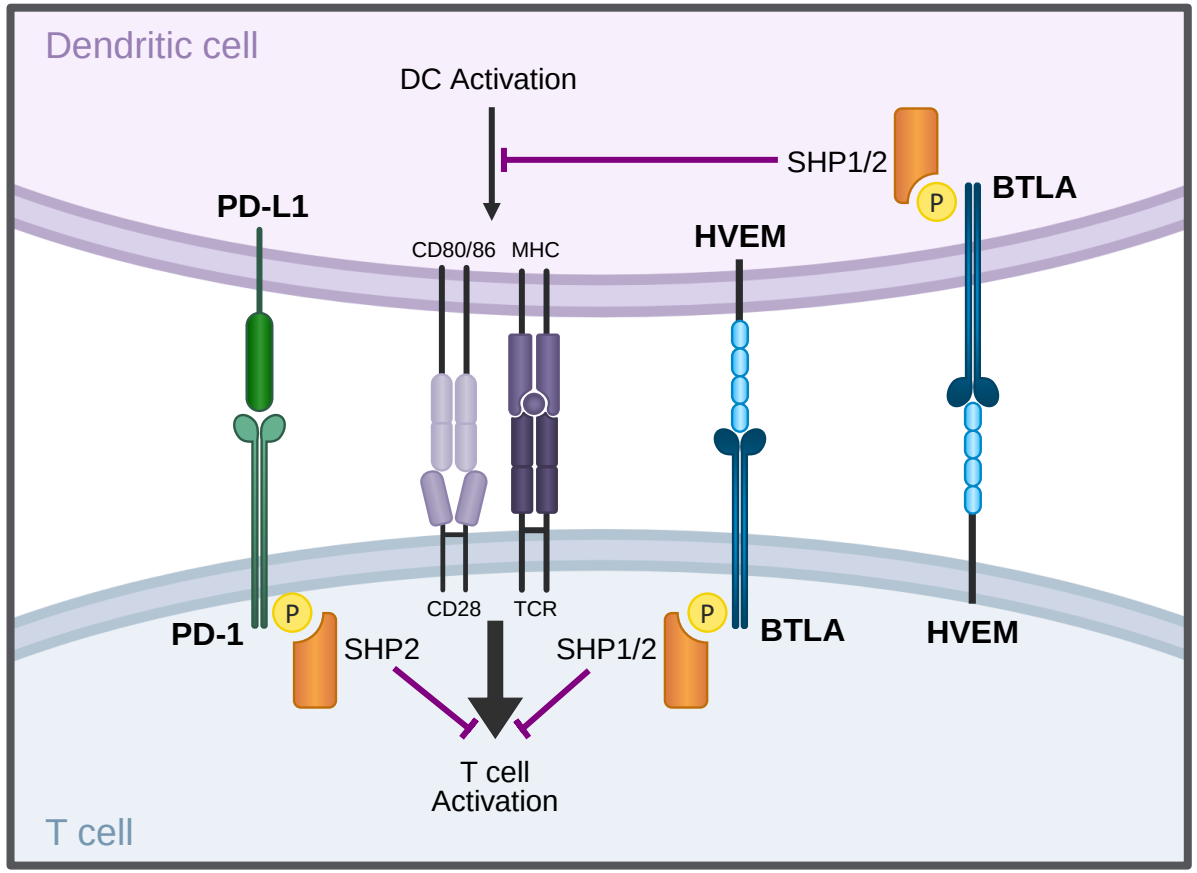








BTLA is Key Node of Immune Regulation



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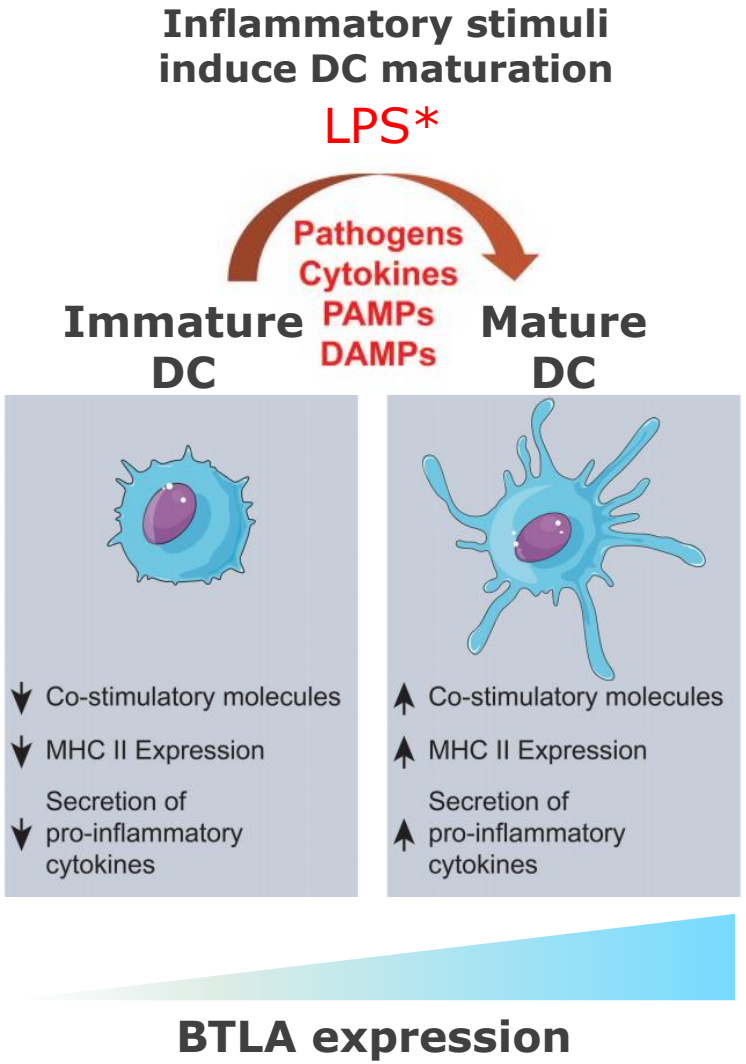
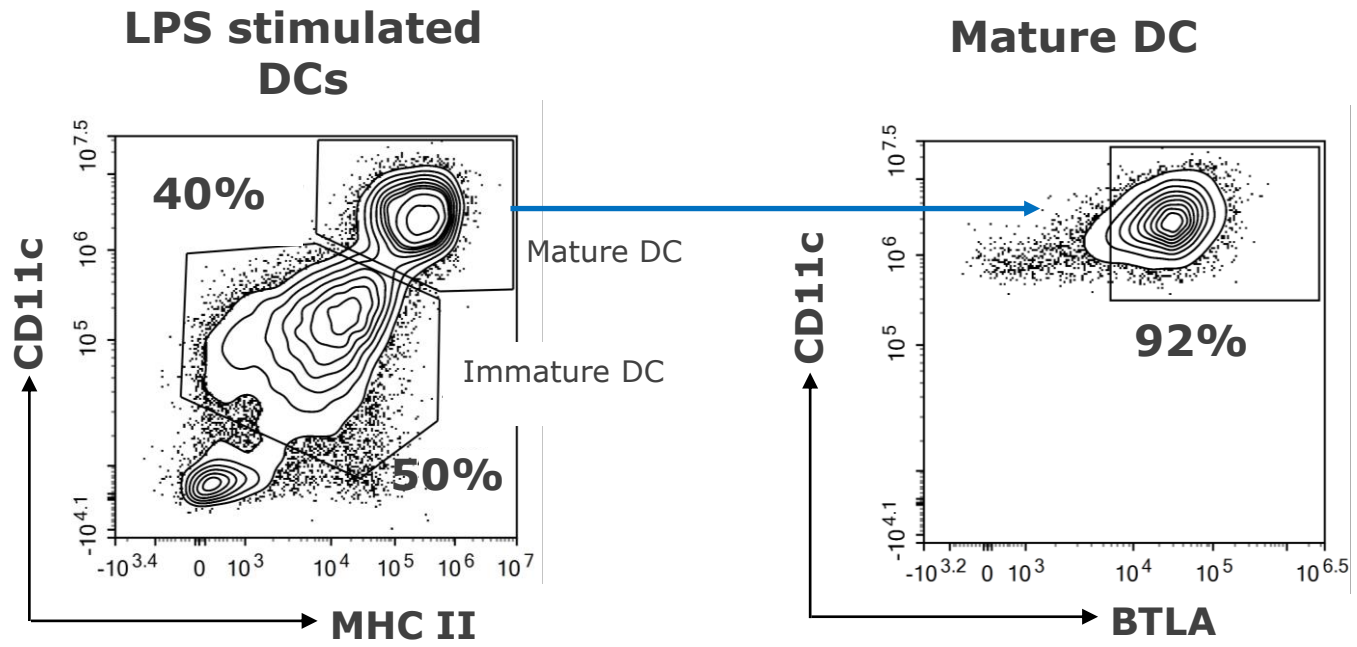
Adapted from Xu et al, J. Cell Biol. 2020 Vol. 219 No. 6

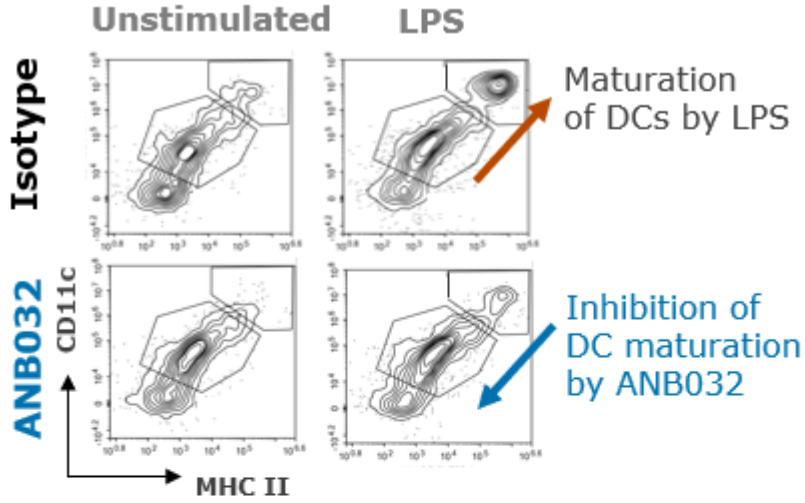
BTLA is Highly Expressed on Mature DCs

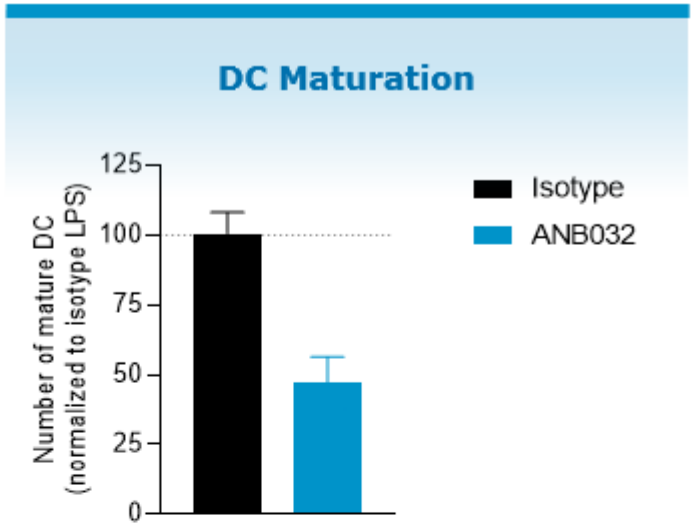


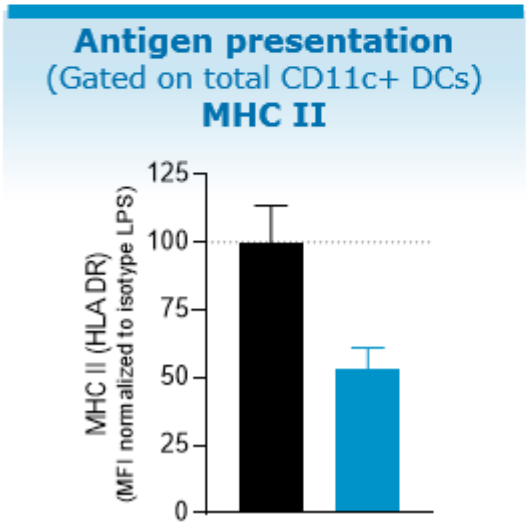


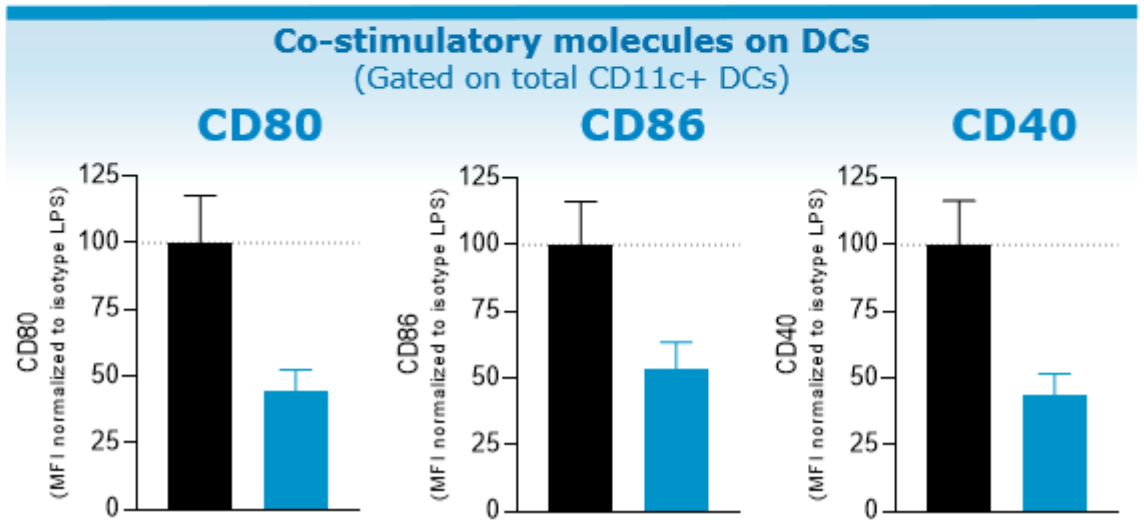
Adapted from Frontiers in Immunology. 2019 Jan 21;9:3176 ; * LPS=Lipopolysaccharide (a TLR4 agonist).

ANB032 Reduced DC Maturation, Antigen Presentation, and Co-stimulatory Molecule Expression



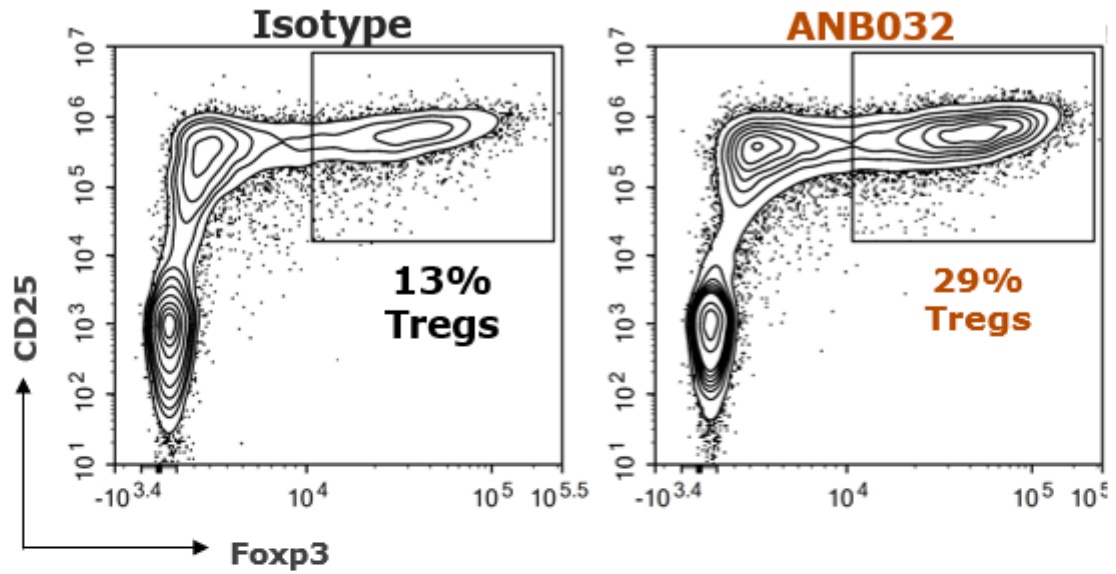


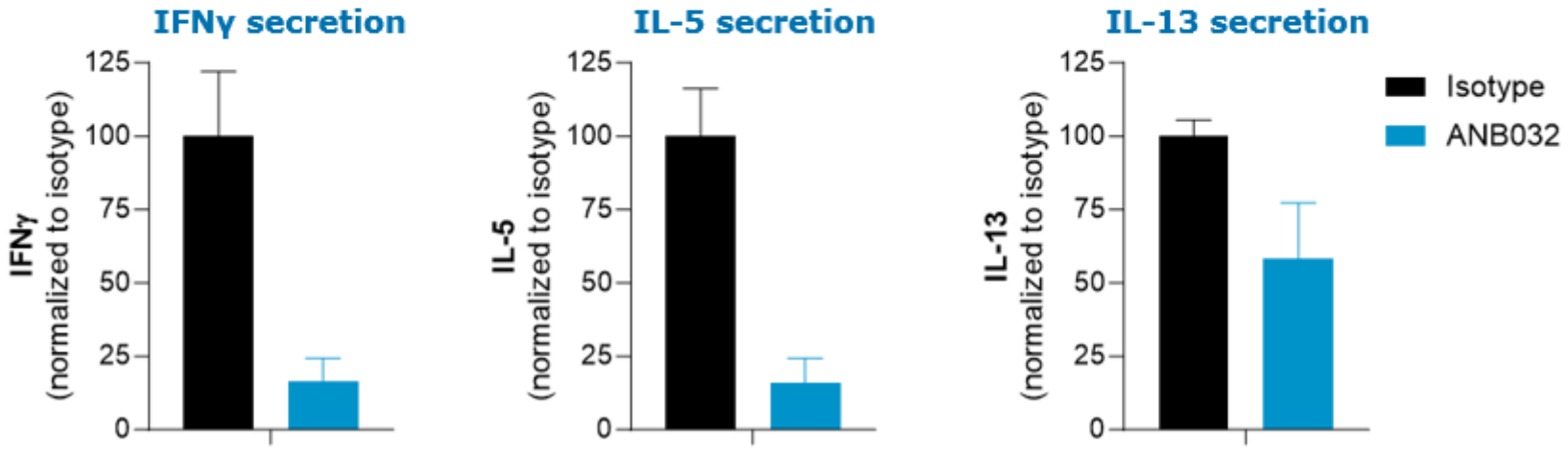




Muench, et al. Presented at the American Academy of Dermatology Meeting, San Diego, March 8-12, 2024.

ANB032-treated DCs Induced Functional Tregs and Reduced Inflammatory Cytokines in a MLR Assay





Muench, et al. Presented at the American Academy of Dermatology Meeting, San Diego, March 8-12, 2024.

Conclusion

- 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
- ANB032 reduced Th1, Th2, Th17 and Th22 inflammatory cytokine secretion from atopic dermatitis patient-derived PBMCs
- ANB032 reduced the maturation of DCs, reduced MHC II, reduced co-stimulatory molecules and induced Tregs in vitro
- ANB032 is currently being evaluated in an ongoing Phase 2 study in moderate-to-severe atopic dermatitis (NCT05935085)