AnaptysBio

Discovery of a PD-1 Checkpoint Agonist Antibody for Autoimmune/Inflammatory Disease

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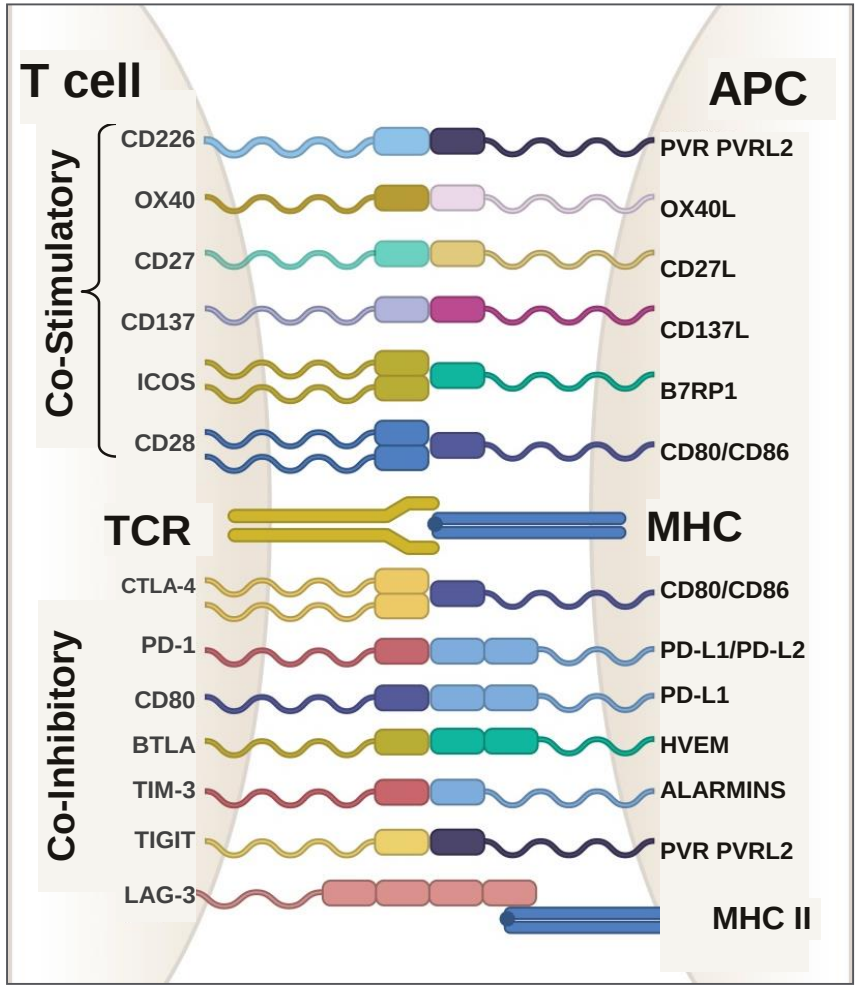
PD-1 is an Inhibitory Checkpoint Molecule that Down-modulates T Cell Responses



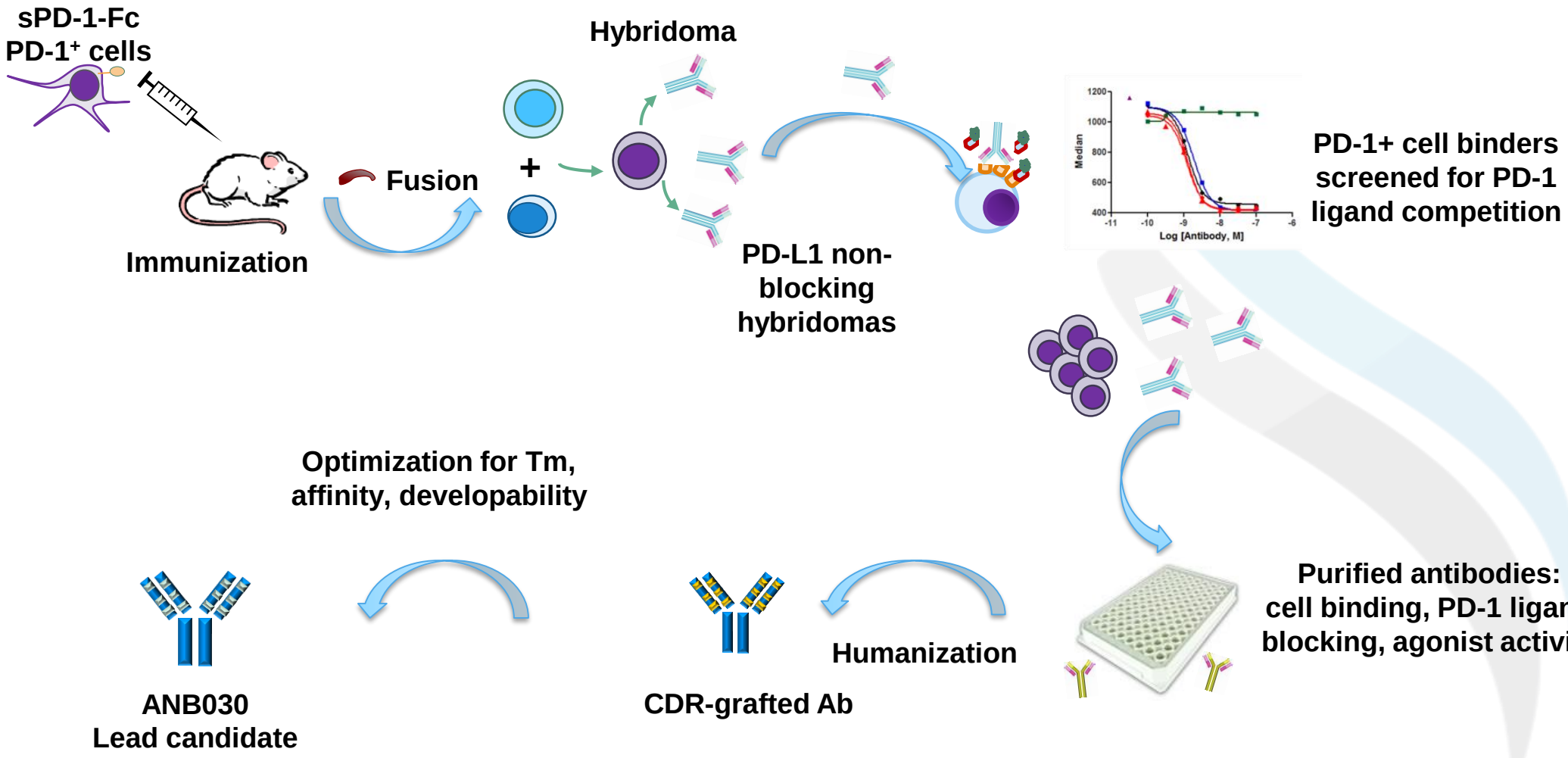
- **Immune checkpoint receptor-ligand interactions are essential for down-regulating immune responses and maintaining self-tolerance**
- **Functional antagonist antibodies to PD-1 and CTLA-4, major checkpoints on activated T cells, enhance existing immune responses and are approved therapeutics for immuno-oncology**
- **Genetic mutations in the PD-1 pathway have been shown to increase susceptibility to various autoimmune and inflammatory diseases***
- **We hypothesize that many human autoimmune diseases occur due to dysregulated PD-1 signaling, leading to uncontrolled T cell responses**
- **Agonist antibodies to PD-1 that mimic the function of natural ligands and augment PD-1 signaling have the potential to suppress human autoimmune/inflammatory diseases and reinstate tolerance**

Agonist antibodies that negatively signal are expected to restore natural immune modulation

*Okazaki T and Honjo T (2007) *Int. Immunol.* **19**:813.

PD-1 Agonist Antibody Discovery

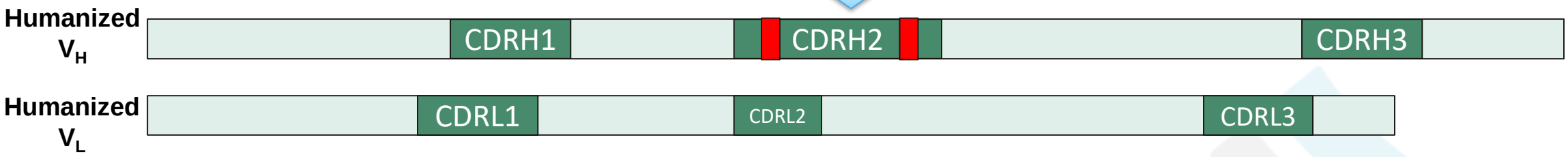
ANB030 is a Humanized IgG1,κ Hybridoma-derived PD-1 Agonist Antibody



ANB030 is PD-L1 non-blocking, optimized for affinity and functional activity, and has no ADCC activity

Optimization of ANB030 V_H Sequence for Stability and Affinity

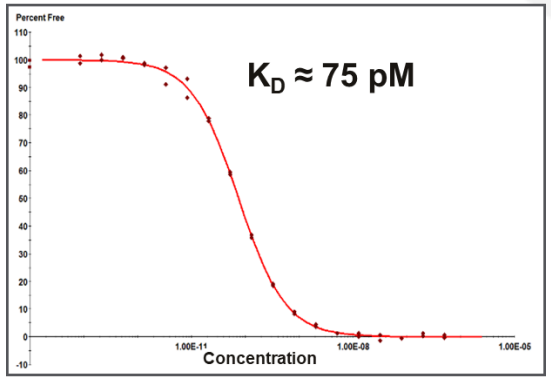
Saturating Mutagenesis at 4 V_H Positions



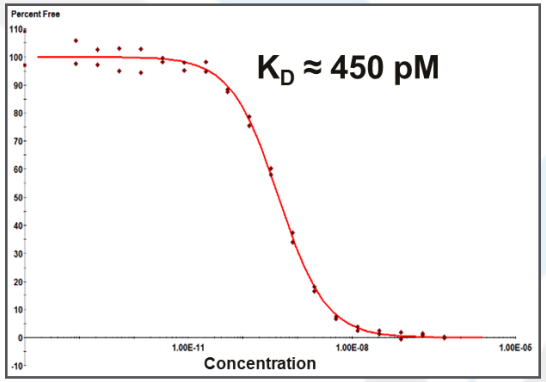
ANB030 Variant Antibody	K_D Human PD-1	K_D Cynomolgus PD-1	Fab Tm
Mouse chimeric	4.3 nM	n.d.	n.d.
CDR-grafted	0.19 nM	1.00 nM	61.2°C
CDR-grafted Optimized (ANB030)	0.075 nM	0.45 nM	66.1°C

Affinity by KinExA

Human PD-1

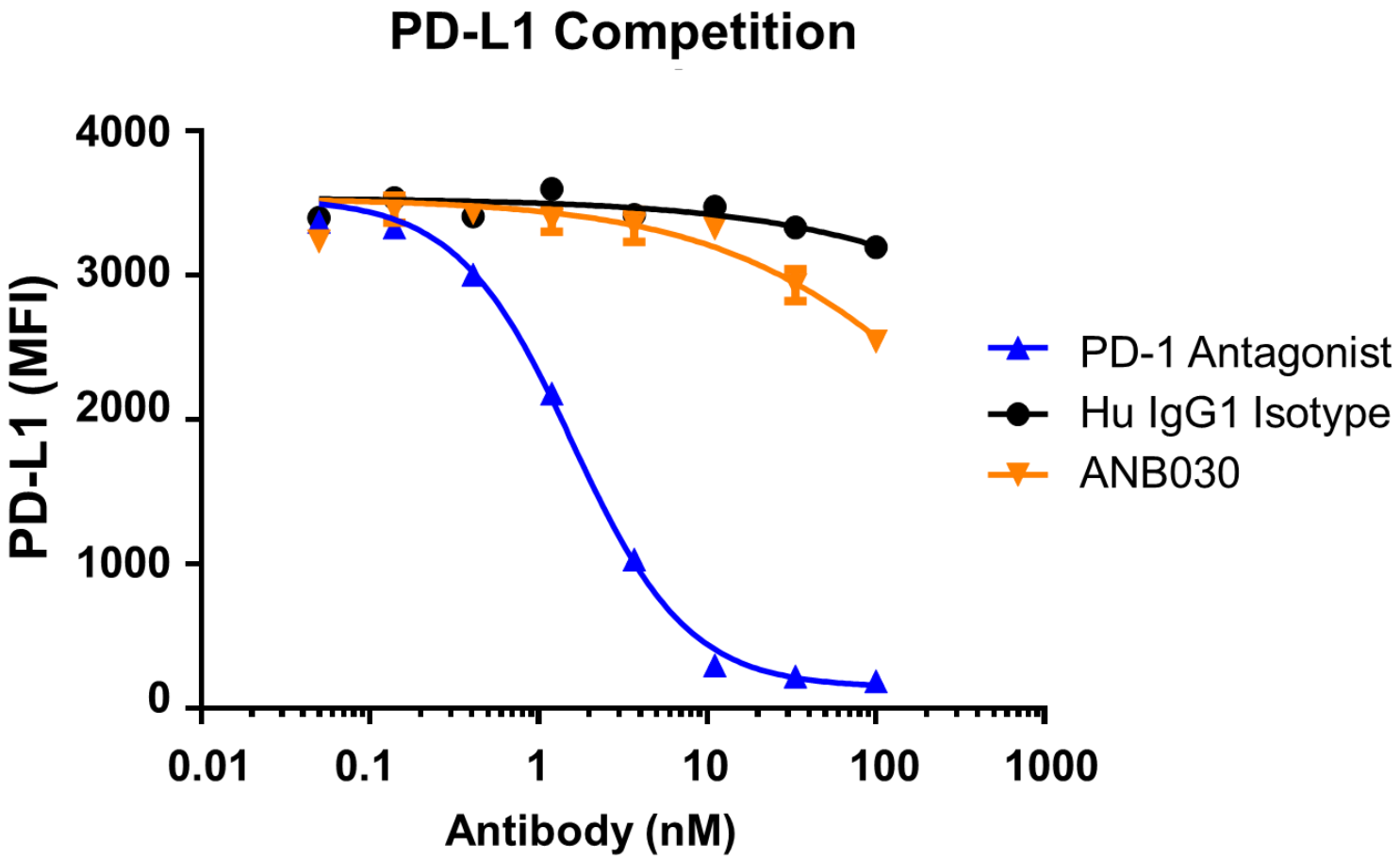


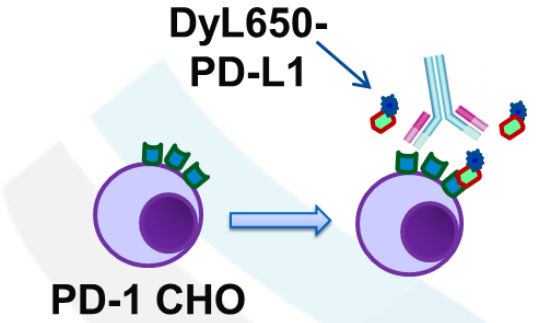
Cynomolgus Monkey PD-1



Optimized for thermal stability and affinity with 2 V_H substitutions
Additionally de-risked in 28-day accelerated stability and pre-formulation studies at 100 mg/ml

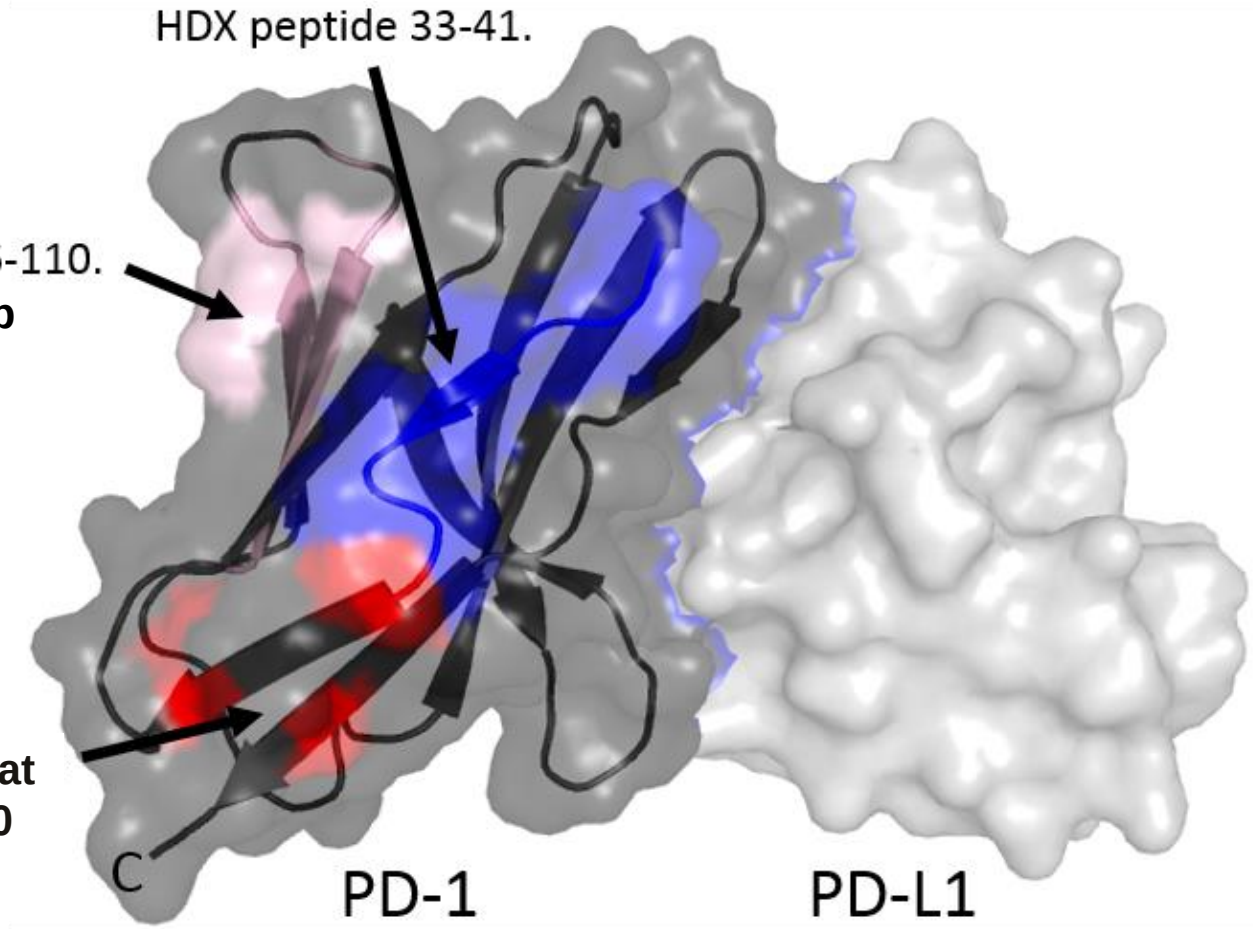
ANB030 Binding to PD-1 Does Not Compete with PD-L1 Binding





Ligand non-blocking is desirable to avoid potential antagonist activity

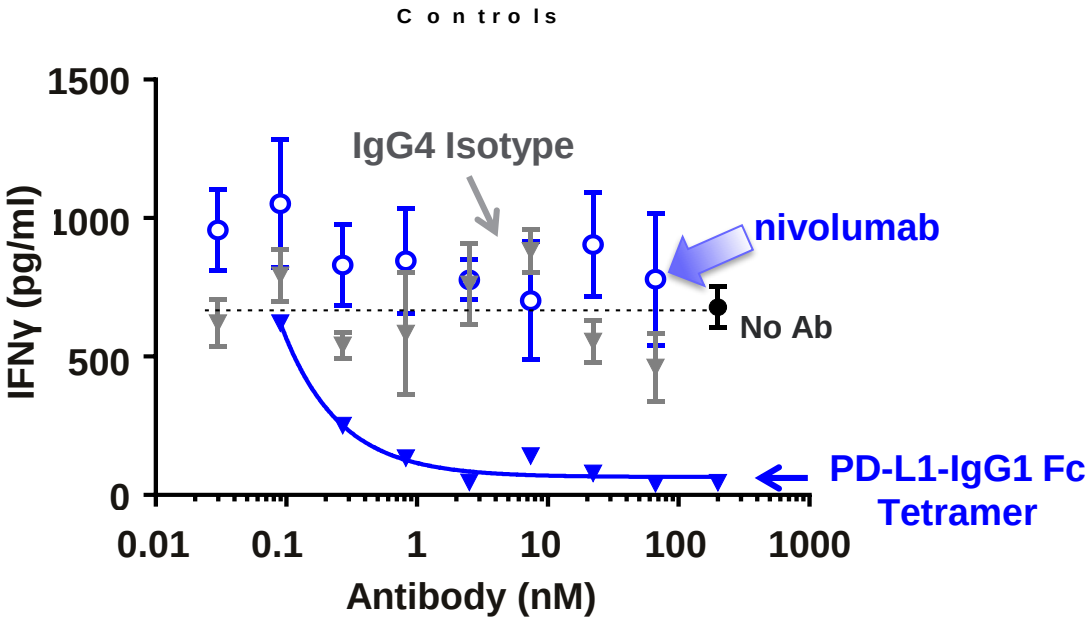
Model of the Epitope Bound by PD-1 Agonist Antibody ANB030

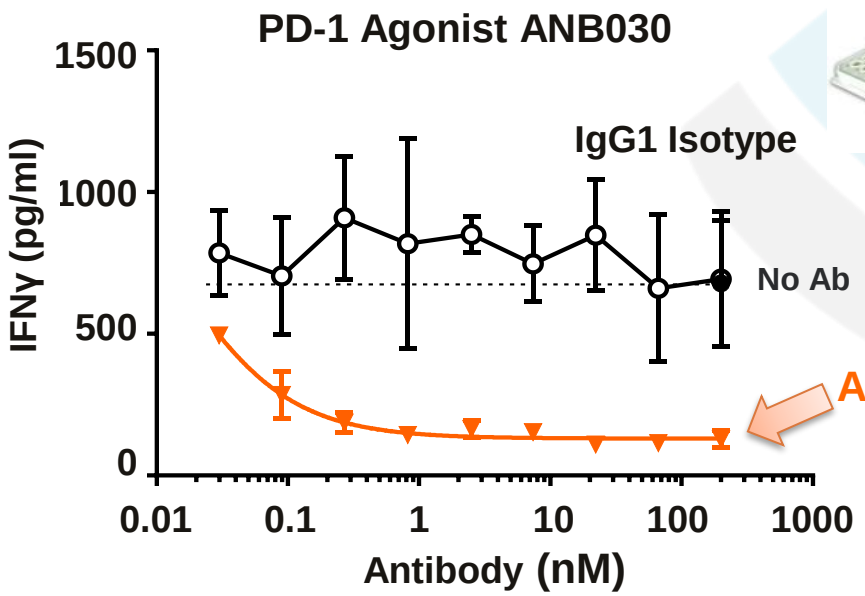


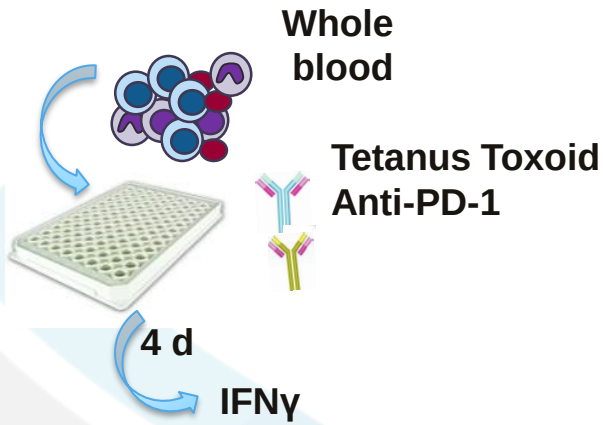
- **Regions on PD-1 bound by ANB030 were identified by hydrogen-deuterium exchange (pink, blue) and PD-1 surface point mutations (red)**
- **Both datasets generated a consistent model of the ANB030 epitope**

ANB030 binds PD-1 on a more membrane-proximal region opposite the PD-L1 binding site

ANB030 in Solution Potently Inhibits Whole Blood Tetanus Toxoid Recall Response

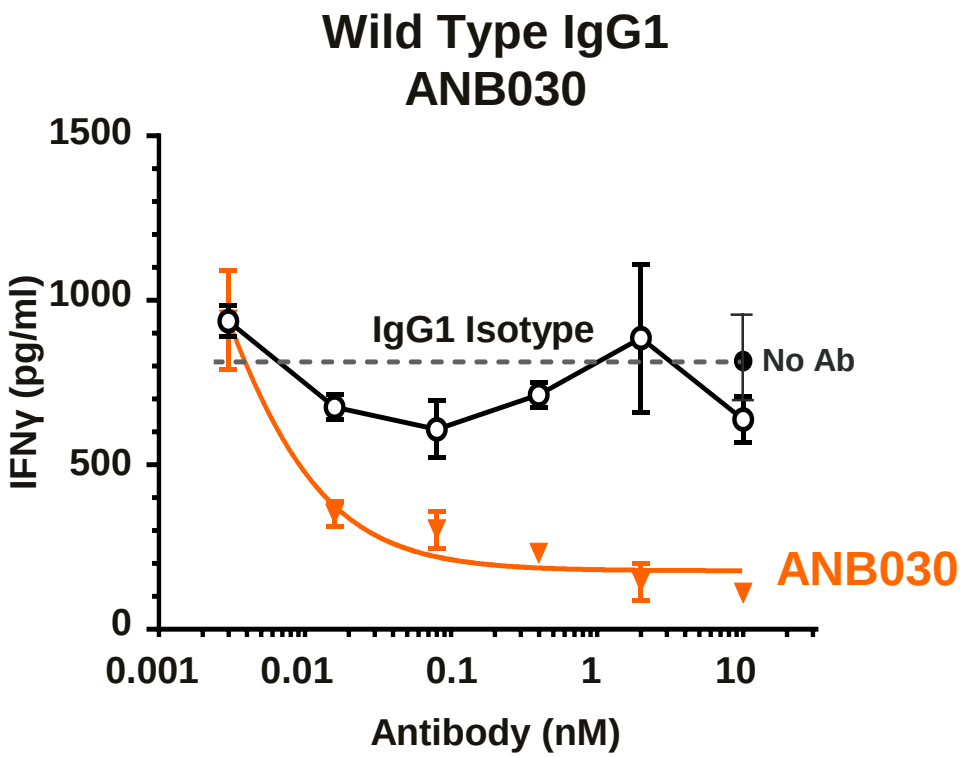


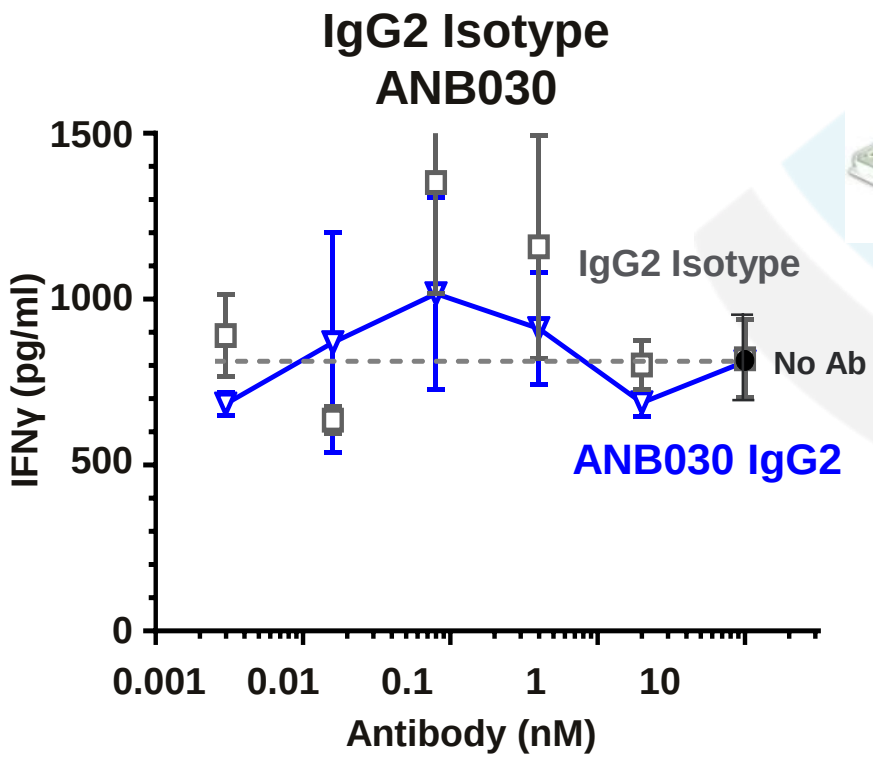


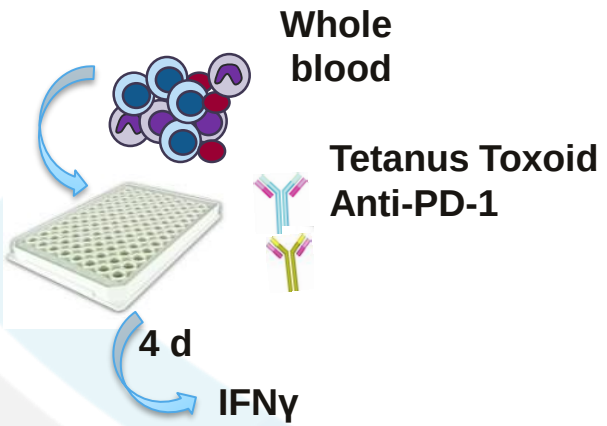


PD-1 agonist antibody inhibits tetanus toxoid IFNγ recall response in whole blood
PD-1 antagonist antibody nivolumab shows no inhibition

ANB030 Functional Agonist Activity in Whole Blood is Dependent on FcγR Engagement







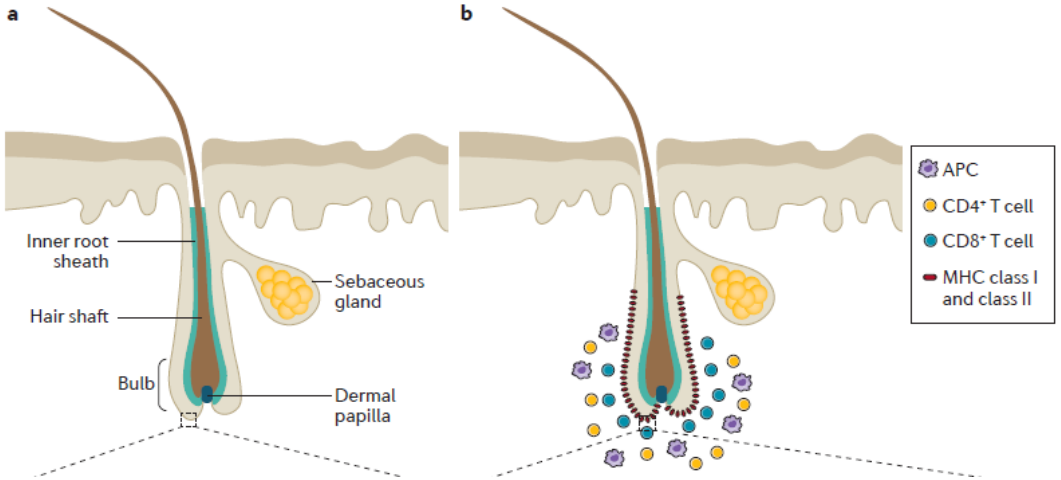
ANB030 human IgG2, IgG4, or IgG1(L234A,L235A) isotypes lack agonist activity in solution

Alopecia Areata Is An Immune-Mediated Disease

Alopecia areata is an immune-mediated form of hair loss resulting from breakdown of immune privilege that is driven by keratinocyte and melanocyte antigen-specific T cells producing IFNγ

Healthy Hair Follicle

- Low MHC class I
- Locally immune suppressed

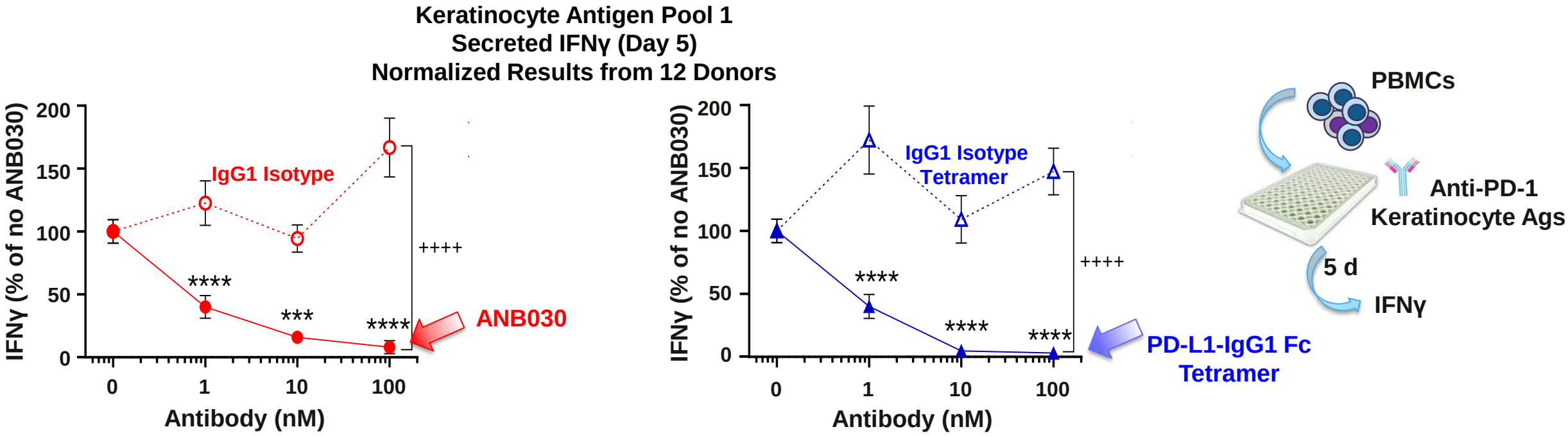


Alopecia areata Hair Follicle

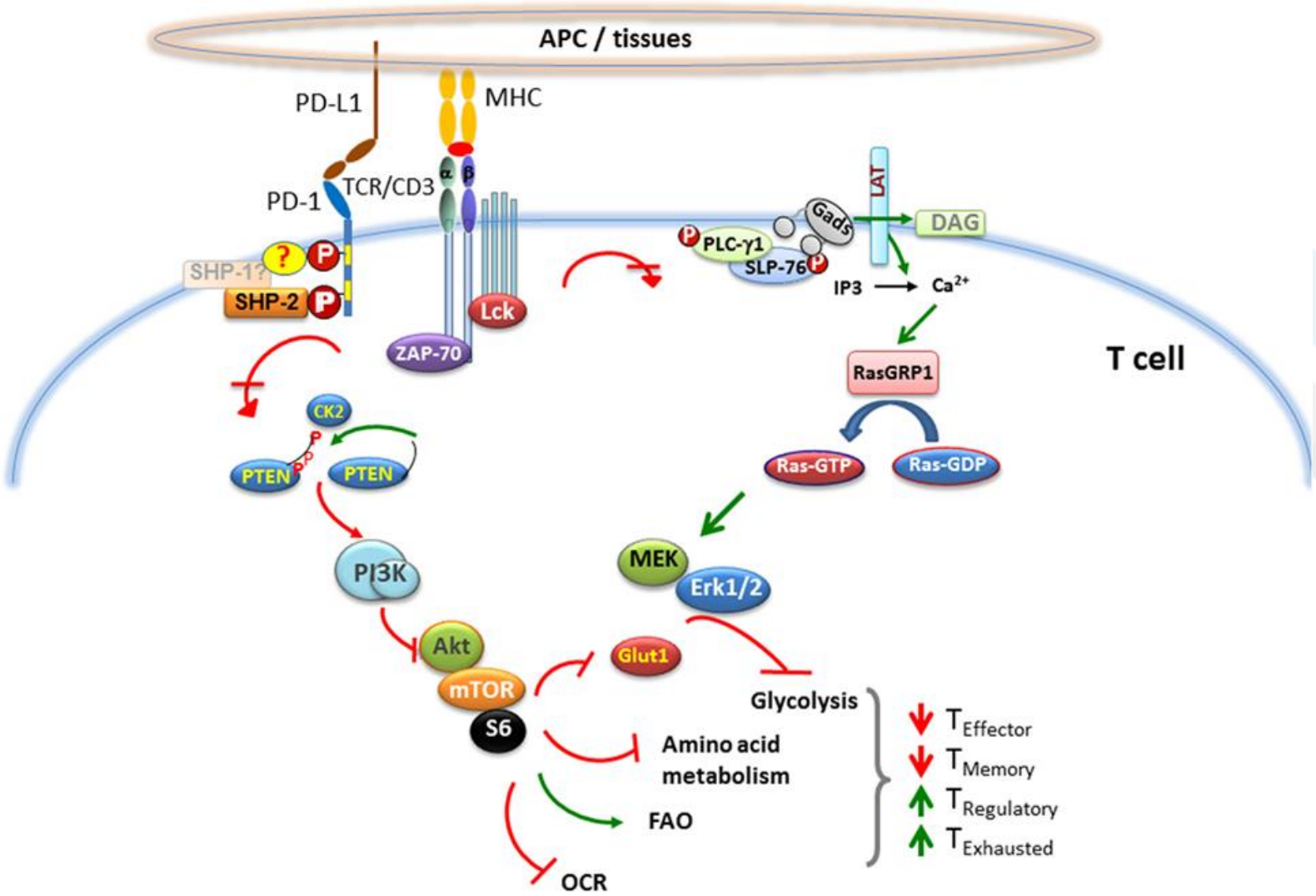
- NKG2D+CD8+ infiltrate into hair follicle root sheaths
- Excessive IFNɣ production by activated T cells leads to loss of hair follicle immune privilege
- Abnormal expression of MHC class I and II molecules
- Subsequent destruction of hair follicle cells & hair loss

Inhibition of IFNγ Production by ANB030 in Human Alopecia Areata PBMCs Stimulated with Keratinocyte Peptide Antigens

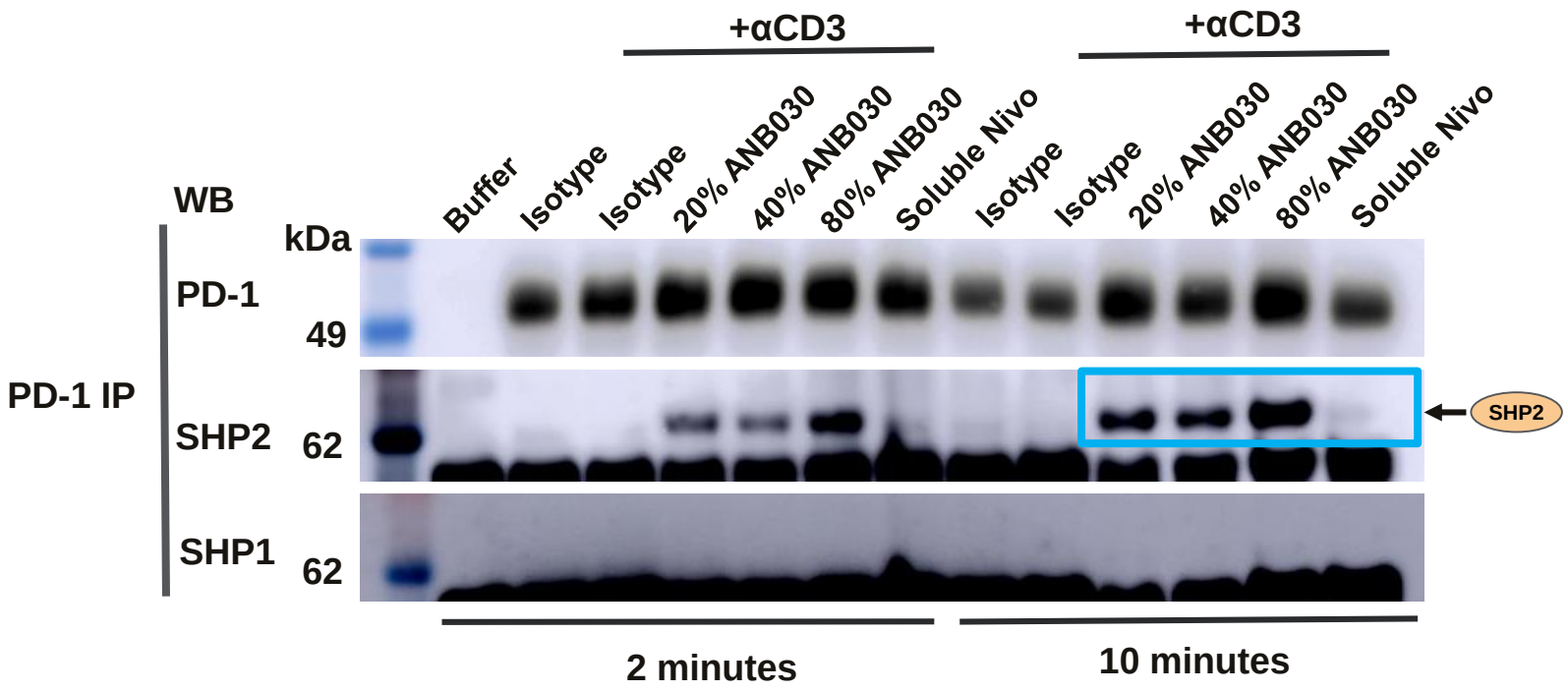
Alopecia areata is an immune-mediated form of hair loss resulting from breakdown of immune privilege that is driven by keratinocyte and melanocyte antigen-specific T cells producing IFNγ

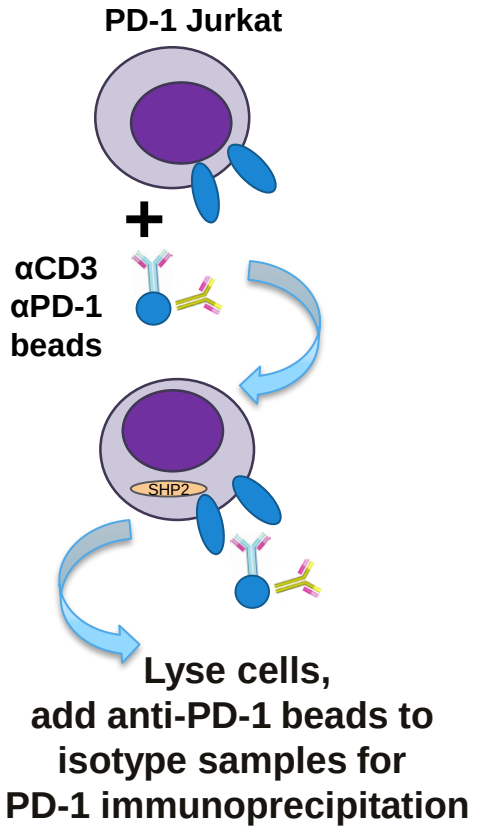


PD-1 Signaling in T Cells



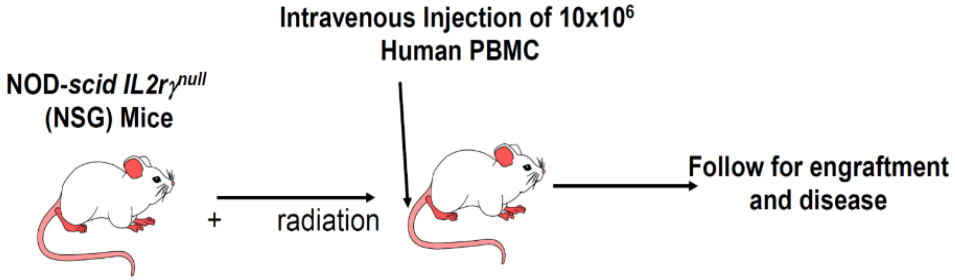
ANB030 Induces SHP2 but not SHP1 Recruitment to PD-1 after Activation of Jurkat PD-1 Cells





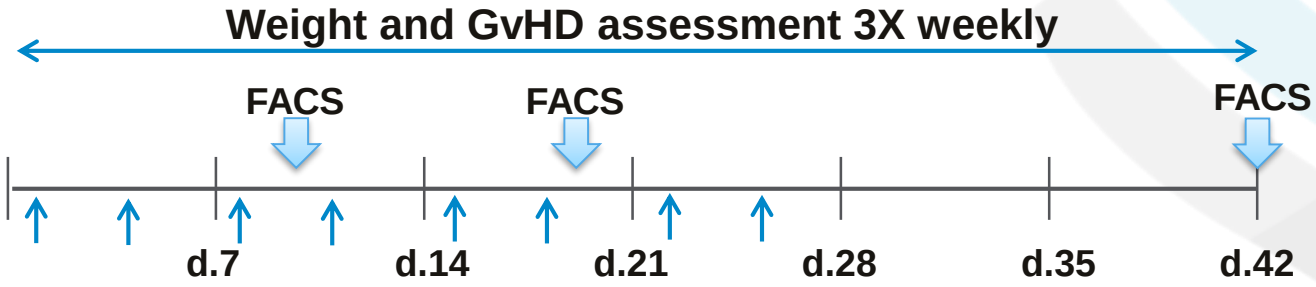
ANB030 had no effect on signaling pathways in the absence of T cell activation

NSG/Hu-PBMC Graft vs. Host Disease Model



Endpoints:

- Weight loss (-20%)
- Death
- GvHD scores



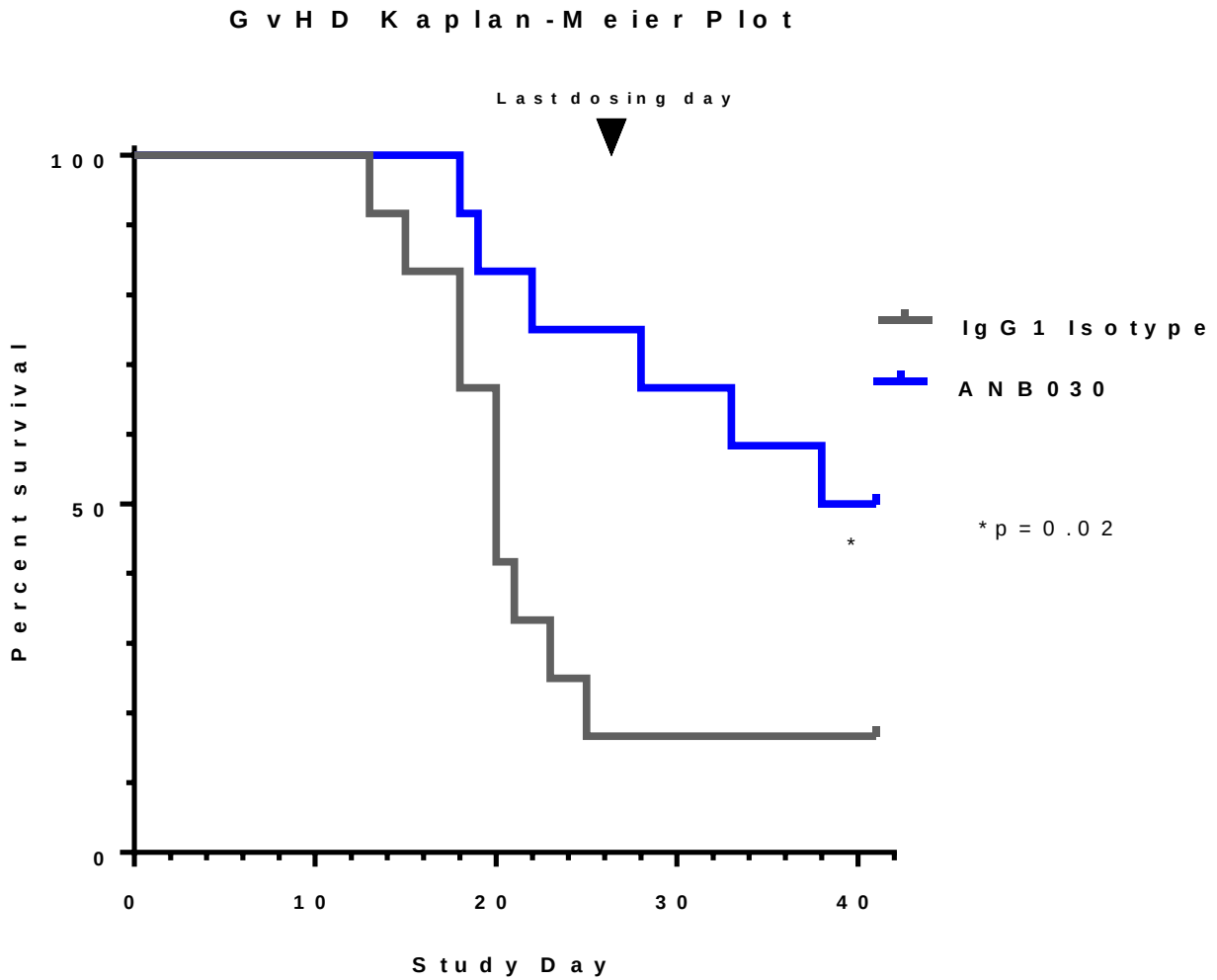
Day 0: Irradiation
0.9 x 10^7 hu PBMC i.v.
mAb dosing 24-hr-post PBMC

Groups:

1. Isotype control IgG1
2. Anti-PD-1 agonist IgG1 (ANB030)
3. CTLA-4-Ig (positive control)

↑ Dosing days

PD-1 Agonist Antibody ANB030 is Efficacious in an Acute Xenogeneic Graft vs. Host Disease Model



Treatment	Median Survival
Isotype	20 days
ANB030	39.5 days

Efficacy of ANB030 in the model is dependent on its IgG1 isotype

Summary:
PD-1 Agonist Discovery for Autoimmune/Inflammatory Disease

- A functional agonist anti-PD-1 antibody that down-regulates antigen-specific immune responses and lacks antagonist activity has been discovered and optimized
- ANB030 is a humanized IgG1/κ anti-PD-1 agonist antibody that is non-blocking for PD-L1 binding and requires Fcγ receptor engagement for its functional activity in solution
- Signaling mechanism studies show similar PD-1-dependent effects for ANB030 and PD-L1-Fc
- ANB030 demonstrated efficacy in a xenogeneic NSG-Human-PBMC graft vs. host disease model
- An IND for ANB030 has been filed and Phase 1 clinical trial initiation is anticipated in H1 2020
- Anti-PD-1 antibodies that mimic activity of natural ligands and down-modulate T cell responses have the potential to restore and maintain immune balance in autoimmune and inflammatory diseases

Acknowledgments

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