Discovery and Characterization of PD-1 Agonist Antibodies

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ABSTRACT

T cell function is regulated by complex signaling networks of interconnected activators and inhibitors. Blockade of inhibitory receptors such as PD-1 has emerged as a novel treatment for multiple forms of cancer. Rather than block the interaction of PD-1 with its endogenous ligands, we sought to develop antibodies that can activate this pathway to inhibit T cell activation and function.

We conducted our antibody screen against human, cynomolgus monkey, and mouse PD-1 to select for antibodies with species cross-reactivity. We then conducted functional screens for antagonism and agonism using a human PD-1 Jurkat reporter cell-based screen. We identified several classes of PD-1-specific antibodies with a range of functional activities. Approximately one-third of antibodies exhibited antagonist activity when in solution, but agonist activity when immobilized. Less than 1% of antibodies were antagonists in solution with no agonist activity. The observation that most PD-1 antibodies that are antagonists in solution also function as agonists when immobilized suggests a fundamental relationship between agonism and antagonism of the PD-1 pathway. Of particular interest to us were antibodies that displayed agonist activity yet did not antagonize PD-1/PD-L1 interactions. We further characterized the ability of this subset of antibodies to inhibit function of primary T cells.

In summary, we identified several classes of PD-1 antibodies that antagonize and/or agonize human and mouse PD-1 when in solution and/or when immobilized. We believe the subset of antibodies that can agonize PD-1 and attenuate T cell activation create an opportunity for developing new therapeutics for autoimmune and inflammatory diseases.

INTRODUCTION

- PD-1 is a critical immune regulator found on multiple cell types, most notably CD4+ and CD8+ T cells (*1*).
- The PD-1/PD-L1 axis has emerged as a prominent immunotherapeutic target that can be blocked by monoclonal antibodies. This approach is efficacious in treating multiple forms of cancer (*2*).
- Blockade of the PD-1/PD-L1 axis frequently results autoimmune complications (*3, 4*).
- Data from mice lacking either PD1-1 or PD-L1 indicates that loss of this immunoregulatory axis results in abnormal immune responses (*5*).
- Based on these observations we sought to make a novel class of immunomodulatory agents that would specifically agonize the PD-1 pathway

RESULTS

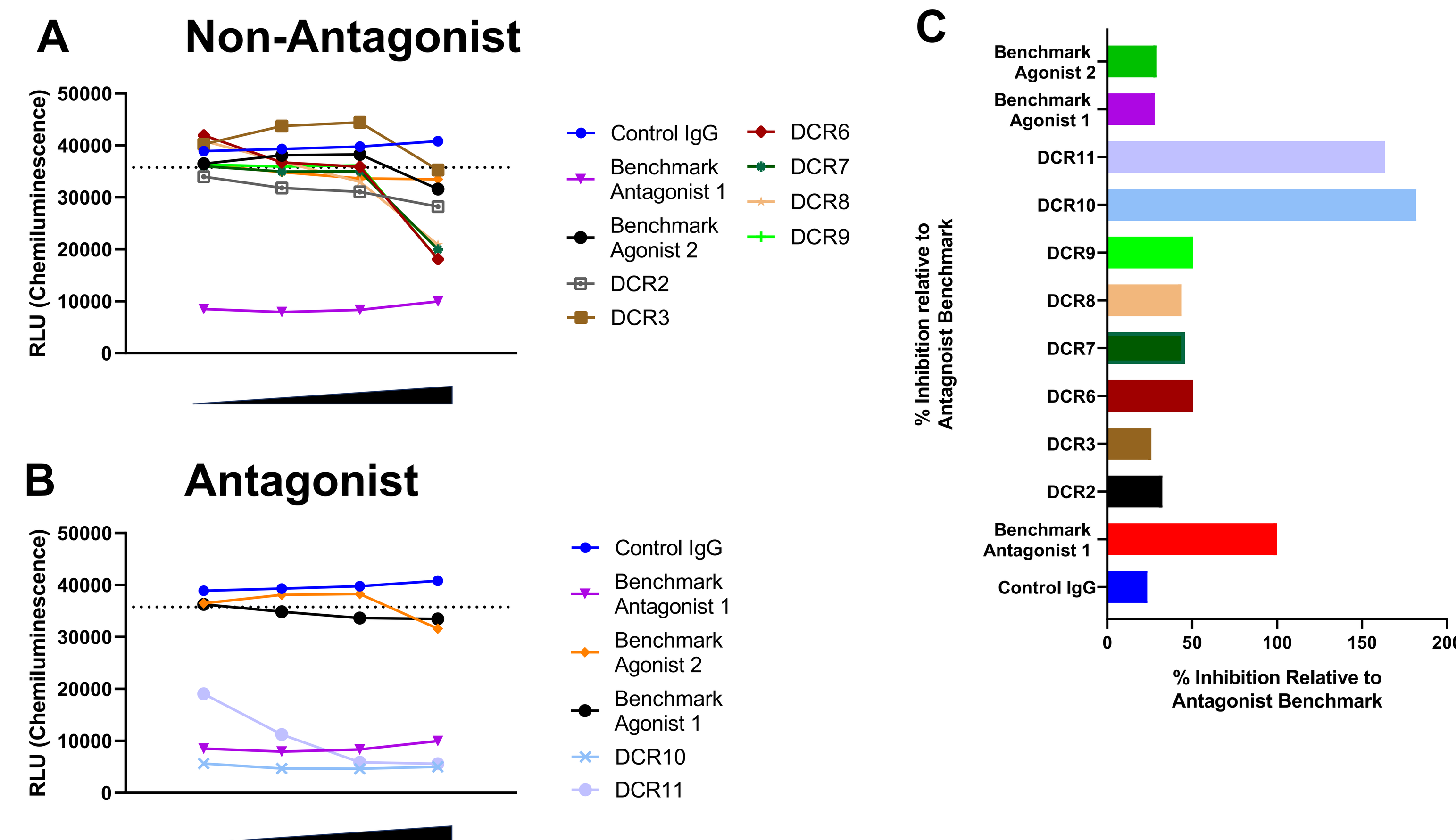

Figure 1 – Identification of PD-1 Antagonist Antibodies

Representative dose responses of experimental antibodies that were classified as PD-1/PD-L1 Non-Antagonists (A), or Antagonists (B). (C) Data from the highest antibody doses in (A) and (B) plotted together.

RESULTS

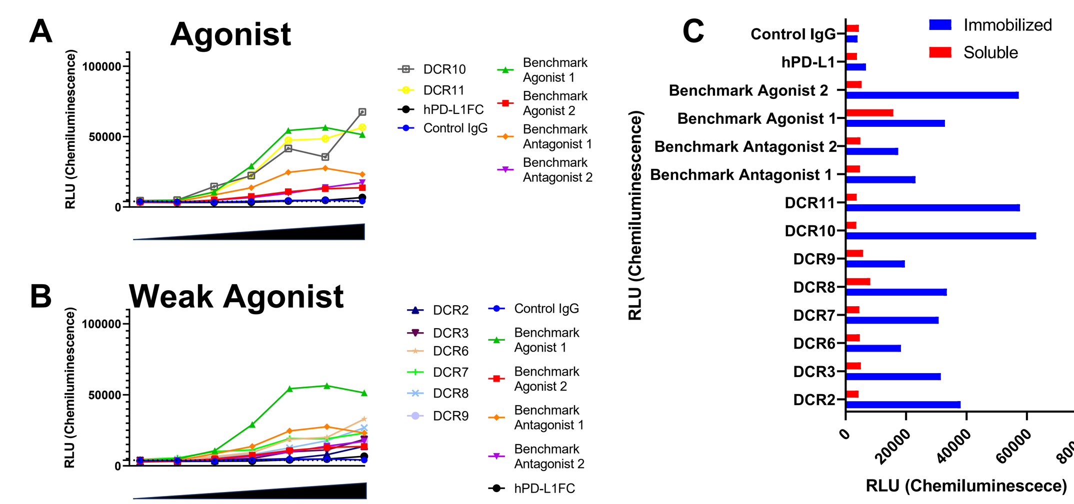

Figure 2 - Identification of PD-1 Agonist Antibodies

Representative dose responses of experimental antibodies that were classified as PD-1 agonists (A) or weak agonists (B). (C) Data from the highest dose of antibody plotted in A and B, as well as the same high dose of antibody assayed in solution plotted together.

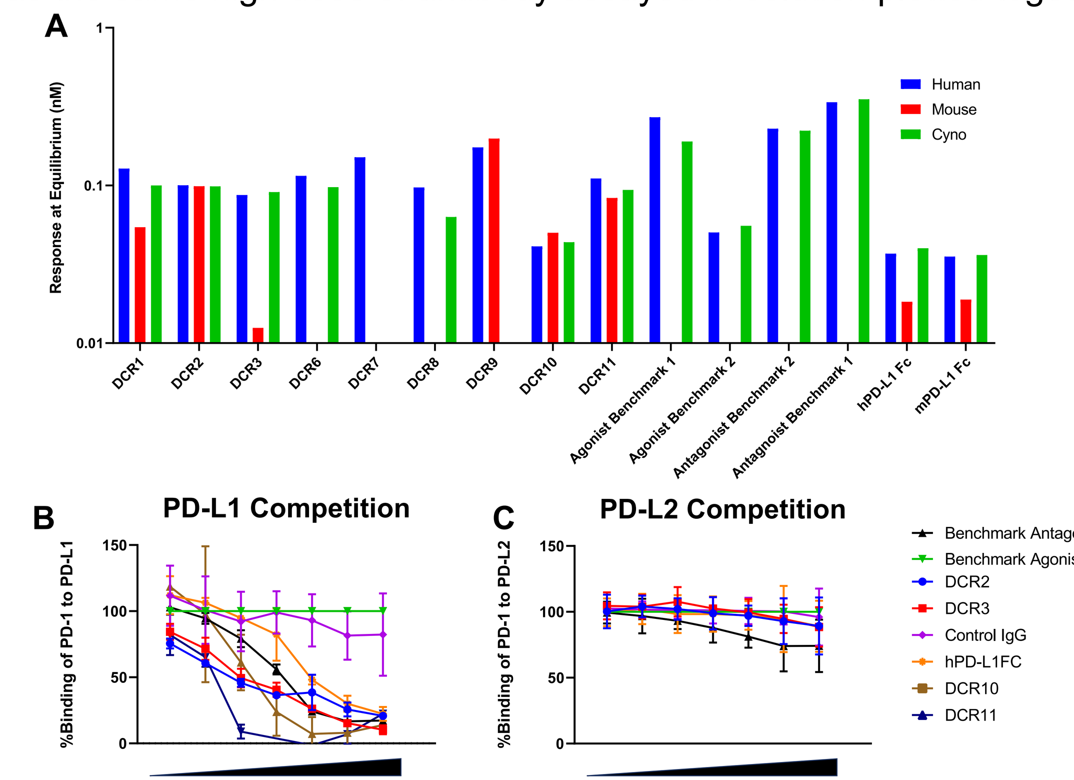

Figure 3 – Cross Species Reactivity and Ligand Competition

Binding of experimental antibodies and benchmarks to human, mouse and cynomolgus monkey PD-1 was determined using an Octet Biosensor (A). Competition ELISA for PD-1 biding to PD-L1 (B) or PD-L2 (C) in the presence of experimental antibodies.

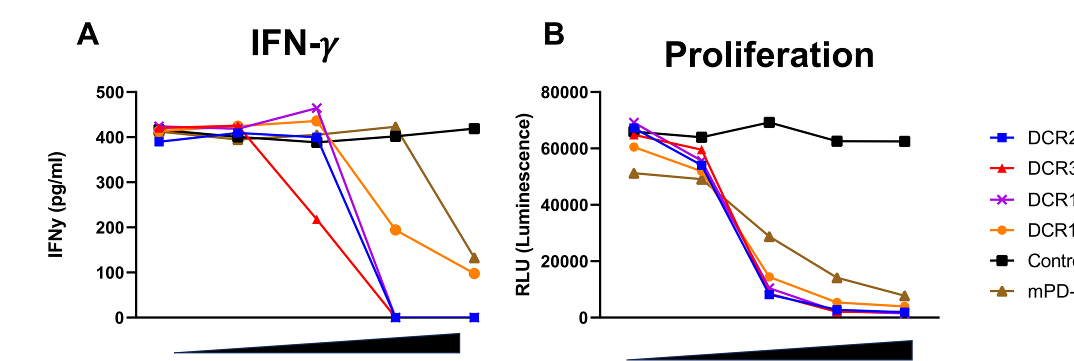

Figure 4 – PD-1 Agonist Antibodies Inhibit Mouse T Cell Activation

Total mouse T cells were assessed for their ability to respond to anti-CD3 in the presence of immobilized antibodies. IFN-γ production was determined via HTRF after 24hr in (A). Cell proliferation was determined by CellTiter Glo Assay (CTG) in (B) after 48 hr in culture.

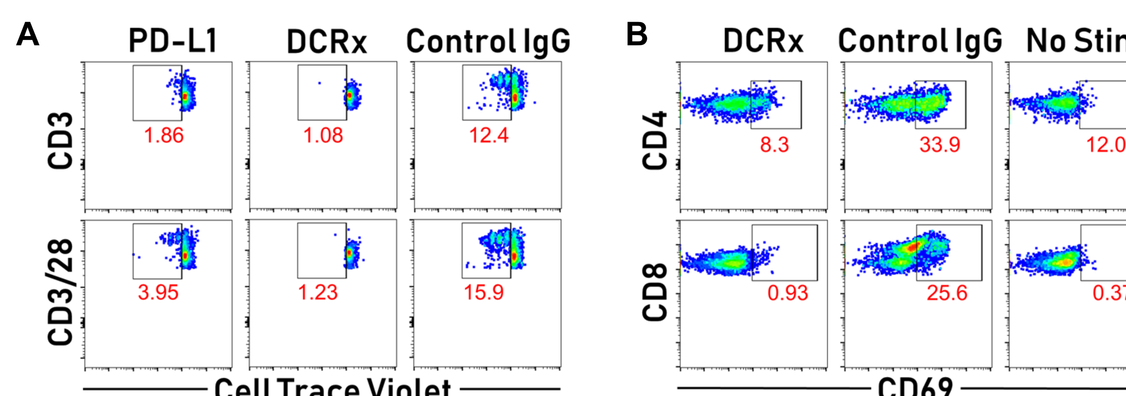

Figure 5 – PD-1 Agonist Antibodies Inhibit T Cell Proliferation and Early Activation

Total CD4+ T cells labeled with CellTrace Violet were stimulated in the presence of plate bound anti-CD3 +/- soluble anti-CD28 and anti-hIgG1 captured experimental antibody for 72 hr (A) or 12 hr (B). FACS plots are pre-gated on singlets, lymphocyte gate and live cells (A).

RESULTS

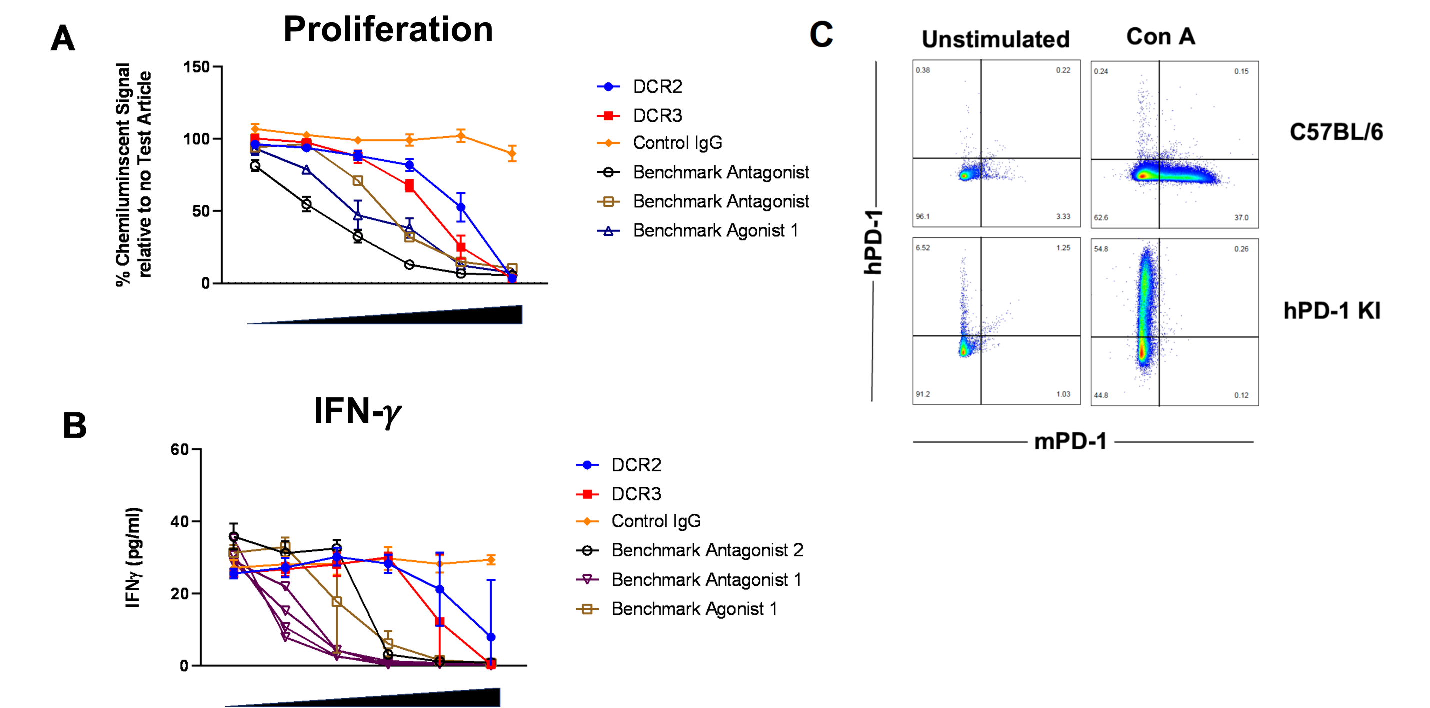

Figure 6 – Human PD-1 Expressing Mouse T Cells can be Functionally Inhibited by PD-1 Agonist Antibodies

Total T cells from human PD-1 knock-in mice were assessed for their ability to respond to anti-CD3 in the presence of immobilized test article. Cell proliferation was determined by CellTiter Glo Assay (CTG) in (A) after 48 hr in culture. IFN-γ production was determined via HTRF after 24 hr in (B). (C) Representative flow plots of PD-1 expression on hPD-1 KI and C57BL/6 mice.

SUMMARY TABLE

Clone ID	Soluble antagonist	Soluble Agonist	Immobilized agonist	H/M/C Cross Reactivity	PD-L1 Competition	PD-L2 competition	mouse agonism	hPD1KI agonism
DCR1	yes	no	no	HMC	NA	NA	NA	NA
DCR2	no	no	yes	HMC	yes	yes	yes	yes
DCR3	no	no	yes	HMC	yes	yes	yes	yes
DCR6	yes	no	weak	HC	NA	NA	NA	NA
DCR7	yes	no	yes	HC	NA	NA	NA	NA
DCR8	yes	weak	yes	HC	NA	NA	NA	NA
DCR9	yes	no	weak	HC	NA	NA	NA	NA
DCR10	yes	no	yes	HMC	yes	yes	yes	NA
DCR11	yes	no	yes	HMC	yes	yes	yes	NA
Agonist Benchmark 1	no	no	yes	HC	no	no	no	yes
Agonist Bechnmark 2	no	no	yes	HC	NA	NA	no	yes
Antagonist Benchmark 1	yes	no	yes	HC	yes	yes	no	yes
Antagonist Benchmark 2	yes	no	Yes	HC	yes	yes	no	yes
Control IgG	no	no	no	no	no	no	no	no

Legend
Has Function
Does not Have
Intermediate
Not Assesesed

CONCLUSION

We were able to successfully generate a diverse panel of PD-1 agonist antibodies. Several of these antibodies were cross-reactive against human, cynomolgus monkey and mouse. These cross-species functional antibodies will facilitate further investigation into the basic biology of PD-1. We observed that the vast majority of PD-1 antagonist antibodies also functioned as PD-1 agonists when immobilized, suggesting a fundamental link between PD-1 agonism and antagonism. Functionally, we determined that these PD-1 agonist antibodies were able to inhibit mouse T cell proliferation, cytokine production and early activation. Using cells isolated from mice expressing hPD-1 we validated that these antibodies were able to inhibit several facets of T cell activation downstream of PD-1 in a primary cell assay system. We believe that these antibodies will allow us to specifically manipulate the PD-1/PD-L1 axis to inhibit T cell responses in a variety of autoimmune and inflammatory disorders.

Methods and Select References

Generation of Antibodies - Antibodies were generated using the Expi293 expression system according to the manufacturer's specification (Gibco / Life Technologies). Proteins were affinity purified from the resulting conditioned media using Protein A resin followed by cation exchange chromatography and desalting into the final formulation buffer.
PD-L1/L2 Competition ELISA - Antibody competition ELISA was performed by coating plates with human PD-L1 or PD-L2 overnight before blocking. PD-1 and the test articles were incubated for 1 hour before addition to PD-L1 or PD-L2 coated plates. Bound PD-1 was detected using an HRP conjugated polyclonal anti-PD-1 antibody.
T Cell PD-1 Agonism and Antagonism Assays – Mouse: Bulk mouse splenocytes isolated from either C57BL/6 mice (Jackson) or human PD-1 knock-in mice (BioCytogen) were stimulated for 3 days in the presence of 0.5 µg/ml of Concanavalin A (Sigma) and 30 U/mL mIL-2 (Life Technologies). After 3 days T cells were isolated via negative selection (StemCell mouse T cell isolation kit) and stimulated for 3 days in U-bottom plates coated with anti-CD3 at a concentration of 0.5 µg/mL. After 3 days cell proliferation was measured by CellTiter Glo assay (Promega) and IFN-γ production was assessed via HTRF (CisBio). Human: Jurkat PD-1 reporter cells were incubated either with plate bound test article with or with PD-L1 expressing cells. Reporter signal was determined via chemiluminescent readout.
CellTrace Violet Dilution Assay – Mouse T cells were isolated from 8 week old C57BL/6 female mice via magnetic negative selection (StemCell). Cells were labeled with CellTrace Violet per manufacturer's instructions (Life Technologies). T cells were stimulated for 3 days in U-bottom plates coated with anti-CD3 at a concentration of 0.5 µug/mL and anti-CD28 at 0.5 µg/mL in solution where indicated.
CD69 induction assay - Mouse T cells were isolated from 8 week old C57BL/6 female mice via magnetic negative selection (StemCell). T cells were stimulated for 12hr in U-bottom plates coated with anti-CD3 at a concentration of 0.5 µg/mL and anti-CD28 at 0.5 µg/mL. After 12hr cells were stained with CD69-PE-CY7, CD4-PE , and CD8-BV605 (BioLegend).
Octet binding assays -Test article was immobilized onto an anti-AHC biosensor tips, and binding of monomeric PD-1 to the captured test article was measured.

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