Discovery and Characterization of PD-1 Agonist Antibodies

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ABSTRACT

T cell function and activation are regulated by a complex signaling network that consists of interconnected activators and inhibitors. Recently, significant attention has been given to the therapeutic blockade of co-inhibitory receptors, such as PD-1, for the treatment of multiple forms of cancer. Taking lessons learned from these immune-oncology focused endeavors we sought to develop antibodies that agonize, rather than antagonize, the PD-1 pathway and mimic the effects of ligation of PD-1 by its endogenous ligands PD-L1 and PD-L2 in order 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. For instance, one class, constituting approximately one-third of the antibodies, exhibited antagonist activity when in solution, but demonstrated agonist activity when immobilized. Less than 1% of antibodies were antagonists in solution with no agonist function at all. The observation that the majority of PD-1 antibodies that are antagonists in solution also function as agonists when immobilized confirms data previously reported in Bennet et al (*1*). and suggests a fundamental relationship between agonism and antagonism of the PD-1 pathway. Of particular interest to us were the antibodies that displayed agonist activity yet did not antagonize PD-1/PD-L1 interactions. We next characterized the ability of this subset of antibodies to exert PD-1 agonist activity on mouse T cells and identified several clones that exhibited similar functional activity across species.

In conclusion we identified several classes of PD-1 antibodies that were able to 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 to treat autoimmune and inflammatory diseases.

INTRODUCTION

•PD-1 is a critical immune regulator found on numerous cell types, most notably CD4+ and CD8+ T cells (*2*).
•The PD-1 / PD-L1 axis has emerged as a prominent immunotherapeutic target that can be blocked by monoclonal antibodies, and have proven efficacious in treating multiple forms of cancer.(*3*)
•Blockade of the PD-1 / PD-L1 axis frequently results autoimmune complications. (*4, 5*)
•Adverse immune events that occur after PD-1 or PD-L1 blockade are common, and manifest as a variety of autoimmune disorders. Additionally, data from mice lacking either PD1-1 or PD-L1 indicates that loss of this immunoregulatory axis results in abhorrent immune response (*6*).
•Based on these observations we sought to make a novel class of immunomodulatory agents that would have the ability to specifically agonize the PD-1 pathway

RESULTS

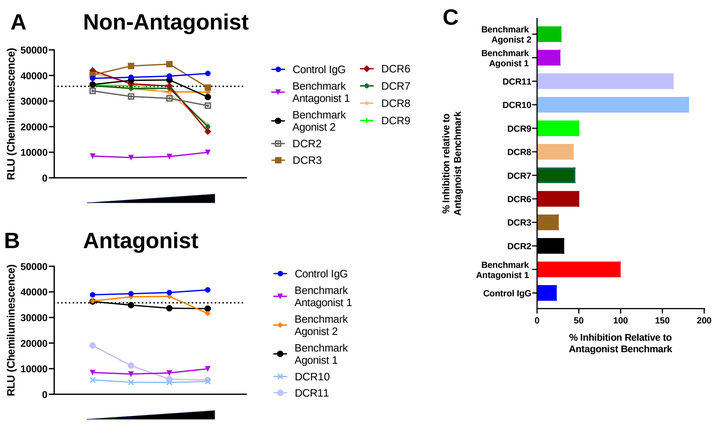

Figure 1 – Identification of PD-1 Antagonist Antibodies

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

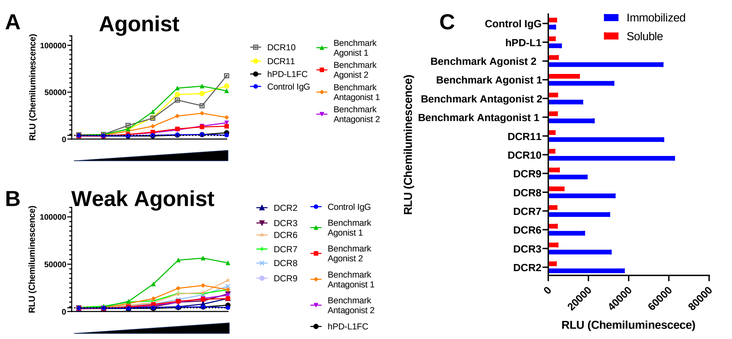

Figure 2- Identification of PD-1 Agonist Antibodies

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 does of antibody assayed in solution plotted together.

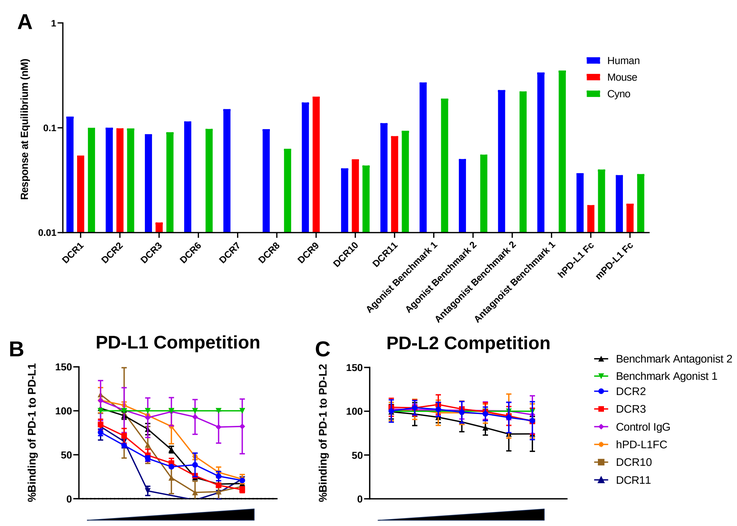

Figure 3 – Cross Species Reactivity and Ligand Competition

Binding of experimental antibodies and benchmarks to human, mouse and cyno PD-1 was determine 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.

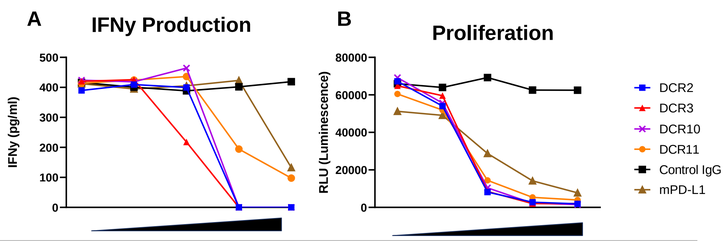

Figure 4 – PD-1 Agonist Antibodies Inhibit Mouse T Cell Activation

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

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

Has Function
Does not Have
Intermediate
Not Assesesed

RESULTS

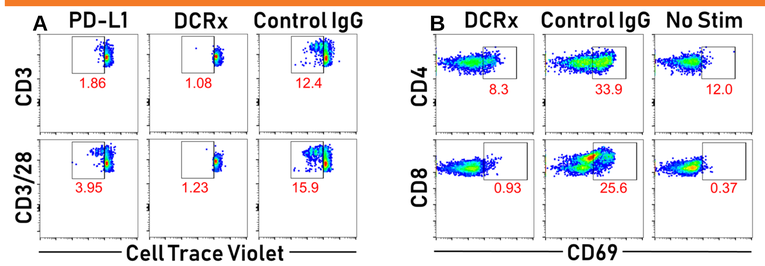

Figure 5 – PD-1 Agonist Antibodies Inhibit T Cell Proliferation and Early Activation

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

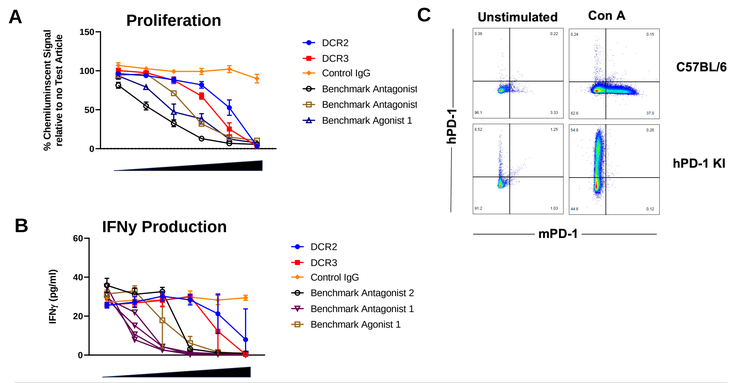

Figure 6 – Human PD-1 Expressing Mouse T Cells can be Functionally Inhibited by PD-1 Agonist Antibodies

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 Cell Titer Glo Assay (CTG) in (A) after 48hr in culture. IFNy production was determined via HTRF after 24hr in (B). (C) Representative flow plots of PD-1 expression on hPD-1 KI and C57BL/6 mice

CONCLUSION

In 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 agonist 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 cell 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 based 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 transfected and expressed 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 overnight before blocking. PD-1 and the test articles were incubated for 1 hour before addition to PD-1 coated plates and PD-1/PD-L1 binding 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 0.5ug/ml of ConA (Sigma) and 30 U/mL mIL-2 (Life Technologies). After 3 days T cell were isolated via negative isolation (Stemcell mouse T cell isolation kit) and stimulated for 3 days in U-bottom plates coated with anti-CD3 at a concentration of 0.5ug/mL. After 3 days cell proliferation was measured by CTG assay (Promega) and IFNy 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.
Cell Trace Violet Dilution Assay – Mouse T cell were isolated from 8 week old C57BL/6 female mice via magnetic negative selection (Stemcell). Cells were labeled with Cell Trace Violet per manufacturers instructions (Life Technologies). T cells were stimulated for 3 days in U-bottom plates coated with anti-CD3 at a concentration of 0.5ug/mL and anti-CD28 at 0.5 ug/mL in solution where indicated.
CD69 induction assay - Mouse T cell 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 0.5ug/mL and anti-CD28 at 0.5 ug/mL. After 12hr cells were stained with CD69-PECY7, CD4-PE , and CD8-BV605 (BioLegend)
Octet binding assays -Test article was immobilized onto an anti-AHC biosensor tips, we then measured the binding of monomeric PD-1 to the captured test article.

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