HUMAN PD-L2 TRIGGERS A UNIQUE T CELL INHIBITORY PROGRAM THROUGH PD-1 ENGAGEMENT DISTINCT FROM THAT OF PD-L1

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Background PD-1/PD-L1 blockade is responsible for the majority of the success of cancer immunotherapy.¹ However, only 14% of patients eligible to receive checkpoint blockade achieve objected clinical responses.^{2,3} The reason for the failure of PD-L1 blockade may be attributed to the recently appreciated widespread expression of PD-L2 across human cancers and its immunosuppressive stromal cells.⁴ PD-L2 expression was shown to be as or more predictive of response to PD-1 blockade than PD-L1. PD-L2 traditionally was dismissed as functionally redundant to PD-L1 varying only in pattern of expression. We hypothesize that PD-L2 engages PD-1 to generate a distinct inhibitory signal from that of PD-L1, and antibody mediated blockade and depletion of PD-L2 + cells may promote anti-tumor immunity that is superior to PD-L1 blockade alone.

Methods Cell based bioluminescent assay demonstrated the nature of regulation mediated by human PD-L2 through the PD-1 co-receptor. RNA-sequencing identified key differences in the signaling pathways generated in Jurkat T cells by PD-1 binding to PD-L1 or PD-L2. Multidimensional flow cytometry determined the differential effects of PD-L1 and PD-L2 on human T cell proliferation and effector function. Western blot elucidated the temporal kinetics of inhibition mediated by PD-L1 and PD-L2. Survival studies in murine syngeneic lymphoma model evaluated the efficacy of antibody mediated blockade and depletion of PD-L2+ cells.

Results We validated that human PD-L2, unlike murine PD-L2, generates a purely co-inhibitory signal in human T cells, albeit with a reduced inhibitory potential relative to PD-L1. We discovered significant differences in downstream T cell signaling pathways generated by PD-L1 versus PD-L2 through PD-1 engagement. Human PD-L1 and PD-L2 differentially modulated T cell effector function and proliferation with PD-L2 preferentially arresting T cells in S-phase of cell cycle. PD-L1 and PD-L2 also differed in the temporal kinetics of dephosphorylation of the membrane proximal proteins in the TCR-CD3 signaling complex. We observed that combination blockade of PD-L1 and PD-L2 improves on blockade of PD-L1 alone resulting in increased production of IL-2 and IFN γ in primary human mixed lymphocyte reactions. Our data in a syngeneic murine model of EL4 showed that effector-function capable PD-L2 blocking antibodies are therapeutically superior to PD-L1 or PD-L2 blockade alone.

Conclusions We are the first to report on T cell immunoregulatory functions of PD-L2 which are distinct from those of PD-L1, and demonstrate that the more tumor-selective expression pattern of PD-L2 relative to PD-L1 provides a therapeutic advantage to effector-function capable PD-L2 antibodies.

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REFERENCES

- 1.. Ribas A, Wolchok JD (2018). Cancer immunotherapy using checkpoint blockade. *Science* **359**:1350–1355.
- 2.. Cristescu R, Mogg R, Ayers M, Albright A, Murphy E, Yearley J, Sher X, Liu XQ, Lu H, Nebozhyn M, Zhang C, Lunceford JK, Joe A, Cheng J, Webber AL, Ibrahim N, Plimack ER, Ott PA, Seiwert TY, Ribas A, McClanahan TK, Tomassini JE,

Loboda A, Kaufman D (2018). Pan-tumor genomic biomarkers for PD-1 checkpoint blockade-based immunotherapy. *Science* **362**.

- 3.. Haslam A, Prasad V (2019). Estimation of the percentage of US patients with cancer who are eligible for and respond to checkpoint inhibitor immunotherapy drugs. *JAMA Netw Open* **2**:e192535.
- 4.. Yearley JH, Gibson C, Yu N, Moon C, Murphy E, Juco J, Lunceford J, Cheng J, Chow LQM, Seiwert TY, Handa M, Tomassini JE, McClanahan T (2017). PD-L2 expression in human tumors: relevance to anti-PD-1 therapy in cancer. *Clin Cancer Res* **23**:3158–3167.

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