

PD-L1/PD-L2 DUAL-SPECIFIC CYTOTOXIC ANTIBODIES AUGMENT SURVIVAL AND MEDIATE TUMOR REGRESSION ACROSS IMMUNE "HOT" AND "COLD" MURINE CANCERS

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Background Inhibition of T cell activation and effector function via engagement of the co-inhibitory receptor PD-1 is a critical mechanism enabling tumors to evade host immunity. The two ligands for PD-1, PD-L1 and PD-L2, are expressed by a variety of immunosuppressive stromal cells, particularly the myeloid stroma and tumor endothelium, and by tumors themselves. In addition to PD-1, PD-L1 engages B7-1 in an additional co-inhibitory interaction. Blocking only PD-1 or only PD-L1 thus does not relieve all inhibitory components of this pathway. We hypothesized that dual-specific antibodies blocking both PD-L1 and PD-L2 could more fully restore tumor-specific T cell activation and potentiate anti-cancer immunotherapy. Furthermore, we speculated that enhancing the cytotoxic effector function of these antibodies might further enhance their efficacy through direct depletion of tumor cells and supportive stroma.

Methods We investigated the capacity of monoclonal antibodies capable of bivalent binding to both PD-L1 and PD-L2 to restore the function of PD-1-suppressed T cells *in vitro*. To assess the *in vivo* therapeutic efficiency of bispecific PD-Ligand antibodies with ADCC capacities, mouse IgG2a and effector enhanced human IgG1 versions were generated. Anti-tumor ADCC activity was assessed *in vitro* using a bioluminescent reporter assay, and therapeutic efficiency measured *in vivo* in syngeneic or human xenograft cancer models.

Results The dual-specific antibodies we generated restore the function of PD-1-suppressed T cells *in vitro* with equivalent efficiency to the FDA approved PD-1 antibody pembrolizumab. Moreover, our modified human dual-specific antibodies drive significantly higher FcγRIIa and FcγRIIIa activation and induction of NK cell ADCC versus FDA-approved PD-L1 antibodies *in vitro*. In syngeneic models of PD-L1/PD-L2 double positive colon carcinoma and melanoma, ADCC-capable PD-Ligand dual-specific antibodies demonstrate superiority to PD-1 blocking antibodies to limit tumor growth and increase survival. Furthermore, treatment with our dual-specific antibodies increases T cell proliferation and cytotoxicity and reduces density of immunosuppressive myeloid stroma *in vivo*. Our antibodies also suppress the growth of U2940 lymphoma in immunodeficient mice more efficiently than Rituximab.

Conclusions ADCC-capable PD-Ligand dual-specific antibodies display higher therapeutic potential than existing anti-PD-1 antibodies and represent a new class of PD-1 pathway therapeutics with significant potential to extend the efficacy of checkpoint immunotherapy to "cold" tumor patients.

<http://dx.doi.org/10.1136/jitc-2022-SITC2022.0462>