PD-1 Probody™ Therapeutic Anti-tumor Efficacy and Protection Against Autoimmunity in Preclinical Models

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ABSTRACT

Probody therapeutics (Pb-Txs) are recombinant antibody prodrugs that have the potential to meaningfully widen therapeutic index. Pb-Txs are preferentially activated in the tumor microenvironment by cancer-associated proteases but are substantially inactive systemically[1]. Probody technology has been successfully applied to several antibody formats, with efficacy and increased safety windows demonstrated preclinically for an antibody to the tumor expressed checkpoint inhibitor PD-L1, antibody drug conjugates to highly expressed tumor antigens including CD166 and CD71, and T-cell engaging bispecific antibodies[2]. Here we extend the Probody platform to generate Pb-Txs that target the T cell expressed inhibitory checkpoint receptor PD-1.

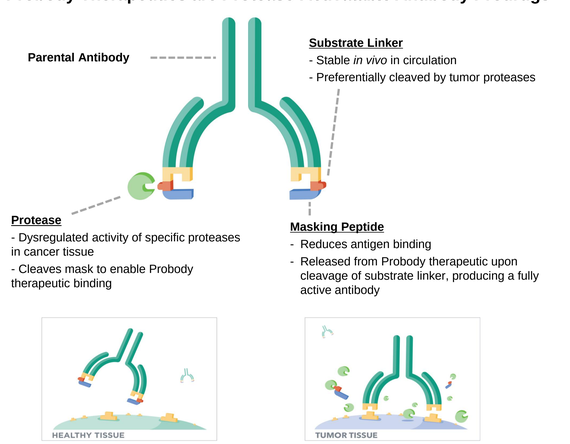
Antibodies blocking the T cell resident inhibitory checkpoint molecules CTLA-4 and PD-1 have transformed the treatment of multiple advanced cancers, showing over 50% objective response rates in combination in metastatic melanoma[3]. These increased response rates have concomitant increases in immune-related Grade 3/4 adverse events, in many cases resulting in alteration or termination of treatment. Probody therapeutics targeting T cell checkpoint receptors have the potential to retain combination checkpoint therapy response rates while significantly reducing systemic autoimmune adverse events.

For preclinical assessment of PD-1 as a Probody target, an anti-mouse PD-1 antibody[4] was engineered to produce mPD-1 Pb-Tx. The mPD-1 Pb-Tx showed comparable anti-tumor potency to the parental antibody as a single agent and in combination with an anti-CTLA-4 antibody in mice bearing established MC38 syngeneic tumors. In contrast, the mPD-1 Pb-Tx was up to 10 times less potent than the parental antibody in inducing autoimmune diabetes in NOD mice.

PD-1 Ab, a proprietary PD-L1/L2 blocking anti-human PD-1 antibody with potent *in vitro* activity, was engineered with the same protease-cleavable linker as mPD-1 Pb-Tx to produce PD-1 Pb-Tx. Biophysical, functional and pharmacokinetic evaluation of PD-1 Pb-Tx supports its further development as an immune oncology Probody therapeutic.

INTRODUCTION

Probody Therapeutics are Protease-Activatable Antibody Prodrugs



RATIONALE

% of Patients Reporting Event	Nivo + Ipi		Nivo (PD-1)		Ipi (CTLA4)	
	All	Gr 3-4	All	Gr 3-4	All	Gr 3-4
Treatment related adverse event (AE)	95.5	**55.0**	82.1	16.3	86.2	27.3
Discontinuation due to treatment-related AE	**36.4**		7.7		14.8	

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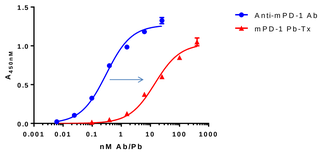
Combination immunotherapy produces both increased efficacy and increased toxicity. Probody therapeutics are engineered to avoid systemic toxicities while retaining activity in the tumor.

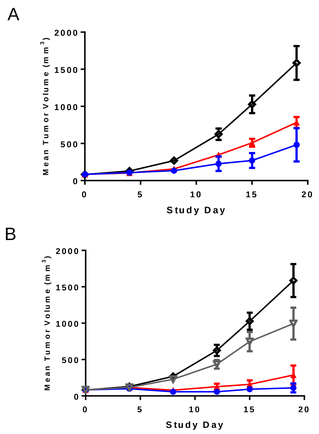
RESULTS: Preclinical POC with mPD-1 Pb-Tx

Figure 1: mPD-1 Pb-Tx binds mouse PD-1 with a decreased affinity relative to parental mPD1 Ab



The anti-mouse PD-1 antibody engineered to contain a protease substrate and mask (mPD-1 Pb-Tx) binds to immobilized mouse PD-1 with decreased affinity relative to parental Ab by standard plate ELISA.

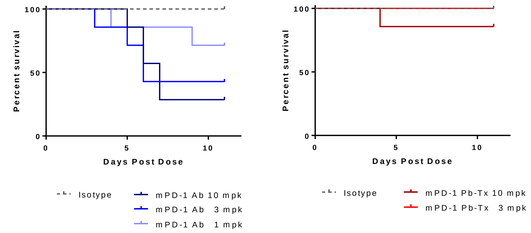
Figure 2: mPD-1 Pb-Tx provides anti-tumor efficacy comparable to parental mPD-1 Ab as a single agent and in combination with CTLA-4 antibody in an MC38 model



C57BL/6 mice at 7-9 weeks of age were implanted subcutaneously with 1.5e6 MC38 colon carcinoma cells. Antibodies and mPD-1 Pb-Tx were dosed at 10 mg/kg, b.i.w. x 3 weeks in mice bearing MC38 tumors of ~80mm^3 (n=10/group) starting at Study Day 0.

Shown are mean tumor volumes for (A) single agent mPD-1 Ab and mPD-1 Pb-Tx groups and (B) single agent anti-CTLA-4 plus combination mPD-1 Ab + anti-CTLA-4 and mPD-1 Pb-Tx + anti-CTLA-4 groups.

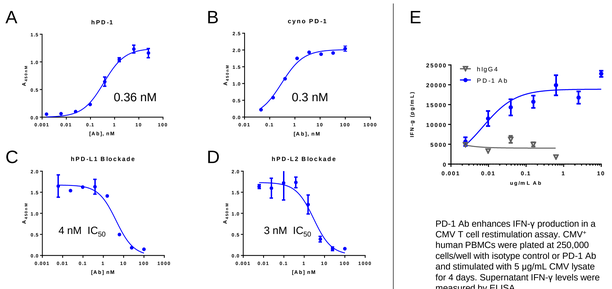
Figure 3: mPD-1 Pb-Tx protects against systemic autoimmunity by up to 10-fold compared to parental antibody in NOD mice



Ten-week old female NOD mice were administered mPD-1 Ab or mPD-1 Pb-Tx at the indicated doses on day=0. Mice were monitored daily for the induction of diabetes by glucosuria plus confirmation of two consecutive blood glucose levels ≥ 250mg/dL. Monitoring continued for a minimum 7 days until 48 hours passed with no new incidents of glucosuria.

RESULTS: Human PD-1 Ab and Pb-Tx

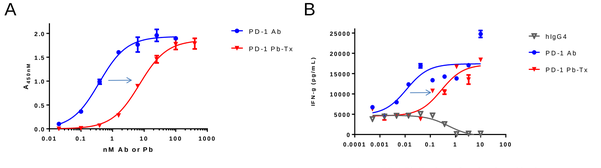
Figure 4: Proprietary anti-human PD-1 Ab blocks PD-L1/L2 binding to PD-1 and potently activates T cells in an antigen recall assay



Binding of proprietary anti-human PD-1 Ab to immobilized (A) human and (B) cyno PD-1 by ELISA. Inhibition by PD-1 Ab of (C) biotin-PD-L1 and (D) biotin-PD-L2 binding to immobilized PD-1 by ELISA.

PD-1 Ab enhances IFN-γ production in a CMV T cell restimulation assay. CMV+ human PBMCs were plated at 250,000 cells/well with isotype control or PD-1 Ab and stimulated with 5 µg/mL CMV lysate for 4 days. Supernatant IFN-γ levels were measured by ELISA.

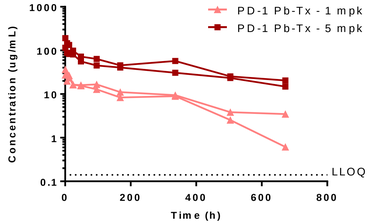
Figure 5: PD-1 Pb-Tx binds human PD-1 with decreased affinity relative to parental PD-1 Ab and shows functional masking in a T cell antigen recall assay



Intact PD-1 Pb-Tx binds PD-1 with a decreased affinity relative to PD-1 Ab.

Intact PD-1 Pb-Tx shows functional masking in a CMV T cell restimulation assay.

Figure 6: PD-1 Pb-Tx demonstrates favorable PK and dose proportionality comparable to the parental antibody in cynos



Female cynomolgus monkeys (n=2/group) were dosed with a single IV bolus dose of either PD-1 Ab (parental antibody, data not shown) or PD-1 Pb-Tx at 1 or 5 mg/kg. Plasma samples were analyzed for PD-1 Ab or PD-1 Pb-Tx concentrations by a qualified anti-human sandwich ELISA. Each line represents one individual. Mean PK parameters shown for both PD-1 Ab and PD-1 Pb-Tx (n=2/group).

Test Article	Dose (mpk)	$t_{1/2}$ (days)	C_{max} (ug/mL)	AUC_{last} (day*ug/mL)
PD-1 Pb-Tx	1	8.5	32.1	228.8
	5	14.1	153.1	1132.2
PD-1 Ab	1	8.3	34.1	184.1
	5	8.7	150.8	544.0

SUMMARY/CONCLUSIONS

- **Probody therapeutic mPD1 Pb-Tx, targeting the T cell inhibitory checkpoint receptor PD-1, demonstrates potent antitumor activity as a single agent and in combination with anti-CTLA4 in the MC38 murine tumor model.**
- **mPD1 Pb-Tx provides 10-fold dose protection from induction of autoimmune diabetes in NOD mice relative to the parental Ab.**
- **Proprietary anti-human PD-1 Ab binds to PD-1 with sub-nM affinity, blocks binding to PD-L1/PD-L2 and enhances T cell responses *in vitro*.**
- **As with PD-L1, our work on PD-1 supports the hypothesis that localized activity of checkpoint inhibition can result in anti-tumor efficacy.**
- **The *in vitro* biophysical and functional assessment and cyno pharmacokinetic profile of the anti-human PD-1 Pb-Tx support its further development.**

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