Probody Therapeutics are Protease-Active Antibody Prodrugs

**INTRODUCTION**

Probody Therapeutics are recombinant antibody prodrugs that have the potential to meaningfully widen therapeutic index. Pro-Txs are preferentially activated in the tumor microenvironment by cancer-associated proteases but are substantially inactive systemically. 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. Here we extend the Probody platform to generate Pb-Txs, target the T-cell expressed 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. These increased response rates have concomitantly increased 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 receptor engagement while significantly reducing systemic autoimmune adverse events.

For preclinical assessment of PD-1 as a Probody target, an anti-mouse PD-1 antibody was engineered to produce Pb-Tx. The mPD-Tx showed comparable anti-tumor potency to the parental antibody as a single agent and in combination with an anti-CTLA-4 antibody in multiple advanced cancer models, including a melanoma model where PD-1 is expressed less than the parental antibody in inducing autoimmune diabetes in NOD mice. Pb-Txs, a proprietary PD-1/L2 blocking anti-mouse PD-1 antibody with potent in vitro activity, was engineered with the same protease-cleavable linker as mPD-Tx to produce Pb-Tx. Biophysical, functional and pharmacodynamic study of Pb-Tx supports its further development as an immuno-oncology Probody therapeutic.

**Rationale**

**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. 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. Here we extend the Probody platform to generate Pb-Txs, target the T-cell expressed checkpoint receptor PD-1.

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**RESULTS:** Preclinical POC with mPD-1 Pb-Tx

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

**Figure 2:** mPD-1 Pb-Tx provides anti-tumor efficacy comparable to parental mPD1 Ab as a single agent and in combination with CTLA-4 antibody in an MC38 model

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

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

**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

**Figure 6:** PD-1 Pb-Tx demonstrates favorable PK and dose proportionality compared to the parental antibody in cynomolgus monkeys

**SUMMARY/CONCLUSIONS**

Probody therapeutic mPD-1 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.

- mPD-1 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 local activity of checkpoint inhibition can result in anti-tumor efficacy.

The in vitro biophysical and functional assessment and in vivo pharmacokinetic profile of the anti-human PD-1 Pb-Tx supports its further development.

**REFERENCES**


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