Probody™ Therapeutic Targeting PD-1 Provides Preclinical Anti-tumor Efficacy While Minimizing Induction of Autoimmunity as a Single Agent and in Combination with CTLA-4 Blockade

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ABSTRACT

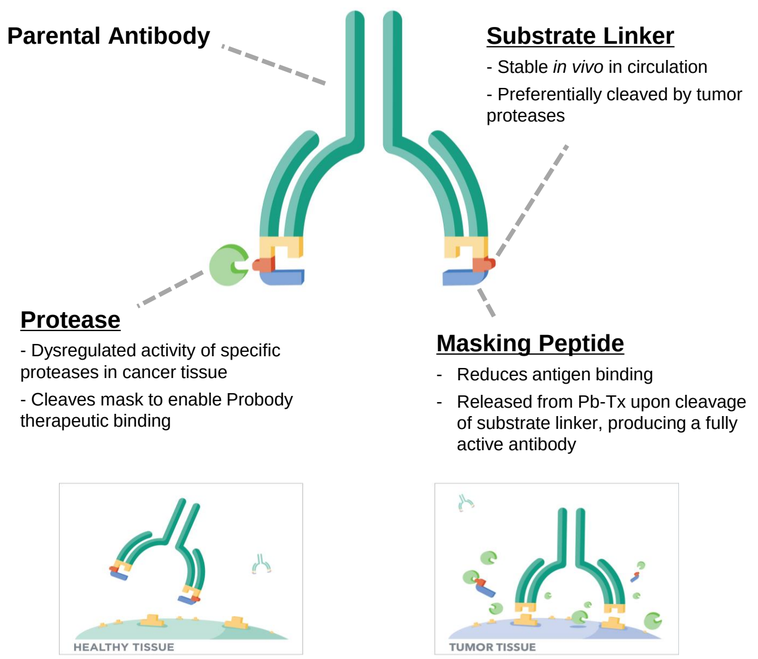
Immunotherapy has transformed cancer treatment by unleashing potent and durable anti-tumor immunity against many cancers. However, these therapies can also induce systemic autoimmunity by activating autoreactive T cells in normal tissues. Combinations of checkpoint inhibitors targeting PD-1 and CTLA-4 increase clinical response rates but similarly increase toxicities, thereby reducing their clinical potential. New approaches are therefore needed that provide anti-tumor activity without dysregulating systemic immunity.

CytomX has developed Probody therapeutics (Pb-Tx), which are proteolytically-activated antibodies (Abs) designed to widen the therapeutic index by minimizing drug interaction with normal tissue while retaining anti-tumor activity. Pb-Tx are “masked” to attenuate binding to target in healthy tissue but can become “unmasked” in the tumor microenvironment by tumor-specific protease activity.

In vitro, the masked PD-1 Pb-Tx had reduced affinity for mouse PD-1 relative to the parental antibody (Ab). In mice, single-agent Abs to CTLA-4 and to PD-1 as well as the PD-1 Pb-Tx induced 10%, 30%, and 20% complete tumor regressions (CRs) against established MC38 tumors, respectively. In combination with CTLA-4 Ab, both PD-1 Ab and PD-1 Pb-Tx induced 80% CRs and generated effective T cell memory against tumor re-challenge. In 10-week-old NOD mice, a 1 or 10 mpk single dose of anti-PD-1 antibody induced diabetes in 43% and 57% of mice, respectively, while a 10 mpk dose of PD-1 Pb-Tx yielded only 14% disease incidence with delayed onset. In younger NOD mice, the CTLA-4/PD-1 antibody combination induced diabetes in 50% of mice. In contrast, mice administered the PD-1 Pb-Tx/CTLA-4 antibody combination were completely protected.

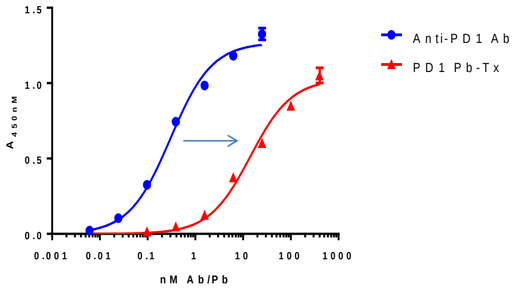
INTRODUCTION

Probody Therapeutics are Protease-Activatable Antibody Prodrugs



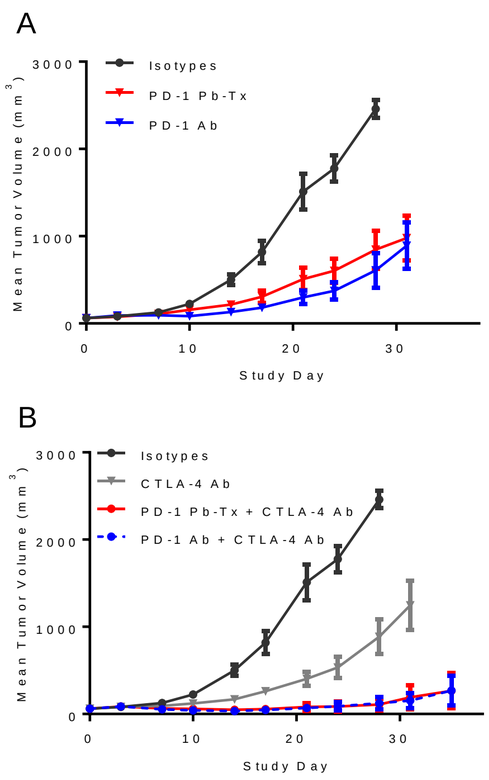
RESULTS

Figure 1: PD-1 Pb-Tx is functionally masked and binds mouse PD-1 with decreased affinity relative to parental PD-1 Ab.



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

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



C57BL/6 mice at 7-9 weeks of age were implanted subcutaneously with 1.5e6 MC38 colon carcinoma cells. Antibodies and PD-1 Pb-Tx were dosed at 10 mg/kg, b.i.w. x 3 weeks in mice bearing ~60mm^3 MC38 tumors (n=10/group) starting at Study Day 0.

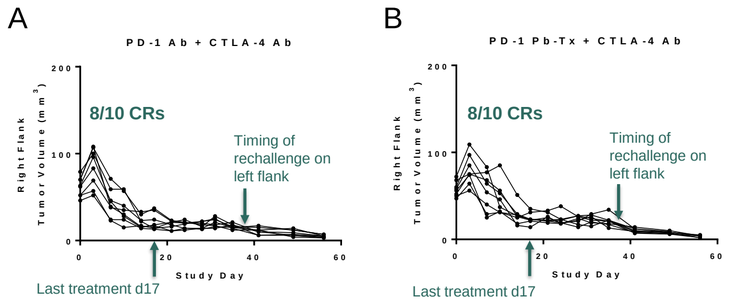
Shown are mean tumor volumes for (A) single agent PD-1 Ab and PD-1 Pb-Tx groups and (B) single agent anti-CTLA-4 Ab and combination PD-1 Ab + anti-CTLA-4 Ab and PD-1 Pb-Tx + anti-CTLA-4 Ab groups.

Complete responses

	% CRs:
Isotypes	0
PD-1 Ab	30
PD-1 Pb-Tx	20
CTLA-4 Ab	10
PD-1 Ab + CTLA-4 Ab	80
PD-1 Pb-Tx + CTLA-4 Ab	80

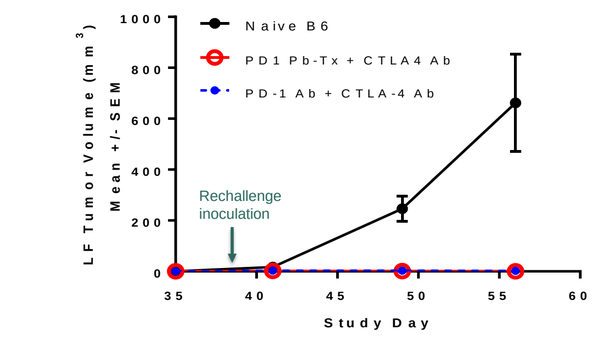
Figure 3: PD-1 Pb-Tx in combination with CTLA-4 Ab elicits a durable anti-tumor memory response that makes mice resistant to rechallenge with MC38 tumor cells.

First challenge (Right Flank)



Individual complete anti-tumor responses to treatment with (A) PD-1 Ab + CTLA-4 Ab and (B) PD-1 Pb-Tx + CTLA-4 Ab as described in Figure 2B.

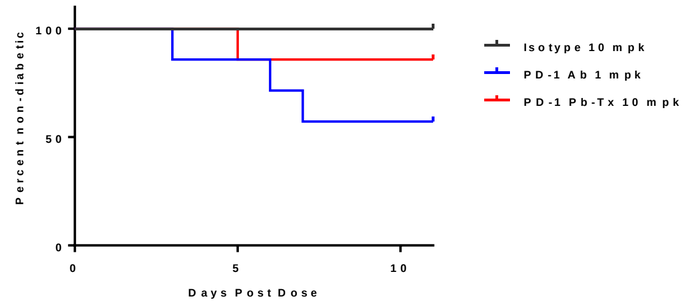
Rechallenge (Left Flank)



C57BL/6 complete responders to PD-1 Ab + CTLA-4 Ab and PD-1 Pb-Tx + CTLA-4 Ab were implanted on Day 38 with 1.5e6 MC38 cells in the opposite (left) flank to the original implantation. As a control for tumor inoculation and viability, naive C57BL/6 (B6) were implanted with 1.5e6 MC38 cell in the left flank on study day 38. Mean +/- SEM tumor volume. Tumor volume was monitored until day 56 of study.

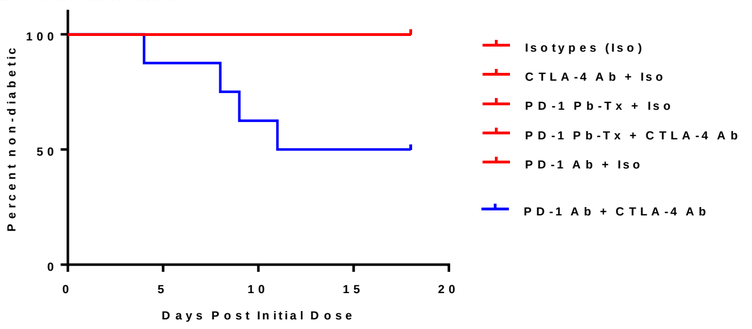
Figure 4: PD-1 Pb-Tx protects against systemic autoimmunity in NOD mice at 10 times the dose of PD-1 Ab as single agent. When dosed in combination with CTLA-4 Ab in NOD mice, PD-1 Ab induces autoimmune diabetes in 50% of mice while the CTLA-4 Ab + PD-1 Pb-Tx combination induces no diabetes.

A Single Agent



Ten-week old female NOD mice were administered PD-1 Ab or PD-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.

B Combination



Five-week old female NOD mice were administered CTLA-4 Ab, PD-1 Ab, PD-1 Pb-Tx and/or isotype controls in the listed combinations at 10mpk each article on days 0, 4 and 7. 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.

CONCLUSIONS

- **A Probody therapeutic targeting the T cell inhibitory checkpoint receptor PD-1 (PD-1 Pb-Tx) demonstrates potent antitumor activity as a single agent and in combination with anti-CTLA-4 in the MC38 murine tumor model.**
  - **Combination treatment generated systemic and durable anti-tumor immunity that was fully effective against tumor rechallenge.**
- **As a single agent, PD-1 Pb-Tx provides 10-fold dose protection from induction of autoimmune diabetes in NOD mice relative to the parental Ab and protects against diabetes in younger mice when combined with anti-CTLA-4.**
- **Systemic delivery of PD-1 Pb-Tx provides effective localized anti-tumor activity with improved peripheral safety when combined with CTLA-4 blockade in preclinical studies, demonstrating the promise of Probody technology for enabling effective yet safer combination immunotherapies for cancer treatment.**

REFERENCES

1. Wong et al. CRI-CIMT-EATI-AACR 2015
2. Agata et al. Int Immunol. 1996 May;8(5):765-72.