A PD-L1-targeted Probody™ Therapeutic Provides Anti-Tumor Efficacy While Minimizing Induction of Systemic Autoimmunity in Preclinical Studies

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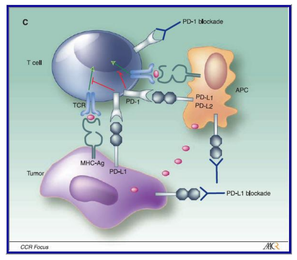
INTRODUCTION

Antibodies to T cell checkpoint molecules can unleash potent and durable anti-tumor immunity in many cancer types[1-3]. However, because similar mechanisms control anti-tumor immunity and self-tolerance, they can also induce systemic autoimmunity. Combinations of checkpoint inhibitors greatly increase clinical responses, but similarly increase these toxicities, thereby limiting their clinical utility [4-5]. New approaches are therefore needed that provide anti-tumor activity without deregulating systemic immunity.

CytomX has developed a new class of antibodies called Probody Therapeutics that are recombinant, protease-activatable antibody prodrugs, designed to widen the therapeutic window by minimizing interaction with normal tissue and maximizing interaction with tumor tissue[6,7]. Probody Therapeutics are "masked" to prevent binding to antigen in healthy tissue, but can become "unmasked" in the tumor microenvironment by tumor-specific protease activity.

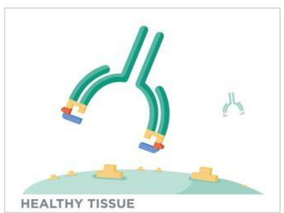
Here we demonstrate the ability of a Probody therapeutic (Pb-Tx) targeting PD-L1, to provide equivalent anti-tumor activity in mice to that of its parental antibody, while minimizing induction of systemic autoimmunity in preclinical studies. By localizing its activity to the tumor microenvironment, the PD-L1 Pb-Tx is expected to expand clinical opportunities for targeting the PD-1/PD-L1 pathway in combination therapies.

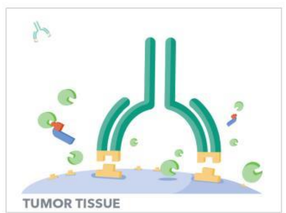
PD-1 Pathway Inhibitors: Broad Anti-tumor Activity, but Toxicities Can Limit Combinations



- PD-L1 negatively regulates T cell function via its interaction with PD-1, an inhibitory receptor expressed on activated T cells[8]
- Blockade of PD-L1 or PD-1 can relieve T cell suppression and enable potent and durable anti-tumor immunity in different cancer types[2,3]
- Combination checkpoint therapies can increase clinical response, but can also enhance systemic autoimmune side effects, causing some patients to discontinue treatment[4-5]

Probody Therapeutics are Designed to Bind Target in Tumors But Not Healthy Tissue





- The antigen-binding site of a Probody therapeutic is blocked by a masking peptide that extends from the amino terminus of the light chain
- The peptide mask, joined to the antibody by a protease-cleavable linker, can be removed by proteases that are active primarily in the tumor microenvironment

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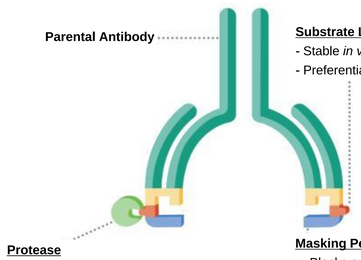
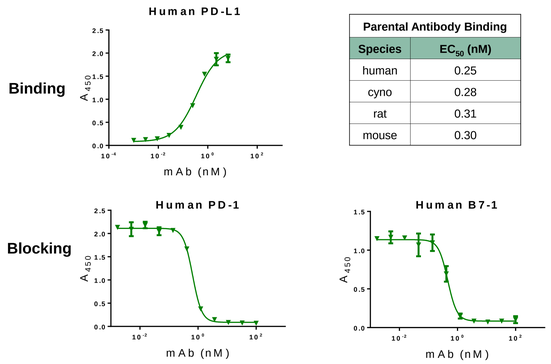
Probody Therapeutics are Protease Activated Antibody Pro-Drugs



RESULTS AND DISCUSSION

Figure 1: CytomX Anti-PD-L1 Antibody is a Cross-reactive, Blocking Antibody



Parental Antibody Binding	
Species	EC_{50} (nM)
human	0.25
cyno	0.28
rat	0.31
mouse	0.30

CytomX-developed anti-PD-L1 antibody:
- Phage-derived, fully human antibody
- High affinity binding to mouse, rat, cyno and human PD-L1 (ELISA)
- Blocks PD-L1 interactions with both PD-1 and B7.1 (ELISA)

Figure 2: Parental anti-PD-L1 Antibody Enhances Antigen-specific T cell Responses and Shows Efficacy in the MC38 Mouse Tumor Model

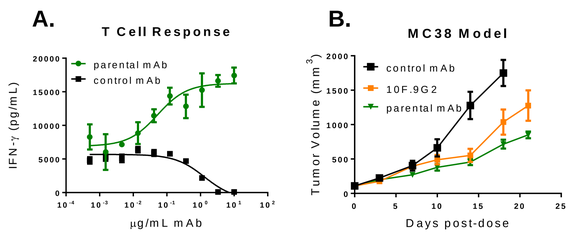
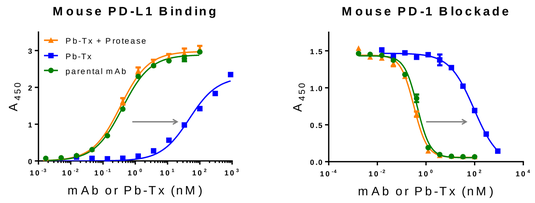


Figure 2A: Anti-PD-L1 antibody enhances IFN-γ production in a CMV T cell restimulation assay. CMV+ human PBMCs were plated at 350,000 cells/well and stimulated with 4 µg/mL CMV lysate for 4 days. IFN-γ was measured by ELISA

Figure 2B: Anti-PD-L1 antibody* achieves similar tumor growth inhibition of established MC38 tumors as a reference anti-PD-L1 antibody 10F.9G2[9]. (n=10/group, when tumors reached ~100 mm3 - dosed at 10 mg/kg, t.i.w. x 2 weeks)

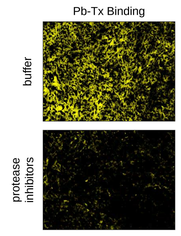
* all murine studies used human/mIgG2a chimera

Figure 3: PD-L1 Probody Tx Exhibits Protease-dependent Binding and Blocking Activity *in vitro*



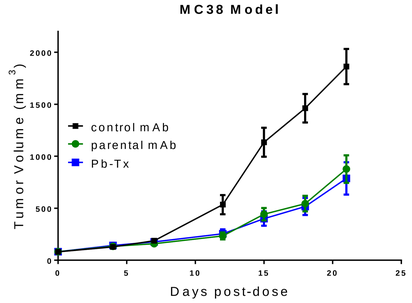
- Pb-Tx (blue line) demonstrates higher EC_{50}s for PD-L1 binding and PD-1 blocking relative to the parental antibody (green line) as measured by ELISA
- Activation of Pb-Tx by matriptase cleavage of its linker (orange line) fully restores binding and blocking to that of the parental antibody

Figure 4: Anti-PD-L1 Antibody and Probody Tx Provide Comparable Tumor Efficacy in MC38 model



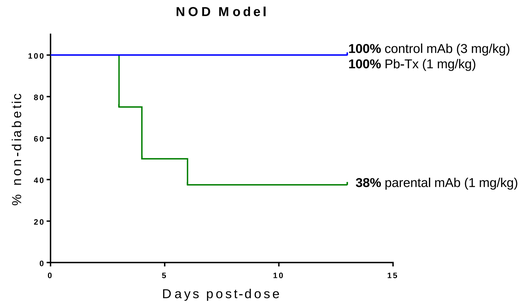
Proteases in MC38 Mouse Tumors Activate Pb-Tx *in vitro*

- Fluorescently-labeled Pb-Tx is activated by proteases present in a frozen PD-L1+ MC38 tumor section (1 hour incubation in protease-compatible buffer)
- Addition of broad spectrum protease inhibitors prevents the majority of Pb-Tx binding, demonstrating its protease dependence



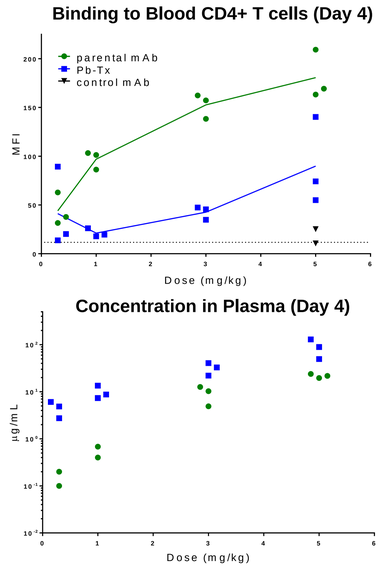
Comparable anti-tumor activity of antibody and Pb-Tx
- Antibody and Pb-Tx were dosed at 5 mg/kg, b.i.w. x 3 weeks in mice bearing MC38 tumors of ~80mm3. (n=9/group). $p \leq 0.05$ for both parental mAb and Pb-Tx vs control at day 18 (one way ANOVA)

Figure 5: Pb-Tx Protects from Induction of Diabetes in NOD Mice



- A single 1 mg/kg dose of the anti-PD-L1 antibody induced diabetes in 5/8 non-obese diabetic (NOD) mice (green)
- Mice treated with a comparable dose of Pb-Tx remained disease free (blue)
- A single 3 mg/kg dose of the Pb-Tx induced only a moderate incidence of diabetes (2/8 mice), providing >3-fold safety margin relative to its parental antibody. (n=8/group)

Figure 6: Probody Tx Demonstrates Reduced Peripheral PD-L1 Binding in Tumor-Bearing Mice



- Reduced binding of Pb-Tx to blood T cells from tumor-bearing mice is observed relative to binding of the parental antibody at all doses, despite its higher plasma concentrations
- Data demonstrate that the presence of tumor-derived proteases capable of cleaving the Pb-Tx does not lead to high levels of activated Pb-Tx in blood
- Method: Mice bearing MC38 tumors between 100-200mm3 were treated with a single dose of test article as indicated above and blood analyzed for surface bound antibody by flow cytometry 4 days after dosing. Plasma concentrations of test articles were determined by ELISA.

SUMMARY/CONCLUSIONS

Preclinical results demonstrate equivalent efficacy with an improved safety profile and provide validation of the Probody concept for a T cell checkpoint target

- CytomX-derived, high affinity anti-PD-L1 antibody blocks PD-L1 interactions with both PD-1 and B7-1 *in vitro*
- PD-L1 Pb-Tx demonstrated reduced PD-L1 binding and blocking relative to its parental antibody – activity was fully restored upon cleavage of its linker by tumor-associated proteases
- Pb-Tx administered systemically to mice bearing MC38 tumors induced comparable anti-tumor responses to those of the parental antibody at the same dose
- In contrast, Pb-Tx provided protection from induction of autoimmune diabetes in NOD mice at doses sufficient to induce maximal diabetes with the parental antibody
- PD-L1 occupancy by the Pb-Tx on blood T cells was reduced compared to that of the parental antibody in tumor-bearing mice, consistent with its ability to reduce the incidence of diabetes
- Based on these data, clinical development of a PD-L1-targeted Probody Therapeutic is warranted

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