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. However, because similar mechanisms control anti-tumor immunity and self-tolerance, they can also induce systemic autoimmune. Combinations of checkpoint inhibitors greatly increase clinical responses, but similarly increase these toxicities, thereby limiting their clinical utility. 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 produgs, designed to widen the therapeutic window by minimizing interaction with normal tissue and maximizing interaction with tumor tissue. 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), to provide selective antitumor 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 Pb-Tx is expected to expand clinical opportunities for targeting the PD-1/PD-L1 pathway in combination therapies.

**RESULTS AND DISCUSSION**

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

- Probody Therapeutics are Prostate Activated Antibody Pro-Drugs

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

- Pb-Tx (blue line) demonstrates higher EC50 for PD-L1 binding and PD-1 blocking relative to the parental antibody (green line) as measured by ELISA
- Activation of Pb-Tx by multistep cleavage of its linker (orange line) fully restores binding and blocking to that of the parental antibody

**Figure 3: PD-L1 Probody Tx Exhibits Prostate-dependent Binding and Blocking Activity 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 prostate dependence

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

- Reduced binding of Pb-Tx to ‘blind’ 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

**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 (gray)
- 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)

**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 affinty 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

**REFERENCES**

10. CytomX Therapeutics, Inc. © 2015 CytomX Therapeutics, Inc.