CD3-EGFR Probody™ T Cell-engaging Bispecific Therapeutic Induces Tumor Regressions and Substantially Increases Safety Window in Preclinical Studies

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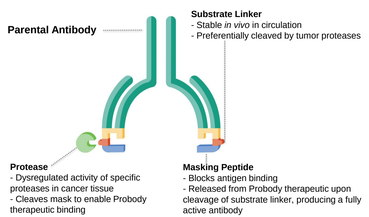
INTRODUCTION

T cell-engaging bispecific antibodies (TCBs) represent a highly potent modality to direct the activity of cytotoxic T cells to tumors. TCBs have shown clinical activity in hematologic malignancies (e.g. blinatumomab, a CD19xCD3 bispecific), but their development for non-hematologic cancers has been challenging, due in part to toxicity[1,2] that results from the inability of these highly potent modalities to discriminate between tumor and healthy cells expressing target antigen. Therefore, new approaches are needed that enable the potent anti-tumor activity of TCBs without on-target damage to normal tissues.

CytomX has developed a new class of antibody therapeutics called Probody Therapeutics that are recombinant, proteolytically activatable antibody prodrugs. Probody therapeutics are designed to widen the therapeutic window by minimizing interaction with normal tissue and maximizing interaction with tumor tissue[3,4]. 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.

We have applied Probody technology to this highly potent bispecific modality. Here we demonstrate the ability of a Probody T cell-engaging Bispecific (Pb-TCB) targeting CD3 and Epidermal Growth Factor Receptor (EGFR), to provide equivalent anti-tumor activity in NSG mice to its corresponding antibody bispecific (Ab-TCB), while increasing the maximum tolerated dose by at least 30-fold in cynomolgus monkeys. By localizing their activity to the tumor microenvironment, Pb-TCBs have the potential to expand clinical opportunities for T cell-engaging bispecific therapies in solid tumors that are currently limited by on-target toxicities.

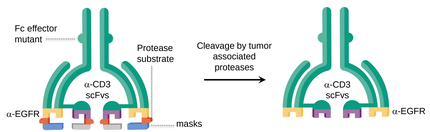
Figure 1: Probody Therapeutics are Protease-Activatable Antibody Pro-Drugs



Designed to Bind Target in Tumors But Not Healthy Tissue

PROBODY is a trademark of CytomX Therapeutics, Inc.

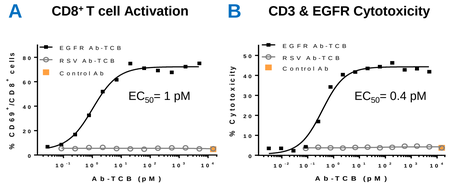
Figure 2: CytomX Probody T Cell-Engaging Bispecific (Pb-TCB) Format



- Full IgG bispecific format to maximize exposure and half-life
- Fc-effector impaired to minimize ADCC

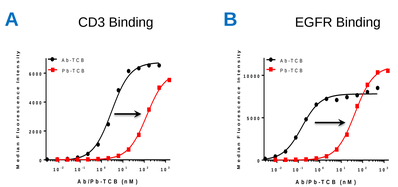
RESULTS

Figure 3: CD3/EGFR Ab-TCB Induces Potent, Target-dependent T cell Activation and Cytotoxicity *in vitro*



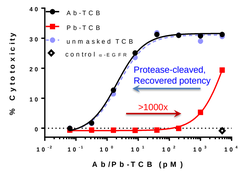
T cell activation and T cell-mediated targeted cell killing by the Ab-TCB requires co-engagement of EGFR and CD3.
Activation of primary human CD8+ T cells (measured by induction of CD69) was assessed by flow cytometry following a 24 hour co-incubation of CD3+ T cells and EGFR+ HT-29Luc2 cells at a ratio of 5:1. Target cell cytotoxicity was quantified by CytotoxGlo assay following a 48 hour incubation of CD3+ T cells and target cells at a 5:1 ratio. The CD3/EGFR Ab-TCB, but not an Ab-TCB targeting an irrelevant antigen derived from respiratory syncytial virus (RSV Ab-TCB), induced CD69 expression (A) and killing of HT-29Luc2 cells (B).

Figure 4: Pb-TCB Demonstrates Reduced Binding to Both CD3 and EGFR *in vitro*, as designed



Binding of the Pb-TCB to CD3 (Jurkat T cells) (A) and EGFR (HT-29Luc2 cells) (B) was assessed by flow cytometry.

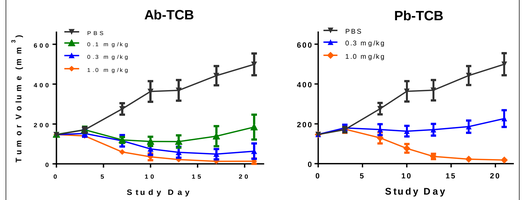
Figure 5: Pb-TCB Provides > 1000-fold Protection From Target-dependent Cytotoxicity Under Protease-Deficient Conditions *in vitro*



Masking of Pb-TCB shifts the cytotoxicity EC_{50} more than 1000-fold relative to the unmasked Ab-TCB. Cytotoxicity was quantified by CytoToxGlo assay following a 48 hour incubation of PBMCs and HT-29Luc2 target cells at a 10:1 ratio. Cleavage of the masks from Pb-TCB with recombinant uPA protease fully restores its killing activity, as evidenced by the indistinguishable cytotoxicity curves of Ab-TCB and protease-activated unmasked Pb-TCB.

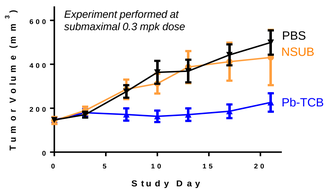
Figure 6: EGFR/CD3 Probody TCB Induces Regressions of Established HT-29 Tumors in T Cell-Engrafted NSG mice

A Ab-TCB & Pb-TCB demonstrate comparable anti-tumor activity @1mpk.



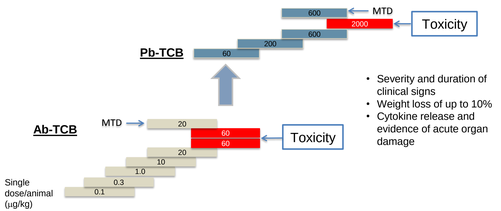
Potency of Probody TCB is within 2-3 fold of antibody TCB

B Protease activation of Pb-TCB is required for anti-tumor activity.



Female NSG mice (n=8/group) were implanted SC with 2 million HT-29Luc2 cells on day -15. Three days later, mice were injected IP with human PBMCs at a T cell/tumor inoculum ratio of 1:1. At onset of dosing on Day 0, the average tumor volume (TV) was 147 mm^3. Test and control articles were administered weekly IV as depicted in the above schematic. NSUB is a Pb-TCB devoid of a cleavable substrate sequence (No-SUBstrate). TV was mean ±SEM.

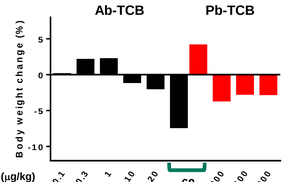
Figure 7: Dose-Escalating, Range-Finding Tolerability Study in Cynomolgus Monkeys



- Severity and duration of clinical signs
- Weight loss of up to 10%
- Cytokine release and evidence of acute organ damage

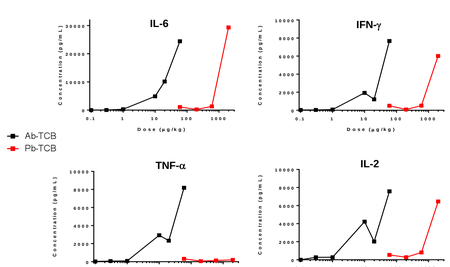
Figure 8: Probody TCB Provides > 30 Fold Improvement In Safety Versus Ab-TCB in Cynomolgus Monkeys

A Weight loss as a measure of overt toxicity was < 5% for Pb-TCB at doses up to 33-fold above the Ab-TCB toxic dose of 60 μg/kg.



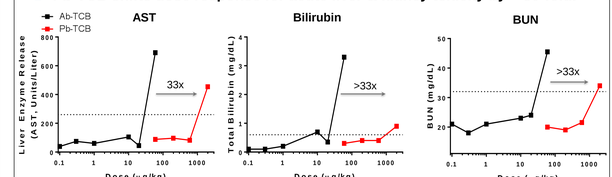
Body weight was measured prior to dosing and 7 days post-dose.

B Pb-TCB shifts dose-response for cytokine release.



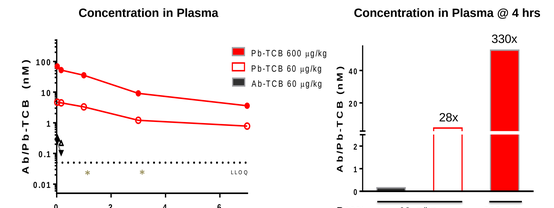
Cytokine analysis was performed with a Luminex® suspension array system on serum samples collected pre-dose, 1, 4 and 24 hours post-dose. Data presented were obtained at 1 hour (TNF-α) or 4 hours (IL-2, IL-6, IFN-γ) post-dose.

C Pb-TCB shifts dose response for acute liver & kidney toxicity by > 30-fold.



Hematology and serum chemistry was assessed pre-dose, 48 and 158 hours post-dose. Data presented were acquired 48 hours post-dose. Dotted line represents the upper limit of normal for cynomolgus Mauritius male monkeys[5].

Figure 9: Tolerated Pb-TCB Exposure is > 300-fold Higher than Toxic Ab-TCB Exposure in Cynomolgus Monkeys



*below level of quantitation

- Pb-TCB clearance is significantly slower than for Ab-TCB, presumably due to absence of target-mediated clearance.
- 4 hrs post-dose, Pb-TCB is 28x higher concentration than Ab-TCB at the toxic Ab-TCB dose (60 μg/kg), and 330x higher at its MTD (600 μg/kg).

CONCLUSIONS

- CD3/EGFR antibody-TCB mediates potent EGFR-dependent T cell activation and tumor target cell killing at single-digit pM concentrations *in vitro*.
- Probody-TCB attenuates EGFR and CD3 binding and reduces targeted T cell cytotoxicity by over 1000-fold *in vitro*.
- Pb-TCB activation by proteases restores full potency to Ab-TCB levels
- Pb-TCB and Ab-TCB eliminated established EGFR+ HT-29 colorectal tumors in human T cell engrafted NSG mice. Pb-TCB potency is within 2-3 fold of the Ab-TCB.
- In cynomolgus monkeys, masking increased the Pb-TCB maximum tolerated dose by 30-fold relative to Ab-TCB. Masking also reduced target-mediated clearance, enabling safe achievement of a serum drug concentration >300 times that of the Ab-TCB at its toxic dose.
- The demonstration of anti-tumor efficacy with a 30-fold improvement in safety validates the advantage of Probody technology for T cell-engaging bispecific therapies, and potentially enables the wider use of TCBs for solid tumor targets.

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