## EGFR-CD3 Bispecific Probody<sup>™</sup> Therapeutic Induces Tumor Regressions and Increases Maximum Tolerated Dose >60 fold in Preclinical Studies

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## INTRODUCTION

T cell-engaging bispecific antibodies (TCBs) are highly potent therapeutics which direct the activity of cytotoxic T cells to tumors. TCBs have shown clinical activity in hematologic malignancies, but development of TCBs for solid tumor indications is proving more challenging. Due to their high potency, TCBs can target normal tissues with low antigen expression, resulting in significant ontarget, off-tumor toxicity that can limit dosing to low levels. As a result, it has been difficult to reach the level of drug exposure required for efficacy without excessive toxicity. Therefore, novel methods are needed to enable the potent anti-tumor activity of TCBs while minimizing toxicity due to cytokine release and damage to healthy tissues.

CytomX has developed a new class of recombinant, proteolytically activated antibody prodrugs (Probody<sup>™</sup> therapeutics) that are "masked" to reduce binding to antigen in healthy tissue, but can become "unmasked" by proteases that are preferentially activated in the tumor microenvironment. In this way, Probody therapeutics are designed to increase therapeutic index by maximizing efficacy and minimizing on-target toxicity in normal tissues. Here we describe a T cell-engaging Bispecific Probody therapeutic (Pb-TCB) targeting Epidermal Growth Factor Receptor (EGFR) and CD3 that has been optimized for affinity, effector function, masking, and cleavability. In vitro, under protease-deficient conditions, we demonstrate that the unmasked EGFR-CD3 TCB has potent, EGFR-dependent tumor cell killing, while the doubly-masked EGFR-CD3 Pb-TCB reduces target-dependent cytotoxicity by more than 100,000-fold. However, in established tumor models where tumor-resident proteases are expected to be active, we demonstrate that Pb-TCBs potently induce tumor regressions. Further, in non-human primates, the maximum tolerated dose (MTD) of the EGFR-CD3 Pb-TCB is more than 60-fold higher than the MTD of the unmasked TCB, and the tolerated exposure (AUC) is more than 10.000-fold higher. Finally, even at a 60-fold higher dose, transient serum cytokine and AST/ALT increases observed in non-human primates treated with the Pb-TCB are still lower than those induced by the TCB. By localizing activity to the tumor microenvironment, Pb-TCBs have the potential to expand clinical opportunities for T cell-engaging bispecific therapies that are limited by on target toxicities, especially in solid tumors. Moreover, an EGFR-CD3 Pb-TCB has the potential to address EGFR-expressing tumors that are poorly responsive to existing EGFR-directed therapies.

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







- Full IgG bispecific format to maximize exposure and half-life
- Fc-effector impaired to minimize cross linking to FcγR bearing cells
- Format optimized for a-CD3 affinity, mask strength and cleavable substrates
- act-TCB represents protease activated, unmasked TCB

## RESULTS Figure 3: Pb-TCB Demonstrates Reduced Binding to EGFR+ and CD3+ Cells in vitro



### Binding of the Pb-TCB and act-TCB to EGFR+ HT29 cells (A) and CD3+ Jurkat cells (B) was evaluated by flow cytometry. Masking efficiency (shift in apparent Kd of Pb-TCB relative to act-TCB) of the EGFR mask is approx. 1000 and is >5000 for the CD3 mask.

## Figure 4: Pb-TCB Offers Substantial Protection in *in* vitro Functional Assays



A. Pb-TCB shifts EC<sub>50</sub> of T cell mediated cell killing >300,000x. No cytotoxicity is observed when a non-EGFR binding Pb-TCB is used demonstrating that target engagement is required for activity. Cytotoxicity was quantified using Promega OneGlo assay following a 48 hour incubation of PBMCs and HT29 Luc2 target cells at a 10:1 ratio. Co-cultures were treated with Pb-TCB or act-TCB at the concentrations shown above.

## B. The Pb-TCB demonstrates reduced T cell activation relative to the unmasked TCB.

The dose response curve for CD8+ T cell activation is shifted for the Pb-TCB relative to the act-TCB. A ~300x higher concentration of the masked molecule is required to activate T cells relative to the unmasked molecule. CD8+ T cell activation was measured by flow cytometry as the percentage of CD69+CD8+ T cells following a 48 hour incubation of PBMCs and U266 cells at a 10:1 ratio. Co-cultures were treated with Pb-TCB or act-TCB at the concentrations shown above.



## Figure 5: Pb-TCB Sensitivity to Protease Cleavage **Correlates with Tumor T cell Infiltration and** Efficacy in PBMC Engrafted NSG Mice

A Pb-TCB with higher protease sensitivity leads to greater T cell infiltration

# CD3+ cells (brown staining) in HT29 tumors harvested 7 days after a 1 mg/kg dose



**B** Pb-TCB with higher protease sensitivity leads to greater efficacy



Female NSG mice (n=8/group) were implanted SC with 2 million HT29Luc2 cells on day -15. Three days later, mice were injected IP with human PBMCs at a T cell/tumor inoculum ratio of 1:1. Test and control articles were administered at 0.3 mg/kg, weekly IV as shown in the above schematic. \*NSUB is a Pb-TCB devoid of a cleavable substrate sequence (No-SUBstrate). TV is presented as mean ±SEM.

## Figure 6: EGFR/CD3 Pb-TCB Induces Regressions of Established HT-29 Tumors in PBMC Engrafted **NSG** mice



Female NSG mice (n=7/group) were implanted SC with 2 million HT29Luc2 cells on day -15. Three days later, mice were injected IP with human PBMCs at a T cell/tumor inoculum ratio of 1:1. Test and control articles were administered at 0.5 or 1.5 mg/kg, weekly IV. TV is presented as mean ±SEM.



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