A Probody™ Drug Conjugate Targeting CD166 (ALCAM) Enhances Preclinical Antitumor Activity of a Probody Therapeutic Targeting PD-1

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ABSTRACT

Immune checkpoint blockade therapies have been shown to induce potent and durable anti-tumor immunity in many cancer types. Nevertheless, not all patients benefit from immunotherapy, and immune-related adverse events remain a problem. Recently, it has been demonstrated that Antibody Drug Conjugates (ADCs) are not only capable of killing cancer cells but also can act to induce the immunogenic cell death of tumor cells as well as directly activate dendritic cells. These results provided a rationale to combine ADCs with immunotherapy to enhance the potential of immune checkpoint blockade therapies in a broader population of patients.

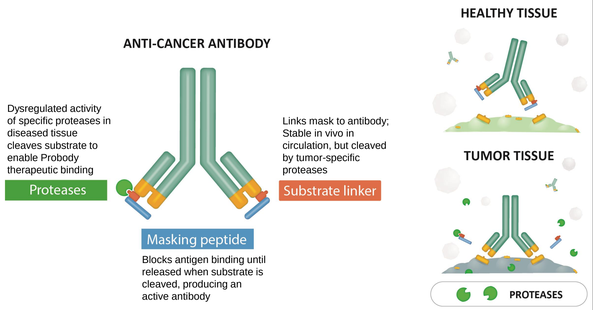
CytomX Therapeutics has developed a new class of antibodies called Probody™ therapeutics, designed to widen the therapeutic window by minimizing binding to target in healthy tissue while being specifically activated in the tumor microenvironment (TME) by tumor-associated proteases. Probody technology has been evaluated in preclinical studies in several antibody formats, with efficacy and increased safety windows observed for Probody therapeutics targeting the PD-1 pathway, Probody drug conjugates (PDCs) targeting highly expressed tumor antigens, and T-cell engaging bispecific Probody therapeutics. Here we extend our evaluation of the Probody platform to the combination of CX-2009, an investigational PDC targeting human CD166, with an investigational Probody therapeutic targeting PD-1.

To evaluate the anti-tumor activity of PDC CX-2009 in a syngeneic mouse model, human CD166 was overexpressed on the surface of the CT-26 murine colon carcinoma cell line. The combination treatment of CX-2009 with a surrogate mouse anti-PD-1 Probody molecule significantly inhibited tumor growth in human CD166 positive CT-26 tumor-bearing mice as compared to CX-2009 or anti-PD-1 Probody molecule alone. Tumor rejection is partially dependent on CD8$^+$ T cells as illustrated by the evidence of a CD8$^+$ memory T cell response in a re-challenge assay, and a reduced activity of CX-2009 alone or in combination with a mouse anti-PD-1 Probody molecule after CD8$^+$ T cell depletion. The immunogenic potential of CX-2009 was further evaluated in multiple *in vitro* assays using human cancer cells and human PBMCs. In contrast to its cytotoxic activity towards CD166$^+$ tumor cells, CX-2009 spares T cells and may enhance T cell priming.

These preclinical data demonstrate the potential utility of a combination of PDC CX-2009 with a Probody therapeutic targeting the PD-1 pathway. Generally, these data highlight the potential to combine ADCs or PDCs with immune checkpoint blockade therapies.

INTRODUCTION

Figure 1: Probody Therapeutics are Protease-Activatable Antibody Prodrugs



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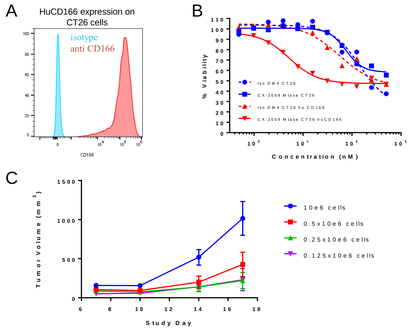
RESULTS

Figure 2: Establishment of CT-26 human CD166 syngeneic tumor mouse model

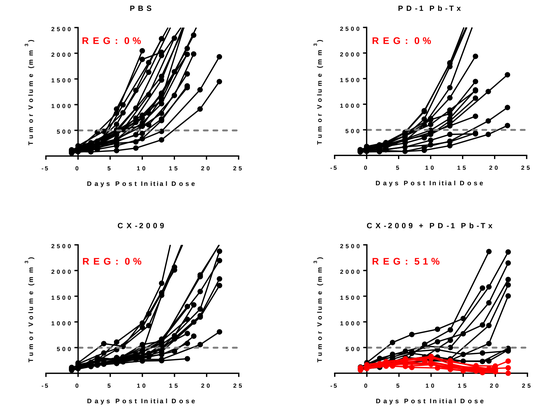


A) CT26 huCD166 cells express high levels of human CD166. CT26 cells transduced to express human CD166 were analyzed by flow cytometry to detect CD166 expression.

B) CT26 huCD166 cells are sensitive to CX-2009 cytotoxic activity. Parental CT26 cells and CT26 huCD166 cells were cultured for four days in the presence of an isotype conjugated to DM4 or CX-2009 activated by Matriptase (Mtase). Cell viability was measured using Cell Titer Glo.

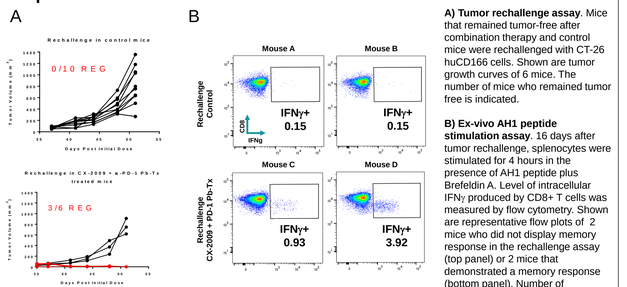
C) CT26 huCD166 cells form tumors in Balb/c mice. Balb/C mice at 7 weeks of age were implanted with various numbers of CT26 huCD166 cells. Tumor volume was monitored for 10 days.

Figure 3: CX-2009 enhances the anti-tumor activity of a mouse anti-PD-1 Pb-Tx in the CT26 HuCD166 syngeneic model



Balb/C mice at 7 weeks of age were implanted with 10e^6 CT-26 human CD166 syngeneic tumor cells. When tumor size reached 100-175 mm^3, an anti-mouse PD-1 Pb-Tx was dosed intraperitoneally at 10mg/kg b.i.w. x 2.5 weeks, and CX-2009 was dosed intravenously at 5mg/kg Q1Wx2.
Shown are tumor growth curves of individual mice from 3 independent studies. A comparison of CX-2009 and CX-2009 + anti-PD-1 Probody therapeutic (Pb-Tx) in tumor volume (mm^3) was performed at the last tumor measurement. A repeated measures, mixed effects model using an unstructured covariance structure was fit to the data with study, time, treatment, and time-by-treatment as covariates. There was a statistically significant reduction in the tumor volume at the last tumor measurement for mice treated with CX-2009 + anti-PD-1 Pb-Tx compared with CX-2009 alone ($p < 0.0001$).
Percent Regression (REG): defined as % of mice with a tumor volume at day 20 post initial dose lower than the volume at randomization (colored in Red)

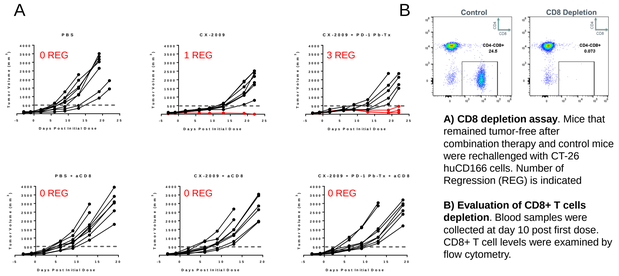
Figure 4: CX-2009 + mouse anti-PD-1 Pb-Tx induces memory T cell response in mice



A) Tumor rechallenge assay. Mice that remained tumor-free after combination therapy and control mice were rechallenged with CT-26 huCD166 cells. Shown are tumor growth curves of 6 mice. The number of mice who remained tumor free is indicated.

B) Ex-vivo AH1 peptide stimulation assay. 16 days after tumor rechallenge, splenocytes were stimulated for 4 hours in the presence of AH1 peptide plus Brefeldin A. Level of intracellular IFNγ produced by CD8+ T cells was measured by flow cytometry. Shown are representative flow plots of 2 mice who did not display memory response in the rechallenge assay (top panel) or 2 mice that demonstrated a memory response (bottom panel). Number of Regression (REG) is indicated.

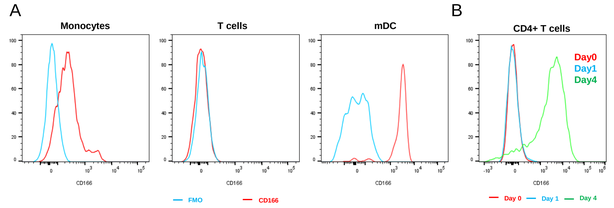
Figure 5: Depletion of CD8+ T cells decreases CX-2009 and CX-2009 + anti-PD-1 Pb-Tx antitumor activity in mice



A) CD8 depletion assay. Mice that remained tumor-free after combination therapy and control mice were rechallenged with CT-26 huCD166 cells. Number of Regression (REG) is indicated

B) Evaluation of CD8+ T cells depletion. Blood samples were collected at day 10 post first dose. CD8+ T cell levels were examined by flow cytometry.

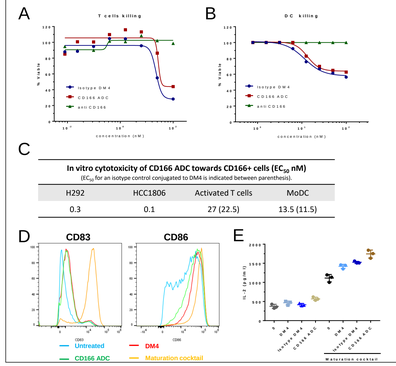
Figure 6: CD166 expression on immune cells



A) CD166 expression on immune cells. CD166 expression on the surface of immune cells (Human PBMCs) was analyzed by flow cytometry. Shown are representative plots of CD166 expression on monocytes, T cells and Dendritic cells.

B) Expression of CD166 on activated T cells. CD4+ T cells isolated from human PBMCs were stimulated with aCD3/aCD28 beads for 4 days. Cells were analyzed by flow cytometry to detect CD166 expression.

Figure 7: CD166 ADC does not induce specific killing of CD166+ immune cells, and may enhance T cells priming through DC



In vitro cytotoxicity of CD166 ADC towards CD166+ cells (EC_{50} nM)
(EC_{50} for an isotype control conjugated to DM4 is indicated between parenthesis)

H292	HCC1806	Activated T cells	MoDC
0.3	0.1	27 (22.5)	13.5 (11.5)

A) CD166 ADC cytotoxic activity towards human Monocytic derived dendritic cells (MoDC). Fully activated MoDC were incubated with the indicated treatments for 48hrs. Cell viability was measured using Cell Titer CytoTox Glo.

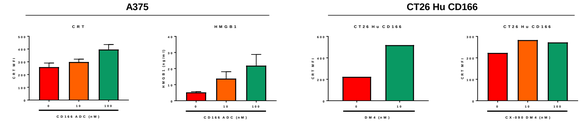
B) CD166 ADC cytotoxic activity towards activated human T cells. T cells were stimulated for 6 hours with aCD3/CD28 beads. For the last 72 hours, cells were incubated with the indicated treatment. Cell viability was measured using Cell Titer Glo

C) In vitro cytotoxicity of CD166 ADC towards CD166+ cancer cells or CD166+ immune cell

D) Effect of CD166 ADC on MoDC maturation. Expression of DC markers of maturation, CD83 and CD86, after 48 hours of exposure to the indicated drugs (10nM) or a maturation cocktail was assessed by flow cytometry.

E) T cell stimulatory capacity of MoDC matured with CD166 ADC. MoDC pretreated as indicated in (C) were used to stimulate allogeneic CD4+ T cells in a mixed lymphocyte reaction for 48hrs. Levels of IL-2 produced by CD4+ T cells was measured by ELISA.

Figure 8: CD166 ADC induces immunogenic cell death of A375 and CT26 Hu CD166 cells



Cells were treated with CD166 ADC or DM4 for 48 hours (A375) or 72 hours (CT26 Hu CD166) and immunogenic cell death markers were measured by flow cytometry (calreticulin) or ELISA (HMGB1).

SUMMARY/CONCLUSIONS

- **CX-2009 (a CD166 targeting Probody Drug Conjugate) enhances the preclinical antitumor activity of a Probody therapeutic targeting PD-1**
- **The underlying MOA is partially dependent on CD8+ T cells.**
- **In vitro, in addition to its cytotoxic activity towards CD166+ tumor cells, a CD166 Antibody Drug Conjugate:**
  - **induces immunogenic cell death of cancer cells**
  - **spares T cells and may enhance T cell priming.**
- **These data demonstrate the potential utility of combining a PDC (CX-2009) with a Probody therapeutic targeting the PD pathway, such as CX-072**

References and Acknowledgements

1. Desnoyers et al. Sci Transl Med. 2013 Oct 16;5(207):207ra144.
2. Rios-Doria et al., Cancer Res., 2018.
3. Mueller et al, Oncoimmunol, 2014.

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