Novel Conditionally Active Bispecific HER2 x CD3 T-Cell Engager Targeting Solid Tumors

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INTRODUCTION

Human epidermal growth factor receptor 2 (HER2) is overexpressed in multiple cancers and is associated with poor prognosis. Despite the outstanding improvement in survival with the introduction of anti-HER2 therapies, therapeutic benefit is limited by many resistance mechanisms and toxicities. Monoclonal antibodies targeting HER2 are ineffective in HER2-low cancers, and even though promising results have been obtained using antibody drug conjugates, the impact on survival endpoints has not been established. In addition, clinical trials of therapeutics redirecting T-cell activity to HER2+ tumors have highlighted the apparent risk of on-target/off-tumor adverse effects, as HER2 is also expressed in normal epithelia. The generation of new therapies targeting HER2 that are safe, promote killing of cancers that are refractory to current therapies and are effective against HER2-low expressing cancers is an unmet medical need.

RATIONALE

Using BioAtla's Conditionally Active Biologic (CAB) technology[1] we developed a Mono-CAB HER2/CD3 (WT-HER2 x CAB-CD3) bispecific antibody that binds with high affinity to recombinant CD3 and induces T cell activation under conditions that mimic the tumor microenvironment (acidic conditions), but with lower affinity in physiological conditions (normal alkaline microenvironment).

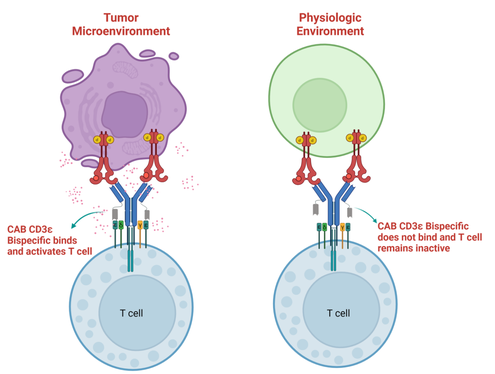

Figure 1: WT-HER2 x CAB-CD3 bispecific antibody activates T cells in the tumor microenvironment and is less efficient in inducing T cell activation under physiological conditions.

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The WT-HER2 x CAB-CD3 bispecific antibody is more potent at inducing cytotoxicity of HER2+ cancer cells *in vitro* under acidic conditions, and less potent under physiological conditions. Humanized xenograft mouse models of human breast and colorectal cancers treated with WT-HER2 x CAB-CD3 bispecific antibody exhibited complete tumor regression *in vivo*. Additionally, the anti-tumor activity of WT-HER2 x CAB-CD3 antibody was observed in both HER2-high expressing BT474 human breast cancer and HER2-low expressing HCT116 human colorectal cancer. A single intravenous bolus administration of WT-HER2 x CAB-CD3 bispecific antibody was well tolerated and overall safe at 0.1 mg/kg in Non-human Primate (NHP) toxicity studies, with mild clinical pathology changes and cytokine release in contrast to a non-CAB Benchmark HER2/CD3 bispecific antibody.

1. Chang HW, Frey G, Liu H, Xing C, Steinman L, Boyle WJ, Short JM. Proc Natl Acad Sci U S A. 2021 Mar 2;118(9).3

RESULTS

Figure 2: Differential binding of WT-HER2 x WT-CD3 and WT-HER2 x CAB-CD3 at pH 6.0 (A, Tumor Microenvironment) and pH 7.4 (B, Normal Physiological pH). pH Affinity ELISA Assay using recombinant hCD3 as coating antigen, binding of the antibodies to hCD3 at pH 6.0 and 7.4 was determined with hHER2-mFc and anti-mIgG-HRP conjugated antibody.

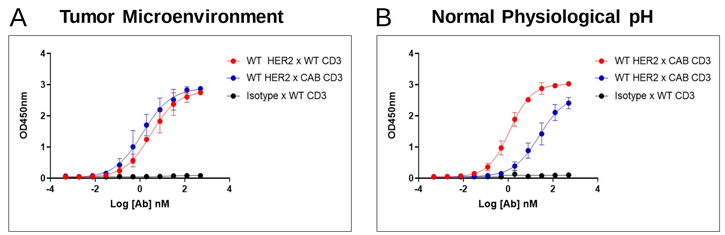

Figure 3: HER2 x CAB-CD3 Antibody induces complete tumor regression *in vivo*

- Figure 3A: BT474 human breast cancer cell line expresses high levels of HER2, and HCT116 human colorectal cancer cell line expresses low levels of HER2 antigen.
- Figure 3B: HER2 x CAB-CD3 bispecific antibody dosed at 0.1 mg/kg biweekly for four weeks led to complete tumor regression in a cancer cell line derived MiXeno model of human breast carcinoma.
- Figure 3C: HER2 x CAB-CD3 bispecific antibody dosed at 1 mg/kg biweekly led to complete tumor regression in a cancer cell line derived MiXeno model of human colorectal carcinoma, a cell line that expresses low levels of HER2 antigen.

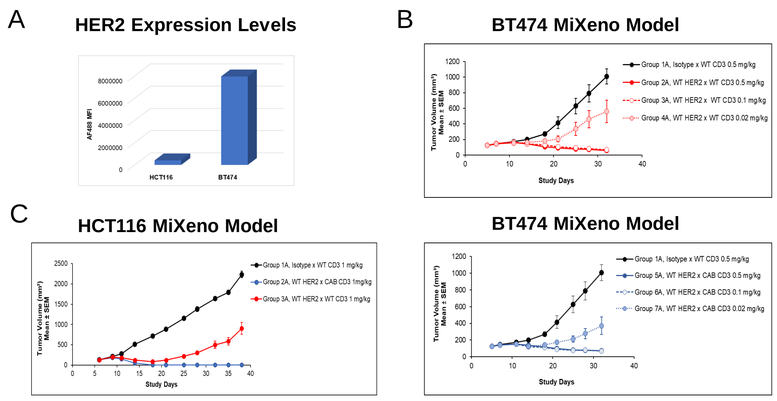

Figure 3. HER2 Expression on HCT116 and BT474 cancer cells (A). *In vivo* efficacy studies: Triple immunodeficient mice were engrafted with human PBMCs and inoculated with BT474 **(B)** or HCT116 **(C)** cells. Tumor bearing animals were randomized to treatment groups when the tumor volume reached approximately 80-120 mm^3. Following randomization, animals were dosed with Isotype, WT-HER2 x WT-CD3, WT-HER2 x CAB-CD3 bispecific antibodies at different doses biweekly for four weeks.

RESULTS

Figure 4: WT-HER2 x CAB-CD3 is more potent in inducing cytotoxicity of cancer cells at pH 6.0 **(A, Tumor Microenvironment)** and less potent in physiological pH 7.4 **(B, Normal Physiological pH).**

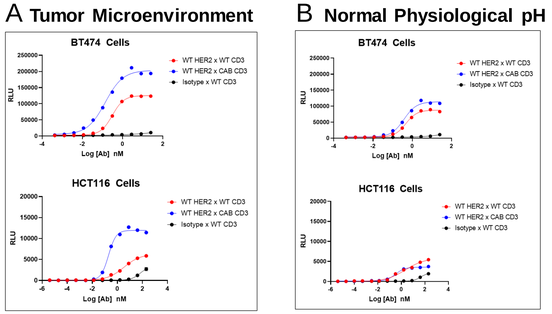

Figure 4: HER2 x CD3 bispecific antibodies induce T-cell activation *in vitro*. BT474 and HCT116 cells were cocultured with TCR/CD3 Jurkat effector cells that express a luciferase reporter driven by NFAT-response element (Promega T-cell Activation Assay). Cocultures were incubate in the presence of HER2 x CD3 bispecific antibodies at pH 6.0 and pH 7.4.

Figure 5: Single Dose Finding Study in NHP

In NHP WT-HER2 x WT-CD3 and WT-HER2 x CAB-CD3 were well tolerated and safe at a dose of 0.1 mg/kg. The WT-HER2 x WT-CD3 bispecific antibody administered at 0.1 mg/kg induced high levels of cytokines, while the WT-HER2 x CAB-CD3 bispecific antibody induced only mild cytokine response at the same dose level.

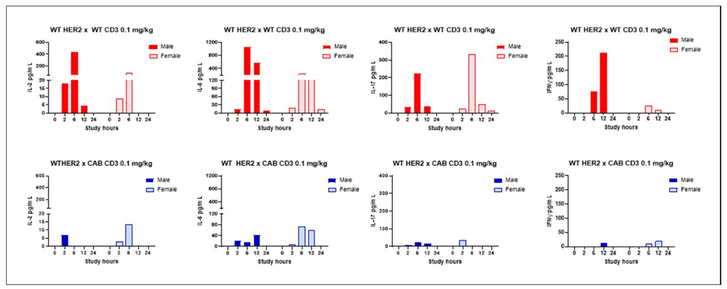

Figure 5 cont.: Cytokine levels in the serum of NHP treated with HER2 x CD3 bispecific antibodies. Cynomolgus monkeys received a single intravenous administration of WT-HER2 x WT-CD3 or WT-HER2 x CAB-CD3 bispecific antibodies. Serum was collected at different time points for cytokine analysis.

CONCLUSIONS

- WT-HER2 x CAB-CD3 bispecific antibody have strong **binding** to CD3 under **tumor conditions** compared to low binding under normal physiological conditions.
- WT-HER2 x CAB-CD3 bispecific antibody is more potent in controlling **tumor growth *in vivo*** compared the WT-HER2 x WT-CD3 bispecific antibody.
- WT-HER2 x CAB-CD3 bispecific antibody induced significant **less cytokine** in NHP compared to WT-HER2 x WT-CD3 bispecific antibody.
- The BioAtla CAB platform offers the potential to **transform bispecific solid tumor therapies** through the widening of the therapeutic index.

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