Novel Conditionally Active Bispecific EpCAM x CD3 T Cell Engagers Targeting Solid Tumors

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INTRODUCTION

Epithelial cell adhesion molecule (EpCAM) is a multi-functional transmembrane protein that mediates Ca2+-independent homotypic cell–cell adhesion during cell signaling, migration, proliferation, and differentiation. EpCAM is expressed at high levels exclusively in epithelia and epithelial-derived neoplasms, making it a suitable target for many important solid tumor types and cancer stem cells. EpCAM expression on normal tissues limits its utility as a target for therapeutic antibodies and Antibody Drug Conjugates (ADCs) due to the potential effects on normal epithelial tissues throughout the body. Treatment of EpCAM-positive solid tumors with EpCAM-specific T cell engagers has been associated with dose-limiting toxicities, precluding their therapeutical use. Eliminating the on-target off-tumor toxicity associated with the EpCAM target would enable a powerful, broad spectrum anti-tumor target.

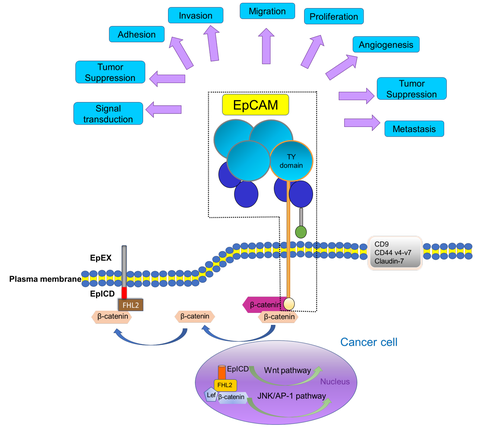

Figure 1: Modified from: Biology of EpCAM. Cancer Metastasis Rev **31**(3-4): 779-791.

RATIONALE

Conditionally Active Biologic (CAB) technology[1] is a proprietary platform that generates bispecific antibodies which have little to no binding to CD3 and the target EpCAM antigen (dual CAB; CAB EpCAM x CAB CD3) in healthy tissue (normal alkaline microenvironment). However, in acidic conditions that mirror the tumor microenvironment (high glycolysis) the binding of the antibodies to their target molecules is strong. These molecules bind to both recombinant EpCAM/CD3 and EpCAM/CD3 expressing cells under acidic conditions that are present in the tumor microenvironment, but not in normal tissues. Both EpCAM x CAB CD3 and CAB EpCAM x CAB CD3 bispecific antibodies were active against EpCAM positive human tumor xenografts. Importantly, complete tumor regression was observed upon treatment with these CAB bispecific antibodies. In contrast to the non-CAB bispecific antibodies, the CAB bispecific antibody demonstrated that a single intravenous bolus administration of CAB EpCAM x CAB CD3 antibody was well tolerated and overall safe in Non-human Primate (NHP) toxicity studies. Mild cytokine response and low levels of toxicity was observed only at 2.5mg/kg, a dose that is 50-fold higher than EpCAM x CD3 control antibody. Reversible CAB bispecifics yield a superior therapeutic index relative to other formats, including prodrugs.

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).

RESULTS

- **Figure 2A and 2B:** EpCAM x CAB CD3 and CAB EpCAM x CAB CD3 demonstrated a differential pH binding profile to hEpCAM and hCD3, with higher affinity at pH 6.0 **(A, tumor microenvironment)** and low affinity in physiological pH 7.4 **(B, physiological pH)**.

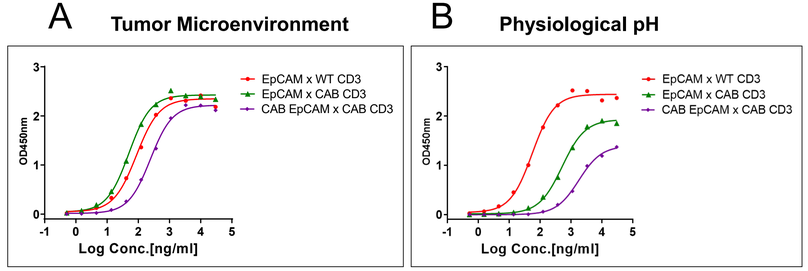

Figure 2. Differential binding of EpCAM x CD3 non-CAB and CAB variants at pH 6.0 (A, tumor microenvironment) and pH 7.4 (B, physiological pH). pH Affinity ELISA Assay using recombinant hCD3 as coating antigen, binding of the antibodies to hCD3 at pH 6.0 and 7.4 was determined with hEpCAM-mFc and anti-mIgG-HRP conjugated antibody.

Results Summary of Figures 3A and 3B:

- Figure 3A- CAB bispecifics demonstrate pH dependent binding (ELISA).
- Figure 3B- EpCAM x CD3 CABs bispecifics dosed at 1mg/kg BIW led to complete tumor regression in a cancer cell line derived MiXeno model of human colon carcinoma. EpCAM x CD3 CAB bispecific antibodies show similar efficacy in a HCT116 MiXeno model compared to EpCAM x CD3 non-CAB benchmark antibody.

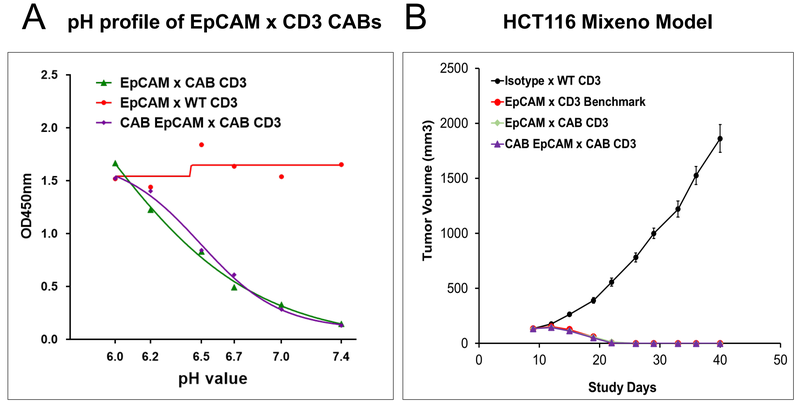

Figure 3. pH range ELISA assay (A). *In vivo* efficacy of EpCAM x CD3 CABs bispecific molecules (B). HCT116 cells were implanted in triple immunodeficient mice engrafted with human PBMCs. Tumor bearing animals were randomized to treatment groups when the tumor volume reached approximately 120 mm3. Following randomization, animals were dosed with Isotype, benchmark, EpCAM x CAB CD3 or CAB EpCAM x CAB CD3 bispecific antibodies at 1mg/kg BIW x 4 weeks.

RESULTS

- In NHP (see Figures 4A and B), EpCAM x CD3 Benchmark antibody was not tolerated at a dose of **0.05mg/kg.** Animals developed acute inflammatory response characterized by high levels of cytokines, marked increase of liver enzymes, with impacted GI, liver and kidney functions.
- EpCAM x CAB-CD3 bispecific antibody was tolerated at a **5x higher dose of 0.25mg/kg.** At this dose cytokine response and mild to moderate increase in liver enzymes was observed.
- The dual bispecific CAB EpCAM x CAB CD3 was tolerated at a **50x higher dose of 2.5mg/kg**, which was the highest dose tested. At 2.5mg/kg CAB EpCAM x CAB CD3 antibody was overall safe and well-tolerated, inducing only mild cytokine response and mild increase in liver enzymes with quick recovery.

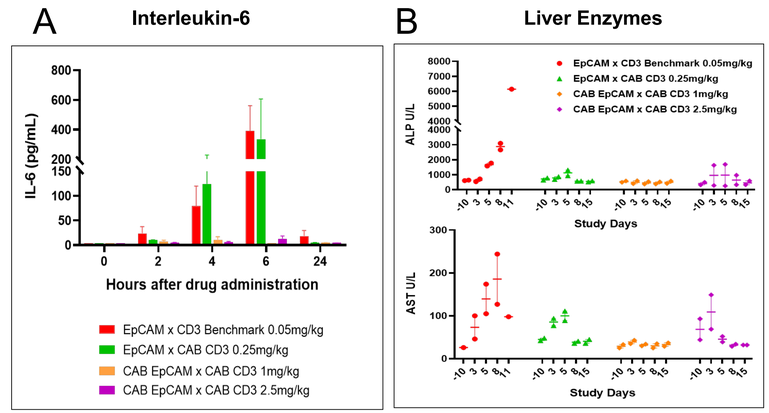

Figure 4. Single-dose finding study in NHP. Cynomolgus monkeys received a single intravenous administration of EpCAM x CD3 Benchmark antibody, EpCAM x CAB CD3 or CAB EpCAM x CAB CD3 antibodies. Different doses of each compound were administered (0.05-2.5mg/kg). Serum was collected at different time points for cytokine and serum chemistry analysis. (**A) Levels of IL-6 in the serum animals after drug administration. (B) Levels of liver enzymes at different time points after treatment.**

CONCLUSIONS

- CAB EpCAM x CAB CD3 bispecific antibodies have increased **binding** to EpCAM under **tumor conditions** compared to normal conditions.
- CAB EpCAM x CAB CD3 bispecific antibodies have **similar efficacy** in cancer cell line derived MiXeno models *in vivo* compared the non-CAB benchmark antibody.
- CAB EpCAM x CAB CD3 antibody demonstrates **substantially higher tolerance *in vivo*;** only inducing low levels of cytokines with very mild toxicity at 2.5mg/kg.
- The BioAtla CAB platform offers the potential to **transform bispecific solid tumor therapies** through the widening of the therapeutic index.

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