Novel conditionally active biologic (CAB) antibodies targeting EpCAM demonstrate anti-tumor efficacy *in vivo*

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ABSTRACT

Epithelial cell adhesion molecule (EpCAM) is a transmembrane glycoprotein mediating 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 target for therapeutic antibodies and ADCs due to the potential effects on normal epithelial tissues throughout the body.

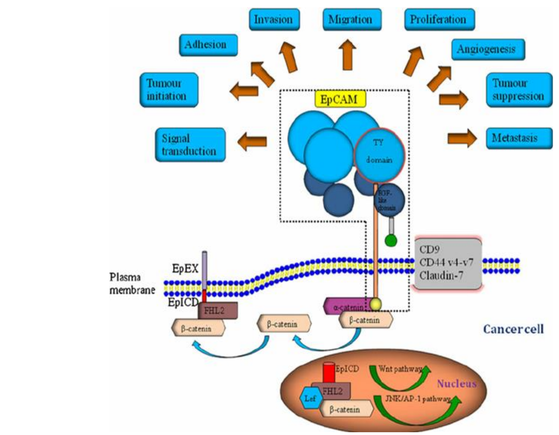

Figure 1:Biology of EpCAM; modified from Ni, J., et al. (2012). "Role of the EpCAM (CD326) in prostate cancer metastasis and progression." Cancer Metastasis Rev **31**(3-4): 779-791

RATIONALE

Using our CAB technology, we have developed CAB antibodies to EpCAM that reversibly bind to recombinant EpCAM and EpCAM expressing cells under select conditions that are present in the tumor microenvironment, but not in normal tissues. CAB-EpCAM antibodies were then conjugated to a model toxin payload to generate CAB-EpCAM-ADC. Similarly, we also developed CAB antibodies to CD3 molecule to generate EpCAM x CAB-CD3 bispecific antibodies. Both EpCAM-ADCs and EpCAM x CAB-CD3 bispecific antibodies were active against EpCAM positive human tumor xenografts with complete tumor regressions observed. Our data suggest that conditionally active EpCAM-ADCs and bispecific antibodies generated using the CAB technology will provide drug candidates that have an increased safety margin and therapeutic index in the clinic.

RESULTS

- Anti-EpCAM CAB ADCs bind to recombinant human EpCAM ECD with higher affinity in conditions mimicking the tumor environment pH (pH 6.0, A) compared to conditions mimicking the normal tissue pH (pH 7.4, B)

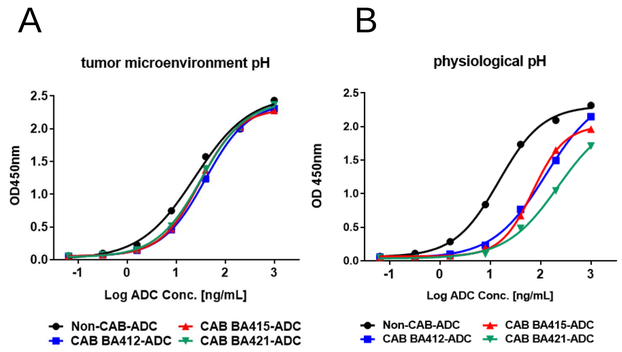

Figure 2. Differential binding of anti-EpCAM CAB ADCs at pH6.0 (A, tumor microenviroment) and pH 7.4 (B, physiological pH)

- Anti-EpCAM CABs induce greater cell cytotoxicity of EpCAM expressing cells (Colo205) in *in vitro* cell killing assay in conditions mimicking the tumor environment pH (A) compared to conditions mimicking the normal tissue environment pH (B).
- Anti-EpCAM CAB ADCs dosed at 3mg/kg Q4Dx4 lead to a complete tumor regression in a Colo205 colon cancer xenograft model (C)

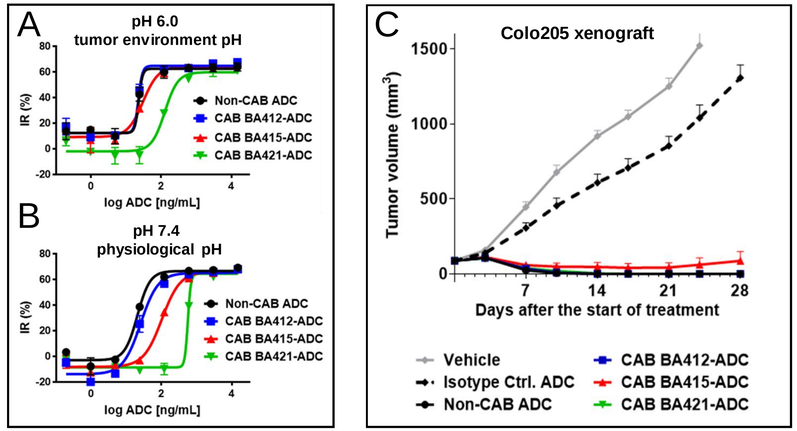

Figure 3. *In vitro* cell-killing and *in vivo* efficacy of anti-EpCAM CAB ADCs in cell line derived xenograft models. C-Colo205 cells were implanted in immunodeficient mice. Tumor-bearing animals were randomized to treatment groups when the tumor volume reached approximately 80-100 mm^3. Following randomization, animals (n=8 per group) were dosed with the indicated test article (3 mg/kg; Q4D x 4).

RESULTS

- EpCAM x CAB-CD3 bispecific dosed at 2.5mg/kg bi-weekly led to complete tumor regression in cell line derived MiXeno models (Crown Bioscience).
- EpCAM x CAB-CD3 bispecific antibody shows similar efficacy in HCT116 MiXeno model compared to EpCAM x CD3 non-CAB bench mark.
- EpCAM x CAB-CD3 bispecific antibody induces significantly reduced T-cell activation in the periphery compared to EpCAM x CD3 non-CAB bench mark antibody.

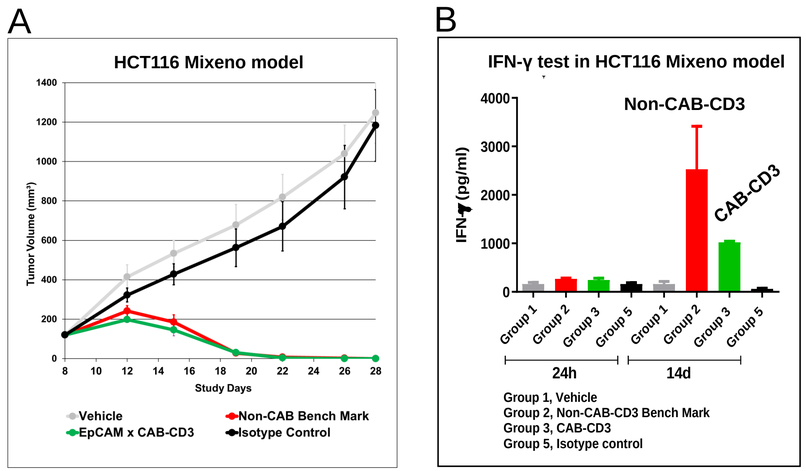

Figure 4. *In vivo* efficacy of EpCAM x CAB-CD3 bispecific molecules. A. Colon cancer cell line HCT116 cells were implanted in triple immunodeficient mice engrafted with human PBMCs. Tumor bearing animals were randomized to treatment groups when the tumor volume reached approximately 150 mm^3. Following randomization, animals were dosed with vehicle, control or test antibodies at 2.5 mg/kg BIW x 4 weeks. B. Serum IFN- γ was measured by MSD assay.

CONCLUSIONS

CAB technology enhancement of therapeutic index to achieve greater efficacy and safety is supported by the following conclusions:

- Anti-EpCAM CAB antibodies have increased binding to EpCAM under tumor conditions compared to normal conditions.
- Anti-EpCAM CAB ADCs have reduced cytotoxicity to EpCAM expressing cells in normal conditions compared to tumor conditions.
- Anti-EpCAM CAB ADCs have similar efficacy in cell line derived xenograft models *in vivo* as the non-CAB bench mark antibody.
- EpCAM x CAB-CD3 bispecific has similar efficacy in cell line derived MiXeno models *in vivo* compared to the non-CAB benchmark antibody.

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