Novel Conditionally Active Bispecific T Cell Engagers Targeting Solid Tumors

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INTRODUCTION

B7H3 (CD276) is an immune checkpoint in the B7 family of molecules, many of which interact with known checkpoint markers including CTLA4, PD-1 and CD28. B7H3 is also overexpressed in many solid cancers and its overexpression has been correlated with disease severity. Targeting B7H3 in cancer treatment can reduce cell proliferation, progression, and metastasis. Potent therapies target B7H3 designed with reduced on-target off-tumor toxicity are expected to lead to improved therapeutic options and better clinical outcomes.

Nectin4 is a predictive marker for cancer diagnosis and is a validated therapeutic target. It is believed to play a mechanistic role in cancer metastasis and angiogenesis of several types of primary tumors, as well as being a general target for adenocarcinomas. Nectin4 expression has a significant correlation with tumor grade and stages associated to tumor progression. Next generation Nectin4 therapies offer the potential to improve patient survival.

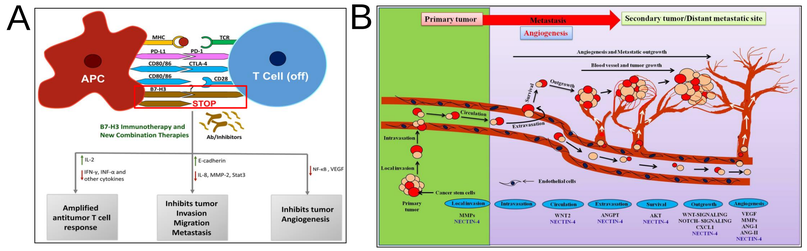

Figure 1: B7H3 and Nectin4 in the tumor tissues and downstream pathways.

A. B7H3 immunotherapy and new combination therapies from Castellanos JR., et al. (2017). AM J Clin Exp Immunol. 6 (4):66-75

B. Schematic representation of metastasis and angiogenesis processes of Nectin4. Sethy C. et al., (2020). J. Cancer Res. Clin. Oncol. 146(1): 245-259

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 antigen in healthy tissue (normal alkaline microenvironment). However, in acid conditions that mirror the tumor microenvironment (high glycolysis) the binding of the antibodies to their target molecule is strong. These molecules bind to both recombinant tumor associated antigen-TAA/CD3 and TAA/CD3 expressing cells under acidic conditions that are present in the tumor microenvironment, but not in normal tissues. We have developed dual-CAB (CAB TAA x CAB CD3) bispecific antibodies targeting two well-established tumor associated antigens, B7H3 and Nectin4. These bispecific antibodies were active against target positive human tumor xenografts. Importantly, complete tumor regression was observed upon treatment with these CAB bispecific antibodies. Reversible CAB bispecifics yield a superior therapeutic index relative to other formats, including prodrugs.

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

RESULTS

- CAB B7H3 x CAB CD3 bispecific antibody binds to recombinant human B7H3 ECD and CD3 epsilon/delta heterodimer protein with higher affinity in conditions mimicking the tumor microenvironment pH compared to conditions mimicking the normal tissue pH (A)
- CAB B7H3 x CAB CD3 pH profile showed that the affinity to human CD3 and human B7H3 were higher in acidic tumor microenvironment pH (6.0-6.5), lower in physiological pH (7.4) (B).
- CAB B7H3 x CAB CD3 dosed at 2mg/kg BIW x4 led to a complete tumor regression in a Detroit 562 MiXeno model, comparable to WT B7H3 x WT CD3 at the same dose (C).

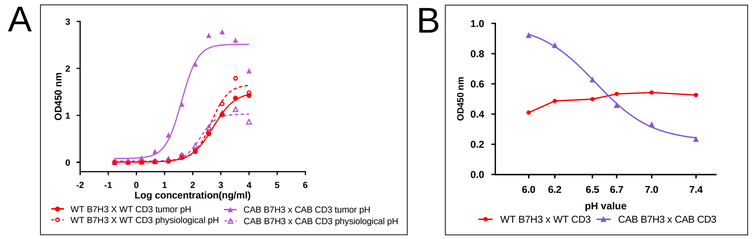

Figure 2. Differential affinity binding (A), pH range ELISA (B) and *in vivo* efficacy (C).

A. pH affinity ELISA applied human CD3 as capture antigen, human B7H3-mFc as detection followed by anti-mouse IgG HRP conjugated antibody. CAB B7H3 x CAB CD3 showed higher affinity in tumor microenvironment pH, but lower binding under the physiological pH.

B. CAB B7H3 x CAB CD3 demonstrated a differential binding with human CD3 as capture antigen, human B7H3-mFc as detection following with anti-mouse IgG HRP conjugated antibody with the pH range 6.0-7.4. The affinity binding of WT B7H3 x WT CD3 remained at a similar level.

C. Tumor-bearing animals were randomized to treatment groups when the tumor volume reached approximately 100-150mm^3. Following randomization, animals were dosed with the indicated test article (2 mg/kg; BIW x4). CAB B7H3 x CAB CD3 demonstrated a comparable tumor regression as WT B7H3 x WT CD3.

RESULTS

- CAB Nectin4 x CAB CD3 bispecific antibody shows high affinity to recombinant human Nectin4 ECD and CD3 epsilon/delta heterodimer protein like WT Nectin4 x WT CD3 in tumor microenvironment pH, but shows lower affinity in the physiological pH (A).
- CAB Nectin4 x CAB CD3 pH profile showed that the affinity to human CD3 and human Nectin4 were higher in acidic tumor microenvironment pH (6.0-6.5) and lower in physiological pH (7.4) (B).
- CAB Nectin4 x CAB CD3 dosed at 2.5mg/kg BIW x4 led to similar tumor regression as WT Nectin4 x WT CD3 in NCI-H358 MiXeno model at the same dose (C).

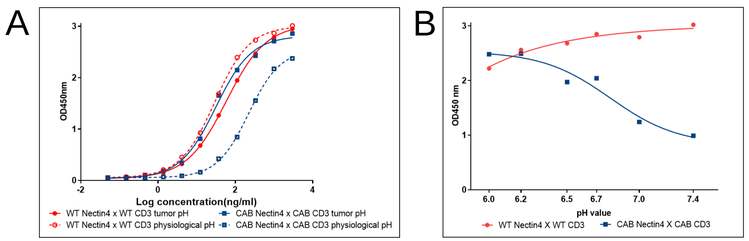

Figure 3. Differential affinity binding (A), pH range ELISA (B) and *in vivo* efficacy (C).

A. pH affinity ELISA applied human CD3 as capture antigen, human Nectin4-mFc as detection followed by anti-mouse IgG HRP conjugated antibody. CAB Nectin4 x CAB CD3 showed higher affinity in tumor microenvironment pH, but lower binding under the physiological pH.

B. CAB Nectin4 x CAB CD3 demonstrated a differential binding with human CD3 as capture antigen, human Nectin4-mFc as detection following with anti-mouse IgG HRP conjugated antibody with the pH range 6.0-7.4. The affinity binding of WT Nectin4 x WT CD3 remained at a similar level.

C. The *in vivo* efficacy study showed that CAB Nectin4 x CAB CD3 dosed at 2.5mg/kg BIW x4 demonstrated a comparable to superior tumor regression in NCI-H358 MiXeno model relative to WT Nectin4 x WT CD3.

CONCLUSIONS

- CAB B7H3 x CAB CD3 and CAB Nectin4 x CAB CD3 bispecific antibodies have increased **binding** under **tumor conditions** compared to normal conditions. The pH profile ELISA confirmed the differential affinity with the pH ranges from 6.0 to 7.4, which should translate to reduced on-target, off-tumor toxicity in non-human primates (studies in process).
- CAB B7H3 x CAB CD3 and CAB Nectin4 x CAB CD3 bispecific antibodies have **similar efficacy** in cancer cell line derived MiXeno models *in vivo* compared to the non-CAB benchmark antibodies.
- The BioAtla CAB platform offers the potential to **transform bispecific solid tumor therapies** through the widening of the therapeutic index.

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