A Novel Dual-CAB Nectin-4 x CD3 Bispecific Antibody Targeting Solid Tumors

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INTRODUCTION

Nectin-4 is a predictive marker for cancer diagnosis and a validated therapeutic target. It is believed to play a mechanistic role in cancer metastasis and angiogenesis of several types of primary tumors. Nectin-4 expression has a significant correlation with tumor grade and tumor progression. The next generation of anti-cancer therapies targeting Nectin-4 offer the potential to improve patient survival, while reducing harmful side effects.

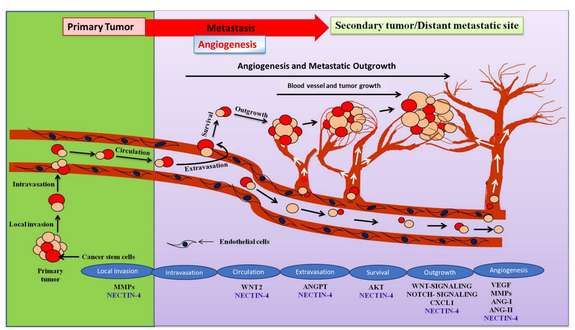

Figure 1. Nectin-4 in the tumor tissues and downstream pathways.
Schematic representation of metastasis and angiogenesis processes of Nectin-4. Sethy C. et al., (2020). J. Cancer Res. Clin. Oncol. 146(1): 245-259

RATIONALE

Many therapies targeting Nectin-4 are being developed due to its ubiquitous expression in several types of cancers. These therapies, however, display a high rate of drug related toxicities (due to binding in healthy tissue) accentuating the need for alternative approaches of anti-cancer therapeutics targeting Nectin-4.

Conditionally Active Biologic (CAB) technology[1] is a proprietary platform that allows the generation of bispecific antibodies which have little to no binding to CD3 or the target antigen in healthy tissue (normal alkaline microenvironment). However, in acid conditions that mirror the tumor microenvironment (high glycolysis), the binding of these antibodies to their target molecules is strong. By utilizing BioAtla's CAB platform, we developed a CAB-Nectin-4 x CAB-CD3 bispecific antibody to redirect T-cell activity to the tumor microenvironment.

The CAB-Nectin-4 x CAB-CD3 bispecific antibody was active against Nectin-4 positive human tumor xenografts. Importantly, complete tumor regression was observed upon treatment with CAB-Nectin-4 x CAB-CD3 bispecific antibody. A single intravenous bolus administration of CAB-Nectin-4 x CAB-CD3 was well tolerated and overall safe in non-human primate toxicity studies, with no clinical signs of toxicity.

Reversible CAB bispecific antibodies 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

Figure 2. Differential binding profile of non-CAB and dual-CAB Nectin-4 x CAB CD3 by affinity and pH range ELISA.

CAB-Nectin-4 x CAB-CD3 demonstrated a differential pH binding profile to human Nectin4 and CD3 epsilon/delta heterodimer, with higher affinity at pH 6.0 and low affinity in physiological pH 7.4.

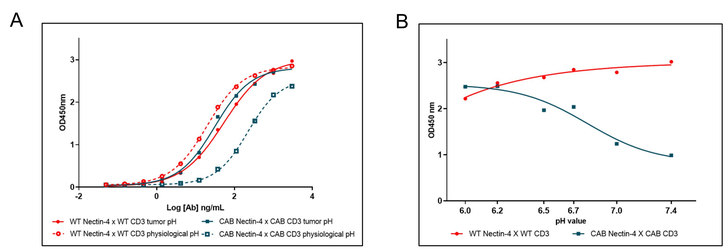

Figure 2

- Figure 2A: 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 hNectin-4-mFc and anti-mIgG-HRP conjugated antibody.
- Figure 2B: CAB-Nectin-4 x CAB-CD3 demonstrated differential binding with human CD3 as capture antigen, human Nectin-4-mFc as detection followed by anti-mouse IgG HRP conjugated antibody with the pH range 6.0-7.4. The binding affinity of WT-Nectin-4 x WT-CD3 remained at a similar level independent of pH.

Table 1. Nectin-4 x CD3 bispecific antibodies induce T cell activation *in vitro*.

CAB-Nectin-4 x CAB-CD3 is more potent at inducing elimination of Nectin-4 expressing cells at pH 6.0 and less potent at normal physiological pH 7.4.

Antibody	Cell line	EC50 (ng/mL)		
		pH6.0	pH7.4	pH7.4/pH6.0
WT Nectin4 x WT CD3	CHO hNectin-4	14.5	17.2	1.2
	CHO cynoNectin-4	9.0	7.8	0.9
	BT474	5.4	4.1	0.8
	NCI-H358	10.2	9.9	1.0
CAB Nectin4 x CAB CD3	CHO hNectin-4	3.2	**6.0**	**1.9**
	CHO cynoNectin-4	1.3	**3.4**	**2.5**
	BT474	3.4	**13.5**	**4.0**
	NCI-H358	6.8	**37.7**	**5.6**

Table 1. Nectin-4 expressing 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 incubated in the presence of Nectin-4 x CD3 bispecific antibodies at pH 6.0 and pH 7.4.

RESULTS

Figure 3. Dose-range efficacy study of the Nectin-4 x CD3 bispecific antibodies using the BT474 MiXeno model of human breast cancer.

CAB-Nectin-4 x CAB-CD3 exhibited a similar dose-dependent tumor regression as WT-Nectin-4 x WT-CD3 in breast cancer BT474 MiXeno model.

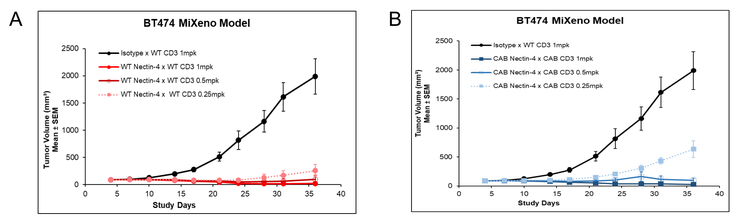

Figure 3. Triple immunodeficient mice were engrafted with human PBMCs and inoculated with BT474 cells. Tumor bearing animals were randomized to treatment groups when the tumor volume reached approximately 80-120 mm^3. Following randomization, animals were dosed with Isotype (A,B), WT-Nectin-4 x WT-CD3 (A) and CAB-Nectin-4 x CAB-CD3 (B) at 0.25, 0.5 and 1 mg/kg (mpk) biweekly for 4 weeks.

Figure 4. IL-6 cytokine levels in the serum of NHP treated with the Nectin-4 x CD3 bispecific antibodies. In non-human primates (NHP), WT-Nectin-4 x WT-CD3 and CAB-Nectin-4 x CAB-CD3 were well tolerated at a dose of 5 mg/kg. WT-Nectin4 x WT-CD3 bispecific antibody induced high levels of IL-6, while the CAB-Nectin-4 x CAB-CD3 bispecific antibody induced only a mild IL-6 cytokine response at the highest dose level.

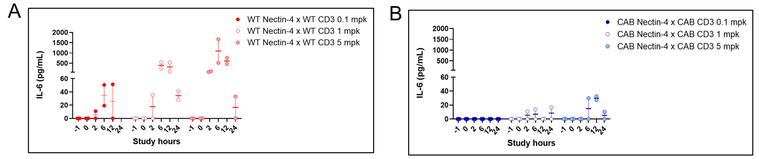

Figure 4. Cynomolgus monkeys received a single intravenous administration of (A) WT-Nectin-4 x WT-CD3 or (B) CAB-Nectin-4 x CAB-CD3 bispecific antibodies. Serum was collected at different time points for cytokine analysis.

CONCLUSIONS

- CAB-Nectin-4 x CAB-CD3 bispecific antibodies have **increased binding** under **tumor conditions** compared to normal conditions. The pH profile ELISA confirmed the differential affinity with pH ranges from 6.0 to 7.4.
- CAB-Nectin-4 x CAB-CD3 bispecific antibodies have **similar efficacy** in cancer cell line derived MiXeno models *in vivo* compared to the non-CAB benchmark antibodies.
- BioAtla's CAB platform offers the potential to **transform bispecific solid tumor therapies** through the widening of the therapeutic index.

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