CT224: A biparatopic VHH-based CDH17×CD3 T cell engager with optimized safety and efficacy for colorectal cancer

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Abstract #958

I. INTRODUCTION

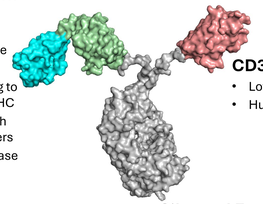
Colorectal cancer (CRC) remains a leading cause of cancer mortality with limited effective immunotherapies. Cadherin-17 (CDH17) is highly expressed in most CRCs and correlates with poor prognosis. In normal intestinal tissue, CDH17 localizes to tight junctions, but its aberrant overexpression and mislocalization in tumors provide a therapeutic opportunity. Targeting CDH17 using biparatopic VHH-based TCEs with affinity-tuned CD3 arm may enhance tumor specificity and broaden the therapeutic index in CRC.

A broad panel of humanized anti-CDH17 VHHs and anti-CD3 VHHs were generated by using our proprietary **VHHMAb®** platform, covering diverse epitopes, which enabled AI-assisted construction of biparatopic CDH17 TCEs with optimized molecular geometry. We identified the lead candidate **CT224**, a novel biparatopic CDH17 TCE that combines potent antitumor efficacy, favorable safety profile, and small molecular size for improved tumor penetration, addressing limitations of TCEs in solid tumors. Its cross-species reactivity, favorable pharmacokinetics, and strong developability profile support ongoing preclinical development and potential clinical translation as a best-in-class TCE for CRC.

II. CT224, a Biparatopic CDH17 TCE

CDH17 biparatopic arm

- High affinity binding
- Humanized VHHs
- Cross-reactive to cyno/mice
- Safety profile:
  - No nonspecific binding to 34 normal tissues by IHC
  - No cross-reactivity with other cadherin members
- Biparatopic design to increase avidity and efficacy
- Structural optimization:
  - Binder combination
  - Binder orientation
  - Linker between binders



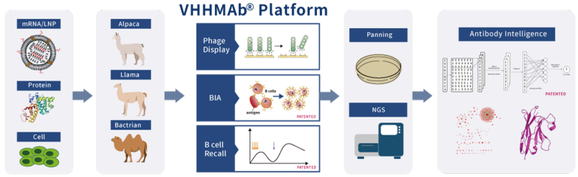
CD3 arm

- Low affinity binding
- Humanized VHH

Silenced Fc

- Optimized hinge
- No Fc-related ADCC/ADCP

III. METHODS



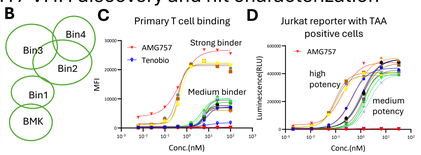
VHHMAb® Platform: Schematic representation of the VHHMAb® platform workflow.

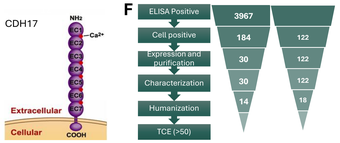
IV. RESULTS

1. Anti-CD3/anti-CDH17 VHH discovery and hit characterization

A

Name	SPR		
	ka (1/Ms)	kd (1/s)	KD (M)
CD3_#01	5.58E+04	6.30E-04	1.10E-08
CD3_#02	5.05E+04	2.33E-03	4.63E-08
CD3_#03	4.78E+04	5.13E-04	1.07E-08
CD3_#04	4.17E+04	8.53E-03	2.05E-08
CD3_#05	9.48E+04	1.01E-02	1.16E-07
CD3_#06	2.78E+04	4.90E-04	1.76E-07
CD3_#07	4.61E+04	1.02E-04	4.10E-09
CD3_#08	2.17E+04	2.19E-03	1.01E-07





G

Name	SPR		
	ka (1/Ms)	kd (1/s)	KD (M)
CDH17_#01	1.57E+05	1.04E-03	6.58E-09
CDH17_#02	1.61E+05	1.25E-03	7.74E-09
CDH17_#03	1.78E+05	5.11E-04	2.87E-09
CDH17_#04	3.92E+04	1.25E-04	3.20E-09
CDH17_#05	4.89E+04	6.87E-04	1.41E-08
CDH17_#06	3.18E+04	6.07E-05	1.91E-09
CDH17_#07	1.65E+04	2.39E-07	1.45E-11

Domains	EC1	EC2	EC3	EC4	EC5	EC6	EC7
# of binders	20	10	69	36	18	34	27

Fig 1. (A-D) Anti-CD3 VHH discovery, (E-G) Anti-CDH17 VHH discovery. (A) CD3 VHH campaign starts from ~10,000 binders. Affinity of CD3 VHHs ranges from nM to hundreds of nM. (B) Selected VHHs are categorized into 4 binding bins. (C) T cell binding of CD3 VHHs. (D) CD3 VHHs display varying T cell activation in TCE format. (E) CDH17 has 7 extracellular domains. (F) Flowchart of CDH17 VHH discovery. (G) Affinity of CDH17 VHHs ranges from nM to pM. Characterized VHHs cover all 7 ECs.

2. Mono-CT224 with desired low CD3 binding shows modest efficacy

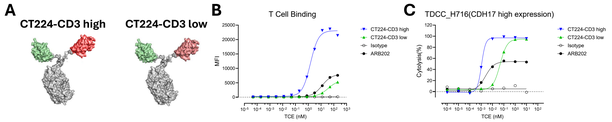


Fig 2. (A) Anti-CD3 VHHs with high or low potency were used for CT224 mono-TCEs. (B) CT224 with high potency CD3 shows high T cell binding activity. (C) Preferred CT224 with low CD3 binding has modest efficacy in CD8 T-cell dependent cellular cytotoxicity (TDCC) assays.

3. CT224 biparatopic has superior efficacy over mono-targeting TCE

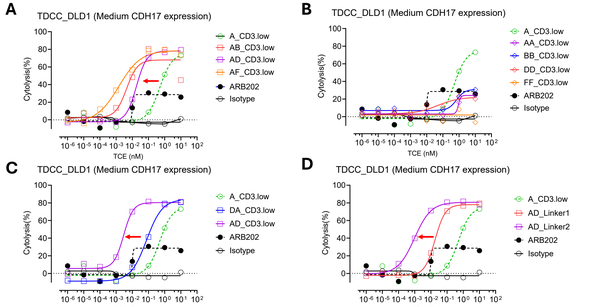


Fig 3. Biparatopic CT224 (CT224BP) structural fine-tuning. (A) Biparatopic design achieves 10-100 fold improvement in cytotoxicity (AB/AD/AF are biparatopic with 2 different VHHs). (B) Bivalent format does not enhance cytotoxicity (AA/BB/DD/FF are bivalent with the same VHH). (C) Orientation optimization improves cytotoxicity of CT224. (D) Optimization of linkers between VHHs improves CT224 cytotoxicity.

4. CT224BP candidates (various formats) for better safety/efficacy

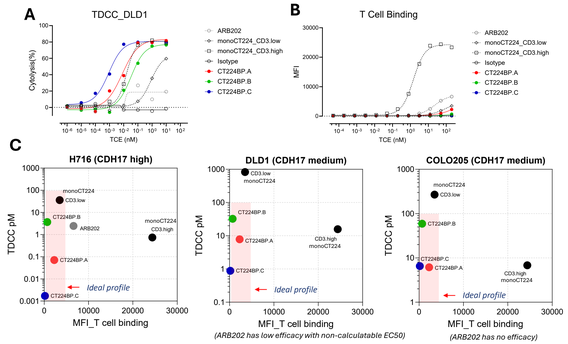


Fig 4. CT224BP TCE geometry optimization. CT224BP.A/B/C represent three specific geometries built from the same modules. (A) All three CT224BPs have decent killing potency in TDCC assay, with (B) reduced CD3 binding activity. (C) 2D plots of TDCC EC50 vs T cell binding activity show the three CT224BPs having similar, ideal profiles across three different CDH17-expressing cell lines.

5. CT224BPs do not mediate target-independent T cell activation

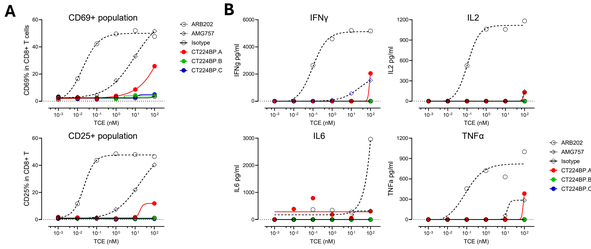


Fig 5. CT224BPs do not mediate target-independent T cell activation in PBMC co-culture assays. CT224 and benchmarks were co-cultured with PBMC for 24h. (A) T cell activation markers of CD8+ T cells, and (B) released cytokines were measured accordingly.

6. CT224BPs exhibit superior efficacy with good safety *in vivo*

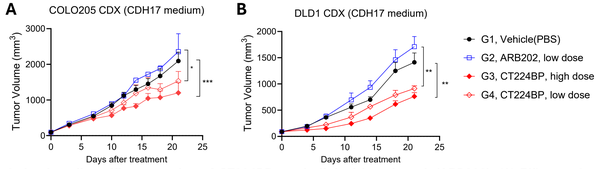


Fig 6. ***In vivo*** **pilot efficacy testing of CT224BPs and clinical benchmark (ARB202).** (A) Efficacy of CT224BPs in COLO205 CDX PBMC-humanized mice model; QWx4 doses. (B) Efficacy of CT224BPs in DLD1 CDX HSC-humanized mice model; QWx4 doses. CT224 outperforms ARB202 at same dose level. *Data presented as mean ± SEM (*P < 0.05, **P < 0.01, ***P < 0.001; Two-way ANOVA)*

7. CT224BP shows favorable PK in cynomolgus monkeys

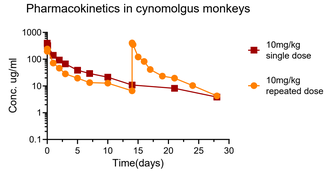


Fig 7. PK profile of CT224BP in cyno. CT224BP exhibits IgG-like PK profile with half-lives ~6-7 days. CT224BP levels were measured over time after single- or repeat-doses to determine PK profiles. Exposure metrics and systemic levels of CT224BP were evaluated, including clearance over the study period. (Note: CDH17 VHHs are cyno cross-reactive, whereas the CD3 VHH is not.)

8. CT224BPs have excellent developability properties

Molecule	Titer (mg/L)	Heterodimer/purity SEC (%)	BVP score	Tm (°C)
CT224BP. A	1751.8	100	2.6	65.7
CT224BP.B	1046.5	97.5	1.5	66.2
CT224BP.C	1137.5	94.3	2.5	66.4

Table 1. CT224BPs have good developability properties, high titer in transient expression system, high purity after one-step purification, low polyreactivity (BVP), and good thermostability (Tm).

SEC%		40°C incubation				50°C incubation	
	0w	1w	2w	3w	4w	1w	2w
CT224BP. A	100	100	100	99.6	99.2	100	98.7
CT224BP.B	99.2	99.4	99.6	99.3	99.2	100	96.9
CT224BP.C	94.1	92.8	92.8	92.1	91.8	92.2	89.5

Table 2. CT224BPs are stable in accelerated thermostability test, 40°C for 4 weeks and 50°C for 2 weeks.

V. CONCLUSIONS

- Biparatopic CT224 (CT224BP) TCE candidates exhibit optimal efficacy and safety profile
  - Superior tumor cell killing with lower CD3 T cell binding affinity compared to mono-targeting TCEs
  - Potent cytotoxicity against multiple CDH17+ tumor cell lines
  - Outperforming ARB202 in two *in vivo* efficacy models
  - Minimal background T cell activation and low cytokine release in PBMC assays
- Robust manufacturability and translational readiness
  - Cross-reactivity to human, mouse, and cyno CDH17, enabling seamless preclinical and NHP studies
  - Strong developability profile, including high expression titer, high purity, robust thermostability, and low polyreactivity

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