# A novel CD3 VHH-based multi-specific T cell engager platform and multiple leads for colorectal cancer and auto-immune diseases

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Abstract # 5465

## Summary

T cell engagers (TCEs) have recent approvals and great potential in the treatment of various diseases such as cancer and auto-immune diseases. The challenge of TCE in solid tumor is the therapeutic window due to limited tumor specific targets, CD3 arm tuning, and tumor penetration.

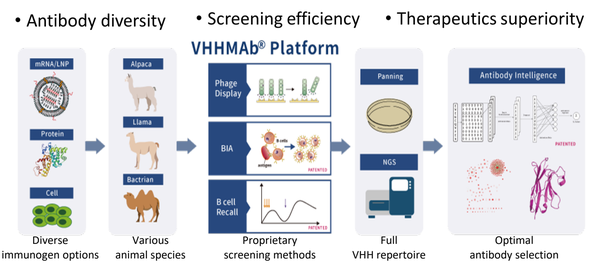
VHHs being single domain, are ideal for bi- and multi-specific molecules. We have identified anti-CD3 VHHs with **diverse portfolio** using in house llama, alpaca, and camel immunization with cells, protein and mRNA. We utilized **VHHMAb® platform to capture full repertoire** of the binders. The humanized anti-CD3 VHHs are diverse in epitope, affinity, T cells activation and TDCC. We utilized *in house* developed **AI-powered lead design to** optimize TCE structure and to improve developability attributes such as expression, solubility, aggregation, poly-reactivity and stability. Our TCEs generated **with all single-domain VHHs** (anti-CD3 and TAA targeting) **are first-in-class** for superior therapeutics.

Colorectal cancer (CRC) has huge unmet clinical need as current treatments have limited clinical efficacy. **CDH17 is differentially** and **restrictively expressed in CRC** compared with healthy tissues. The **membrane proximal domains** are desirable for TCE. With the VHHMAb® platform we have **identified sets of VHHs** that target different domains of CDH17. These VHHs are cross species (human, cyno and mouse) binders that provide seamless path of pre-clinical to clinical development. With the VHH binders of diverse epitopes on CDH17 target, and various affinity and potency on CD3, we have identified **CT224 TCE (CDH17xCD3)** candidates that meet optimal T cell binding and tumor killing profile, **BIC** with potential to be FIC CDH17xCD3, demonstrating *in vitro* cytotoxicity better than BMK and good developability properties.

Therapeutic efficacy, durability and safety are unmet medical needs in **auto-immune diseases**. For single target therapeutics, relapse due to antigen escape is one of the major problems. We have identified VHHs with NHP cross binding for **BCMA and CD19 as B cell targets** of auto-immune diseases, and generated **CT222 (BCMAxCD19xCD3) TCEs** with multi-targeting for better efficacy and durability potentially overcoming the relapse (data not shown in this poster).

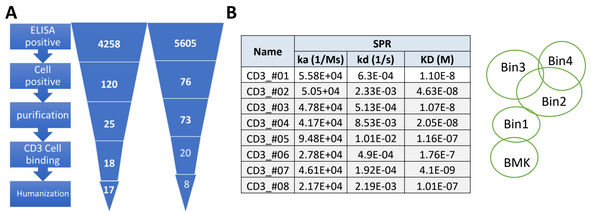
In summary, we have identified diverse humanized VHHs for CD3 and various TAAs. Leveraging modular nature of VHHs, we have generated multiple all-VHH lead TCEs with FIC/BIC potential, for CRC and auto-immune diseases.

## VHHMAb® platform




## Results

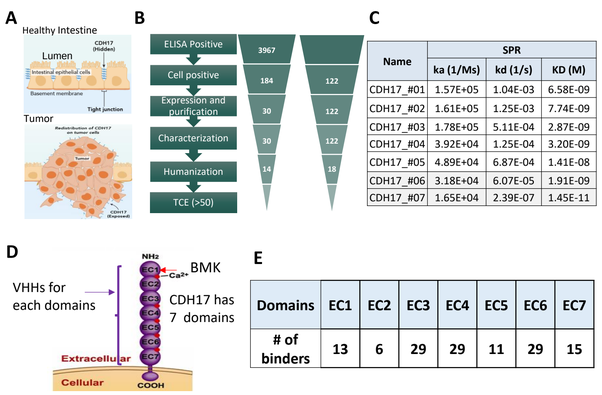
### I. Anti-CD3 VHH discovery and hit characterization




| Name | SPR | | |
|---|---|---|---|
| | ka (1/Ms) | kd (1/s) | KD (M) |
| CD3_#01 | 5.58E+04 | 6.3E-04 | 1.10E-8 |
| CD3_#02 | 5.05+04 | 2.33E-03 | 4.63E-08 |
| CD3_#03 | 4.78E+04 | 5.13E-04 | 1.07E-8 |
| 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 | 4.9E-04 | 1.76E-7 |
| CD3_#07 | 4.61E+04 | 1.92E-04 | 4.1E-09 |
| CD3_#08 | 2.17E+04 | 2.19E-03 | 1.01E-07 |

**Figure 1.** (A) VHHs discovery funnel starting from around 10,000 ELISA binding clones. (B) Affinity of anti-CD3 VHHs ranging from nM to hundreds of nM, and are categorized into different binding bins (8 hit examples shown). (C) Binding profiles of anti-CD3 VHHs to T cells. (D, E) T cell activation in a TCE format with antigen-positive (D) and antigen-negative (E) cells. Identified anti-CD3 VHHs display varying T cell activation potencies.

### II. Anti-CDH17 VHHs discovery and hit characterization




| 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 | 13 | 6 | 29 | 29 | 11 | 29 | 15 |

**Figure 2.** (A) CDH17 is overexpressed in tumors and loses its polarized distribution seen in healthy intestines. (B) VHHs discovery funnel starting from around 4000 ELISA binding clones. (C) Affinity of anti-CDH17 VHHs ranging from nM to pM. (D) CDH17 consists of 7 extracellular domains EC1-EC7. (E) Selected VHHs covering all 7 domains of CDH17 and are cross-reactive with human, cyno, and mouse CDH17.

### III. Design of CT224 TCEs

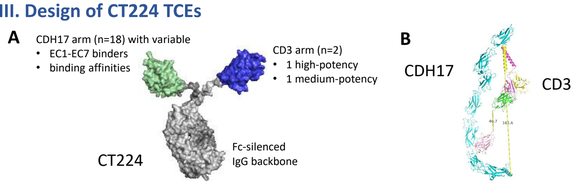



**Figure 3.** VHHs with different CDH17-domain binding and CD3 potency are used to design the CT224 TCEs. For optimal synapse and good developability, ML/AI powered designs are used.

### IV. T cell-dependent cellular cytotoxicity for TCE leads selection

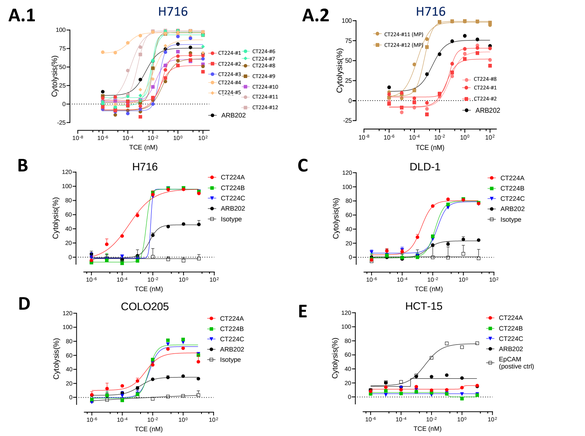



**Figure 4.** (A) Screening for potent CT224 by TDCC assay. (A.1) Range of cytolysis by different TCEs with CDH17 high (H716) cells. (A.2) TCEs targeting membrane proximal (MP) domains showed higher killing capacity than the membrane distal (MD) domains. (B-E) Leads CT224A/B/C were selected for further validation. Leads have broader killing of CDH17 positive cells H716(B), DLD-1 (C) and COLO-205 (D) and no cytotoxicity on CDH17-low/negative HCT-15 (E).

### V. CT224 Leads show superior tumor control in xenograft model.

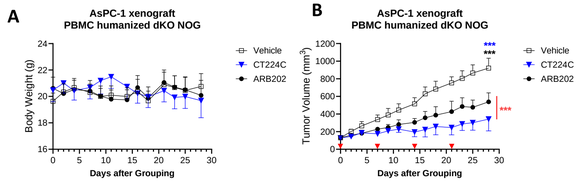



**Figure 5.** PBMC humanized MHC dKO NOG mice bearing AsPC-1 tumors were treated intraperitoneally (i.p.) once weekly (QW) with 2 mg/kg CT224C and 5 mg/kg ARB202 (equimolar). (A) Body weight and (B)Tumor volume were monitored twice a week, calculated by a two-way ANOVA test, ***p<0.001.

### VI. CT224 TCEs are human/cyno/mouse cross-reactive and specific to CDH17, have good developability

A

| Name | Human | | | Cyno | | | mouse | | |
|---|---|---|---|---|---|---|---|---|---|
| | ka (1/Ms) | kd (1/s) | KD (M) | ka (1/Ms) | kd (1/s) | KD (M) | ka (1/Ms) | kd (1/s) | KD (M) |
| CT224A | 6.75E+04 | 2.26E-05 | 3.34E-10 | 3.47E+04 | 3.63E-07 | 1.05E-11 | 5.83E+04 | 9.26E-04 | 1.59E-08 |
| CT224B | 5.04E+04 | 3.92E-04 | 7.77E-09 | 4.27E+04 | 4.42E-04 | 1.04E-08 | 3.08E+04 | 4.71E-04 | 1.53E-08 |
| CT224C | 6.44E+04 | 8.90E-04 | 1.38E-08 | 6.95E+04 | 5.65E-04 | 8.14E-09 | 3.44E+04 | 7.22E-04 | 2.1E-08 |

B

| Name | CDH17 | CDH2 | CDH4 | CDH6 | CDH13 | CDH16 | Control 1 | Control 2 |
|---|---|---|---|---|---|---|---|---|
| CT224A | 2.9721 | 0.0469 | 0.0457 | 0.0504 | 0.0426 | 0.0503 | 0.0515 | 0.0432 |
| CT224B | 2.9162 | 0.0402 | 0.041 | 0.0474 | 0.0396 | 0.0463 | 0.0478 | 0.0404 |
| CT224C | 2.7932 | 0.0411 | 0.0418 | 0.0467 | 0.0412 | 0.0484 | 0.0473 | 0.0416 |

C

| Name | Yield (mg/L) | Purity SEC (%) | BVP score | Tm (Celsius) |
|---|---|---|---|---|
| CT224A | 415 | 91 | 2.83 | 56.5 |
| CT224B | 423 | 100 | 4.44 | 61.6 |
| CT224C | 450 | 100 | 3.14 | 61.05 |

**Figure 6.** (A) Binding to human, cyno and mouse CDH17 using BLI method. (B) CT224 TCEs specifically bind CDH17 and not other CDH family members by ELISA. (C) CT224 TCEs have good developability properties, high yield in transient expression system, high purity in a one-step purification, low polyreactivity (BVP score), and good Thermostability (Tm).

### VII. The differentiation of CT224 TCEs compared to benchmark

| | Membrane proximal binding of TAA arm | Cyno mouse CDH17 binding | Monovalent CD3 arm | Tuned potency of CD3 arm | All VHHs (small size advantage) |
|---|---|---|---|---|---|
| CT224 | ✓ | ✓ | ✓ | ✓ | ✓ |
| Competitors (ARB202) | ✗ | ✗ | ✗ | ✗ | ✗ |

## Conclusions

1. Using integrated and robust VHHMAb® platform, we have discovered a panel of anti-CD3 VHHs with distinct binding kinetics and T cell activation profiles, as well as anti-TAA VHHs exhibiting diverse affinity ranges and various domain targeting capabilities.
2. CT224 TCEs are all-VHHs, superior to BMK in tumor cell killing potency depending on different CDH17 expression levels, demonstrating FIC/BCI potential.
3. CT224 TCEs are mouse and cyno CDH17 binders for seamless pre-clinical and NHP studies and have good developability properties.

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