D-Domain Binder in Anitocabtagene Autoleucel Shows Absence of Tonic Signaling and Cross-Reactivity Profile

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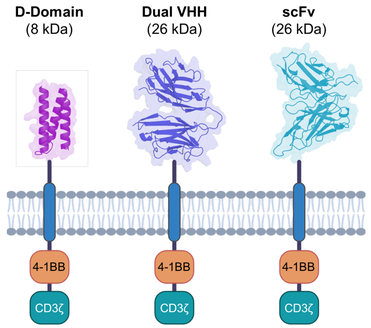
Background

BCMA-directed CAR-T cell therapies are effective in treating relapsed and/or refractory multiple myeloma, with potential for deep, durable responses. Anitocabtagene autoleucel (anito-cel) has demonstrated an encouraging efficacy and safety profile, with no cases of delayed neurotoxicities such as parkinsonism and cranial nerve palsies or immune effector cell-associated enterocolitis (IEC-EC) observed to date.[1] This profile differentiation may be attributed to the different BCMA-targeting binders, as current standard-of-care BCMA-directed CAR-T cell therapies either demonstrate limited durability of response with idecabtagene vicleucel (ide-cel), or a risk of delayed toxicities with ciltacabtagene autoleucel (cilta-cel). The anito-cel CAR construct utilizes a novel D-Domain binder that displays a fast off-rate and minimal antigen-independent aggregation (Figure 1).[2,3]

Fig 1. Comparison of BCMA-directed chimeric antigen receptors (CARs)



- Small D-Domain construct facilitates high transduction efficiency and CAR positivity
- The D-Domain CAR is stable, has a fast off-rate for BCMA binding, and high CAR expression[2]

Claudin family proteins have been implicated in maintaining structural and functional integrity of the blood-brain and gut-vascular barriers; and disrupted claudin function is linked to inflammatory, neurodegenerative, and gut disorders.[4] Claudin-9 (CLDN9) is a member of the Claudin family and is expressed at the tight junctions of endothelial and epithelial barriers across various tissues, including follicular-stellate cells in the anterior pituitary gland and cerebellum.[5] Therefore, binding of CLDN9 in addition to BCMA could increase the risk of off-target toxicities.

Objectives

This study aims to further explore the contribution of binder attributes towards specificity of BCMA-directed CAR-T cells.

Methods

CAR constructs representative of cilta-cel (dual VHH), ide-cel (scFv), and anito-cel (D-Domain) were transduced into healthy donor T cells (Fig. 1). In the absence of antigen-expressing cells, T cell phenotype and IFN-γ release were assessed across surrogate CAR-T cells with matched transduction (56-74% CAR+) and vector copy number (1.2-2 copies/cell) when expression was driven by the full-length EF1α promoter (Fig. 2), as used in cilta-cel (EPAR EMA/594558/2022).[6] Surrogate CAR-T cells under the weaker EFS (EF1a-short) promoter were used to evaluate the impact of CLDN9-induced activity while minimizing the impact of tonic signaling (Fig. 3-5). Off-target activity against claudin-9 (CLDN9)+ HEK293 cells was assessed by cytotoxicity and T cell activation based on previous reports that cilta-cel binds CLDN9 in addition to BCMA when assayed in a membrane surface protein array (EPAR).[6]

Results

Fig 2. With high surface expression, Dual VHH and scFv, but not D-Domain, CAR-T cells demonstrate tonic signaling phenotype and function in absence of antigen

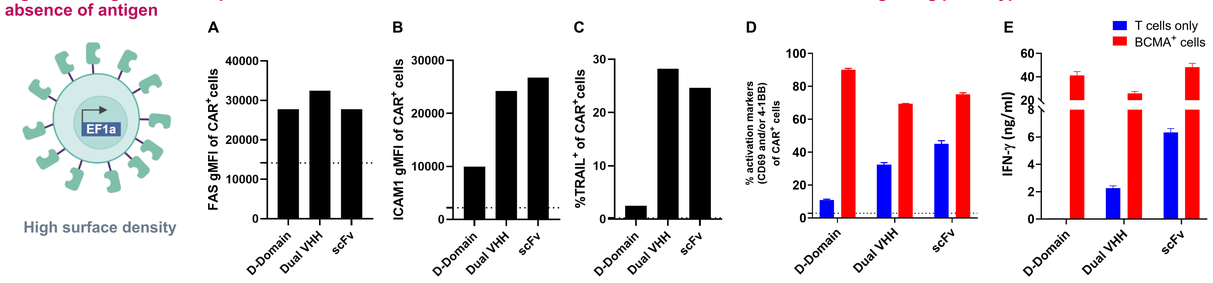


Figure 2: Dual VHH and scFv CAR-T cells show comparable expression of (A) FAS, with increased expression of (B) ICAM and (C) TRAIL compared to D-Domain CAR-T cells, in the absence of antigen-expressing cells suggesting a tonic signaling phenotype. (D) Dual VHH and scFv CAR-T cells show elevated expression of activation markers (CD69+, 4-1BB+, and CD69+4-1BB+) and IFN-γ release (E), compared to D-Domain CAR-T cells in the absence of antigen-expressing cells (blue), demonstrating tonic signaling activity. H929 (BCMA+) cells were used as a positive control. All CARs expressed under EF1a promoter. Dotted line represents transduced non-targeting CAR control.

Fig 3. Dual VHH, but not scFv or D-Domain, CAR-T cells show upregulation of activation markers and increased IFN-γ release after overnight culture with claudin-9 over-expressing cells

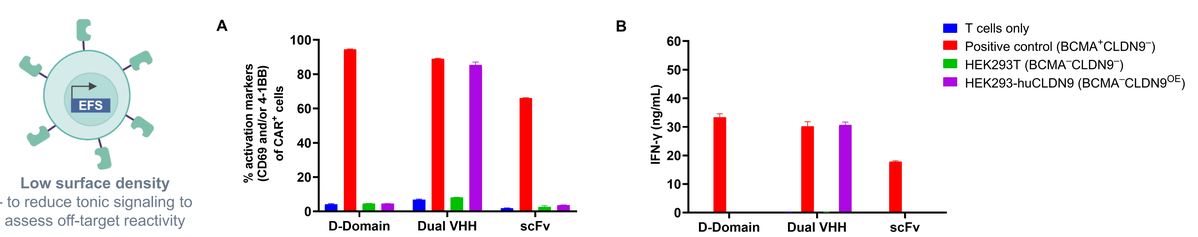


Figure 3: Dual VHH CAR-T cells stimulated with CLDN9 over-expressing (OE) HEK293 cells demonstrated up-regulation of (A) activation markers (CD69+, 4-1BB+, and CD69+4-1BB+) and (B) IFN-γ release. CLDN9-induced activity was not observed with D-Domain or scFv CAR-T cells. H929 (BCMA+CLDN9−) cells were used as a positive control. All CARs expressed under EFS promoter.

Fig 4. Dual VHH, but not scFv or D-Domain CAR-T cells, induce cytotoxicity of claudin-9 over-expressing cells

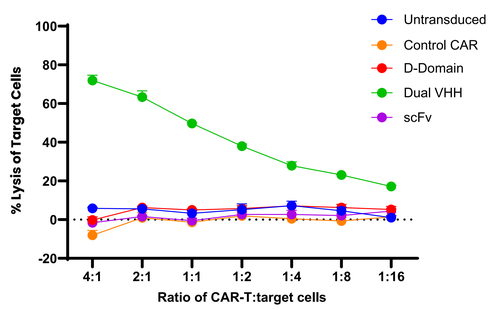


Figure 4: Dual VHH CAR-T cells co-cultured with CLDN9 over-expressing HEK293 cells induced cytotoxicity of target cells that was not observed with D-Domain or scFv CAR-T cells. All CARs expressed under EFS promoter.

Fig 5. Neither Dual VHH nor D-Domain CAR-T cells upregulate activation markers after incubation with CLDN6 or CLDN4 over-expressing cells

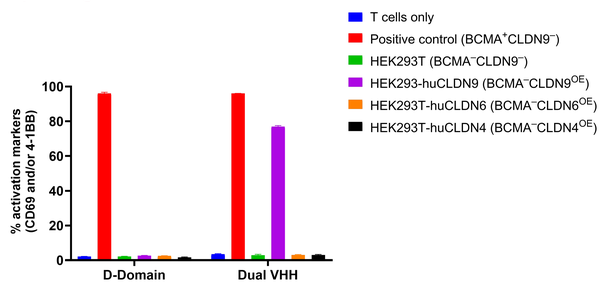


Figure 5: (A) Sequence alignment of extracellular loop 1 from CLDN9 and similar CLDN family members. (B) Only dual VHH CAR-T cells stimulated with CLDN9, but not CLDN6 or CLDN4 over-expressing (OE) HEK293T cells, demonstrated up-regulation of activation markers (CD69+, 4-1BB+, and CD69+4-1BB+). H929 (BCMA+CLDN9−) cells were used as a positive control. All CARs expressed under EFS promoter.

Conclusions

- Tonic signaling phenotype and function were observed with dual VHH and scFv, but not D-Domain CAR-T cells, as previously reported in absence of antigen.[7]
- Off-target activity with dual VHH, but not D-Domain or scFv CAR-T cells, was seen with CLDN9. CAR-T cell products were also tested under the EF1a promoter with activation against CLDN9 demonstrated only with dual VHH CAR-T cells.
- Despite the high similarity in the extracellular loops among members of the CLDN family, the activation of dual VHH CAR-T cells was noted only in response to cells overexpressing CLDN9, and not in those overexpressing CLDN6 or CLDN4.
- Other features of the D-Domain previously reported[2,3] such as fast off-rate and lack of self-aggregation, as well as reduced cytokine profile, may contribute to lack of tonic signaling and enhanced target specificity differentiating it from the dual VHH and scFv binders.
- As CLDN9 is expressed in tight junctions across various tissues, including follicular-stellate cells in the anterior pituitary gland and cerebellum, there is a possibility that the dual VHH binder could lead to off-tumor binding and toxicities.[5]

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Figures 1,2,3 Created with Biorender.com

REFERENCES

1. Patel K et al. Phase 2 registrational study of anitocabtagene autoleucel for the treatment of patients with relapsed and/or refractory multiple myeloma: Updated results from iMMagine-1. *Blood 2025; 146 (Supplement 1):256*
2. Hart K et al. The fast off-rate of anito-cel's D-Domain binder contributes to its distinctive pharmacology profile in preclinical models of multiple myeloma. *Blood 2025; 146 (Supplement 1):7644*
3. Buonato JM et al. Preclinical Efficacy of BCMA-Directed CAR T Cells Incorporating a Novel D Domain Antigen Recognition Domain. *Mol Cancer Ther. 2022 Jul 5;21(7):1171-1183.*
4. Scalise, AA et al. The blood-brain and gut-vascular barriers: from the perspective of claudins. *Tissue* Barriers 2021; 21;9(3):1926190
5. Higashi A et al. Claudin-9 constitutes tight junctions of folliculo-stellate cells in the anterior pituitary gland. *Scientific Reports 2021; 11: 21624*
6. European Medicines Agency: EMA/594558/2022 – Assessment Report (https://www.ema.europa.eu/en/documents/assessment-report/carvykti-epar-public-assessment-report_en.pdf; Accessed December 2025).
7. Atanackovic D. et al. Immune correlates of anti-BCMA CAR-T products idecabtagene vicleucel and ciltacabtagene autoleucel in a real-world cohort of patients with multiple myeloma. *Nature Communications 2025; 16:6154*

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Authors had relevant financial disclosures, please refer to abstract for the full list of financial disclosures for study authors

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