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702. CAR-T CELL THERAPIES: BASIC AND TRANSLATIONAL

The fast off-rate of anito-cel's D-Domain binder contributes to its distinctive pharmacology profile in preclinical models of multiple myeloma

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Abstract **Introduction:** B cell maturation antigen (BCMA) directed CAR T-cell therapies have transformed the multiple myeloma (MM) treatment landscape with idecabtagene vicleucel and ciltacabtagene autoleucel approvals in relapsed/refractory MM (RRMM). Despite these successes, late-onset toxicities associated with these treatments emphasize the continued need for improved safety and efficacy in patients with RRMM. Anitocabtagene autoleucel (anito-cel) is an autologous CAR T-cell therapy utilizing a novel synthetic binder, the D-Domain, to engage BCMA on myeloma cells and triggers T-cell mediated tumor killing through 4-1BB and CD3ζ intracellular signaling. The simplicity and structural design of the D-Domain facilitates unique functional attributes including high transduction efficiency and high CAR density with limited tonic signaling that may impact the manufacturability and clinical profile of anito-cel. Clinically, anito-cel has demonstrated deep and durable efficacy in 4L+ RRMM, with a predictable and manageable safety profile (Kaur et al., EHA 2025). Specifically, no delayed or non-immune effector cell-associated neurotoxicity syndrome (ICANS) neurotoxicities or immune effector cell-associated enterocolitis have been observed to date. We hypothesize that the BCMA-D-Domain binding dynamics confers an improved safety profile compared to conventional antibody-derived binding domains. We evaluated and compared the preclinical attributes of the D-Domain binder (ddBCMA) to a dual camelid-derived variable heavy domain of heavy chain only (VHH) antibody.

Methods: We compared the biophysical binding properties of the ddBCMA binder to a dual VHH BCMA binder resembling ciltacabtagene autoleucel by biolayer interferometry (BLI) and size-exclusion chromatography (SEC). ddBCMA or dual VHH CAR T-cells were co-cultured with BCMA$^+$ tumor cell lines or MM patient bone marrow mononuclear cells (BMMCs) for assessments of cytotoxic activity and cytokine release. Imaging flow cytometry was performed to quantify immune synapse architecture via F-actin staining. Cytotoxicity of anito-cel was also evaluated against cells expressing identified BCMA mutations observed in patients treated with BCMA-directed T cell engagers (TCE) (Lee et al, 2024).

Results: SEC revealed that ddBCMA binds BCMA at 1:1 stoichiometry while the dual VHH demonstrates multivalent binding to BCMA likely due to its two unique binders. BLI studies revealed that the on-rate was comparable between the 2 binders (ddBCMA $k_{on} = 1.2 \times 10^6$ Ms^{-1}; dual VHH $k_{on} = 1.1 \times 10^6$ $Ms\text{-}^1$). However, there was a ~6-fold difference in the off-rate of ddBCMA ($k_{off} = 0.028s^{-1}$) compared with dual VHH ($k_{off} = 0.0044s^{-1}$). This difference in the off-rate between the 2 binders was further amplified when avidity was introduced in the assay. Across multiple donors, image cytometry showed 2-fold higher F-actin recruitment to the immune synapse with the dual VHH CAR T compared to ddBCMA CAR T. In parallel, the dual VHH CART consistently released higher levels of cytokines including GM-CSF (2-11 fold), IL-2 (4-8 fold) and IFNγ (2-18 fold) suggestive of increased toxicity potential when compared to the ddBCMA CART after 24hr co-culture with MM cell lines (H929, MM1.S and OPM2) and MM patient BMMC samples. In the same assays, cytotoxicity (ddBCMA 70-90%; dual VHH 63-86%) was comparable between the BCMA CAR Ts. In examining BCMA variants implicated in TCE treatment resistance, anito-cel further sustained cytotoxic function against K562 cells expressing either wildtype BCMA (74-87%) or BCMA mutations (A54T 67-84%, ΔP34 73-87%, ΔS30 66-76%, P33S 72-89%, R27P 84-91%).

Conclusions: We determined that anito-cel's D-Domain binder has a faster off-rate, bound to BCMA$^+$ target cells with a distinct immune synapse architecture and demonstrated decreased cytokine production while maintaining similar cytotoxicity relative to a dual VHH BCMA CAR T. Anito-cel also maintains its ability to target BCMA variants acquired post BCMA TCEs. Combined, these structural and functional data highlight anito-cel's unique ability to transiently engage BCMA, potentially

conferring tumor killing without prolonged inflammation. Emerging crystallography and epitope mapping studies will further substantiate this model. Collectively, these data reveal an additional differentiating attribute for anito-cel's D-Domain binder and provide a mechanistic rationale for the efficacy and safety profile observed with anito-cel in the clinic to date.

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