A GPRC5D and BCMA Bispecific CAR-T Product Demonstrated Dual Antigen Targeting Capability and Efficacy Against Multiple Myeloma Cells Bearing BCMA Mutations Associated with Treatment Resistance

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Abstract

Background

Although B-cell Maturation Antigen (BCMA)-targeting immunotherapies greatly improve survival in multiple myeloma (MM) patients, antigen escape remains a significant challenge, preventing long-term tumor control [1,2]. Patients who are resistant or relapsed to anti-BCMA treatment often exhibit mutations, biallelic loss, and downregulation of BCMA gene expression [3]. We previously reported the development of a G Protein-Coupled Receptor Class C Group 5 Member D (GPRC5D)/BCMA bispecific CAR that demonstrated robust efficacy against MM tumor lines both *in vitro* and *in vivo* [4]. Here, we present a dual-targeting CAR that successfully addresses antigen escape challenges, providing an alternative treatment option for refractory/relapsed (R/R) MM.

Methods

The GPRC5D/BCMA bispecific CAR was developed with novel binders with high binding affinity and specificity. These CARs were optimized for binder combination, binder order, and CAR backbone structure. To assess binder affinity and CAR-T killing efficacy, flow cytometry and *in vitro* long-term cytotoxicity assays were utilized against tumor cells expressing wild-type and mutant BCMA genes.

Results

The GPRC5D/BCMA bispecific CAR was designed to address antigen escape resulting from loss or downregulation of antigens, or reduced binding of BCMA-targeting therapy to the mutated BCMA. To confirm the dual antigen cytotoxicity of the CAR, we expressed GPRC5D, BCMA, or both in HEK293 cells for in vitro killing assay. Notably, no significant cytotoxicity was observed in parental HEK293 cells, which lack expression of either antigen. Importantly, the GPRC5D or BCMA mono-targeting control CARs selectively controlled HEK293-GPRC5D and HEK293-BCMA cells, respectively. In comparison, the dual-targeting CAR demonstrated remarkable efficacy against HEK293 cells expressing either single or dual antigens. Recently, alterations in BCMA (R27P, P33S, S30del, and P34del) have been reported in MM patients relapsed after BCMA-targeting T cell engagers (TCEs) treatment. The BCMA binder in the bispecific CAR exhibited nanomolar-level EC50s, substantially lower than TCE controls, for all four mutant proteins. Additionally, in vitro cytotoxicity assay demonstrated that the bispecific CAR effectively suppressed the growth of cells expressing BCMA mutant proteins.

Conclusions

In our current investigation, we elucidated the dual-targeting capacity of the previously reported GPRC5D/BCMA bispecific CAR. Moreover, the BCMA binder within the bispecific CAR exhibits a high binding affinity to BCMA mutants associated with resistance to BCMA TCEs. Notably, the bispecific CAR also demonstrated robust cytotoxicity against cells expressing these BCMA mutants. These compelling findings strongly suggest that the GPRC5D/BCMA bispecific CAR holds promise in addressing antigen escape, meeting the unmet medical needs of R/R MM patients, and potentially improving survival rate.

Reference:

1. Paula Rodriguez-Otero, et al. Ide-cel or Standard Regimens in Relapsed and Refractory Multiple Myeloma. N Eng J Med. 2023;388:1002-1014.
2. Jesús San-Miguel, et al. Cilta-cel or Standard Care in Lenalidomide-Refractory Multiple Myeloma. N Engl J Med. 2023;389(4):335-347
3. Holly Lee, et al. Mechanisms of antigen escape from BCMA- or GPRC5D-targeted immunotherapies in multiple myeloma. Nature Medicine. 2023;29:2295–2306.
4. Chia-Wei Chang, et al. G Protein-Coupled Receptor Class C Group 5 Member D (Gprc5d) and B-Cell Maturation Antigen (BCMA) Bi-Specific Dual Chimeric Antigen Receptors (CARS) Effectively Address Antigen Escape and Tumor Heterogeneity Challenge in Multiple Myeloma (MM). ASGCT 27th Annual Meeting. Molecular Therapy 2024;32:4S1:205-206

GPRC5D and BCMA Binders Demonstrated High Binding Affinity with BCMA or GPRC5D Expressing MM Tumor Cell Lines

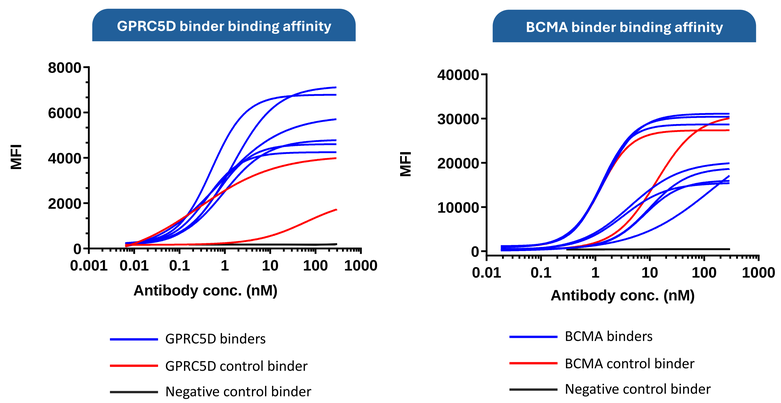

Figure 1: GPRC5D and BCMA binders of bispecific CAR show strong binding affinity against antigen expressing MM tumor cells lines MM1.S (for GPRC5D binder) or NCI-H929 (for BCMA binders) in flow-based binding assay.

BCMA Binders Shows Strong Binding Activity Against Wild Type and Mutant BCMA Proteins Identified in BCMA TCE*-Relapse MM Patients

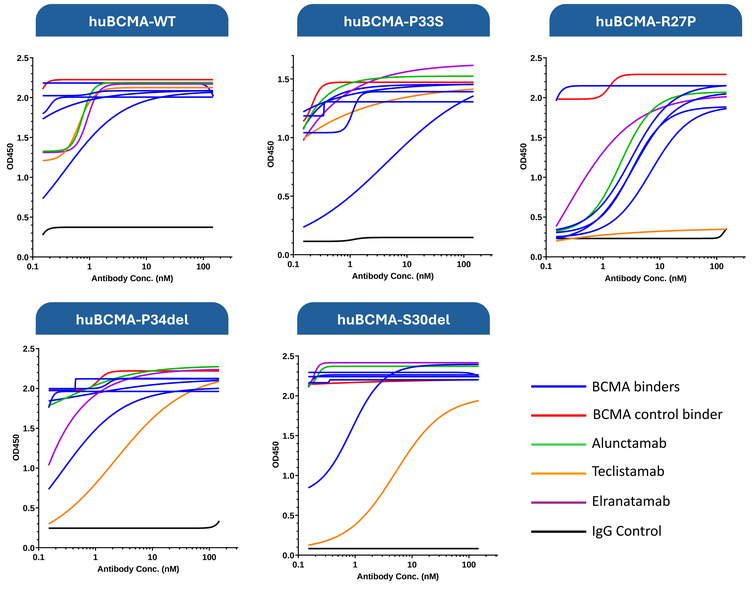

Figure 2: HEK293 cells were transduced with lentivirus carried wild type (WT) or mutant (P33S, R27P, P34del, S30del) BCMA proteins. Transgenes expressed HEK293 cells were selected by G418 treatment. To measure the antibody and antigen binding affinity, flow cytometry based interacting assay were performed with antibodies concentration range between 0.005nM to 100nM. Medium Fluorescence Intensity from each data points were plots in the figures.

*T cell engager or bi-specific antibody

Dual Targeting Capability of GPRC5D and BCMA Bispecific CAR-T Cells

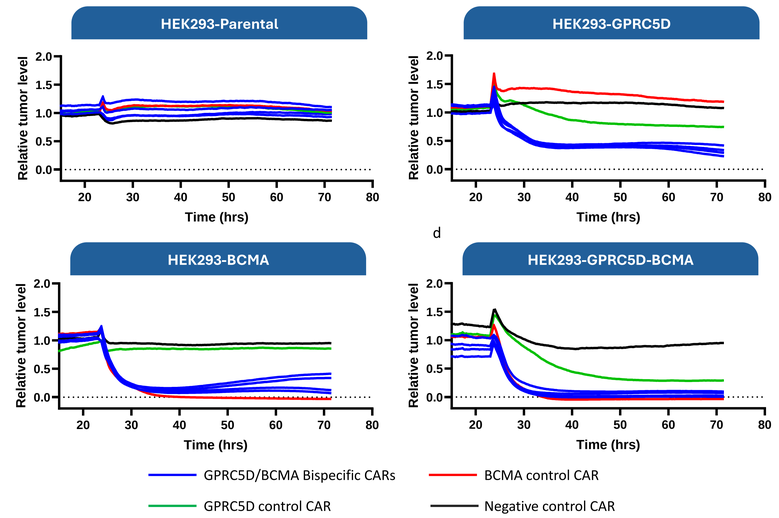

Figure 3: HEK293 parental or HEK293 expressing GPRC5D, BCMA or GPRC5D and BCMA were tested for *in vitro* impedance based killing assay upon co-cultured with T cells carrying GPRC5D/BCMA bispecific CAR, GPRC5D control CAR, BCMA control CAR or Negative control CAR. Bispecific GPRC5D/BCMA CAR effectively suppress target cells proliferation in all single-antigen or dual-antigen expressing cells.

Bispecific CAR Suppress Proliferation of HEK293 Cells Expressing Mutant BCMA Proteins identified in BCMA TCE-replapsed MM patients

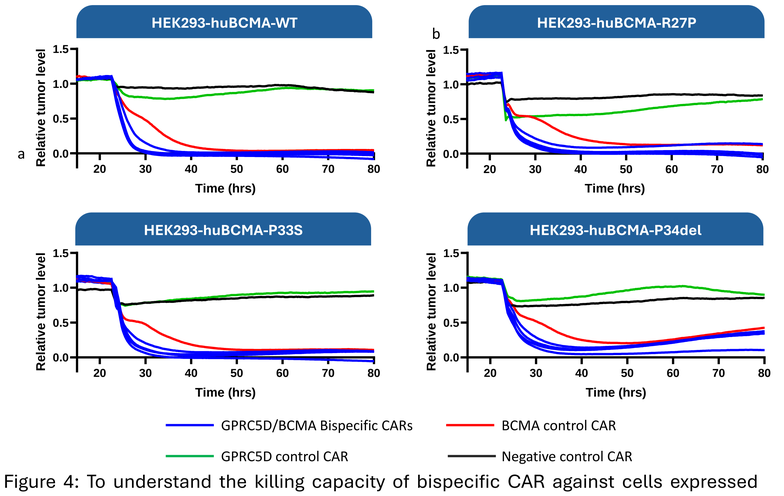

Figure 4: To understand the killing capacity of bispecific CAR against cells expressed mutant BCMA proteins associated with resistance to BCMA TCEs, HEK293 cells transduced with wild type (WT) or mutant BCMA genes (R27P, P22S, P34del) were co-cultured with indicated CAR-T cells in 1:1 ratio. Relative tumor level were measured with impediance based platform and normalized to tumor only group. All bispecific CAR-Ts demonstrated robust killing of HE293 cells expressing WT or mutant BCMA antigens.