ELSEVIER

Blood 142 (2023) 2089–2090

The 65th ASH Annual Meeting Abstracts

POSTER ABSTRACTS

703.CELLULAR IMMUNOTHERAPIES: BASIC AND TRANSLATIONAL

DAP10 Co-Stimulation Imparts Memory-like Features to CD5 Targeting Cord Blood Derived CAR-NK Cells

Rafet Basar, MD[1], *May Daher, MD*[2], *Nadima Uprety*[1], *Emily Ensley*[1], *Ana K. Nunez Cortes, MD*[1], *Sunil Acharya*[3], *Rejeena Shrestha*[4], *Bin Liu, PhD*[1], *Mayra Shanley, PhD*[1], *Ye Li, MDPhD*[1,5], *Francia Silva Reyes*[6], *Merve Dede*[7], *Hind Rafei, MD*[1,8], *Hila Shaim, MD*[7], *Mecit Kaplan*[1], *Paul Lin, MD PhD*[1], *Pinaki Banerjee, PhD*[1], *Vakul Mohanty, PhD*[9], *Jinhuang Dou*[10], *Mayela Carolina Mendt, PhD*[1], *Qi Miao*[9], *Mariam Ammari*[4], *Enli Liu, MD*[1], *Xingliang Guo*[7], *Bijender Kumar*[6], *Alexander Biederstädt, MD*[1], *Xin Ru Jiang*[1], *Sonny Ang*[1], *Navin Varadarajan, PhD*[11], *Richard E. Champlin, MD*[1,12], *Ken Chen, PhD*[9], *David Marin, MD*[1], *Elizabeth J. Shpall, MD*[1], *Katayoun Rezvani, MDPhD*[12]

[1] Department of Stem Cell Transplantation and Cellular Therapy, The University of Texas MD Anderson Cancer Center, Houston, TX
[2] Stem Cell Transplantation and Cellular Therapy, MD Anderson Cancer Center, Houston, TX
[3] UT MD Anderson Cancer Center, Houston
[4] UT MD Anderson Cancer Center, Houston, TX
[5] MD Anderson, Houston, TX
[6] The University of Texas MD Anderson Cancer Center, Houston
[7] The University of Texas MD Anderson Cancer Center, Houston, TX
[8] Department of Stem Cell Transplantation and Cellular Therapy, The University of Texas MD Anderson Cancer Center, Missouri City, TX
[9] Department of Bioinformatics and Computational Biology, The University of Texas MD Anderson Cancer Center, Houston, TX
[10] UT MD ANderson Cancer Center, Houston, TX
[11] Department of Chemical and Biomolecular Engineering, University of Houston, Houston, TX
[12] MD Anderson Cancer Center, Houston, TX

Relapsed/refractory T-cell malignancies have a particularly poor prognosis and novel therapies are direly needed. CD5 is a great candidate for adoptive cellular therapy to target T-cell malignancies since it is ubiquitously expressed on T cells with restricted expression on other hematopoietic cells. NK cells are an attractive platform for CAR engineering to target CD5 since, unlike T cells, they do not express CD5 on their surface, which eliminates the risk of fratricide. Another advantage of NK cells for CAR engineering is their safety profile; in contrast to T cells, they do not cause cytokine release syndrome (CRS) or immune effector cell-associated neurotoxicity syndrome (ICANS) and they are not associated with graft-versus-host disease (GVHD) in the allogeneic setting, opening the potential for a completely off-the-shelf cellular product to be used at point of care. Therefore, we sought to develop CD5 targeting CAR-NK cells for the treatment of T-cell malignancies.
Multiple studies have demonstrated that the choice of co-stimulatory domain in CAR-T cells influences their function, persistence and metabolic profile. In the field of CAR-NK cells, the first constructs tested in the clinic have incorporated CD28, a T cell specific co-stimulatory domain, borrowing from the design of CAR-T cells. There is a sparsity of data regarding the impact of co-stimulatory domains on the proliferation, transcriptomic and proteomic profile, polyfunctionality, metabolism and fitness of CAR-NK cells. In this study, we aimed to do so, by comprehensively studying the impact of co-stimulatory domains including some that are more relevant for NK cell biology, namely DNAX-activating Protein 10 (DAP10), a major adaptor protein and the exclusive signaling intermediate of NKG2D in human NK cells, DNAX-activating Protein 12 (DAP12), an important adaptor molecule that associates with multiple activating receptors (e.g. NKG2C, NKp44, activating KIRs) and NKG2D, one of the most potent NK cytotoxicity receptors which is essential for anti-tumor immunity.
Our results show that CD5 CAR-NK cells with DAP10 co-stimulatory domain show enhanced cytotoxicity against CD5+ T-cell leukemia targets even after multiple tumor rechallenges in an Incucyte live-cell imaging assay. They also show augmented polyfunctionality compared to CD5 CAR-NK cells with other co-stimulatory domains in an Isoplexis single-cell secretome assay. Moreover, DAP10 co-stimulation endowed CD5 CAR-NK cells with enhanced metabolic fitness as evidenced by increased oxidative phosphorylation compared to other co-stimulatory molecules. At the epigenetic level, CD5 CAR-NK cells

with DAP10 co-stimulation interrogated by sc-ATACseq show enrichment in AP-1 complex and BATF transcription factors related to memory formation and exhaustion resistance. This translates to better in vivo performance as CD5 CAR-NK cells with DAP10 co-stimulatory domain significantly improve tumor control and survival in an NSG mouse model of CD5+ T-cell leukemia (CCRF-CEM) and show evidence of recall response following tumor rechallenge.
In conclusion, our data show that DAP10 co-stimulation induces epigenetic reprogramming of CD5 CAR-NK cells leading to enhanced cellular fitness and memory formation ensuing better anti-tumor potential. Based on these preclinical data, a Phase I/II clinical trial evaluating the safety and efficacy of CD5 CAR-NK cells for the treatment of CD5+ malignancies is in preparation.

Disclosures Basar: *Affimed:* Other: R. B. and The University of Texas MD Anderson Cancer Center have an institutional financial conflict of interest with Affimed GmbH. ; *Takeda:* Other: R. B. and The University of Texas MD Anderson Cancer Center have an institutional financial conflict of interest with Takeda Pharmaceutical. . **Daher, MD:** *Takeda:* Patents & Royalties. **Uprety:** *Takeda:* Other: N.U. and The University of Texas MD Anderson Cancer Center have an institutional financial conflict of interest with Takeda Pharmaceutical. . **Acharya:** *Takeda:* Patents & Royalties. **Li:** *Takeda:* Patents & Royalties. **Rafei:** *Takeda:* Other: H. R. and The University of Texas MD Anderson Cancer Center have an institutional financial conflict of interest with Takeda Pharmaceutical. . **Lin:** *Takeda:* Patents & Royalties, Research Funding. **Banerjee:** *Takeda:* Patents & Royalties, Research Funding. **Liu:** *Takeda:* Patents & Royalties. **Ang:** *Takeda:* Patents & Royalties. **Champlin:** *Takeda Corporation:* Patents & Royalties; *Kadmon:* Consultancy; *Orca Bio:* Consultancy; *Johnson & Johnson/Janssen:* Consultancy; *Arog:* Consultancy; *Cell Source:* Research Funding; *Actinium Pharmaceuticals:* Consultancy; *Omeros:* Consultancy. **Marin:** *Affimed:* Patents & Royalties; *Takeda:* Patents & Royalties. **Shpall:** *Takeda:* Other: License agreement; *Axio:* Membership on an entity's Board of Directors or advisory committees; *Fibrobiologics:* Membership on an entity's Board of Directors or advisory committees; *Affimed:* Other: License agreement; *Celaid Therapeutics:* Membership on an entity's Board of Directors or advisory committees; *NY Blood Center:* Membership on an entity's Board of Directors or advisory committees; *Adaptimmune:* Membership on an entity's Board of Directors or advisory committees; *Navan:* Membership on an entity's Board of Directors or advisory committees; *Syena:* Other: License agreement. **Rezvani:** *Takeda:* Patents & Royalties.

https://doi.org/10.1182/blood-2023-187665