ELSEVIER

Blood 142 (2023) 6820–6821

The 65th ASH Annual Meeting Abstracts

ONLINE PUBLICATION ONLY

703.CELLULAR IMMUNOTHERAPIES: BASIC AND TRANSLATIONAL

Exploring BiTE-Integrated CAR T-Cell Therapy to Overcome Tumor Antigen Escape and Reinforce CAR-T Therapy in Mantle Cell Lymphoma

Grace Glass Crutchfield[1], *Yang Liu, PhD*[1], *Fangfang Yan, PhD*[1], *Xiaolin Li, PhD*[1], *Heng-Huan Lee, PhD*[1], *Michael Wang, MD*[1], *Yixin Yao, PhD*[2]

[1] Department of Lymphoma and Myeloma, The University of Texas MD Anderson Cancer Center, Houston, TX
[2] Department of Lymphoma and Myeloma, MD Anderson Cancer Center, Houston, TX

Background
T cell engagers (BiTEs) are bispecific T-cell engaging antibodies that redirect T cells to target antigen-expressing tumor cells. Combining a second antigen-targeted bispecific T cell engager (BiTE) with CAR T cells or incorporating bispecific engaging antibody-secreting T cells (engager-T cells) is expected to augment T-cell activation and proliferative capacity, and antitumor cytotoxicity, even towards tumor cells expressing tumor antigens heterogeneously. Here, we report a BiTE-integrated T-cell therapeutic strategy to overcome tumor antigen escape and reinforce the efficacy of CAR-T therapy in mantle cell lymphoma (MCL).

Methods
CD20-directed bispecific T-cell engager (CD20-BiTE) was constructed with a publicly available VHH fragment from an anti-CD20 single-domain antibody, and anti-CD3 scFv from OKT3 monoclonal antibody. CD19-directed CAR construct is comprised of anti-CD19 scFv from publicly available FMC63 monoclonal antibody, CD8 transmembrane-, 4-1BB costimulatory-, and CD3ζ signaling-domains. Human primary pan-T cells were isolated from PBMC derived from normal human donors (MD Anderson Blood Bank) or patients with MCL. Activated T cells were used for effector functional assay or transduced with lentivirus to produce CAR T or engager-T cells. Effector function of bispecific antibodies and T cells was determined via T-cell activation, cytokine release, and T-cell-dependent cytotoxicity assay in a co-culture model.

Results
Treatment with CD20-BiTE enhances the effector function of naïve T cells: the BiTE-Fc fusion protein was ectopically expressed in Expi293 and purified by immobilized Protein-A. The binding specificity of scFv(s) of CD20-BiTE was confirmed to bind strongly and specifically to CD20-expressing cells. *In vitro* functional analyses demonstrated that co-culture of naïve T cells with CD20-exressing cells in the presence of CD20-BiTE induced antigen-specific activation of the T cells (increase in the expression of CD25 and CD69). In the presence of naïve T cells and antigen-positive MCL cells, CD20-BiTE enhanced the secretion of cytokines (IFNγ and TNFα, > 2-fold increase) and CD8 T-cell cytotoxic molecule granzyme B by T cells, in a BiTE-dependent and CD20-specific manner. The enhanced effector function translated into improved antitumor cytotoxicity, as activated T cells treated with CD20-BiTE exerted 43- 72% increase in the killing efficacy of JeKo-1 BTK-KD cells.
CD20-BiTE-combined CAR T cells endowed with enhanced antitumor activity overcome tumor antigen escape and resistance to current CD19-CAR T cell therapy: when primary T cells and CD19-CAR T cells were co-cultured with MCL cells, addition of CD20-BiTE potentiated T cell-dependent cytotoxicity ($p < 0.001$). Since both CD19-dependent and -independent CAR T cell-resistance often develop during adoptive cell therapy in MCL, we sought to overcome the resistance in MCL by combining a second antigen-targeted CD20-BiTE with CAR T cells or incorporating CD20-BiTE secreting T cells (engager-T cells). Remarkably, when combined with CD19-CAR T cells, CD20-BiTE mediates significant increase in the antitumor cytotoxicity, compared to T cells alone ($p \leq 0{,}001$ and $p < 0.001$ for 2nM and 5nM of the BiTE, respectively), in a CD20-specific fashion, in both MCL cells with diminished CD19 expression, and CD19-CAR T-resistant primary MCL cells. Importantly, in a transwell assay, CD19-CAR T cells demonstrated restored potent killing (>3-fold increase over CAR T cells alone) of the CD19-low or CAR T therapy resistant tumor cells, but only when the engager-T cells were present in the insert, suggesting the engaging molecules were able to redirect CD19-CAR T cells to CD20-positive tumor cells regardless of their CD19 expression. The anti-lymphoma efficacy of engager-CAR T cell therapy will be further evaluated in our well-established unique PDX model for MCL.

In summary, we have successfully generated a CD20-directed bispecific T-cellengaging antibody and CD20-BiTE engager-T cells that could be combined with CAR T cell therapy, which was demonstrated effective in overcoming tumor antigen escape and resistance to current CD19-CAR T cell therapy in MCL.

Disclosures No relevant conflicts of interest to declare.

https://doi.org/10.1182/blood-2023-190167