Check for updates

ELSEVIER

Blood 142 (2023) 3438–3439

The 65th ASH Annual Meeting Abstracts

POSTER ABSTRACTS

703.CELLULAR IMMUNOTHERAPIES: BASIC AND TRANSLATIONAL

Trogocytosis-Resistant CAR T Cells Exhibit Increased Persistence and Prevent Antigen Escape

Kenneth A. Dietze, BS[1], *Michael L. Olson, PhD*[2], *Etse Gebru, BS*[3], *Djordje Atanackovic, MD*[3], *Aaron P. Rapoport, MD*[3], *Tim Luetkens, MD*[3,1]

[1] Department of Microbiology and Immunology, University of Maryland School of Medicine, Baltimore, MD
[2] Division of Hematologic Neoplasia, Dana Farber Cancer Institute, Boston, MA
[3] University of Maryland Greenebaum Comprehensive Cancer Center, Baltimore, MD

Chimeric Antigen Receptor (CAR) T cell therapy is a highly effective treatment for cancer patients in which T cells are genetically modified to express a CAR targeting an antigen expressed on the surface of cancer cells. While CAR T cells have shown remarkable efficacy, many patients receiving CAR T cell therapy relapse within five years of treatment. It has recently been shown that tumor cells can escape killing by CAR T cells using a mechanism called trogocytosis. CAR-mediated trogocytosis (CMT) involves the transfer of cell surface proteins from tumor cells to CAR T cells. Antigen transfer by CMT results in antigen-negative tumor cells and reduced CAR T cell persistence due to increased CAR T cell exhaustion and fratricide. To date, the molecular processes causing CMT remain poorly understood and there are currently no approaches to therapeutically target CMT.

To track CMT in real-time, we first developed a custom luciferase complementation assay with a detection limit of approximately 100 CAR T cells displaying the target antigen (CompLuc). Using this assay, we found that CMT is effector-target ratio and time-dependent and can be modulated by inhibiting actin polymerization, a process essential for key T cell functions including vesicle formation, protein transport, and target cell killing.

To develop a universal approach to inhibit CMT in CAR T cells without altering tumor cell killing, we investigated the effect of inhibiting adhesion, endocytosis, vesicle formation, and proteolytic degradation using various small-molecule inhibitors. We found that inhibition of the cysteine protease Cathepsin B with the small molecule inhibitor Ca-074-Me significantly reduced CMT as determined by CompLuc assay and flow cytometry. Performing a luciferase-based cytotoxicity assay, we found that Cathepsin B inhibition does not affect tumor cell killing. Based on these data, we hypothesized that Cathepsin B inhibition could be a potential therapeutic approach to limit CMT.

Cathepsin B activity in human cells is regulated by the human proteins Cystatin A and Cystatin B. Structural data of Cathepsin B in complex with Ca-074-Me or Cystatin A show that both molecules appear to sterically block access of substrates to the active site cysteine of Cathepsin B. We therefore explored if overexpression of cystatins in CAR T cells could be an efficient approach to inhibit CMT. We found that overexpression of Cystatin A significantly reduced Cathepsin B activity in CAR T cells and limited transfer of tumor antigen to CAR T cells. We also observed that cystatin overexpression reduced loss of antigen from the tumor cell surface, indicating that Cathepsin B inhibition blocks CMT at an early stage of the trogocytic process. Importantly, Cystatin A overexpression did not alter CAR T cell expansion, tumor cell killing, or CAR T cell phenotype, indicating that this approach selectively inhibits CMT and that key CAR T cell functions remain intact. Cystatin overexpression also led to significantly increased tumor-specific CAR T cell numbers (78% of non-targeting CAR T cells) compared to wild-type CAR T cells (61% of non-targeting CAR T cells, $p = 0.0298$) after 24 h exposure to tumor cells, indicating that selective inhibition of CMT prevents CAR T cell fratricide and/or exhaustion.

To further reduce presence of antigen on CAR T cells following CMT, we next developed a system to actively degrade the trogocytosed protein in CAR T cells. Specifically, we developed a fusion protein combining the SH2 domain of the Src kinase Lyn, which binds with high affinity to the intracellular domain of CD19, with the von Hippel-Lindau E3 ubiquitin ligase. We show that expression of this fusion protein within CAR T cells rapidly targets trogocytosed CD19 for degradation and eliminates its presence on the surface of CAR T cells. Trogocytosed antigen degradation also increased CAR T cell survival at the end of in vitro co-culture, indicating reduced CAR T cell fratricide and/or exhaustion.

Taken together, we provide the first preclinical proof-of-concept that, using a fully human genetic engineering approach, CMT can be targeted without compromising essential CAR T cell functions. This represents a promising approach to improve CAR T cell efficacy and limit the occurrence of relapse in patients receiving CAR T cell therapy.

Downloaded from http://ashpublications.net/blood/article-pdf/142/Supplement 1/3438/2190627/blood-40-main.pdf by guest on 21 May 2024

© 2023 by The American Society of Hematology

Disclosures No relevant conflicts of interest to declare.

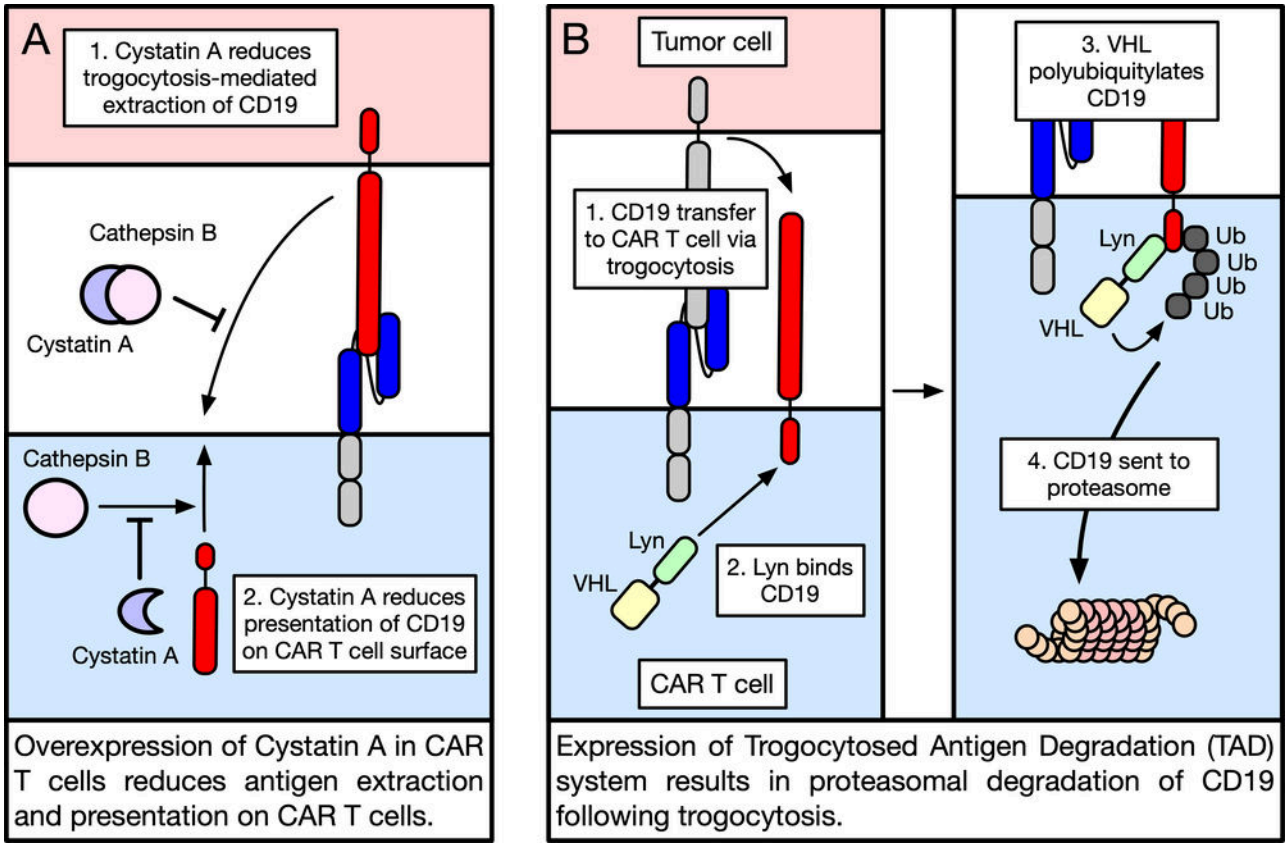

Figure 1

https://doi.org/10.1182/blood-2023-187421

Downloaded from http://ashpublications.net/blood/article-pdf/142/Supplement 1/3438/2190627/blood-40-main.pdf by guest on 21 May 2024