ELSEVIER

Blood 142 (2023) 354–355

The 65th ASH Annual Meeting Abstracts

ORAL ABSTRACTS

703.CELLULAR IMMUNOTHERAPIES: BASIC AND TRANSLATIONAL

Enhancing MHC-Independent T Cell Receptors Using Rational Protein Engineering

Jufang Chang[1], *Jack Landmann*[2], *Yangdon Tenzin*[1], *John Warrington*[3], *Mehmet Emrah Selli, PhD*[1], *Tien-Ching Chang*[3], *Michael Slade, MDMSCI*[4], *Deepesh Gupta, PhD*[3], *Nathan Singh, MD*[1]

[1]Washington University School of Medicine, Saint Louis, MO
[2]Washington University School of Medicine, St. Louis, MO
[3]Washington University, St Louis, MO
[4]Division of Oncology, Washington University School of Medicine, Saint Louis, MO

The paradigm of synthetic receptors, chimeric antigen receptors (CARs), combine MHC-independence with T cell activation. While T cells engineered to express CARs are successful for some patients, the majority are not cured and clinical failures often result from CAR-driven T cell dysfunction (Lynn R, *Nature* 2019; Selli ME, *Blood* 2023). Simultaneously, many patients develop cytokine release syndrome, a hyperinflammatory state resulting from uncontrolled CAR-driven immune activation, indicating that CARs can drive both failure and toxicity. We hypothesized that these limitations result from CAR design which, in its simplicity, bypasses natural TCR regulation.

To re-engage endogenous T cell regulatory circuits, we replaced the variable region of the TCR α and β chains with the CD19 binding domain used in several clinical CAR constructs. As shown recently by others (Liu Y, *Sci Transl Med* 2021; Mansilla-Soto *Nature Medicine* 2022), we found that this format of synthetic receptor expressed on the cell surface, clustered with endogenous CD3 molecules, and drove T cell cytotoxicity against CD19+ leukemia (Nalm6). Intriguingly, surface expression of these MHC-independent TCRs (miTCR) was markedly reduced in comparison to transgenic full-length TCRs and CARs. Examination of the chimeric interface created by joining the antibody and TCR parent chains revealed significant biochemical conflicts that, based on predictive modeling, disrupted naturally-occurring hydrostatic interactions and associated hydrogen bonds, collectively resulting in steric "strain" on the miTCR. We generated a series of miTCR mutants which resolved these conflicts in a step-wise fashion to more closely approximate the "relaxed" state of endogenous TCRs. We found that most mutations improved miTCR surface expression, and more importantly that some mutations significantly boosted T cell functionality as compared to the wild-type miTCR. Impressively, one mutant that closely approximated endogenous TCR structure (mut035) enabled functionality similar to CD19 CARs in short-term cytotoxicity assays.

To further evaluate the functional implications of targeted interface mutation, we subjected miTCRs to chronic *in vitro* stimulation assays in which engineered T cells are exposed to high quantities of Nalm6 for prolonged periods until T cell lose function. We observed that mut035 outperformed all other miTCRs, but all miTCR T cells lost the ability to kill targets earlier than CAR T cells. To determine if a lack of costimulation was the cause of this reduced efficacy we engineered Nalm6 cells to express ligands for CD28 (CD80) and 41BB (41BBL). In the presence of these ligands, long-term functionality all miTCR T cells was profoundly improved. Notably, mut035 T cells persisted longer and killed Nalm6 better than CD19 CAR T cells under conditions of persistent and high-intensity stimulation.

Finally, to determine if CD28 or 41BB engagement was most supportive of miTCR function we designed a "priming" experiment. miTCR T cells were initially cultured with Nalm6 expressing either no, one or both costimulatory ligands and then, after tumor clearance, were re-challenged with WT Nalm6, mirroring endogenous immune responses to infection. mut035 T cells initially exposed to CD80 were unable to clear WT cells on re-challenge, however priming with 41BBL enabled potent and prolonged co-stimulation-independent killing of large Nalm6 burdens, suggesting engagement of endogenous T cell functionality.

These data demonstrate that classical approaches to combine protein domains can lead to biochemical conflicts that restrain chimeric protein function. Targeted mutation significantly enhanced miTCR function to exceed CAR under conditions of high-burden, high-antigen tumors.

Disclosures Slade: *SecuraBio:* Research Funding.

https://doi.org/10.1182/blood-2023-182154