The 65th ASH Annual Meeting Abstracts

ORAL ABSTRACTS

703.CELLULAR IMMUNOTHERAPIES: BASIC AND TRANSLATIONAL

Discovery and Development of CD70 As a Cellular Therapy Target in High Risk Multiple Myeloma

Corynn Kasap, MD PhD[1], *Adila Izgutdina, MD*[2], *Bonell Patino Escobar, MD*[2], *Yu-Hsiu Tony Lin, BA*[2], *Huimin Geng, PhD*[3], *Gianina Wicaksono, BA*[2], *Daphne Dupere-Richer, PhD*[4], *Emilio Ramos, MD PhD*[3], *Abhilash Barpanda, PhD*[2], *Anjanaa Vijayanarayanan, MD*[2], *Fernando Salangsang*[5], *Paul Phojanakong, BA*[5], *Juan Antonio Camara Serrano, PhD*[6], *Veronica Steri, PhD*[5], *William Karlon, MD PhD*[2], *Mala Shanmugam*[7], *Lawrence H. Boise*[8], *Benjamin G. Barwick*[7], *Jonathan D. Licht, MD*[4], *Arun P Wiita, MD PhD*[2]

[1] Department of Medicine, Division of Hematology Oncology, University of California San Francisco, San Francisco, CA
[2] Department of Laboratory Medicine, UCSF, San Francisco, CA
[3] Department of Laboratory Medicine, University of California, San Francisco, San Francisco, CA
[4] University of Florida, Gainesville, FL
[5] Preclinical Therapeutics Core, Helen Diller Family Comprehensive Cancer Center, University of California San Francisco, San Francisco, CA
[6] Preclinical Therapeutics Core, Helen Diller Family Comprehensive Cancer Center, University of California San Francisco, San Francisco, CA
[7] Department of Hematology and Medical Oncology, Winship Cancer Institute, School of Medicine, Emory University, Atlanta, GA
[8] Department of Hematology and Medical Oncology, Winship Cancer Institute, Emory University School of Medicine, Atlanta, GA

Background:
Even in the BCMA CAR-T era, multiple myeloma (MM) patients with high-risk genotypes have the poorest outcomes. We thus leveraged MMRF CoMMpass data to identify potential novel surface immunotherapeutic targets in high-risk MM. As described below, we focused on CD70, an immunotherapeutic target known to be upregulated on many hematologic and solid tumors but minimally expressed on normal tissue. While CD70 was previously explored in MM (McEarchern et al, Clin Cancer Res (2008)), it was not further investigated due to heterogeneous expression in newly diagnosed samples. Here we revisit this target to suggest cellular therapies against CD70 could be highly beneficial in high-risk MM patients, currently the greatest unmet need in the field.

Methods:
Primary patient samples were obtained under an IRB-approved protocol. CAR-T lentiviral transduction was performed on paired CD4/CD8 human T cells from at least 3 healthy donors. Effector function was assessed by cytotoxicity against MM cell lines and CAR-T profiling for memory and exhaustion markers. *In vivo* efficiency was assessed using bioluminescence. ATAC-seq peaks were called using Promo and HOMER (data from Jin et al. Blood (2018)). Machine learning was performed using XGBoost approach applied to CoMMpass RNA-seq data with 80% of samples for training and 20% as test set.

Results:
Analyzing CoMMpass mRNA data (release IA19) we first evaluated surface protein encoding-genes upregulated in tumors at relapse compared to diagnosis. Among >100 significantly upregulated genes, we focused on *CD70* given known promise as an immunotherapy target. Further analysis across genomic subtypes revealed a specific, marked upregulation of *CD70* in t(4;14) and gain 1q (**Fig. 1**), as well as R-ISS 3 at diagnosis.

Flow cytometry profiling of 8 cell line models, including t(4;14) models KHM11, KMS26, and LP1, revealed consistent CD70 protein expression with antigen density similar to BCMA. Profiling of relapsed primary MM samples confirmed that 19 of 28 samples had CD138+ plasma cells positive for CD70 expression. Of 5 patients in whom both BCMA and CD70 were measured, antigen densities were also similar.

To target CD70, we then designed 10 unique anti-CD70 CAR-T constructs. 9 of these were scFv-based binders derived from therapeutic antibody fragments currently in preclinical or clinical development. By a structure-guided design based on the CD70:CD27 co-crystal structure (PDB: 7KX0), we also derived a unique truncated fragment of CD27 as an alternative CD70

binding element. All CD70 CARs were efficacious in *in vitro* cytotoxicity assays targeting MM cell lines AMO1, MM1.S, LP1, and RPMI8226. We then selected the top 3 performing scFv based-CARs as well as our natural ligand (CD27) CAR-T for *in vivo* studies. All 4 CD70 CAR-Ts prolonged survival of MM.1S-bearing mice compared to empty CAR (n=5 mice/arm, p<0.005 by log-rank), similar to BCMA CAR-T controls. Remarkably, the CD27-based CAR showed superior expansion (50-85x, p< 0.0001 by ANOVA) and persistence compared to scFv-based (**Fig. 2**). These results underscore that targeting CD70 using a natural ligand-based CAR-T represents a promising preclinical candidate for treatment of relapsed and high risk MM.

We further investigated why CD70 may be upregulated in high risk MM. Analysis of ChIP-seq in ENCODE and primary MM tumor ATAC-seq was used to nominate a set of transcription factors that bind the *CD70* locus. Extending from CoMMpass data, we built a predictive machine learning model for *CD70* expression as a function of transcription factor gene expression, nominating key transcription factors TFAP2A, LEF1, MYB, and PAX5 as CD70 regulators; validation is now underway. Additionally, we showed that knockout of *NSD2* in t(4;14) models KMS34 and KMS11 led to significantly decreased CD70 surface expression, implicating this central epigenetic driver in CD70 overexpression in this high-risk MM subtype. Finally, we also demonstrated that treatment with the hypomethylating agent azacytidine can drive increased CD70 expression, similar to that shown in AML models.

Conclusion:

Taken together, targeting CD70 appears to be a highly promising cellular therapeutic strategy for MM, with particularly high utility in high-risk patients after BCMA CAR-T relapse. We are continuing preclinical and future clinical development of this CAR-T strategy, using our natural ligand CD27 design with extended *in vivo* persistence.
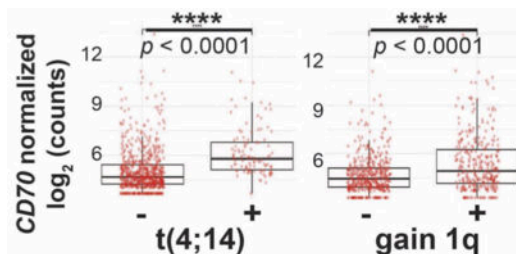
Figure 1: High-Risk Myeloma Patients Express Higher CD70. Data from MMRF CoMMpass (release IA19) demonstrating increased CD70 expression in CD138+ tumor cells as a function of high-risk genotype. *p*-value by two-tailed *t*-test.
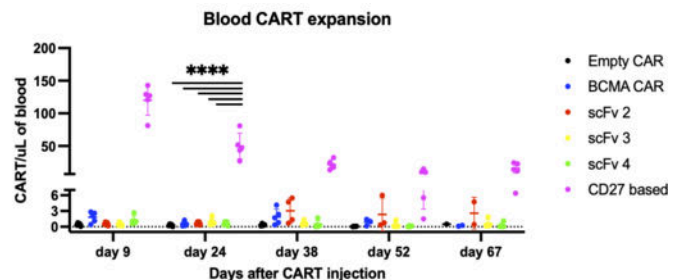
Figure 2: Expansion of natural ligand (CD27) based CAR-T in vivo. Quantitative analysis of number of CAR-T per microliter murine peripheral blood for each treatment group. Data are the mean ± standard deviation (SD) of 5 animals per treatment group. CD27-based CAR-T expansion was compared to other CD70 scFv-based binders and BCMA CAR-T on day 24 by ANOVA; ****p< .0001.

Figure 1

https://doi.org/10.1182/blood-2023-182578