KPCRTF State Funded Projects Reporting Template

University of Louisville – Project #1
Anti-CD33/CD123 compound CAR-T cells, a novel and promising immunotherapy for the treatment of pediatric acute myeloid leukemia
Principal Investigator: William Tsun-Yan Tse

Reporting Period: __April 2021 - June 2021__

Below please provide a brief summary of the status of the Project listed as well as for each Aim listed below. Include any barriers, how and if they were overcome, and successes achieved.

Summary of Status of Project:

In this project, we have continued to move towards our goal of bringing effective CAR-T therapies to fight high-risk hematological malignancies in children. To lay the groundwork for conducting a clinical trial of the anti-AML compound CAR-T cells, we are refining a clinical protocol to test the functionality and persistence against pediatric ALL of a novel CAR-T construct that also co-expresses an immune cell enhancing modulator. Our collaborator, the UofL Cellular Therapy Laboratory, is finishing the upgrading of the facility and are conducting engineering runs of CAR-T cell production. To dissect the optimal conditions required to manufacturing CAR-T cells, we are developing single-cell transcriptomic methodologies to study the phenotype of CAR-T cells grown under different culture conditions. We showed that CAR-T cells are heterogeneous in phenotype and can be grouped into clusters that correspond to T cell subsets at different stages of development, such as central memory, effector memory and terminal effector T cells. Different culture conditions contribute to distinctive cluster composition. Basing on these findings, we calculated for each cluster its signaling entropy rate (SR), a quantitative measure of differentiation potency or lineage promiscuity. We showed that differentiated clusters have a lower SR and primitive clusters have a higher SR. We are now correlating gene expression pattern, cell surface epitope distribution, and SR values of each cluster to construct gene signatures that can be used to characterize the CAR-T cell clusters. These studies provide important concepts underlying CAR-T biology that will help formulate compound CAR-T cells that will effectively work against high-risk AML in children.

Project #1: Anti-CD33/CD123 compound CAR-T cells, a novel and promising immunotherapy for the treatment of pediatric acute myeloid leukemia

Aim 1: Improve the safety profile of anti-CD33/CD123 compound CAR-T cells by incorporating a safety switch

Working with our biotech industry collaborator, iCell Gene Therapeutics, we have made DNA constructs designed to express on human T cells compound CAR (chimeric antigen receptors) that target both CD33 and CD123 antigens on acute myeloid leukemia cells. We demonstrated the ability to eliminate the CAR T cells by treatment with the monoclonal antibody alemtuzumab as a safety switch.

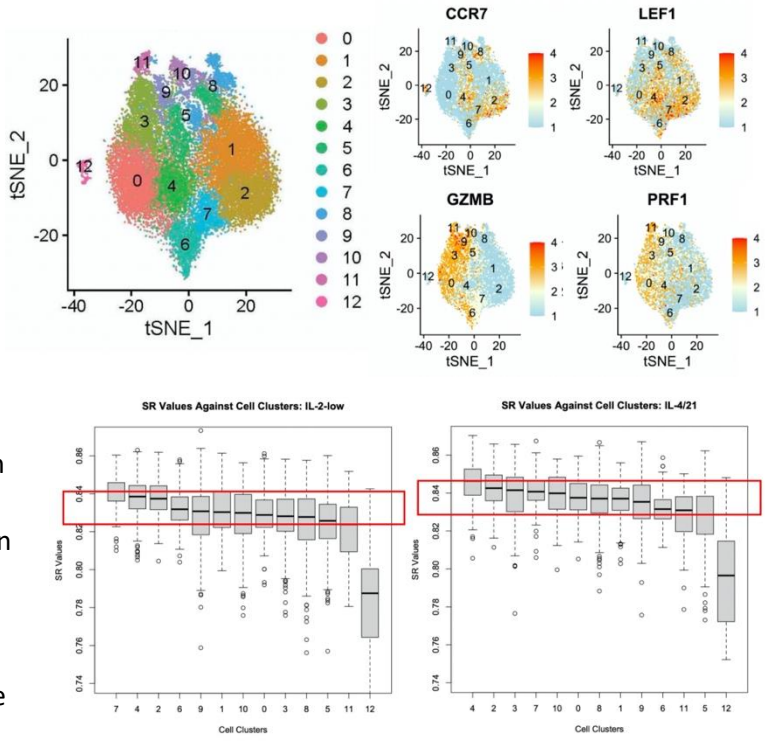
To lay the groundwork for a clinical trial to evaluate the use of compound anti-CD33/CD123 CAR-T cells to treat pediatric AML, we are refining a draft clinical protocol to evaluate the safety and

feasibility of the use of a novel anti-CD19 CAR-T cell product to treat pediatric ALL, which co-express both the leukemia-targeting CAR and an Immune Cell Enhancing Modulator (ICEM). This CAR-T product, designated CD19-ICEM CAR-T cells, has been shown in pre-clinical studies to exhibit increased persistence *in vivo* and enhanced antileukemia functionality. After revision of the protocol is completed, it will be submitted to IRB shortly for review and approval. With upgrading of the UofL GMP facility for CAR-T cell production close to completion, engineering runs are being planned to produce the CD19-ICEM CAR-T cells in preparation for clinical trials. Once the anti-ALL protocol has been submitted for IRB review, a similar protocol will be drafted to evaluate the use of the compound anti-CD33/CD123 CAR-T cells to treat pediatric AML.

Aim 2: Optimize the manufacturing processes to produce clinical-grade, anti-CD33/CD123 compound CAR-T cells

We are now close to completing the upgrading of the UofL GMP Laboratory to improve compliance with strict GMP requirements. WorkingBuildings, a laboratory quality consultant company, is performing ongoing maintenance of the GMP facility and revision of its working protocols. Standard Operating Procedures have been revised. Engineering runs of CAR-T cell production are ongoing.

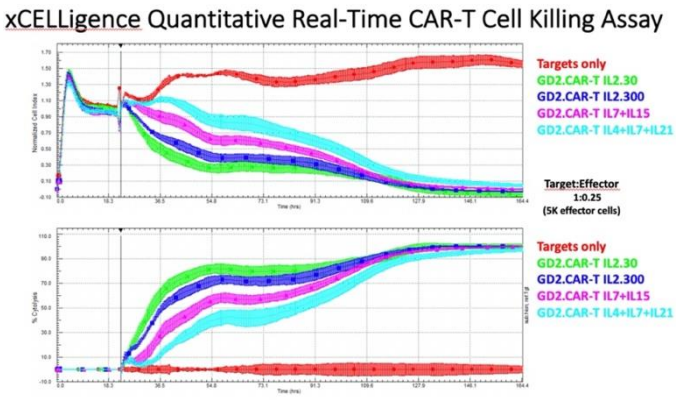
Using single-cell RNA sequencing (scRNA-seq) technology, we have shown that CAR-T cells are highly heterogeneous in nature and can be clustered into distinct subsets that reflect different stages of T cell development. The clusters span the developmental continuum ranging from primitive central memory T cells (e.g., positive for CCR7 and LEF1) to terminally differentiated effector T cells (e.g., positive for GZMB and PRF1). To evaluate the developmental potential associated with each cluster, we calculated the signaling entropy rate (SR) of clusters cultured under different conditions. SR measures the differentiation potency of cells by correlating gene expression patterns with connectivity of protein interacting networks. As expected from the definition of SR, clusters that contain differentiated effector cells are shown to have a low SR whereas clusters that contains primitive cells with high differentiation potency are shown to have a high SR. Samples that were cultured in IL-4/IL-21 have higher SRs across the board, suggesting that this culture condition promotes the production of CAR-T cells that have a high differentiation potency. We are currently correlating the gene expression patterns of the clusters with the culture conditions to derive gene signatures that can characterize different subsets of CAR-T cells. Such analyses will facilitate definition of



specific culture conditions that can promote the production of CAR-T cells with high functionality and persistence.

Aim 3: Characterize quantitatively the cytotoxic activity of anti-CD33/CD123 compound CAR-T cells directed against human AML cell lines and primary isolates

To enable quantitative measurement of the cytotoxicity function of CAR-T cells, we have set up an xCELLigence Real-Time Cell Analysis System, which works by providing continuous monitoring of target cell viability through measurement of electrical impedance. This system was used to measure the rate of tumor cell killing by CAR-T cells by culturing the 2 cell types together in a chamber slide. Using specific CAR-T cells and target tumor cells, we have continued to calibrate the xCELLigence system and use to system to compare the relative abilities of different types of CAR-T cells to kill tumor cells. We showed that the tumor cell-killing data acquired using the xCELLigence assay provide a reliable quantification of the functionality of CAR-T cells and that this new assay is an improvement over the conventional chromium-release cytotoxicity assay.



We continued to use the xCELLigence system to quantitatively evaluate the anti-tumor functionality of CAR-T cells cultured under different conditions: 1) low-dose IL-2 alone; 2) high-dose IL2 alone; 3) IL-4, IL-7 and IL-21; or 4) IL-7 and IL-15. The resulting CAR-T cells were co-cultured with target tumor cells and the killing of target cells monitored using the xCELLigence system. We showed that the tumor-killing capacities of these CAR-T cell preparations differ, likely reflecting the different numbers of cytotoxic T cells being produced under the various conditions. We are now using this quantitative cytotoxicity assay to define the culture condition that can produce CAR-T cells with the best anti-tumor capability.

Aim 4: Generate data on the viability, purity, stability, activity, and safety of anti-CD33/CD123 compound CAR-T cells produced in compliance with GMP requirements

As discussed above, we have upgraded our GMP laboratory to enhance its capacity to produce clinical-grade cellular products necessary for clinical trials. Once the laboratory preparation work and process optimization studies have been completed, large-scale engineering runs will be performed to produce CAR T cells for testing of viability, purity, stability, activity and safety.

Timeline:

Aim #1 (check when completed)	Month 1-6	Month 7-12	Month 13-18	Month 19-24	√
Improve CAR construct with safety switch	√	√			√
Optimize production process for CAR-T cells	√	√	√		√
Analyze cytotoxicity profile of CAR-T cells		√	√	√	√
Obtain safety data for CAR-T cells		√	√	√	√

Deliverables:

Check when deliverable is completed:	√
Completed validation of the effectiveness of an anti-CD33/CD123 compound CAR-T cell design, with improvement of the safety profile by incorporation of a tEGFR safety switch	√
Completed optimization of the process for the production of the anti-CD33/CD123 compound CAR-T cells	√
Completed quantitative analysis of the cytotoxicity profile of the anti-CD33/CD123 compound CAR-T cells	√
Completed characterization of the safety profile of the anti-CD33/CD123 compound CAR-T cells produced in a cGMP-compliant environment	√
Completed collection of pre-clinical data for an IND application and initiation of the IND submission process	√

Reports should be returned to:
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