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ORAL ABSTRACTS

703.CELLULAR IMMUNOTHERAPIES: BASIC AND TRANSLATIONAL

Single-Cell Multi-Omics Reveals Type-2 Functionality in Maintaining CAR T Cell Longevity Associated with 8-Year Leukemia Remission

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Despite a high response rate in chimeric antigen receptor (CAR) T therapy for acute lymphocytic leukemia (ALL), ~50% of patients relapse within the first year, representing an urgent question to address in the next stage of cellular immunotherapy. The pioneering clinical trials conducted at Upenn/CHOP provide a unique opportunity to examine the molecular determinants of ultra-long CAR T persistence.

We performed single-cell multi-omics profiling of ~700k pre-infusion CD19-targeted 4-BB CAR T cells from 82 pediatric ALL patients and 6 healthy donors. Patient demographics were collected between Sep-2012 to July-2022. To uncover the hallmarks of CAR T longevity, we correlated the single-cell atlas with the duration of B-cell aplasia (BCA), a widely used pharmacodynamic measurement of CAR T persistence, and classified all the patients into 5 groups (Table 1). Five patients in our cohort have exhibited a median BCA duration of 8.4 years (BCA-L group) and another 11 patients continue to have BCA with a median time of 5.1 years (BCA-O group). To remove potential confounding variables between trial design, 42 patients including the 5 BCA-L were analyzed as the Discovery Cohort, while the other 40 patients including the 11 BCA-O were used as Validation Cohort.

Our analysis of CD19-specific stimulated CAR T cells from the Discovery Cohort revealed a prominent role of type-1 function, which was highly represented but had no discernible correlation with long-term persistence. Unexpectedly, we identified that elevated type-2 functionality was significantly associated with BCA-L patients maintaining CAR T persistence of 8.4 years, and this signature was robustly presented in the BCA-O patients in our Validation Cohort. Higher type-2 cytokine level of long-term persistent CAR T cells was independently validated using both flow cytometry and multiplexed secretomic assay. Through ligand-receptor interaction analysis, type-2 cytokines were found to regulate a cluster of Tim-3+terminal effector cells showing over activation of cytotoxicity, impaired immune function, elevated exhaustion signature and diminished proliferative potentiality, and CAR T cells from BCA-L patients showed reduced dysfunctional hallmarks.

We next conducted in vitro functional studies to assess the impact of adding type-2 cytokine (IL-4) during CAR-specific activation on CAR T fitness. The addition of 10ng/mL IL-4 significantly boosted the proliferation and mitigated dysfunction of CAR T cells from 6 patients in the BCA2 group that only mediated short-term response (~3 months) and had negligible impact on their type-1 functionality. Remarkably, the functional profile of short-term BCA2 CAR T cells after IL-4 supplementation exhibited a comparable pattern to that of long-term BCA-L CAR T cells, particularly in terms of type-2 pathway, oxidative phosphorylation metabolism, PI3K/AKT signaling, mTOR signaling, and cell cycle regulation.

To investigate the response of the host following CAR T infusion, we performed a comprehensive proteomic profiling of post-infusion serum proteins in a total of 41 patients. Baseline measurements (-2 to 0 days before CAR T infusion) showed a significantly higher level of type-2 cytokines in long-term patients. From the rapid expansion period (day 6-8) until 11 days post-infusion, we consistently observed significantly higher levels of circulating type-2 cytokines in 5-year or 8-year relapse-free responders. Conversely, no difference was observed in type-1 cytokines.

In a leukemic NSG mouse model, both type-2 high and low CAR T cells were highly effective in reducing tumor burden in the first two weeks. However, type-2 high CAR T had significantly superior expansion, and the absolute CAR+ cell counts

in peripheral blood were 10-fold higher than type-2 low CAR T at day 8 and day 12. To mimic tumor cell relapse, a second dose of tumor cells were injected into mice on day 17. Notably, type-2 high CAR T cells demonstrated a potent capability to elicit recall responses upon leukemia rechallenge, significantly prolonging the survival of tumor-bearing mice compared to type-2 low CAR T. The superior performance could be associated with their decreased exhaustion and increased memory phenotype.

Our findings provide key insights into the mediators of CAR T longevity and suggest a potential therapeutic strategy to maintain long-term remission by enhancing type-2 functionality in CAR T cells.

Disclosures Tang: *Leman Biotech:* Current holder of *stock options* in a privately-held company, Membership on an entity's Board of Directors or advisory committees. **June:** *AC Immune:* Membership on an entity's Board of Directors or advisory committees; *BluesphereBio:* Membership on an entity's Board of Directors or advisory committees; *Cabaletta:* Membership on an entity's Board of Directors or advisory committees; *Carisma:* Membership on an entity's Board of Directors or advisory committees; *Cartography:* Membership on an entity's Board of Directors or advisory committees; *Cellares:* Membership on an entity's Board of Directors or advisory committees; *Celldex:* Membership on an entity's Board of Directors or advisory committees; *Decheng:* Membership on an entity's Board of Directors or advisory committees; *Poseida:* Membership on an entity's Board of Directors or advisory committees; *Verismo:* Membership on an entity's Board of Directors or advisory committees; *WIRB-Copernicus:* Membership on an entity's Board of Directors or advisory committees; *Danaher:* Membership on an entity's Board of Directors or advisory committees; *Kite Pharma:* Consultancy, Membership on an entity's Board of Directors or advisory committees, Research Funding. **Grupp:** *Kite:* Research Funding; *Servier:* Research Funding; *CBMG:* Consultancy, Membership on an entity's Board of Directors or advisory committees; *Adaptimmune:* Consultancy, Membership on an entity's Board of Directors or advisory committees; *Cellectis:* Consultancy, Membership on an entity's Board of Directors or advisory committees; *Juno:* Consultancy, Membership on an entity's Board of Directors or advisory committees; *Allogene:* Consultancy, Membership on an entity's Board of Directors or advisory committees; *Cabaletta:* Consultancy, Membership on an entity's Board of Directors or advisory committees; *Jazz:* Consultancy, Membership on an entity's Board of Directors or advisory committees, Research Funding; *Vertex:* Consultancy, Research Funding; *Novartis:* Consultancy, Research Funding. **Fan:** *AtlasXomics:* Membership on an entity's Board of Directors or advisory committees; *Singleron Biotechnologies:* Membership on an entity's Board of Directors or advisory committees; *IsoPlexis:* Membership on an entity's Board of Directors or advisory committees.

Table 1. Patient demographics and clinical documentation.

Demographics	BCA-L (n=5)	BCA-O (n=11)	BCA3 (n=11)	BCA2 (n=38)	BCA1 (n=17)
Cohort group, No.					
Discovery Cohort	5	N/A	5	21	11
Validation Cohort	N/A	11	6	17	6
Age in years	14.5±1.4	13.3±2.0	13.3±1.9	9.7±1.0	10.4±1.2
Median±SEM (Range)	(9.3-16.2)	(6.0-29.1)	(5.0-24.5)	(1.7-27.2)	(3.3-21.5)
BCA observed	Yes	Yes	Yes	Yes	Partial (11/17)
BCA duration in months	101±5.0	61±1.8	18±2.9	4±0.4	1±0.2
Median±SEM (Range)	(82-106)	(48-72)	(12-43)	(3-11)	(0-2)
Relapse observed	No	No	Yes	Yes	Yes
Relapse type, No. (%)					
CD19-postive	N/A	N/A	6 (54.5)	13 (34.2)	4 (23.5)
CD19-negative			4 (36.4)	19 (50.0)	5 (29.4)
Time to relapse in months	N/A	N/A	18.5±3.3	8.5±2.2	2.1±1.1
Median±SEM (Range)			(12.0-45.6)	(2.5-60.2)	(1.0-9.3)
Follow up months	101±5.0	61±1.4	51±5.7	46±4.3	4±7.0
Median±SEM (Range)	(82-106)	(54-72)	(17-78)	(4-91)	(1-78)

Patients are divided into 5 persistence groups based on their durations of B-cell aplasia (BCA). The Discovery Cohort includes 42 patients from clinical trial NCT01626495, and the Validation Cohort consists of 40 patients from clinical trial NCT02906371.

Figure 1

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