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POSTER ABSTRACTS

704.CELLULAR IMMUNOTHERAPIES: EARLY PHASE AND INVESTIGATIONAL THERAPIES

Transcriptional Profiling Associated with CD22 CAR T Cell Clinical Response in LBCL

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Introduction

We have previously presented outcomes of a phase 1 trial (NCT04088890) in which 38 patients with CAR19-refractory LBCL received a single infusion of a CD22-directed CAR T cell (CAR22) at either 1 (DL1) or 3 (DL2) million CAR+ cells/kg. The overall objective response rate (ORR) and complete response (CR) rate were 68% (95% confidence interval [CI], 51 to 83) and 53% (95% CI 36 to 69), respectively. The impact of CAR22 product characteristics on outcomes and CAR22 expansion is unknown and our objective is to identify CAR T cell intrinsic factors associated with treatment outcomes.

Methods

Comprehensive phenotyping and exhaustion profiling were performed on manufacturing samples during apheresis, enrichment, and final product harvest. Additionally, quantification of lymphocyte subsets (CAR+ and CAR-) in all patients was carried out using flow cytometry/qPCR on peripheral blood samples. For in-depth analysis, raw single-cell RNA-seq (scRNA-seq) data from cryopreserved aliquots of 14 CAR22 products was processed through the 10× Genomics platform.

Results

The OR (78% vs 66%) and CR rates (56% vs 52%) were similar between DL1 and DL2, and peripheral blood CAR+ T cells peaked at a median of 71 and 360 CAR+ T cells/mL at DL1 and DL2, respectively, at a median of 14 days (range, 7 to 22) post-infusion.

Subjects with a response vs. those without a response (median 141 vs 9.2, P=0.0022); subjects with grade 2 or 3 CRS vs. those with grade 1 or no CRS (median 203 vs 46, P=0.026); and subjects who required treatment for IEC-HS vs. those who did not develop IEC-HS (median 1884 vs 90, p-value 0.0016) had significantly higher circulating CAR22 T-cells at peak expansion, as well as higher cumulative levels of CAR+ T cells measured by AUC over the first 28 days (Figure 1).

Characterization by flow cytometry analysis identified a predominance of CD4+ CAR T cells in all CAR22 products. Despite this, CD8+ T-cells represented the majority of CAR+ T cells post-infusion. We obtained matched single-cell sequencing data from 14 products for transcriptome, surface protein expression, and TCR sequence. Analyzed were 242,571 cells, of which 141,152 expressed the CAR22 transgene. Products were representative of the study population in terms of age, sex, cell dose, adverse events, and clinical outcome. Higher TCR diversity was found in products of patients who achieved a CR. We applied the propeller method to calculate significant differences in cell type proportions related to response, performing transformation on cell type proportions using linear modelling, and found a significantly higher proportion of terminal effector memory cells (TEMRA) in the products of patients who progressed. Dividing our cohort into subgroups based on high percentage

(≥median) or low percentage (<median) of CD4+ TEMRA cells in products by flow cytometry, there was a significantly better progression free survival in the latter.

Differential gene expression analysis revealed that CAR $^{+}$ T cells in the products of patients achieving CR expressed higher levels of FOS and JUN, transcription factors of the AP-1 family (Figure 2). In contrast, cells from patients who progressed showed higher levels of multiple immunomodulatory killer cell immunoglobulin like receptors (KIRs).

Discussion

This study demonstrates that CAR T cell expansion correlates with clinical response and toxicities of the CAR22 therapy. However, little is known about the clonal composition of CAR22 products and how distinct transcriptional signatures in the CAR T products might affect cell performance *in vivo*. In this exploratory analysis, we identified an association TEMRA cells with poor response. In addition, we observed higher expression of FOS and JUN in CAR22 products of patients achieving CR. The classic AP-1 heterodimer c-Fos-c-Jun drives transcription of IL2 and has also been described to contribute to exhaustion resistance in CD19 targeting CAR T cells. This study identifies specific cell features that are associated with the clinical outcome CAR22 therapy, which has implications for optimizing CAR22 products for improved clinical outcomes. Further analyses are ongoing to examine the clonal dynamics of the CAR22 T cells *in vivo* by including patient samples at later timepoints to our scRNA-seq dataset.

Disclosures Hamilton: *Kite Pharma:* Other: Advisory Board. **Feldman:** *FreshWind Bio:* Membership on an entity's Board of Directors or advisory committees; *Alaunos:* Membership on an entity's Board of Directors or advisory committees; *MicroFluidX:* Membership on an entity's Board of Directors or advisory committees; *Autolomous:* Membership on an entity's Board of Directors or advisory committees. **Baird:** *Kite Pharma-Gilead:* Research Funding, Speakers Bureau; *Genentech-Roche:* Research Funding; *Regeneron Pharmaceuticals:* Research Funding; *Cellular Biomedicine Group:* Research Funding. **Muffly:** *amgen:* Consultancy; *pfizer:* Consultancy; *kite:* Consultancy, Honoraria, Research Funding; *autolus:* Consultancy; *astellas:* Consultancy, Research Funding; *jasper:* Research Funding; *bms:* Research Funding; *adaptive:* Membership on an entity's Board of Directors or advisory committees, Research Funding; *orca bio:* Research Funding. **Mackall:** *Lyell Immunopharma:* Current equity holder in private company, Research Funding; *Mammoth:* Consultancy, Current equity holder in private company; *Adaptimmune:* Consultancy; *Immatics:* Consultancy; *CARGO:* Consultancy, Current equity holder in private company, Membership on an entity's Board of Directors or advisory committees; *Link Cell Therapies:* Consultancy, Current equity holder in private company, Membership on an entity's Board of Directors or advisory committees. **Miklos:** *Kite, a Gilead Company:* Consultancy, Research Funding; *Adicet:* Research Funding; *Miltenyi:* Consultancy, Research Funding; *Bioline Rx:* Membership on an entity's Board of Directors or advisory committees; *Fate Therapeutics:* Research Funding; *Pharmacyclics:* Consultancy, Honoraria; *Umoja:* Consultancy, Honoraria; *NA:* Patents & Royalties: cGVHD patent holder for Ibrutinib as cGVHD therapy but no compensation; *A2 Biotherapeutics:* Consultancy, Current holder of *stock options* in a privately-held company, Honoraria; *Amgen:* Consultancy, Honoraria; *Novartis:* Consultancy, Honoraria; *Navan Technologies:* Consultancy, Current holder of *stock options* in a privately-held company, Honoraria; *Mustang Bio:* Consultancy, Honoraria; *MorphoSys:* Consultancy, Honoraria; *Legend Biotech:* Consultancy, Honoraria; *Juno Therapeutics:* Consultancy, Honoraria, Patents & Royalties: rights to royalties from Fred Hutch for patents licensed to Juno, Research Funding; *Gilead Sciences:* Consultancy, Honoraria; *Incyte:* Consultancy, Honoraria; *Celgene:* Consultancy, Honoraria, Membership on an entity's Board of Directors or advisory committees, Research Funding; *Genentech:* Consultancy, Honoraria; *Bristol-Myers Squibb:* Consultancy; *2Seventy Bio:* Research Funding; *Adaptive Biotechnologies:* Consultancy; *Allogene:* Research Funding; *Janssen:* Consultancy, Honoraria, Other: Travel support. **Frank:** *Kite, a Gilead Company:* Research Funding; *BRVLH:* Consultancy; *Allogene:* Consultancy; *Adaptive Biotechnology:* Consultancy; *Gilead Sciences:* Consultancy, Other: Travel Support; *Roche/Genentech:* Current holder of *stock options* in a privately-held company; *EcoR1:* Consultancy; *Cargo Therapeutics:* Consultancy, Other: Travel Support.

Figure 1| CAR T-Cell Expansion and Correlations with Response

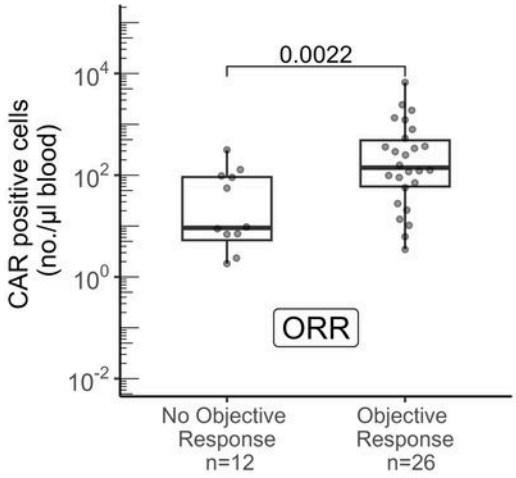

Figure 2| Differential gene expression analysis of CAR+ T cells in CAR22 products of patients achieving CR

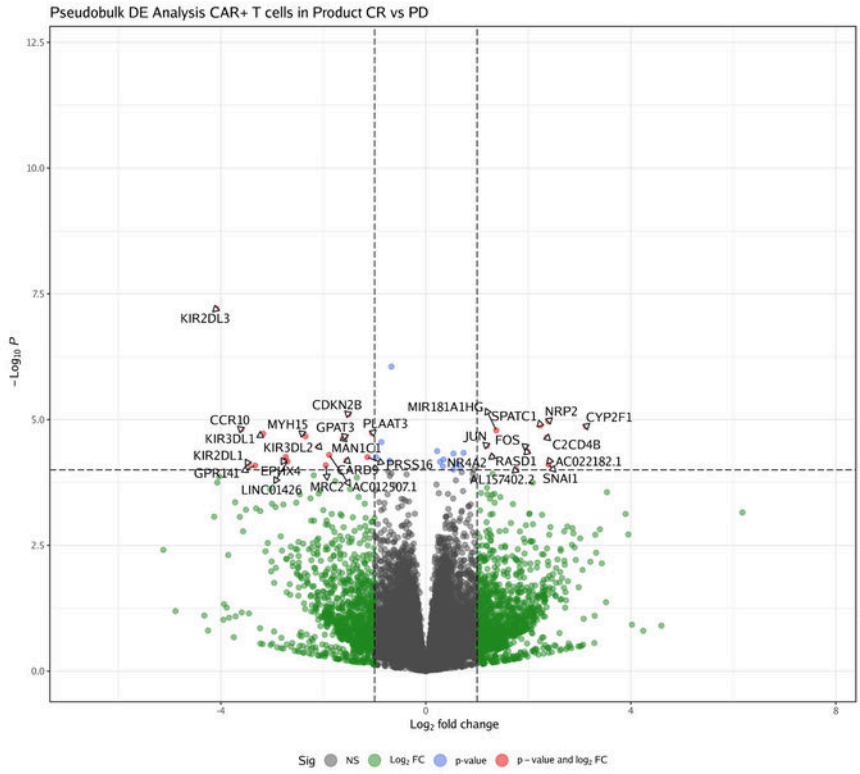

Figure 1

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