Joint Meeting
SFI & DGfI
26-29 SEPTEMBER 2023
Palais de la Musique et des Congrès STRASBOURG

**O30**

## Transcriptional signatures associated with persisting CD19 CAR T-cells in children with leukaemia

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Over the past decade, chimeric antigen receptor (CAR)-modified T-cells have become established as an effective treatment of haematological cancers. CD19 targeting CAR T-cells often induce durable remissions in relapsed and refractory childhood pre-B cell acute lymphoblastic leukaemia (B ALL). In this context, the persistence of CAR T-cells is one of the most critical determinants of achieving a durable response.
In this study, we systematically analysed CD19 CAR T-cells of 10 children with relapsed or refractory B ALL enrolled in the CARPALL trial (NCT02443831). We performed high throughput single-cell gene expression and T-cell receptor (TCR) sequencing, and flow cytometry immunophenotyping of 2 infusion products and 71 serial blood and bone marrow samples up to six years post-infusion.
Our key finding was that long lived CAR T-cells developed a TCR??- CD4/CD8 double-negative (DN) phenotype characterised by GZMK+ exhausted-like memory state, including expression of the co-inhibitory receptors TIGIT and LAG3, and the exhaustion regulator TOX. We observed the dominance of this distinctive persistence phenotype in all children with a long-lived treatment response. The phenotype emerged across clonotypes and subsets of T-cells, indicating that CAR T-cells converge transcriptionally during a durable clinical response. Remarkably, we also detected this persistence signature in recipients of a different CD19 CAR T-cell product that maintained decade long remissions in two adults with chronic lymphocytic leukaemia. Examination of single T-cell transcriptomes from a wide range of healthy and diseased tissues across children and adults indicated that the persistence signature is rarely encountered in other settings. Accordingly, the persistence signature we found appears to be independent of infusion product, patient age, and leukaemia type.
These findings raise the possibility that a universal transcriptional signature of clinically effective, persistent CD19 CAR T-cells exists. It may provide a basis for the identification of biomarkers of persistence and guide refinement of manufacturing methods.