CLONAL EXPANSION OF CD22 CAR T-CELLS FOLLOWING LENTIVIRAL VECTOR INTEGRATION IN THE PWWP3A GENE

1Alka Dwivedi*, 2Ling Su, 1Justin Mirazee, 1Mehdi Benzaoui, 1Christopher Chien, 1Nirali Shah, 1Xiaolin Wu, 1Naomi Taylor. 1National Cancer Institute, Rockville, MD, USA; 2Frederick National Laboratory for Cancer Research, Frederick, MD, USA.

Background
A 17-year-old female with multiply relapsed B-cell ALL following CD19 CAR T-cells and blinatumomab was treated with CD22 CAR T-cells (NCT02315612) for marrow and extramedullary disease (EMD). Following development of grade 2 cytokine release syndrome (on day +10) and resolution, she experienced a rapidly rising lymphocyte count at day 21 that was further evaluated.

Methods
Following parental permission and minor assent, the patient was treated on a National Cancer Institute IRB approved protocol for a phase 1 trial of CD22 CAR T-cells. To evaluate for clonal expansion, TCR sequencing and integration site analysis were performed by ImmunoSEQ hsTCRB sequencing and a ddPCR assay, respectively. Evaluation of the mutant CAR and the novel endogenous protein generated by integration of the lentiviral vector was performed following their introduction into a lentiviral vector. Functional assays included cytotoxicity analyses, expansion analyses, and high throughput cytokine analyses.

Results
The patient experienced full resolution of her clonal expansion and achieved a minimal residual disease negative CR in the bone marrow, with partial response at sites of EMD. TCR sequencing and integration site analyses revealed a single dominant T cell clone with lentivector integration in the PWWP domain-containing DNA repair factor 3A (PWWP3A) gene on chromosome 19 (figure 1) resulting in a fusion transcript, generated by a read-through from the EF-1a-driven CAR transcript. Additionally, a single glycine to serine mutation at position 51 (G51S) of the single chain variable fragment of CD22 CAR was detected. T cells engineered to express the mutant G51S CD22 CAR efficiently killed leukemic cells overexpressing CD22 but exhibited lower cytotoxicity against CD22+ leukemic cells. Furthermore, a high throughput dynamic evaluation of cytokine secretion revealed a significantly reduced secretion of IFN-g, IL-2, IL-10, IL-4, IL-8 and TNF-a of G51S CD22 CAR T-cells in response to CD22 antigen. Notably though, T cells engineered to express the G51S CD22 CAR exhibited an augmented cytokine-driven proliferation as compared to WT CD22 CAR T-cells.

Conclusions
This is the second report of clonal expansion in the context of the CD22-CAR trial. Interestingly, in both this case and the previous one (PMID: 31387880), clonal expansion occurred in the setting of extramedullary disease and spontaneously resolved. In this patient, clonal expansion occurred secondary to a G51S CAR mutation and generation of a PWWP3A fusion transcript. The relative impact of these two events in promoting clonal T cell expansion in this CD22 CAR T-cell treated patient will be discussed.

Ethics Approval
All human samples were obtained with informed consent and following institutional guidelines under protocols approved by the National Cancer Institute institutional review board and the National Institutes of Health Recombinant DNA Advisory Committee. The subject was also enrolled on an institutional review board-approved genomics protocol, which allowed for additional testing of blood samples. This trial was registered at clinicaltrials.gov as NCT02315612.