Background Expansion and persistence of CAR-T products has historically correlated with patient outcomes. CART expansion and pharmacokinetics (PK) are typically monitored by flow cytometry or by transgene qPCR, using detection reagents that are bespoke to individual products, limiting universal application across products or platforms. In monitoring allogeneic CART products, these methods also usually lack capabilities for distinguishing multi-donor origin. Here, we present qualification and clinical application of a quantitative and universal solution (AlloCell) for monitoring of ADI-001, a first-in-class CD20-targeted allogeneic γδ1 CAR T cell therapy.1

Methods AlloCell was analytically validated for 2-genome-based (1 donor) and 3-genome-based (2 donors) analyses. To additionally qualify AlloCell for ADI-001 monitoring, genomic DNA (gDNA) mixtures were prepared using 1) gDNA from peripheral blood of two healthy individuals, or 2) gDNA from 2 FFPE blocks (to establish feasibility for application in solid tissues). The mixtures mimicked cell product levels of 3%, 1%, 0.3%, 0.1%, and 0.03%.

Applying this method, clinical blood samples from the Phase 1 evaluation of ADI-001 (NCT04735471)2 were processed and gDNA was extracted for analysis. gDNA samples were analyzed using the AlloCell assay workflow. Briefly, quality control (QC) was performed using Nanodrop and Qubit. Next, gDNA was used in NGS library preparation and sequenced using NextSeq technology. Sequencing data were analyzed using the AlloCell bioinformatics pipeline.

Results The levels of cell product measured for qualification closely matched the expected levels of donor-derived gDNA in cell mixtures, with a high degree of linearity (n=3, R²=1), and was reproduced in FFPE samples (n=3, R²=0.996). We observed high precision across triplicates, with CVs ranging from 0.31% for 3% mixture, to 5.3% for the 0.03% mixture, or for FFPE samples, from 0.93% for 1% mixture to 23.6% for the at the lowest bound mixture of 0.03%.

Data from ADI-001 patient samples demonstrated AlloCell’s ability to track cell product kinetics in a clinical setting and determined dose-dependent exposure for ADI-001 across four dose levels evaluated. Finally, in one patient receiving two separate and sequential doses of ADI-001, each manufactured from distinct donors, AlloCell successfully distinguished independent expansions of the first and second donor-sourced cell products.

Conclusions AlloCell was validated as a precise and sensitive analytical platform that can quantitatively monitor PK of allogeneic cell therapies. Application of AlloCell to monitor clinical exposure of ADI-001 successfully demonstrated dose-dependent expansion and persistence across multiple dose levels and resolved donor origin for distinct ADI-001 expansions associated with a multiple dosing regimen.

Trial Registration NCT04735471: A Safety and Efficacy Study of ADI-001, an Anti-CD20 Allogeneic Gamma Delta CAR-T, in Subjects With B Cell Malignancies (GLEAN-1)