Assay qualification and quantitative detection of ADI-001, a CD20-targeted γδ1 CAR T therapy, using AlloCell, a universal assay for monitoring of off-the-shelf allogeneic cell therapies

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Background

ADI-001 is a first-in-class CD20-targeted allogeneic γδ1 CAR (chimeric antigen receptor) T cell therapy[1]. ADI-001 consists of allogeneic healthy donor peripheral blood mononuclear cell (PBMC)-derived Vδ1 γδ T cells that have been ex vivo activated, expanded, and genetically engineered to express the CD20 CAR. γδ T cells as a platform for CAR T cell therapy have potential advantages to traditional CAR mediated anti-tumor activity due to innate anti-tumor activity targeted towards multiple receptors that mark tumor cells for killing, tissue-specific tropism, adaptive anti-tumor activity via γδ TCR (T cell receptor), and expression of MHC independent γδ TCR, which lowers GvHD risk without the need for gene editing. ADI-001 is also a readily available, “off-the-shelf” product candidate generated with a scalable cGMP manufacturing process.

Expansion and persistence of CAR-T products has historically correlated with patient response. CAR T expansion and cellular kinetics (CK) are typically monitored by flow cytometry or by transgene qPCR, using detection reagents that are bespoke to individual products, thus limiting universal application across products or platforms. In monitoring allogeneic CAR-T products, these methods usually lack capabilities for distinguishing between distinct exposures from more than one donor origin. Here, we present qualification and clinical application of a quantitative and universal next generation sequencing (NGS)-based solution (AlloCell, CareDx) for monitoring exposure of ADI-001. Qualification studies demonstrate accurate quantification of ADI-001 in PBMC and FFPE matrices, while clinical monitoring by AlloCell precisely quantified ADI-001 cell expansion and persistence in patient blood via donor-specific signatures determined through query of 405 single nucleotide polymorphisms (SNPs) distributed across the human genome.

Methods

AlloCell had been analytically validated for 2-genome-based (1 donor) and 3-genome-based (2 donors) analyses (CareDx, report on file). To additionally qualify AlloCell for ADI-001 monitoring, genomic DNA (gDNA) mixtures were prepared using 1) gDNA from the cells of two healthy individuals, or 2) gDNA from two 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%, as a proportion of total cell input.

Applying this method, clinical blood samples from the Phase 1 evaluation of ADI-001 (NCT04735471) 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, R^2 =1), which was reproduced in FFPE samples (n=3, R^2 =0.998). We also 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% at the lowest 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 a distinct donor, AlloCell successfully distinguished and independently quantified expansions of the first and second donor-sourced cell products.

Manufacture of Off-The-Shelf Allogenic ADI-001

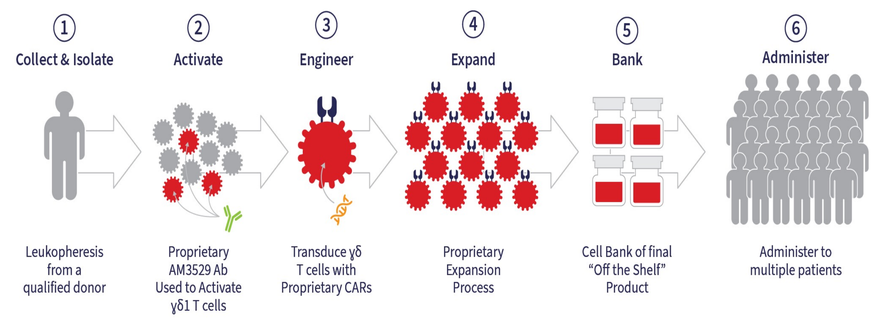
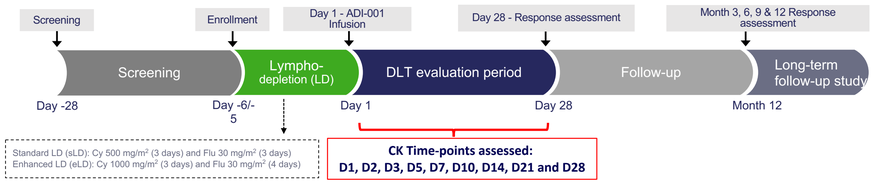


Figure 1. ADI-001 is manufactured with a proprietary AM3529 activating antibody, designed to expand γδ1 T cells, proprietary vectors and a proprietary scalable process.

GLEAN: ADI-001 First-in-Human Study (CD20 CAR+ γδ T cells)



ADI-001 Dose (CAR+ Cells) (3 + 3 escalation design)*

DL1	DL2	DL3	DL4
3E7	1E8	3E8	1E9

Primary endpoint:
- Number of DLTs
- Treatment emergent and treatment-related AEs

Secondary endpoint:
- ORR, DOR, PFS, TTP, and OS
- CK, immunogenicity

Key eligibility criteria:
- R/R high grade B-cell lymphomas (indolent lymphomas, such as FL, were not enrolled)
- At least 2 prior regimens, including anti-CD20 Ab and anthracycline based chemotherapies for DLBCL
- Measurable disease by Lugano 2014
- >18 years; ECOG 0 or 1
- Prior CAR T therapies allowed

*Protocol part 1b includes patients receiving a single lymphodepletion and two infusions at DL3 (2 × 300 million CAR+ cells on day 1 and 7).

Figure 2. Study Overview. Of the 24 efficacy-evaluable patients, 3 received ADI-001 at dose level 1 (DL1) (30 million CAR+ cells), 3 received ADI-001 at dose level 2 (DL2) (100 million CAR+ cells), 6 received ADI-001 at dose level 3 (DL3) (300 million CAR+ cells), 4 received two infusions of ADI-001 at DL3 (two doses of 300 million CAR+ cells, one on day 1 and the second dose on day 7 following a single lymphodepletion), and 8 received ADI-001 at dose level 4 (DL4) (1 billion CAR+ cells). AEs= Adverse events; Cy= Cyclophosphamide; DLBCL=Diffuse large B-cell lymphoma; DL= Dose level; DLT= Dose limiting toxicity; DOR= Duration of response; ECOG= Eastern Cooperative Oncology Group; Flu= Fludarabine; GLEAN= Gamma Delta adoptive therapy for Nhl-1; OS= Overall survival; PFS= Progression-free survival; R/R= Relapsed or refractory; TTP= Time to progression

AlloCell Quantitatively Interrogates Proportion of Donor:Product Signatures Defined Over 405 SNPs Throughout the Genome

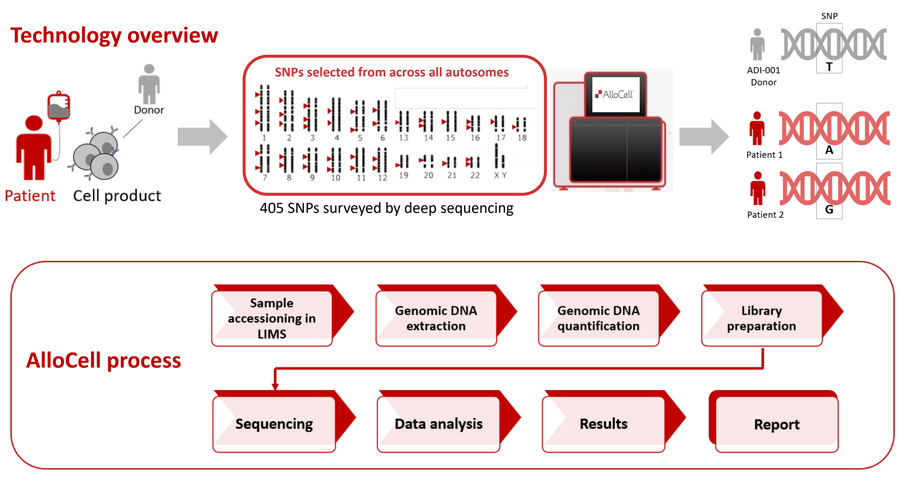
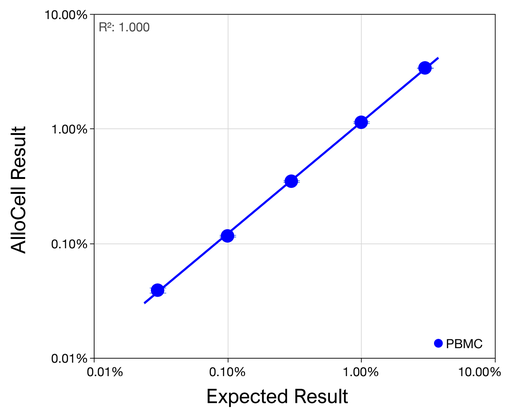


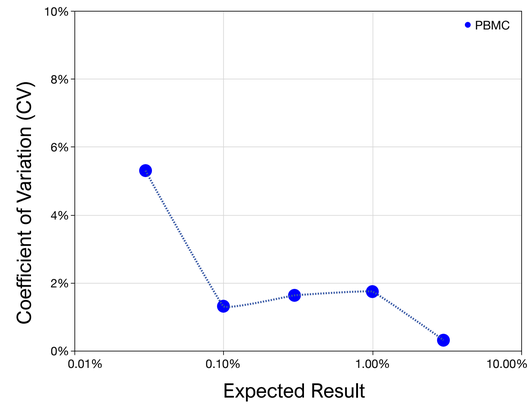
Figure 3. The AlloCell test quantifies the cell therapy in patient blood or gDNA samples provided to CareDx. The tests are performed in a GLP laboratory. Samples are accessioned, gDNA is extracted (if necessary), and quantified. During library preparation, samples are amplified, barcoded, and sequenced to assess relative allele contributions at SNP positions. The % Cell Product results are computed using a validated analysis pipeline and summarized in a report.

Qualification of Accurate and Precise Quantification in Cells

A Evaluation of Linearity Using Donor Cells



B Evaluation of Precision Using Donor Cells



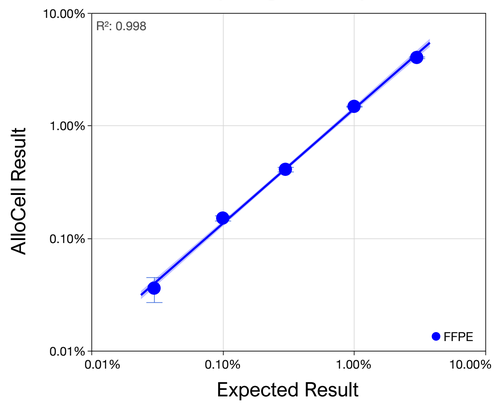
C Evaluation of Precision and Sensitivity Measurements using Cells

Sample Condition	gDNA Input (ng)	Replicate 1	Replicate 2	Replicate 3	Mean	Std	CV	Product Signal
3% Donor gDNA in Recipient gDNA	101	3.387%	3.378%	3.399%	3.388%	0.011%	0.31%	Yes
1% Donor gDNA in Recipient gDNA	119	1.133%	1.158%	1.119%	1.137%	0.020%	1.74%	Yes
0.3% Donor gDNA in Recipient gDNA	116	0.353%	0.342%	0.350%	0.348%	0.006%	1.63%	Yes
0.1% Donor gDNA in Recipient gDNA	102	0.116%	0.115%	0.118%	0.116%	0.002%	1.31%	Yes
0.03% Donor gDNA in Recipient gDNA	121	0.040%	0.041%	0.037%	0.039%	0.002%	5.30%	Yes

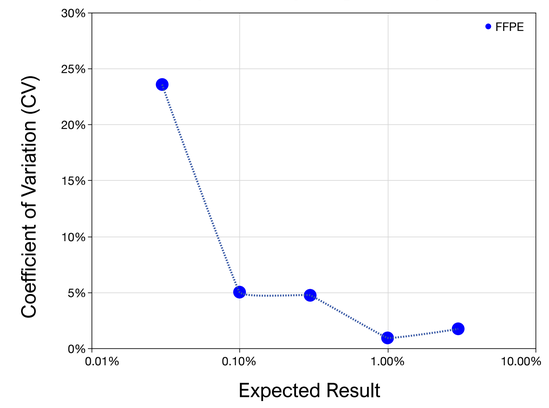
Figure 4. ADI-001 gDNA spiked into gDNA isolated from donor PBMCs (ADI-001 naïve) demonstrates accurate quantification of ADI-001. **(A)** linearity (R^2 = 1) and **(B)** precision measured by coefficient of variation over [0.03% to 3%] range. **(C)** Donor-derived genomic DNA mixtures from cells ranging from 3% to 0.03% (donor gDNA) show high precision across triplicates, with acceptable CVs ranging from 0.31% to 5.3%

Demonstrated Assay Performance in FFPE Blocks

A Evaluation of Linearity Using FFPE Samples



B Evaluation of Reproducibility Using FFPE Samples



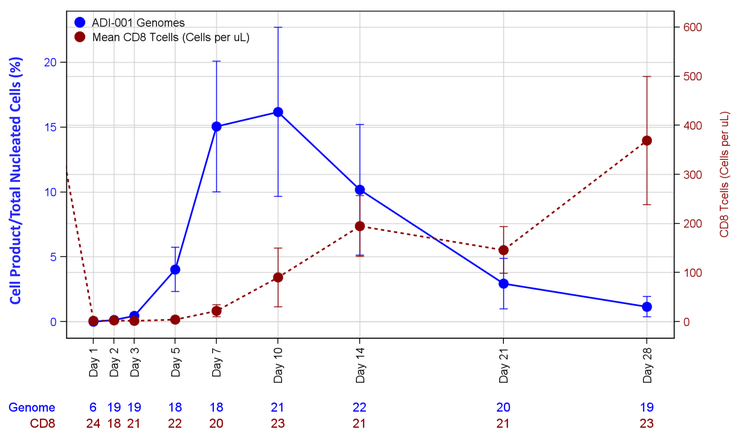
C Evaluation of Precision and Sensitivity Measurements using FFPE Blocks

Sample Condition	gDNA Input (ng)	Replicate 1	Replicate 2	Replicate 3	Mean	Std	CV	Product Signal
3% Donor gDNA in Recipient gDNA	69	4.074%	3.948%	4.064%	4.028%	0.070%	1.740%	Yes
1% Donor gDNA in Recipient gDNA	73	1.488%	1.466%	1.491%	1.482%	0.014%	0.927%	Yes
0.3% Donor gDNA in Recipient gDNA	76	0.431%	0.400%	0.396%	0.409%	0.019%	4.735%	Yes
0.1% Donor gDNA in Recipient gDNA	73	0.160%	0.149%	0.145%	0.151%	0.008%	5.018%	Yes
0.03% Donor gDNA in Recipient gDNA	53	0.043%	0.039%	0.027%	0.036%	0.009%	23.575%	Yes

Figure 5. To demonstrate accurate quantification of ADI-001 in formalin-fixed paraffin embedded (FFPE) samples, ADI-001 product lot cells were pelleted, formalin-fixed, and embedded into FFPE blocks. ADI-001 gDNA isolated from the FFPE blocks was spiked into ADI-001 naïve gDNA (isolated from FFPE tumor tissue from a pre-dose DLBCL patient). Results showed accurate quantification of ADI-001 in FFPE samples **(A)** linearity (R^2 = 0.998) and **(B)** precision measured by coefficient of variation over [0.03% to 3%] range. **(C)** For FFPE samples, AlloCell also showed a high precision, with CV = 0.93% for the 1% mixture, to a reasonable precision of CV = 23.6% at the lowest mixture level of 0.03%.

Robust Expansion of ADI-001 Measured in the Peripheral Blood

A Proportion of ADI-001 in Peripheral Blood as a Function of Lymphocyte Recovery



B Robust Dose-Dependent Expansion of ADI-001 Measured in the Peripheral Blood

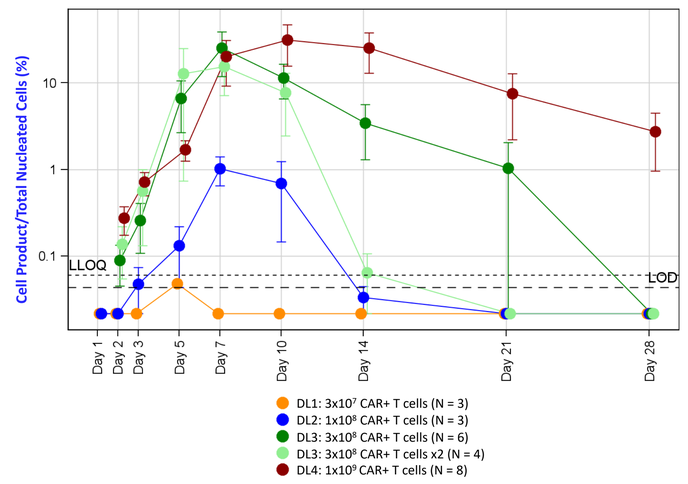


Figure 6. (A) The gross dynamic range of average ADI-001 proportionality across dose levels is shown versus the average recovery of patient CD8+ T cells. The number of samples analyzed at each timepoint are shown below the collection day as Genome (AlloCell) or CD8 (Flow). **(B)** The ADI-001 genomes were graphed by cohort using a log scale to show the sensitivity of the assay at residual exposure levels. Horizontal lines (dashed) represent the LLOQ and LLOD values of the AlloCell assay. For each dose level the number of subjects varies due to sample availability.

Detection of Distinct Expansions From Two ADI-001 Lots Derived from Distinct Donors in a Single Patient Using 3-Genome Analysis

Figure 7. Representative data from a single DLBCL patient showing distinction of Donor 1 and Donor 2. Following completion of a first cycle of enhanced lymphodepletion (eLD) and dosing with Donor 1-derived product, the patient received a second cycle of standard lymphodepletion (sLD) followed by dosing with Donor 2-derived product. Both product lots were at dose level 3 (DL3) 3 x 10^8 CAR+ cells. Results demonstrate unique detection of the first and second donor-sourced cell products using 3-genome analysis.

Summary & Conclusions

- Here we present qualification and clinical application of a quantitative and universal next generation sequencing solution (AlloCell) for monitoring of ADI-001, a first-in-class CD20-targeted allogeneic γδ1 CAR T cell therapy
- We show robust quantitation of dose-dependent expansion of ADI-001 in the peripheral blood using this method
- We demonstrate assay performance assessments in both PBMC and tissue biopsy (FFPE) samples.
- We present successful distinction and quantification of two separate and distinct doses of ADI-001 infused in a single patient using 3-genome analysis

References

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2. Neelapu SS, Stevens DA , Hamadani M, Matthew JF, Holmes H, Jacobovits A, Hinkle J, Kennedy-Wilde J, Maller O, Weinstein B, Galimi F, Lai RK, Miklos DB. A Phase I Study of ADI-001: Anti-CD20 CAR-Engineered Allogeneic Gamma Delta 1 T cells in Adults with B-cell Malignancies. Blood 140 (Supplement 1): 4617-1619 (2022). https://doi.org/10.1182/blood-2022-157400