Expansion, Persistence and Pharmacodynamic Profile of ADI-001, a First-in-Class Allogeneic CD20-targeted CAR Gamma Delta T Cell Therapy, in Patients with Relapsed/Refractory Aggressive B-cell Non-Hodgkin's Lymphoma

M. Moreno, PhD, J. Kennedy-Wilde, MS, N. Jahchan, PhD, A. Wrong, MS, F. Galimi, MD, PhD, Y. Ye, PhD, J. Wu., PhD, L. Wei, MS, R. Lai, MD, B. T. Aftab, PhD
Adicet Bio, Inc. Redwood City, CA 94065

Abstract

ADI-001 is a first-in-class allogeneic gamma delta (γδ) CAR T cell therapy targeting the B cell antigen, CD20. Expansion and persistence of cell therapy products and release of functional cytokines have historically correlated with patient outcomes. Here we report cellular kinetic and pharmacodynamic correlates from a phase 1, multicenter, open-label, dose escalation study to evaluate ADI-001 in R/R B cell NHL.

Cellular kinetics of ADI-001 were measured using three orthogonal methods, including quantitative SNP profiling of cell product (AlloCell), flow-cytometry for CAR+ Vδ1 γδ T cells, and droplet digital PCR (ddPCR) quantification of CAR transgene copies. Using these methods, expansion of ADI-001 was assessed in the peripheral blood for 24 DLT evaluable patients across four dose levels in association with a phase 1 dose-escalation trial (NCT04735471). Relationships between ADI-001 cellular kinetics and radiographic clinical responses were also examined. Overall, ADI-001 was well tolerated throughout the dose escalation including in patients dosed at DL4, particularly in comparison with the safety profile of approved autologous CD19 CAR T. Serum biomarkers related to host immune cell recovery during lymphodepletion, and cytokine release were monitored for pharmacodynamic purposes. Other correlative characteristics were also evaluated, including degree of shared HLA alleles between patient and ADI-001 product in relation to response and/or ADI-001 expansion and persistence.

ADI-001 was detected at all dose levels tested using ddPCR and flow cytometry to quantify CAR transgene copies and CAR+ Vδ1 γδ T cells, respectively. Treatment at the highest dose level (1E9, DL4), achieved a mean Cmax of 201,666 copies/μg or approximately 364 cells/μL, a mean day 28 persistent exposure of 16,553 copies/μg or approximately 27 CAR+ cells/μL, and a mean time to peak (Tmax) of 8.1 days. Cellular kinetics of consolidation dosing (following a second LD) exhibited a notable and distinguishable second expansion and exposure of ADI-001. Additional measures of ADI-001 exposure (AlloCell) further demonstrated a robust dose-dependent expansion profile of ADI-001 in the peripheral blood. Subjects with a BOR of CR or PR were observed to have a mean peak of 180,107 CAR copies/μg versus a mean peak of 20,950 CAR copies/μg in subjects with a BOR of SD or PD. Additionally, as expected following lymphodepletion, a transient increase in the homeostatic cytokine IL-15, coinciding with ADI-001 expansion and host-mediated immune cell recovery, was observed. Using high-resolution HLA typing, the degree of shared HLA alleles between allogeneic ADI-001 product and patients did not associate with degree of cellular expansion or response rate.

Manufacture of Off-The-Shelf Allogeneic ADI-001

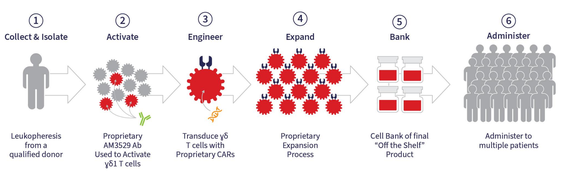
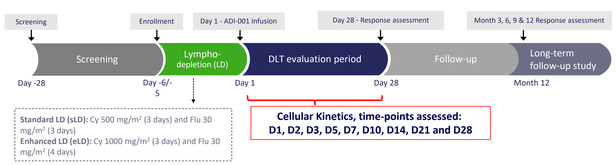


Figure 1. ADI-001 is manufactured from qualified donor leukopheresis using a proprietary activating antibody, designed to expand the Vδ1 subset of γδ T cells. CAR expression is achieved following transduction with proprietary vectors in a proprietary scalable process.

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

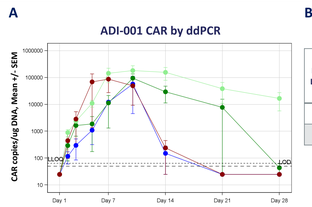
Secondary endpoint:
- ORR, DOR, PFS, TTP, and OS
- CK, immunogenicity

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).

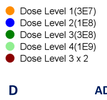
Figure 2. Study Overview. Of the 24 DLT-evaluable patients, 3 received ADI-001 at dose level 1 (DL1) (30 million CAR+ cells), 3 received ADI-001 at dose level 2 (DL2) (100 million CAR+ cells), 6 received ADI-001 at dose level 3 (DL3) (300 million CAR+ cells), 4 received two infusions of ADI-001 at DL3 (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

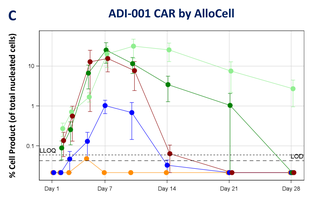
Robust Expansion and Cellular Kinetics of ADI-001 Measured in Peripheral Blood

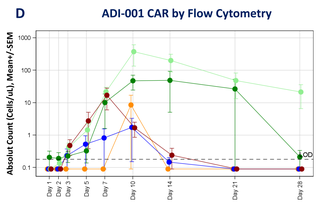


B Summary of Cellular Kinetics by ddPCR

Dose Level	Cmax		AUC_{0-28}	Tmax	Day 28 Persistence	
	CAR copies/μg	CAR T cells/μL	Days × CAR copies/μg	Days	CAR copies/μg	CAR T cells/μL
DL3	98,177	56.34	301,800	9.2	44	0.04
DL4	201,666	363.80	915,075	8.1	16,553	26.51







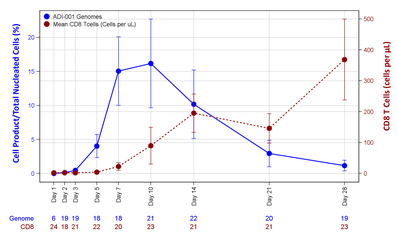


Figure 3. Cellular kinetics of ADI-001. ADI-001 was measured by **(A)** droplet digital PCR (ddPCR) with **(B)** cellular kinetic parameters summarized for DL3 and DL4 showing mean Cmax, Area under the curve (AUC_{0-28}), Tmax and Day 28 persistence. Mean DL3 and DL4 Cmax by ddPCR aligns with what was previously reported by internal flow cytometry analysis. AUC_{0-28} was calculated using a model-based cellular kinetics analysis for CAR T cells for the first 28 days after infusion (units of days*CAR copies/ug DNA). CAR cells/μL were derived from ddPCR and consider product VCN. LLOQ (64.5 CAR copies/μg) and LOD (49.5 CAR copies/μg) are shown (y-axis, dashed lines). N= 20 patients were assessed by ddPCR; N = 16 patients were assessed for AUC_{0-28}. **(C)** Quantitative SNP profiling of cell product (AlloCell) was assessed in 24 patients and plotted as Mean ±SEM. **(D)** Whole blood (WB) from 24 patients across multiple timepoints was evaluated for the presence of CAR+ Vδ1+ T cells by flow cytometry and expressed as absolute cell counts (cells/μL blood). The lower limit of quantification (LLOQ) for this assay is 3.2 cells/μL blood with a lower limit of detection (LOD) of 0.18 cells/μL blood. CAR+ Vδ1+ T cells detected below LLOQ were graphed at ½ the LOD of 0.18 cells/μL blood; internal paired flow analysis subject to verification **(E)** The gross dynamic range of average ADI-001 genomes (measured by AlloCell and expressed as % ADI-001 cells per total nucleated cells) across all dose levels is shown versus the average recovery of patient CD8+ T cells by flow cytometry (N = 24). For all measures, detection of ADI-001 returns to <LOD by the month 3 assessment.

Consolidation Dosing Regimen Results in 2nd Distinct Expansion and Additional Exposure for ADI-001

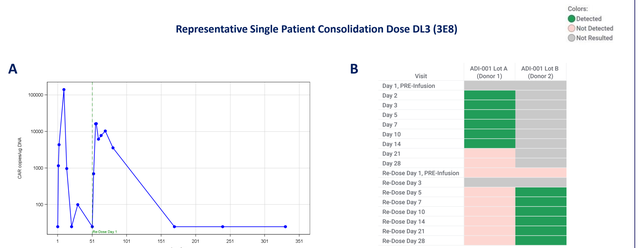


Figure 4. Representative data from a single DL3 (3E8) DLBCL patient showing distinction of product expansion and detection for two products generated from two independent donor sources (Donor 1 and Donor 2). Following completion of a first cycle of enhanced lymphodepletion (eLD) and dosing with Donor 1-derived product, the patient received a second cycle of standard lymphodepletion (sLD) followed by dosing with Donor 2-derived product. **(A)** Representative cellular kinetics by ddPCR of consolidation dosing exhibit a notable second expansion and exposure of ADI-001. **(B)** Results demonstrate unique detection of the first and second donor-sourced cell products using a single nucleotide polymorphism (SNP) 3-genome analysis. Both product lots dosed in a single patient were dosed at DL3 (3E8).

ADI-001 C_{max} and AUC_{0-28} are Associated with Clinical Response

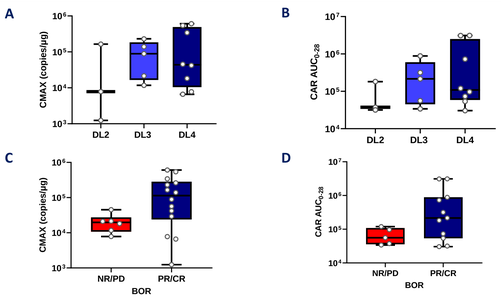


Figure 5. Relationship of ADI-001 Cellular Kinetics with Dose and Clinical Response. Relationship of **(A)** Cmax and **(B)** AUC_{0-28} with dose level. **(C and D)** Relationship of Cmax and AUC_{0-28} with best overall response (BOR) in patients. Those subjects whose BOR was complete response (CR) or partial response (PR) appear to associate with higher **(C)** Cmax (Wilcoxon Rank Sum test, two-sided P-value, N=20, P=0.0757) and **(D)** AUC_{0-28} (Mann-Whitney test, N = 16, P=0.1804) than those who had a stable disease (SD) or progressed disease (PD) as BOR. DL1 was not assessed by ddPCR due to insufficient or unavailable sample material; N = 20 patients total were assessed by ddPCR. AUC_{0-28} was calculated using a model-based cellular kinetics analysis for CAR T cells for the first 28 days after infusion (units of days*CAR copies/ug DNA).

Stimulation and Proliferation of ADI-001 is Associated with Clinical Response and Elevated Polyfunctional Serum Cytokines

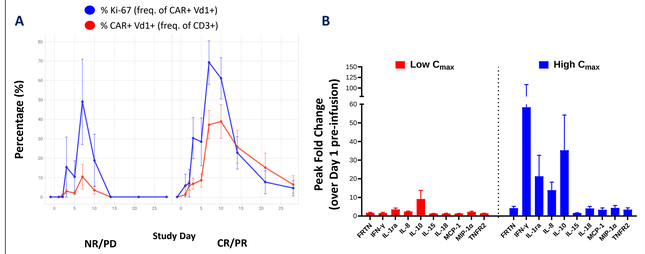


Figure 6. ADI-001 cell expansion (% of CAR+ Vd1+ cells) and proliferation (% of CAR+ Vd1+ cells expressing Ki67) were assessed in whole blood from 24 DLT evaluable patients across multiple timepoints (Day 1 pre-infusion to Day 28) using flow cytometry and shown as mean ± SEM. **(A)** Subjects whose BOR was CR or PR (N=17) appear to have higher expansion and stimulation/proliferation of CAR+ cells (%Ki-67+ CAR+ cells) than those subjects with NR/PD as BOR (N=7); Mann-Whitney, p=0.005 for Day 10 and p=0.045 for Day 14. Time-points with less than a total cell count of 10 CAR+ Vd1+ cells were plotted as 0 for % of CAR+ Vd1+ cells and % of Ki67+ cells; internal paired flow analysis subject to verification. **(B)** Relationship of ADI-001 Cmax at DL2, DL3, DL3x2, and DL4 with serum cytokine/chemokine levels represented as mean ± SEM of the peak fold change over Day 1 pre-infusion. Subjects whose Cmax was above 20,000 CAR copies/μg (High Cmax, blue bars, N=14) appear to have a higher mean peak fold change of serum cytokines/chemokines than subjects with Cmax below 20,000 CAR copies/ug (Low Cmax, red bars, N=6); Mann-Whitney, p=0.05 for IL-8, p=0.04 for IL-18, and p=0.02 for MCP-1.

Production of Polyfunctional Serum Cytokines is Associated with Clinical Response

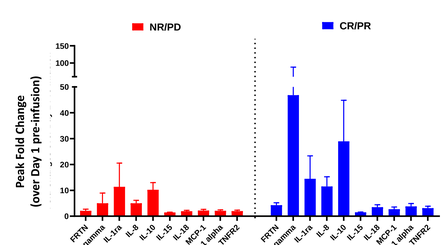


Figure 7. Relationship of ADI-001 serum cytokines/chemokines levels with BOR, represented as mean ± SEM of the peak fold change over Day 1 pre-infusion. Subjects whose BOR was CR or PR (blue bars, N=17) appear to have higher polyfunctional cytokines/chemokine mean peak fold change than subjects who had a SD or PD as BOR (red bars; N=7).

Induction of Endogenous Cytokines May Contribute to Expansion and Response

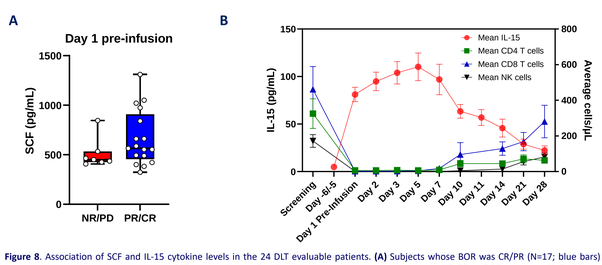
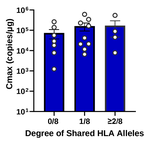


Figure 8. Association of SCF and IL-15 cytokine levels in the 24 DLT evaluable patients. **(A)** Subjects whose BOR was CR/PR (N=17; blue bars) show a trend of higher stem cell factor (SCF) production on Day 1 pre-infusion than subjects who had a NR/PD as BOR (red bars; N=7); Mann-Whitney, p=0.1823. **(B)** Interleukin-15 (IL-15) serum levels (y-axis left side, pg/mL) were examined across multiple timepoints, and immune cell reconstitution was assessed using flow cytometry (y-axis right side, cells/μL). Each timepoint represents the Mean ± SEM. As expected, following lymphodepletion, there is a transient increase in the homeostatic cytokine IL-15, coinciding with ADI-001 expansion and host-mediated immune cell recovery, which dropped close to baseline levels between D14 and D28 in all patients and for all LD regimens evaluated.

Cellular Kinetics and Response is Not Associated With the Degree of Shared HLA Alleles



B

Shared HLA Alleles	CR/PR Rate n/N (%)
0/8	7/8 (87.5)
1/8	7/11 (63.6)
≥2/8	3/5 (60.0)

Figure 9. Relationship of the degree of shared HLA alleles with cellular kinetics and response. **(A)** Relationship of Cmax with degree of shared HLA alleles between ADI-001 product and patients. The degree of shared HLA alleles does not associate with changes in Cmax. Each high expression HLA gene loci contains two alleles for a total of 8 alleles (A, B, C, and DRB1)[5]. Degree of shared HLA alleles are shown as 0/8, 1/8 or ≥2/8. **(B)** Relationship of degree of shared HLA alleles with CR/PR as best overall response (BOR). The degree of shared HLA alleles does not associate with response. n = number of patients with CR/PR as BOR. N = total number of patients in the specified shared HLA allele grouping.

Key Summary and Conclusions

- Using three orthogonal measures of exposure, we show for the first-time, robust dose-dependent expansion and persistence of an allogeneic CAR γδ T cell therapy, ADI-001.
- At DL3 and DL4, ADI-001 Cmax and Tmax were comparable to, or exceeded those demonstrated by approved autologous CD19 CAR T therapies [1-4]. Additionally, DL4 demonstrated a notable mean Day 28 persistent exposure, measured as 16,533 copies/μg or approximately 27 cells/μL.
- Higher ADI-001 Cmax and AUC_{0-28} were associated with clinical response.
- Higher ADI-001 Cmax associated with higher stimulatory and proliferative flux of CAR+ cells and coincided with production of functional serum cytokines/chemokines and increased clinical response.
- Endogenous cytokines, including SCF and IL-15, may contribute to both ADI-001 expansion dynamics and clinical response.
- Degree of shared HLA alleles between patient and ADI-001 product did not associate with differences in ADI-001 exposure or clinical response.
- Taken together ADI-001 demonstrates a robust exposure profile and is positively associated with both pharmacodynamic correlates and clinical response.

References

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