YMTHE, Volume *29*

Supplemental Information

CART19-BE-01: A Multicenter Trial of ARI-0001 Cell Therapy in Patients with CD19^{+} Relapsed/Refractory Malignancies

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SUPPLEMENTAL INFORMATION

SUPPLEMENTAL TABLES

Table S1: Adverse events (any grade) in patients with acute lymphoblastic leukemia

	Cohort 1-2 (single infusion, n=15) Occurrences/ Subjects (%)	**Cohort 3 (fractionated infusion, n=23)** Occurrences/ Subjects (%)	**All patients (n=38)** Occurrences/ Subjects (%)
Neutropenia	15/15 (100%)	24/22 (95.7%)	39/37 (97.4%)
Anemia	14/14 (93.3%)	19/18 (78.3%)	33/32 (84.2%)
Hypogammaglobulinemia	10/10 (66.7%)	22/20 (87.0%)	32/30 (78.9%)
Thrombocytopenia	15/13 (86.7%)	16/16 (69.6%)	31/29 (76.3%)
Lymphopenia	19/11 (73.3%)	17/17 (73.9%)	36/28 (73.7%)
Cytokine release syndrome	11/11 (73.3%)	10/10 (43.5%)	21/21 (55.3%)
AST increased	14/10 (66.7%)	11/9 (39.1%)	25/19 (50.0%)
ALT increased	15/9 (60.0%)	9/9 (39.1%)	24/18 (47.4%)
GGT increased	9/7 (46.7%)	8/8 (34.8%)	17/15 (39.5%)
AlkPhos increased	9/7 (46.7%)	7/7 (30.4%)	16/14 (36.8%)
Vomiting	6/5 (33.3%)	8/5 (21.7%)	14/10 (26.3%)
Febrile neutropenia	5/3 (20.0%)	6/6 (26.1%)	11/9 (23.7%)
Viral infection	11/7 (46.7%)	2/2 (8.7%)	13/9 (23.7%)
Headache	6/4 (26.7%)	7/5 (21.7%)	13/9 (23.7%)
Pyrexia	4/3 (20.0%)	3/3 (13.0%)	7/6 (15.8%)
Erythema	6/5 (33.3%)	1/1 (4.3%)	7/6 (15.8%)
Tachycardia	3/3 (20.0%)	2/2 (8.7%)	5/5 (13.2%)
Decreased appetite	2/2 (13.3%)	3/3 (13.0%)	5/5 (13.2%)
Arthralgia	1/1 (6.7%)	6/4 (17.4%)	7/5 (13.2%)
Hypotension	4/4 (26.7%)	1/1 (4.3%)	5/5 (13.2%)
Herpes zoster	3/3 (20.0%)	2/2 (8.7%)	5/5 (13.2%)
Eyelid edema	1/1 (6.7%)	5/3 (13.0%)	6/4 (10.5%)
Nausea	3/3 (20.0%)	1/1 (4.3%)	4/4 (10.5%)

	Cohort 1-2 (single infusion, n=15) Occurrences/ Subjects (%)	**Cohort 3 (fractionated infusion, n=23)** Occurrences/ Subjects (%)	**All patients (n=38)** Occurrences/ Subjects (%)
Bone pain	2/2 (13.3%)	3/2 (8.7%)	5/4 (10.5%)
Dysgeusia	0/0 (0.0%)	4/4 (17.4%)	4/4 (10.5%)
Coagulopathy	2/2 (13.3%)	0/0 (0.0%)	2/2 (5.3%)
Abdominal pain	2/2 (13.3%)	0/0 (0.0%)	2/2 (5.3%)
Toothache	2/2 (13.3%)	0/0 (0.0%)	2/2 (5.3%)
Bilirubin increased	2/2 (13.3%)	0/0 (0.0%)	2/2 (5.3%)
Acidosis	2/2 (13.3%)	0/0 (0.0%)	2/2 (5.3%)
Hypoalbuminemia	2/2 (13.3%)	0/0 (0.0%)	2/2 (5.3%)
Water intoxication	2/2 (13.3%)	0/0 (0.0%)	2/2 (5.3%)
Dizziness	2/2 (13.3%)	0/0 (0.0%)	2/2 (5.3%)
Epistaxis	4/2 (13.3%)	0/0 (0.0%)	4/2 (5.3%)
Ecchymosis	2/2 (13.3%)	0/0 (0.0%)	2/2 (5.3%)

AEs occurring in more than 10% of patients per cohort are depicted in the Table.

Table S2: Adverse events (any grade) in patients with non-Hodgkin's lymphoma

	Cohort 1-2 (single infusion, n=3) Occurrences/ Subjects (%)	**Cohort 3 (fractionated infusion, n=5)** Occurrences/ Subjects (%)	**All patients (n=8)** Occurrences/ Subjects (%)
Neutropenia	7/3 (100%)	6/5 (100%)	13/8 (100%)
Lymphopenia	6/3 (100%)	5/5 (100%)	11/8 (100%)
Anemia	7/3 (100%)	4/4 (80.0%)	11/7 (87.5%)
Thrombocytopenia	6/3 (100%)	5/4 (80.0%)	11/7 (87.5%)
Cytokine release syndrome	3/2 (66.7%)	5/5 (100%)	8/7 (87.5%)
Hypogammaglobulinemia	2/2 (66.7%)	4/4 (80.0%)	6/6 (75.0%)
GGT increased	5/3 (100%)	3/3 (60.0%)	8/6 (75.0%)
ALT increased	2/1 (33.3%)	2/2 (40.0%)	4/3 (37.5%)
AST increased	2/1 (33.3%)	2/2 (40.0%)	4/3 (37.5%)
Nausea	1/1 (33.3%)	1/1 (20.0%)	2/2 (25.0%)
Infusion site reaction	4/1 (33.3%)	1/1 (20.0%)	5/2 (25.0%)
Pneumonia	1/1 (33.3%)	1/1 (20.0%)	2/2 (25.0%)
Bilirubin increased	1/1 (33.3%)	1/1 (20.0%)	2/2 (25.0%)
AlkPhos increased	4/2 (66.7%)	0/0 (0.0%)	4/2 (25.0%)
Headache	0/0 (0.0%)	2/2 (40.0%)	2/2 (25.0%)
Syncope	0/0 (0.0%)	2/2 (40.0%)	2/2 (25.0%)
Cough	1/1 (33.3%)	1/1 (20.0%)	2/2 (25.0%)
Erythema	1/1 (33.3%)	1/1 (20.0%)	2/2 (25.0%)
Bradycardia	1/1 (33.3%)	0/0 (0.0%)	1/1 (12.5%)
Cardiac arrest	0/0 (0.0%)	1/1 (20.0%)	1/1 (12.5%)
Iridocyclitis	1/1 (33.3%)	0/0 (0.0%)	1/1 (12.5%)
Aphthous ulcer	0/0 (0.0%)	1/1 (20.0%)	1/1 (12.5%)
Diarrhea	0/0 (0.0%)	1/1 (20.0%)	1/1 (12.5%)
Dysphagia	1/1 (33.3%)	0/0 (0.0%)	1/1 (12.5%)
Vomiting	0/0 (0.0%)	1/1 (20.0%)	1/1 (12.5%)
Hemorrhoidal hemorrhage	0/0 (0.0%)	1/1 (20.0%)	1/1 (12.5%)
Palatal edema	0/0 (0.0%)	1/1 (20.0%)	1/1 (12.5%)

	Cohort 1-2 (single infusion, n=3) Occurrences/ Subjects (%)	**Cohort 3 (fractionated infusion, n=5)** Occurrences/ Subjects (%)	**All patients (n=8)** Occurrences/ Subjects (%)
Neutropenic colitis	0/0 (0.0%)	1/1 (20.0%)	1/1 (12.5%)
Peripheral edema	1/1 (33.3%)	0/0 (0.0%)	1/1 (12.5%)
Pyrexia	2/1 (33.3%)	0/0 (0.0%)	2/1 (12.5%)
Bacteraemia	0/0 (0.0%)	1/1 (20.0%)	1/1 (12.5%)
Conjunctivitis	1/1 (33.3%)	0/0 (0.0%)	1/1 (12.5%)
Influenza	0/0 (0.0%)	1/1 (20.0%)	1/1 (12.5%)
Cytomegalovirus pneumonia	0/0 (0.0%)	1/1 (20.0%)	1/1 (12.5%)
Cytomegalovirus viraemia	0/0 (0.0%)	1/1 (20.0%)	1/1 (12.5%)
Septic shock	0/0 (0.0%)	1/1 (20.0%)	1/1 (12.5%)
Skin infection	0/0 (0.0%)	2/1 (20.0%)	2/1 (12.5%)
Candida pneumonia	0/0 (0.0%)	1/1 (20.0%)	1/1 (12.5%)
Herpes ophthalmic	0/0 (0.0%)	1/1 (20.0%)	1/1 (12.5%)
Device related infection	1/1 (33.3%)	0/0 (0.0%)	1/1 (12.5%)
Viral rhinitis	0/0 (0.0%)	1/1 (20.0%)	1/1 (12.5%)
Subdural haematoma	0/0 (0.0%)	1/1 (20.0%)	1/1 (12.5%)
Creatinine increased	1/1 (33.3%)	0/0 (0.0%)	1/1 (12.5%)
Uric acid increased	2/1 (33.3%)	0/0 (0.0%)	2/1 (12.5%)
Hyperglycaemia	0/0 (0.0%)	1/1 (20.0%)	1/1 (12.5%)
Hypernatraemia	0/0 (0.0%)	1/1 (20.0%)	1/1 (12.5%)
Hypokalaemia	0/0 (0.0%)	1/1 (20.0%)	1/1 (12.5%)
Arthralgia	1/1 (33.3%)	0/0 (0.0%)	1/1 (12.5%)
Myopathy	0/0 (0.0%)	1/1 (20.0%)	1/1 (12.5%)
Neoplasm skin	0/0 (0.0%)	1/1 (20.0%)	1/1 (12.5%)
Superior vena cava syndrome	1/1 (33.3%)	0/0 (0.0%)	1/1 (12.5%)
Urinary tract obstruction	0/0 (0.0%)	1/1 (20.0%)	1/1 (12.5%)
Acute kidney injury	0/0 (0.0%)	1/1 (20.0%)	1/1 (12.5%)
Penile edema	1/1 (33.3%)	0/0 (0.0%)	1/1 (12.5%)
Catarrh	1/1 (33.3%)	0/0 (0.0%)	1/1 (12.5%)

	Cohort 1-2 (single infusion, n=3) Occurrences/ Subjects (%)	**Cohort 3 (fractionated infusion, n=5)** Occurrences/ Subjects (%)	**All patients (n=8)** Occurrences/ Subjects (%)
Pneumonia viral	0/0 (0.0%)	1/1 (20.0%)	1/1 (12.5%)
Respiratory failure	0/0 (0.0%)	1/1 (20.0%)	1/1 (12.5%)
Seborrheic dermatitis	0/0 (0.0%)	1/1 (20.0%)	1/1 (12.5%)
Toxic epidermal necrolysis	0/0 (0.0%)	1/1 (20.0%)	1/1 (12.5%)
Shock	1/1 (33.3%)	0/0 (0.0%)	1/1 (12.5%)

AEs occurring in more than 10% of patients per cohort are depicted in the Table.

Table S3: Cytokine levels (all patients with results available)

	Day +7 value (pg/mL) (median, range)	**Peak value (pg/mL)** (median, range)	**Day +7/pre-administration ratio** (median, range)
Interferon-gamma	8.3 (0.3-32031)	22.2 (0.3-32031)	1.56 (0.14-10010)
Interleukin-2	3.2 (0.3-37.4)	3.2 (0.3-37.4)	1 (0.4-29.6)
Interleukin-6	3.2 (0.1-10000)	3.2 (0.1-10000)	1.05 (0.059-4673)
Interleukin-8	11.0 (1.7-212.8)	21.9 (1.8-492.6)	1.29 (0.12-81.3)
Interleukin-10	26.4 (0.2-10000)	42.8 (0.2-10000)	1.6 (0.15-915.8)
Interleukin-15	7.9 (0.8-83)	11.5 (3.2-83)	0.56 (0.1-5.5)
Tumor necrosis factor alpha	14.1 (0.5-148.7)	26.7 (1.1-148.7)	1.4 (0.5-59)

SUPPLEMENTAL FIGURES

Figure S1: Cumulative incidence of cytokine release syndrome (any grade) in patients with acute lymphoblastic leukemia according to type of administration [cohort 1-2 (blue curve – single dose) vs. cohort 3 (yellow curve - fractionated)]. This difference was statistically significant (P = 0.025, Gray's test).

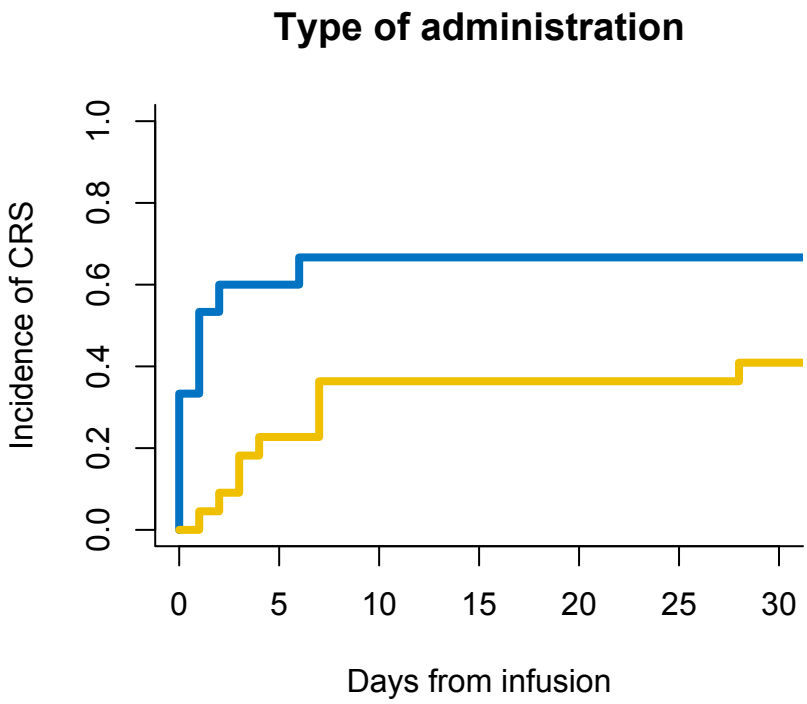


Figure S2: ARI-0001 expansion, normal B-cells, and human anti-murine antibodies (HAMAs) over time in all patients who received a second cell infusion. Patients in panels A-E were diagnosed with acute lymphoblastic leukemia while the patient depicted in panel F was diagnosed with diffuse large B-cell lymphoma. ARI-0001 expansion was measured using quantitative PCR in copies per ng of genomic DNA, normal B-cells were measured as percentage of all lymphocytes and HAMAs were measured as percentage of positive cells.

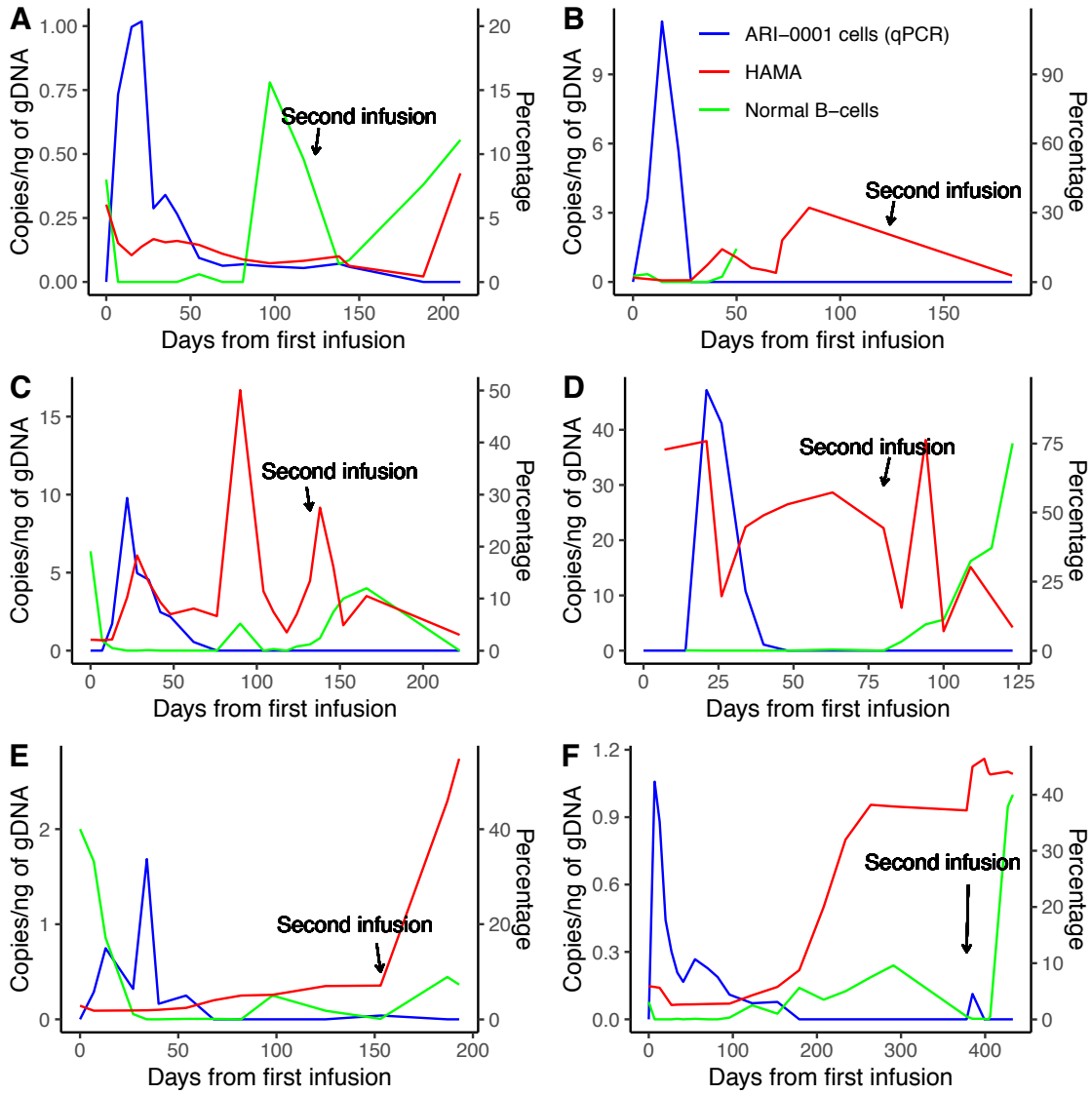


Figure S3: Simon-Makuch curve depicting the impact of B-cell aplasia on progression-free survival in patients with acute lymphoblastic leukemia (blue curve = sustained B-cell aplasia, yellow curve = B-cell recovery). The difference was not statistically significant (P = 0.33, Mantel-Byar test).

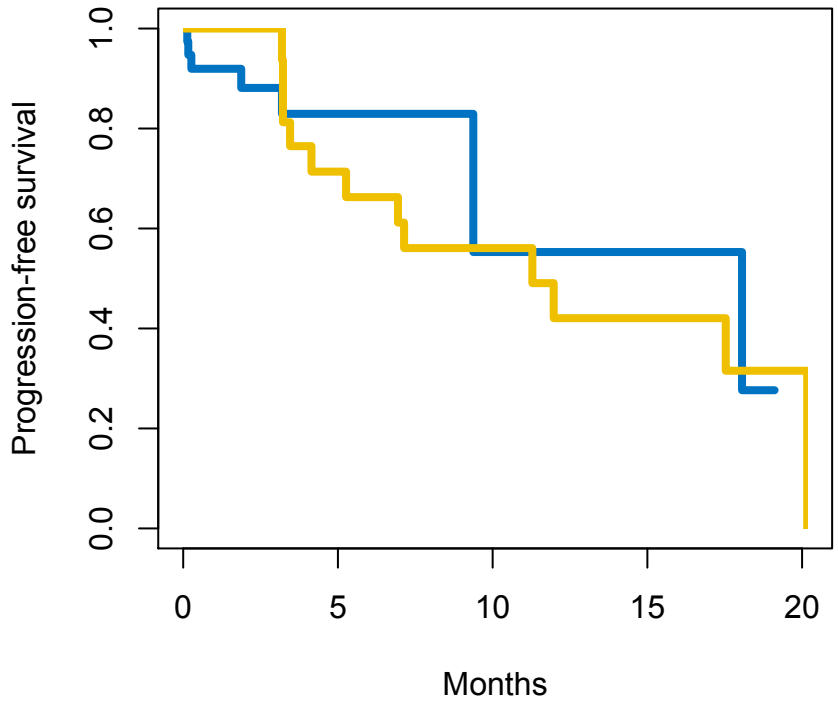
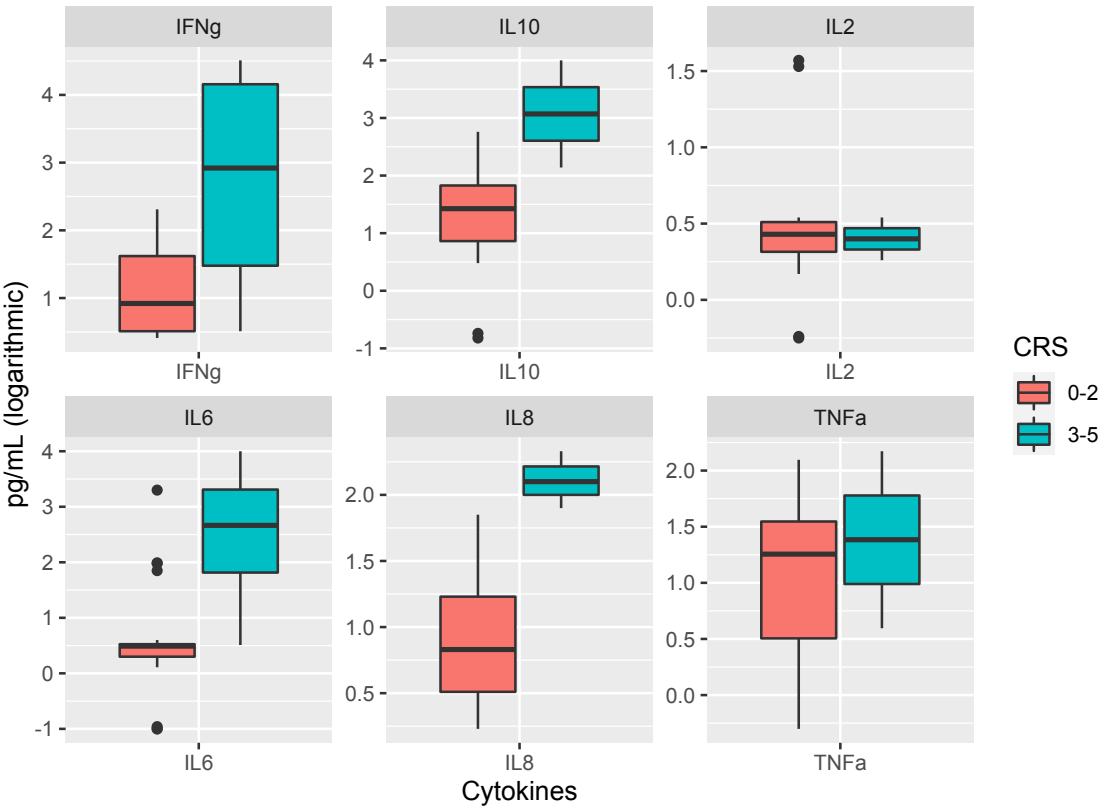
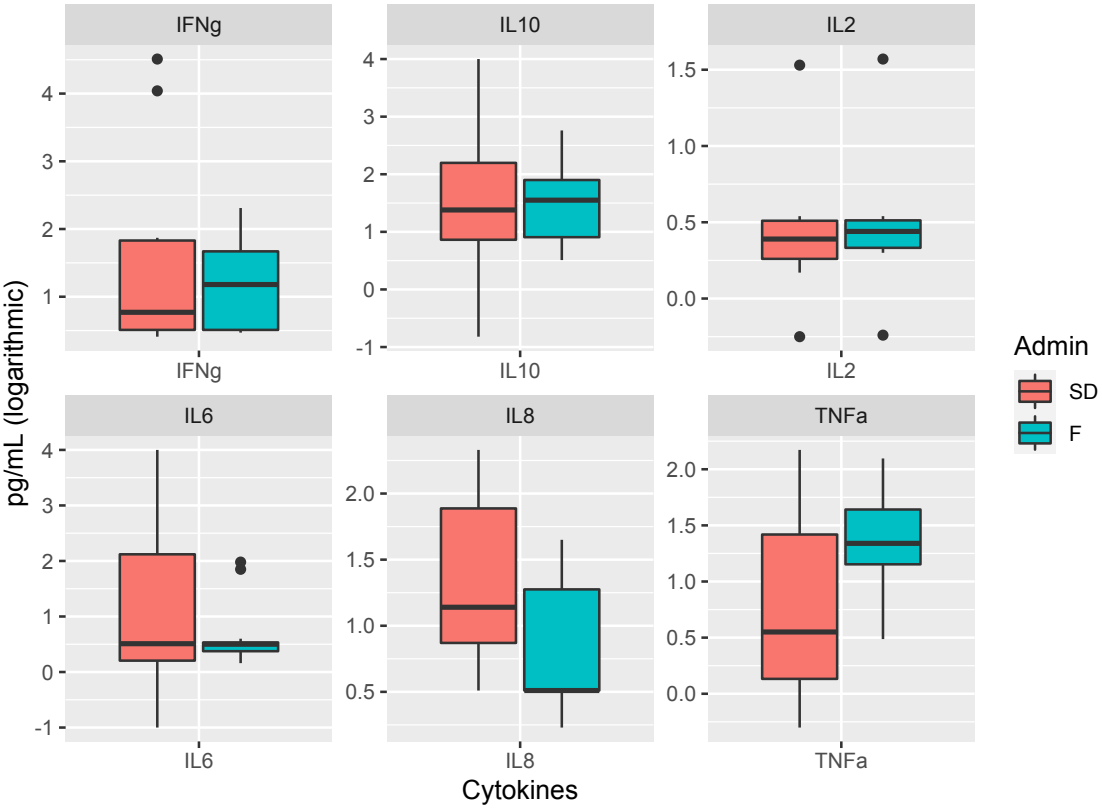


Figure S4: Cytokine levels (patients with acute lymphoblastic leukemia with results available on day +7) according to the presence of cytokine release syndrome (grade 0-2 vs. grade ≥3)



Differences were only statistically significant (Wilcoxon's rank sum test) for IL6 (P = 0.018) and borderline significant for IL8 (P = 0.11) and IL10 (P = 0.065)

Figure S5: Cytokine levels (patients with acute lymphoblastic leukemia with results available on day +7) according to the type of ARI-0001 administration (cohort 1-2 [single dose] vs. cohort 3 [fractionated])



There were no statistically significant differences (Wilcoxon's rank sum test).

Figure S6: ARI-0001 expansion over time, as calculated by quantitative PCR, in patients with acute lymphoblastic leukemia according to age (younger or older than 18 years). Lines represent median values

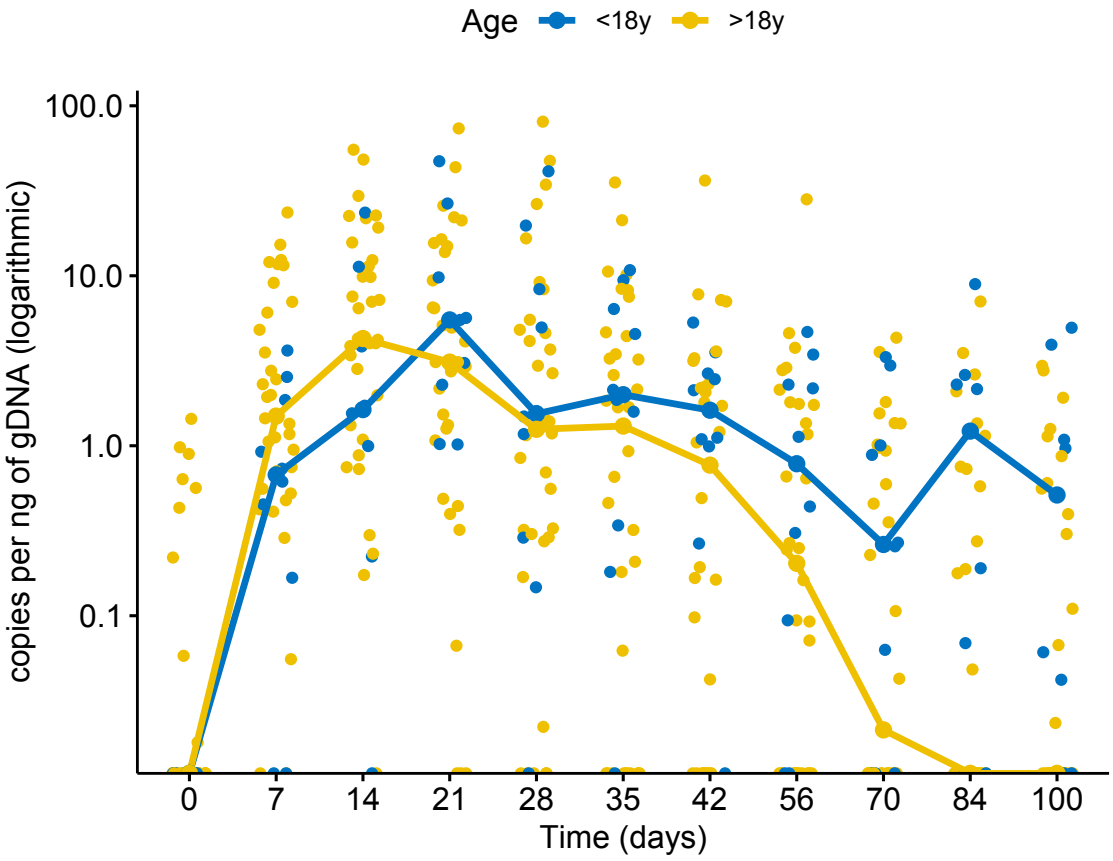
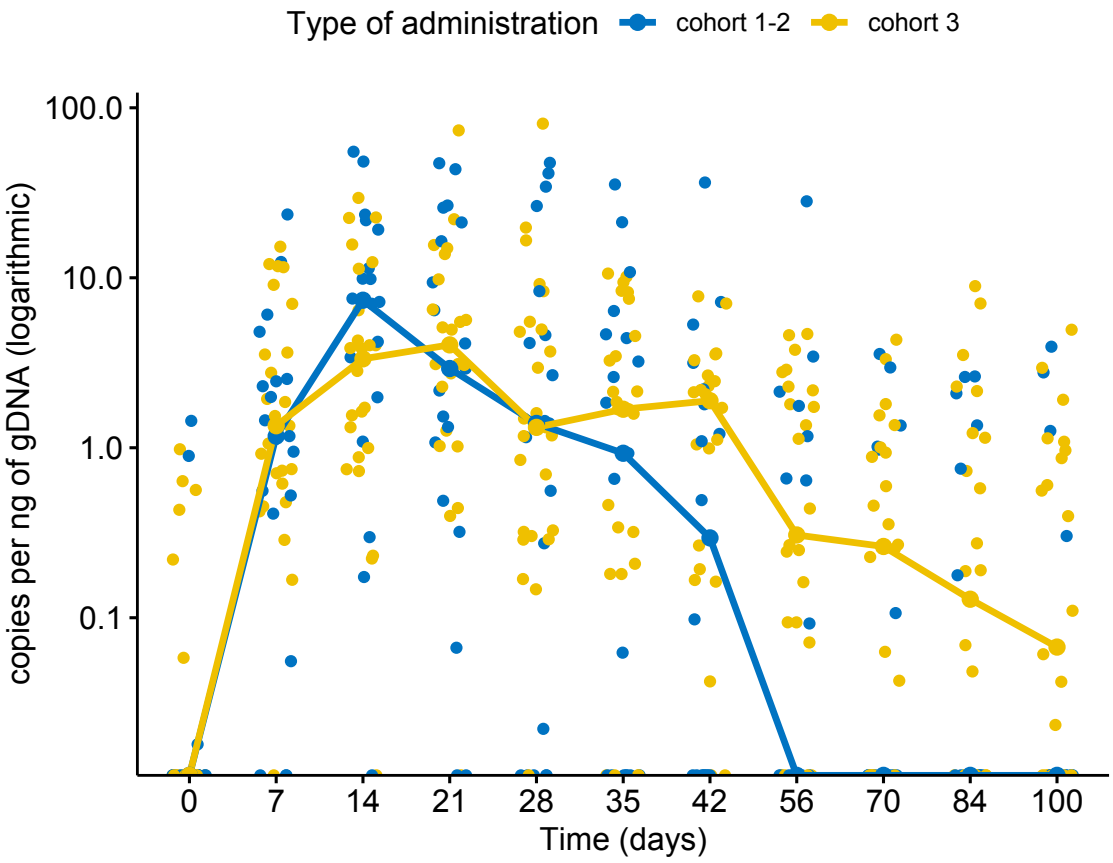


Figure S7: ARI-0001 expansion over time, as calculated by quantitative PCR, in patients with acute lymphoblastic leukemia according to type of administration (single dose [cohort 1-2] vs. fractionated [cohort 3]). Lines represent median values



SUPPLEMENTARY METHODS

Measurement of serum cytokines

Patient serum was extracted on day 0 (prior to ARI-0001 administration), weekly for the first two months and then monthly for the first year. Serum samples were processed and stored at −80°C for subsequent analysis by the Cytokine Human Magnetic 16-Plex Panel for the Luminex platform, specifically designed for quantifying human IFN-γ, IL2, IL6, IL8, IL10 and TNF-α (Millipore). All assays were carried out according to the manufacturer's specifications. Quality and assay standard controls were included for independent runs per the manufacturer's protocol. Luminex assays were read using a Luminex 200 system.

Measurement of ARI-0001 cells and CAR19 transgene

ARI-0001 cells presence was evaluated by flow cytometry with an APC-conjugated AffiniPureF(ab')$_2$-fragment goat-anti-mouse IgG monoclonal antibody (goat-anti-mouse IgG, Jackson ImmunoResearch Laboratories). Moreover, a quantitative PCR assay (qPCR) was optimized and validated for monitoring ARI-0001 cell expansion and persistence. The number of transgene copies per ng of genomic DNA (gDNA) was determined using Light Cycler® 480 SYBRGreen® I Master (Roche, Cat. N. 04707516001). Pairs of primers were designed against the GATA2 gene (control, with just one copy per genome) and WPRE sequence (part of the CAR transgene). Primer sequences are as follows: GATA2_F: 5'tggcgcacaactacatggaa 3'; GATA2_R: 5'cgagtcgaggtgattgaagaaga 3'; WPRE_F: 5'gtcctttccatggctgctc 3'; WPRE_R: 5'ccgaagggacgtagcaga 3'. The absolute quantification method was used to determine the number of copies. Standard curves were prepared using 1:10 serial dilutions of plasmids containing GATA2 or transgene. The final number of molecules in the reaction ranged from 10^2 to 10^8. For GATA2 quantification, GATA2 cDNA was cloned in a pCRII-Topo vector (Invitrogen). The pCCL-CAR19 vector was used in the same way to quantify the transgene copy number. The following PCR program was used: 1) Initial denaturalization: 95ºC, 5'; 2) 40 cycles of: 95ºC, 10''; 58ºC, 10''; 72ºC, 5''; 3) melting curve. qPCR values are therefore expressed as copies of transgene per ng of gDNA.

Human anti-murine antibodies

The presence of human anti-murine antibodies (HAMA) was monitored in patient serum (same timings as for cytokine levels) by cell fluorescence. The HEK293T cell line was transduced with the CAR19 lentivirus (multiplicity of infection of 2) in the presence of polybrene (8 mcg/mL, Santa Cruz Biotechnology) for 48 hours at 37ºC. Once CAR19 expression was verified by flow cytometry, the transduced HEK293T cell line was incubated with the patients' serum. The presence or absence of anti-CAR19 antibodies (HAMAs) was determined by flow cytometry using a FITC-conjugated anti-human IgG antibody (Life Technologies). A sample was considered positive when the percentage of positive cells was greater than 20%, and borderline positive when the percentage of positive cells ranged from 10 to 20%.

Analysis of ARI-0001 cell product

As part of standard release criteria, ARI-0001 products were evaluated for anti-CD19 CAR surface expression and percentage of CD3+, CD4+ and CD8+ T cells. More than 20% of ARI-0001 cells and 70% CD3-positive cells were required, with no particular CD4/CD8 ratio. Samples were run through the fluorescence-activated cell sorting (FACS) flow cytometer BD FACSCanto II (BD Biosciences) and data analyzed using the BD FACSDiva Software. Cell viability was measured using the trypan blue exclusion method, and required to be over 70%. The presence of bacteria or adventitious viruses was ruled out by conventional microbiologic or molecular tests. The absence of endotoxin was confirmed using a kinetic chromogenic assay. Detailed results of the first 28 products were published elsewhere (Castella et al. Frontiers in Immunology 2020).

Additional statistical methods

Comparisons between qualitative variables (presence of HAMAs, disease relapse, loss of B-cell aplasia) were performed using Chi-square or Fisher's exact test as appropriate. The association between cytokine levels and the occurrence of CRS or type of ARI-0001 administration (single dose vs. fractionated) was evaluated using Wilcoxon's rank sum test. The area under the curve for ARI-0001 expansion (as per qPCR) was calculated using the 'AUC' function (spline method) from the 'DescTools' package. The cumulative incidence of CRS was plotted using the 'cmprsk' package and the difference between single-

dose and fractionated cohorts was evaluated using Gray's test. The impact of persistence of B-cell aplasia on progression-free or overall survival was evaluated using the Mantel-Byar method, and graphically represented by means of a Simon-Makuch curve (R Foundation for Statistical Computing, Vienna, Austria).