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

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We evaluated the administration of ARI-0001 cells (chimeric antigen receptor T cells targeting CD19) in adult and pediatric patients with relapsed/refractory CD19+ malignancies. Patients received cyclophosphamide and fludarabine followed by ARI-0001 cells at a dose of $0.4–5 \times 10^6$ ARI-0001 cells/kg, initially as a single dose and later split into 3 fractions (10%, 30%, and 60%) with full administration depending on the absence of cytokine release syndrome (CRS). 58 patients were included, of which 47 received therapy: 38 with acute lymphoblastic leukemia (ALL), 8 with non-Hodgkin's lymphoma, and 1 with chronic lymphocytic leukemia. In patients with ALL, grade ≥3 CRS was observed in 13.2% (26.7% before versus 4.3% after the amendment), grade ≥3 neurotoxicity was observed in 2.6%, and the procedure-related mortality was 7.9% at day +100, with no procedure-related deaths after the amendment. The measurable residual disease-negative complete response rate was 71.1% at day +100. Progression-free survival was 47% (95% IC 27%–67%) at 1 year: 51.3% before versus 39.5% after the amendment. Overall survival was 68.6% (95% IC 49.2%–88%) at 1 year. In conclusion, the administration of ARI-0001 cells provided safety and efficacy results that are comparable with other academic or commercially available products. This trial was registered as ClinicalTrials.gov: NCT03144583.

INTRODUCTION

Despite currently available therapies, most patients with relapsed/refractory (R/R) B cell malignancies such as acute lymphoblastic leukemia (ALL) and diffuse large B cell lymphoma (DLBCL) remain incurable. R/R ALL is associated with a complete response rate (CRR) around 30%–45% and a median overall survival (OS) around 4–8 months, depending on age, type of salvage therapy, and response to it.[1–4] The prognosis is particularly poor for patients relapsing after allogeneic hematopoietic cell transplantation (alloHCT).[5,6] Novel agents such as inotuzumab ozogamicin (IO) or blinatumomab have increased the CR rate in this patient population, but responses are

Signed informed consent (n = 58)

Not meeting inclusion criteria (n = 2)
Redirected to commercial product (n = 2)

Apheresis performed and cell
production attempted (n = 54)

Physician's decision (n = 2)
Exclusion criteria (n = 1)
Death (n = 1)
Production failure (n = 3)

ARI-0001 cells infused (n = 47)

Continue on study (n = 19)
Discontinued (n = 28):
- Disease progression/death (n = 19)
- Death without progression (n = 3)
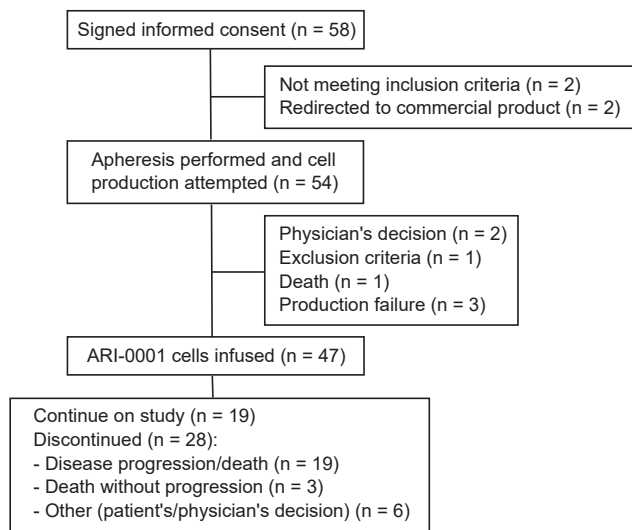- Other (patient's/physician's decision) (n = 6)

Figure 1. Consort Diagram of Patients Included in the CART19-BE-01 Trial

generally not durable.[7,8] Outcomes are also poor in patients with R/R DLBCL, defined as no response to the last line of chemotherapy or relapse within 1 year of autologous hematopoietic cell transplant (autoHCT). A meta-analysis of patients with refractory DLBCL found that these patients achieve an overall response rate of 26% and median OS of 6.6 months.[9]

Adoptive cell therapy with T cells genetically engineered to express chimeric antigen receptors (CAR) targeting CD19 is a well-established approach for the treatment of B cell malignancies.[10] These CAR constructs usually comprise an extracellular single-chain variable fragment (scFv) of a monoclonal antibody (mAb) attached to a transmembrane domain and two or more signaling domains. Two of these cell products (tisagenlecleucel and axicabtagene ciloleucel) were recently approved by the Food and Drug Administration (FDA) and European Medicines Agency (EMA) for the treatment of R/R ALL and DLBCL. Both products share the same scFv (derived from the FMC63 mAb) but have a different composition, including a different co-stimulatory domain and transduction vector, and yet both have remarkable clinical efficacy and a comparable safety profile.[11–13]

In 2013, we started developing our own CAR19 construct. We identified the scFv sequence from our own CD19-A3B1 hybridoma, incorporated a transmembrane CD8 domain, a 4-1BB costimulatory domain, and a CD3z signaling domain next to it, cloned the sequence into a 3rd generation lentiviral vector, and transduced healthy-donor T cells.[14] Once the preclinical tests were completed, we scaled up both the lentiviral and cell production, this leading to the Spanish Agency for Medicines and Health Products (AEMPS) approval of our investigational new drug, called ARI-0001 cells (IMP no 16-187), and also this clinical trial (CART19-BE-01) in May 2017. Here we present the results of this clinical trial.

RESULTS

Baseline Characteristics

58 patients were enrolled in the study, but 4 of them never made it through the screening phase: 2 patients did not comply with inclusion/exclusion criteria and 2 were eventually referred for therapy with commercial products that became available at the time (Figure 1). Out of the remaining 54 patients, 47 received therapy with ARI-0001 cells, 19 in cohorts 1 and 2 (single dose infusion) and 28 in cohort 3 (fractionated infusion). These 47 patients (modified full analysis set [mFAS]) were diagnosed with ALL (38), DLBCL (4, one of them a Richter's transformation from chronic lymphocytic leukemia [CLL]), primary mediastinal B cell lymphoma,[2] follicular lymphoma (FL),[2] and CLL.[1] Baseline characteristics of patients included in the mFAS are shown in Table 1. Median age was 26 years (range, 3–67), and 17 patients (36%) were female. The full details of the only CLL patient were published elsewhere.[15] The data cutoff date was November 5, 2019, when all infused patients had a minimum follow-up of 100 days or had experienced disease relapse or death. At that time, the median follow-up for survivors was 5.5 months (range, 1.9–23.6) from ARI-0001 cell infusion.

Out of 54 patients who proceeded to apheresis, 47 (87%) and 7 (13%) required one and two procedures, respectively. Reasons for a second procedure were bacterial contamination of the cell product[5] and insufficient viral transduction.[2] All infused patients received fludarabine + cyclophosphamide lymphodepletion and received ARI-0001 cells a median of 54 days (range, 34–215) after study inclusion. The median vein-to-vein time was 42 days (range, 25–190) for the entire population. The original target dose ranged from 0.5 to 5 × 10^6 ARI-0001 cells/kg, with the condition imposed by the AEMPS that the first patient had to receive the minimum dose (0.5 × 10^6 ARI-0001 cells/kg). In cohort 3, one patient received 0.4 × 10^6 ARI-0001 cells/kg (i.e., the last fraction was omitted) due to cytokine release syndrome (CRS).

Toxicity

All adverse events (AEs) occurring from study inclusion, even before ARI-0001 cell infusion, were graded and reported (Tables S1 and S2). Grade ≥ 3 AEs were documented in 68.4% of patients with ALL and 75% of patients with non-Hodgkin's lymphoma (NHL) at day +100, whereas serious AEs (SAEs) were observed in 44.7% and 50% of patients with ALL and NHL, respectively (Table 2). Procedure-related mortality (PRM) at day +100 was 7.9% (95% confidence interval [CI] 1.7%–21.4%) for patients with ALL and 0% for patients with NHL. Suspected unexpected serious adverse reactions (SUSARs) were observed in 4 patients: 2 patients (aged 11 and 19, respectively) developed lethal CRS and a 35-year-old patient died of pseudomembranous colitis while recovering from grade 4 CRS. These 3 patients, belonging to cohorts 1 and 2, motivated the second major amendment of the study as previously described. The fourth SUSAR was reported in a patient with FL from cohort 3 who developed grade 4 toxic epidermal necrolysis while recovering from grade 2 CRS. In the last case, causality was investigated but could not be demonstrated (i.e., ARI-0001 cells could not be identified in the skin).

Table 1. Patients' Demographics and Baseline Characteristics

Characteristic	Cohorts 1 and 2 (Single Infusion)	Cohort 3 (Fractionated Infusion)	All Patients
Acute lymphoblastic leukemia	n = 15	n = 23	n = 38
Age (y), median (range)	20 (3–35)	29 (4–67)	24.5 (3–67)
Female sex, n (%)	4 (27)	10 (44)	14 (37)
ECOG performance status ≥ 1, n (%)	4 (31)	5 (22)	9 (25)
Prior regimens, median (range)	4 (3-10)	4 (2–8)	4 (2–10)
Prior inotuzumab, n (%)	4 (27)	9 (39)	13 (34)
Prior blinatumomab, n (%)	3 (20)	6 (26)	9 (24)
Prior allogeneic HCT, n (%)	11 (73)	22 (96)	33 (87)
Bone marrow blast cell count ≥ 5%, n (%)	10 (67)	7 (30)	17 (45)
Non-Hodgkin's lymphoma	n = 3	n = 5	n = 8
Histology:			
● Diffuse large B cell lymphoma	1 (33.3)	3 (60)	4 (50)
● Primary mediastinal B cell lymphoma	2 (66.6)	0	2 (25)
● Follicular lymphoma	0 (0)	2 (40)	2 (25)
Age (y), median (range)	25 (19–51)	50 (22–62)	47.5 (19–62)
Female sex, n (%)	1 (33)	2 (40)	3 (37.5)
ECOG performance status ≥ 1, n (%)	1 (33)	3 (60)	4 (50)
Prior regimens, median (range)	6 (5–7)	6 (4–9)	6 (4–9)
Prior allogeneic/autologous HCT, n (%)	3 (100)	1 (20)	4 (50)

HCT, hematopoietic cell transplantation; NA, not applicable/available.

Regarding AEs of special interest, CRS was reported in 55.3% (13.2% grade ≥ 3) and 87.5% (25% grade ≥ 3) of patients with ALL and NHL, respectively. In patients with ALL, we observed a reduction in the rate of grade ≥3 CRS after the second amendment, dropping from 26.7% (cohorts 1 and 2) to 4.3% (cohort 3; Table 2), this leading to a reduction in tocilizumab administration from 26.7% (cohorts 1 and 2) to 8.7% (cohort 3), and a reduction in corticosteroid use from 20% (cohorts 1 and 2) to 0% (cohort 3). Due to CRS, 1/23 (4%) patients from cohort 3 received only two ARI-0001 cell fractions, while no patient received only one fraction. The occurrence of CRS (any grade) was also reduced and delayed in cohort 3 (Figure S1). Moreover, grade ≥ 3 immune effector-cell associated neurotoxicity syndrome (ICANS) was only observed in 1 (2.6%) patient with ALL. The only grade ≥ 3 s malignancy observed in the study was myelodysplasia in a 7-year-old girl diag-

nosed with ALL who had already received 6 lines of therapy, including IO and alloHCT.

Globally speaking, the most common AEs in patients with ALL were neutropenia (97.4%), anemia (84.2%), hypogammaglobulinemia (78.9%), thrombocytopenia (76.3%), and lymphopenia (73.7%). Liver toxicity was also frequent, including increased AST (50%), increased ALT (47.4%), increased GGT (39.5%) and increased alkaline phosphatase (36.8%), mostly in patients with prior alloHCT (Table S1). Similar figures were observed in patients with NHL (Table S2). Two patients with ALL (2/38, 5%) with prior history of alloHCT and IO therapy developed severe hepatic sinusoidal obstruction syndrome (SOS) that resolved with conventional supportive care.

Efficacy

In patients with ALL, the measurable residual disease (MRD)-negative CRR was 84.2% (95% CI 69%–94%) at day +28 and 71.1% (95% CI 54%–85%) at day +100. All evaluable patients (i.e., excluding those who died prematurely) developed absolute B cell aplasia that lasted for a median of 100 days (95% CI 56–100 days). Progression-free survival (PFS) at 1 year was 47% (27%–67%) for the whole ALL cohort, while the 1-year OS was 68.6% (49%–88%; 78% for children, 65% for adults; Figure 2). The median duration of response (DOR), considering only patients who responded to therapy by day +100, was 14.8 months. Out of 15 patients with progressive disease after ARI-0001 cell infusion, tumor cells expressed CD19 in 13 (87%), while 2 (13%) were CD19-negative. When we considered all patients with ALL recruited into the study, including those who did not receive therapy (n = 43), the 1-year PFS and OS were 43.5% (24%–63%) and 77.7% (62%–93%), respectively.

Subgroup analyses according to type of administration (cohorts 1 and 2 versus cohort 3) and age are depicted in Table 3. The apparent lower response rate observed in the pediatric population at day +100 is due to the early administration of a second ARI-0001 cell dose before that day in 2 patients. Both patients were in MRD-negative CR on day +100 but received the second infusion shortly before this time point due to early B cell recovery. If we count them both as responders, the CRR for pediatric patients would be 72% instead of 55%, and the CRR of the entire population would be 76% instead of 71%. On the other hand, the B cell aplasia lasted for longer in the pediatric population compared to adult patients (48% versus 10.5% at 1 year).

In patients with NHL, the overall response rate at day +100 was 75% (35%–97%), while the CRR was 50% (16%–84%). Full details, including the patient with CLL, are depicted in Figure 3.

Cytokines

Cytokine levels were available from 34 patients, 19 diagnosed with ALL, 5 diagnosed with NHL, and 1 diagnosed with CLL. The most informative cytokine levels were those determined at day +7, but peak values are also displayed for reference. The median absolute and relative values (to the pre-administration level) are depicted in

Table 2. Adverse Events of Special Interest

	Cohorts 1 and 2 (Single Infusion)	Cohort 3 (Fractionated Infusion)	All Patients
Acute lymphoblastic leukemia	n = 15	n = 23	n = 38
Grade ≥ 3 adverse events, n (%)	11 (73.3)	15 (65.2)	26 (68.4)
Severe adverse events, n (%)	8 (53.3)	9 (39.1)	17 (44.7)
Grade ≥ 3 CRS, n (%)	4 (26.7)	1 (4.3)	5 (13.2)
Grade ≥ 3 ICANS, n (%)	1 (6.7)	0 (0.0)	1 (2.6)
Grade ≥ 3 s malignancies, n (%)	1 (6.7)	0 (0.0)	1 (2.6)
Non-Hodgkin's lymphoma	n = 3	n = 5	n = 8
Grade ≥ 3 adverse events, n (%)	3 (100)	3 (60)	6 (75)
Severe adverse events, n (%)	3 (100)	1 (20)	4 (50)
Grade ≥ 3 CRS, n (%)	1 (33.3)	1 (20)	2 (25)
Grade ≥ 3 ICANS, n (%)	0 (0)	0 (0)	0 (0)
Grade ≥ 3 s malignancies, n (%)	0 (0)	0 (0)	0 (0)

CRS, cytokine release syndrome; ICANS, immune effector cell-associated neurotoxicity syndrome.

Table S3. Figure S4 shows the association between cytokine levels on day +7 and the development of CRS for patients with ALL. The only cytokine significantly associated with the occurrence of CRS was IL-6. Figure S5 shows the non-significant impact of fractionated administration of ARI-0001 cells on cytokine levels on day +7, also for patients with ALL.

ARI-0001 Expansion

ARI-0001 cells expanded with a median area under the curve (AUC) of 129.1 copies per cell per day (range: 15.6–4271.3). Peak expansion occurred at a median of 14.5 days after infusion (range: 2–236 days). There was no correlation between the AUC of ARI-0001 expansion and patient's age (younger or older than 18 years), type of administration (cohorts 1 and 2 [single dose] versus cohort 3 [fractionated]), development of CRS, diagnosis (ALL versus NHL), bone marrow blast percentage at study inclusion or response rate. Representative plots are displayed in Figures S6 and S7.

Human Anti-murine Antibodies

In patients with ALL, human anti-murine antibodies (HAMAs) were clearly detected (>20% positive cells by flow cytometry) in 9 patients, but we observed borderline results (10%–20% positive cells) in 5 further patients. Of note, 3 patients already had HAMAs before ARI-0001 cell infusion, which is quite intriguing because 2 of them were pediatric patients who had not received any monoclonal antibodies or an alloHCT. 2 of these patients experienced B cell recovery

1.8 and 3.2 months after ARI-0001 cell infusion but have not experienced disease relapse with current follow-up (one of them received a second infusion and is depicted in Figure S2D). The third patient who had detectable HAMAs before the cell infusion died of severe CRS. Out of the remaining 6 patients with HAMAs, 2 of them developed HAMAs after the second infusion of ARI-0001 cells (Figures S2A and S2E) and 1 before the second infusion of ARI-0001 cells (Figure S2F).

There was no significant association between the presence of HAMAs and B cell recovery or disease relapse (Fisher's exact test). Furthermore, there was no association between persistence of B cell aplasia and PFS (Mantel-Byar test, Figure S3).

Second ARI-0001 Cell Infusion

In cohorts 2 and 3, a second infusion was allowed as per protocol, and this was performed in 6 patients, 2 due to CD19+ relapse and 4 due early B cell recovery. Unfortunately, it only resulted in brief responses and/or B cell aplasia, and also led to the appearance of HAMAs in 3 patients (Figure S2).

DISCUSSION

To our knowledge, this is one of the first European academic clinical trials of CART19-cell therapy in patients with B cell malignancies, and certainly the first with the new construct A3B1:CD8:4-1BB:CD3z. The preliminary results observed in the first 10 patients led to the extension of the trial to include 54 patients in total, most of them diagnosed with ALL. An important caveat is that our study included both pediatric and adult patients with ALL, which are known to have a different prognosis,[16] and this complicates the comparison with other studies. Other important differences are that (1) 87% of our patients with ALL had already received an alloHCT, compared to 33%–62% in other studies;[11,17–21] and (2) response evaluation was performed at day +100, 4–10 weeks later than in other studies.[11,17–21] This late response evaluation was necessary to capture ARI-0001 activity in patients with both ALL and NHL.

The preparation of ARI-0001 cells could be accomplished with one leukocytapheresis procedure in 87% of patients, and the cell production time ranged from 7 to 10 days. Still, the median vein-to-vein time was 42 days (range, 25–190) mostly due to 7 patients who required 2 aphereses and also numerous intervening medical complications that forced us to delay lymphodepleting chemotherapy and cell infusion.

We have observed that the safety profile of ARI-0001 cells is comparable to that of commercially available or other academic products.[11,17–21] Of note, the excessive severity/lethality of CRS observed in the first 19 patients led to a major amendment of the protocol mandating the fractionated administration of ARI-0001 cells (cohorts 1 and 2 versus cohort 3). Consequently, the grade ≥ 3 CRS rate dropped from 27% to 4% and the PRM was reduced from 20% to 0%. These results almost replicate the experience with CTL019 (later known as tisagenlecleucel) in adult patients with ALL, in which the grade ≥ 3 CRS rate was reduced from 50% to 4%, and the PRM
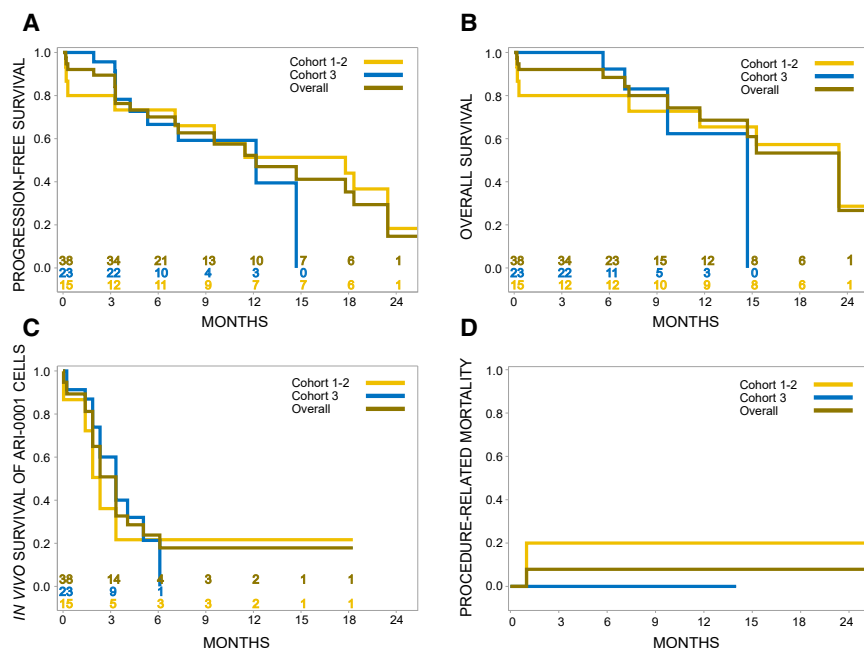
Figure 2. Clinical Outcome of Patients with Acute Lymphoblastic Leukemia

(A–D) Progression-free survival (A), overall survival (B), *in vivo* survival of ARI-0001 cells, as measured by persistence of B cell aplasia (C), and procedure-related mortality (D) of patients with acute lymphoblastic leukemia belonging to the modified full analysis set (n = 38) according to type of administration (cohorts 1 and 2 versus cohort 3).

(14.8 months) and the median OS (20.2 months). If we focus on the survival of ARI-0001 cells, as measured by the persistence of B cell aplasia, it is also comparable to other clinical trials evaluating other CART19 constructs incorporating the 4-1BB costimulatory domain,[18,19] but significantly shorter to what was obtained in the ELIANA trial.[11] Probably, T cell exhaustion accounts for this phenomenon, which also mirrors the tendency toward a longer survival of CART19-cells in pediatric compared to adult ALL populations.[11,19,21]

from 50% to 0%, after the fractionated administration of this drug was introduced into the protocol.[17] Still, the global CRS rate of 55% (13% grade ≥ 3) observed in our trial, considering all patients together, is quite comparable to similar trials of CART19 cells in ALL.[11,18–21] Regarding neurotoxicity, there were no apparent differences before and after the amendment since only one (3%) patient developed grade ≥ 3 ICANS. These results are also comparable (if not better) to those obtained in similar trials of CART19 cells in ALL.[11,17–21] We have no explanation for the reduced incidence of ICANS observed in our trial. Since the cell dose and patient management were comparable with other trials, we hypothesize that this could be a genuine feature of our A3B1-based construct. Moreover, there were no differences in safety according to age: grade ≥ 3 CRS rate was 18% for patients ≤18 years and 11% for those >18 years; and grade ≥3 ICANS was 0% and 4% for the same patient groups. Of note, two patients with ALL developed severe hepatic SOS, which, to the best of our knowledge, has not been reported before in patients receiving CART19 cells. Both patients had in common their relatively advanced age (54 and 67 years), prior alloHCT and prior administration of IO, which are all known risk factors for SOS. As a result, we currently perform a comprehensive liver evaluation in this patient population before the administration of ARI-0001 cells. From these results we can conclude that the fractionated administration of 1×10^6 ARI-0001 cells/kg can be considered safe and merits further evaluation in phase 2 trials.

In this study, ARI-0001 cells achieved a 71% MRD-negative CRR at day +100 with a median PFS of 12 months, which is comparable to results obtained with similar products in pediatric patients,[11,18] and perhaps superior to studies performed exclusively in adults, in which the median PFS ranged from 6 to 8 months.[17,21] Equally comparable are the median DOR of patients who were in remission by day +100

Then, we evaluated the results obtained in cohorts 1 and 2 (single-dose) versus cohort 3 (fractionated administration) and observed that the CRR (67% versus 74%), 1-year PFS (51% versus 39.5%), and 1-year OS (66% versus 62%) were not remarkably different. These comparisons should, however, be handled with care since the follow-up is significantly shorter for patients in cohort 3. On the other hand, we observed some important differences between pediatric and adult patients. The CRR was apparently lower for pediatric patients (55% versus 78%), clearly influenced by the second administration of ARI-0001 cells in two pediatric patients before day +100. In contrast, the 1-year PFS of pediatric patients was significantly higher compared to adults (82% versus 34%), and also the duration of B cell aplasia (48% versus 10.5%), all in keeping with prior experience in pediatric versus adult populations.[11,17–21] Remarkably, almost all (96%) adult patients recruited into this trial had already failed an alloHCT and none underwent a second procedure as consolidation therapy. As such, the effect of ARI-0001 on PFS or OS was not confounded by the effect of a subsequent allograft. On the other hand, most (87%) relapses were CD19+ in keeping to other studies performed in adult ALL,[19,21] but almost the opposite to what was observed with tisagenlecleucel in pediatric patients.[11] Once again, shorter T cell persistence may explain this phenomenon.[11,19,21] Unfortunately, the low number of patients and the trial design prevented us from identifying any relationship between persistence of B cell aplasia and PFS. Finally, second ARI-0001 infusions had little efficacy in reverting early B cell recovery and/or disease relapse as seen by other investigators with similar products.[22]

In conclusion, the administration of ARI-0001 cells, produced in a European academic setting, provided safety and efficacy results that are comparable with other academic or even commercial products.

Table 3. Efficacy of ARI-0001 Cells in Patients with Acute Lymphoblastic Leukemia

Population	n	MRD-CRR at day +28, rate (95% CI)	MRD-CRR at day +100, rate (95% CI)	PFS Median (95% CI) 1-year rate (95% CI)	DOR Median (95% CI) 1-year rate (95% CI)	OS Median (95% CI) 1-year rate (95% CI)
Total	38	84.2 (69–94)	71.1 (54–85)	12.0 mo (4.2–20.2) 47% (27–67%)	14.8 mo (6.0–NA) 59% (34%–83%)	20.2 mo (10.4–NA) 69% (49%–88%)
Cohorts 1 and 2 (single dose)	15	73.3 (45–92)	66.7 (38–88)	17.6 mo (0.3–NA) 51% (25%–77%)	15.7 (3.6–NA) 70% (42%–98%)	20.2 mo (0.3–NA) 65.5% (41%–90%)
Cohort 3 (fractionated)	23	91.3 (72–99)	73.9 (52–90)	12.0 mo (4.2–14.5) 39.5% (4%–75%)	8.7 mo (2.0–NA) NA (NA–NA)	14.5 mo (6.8–14.5) 62% (24%–100%)
Age groups						
≤18 years	11	81.8 (48–98)	54.5 (23–83)	18.1 mo (14.5–NA) 82% (59%–100%)	NA mo (14.8–NA) 100% (100%–100%)	NR (7.1–NA) 78% (50%–100%)
>18 years	27	85.2 (66–96)	77.8 (58–91)	9.4 mo (3.3–20.2) 34% (12%–57%)	8.7 mo (3.9–16.6) 46% (18%–75%)	20.2 mo (12.8–NA) 64.5% (40%–89%)
≤25 years	19	78.9 (54–94)	63.2 (38–84)	17.6 mo (4.2–20.2) 64% (40%–89%)	14.8 mo (6.0–NR) 79% (51%–100%)	20.2 mo (14.5–NA) 82% (63%–100%)
>25 years	19	89.4 (67–99)	78.9 (54–94)	7.2 mo (3.2–NA) 25% (0%–52%)	8.7 mo (2.0–NR) 34% (0%–70%)	NR (6.9–NA) 50.5% (18%–84%)

MRD-CRR, complete response rate with negative measurable residual disease; PFS, progression-free survival; DOR, duration of response; OS, overall survival; mo, months; NA, not available.

In February 2020, a marketing authorization application under the Hospital Exemption Rule was submitted to the AEMPS. This exemplifies how initiatives from Academia and Pharmaceutical companies can be complementary and synergistic in the best interest of patients.

MATERIALS AND METHODS
Patient Population
The CART19-BE-01 study is a single-arm, multicenter, open-label pilot study evaluating the safety and efficacy of ARI-0001 cells in patients with R/R B cell malignancies. Eligible patients had to have all of the following: (1) CD19-positive B cell malignancy, including ALL, DLBCL, CLL, FL, or mantle-cell lymphoma (MCL); (2) age from 2 to 80 years; (3) ECOG performance status 0–2; (4) estimated life expectancy from 3 months to 2 years; and (5) adequate venous access. Patients were eligible if they suffered from (1) ALL in $\geq 2^{nd}$ relapse, either ineligible for or with relapsed disease after alloHCT; (2) DLBCL or MCL in $\geq 2^{nd}$ relapse, ineligible for or with relapsed disease after autoHCT; and (3) CLL or FL who had received a minimum of 2 lines of therapy (including rituximab) and experienced disease progression within 2 years of last therapy. Patients with CLL/FL could be ineligible for or with relapsed disease after alloHCT/autoHCT. Key exclusion criteria included history of other malignancy unless it had been in remission for more than 3 years; severe renal, hepatic, pulmonary, or cardiac impairment; active immunosuppressive therapy; HIV infection; active HBV or HCV infection; and active infection requiring systemic therapy. Of note, neither central nervous system involvement nor prior alloHCT were exclusion criteria for this trial. All patients provided written, informed consent. The AEMPS and Institutional Review Boards/Ethics Committees of each study site approved the trial, which was conducted in accordance with the principles of the Declaration of Helsinki (last updated version, Fortaleza, Brazil, 2013).

Study Design, Endpoints, and Sample Size
The primary endpoint was safety as determined by PRM and grade 3–4 toxicity at day +100 and 1 year. AEs of special interest were CRS, neurotoxicity (currently known as ICANS) and second neoplasia. AEs were graded according to common terminology criteria (CTC), version 4.0. SAEs were defined as those AEs that were fatal, life-threatening, leading to (or prolonging) a stay in hospital or a transfer to the intensive care unit, or resulting in severe disability. For CRS, we used the grading system by Lee et al.[23] Secondary endpoints included objective response rate (ORR) as per conventional criteria;[24–26] PFS, OS, DOR, B cell aplasia duration, and impact of therapy on quality of life. The ORR was assessed at day +100 for all patients (prespecified endpoint) and at day +28 for patients with ALL (exploratory endpoint).

The original sample size was 10 patients (cohort 1). 5 months after study initiation, a major amendment increased the sample size to 39 patients and allowed patients with either normal B cell recovery within 3 months (early B cell recovery), CD19+ disease relapse, or CD19+ refractory disease to receive a second dose of ARI-0001 cells (cohort 2). 12 months after study initiation, with 19 patients already recruited, a second major amendment increased the sample size to a total of 54 patients (cohort 3), mandated the fractionated administration of ARI-0001 cells (10%, 30%, and 60% of the total dose) contingent on the lack of CRS after each fraction, and allowed the early administration of tocilizumab in patients with grade 2 CRS (it was initially reserved to those with grade ≥ 3). This second amendment was motivated by 3 toxic deaths.

ARI-0001 Cells Production and Treatment
Full details of ARI-0001 cell production, including a comprehensive phenotypic characterization of these cells can be found elsewhere.[27]
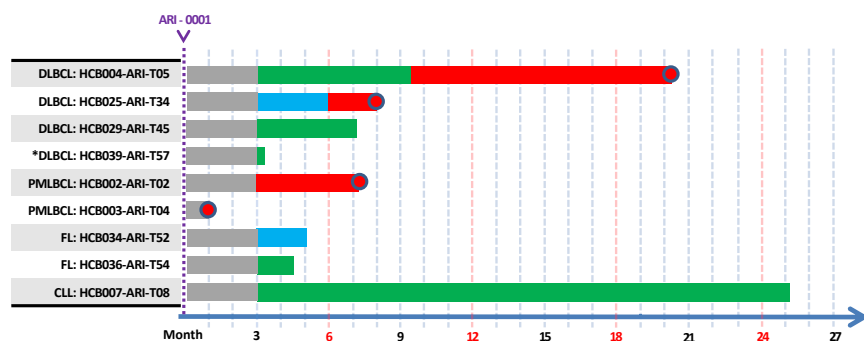
Figure 3. Swimmer Plot of Patients with Non-Hodgkin's Lymphoma and Chronic Lymphocytic Leukemia Belonging to the Modified Full Analysis Set (n = 9)

Green denotes complete remission, blue denotes partial remission, and red denotes refractory disease or disease progression. Sharp edges denote "alive at last follow-up," round edges denote "dead at last follow-up."

Patients were enrolled following screening and confirmation of eligibility, and underwent leukocytapheresis to obtain peripheral blood mononuclear cells (PBMCs). ARI-0001 cells were manufactured using the CliniMACS Prodigy system (Miltenyi). Approximately 100×10^6 T cells were stimulated to expand with anti-CD3 and anti-CD28 antibodies together with IL-7 and IL-15. 24 h after T cell activation, cells were transduced with a lentiviral vector containing the CAR gene construct at a multiplicity of infection of 10. Following expansion, that lasted from 7 to 10 days, the final ARI-0001 cell product was washed, cryopreserved, and tested for identity, potency, sterility, and adventitious agents.

Before ARI-0001 cell infusion, patients received fludarabine at 30 mg/m^2/day plus cyclophosphamide at 300 mg/m^2/day on days −6, −5, and −4 followed by ARI-0001 cells. In cohorts 1 and 2, patients received a single intravenous infusion of ARI-0001 cells, at a dose of 0.5–5 × 10^6 cells/kg, on day 0. Adult patients with ALL received 0.5–1 × 10^6 cells/kg, while pediatric patients with ALL and adult patients with NHL/CLL received 5 × 10^6 cells/kg (if available). In cohort 3, patients received the first fraction (10%) of ARI-0001 cells on day 0, followed by the second (30%) and third (60%) fraction. The second fraction was administered 24–48 h after the first, and the third 24–48 h after the second only if the patient had no signs or symptoms of CRS. Moreover, in cohort 3 all patients with ALL received a maximum ARI-0001 cell dose of 1 × 10^6 cells/kg regardless of age. All patients remained hospitalized to recovery through day +21 or until all procedure-related non-hematological toxicity returned to grade ≤ 1 or baseline. Patients were followed in the post-treatment assessment period and returned to the clinic at weeks 4, 5, 6, 8, 10, 12, and 14 (day +100), and monthly afterward for the first year. All patients completing the first year of follow-up were followed up for survival and disease status every 3 months in years 2 and 3. In case of a second ARI-0001 cell infusion, the entire procedure was repeated, including lymphodepleting chemotherapy, ARI-0001 cell dose (if available), hospital admission, and close follow-up.

The methods used to measure cytokines, ARI-0001 cells and HAMAs are available in the Supplemental Information.

Statistical Analysis

Adverse events and response rates are presented with 95% exact Clopper-Pearson confidence intervals. PRM was calculated as a cumula-tive incidence considering disease relapse as a competing event. OS, PFS, DOR, and persistence of B cell aplasia, were plotted using the Kaplan-Meier method. Due to the open-label non-randomized nature of the study, the statistical analysis was descriptive and no formal comparisons between cohorts are provided. However, for illustrative reasons, some p values are provided, which should be considered informative but not conclusive. Statistical analyses were performed using SAS version 9.4 (SAS Institute, Cary, NC, USA) and R (R Foundation for Statistical Computing, Vienna, Austria). The trial (EUDRA no 2016-2972-29) was registered at https://clinicaltrials.gov/ (ClinicalTrials.gov: NCT03144583).

SUPPLEMENTAL INFORMATION

Supplemental Information can be found online at https://doi.org/10.1016/j.ymthe.2020.09.027.

AUTHOR CONTRIBUTIONS

CONFLICTS OF INTEREST

V.O.-M.: consultant or advisory role (Kite Gilead, Celgene, Novartis), travel grants (Kite Gilead, Celgene, Novartis, Roche, Takeda, Janssen), honoraria (Kite Gilead). S.R.: consultant or advisory role (Novartis, Jazz, Shire/Servier, Amgen), travel grants (Novartis, Jazz, Shire/Servier, Amgen), honoraria (Novartis, Jazz, Shire/Servier, Amgen). A.A.-S.: consultant or advisory role (Novartis), travel grants (Novartis), honoraria (Novartis). T.B.: consultant of advisory role (Janssen), travel grants (Kite Gilead, Abbvie, Janssen), honoraria (Janssen, Abbvie, Roche, Kite Gilead). E.G.-R.: honoraria (Novartis).

REFERENCES
1. Tavernier, E., Boiron, J.-M., Huguet, F., Bradstock, K., Vey, N., Kovacsovics, T., Delannoy, A., Fegueux, N., Fenaux, P., Stamatoullas, A., et al.; GET-LALA Group; Swiss Group for Clinical Cancer Research SAKK; Australasian Leukaemia and Lymphoma Group (2007). Outcome of treatment after first relapse in adults with acute lymphoblastic leukemia initially treated by the LALA-94 trial. Leukemia 21, 1907–1914.

2. Oriol, A., Vives, S., Hernández-Rivas, J.M., Tormo, M., Heras, I., Rivas, C., Bethencourt, C., Moscardó, F., Bueno, J., Grande, C., et al.; Programa Español de Tratamiento en Hematologia Group (2010). Outcome after relapse of acute lymphoblastic leukemia in adult patients included in four consecutive risk-adapted trials by the PETHEMA Study Group. Haematologica 95, 589–596.

3. Kantarjian, H.M., Thomas, D., Ravandi, F., Faderl, S., Jabbour, E., Garcia-Manero, G., Pierce, S., Shan, J., Cortes, J., and O'Brien, S. (2010). Defining the course and prognosis of adults with acute lymphocytic leukemia in first salvage after induction failure or short first remission duration. Cancer 116, 5568–5574.

4. Gökbuget, N., Stanze, D., Beck, J., Diedrich, H., Horst, H.-A., Hüttmann, A., Kobbe, G., Kreuzer, K.A., Leimer, L., Reichle, A., et al.; German Multicenter Study Group for Adult Acute Lymphoblastic Leukemia (2012). Outcome of relapsed adult lymphoblastic leukemia depends on response to salvage chemotherapy, prognostic factors, and performance of stem cell transplantation. Blood 120, 2032–2041.

5. Spyridonidis, A., Labopin, M., Schmid, C., Volin, L., Yakoub-Agha, I., Stadler, M., Milpied, N., Socie, G., Browne, P., Lenhoff, S., et al.; Immunotherapy Subcommittee of Acute Leukemia Working Party (2012). Outcomes and prognostic factors of adults with acute lymphoblastic leukemia who relapse after allogeneic hematopoietic cell transplantation. An analysis on behalf of the Acute Leukemia Working Party of EBMT. Leukemia 26, 1211–1217.

6. Poon, L.M., Hamdi, A., Saliba, R., Rondon, G., Ledesma, C., Kendrick, M., Qazilbash, M., Hosing, C., Jones, R.B., Popat, U.R., et al. (2013). Outcomes of adults with acute lymphoblastic leukemia relapsing after allogeneic hematopoietic stem cell transplantation. Biol. Blood Marrow Transplant. 19, 1059–1064.

7. Kantarjian, H.M., DeAngelo, D.J., Stelljes, M., Martinelli, G., Liedtke, M., Stock, W., Gökbuget, N., O'Brien, S., Wang, K., Wang, T., et al. (2016). Inotuzumab Ozogamicin versus Standard Therapy for Acute Lymphoblastic Leukemia. N. Engl. J. Med. 375, 740–753.

8. Kantarjian, H., Stein, A., Gökbuget, N., Fielding, A.K., Schuh, A.C., Ribera, J.-M., Wei, A., Dombret, H., Foà, R., Bassan, R., et al. (2017). Blinatumomab versus Chemotherapy for Advanced Acute Lymphoblastic Leukemia. N. Engl. J. Med. 376, 836–847.

9. Crump, M., Neelapu, S.S., Farooq, U., Van Den Neste, E., Kuruvilla, J., Westin, J., Link, B.K., Hay, A., Cerhan, J.R., Zhu, L., et al. (2017). Outcomes in refractory diffuse large B-cell lymphoma: results from the international SCHOLAR-1 study. Blood 130, 1800–1808.

10. June, C.H., and Sadelain, M. (2018). Chimeric antigen receptor therapy. N. Engl. J. Med. 379, 64–73.

11. Maude, S.L., Laetsch, T.W., Buechner, J., Rives, S., Boyer, M., Bittencourt, H., Bader, P., Verneris, M.R., Stefanski, H.E., Myers, G.D., et al. (2018). Tisagenlecleucel in Children and Young Adults with B-Cell Lymphoblastic Leukemia. N. Engl. J. Med. 378, 439–448.

12. Schuster, S.J., Svoboda, J., Chong, E.A., Nasta, S.D., Mato, A.R., Anak, Ö., Brogdon, J.L., Pruteanu-Malinici, I., Bhoj, V., Landsburg, D., et al. (2017). Chimeric Antigen Receptor T Cells in Refractory B-Cell Lymphomas. N. Engl. J. Med. 377, 2545–2554.

13. Neelapu, S.S., Locke, F.L., Bartlett, N.L., Lekakis, L.J., Miklos, D.B., Jacobson, C.A., Braunschweig, I., Oluwole, O.O., Siddiqi, T., Lin, Y., et al. (2017). Axicabtagene Ciloleucel CAR T-Cell Therapy in Refractory Large B-Cell Lymphoma. N. Engl. J. Med. 377, 2531–2544.

14. Castella, M., Boronat, A., Martín-Ibáñez, R., Rodríguez, V., Suñé, G., Caballero, M., Marzal, B., Pérez-Amill, L., Martín-Antonio, B., Castaño, J., et al. (2018). Development of a Novel Anti-CD19 Chimeric Antigen Receptor: A Paradigm for an Affordable CAR T Cell Production at Academic Institutions. Mol. Ther. Methods Clin. Dev. 12, 134–144.

15. Delgado, J., Caballero-Baños, M., Ortiz-Maldonado, V., Castellà, M., Magnano, L., Juan, M., and Urbano-Ispizua, Á. (2019). Chimeric Antigen Receptor T Cells Targeting CD19 and Ibrutinib for Chronic Lymphocytic Leukemia. HemaSphere 3, e174.

16. Rafei, H., Kantarjian, H.M., and Jabbour, E.J. (2020). Targeted therapy paves the way for the cure of acute lymphoblastic leukaemia. Br. J. Haematol. 188, 207–223.

17. Frey, N.V., Shaw, P.A., Hexner, E.O., Pequignot, E., Gill, S., Luger, S.M., Mangan, J.K., Loren, A.W., Perl, A.E., Maude, S.L., et al. (2020). Optimizing Chimeric Antigen Receptor T-Cell Therapy for Adults With Acute Lymphoblastic Leukemia. J. Clin. Oncol. 38, 415–422.

18. Gardner, R.A., Finney, O., Annesley, C., Brakke, H., Summers, C., Leger, K., Bleakley, M., Brown, C., Mgebroff, S., Kelly-Spratt, K.S., et al. (2017). Intent-to-treat leukemia remission by CD19 CAR T cells of defined formulation and dose in children and young adults. Blood 129, 3322–3331.

19. Hay, K.A., Gauthier, J., Hirayama, A.V., Voutsinas, J.M., Wu, Q., Li, D., Gooley, T.A., Cherian, S., Chen, X., Pender, B.S., et al. (2019). Factors associated with durable EFS in adult B-cell ALL patients achieving MRD-negative CR after CD19 CAR T-cell therapy. Blood 133, 1652–1663.

20. Lee, D.W., Kochenderfer, J.N., Stetler-Stevenson, M., Cui, Y.K., Delbrook, C., Feldman, S.A., Fry, T.J., Orentas, R., Sabatino, M., Shah, N.N., et al. (2015). T cells expressing CD19 chimeric antigen receptors for acute lymphoblastic leukaemia in children and young adults: a phase 1 dose-escalation trial. Lancet 385, 517–528.

21. Park, J.H., Rivière, I., Gonen, M., Wang, X., Sénéchal, B., Curran, K.J., Sauter, C., Wang, Y., Santomasso, B., Mead, E., et al. (2018). Long-Term Follow-up of CD19 CAR Therapy in Acute Lymphoblastic Leukemia. N. Engl. J. Med. 378, 449–459.

22. Bezerra, E.D., Gauthier, J., Hirayama, A.V., Pender, B.S., Hawkins, R.M., Vakil, A., et al. (2019). Factors associated with response, CAR-T cell in vivo expansion, and progression-free survival after repeated infusions of CD19 CAR-T cells. Blood 134, 201.

23. Lee, D.W., Gardner, R., Porter, D.L., Louis, C.U., Ahmed, N., Jensen, M., Grupp, S.A., and Mackall, C.L. (2014). Current concepts in the diagnosis and management of cytokine release syndrome. Blood 124, 188–195.

24. Alvarnas, J.C., Brown, P.A., Aoun, P., Ballen, K.K., Barta, S.K., Borate, U., Boyer, M.W., Burke, P.W., Cassaday, R., Castro, J.E., et al. (2015). Acute Lymphoblastic Leukemia, Version 2.2015. J. Natl. Compr. Canc. Netw. 13, 1240–1279.

25. Cheson, B.D., Fisher, R.I., Barrington, S.F., Cavalli, F., Schwartz, L.H., Zucca, E., and Lister, T.A.; Alliance, Australasian Leukaemia and Lymphoma Group; Eastern Cooperative Oncology Group; European Mantle Cell Lymphoma Consortium; Italian Lymphoma Foundation; European Organisation for Research; Treatment of Cancer/Dutch Hemato-Oncology Group; Grupo Español de Médula Ósea; German High-Grade Lymphoma Study Group; German Hodgkin's Study Group; Japanese Lymphorra Study Group; Lymphoma Study Association; NCIC Clinical Trials Group; Nordic Lymphoma Study Group; Southwest Oncology Group; United Kingdom National Cancer Research Institute (2014). Recommendations for initial evaluation, staging, and response assessment of Hodgkin and non-Hodgkin lymphoma: the Lugano classification. J. Clin. Oncol. 32, 3059–3068.

26. Hallek, M., Cheson, B.D., Catovsky, D., Caligaris-Cappio, F., Dighiero, G., Döhner, H., Hillmen, P., Keating, M.J., Montserrat, E., Rai, K.R., and Kipps, T.J.; International Workshop on Chronic Lymphocytic Leukemia (2008). Guidelines for the diagnosis and treatment of chronic lymphocytic leukemia: a report from the International Workshop on Chronic Lymphocytic Leukemia updating the National Cancer Institute-Working Group 1996 guidelines. Blood 111, 5446–5456.

27. Castella, M., Caballero-Baños, M., Ortiz-Maldonado, V., González-Navarro, E.A., Suñé, G., Antoñana-Vidósola, A., Boronat, A., Marzal, B., Millán, L., Martín-Antonio, B., et al. (2020). Point-of-care CAR T-cell production (ARI-0001) using a closed semi-automatic bioreactor: experience from an academic phase I clinical trial. Front. Immunol. 11, 482.

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.

	Cohort 1-2 (single infusion, n=3) Occurrences/ Subjects (%)	Cohort 3 (fractionated infusion, n=5) Occurrences/ Subjects (%)	All patients (n=8) Occurrences/ Subjects (%)

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).
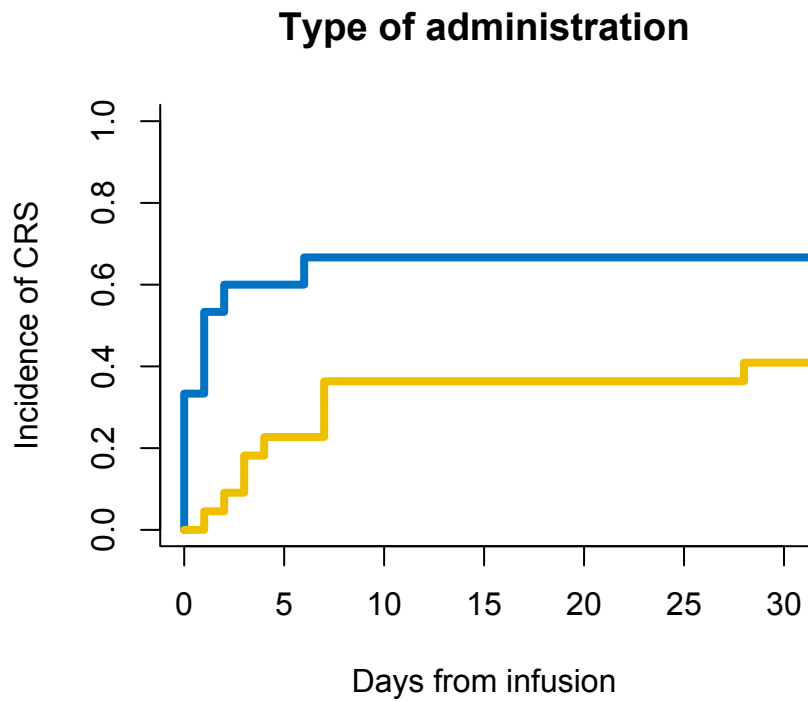
Figure S2: ARI-0001 expansion, normal B-cells, and human anti-murine antibodies (HAMAs) over time in all patients who received a second cell infusion. Patients in panels A-E were diagnosed with acute lymphoblastic leukemia while the patient depicted in panel F was diagnosed with diffuse large B-cell 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.
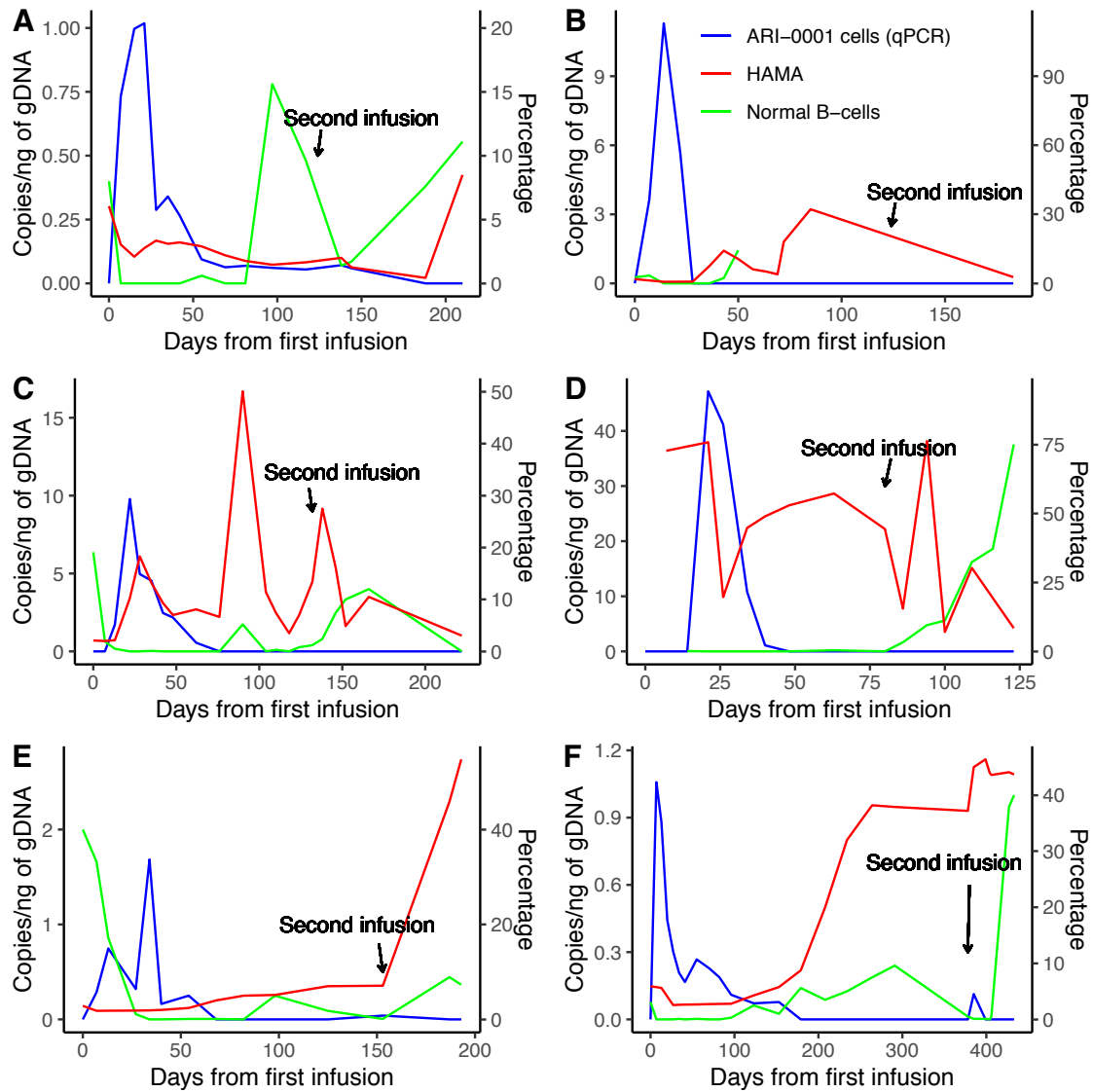
Figure S3: Simon-Makuch curve depicting the impact of B-cell aplasia on progression-free survival in patients with acute lymphoblastic leukemia (blue curve = sustained B-cell aplasia, yellow curve = B-cell recovery). The difference was not statistically significant (P = 0.33, Mantel-Byar test).
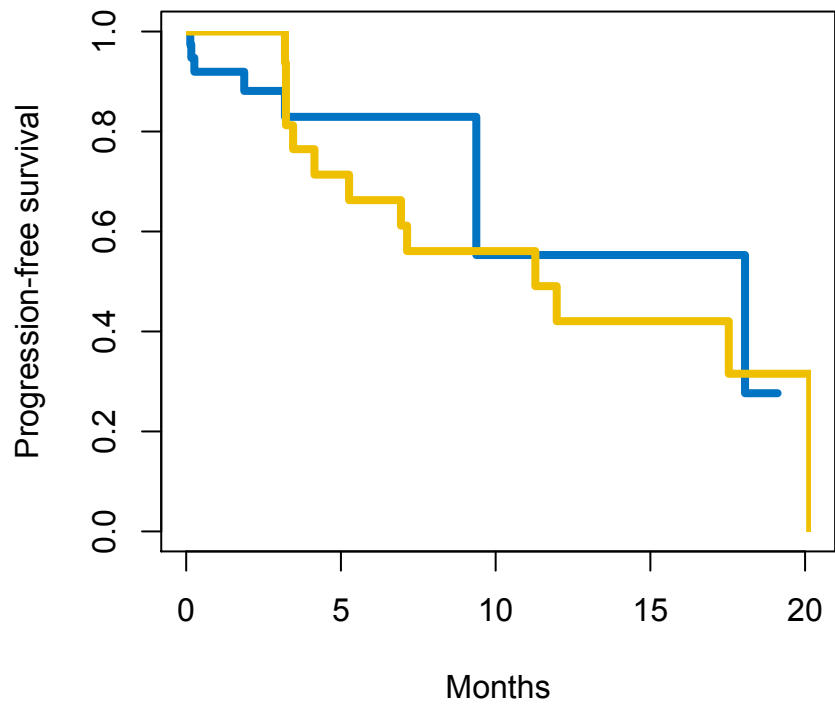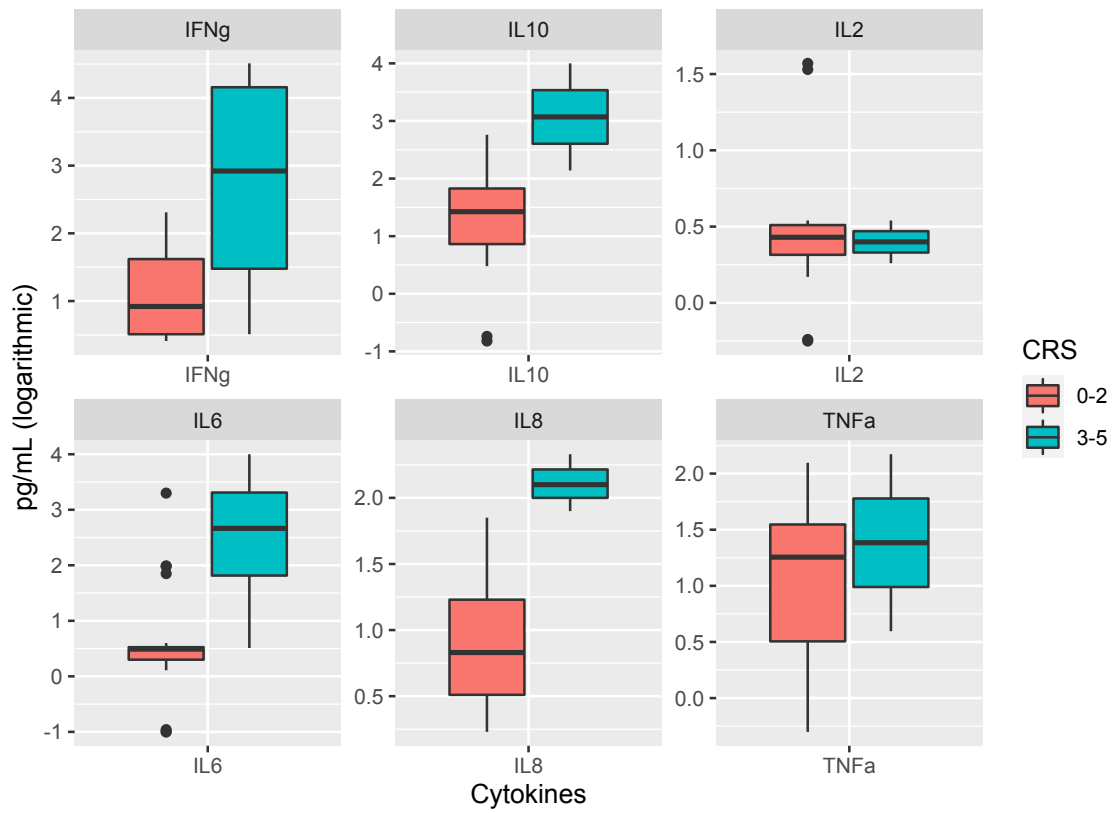
Figure S4: Cytokine levels (patients with acute lymphoblastic leukemia with results available on day +7) according to the presence of cytokine release syndrome (grade 0-2 vs. grade ≥3)

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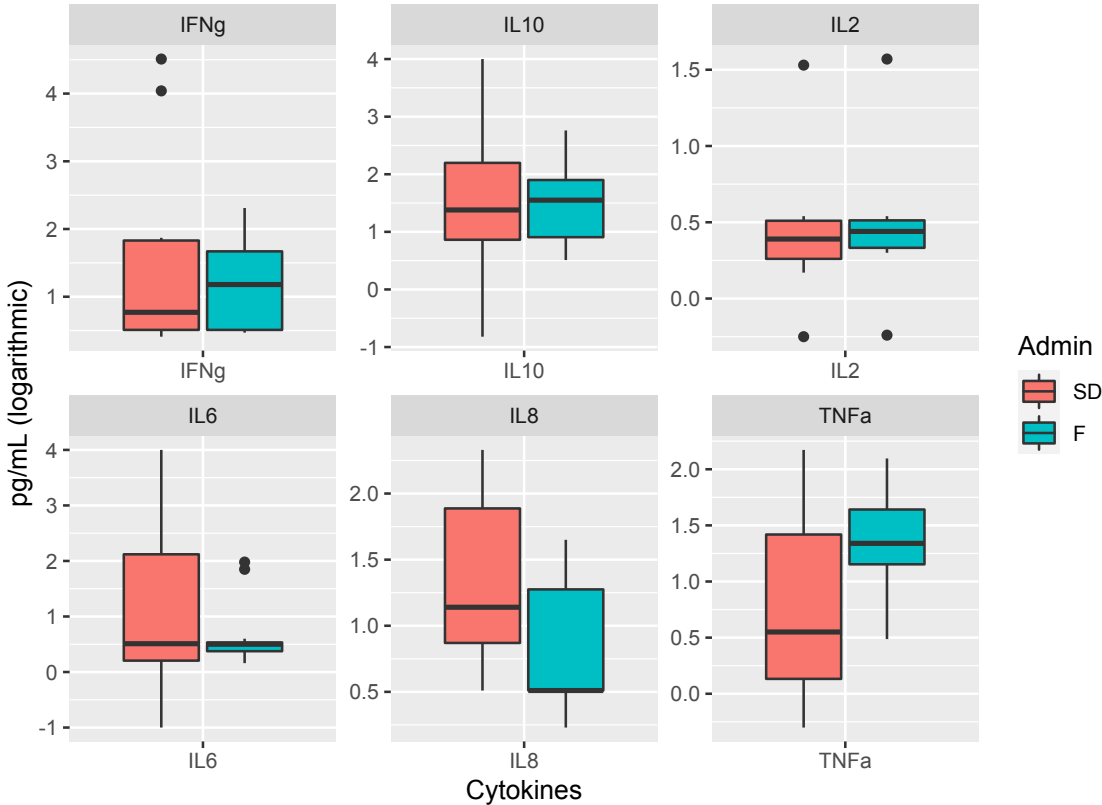
There were no statistically significant differences (Wilcoxon's rank sum test).

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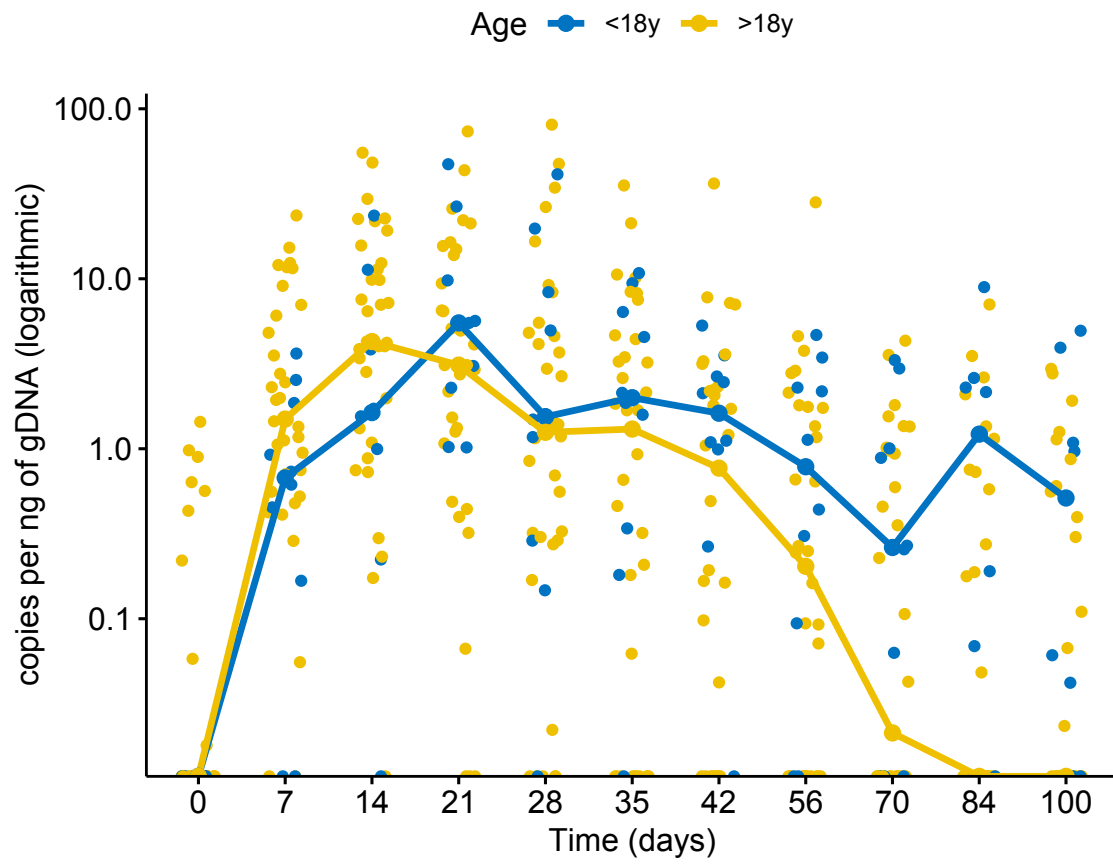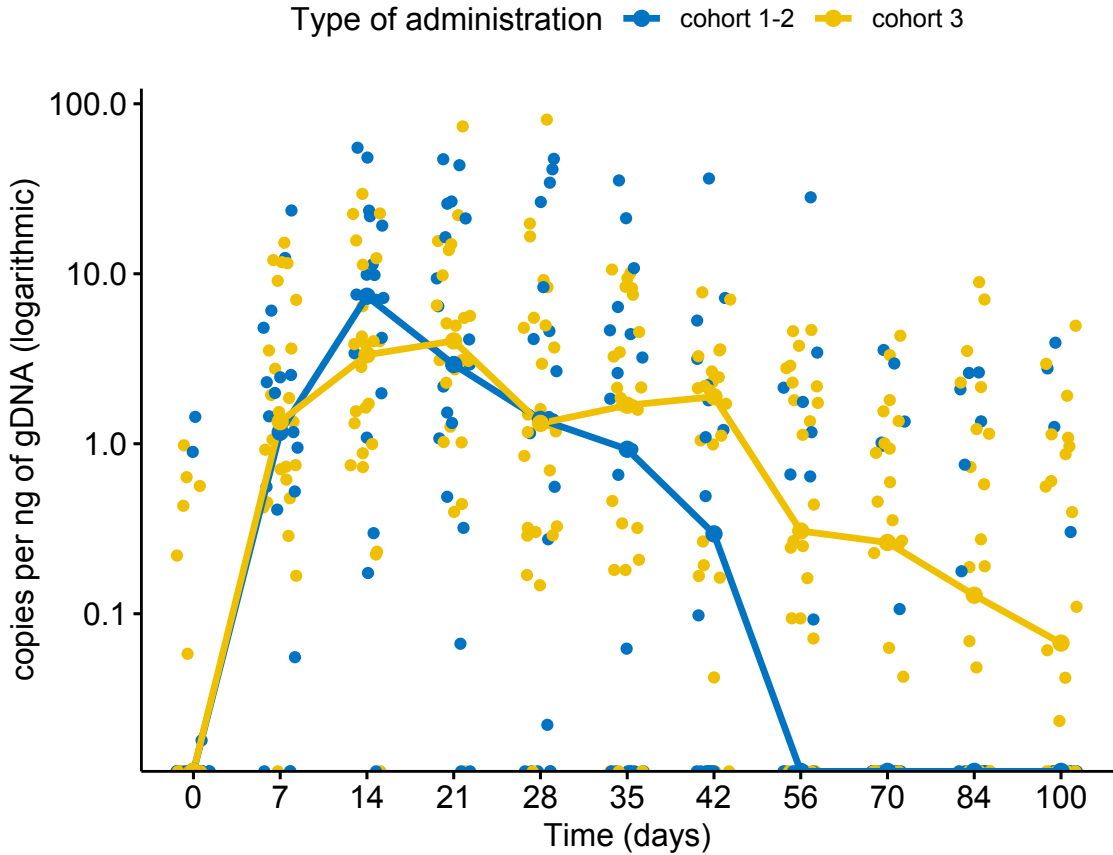
Figure S7: ARI-0001 expansion over time, as calculated by quantitative PCR, in patients with acute lymphoblastic leukemia according to type of administration (single dose [cohort 1-2] vs. fractionated [cohort 3]). Lines represent median values

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'ccgaagggacgtagcagaa 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).