ORIGINAL ARTICLE

Enhanced CAR T-Cell Therapy for Lymphoma after Previous Failure

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ABSTRACT

BACKGROUND

Chimeric antigen receptor (CAR) T cells targeting CD19 have transformed the treatment of B-cell cancers, but many patients do not have long-term remission. We designed an anti-CD19 enhanced (armored) CAR T-cell product (huCART19-IL18) that secretes interleukin-18 to enhance antitumor activity.

METHODS

In this study, we assessed the safety, feasibility, and preliminary efficacy of huCART19-IL18 in patients with relapsed or refractory lymphoma after previous anti-CD19 CAR T-cell therapy. Using a 3-day manufacturing process, we administered huCART19-IL18–positive cells in doses ranging from 3×10^6 to 3×10^8.

RESULTS

A total of 21 patients received huCART19-IL18. Cytokine release syndrome occurred in 62% of the patients (47% with grade 1 or 2), and immune effector-cell–associated neurotoxicity syndrome occurred in 14% (all grade 1 or 2). No unexpected adverse events were observed. Robust CAR T-cell expansion was detected across all dose levels. At 3 months after infusion, a complete or partial response was seen in 81% of the patients (90% confidence interval [CI], 62 to 93) and a complete response in 52% (90% CI, 33 to 71). With a median follow-up of 17.5 months (range, 3 to 34), the median duration of response was 9.6 months (90% CI, 5.5 to not reached).

CONCLUSIONS

In this small study, huCART19-IL18 had a safety profile consistent with other CAR T-cell treatments and showed promising efficacy at low cell doses in patients with lymphoma after the failure of previous anti-CD19 CAR T-cell therapy. (ClinicalTrials.gov number, NCT04684563.)

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Chimeric antigen receptor (CAR) T-cell therapies targeting CD19 have improved treatment outcomes in patients with relapsed B-cell cancers. Currently, four second-generation anti-CD19 CAR T-cell products are commercially available and approved for the treatment of several B-cell lymphomas.[1-9] These therapies incorporate either a 4-1BB (CD137) or a CD28 intracellular costimulatory domain to enhance T-cell activation and persistence. Despite these advancements, more than 50% of patients with lymphoma do not have long-term remission with anti-CD19 CAR T-cell therapy. These patients have limited treatment options and poor prognoses.[10,11]

Most patients who have a relapse after anti-CD19 CAR T-cell therapy or have resistance to such therapy continue to have CD19 expression on malignant B cells.[11] Although CD19 antigen loss can occur in a subgroup of patients, treatment failure is more frequently attributed to T-cell dysfunction, an immunosuppressive tumor microenvironment,[11,12] or both. Retreatment with second-generation CD19-directed autologous or allogeneic CAR T cells has resulted in limited success, a finding that has highlighted the need for new CAR T-cell designs with enhanced (armored) effector functions.[13,14]

A promising strategy to improve CAR T-cell efficacy involves developing fourth-generation armored CAR T cells that secrete proinflammatory cytokines to bolster antitumor activity. This approach is currently being explored in solid tumors according to the hypothesis that cytokine secretion enhances the cytotoxicity of CAR and tumor-infiltrating T cells while modifying the immunosuppressive tumor microenvironment.[15-17] One such cytokine, interleukin-18, is a proinflammatory molecule that is primarily produced by macrophages and dendritic cells. Interleukin-18 enhances the activation of T cells and natural killer cells, promotes the production of interferon-γ, and has potential therapeutic applications.[18-20] Preclinical studies conducted by our group and others have shown that interleukin-18–armored CAR T cells have superior antitumor efficacy and result in prolonged survival in mouse models.[21-23]

Building on this concept, we developed huCART19-IL18, an autologous anti-CD19 CAR T-cell product that constitutively secretes interleukin-18 (Fig. S1 in the Supplementary Appendix, available with the full text of this article at NEJM.org). In addition, huCART19-IL18 is manufactured in a rapid, 3-day process that is designed to preserve stem-cell–like characteristics and reduce exhaustion in T cells.[24,25] To mitigate immunogenicity and improve CAR T-cell persistence, we incorporated a humanized anti-CD19 single-chain variable fragment.[26]

METHODS

TRIAL DESIGN AND OVERSIGHT

This phase 1 trial of huCART19-IL18 involving patients with CD19+ B-cell lymphomas was conducted at the University of Pennsylvania. All the patients had previous failure of anti-CD19 CAR T-cell therapy (except for one patient, for whom cell-product manufacturing had failed twice); there was no prespecified sample size for each lymphoma subtype. The primary objective was to determine the safety and maximum tolerated dose of huCART19-IL18. The secondary objectives were to evaluate manufacturing feasibility, determine preliminary efficacy, and perform correlative studies measuring huCART19-IL18 expansion in peripheral-blood and tissue-biopsy samples, huCART19-IL18 persistence, cytokine levels, and clinicopathological analyses of tissue correlates of response.

We planned to administer huCART19-IL18 at five dose levels, ranging from dose level 1 (DL1) (3×10^6 cells) to dose level 5 (DL5) (3×10^8 cells), with a de-escalated dose level (DL–1) if dose-limiting toxic effects were observed at DL1 (Fig. S2A). We used a Bayesian optimal interval design with accelerated titration to determine the maximum tolerated dose.[27] Additional analyses were performed to determine the recommended dose for expansion.

The reported confidence intervals have not been adjusted for multiplicity and cannot be interpreted as hypothesis tests. Details regarding the trial design and statistical analysis are provided in the Supplementary Appendix.

The trial was approved by the institutional review board at the University of Pennsylvania. It was conducted in accordance with the principles of the Declaration of Helsinki. The trial was overseen by an independent data and safety

monitoring board. All the patients provided written informed consent. All the authors contributed to the writing of the manuscript and vouch for the accuracy and completeness of the data and for the fidelity of the trial to the protocol (available at NEJM.org).

TREATMENT

Autologous T cells were obtained from the patients by means of leukapheresis. Bridging therapy was optional. Manufacturing and cryopreservation of huCART19-IL18 was performed by the Clinical Cell and Vaccine Production Facility at the University of Pennsylvania. Dose levels of huCART19-IL18 between 3×10^6 and 3×10^8 cells were administered as a single intravenous infusion 2 to 5 days after lymphodepleting chemotherapy. Lymphodeletion was performed with either bendamustine (at a dose of 90 mg per square meter of body-surface area) for 2 days or a combination of cyclophosphamide (at a dose of 250 mg per square meter) and fludarabine (at a dose of 25 mg per square meter) for 3 days[28] at the discretion of the investigator (Fig. S2A). Patients who had a clinical benefit after the huCART19-IL18 infusion but who had residual or relapsing disease could receive retreatment with huCART19-IL18.

EFFICACY AND SAFETY MEASURES

The initial response assessment was performed 3 months after the huCART19-IL18 infusion according to the Lugano 2014 response criteria.[29] Subsequent assessments were performed every 3 months for the first year of primary follow-up. Patients were then transitioned to long-term follow-up (Fig. S2A). The grading of cytokine release syndrome and immune effector cell–associated neurotoxicity syndrome (ICANS) was performed according to the consensus criteria of the American Society for Transplantation and Cellular Therapy.[30] Other toxic effects were graded with the use of National Cancer Institute Common Terminology Criteria for Adverse Events, version 5.0.

CORRELATIVE STUDIES

We determined the degree of huCART19-IL18 expansion and persistence by measuring the number of copies of huCART19 transgene per microgram of genomic DNA using real-time quantitative polymerase-chain-reaction (qPCR) assays. Experimental details regarding other correlative studies are provided in the Supplementary Appendix.

RESULTS

PATIENTS

From May 10, 2021, to March 1, 2024, a total of 28 patients were enrolled in the trial. Of these patients, 21 received huCART19-IL18. Six patients were ineligible (3 for lack of CD19 expression on malignant lymphocytes, 2 for a decline in performance status while awaiting treatment, and 1 for active central nervous system disease), and cell-product manufacturing failed for 1 patient (Fig. S2B).

All 21 patients who received infusions were evaluable for safety and efficacy. The characteristics of the patients are described in Table 1 and Table S1. Most patients had subtypes of large B-cell lymphoma, including 8 with diffuse large B-cell lymphoma, 2 with transformed follicular lymphoma, 1 with high-grade B-cell lymphoma, and 1 with T-cell histiocyte-rich B-cell lymphoma; 6 patients had follicular lymphoma, and 3 had mantle-cell lymphoma. Patients had been heavily pretreated with a median of 7 previous therapies (range, 4 to 14). One patient had not received previous anti-CD19 CAR T-cell therapy owing to two unsuccessful attempts at the manufacturing of a commercially available product; thus, he was eligible for our trial, given that no standard CAR options were available.

PREVIOUS RESPONSE AND PREPARATION FOR REPEAT THERAPY

A total of 35% of the patients had no response to previous second-generation anti-CD19 CAR T-cell therapy. The median progression-free survival among the 20 patients who had received previous CAR T-cell therapy was 6.7 months (90% confidence interval [CI], 3.1 to 10.2). The median time from previous CAR T-cell infusion to apheresis was 16 months (range, 3 to 56).

Our trial design allowed for immediate apheresis at the time of enrollment, but because of protocol-specified safety staggers, the median vein-to-vein time was 67 days (range, 26 to 137), despite shortened manufacturing. A total of 90% of the patients received bridging therapy, including 10 patients (48%) who also received radiation. All the patients had received lymphodepleting

chemotherapy except for the first patient, who was treated with huCART19-IL18 alone as a safety measure (as included in the protocol design). Seventeen patients (81%) received bendamustine. Five patients (24%) received retreatment with huCART19-IL18 with lymphodepleting chemotherapy.

MANUFACTURING

The measures of feasibility were successful 3-day product manufacturing that met release criteria and target dose with at least 70% cell viability. Product manufacturing failed for one patient who had circulating lymphoma in the blood (Table S3). Of 21 manufactured products, 8 (38%) did not meet the assigned target dose, but all 21 were above the protocol-defined minimal cell dose and were infused. As expected, manufacturing challenges were greater at higher dose levels, with DL5 deemed to be not feasible since only 2 of 6 attempted products (33%) at this dose level met the target dose.

SAFETY

The most frequent adverse events are listed in Figure 1; all adverse events are shown in Table S2. Cytokine release syndrome was common, occurring in 13 patients (62%). The highest grade of cytokine release syndrome that was observed was grade 3, occurring in 3 patients (14%). The median time until the onset of cytokine release syndrome was 4 days (range, 1 to 11) after infusion. The median duration of cytokine release syndrome was 7 days (range, 3 to 12). Seven patients with cytokine release syndrome (33% of the overall population) were treated with tocilizumab (a monoclonal antibody that blocks interleukin-6), and 2 patients (10%) were treated in the intensive care unit.

ICANS was observed in 3 patients (14%); all ICANS events were grade 1 or 2. The median time until the onset of ICANS was 8 days (range, 7 to 20), and the median duration was 7 days (range, 3 to 7). No trial-related deaths or apparent differences in toxicity according to dose level were observed. Grade 2 and 3 cytokine release syndrome was associated with higher huCART19-IL18 expansion in the blood (Fig. S3). No toxic effects linked to immune effector cell–associated hemophagocytic lymphohistiocytosis–like syndrome were observed on the basis of clinical criteria.[31]

Table 1. Characteristics of the Patients at Baseline.*

Characteristic	Patients (N=21)
Median age (range) — yr	64 (47–74)
Male sex — no. (%)	16 (76)
ECOG performance-status score — no. (%)†	
0	2 (10)
1	19 (90)
Lymphoma subtype — no. (%)	
Large B-cell lymphoma	12 (57)
Diffuse large B-cell lymphoma, not otherwise specified	8 (38)
Transformed follicular lymphoma	2 (10)
High-grade B-cell lymphoma	1 (5)
T-cell histiocyte-rich large B-cell lymphoma	1 (5)
Follicular lymphoma	6 (29)
Mantle-cell lymphoma	3 (14)
Median no. of previous medications (range)	7 (4–14)
Previous therapy or procedure — no. (%)	
Autologous stem-cell transplantation	7 (33)
Allogeneic stem-cell transplantation	1 (5)
Bispecific antibody therapy	7 (33)
Previous CAR therapy — no./total no. (%)	
CD28-based product	10/20 (50)
Axicabtagene ciloleucel	8/20 (40)
Brexucabtagene autoleucel	2/20 (10)
4-1BB–based product	10/20 (50)
Tisagenlecleucel	8/20 (40)
Lisocabtagene maraleucel	2/20 (10)
Response to previous therapy	
Progressive disease — no./total no. (%)	7/20 (35)
Median progression-free survival — mo (90% CI)	6.7 (3.1–10.2)

* A total of 21 trial patients received huCART19-IL18 and were evaluated for efficacy and safety. One patient had not received previous anti-CD19 CAR T-cell therapy owing to two unsuccessful manufacturing attempts. CI denotes confidence interval.

† Scores for Eastern Cooperative Oncology Group (ECOG) performance status range from 0 to 5, with higher scores indicating greater disability.

Three patients (14%) had grade 3 infections, including coronavirus disease 2019. No secondary cancers were observed. Transient pulmonary edema developed in one patient during management of grade 3 cytokine release syndrome, an event that was considered to be a dose-limiting toxic effect that required expansion of the DL3

cohort. No additional dose-limiting toxic effects were observed.

DOSE FINDING

On the basis of the trial design, the highest dose level (3×10^8 transduced huCART19-IL18 cells) would have been the maximum tolerated dose with the isotonic estimate of the dose-limiting frequency of toxic effects that was closest to the target of 30%. However, this dose level met the nonfeasibility end points and was closed after only two patients had been treated. A dose range of 3×10^6 to 7×10^6 of huCART19-IL18–positive cells was selected for the expansion cohort and future trials in this patient population, according to the weighted multicriteria decision analysis. (Details regarding this analysis are provided in the Supplementary Appendix.)

EFFICACY

The frequency of a complete or partial response at 3 months after the initial infusion of huCART19-IL18 was 81% (90% CI, 62 to 93) (Fig. 2A). The percentage of patients with a complete response was 52% (90% CI, 33 to 71), and the percentage of partial response was 29% (90% CI, 13 to 49). Responses were seen across all lymphoma subtypes, with a complete or partial response observed in 67% of the patients with large B-cell lymphoma, in 100% of those with follicular lymphoma, and in 100% of those with mantle-cell lymphoma (Fig. 2A).

Among the patients who had received previous treatment with 4-1BB–based CAR products (8 who had large B-cell lymphoma and 2 who had follicular lymphoma), 60% had a complete or partial response, and 30% had a complete

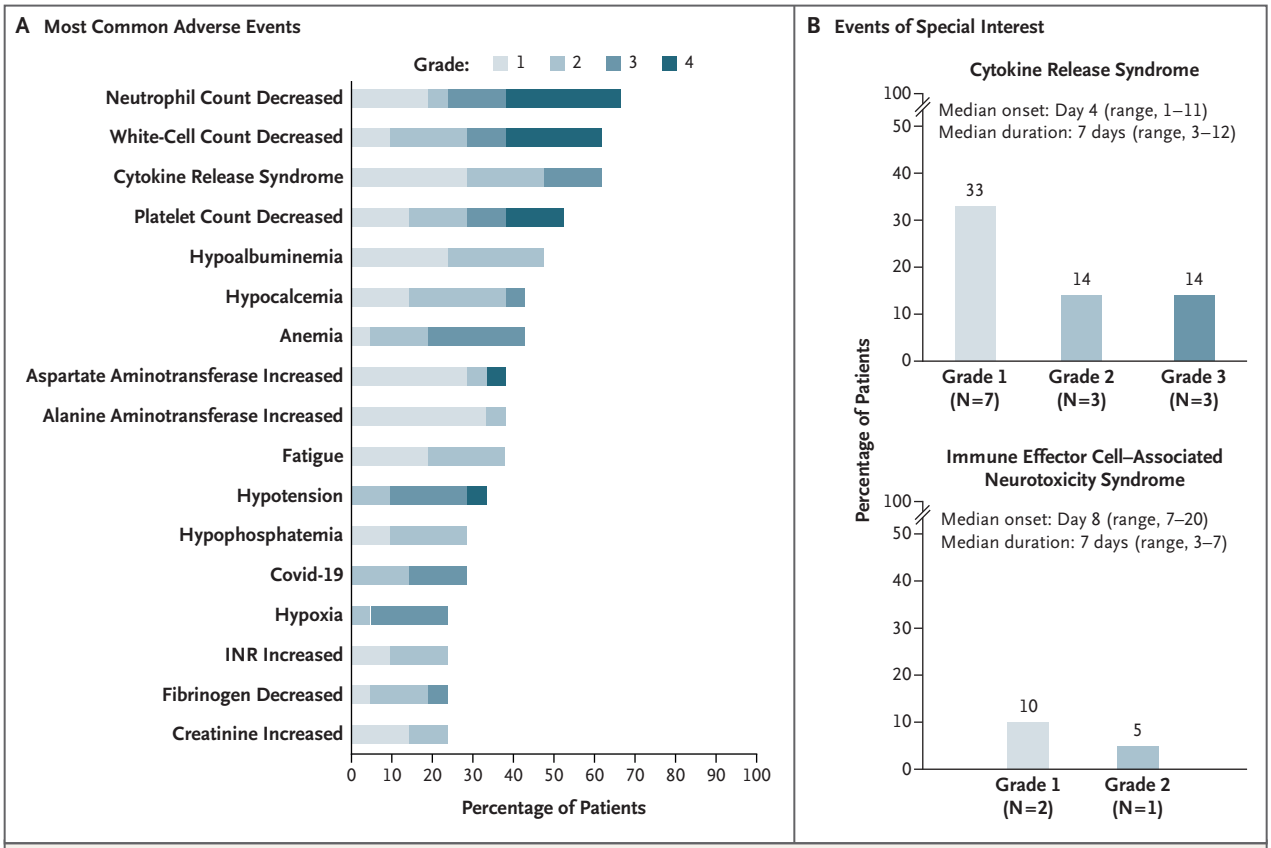


Figure 1. Adverse Events.

Panel A shows the most common adverse events that were identified in more than 20% of the 21 patients who received huCART19-IL18 during the trial. Panel B shows the percentage of patients with cytokine release syndrome and immune cell–associated neurotoxicity syndrome, as graded on the basis of the consensus criteria of the American Society for Transplantation and Cellular Therapy. Covid-19 denotes coronavirus disease 2019, and INR international normalized ratio.

response. This response appeared to be lower than that among the patients who had received previous CD28-based products (4 with large B-cell lymphoma, 4 with follicular lymphoma, and 2 with mantle-cell lymphoma), who had a complete or partial response of 100% and a complete response of 80% (Fig. 3C). No clear effect of cell dose on any of these responses was seen (Fig. 2D and Fig. S5).

At a median follow-up of 17.5 months (range, 3 to 34), the median duration of response was 9.6 months (90% CI, 5.5 to not reached) (Fig. S4). The median duration of progression-free survival was 8.7 months (90% CI, 5.4 to not reached); 10 patients (48%) were alive at 15 months, with an estimated overall survival of 86% (90% CI, 61 to 96) (Fig. 2B and 2C). Some evidence indicated improved progression-free survival in patients who had received previous CD28-based CAR T-cell treatment (Fig. S6). Responses are ongoing, including in 3 patients (2 with large B-cell lymphoma and 1 with follicular lymphoma) with more than 2 years of follow-up (Fig. 2D). Of the 12 patients who had biopsy-proven disease after the initial huCART19-IL18 infusion, only 1 patient had a CD19-negative relapse and 3 were reported to have a dim or weak CD19 level on flow cytometry or immunohistochemical analysis. Detailed data regarding the 5 patients in the retreatment cohort, which resulted in a durable complete response in 2 patients with large B-cell lymphoma, are provided in the Supplementary Appendix.

ARMORED HUCART19-IL18 CHARACTERISTICS AND CORRELATIVE STUDIES

In our trial, huCART19-IL18 showed robust engraftment and expansion at all dose levels, with significantly higher peak expansion in patients who had been previously treated with CD28-based CAR T-cell therapy than in those who had received 4-1BB–based products (Fig. 3A). Durable persistence was observed in peripheral blood and seen in three of four patients (75%) who were assessed at 2 years of follow-up (Fig. 4A and Fig. S8).

Residual second-generation CAR sequences were detected in 100% of the patients with previous exposure to 4-1BB–based CAR therapy, as compared with 40% of those with previous exposure to CD28-based therapy (Fig. 3B). Day 14 core biopsy specimens showed huCART19 transgene in 64% of samples tested with qPCR, and immunohistochemical analysis revealed dense CD3+ T-cell infiltration (Fig. S9B).

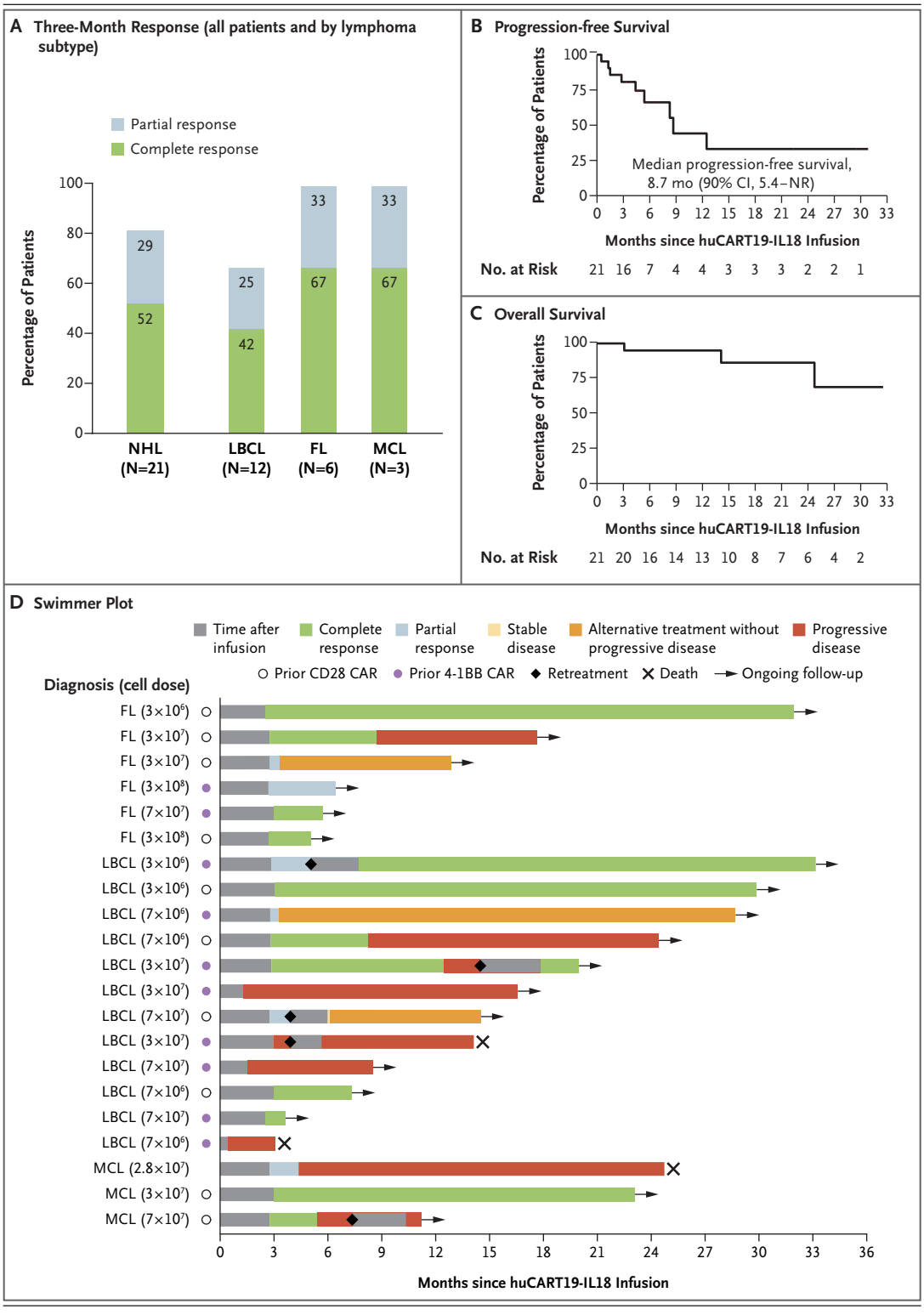
The activity of free interleukin-18 is regulated by circulating high-affinity interleukin-18 binding protein (IL18BP).[20] Serum analyses indicated that although free interleukin-18 levels remained unchanged (probably because of rapid sequestration by IL18BP), total levels of interleukin-18 and interleukin-18–IL18BP complex rose markedly after infusion and correlated strongly with CAR T-cell expansion (Fig. 4C). The increase in levels of interleukin-18–IL18BP complex in serum was unique to huCART19-IL18 and was not observed when we tested historical samples obtained from the same patients after they had received their initial second-generation CAR T cells (Fig. 4B). Furthermore, the huCART19-IL18 product had a higher proportion of naive-like (CD45RA+CCR7+) T cells than the previous conventional anti-CD19 products available for testing in two of the trial patients (Fig. S11).

Preclinical murine studies confirmed that huCART19-IL18 provided superior tumor control and prolonged survival as compared with the nonarmored huCART19 produced by the identical 3-day and 9-day manufacturing processes (Fig. S10). Collectively, these findings support the hypothesis that interleukin-18 armoring enhances local immune activation, CAR T-cell expansion, and antitumor efficacy.

DISCUSSION

In our trial, we found that autologous interleukin-18–armored CAR T cells had promising clinical activity in patients with relapsed or refractory CD19+ lymphomas after the failure of previous anti-CD19 CAR T-cell therapy. The treatment was associated mainly with toxic effects of grade 1 or 2, with no unexpected or delayed effects observed. Our findings indicate that 3-day manufacturing of huCART19-IL18 from autologous T cells is feasible, with 21 of 22 eligible patients receiving the product.

Our goal of using an expedited 3-day manufacturing process was to enrich the product with less differentiated naive-like CAR T cells, which could enhance in vivo expansion and activity. We observed the expansion of huCART19-IL18 with



persistence for more than 2 years in some patients. Such persistence occurred even in patients who had received the lowest dose of huCART19-IL18 (3×10^6), which is lower than the dose used for treatment of lymphomas with currently available second-generation CAR T products by a factor of approximately 20 to 100.[1,3,32] Although the median vein-to-vein time was more than 2 months

Figure 2 (facing page). Clinical Response and Survival.
Shown are the responses at 3 months in all patients (left) and according to lymphoma subtype (right) (Panel A), Kaplan–Meier estimates of progression-free survival (Panel B) and overall survival (Panel C), and a swimmer plot indicating the response to treatment according to lymphoma subtype, huCART19-IL18 cell dose, previous second-generation CD19-directed chimeric antigen receptor (CAR) T-cell therapy, response to initial huCART19-IL18 therapy and retreatment (when applicable), and any alternative therapy that was used in patients without disease progression (Panel D). FL denotes follicular lymphoma, LBCL large B-cell lymphoma, MCL mantle-cell lymphoma, NHL non-Hodgkin's lymphoma, and NR not reached.

in this trial because of the protocol-mandated safety stagger design, a shorter manufacturing process may have the additional benefit of reducing the time required to produce and administer the CAR T-cell therapy.

Interleukin-18 is a proinflammatory cytokine implicated in various inflammatory and autoimmune processes.[33] A phase 1 trial of recombinant human interleukin-18 plus rituximab showed the safety and immunologic activity of this combination in patients with relapsed and refractory lymphomas.[18] A recent report indicates that an elevated level of free interleukin-18 is associated with toxic effects similar to those of hemophagocytic lymphohistiocytosis in patients receiving CD22-directed CAR T-cell therapy.[34] We did not observe any cases of immune effector cell–associated hemophagocytic lymphohistiocytosis–like syndrome or a substantial increase in free interleukin-18 levels in blood in our study, nor any association between free interleukin-18 levels and huCART19-IL18 expansion (Fig. 4B and 4C). This lack of correlation suggests that the effects of interleukin-18 armoring in CAR T cells may be biologically distinct from the effects of elevated systemic levels of endogenous interleukin-18. It also suggests that the secretion of interleukin-18 by huCART19-IL18 may not overwhelm systemic regulation by the endogenous antagonistic IL18BP, a conclusion that is supported by our finding that expansion correlated with levels of interleukin-18–IL18BP complex. These observations suggest that interleukin-18 that is secreted by huCART19-IL18 acts locally within the tumor microenvironment effectively, without disabling systemic immune regulation.

We observed that patients who had received previous treatment with second-generation CAR T cells containing a CD28 intracellular costimulatory domain had higher peak expansion of huCART19-IL18 than those who received 4-1BB–based products (Fig. 3A). We also observed differences in efficacy that were based on previous second-generation CAR exposure (Fig. 3C and Fig. S6). The complete or partial response was 100%, with a complete response reported in 80% of the patients who had previously been treated with CD28-based agents. Those who had been treated with a 4-1BB product had a complete or partial response of 60% and a complete response of 30%, with similar trends in progression-free survival (Fig. 3C and Fig. S6). These findings suggest that both the mean peak expansion and type of previous CAR T-cell therapy may have influenced the efficacy of huCART19-IL18.

The reasons for the difference in outcomes remain uncertain. Specifically, differential persistence between CD28- and 4-1BB–based CAR T cells may reflect distinct resistance mechanisms.[35] In our trial, all 7 patients who were tested and had previously been treated with 4-1BB–based CAR therapy had detectable second-generation residual CAR T cells in their huCART19-IL18 product, including 2 patients who had received previous CAR T-cell therapy more than 3 years earlier. However, only 4 of 10 patients who had received previous CD28-based CAR therapy had any detectable evidence of previous second-generation CAR in the huCART19-IL18 product (Fig. 3B). Although immunogenicity has been considered as a potential factor contributing to nonresponse after CAR retreatment, ongoing investigations are exploring its role alongside other mechanisms that may influence the expansion or activity of huCART19-IL18, despite the persistence of the original 4-1BB–based CAR T cells.[13,36] It is also plausible that intrinsic differences in costimulatory signaling in the initial product lead to distinct T-cell exhaustion profiles or differential interactions with the tumor microenvironment, a hypothesis that merits further investigation.

Our trial was small and had an imbalance of lymphoma subtypes within each group (more patients with large B-cell lymphoma among those with previous 4-1BB product exposure), which confounds interpretation of the efficacy data. We did not see a clear relationship between cell dose and peak expansion and persistence. Standard half-log dose ranges may not be sufficiently spaced for resolving small differences with a limited number of patients. Dose-expansion relationships are complex for CAR T cells. A starting-dose

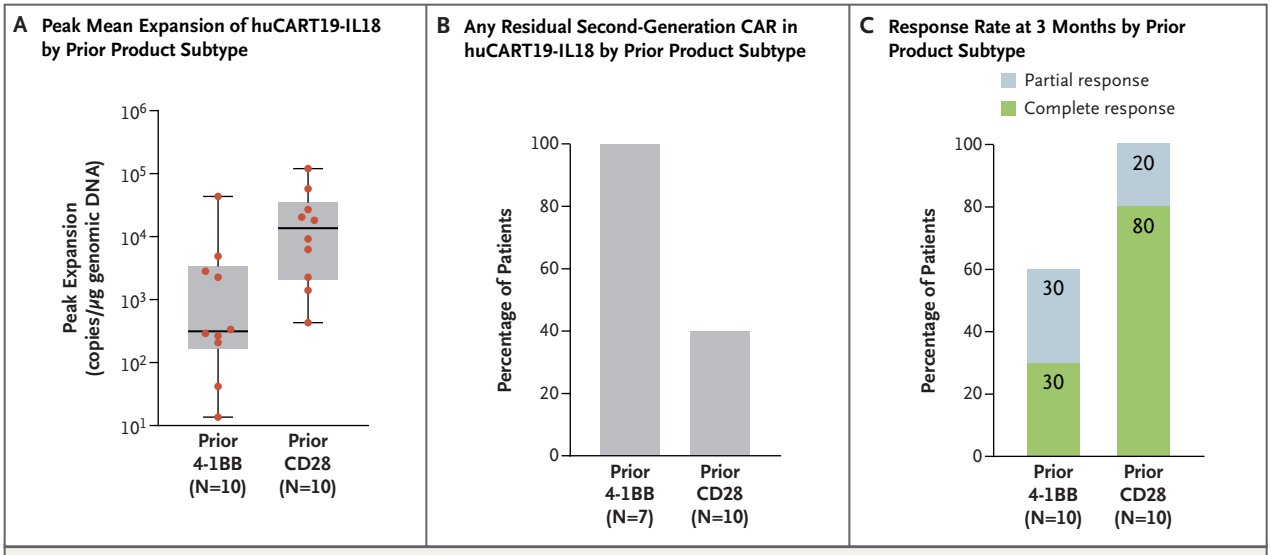


Figure 3. Effect of Previous CD19-Directed CAR T-Cell Therapy on Expansion and Efficacy of huCART19-IL18.

Panel A shows a comparison of values for the peak mean expansion of huCART19-IL18 according to the product subtype that the patient had previously received. The ratio of geometric means for peak expansion of huCART19-IL18 with prior CD28, as compared with prior 4-1BB product, was 16.3 (90% confidence interval [CI], 3.2 to 81.9). The huCART19-IL18 levels were assessed by means of quantitative real-time polymerase chain reaction (qPCR) and reported in copies per microgram of genomic DNA. Panel B shows the assessment of huCART19-IL18 products for the presence of previous second-generation CD19-directed CAR T cells in 17 patients. The assessment was performed with the use of Applied Biosystems TaqMan PCR to detect the sequences of integrated commercial CD19 CAR transgenes. Only 40% of the patients who had received previous treatment with a CD28-based product had detection of residual CAR, as compared with 100% of the patients who had previous treatment with a 4-1BB–based product. The odds ratio for residual CAR detection with prior CD28, as compared with prior 4-1BB product, was 0.1 (90% CI, 0 to 0.42). Panel C shows the differences in response distribution at 3 months according to previous CD19-directed CAR T-cell therapy. The odds ratio for a complete response with prior CD28, as compared with prior 4-1BB product, was 9.3 (90% CI, 1.2 to 84.0).

threshold is probably required, but otherwise the cells do not conform to conventional pharmacokinetic modeling for chemical drugs. Early studies have shown that nonresponse correlates with a failure of CAR T cells to expand, and studies involving patients with chronic lymphocytic leukemia have identified cell-intrinsic determinants of response and resistance.[37,38]

Limitations of this trial include the small number of patients, heterogenous lymphoma subtypes, and differences in previous exposure to anti-CD19 CAR products. One eligible patient had not received a previous commercial CAR T-cell product because of a manufacturing failure, a finding that highlights a real-world limitation of existing technologies — and makes our ability to manufacture a huCART19-IL18 product even more encouraging. We were concerned that the inclusion of this patient might skew the efficacy data in favor of huCART19-IL18. However, a post hoc analysis that excluded data for this patient improved the percentage of patients who had a complete response to 55% (90% CI, 35 to 74). Although one third of the patients had no response to previous anti-CD19 CAR T-cell products, there may also have been a selection bias for patients with lymphoma whose condition was stable enough to permit participation in a clinical trial.

Determining whether the huCART19-IL18 activity in our trial was related to interleukin-18 secretion, shortened manufacturing time, or the humanized anti-CD19 receptor may be difficult. Preclinical murine data have shown that huCART19-IL18 enhances tumor control and extends long-term survival as compared with non-armored anti-CD19 CAR T cells manufactured by the identical 3-day expedited process (Fig. S10). The elevation in interleukin-18–IL18BP levels that was observed after huCART19-IL18 infusion, but not after the infusion of second-generation CAR T-cell products, is also suggestive of the specific role of interleukin-18 in this trial. Although these integrated lines of evidence are suggestive and not conclusive, the burden of the correlative evidence implicates interleukin-18 as a key contribu-

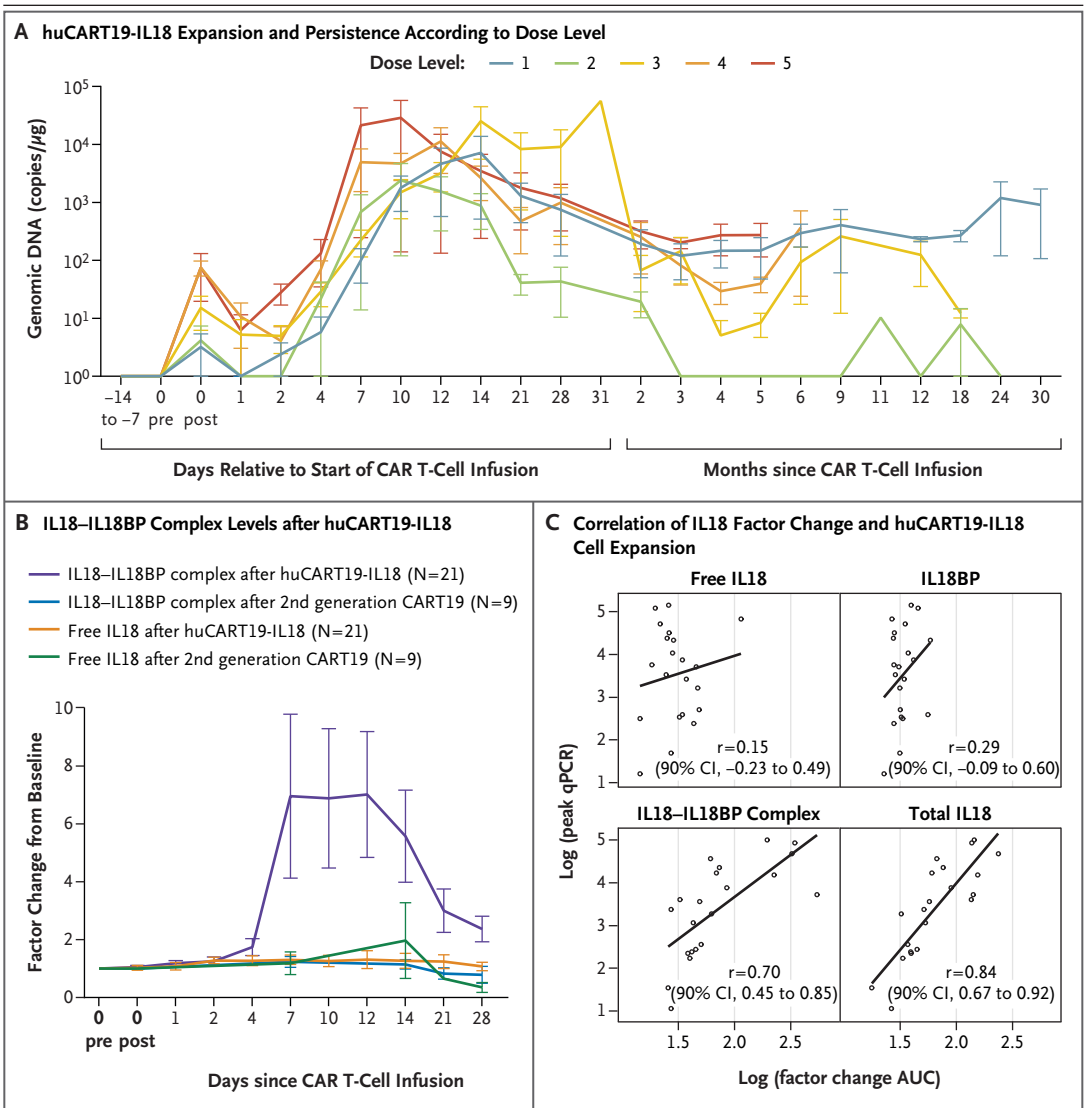


Figure 4. Correlative Studies.

Panel A shows the expansion of huCART19-IL18 T cells in peripheral blood as assessed by qPCR assay and measured as copies per microgram of genomic DNA, separated according to dose level 1 (DL1) through dose level 5 (DL5). Panel B shows the levels of free interleukin-18 (IL18) and interleukin-18 binding protein (IL18BP) complex, as measured by enzyme-linked immunosorbent assay. The amount of free interleukin-18 and interleukin-18 bound to IL18BP is plotted. Although serum levels of free interleukin-18 remained low (probably owing to rapid binding by IL18BP), the substantial increases in total levels of interleukin-18–IL18BP complex may serve as surrogate markers for interleukin-18 production by huCART19-IL18. The elevations in interleukin-18–IL18BP were not seen in historical blood samples after the infusion of second-generation anti-CD19 CAR T cells from the same patients. Panel C shows a scatter plot that illustrates the relationship between the log factor change in interleukin-18 forms (on the x axis) and huCART19-IL18 expansion in blood (on the y axis). Calculations of the Pearson correlation coefficient (r) and corresponding 90% confidence intervals are indicated for each plot. The increases in levels of total interleukin-18 and interleukin-18–IL18BP complex correlate with the expansion of huCART19-IL18 T cells, but no correlation is seen with levels of either free interleukin-18 or free IL18BP. AUC denotes area under the curve.

tor to the improved responses observed with huCART19-IL18 therapy.

In our trial, huCART19-IL18 had toxic effects that were consistent with those associated with other CAR T therapies and had encouraging efficacy in patients with CD19+ lymphomas who did not have a response to previous anti-CD19 CAR T-cell therapy or had a relapse after such

therapy. Some responses were durable, now persisting beyond 2 years, and were observed in patients who had resistance to previous second-generation anti-CD19 CAR T-cell products. These findings indicate that retargeting of CD19 with armored CAR T cells may be an effective strategy for these patients. Our trial provides proof of concept that cytokine-armored CAR T-cell treatment is feasible and may enhance antitumor activity without additional toxic effects. Incorporating cytokine secretion into CAR T-cell design may have broader implications for enhancing cellular therapies beyond hematologic cancers.[39]

Disclosure forms provided by the authors are available with the full text of this article at NEJM.org.

A data sharing statement provided by the authors is available with the full text of this article at NEJM.org.

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