ORIGINAL ARTICLE

Use of CAR-Transduced Natural Killer Cells in CD19-Positive Lymphoid Tumors

Enli Liu, M.D., David Marin, M.D., Pinaki Banerjee, Ph.D., Homer A. Macapinlac, M.D., Philip Thompson, M.B., B.S., Rafet Basar, M.D., Lucila Nassif Kerbauy, M.D., Bethany Overman, B.S.N., Peter Thall, Ph.D., Mecit Kaplan, M.S., Vandana Nandivada, M.S., Indresh Kaur, Ph.D., Ana Nunez Cortes, M.D., Kai Cao, M.D., May Daher, M.D., Chitra Hosing, M.D., Evan N. Cohen, Ph.D., Partow Kebriaei, M.D., Rohtesh Mehta, M.D., Sattva Neelapu, M.D., Yago Nieto, M.D., Ph.D., Michael Wang, M.D., William Wierda, M.D., Ph.D., Michael Keating, M.D., Richard Champlin, M.D., Elizabeth J. Shpall, M.D., and Katayoun Rezvani, M.D., Ph.D.

ABSTRACT

BACKGROUND

Anti-CD19 chimeric antigen receptor (CAR) T-cell therapy has shown remarkable clinical efficacy in B-cell cancers. However, CAR T cells can induce substantial toxic effects, and the manufacture of the cells is complex. Natural killer (NK) cells that have been modified to express an anti-CD19 CAR have the potential to overcome these limitations.

METHODS

In this phase 1 and 2 trial, we administered HLA-mismatched anti-CD19 CAR-NK cells derived from cord blood to 11 patients with relapsed or refractory CD19-positive cancers (non-Hodgkin's lymphoma or chronic lymphocytic leukemia [CLL]). NK cells were transduced with a retroviral vector expressing genes that encode anti-CD19 CAR, interleukin-15, and inducible caspase 9 as a safety switch. The cells were expanded ex vivo and administered in a single infusion at one of three doses (1×10^5, 1×10^6, or 1×10^7 CAR-NK cells per kilogram of body weight) after lymphodepleting chemotherapy.

RESULTS

The administration of CAR-NK cells was not associated with the development of cytokine release syndrome, neurotoxicity, or graft-versus-host disease, and there was no increase in the levels of inflammatory cytokines, including interleukin-6, over baseline. The maximum tolerated dose was not reached. Of the 11 patients who were treated, 8 (73%) had a response; of these patients, 7 (4 with lymphoma and 3 with CLL) had a complete remission, and 1 had remission of the Richter's transformation component but had persistent CLL. Responses were rapid and seen within 30 days after infusion at all dose levels. The infused CAR-NK cells expanded and persisted at low levels for at least 12 months.

CONCLUSIONS

Among 11 patients with relapsed or refractory CD19-positive cancers, a majority had a response to treatment with CAR-NK cells without the development of major toxic effects. (Funded by the M.D. Anderson Cancer Center CLL and Lymphoma Moonshot and the National Institutes of Health; ClinicalTrials.gov number, NCT03056339.)

From the Departments of Stem Cell Transplantation and Cellular Therapy (E.L., D.M., P.B., R.B., L.N.K., B.O., M. Kaplan, V.N., I.K., A.N.C., M.D., C.H., P.K., R.M., Y.N., R.C., E.J.S., K.R.), Nuclear Medicine (H.A.M.), Leukemia (P. Thompson, W.W., M. Keating), Biostatistics (P. Thall), Laboratory Medicine (K.C.), Hematopathology (E.N.C.), and Lymphoma and Myeloma (S.N., M.W.), University of Texas M.D. Anderson Cancer Center, Houston. Address reprint requests to Dr. Rezvani at the Department of Stem Cell Transplantation and Cellular Therapy, University of Texas M.D. Anderson Cancer Center, 1515 Holcombe Blvd., Box 448, Houston, TX 77030, or at krezvani@mdanderson.org.

Drs. Liu, Marin, and Banerjee contributed equally to this article.

This article was updated on February 7, 2020, at NEJM.org.

N Engl J Med 2020;382:545-53.
DOI: 10.1056/NEJMoa1910607

Chimeric antigen receptors (CARs) have been used to redirect the specificity of T cells against a number of hematologic cancers with notable clinical responses. For example, CAR T cells directed against CD19 induce remissions in 68 to 93% of patients with acute B-lymphoblastic leukemia,[1,2] in 57 to 71% of those with chronic lymphocytic leukemia (CLL),[3-5] and in 64 to 86% of those with non-Hodgkin's lymphoma.[6-8] These remissions are durable in a proportion of cases. Two anti-CD19 CAR T-cell products have been approved for clinical use by the Food and Drug Administration (FDA).

Despite their antitumor activity, autologous CAR-modified T cells have some logistic and clinical limitations. CAR T cells are produced on an individual-patient basis, which makes their production complex and expensive. In a number of patients, treatment with CAR T cells has been associated with substantial toxic effects, including cytokine release syndrome and neurotoxicity, which involve treatment in specialized care units.[9-11] An effective allogeneic product with a better safety profile could overcome these limitations.

Natural killer (NK) cells that have been engineered to express a CAR are candidate effectors for cancer treatment. These cells of the innate immune system play a pivotal role in immune surveillance by targeting cancer or virally infected cells that down-regulate HLA class I molecules or express stress markers.[12,13] NK cells from an allogeneic source, such as cord blood, can be safely administered without the need for full HLA matching,[14] which eliminates the need to produce a unique CAR product for each patient. Furthermore, allogeneic NK cells have a proven track record of safety after infusion for adoptive immunotherapy in patients with cancer.[15,16]

Thus, to harness the antitumor potential of NK cells for clinical testing, we used a retroviral vector that expresses genes that encode anti-CD19 CAR, interleukin-15 to enhance the in vivo expansion and persistence of the transduced NK cells,[17] and inducible caspase 9 to trigger apoptosis of the CAR-NK cells in the event of unacceptable toxic effects.[18] In a preclinical model of lymphoma in mice, we found that NK cells that had been derived from cord blood and transduced with anti-CD19 CAR, interleukin-15, and inducible caspase 9 had better antitumor activity than nontransduced control NK cells.[18] On the strength of these findings, we undertook a phase 1 and 2 trial to assess the safety and efficacy of escalating doses of CAR-NK cells for the treatment of relapsed or refractory CD19-positive cancers.

Methods

Study Design and Patients

Here, we report on the first 11 patients in this ongoing study, with a data cutoff of April 2019. (Details regarding enrollment are provided in the Methods section in the Supplementary Appendix, available with the full text of this article at NEJM.org.) Briefly, patients underwent lymphodepleting chemotherapy with fludarabine (at a dose of 30 mg per square meter of body-surface area) and cyclophosphamide (at a dose of 300 mg per square meter) daily for 3 consecutive days, followed by a single infusion of the trial CAR-NK cells at escalating doses of 1×10^5 cells, 1×10^6 cells, and 1×10^7 cells per kilogram of body weight. Postremission therapy was permitted after the day 30 assessment at the treating physician's discretion.

The first 9 patients received a CAR-NK product that was partially matched with the HLA genotype of the recipient (4 of 6 matches at HLA loci A, B, and DRβ1) (Table 1 and Table S1 in the Supplementary Appendix). The protocol was then amended to permit treatment with no consideration for HLA matching, which was the procedure used in Patients 10 and 11. When possible, we selected a cord-blood unit with killer immunoglobulin-like receptor (KIR) ligand mismatch[19] for CAR-NK production. (KIR mismatch between the donor and recipient may enhance the intrinsic [non-CAR–mediated] antitumor activity of NK cells through a process known as missing-self recognition.) Clinical responses to therapy were based on the 2018 criteria of the International Workshop on Chronic Lymphocytic Leukemia[20] and on the 2014 Lugano classification for non-Hodgkin's lymphoma.[21] (Details are provided in the Supplementary Appendix.)

Study Oversight

The study was approved by the institutional review board at the University of Texas M.D. Anderson Cancer Center and was conducted according to the principles of the Declaration of Helsinki. Written informed consent was obtained from all

the patients. The second and last authors wrote the first draft of the manuscript. All the authors vouch for the completeness and accuracy of reported data and adverse events and for the adherence of the study to the protocol, which is available at NEJM.org.

MANUFACTURE OF CAR-NK CELLS FROM CORD BLOOD

Full details regarding the manufacture of the CAR-NK cells are provided in the Methods section in the Supplementary Appendix. Briefly, the cord-blood unit was thawed and NK cells were purified and cultured in the presence of engineered K562 feeder cells and interleukin-2. On day 6, cells were transduced with a retroviral vector encoding the genes for anti-CD19 CAR, the CD28.CD3ζ signaling endodomain, interleukin-15, and inducible caspase 9.[22] The cells were expanded and harvested for fresh infusion on day 15. The efficiency of the final CAR-NK transduction for the infused product was 49.0% (range, 22.7 to 66.5). CAR-NK cells were tested in vitro and killed primary CLL targets in a perforin-dependent manner (Fig. S1). The median CD3-positive T-cell content in the infused product was 500 cells per kilogram (range, 30 to 8000), with a median of 0.01% (range, 0.01 to 0.002) contaminating CAR T cells in the product (Table S2).

STATISTICAL ANALYSIS

We used the Wilcoxon rank-sum test to test the associations between the response to therapy and level of CAR-NK cells. A P value of less than 0.05 was considered to indicate statistical significance.

RESULTS

CHARACTERISTICS OF THE PATIENTS

From June 2017 through February 2019, we enrolled 15 consecutive patients in accordance with the protocol. Of these patients, 4 withdrew before the initiation of treatment owing to disease progression, the development of graft-versus-host disease, the absence of detectable disease, and bacterial contamination of the product (in 1 patient each). Thus, 11 patients received a single dose of CAR-NK cells (Table 1 and Table S1). The median age of the patients was 60 years (range, 47 to 70). The 11 patients had already received a

Table 1. Characteristics of the Patients at Baseline.*

Patient No.	Dose Level	Age	Sex	Diagnosis	Previous Lines of Therapy	Failure of HSCT, Ibrutinib, or Venetoclax	HLA Allelic Match†	KIR Ligand Mismatch‡
	cells/kg	*yr*			*no.*		*no./total no.*	
1	1×10^5	47	Male	Transformed follicular lymphoma	3	Autologous HSCT	4/6	No
2	1×10^5	59	Male	Diffuse large B-cell lymphoma	6	NA	4/6	Yes
3	1×10^5	59	Female	Chronic lymphocytic leukemia	4	Ibrutinib, venetoclax	4/6	No
4	0.25×10^6§	56	Male	Chronic lymphocytic leukemia	5	Ibrutinib	4/6	Yes
5	1×10^6	61	Male	Chronic lymphocytic leukemia with Richter's transformation	5	Ibrutinib	4/6	Yes
6	1×10^6	59	Female	Accelerated chronic lymphocytic leukemia	5	Ibrutinib, venetoclax	4/6	No
7	1×10^6	66	Female	Chronic lymphocytic leukemia	4	Ibrutinib	4/6	Yes
8	1×10^7	64	Male	Transformed follicular lymphoma	11	Autologous HSCT	4/6	No
9	1×10^7	70	Male	Diffuse large B-cell lymphoma	4	Autologous HSCT	4/6	No
10	1×10^7	61	Female	Transformed follicular lymphoma	4	Autologous HSCT	2/6	No
11	1×10^7	60	Male	Follicular lymphoma (focally grade 3B)	4	NA	1/6	Yes

* All 11 patients who received anti-CD19 chimeric antigen receptor natural killer (CAR-NK) cells had disease with high-risk cytogenetic or molecular characteristics. HSCT denotes hematopoietic stem-cell transplantation, and NA not applicable because the patient did not receive any of the listed therapies.
† Listed is the number of HLA matches between the donated cord-blood unit and the patient at HLA loci A, B, and DRβ1.
‡ A mismatch between the donor's killer immunoglobulin-like receptor (KIR) and the recipient's HLA has been associated with a reduced risk of relapse after allogeneic HSCT.
§ Patient 4 received a lower dose than the other patients in the assigned-dose group because of an insufficient number of cells available for transfusion.

median of 4 lines of therapy (range, 3 to 11). Five patients had CLL (including 2 who had Richter's transformation or accelerated CLL), and all had a history of disease progression while receiving ibrutinib plus a minimum of 3 other lines of therapy; all 5 patients had high-risk genetic characteristics. Six patients had lymphoma, including 2 with diffuse large B-cell lymphoma and 4 with the follicular form; 3 of these patients underwent transformation to high-grade lymphoma. Of the 6 patients with lymphoma, 4 had undergone disease progression after autologous hematopoietic stem-cell transplantation and 2 had refractory disease.

SAFETY

After the infusion of CAR-NK cells, none of the patients had symptoms of cytokine release syndrome, neurotoxicity, or hemophagocytic lymphohistiocytosis. Moreover, we did not observe any cases of graft-versus-host-disease, despite the HLA mismatch between the patients and their CAR-NK products. As expected, all the patients had transient and reversible hematologic toxic events, which were mainly associated with the lymphodepleting chemotherapy. We cannot determine whether the infusion of CAR-NK cells contributed to the hematologic toxicity. There were no cases of tumor lysis syndrome or grade 3 or 4 nonhematologic toxicity. The maximum tolerated dose of CAR-NK cells was not reached. Table 2 lists all the adverse events that were observed in the study. No patient was admitted to an intensive care unit (ICU) for management of adverse events associated with CAR-NK cells. However, Patient 2 was admitted to the ICU for treatment of progressive lymphoma and subsequently died. Given the absence of serious toxicity in the study, we did not activate the caspase 9 safety switch (with rimiducid) to eliminate the CAR-NK cells.

TREATMENT RESPONSE

At a median follow-up of 13.8 months (range 2.8 to 20.0), 8 patients (73%) had an objective response, including 7 patients (3 with CLL and 4 with lymphoma) who had a complete response (Fig. 1). An additional patient who had CLL with Richter's transformation (Patient 5) had a complete remission of high-grade lymphoma, according to the absence of lesions with fluorodeoxyglucose uptake on positron-emission tomography–computed tomography (PET-CT) performed 30 days after the CAR-NK infusion, but continued to have cytopenia, with bone marrow infiltration by CLL (Fig. S2). Although this patient eventually had a complete response while receiving postremission therapy (see below), we did not attribute this response to the CAR-NK therapy. In all 8 patients, the response to treatment occurred during the first month after infusion. Of the 11 patients who were treated, 5 received a KIR ligand–mismatched product. It was not possible to assess the effect of KIR ligand mismatch on outcomes in such a small series.

POSTREMISSION THERAPY

Of the 8 patients who had a response to CAR-NK therapy, 5 underwent postremission therapy (Fig. 1). Patient 3 (who had CLL) had subsequent minimal residual disease, as detected on flow cytometry of peripheral blood, 9 months after infusion and received rituximab. Patient 7 (who also had CLL) had a clinical complete response but had persistent minimal residual disease and received lenalidomide as an immunomodulatory agent, beginning 6 weeks after infusion. Patient 8 (who had transformed follicular lymphoma) and Patient 11 (who had follicular lymphoma) underwent hematopoietic stem-cell transplantation after CAR-NK therapy while in complete response without evidence of minimal residual disease. Patient 5 (who had CLL with Richter's transformation) had remission of high-grade lymphoma but had persistent CLL and received venetoclax. All these patients were alive and in complete remission on the date of the last assessment, although Patients 3, 5, and 7 continue to have positive results for minimal residual disease.

B-CELL APLASIA

Since B-cell aplasia has been used as a surrogate for anti-CD19 CAR T-cell activity, we measured the frequencies of CD19-positive B cells in the peripheral blood of patients after the infusion of CAR-NK cells. All the patients except for Patients 1 and 5 had B-cell aplasia associated with previous B-cell–depleting therapies at the time of enrollment. In Patient 1, B-cell aplasia developed after CAR-NK therapy and lymphodepleting chemotherapy. Patient 5 had persistent CLL in peripheral blood, despite having had a complete response with respect to the high-grade transformation, until he received venetoclax. Patient 3 had evidence of B-cell recovery coincident with

recurrent positivity for minimal residual disease. None of the remaining patients had recovery of a normal B-cell count during the follow-up period.

CAR-NK EXPANSION, MIGRATION, AND PERSISTENCE

We used a quantitative real-time polymerase-chain-reaction assay to measure in vivo expansion of CAR-NK cells according to the number of vector transgene copies per microgram of genomic DNA. Expansion was seen as early as 3 days after infusion, with CAR-NK cells persisting for at least 12 months (Fig. 2A and Table S3). The peak CAR-NK copy number was measured 3 to 14 days after infusion and was dose-dependent. Beyond day 14, no dose-related differences were noted in the level of peripheral-blood transcripts or in the persistence of CAR-NK cells. As has been reported in patients treated with CAR-T cells,[5,6,23] patients in our study who had a response to therapy had a significantly higher early expansion of CAR-NK cells than those who did not have a response (Fig. 2B). We did not observe a difference in the persistence of CAR-NK cells according to the degree of HLA mismatch with the recipient (Table 1 and Fig. S3). These results were confirmed by means of flow cytometry (Fig. S4).[24]

In 2 patients with available lymph-node samples, more CAR-NK cells were found in the lymph nodes than in the bone marrow or peripheral blood (Figs. S5 and S6), a finding that supports the notion that CAR-NK cells home in on disease sites. Similar levels of CAR-NK cells were detected in the bone marrow and peripheral blood in the 10 patients with available samples (Fig. S7).

The minimal number of contaminating CAR-expressing T cells in the product did not result in detectable CAR T-cell expansion after infusion, nor did the CD3+ T cells result in the development of graft-versus-host disease (Fig. S8). CAR-NK cells were still detectable at low levels in patients who did not have a response or who had a relapse, despite the expression of CD19 in the tumor cells, which suggests the presence of alternative immune escape mechanisms, such as induction of CAR-NK exhaustion. Functional studies of the residual CAR-NK cells in the patients with relapse have not been performed. The persistent CAR-NK cells did not expand in vivo at the time of relapse.

Table 2. Adverse Events in the 11 Study Patients.*

Adverse Event	Grade 1 or 2	Grade 3	Grade 4
	no. of patients		
Cytokine release syndrome	0	0	0
Graft-versus-host disease	0	0	0
Neurologic event	0	0	0
Hematologic event			
Neutropenia	1	2	8
Lymphopenia	1	0	10
Thrombocytopenia	8	0	0
Anemia	7	2	0
Cardiovascular event			
Chest pain	2	0	0
Hypertension	0	1	0
Atrial fibrillation	1	0	0
Tachycardia	4	0	0
Constitutional event			
Fatigue	3	0	0
Insomnia	2	0	0
Infection			
Bacterial	1	0	0
Viral	1	1	0
Miscellaneous			
Blurred vision	1	0	0
Headache or dizziness	2	0	0
Pleural effusion	1	0	0
Skin discoloration	1	0	0
Muscle or bone pain	2	0	0
Laboratory values			
Elevated creatinine	3	0	0
Elevated alanine aminotransferase	1	0	0
Elevated C-reactive protein	2	0	0
Elevated ferritin	4	0	0
Hyperglycemia	1	0	0
Elevated lactate dehydrogenase	2	0	0
Hypoalbuminemia	2	0	0
Electrolyte abnormality	2	1	0

* Listed are all adverse events that were reported in the 11 patients from the time of infusion of CAR-NK cells until day 40, regardless of whether the investigators attributed the events to the treatment. Abnormalities caused by the original disease are not listed.

ANALYSIS OF SERUM CYTOKINES

The supernatants from serial peripheral-blood samples were measured for inflammatory cytokines as well as for interleukin-15, which was

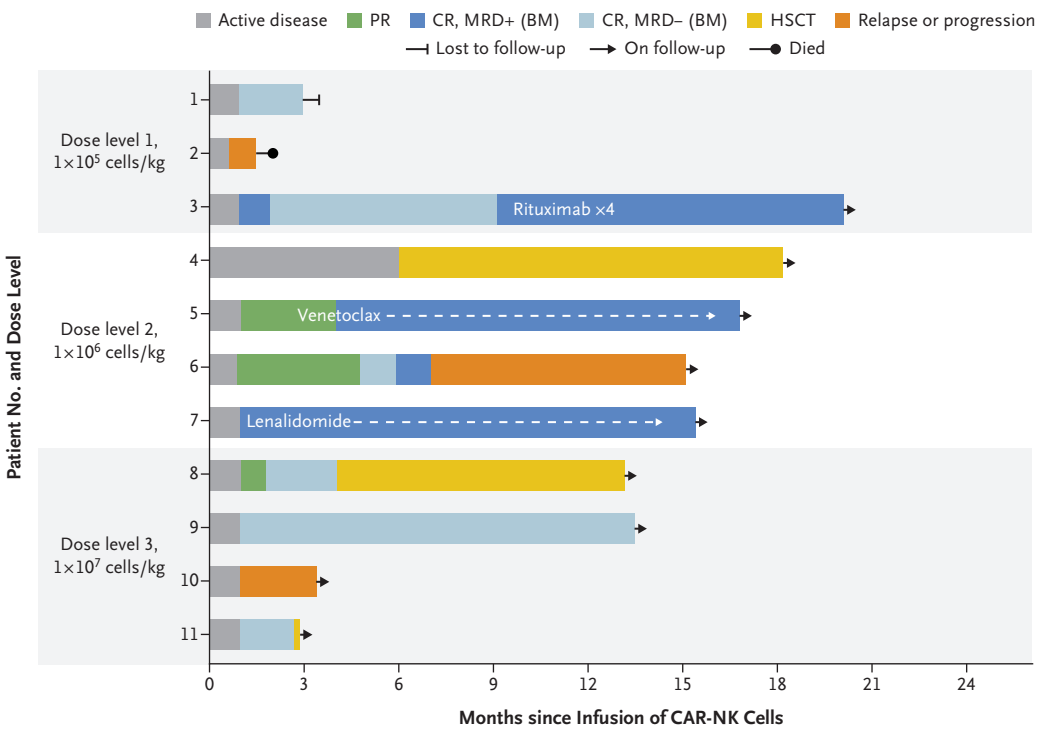

Figure 1. Clinical Response to CAR-NK Therapy and Postremission Treatments.

Shown are the clinical outcomes and subsequent therapies for the 11 patients who were treated with anti-CD19 chimeric antigen receptor (CAR) natural killer (NK) cells in our study. Responses were confirmed and assessed according to the 2018 criteria of the International Workshop on Chronic Lymphocytic Leukemia and the 2014 Lugano classification for non-Hodgkin's lymphoma. The indicated responses include partial response (PR) and complete response (CR); MRD denotes minimal residual disease, as assessed on multiparameter flow cytometry, with or without bone marrow (BM) infiltration. Patient 3 received four doses (×4) of rituximab; for Patients 5 and 7, the dashed white line indicates the duration of postremission therapy. HSCT denotes hematopoietic stem-cell transplantation.

encoded by the retroviral vector that was used to produce the CAR-NK cells. We observed no increase in the levels of inflammatory cytokines (e.g., interleukin-6 and tumor necrosis factor α) as compared with the baseline levels, nor did we find an increase in the systemic levels of interleukin-15 over pretreatment values, which indicated that interleukin-15 was not released to substantial systemic levels by CAR-NK cells in the peripheral blood after infusion (Fig. S9).

INDUCTION OF ALLOIMMUNE ANTIBODY RESPONSES AGAINST THE DONOR

All the patients received HLA-mismatched CAR-NK products. Patients 1 through 9 received a product with partial matching at 4 of 6 HLA molecules, whereas Patients 10 and 11 were recipients of non–HLA-matched CAR-NK cells. Thus, we monitored for the induction of donor-specific HLA antibodies. At all the time points when testing was performed, no antibody induction against the mismatched HLA alleles of the infused product was observed (Table S4). Host cellular responses were not assessed.

DISCUSSION

We present the early results of a phase 1 and 2 study of NK cells that were derived from cord blood and engineered to express anti-CD19 CAR, interleukin-15, and an inducible caspase 9 safety switch. This therapy was tested in heavily pretreated patients with multiply relapsed or refractory CLL or non-Hodgkin's lymphoma (Table 1). At a median follow-up of 13.8 months, 8 of the 11 treated patients (73%) had an objective re-

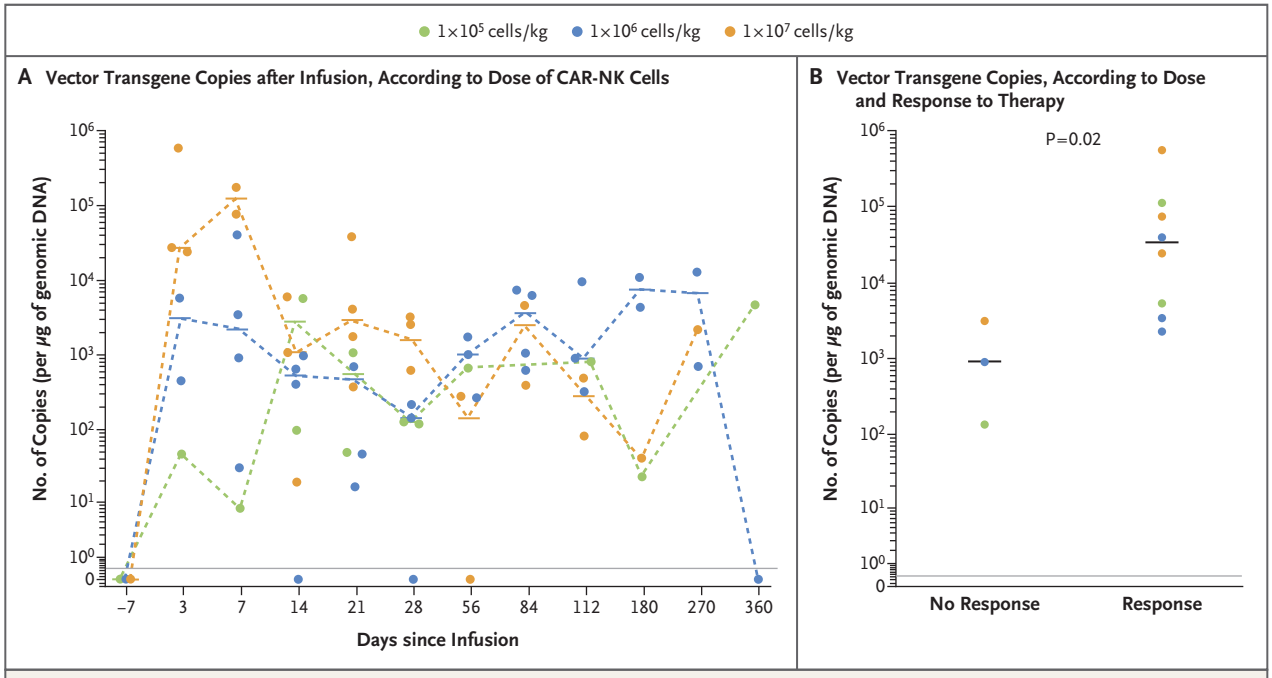

Figure 2. Persistence of CAR-NK Cells after Infusion.

Panel A shows measurements of CAR-NK cells in peripheral-blood samples, as assessed on quantitative polymerase-chain-reaction assay, according to the dose of CAR-NK cells received by the patient. The horizontal gray line at 3 copies per microgram of DNA represents the lower limit of quantification for this assay. The solid horizontal bars indicate the median copy numbers at the various time points for each dose level. After a single infusion of CAR-NK cells, CAR sequences could be detected in all 11 patients. The values increased and remained detectable in peripheral blood for up to 1 year after infusion, regardless of the dose level. No relationship was observed between the administered cell dose and the CAR-NK copy number beyond day 14 after infusion, which suggests that the persistence of CAR-NK cells was driven by in vivo proliferation of the infused cells. The length of follow-up varied among the patients. Panel B shows the peak copy numbers of CAR-NK cells in the first 28 days after infusion for the 11 patients, according to their response to therapy. Patients who had a response at day 30 had a significantly higher copy-number peak of CAR-NK cells after the infusion than those who did not have a response (median value, 31,744 vs. 903 copies per microgram; P=0.02). The black horizontal bars indicate median values.

sponse (4 of 5 patients with CLL and 4 of 6 with non-Hodgkin's lymphoma); 7 of 11 patients (64%) had a complete response. Responses were rapid and seen at all dose levels. Response durations cannot be assessed because of the administration of other therapies, starting as early as 30 days after the infusion of CAR-NK cells. The lymphodepleting chemotherapy regimen used in our trial was similar to that used in most CAR T-cell studies and could have partially contributed to the objective responses, although it is important to note that these patients were considered to have chemotherapy-resistant disease at study entry.

After adoptive immunotherapy, nonengineered NK cells typically disappear by 2 weeks after infusion,[15,18] a property that has limited their clinical utility. We found an expansion of the infused CAR-NK cells and their persistence at low levels for at least 12 months, despite the substantial HLA mismatch between the infused NK cells and the recipient. The inclusion of interleukin-15 in the construct may have played an important role in the persistence and antitumor activity of these CAR-NK cells. Although continuous in vitro exposure to exogenous interleukin-15 was reported to induce NK-cell exhaustion,[25] our preclinical data show that the interleukin-15 in the construct did not lead to exhaustion of CAR-NK cells but instead increased their antitumor activity.[18]

The absence of rejection of the CAR-NK cells by the recipient may be due to multiple factors. In our study, the long-term persistence of HLA-mismatched CAR-NK cells would probably have been mediated by a permissive environment created by the lymphodepleting regimen combined with the ectopic expression of interleukin-15 by

the CAR-NK cells, which led to enhanced expansion and persistence of the transduced cells. Yet these persistent cells were not sufficient to prevent relapse, and relapse did not appear to lead to the subsequent in vivo expansion of the cells.

A proportion of the patients who are treated with anti-CD19 CAR T cells have a subsequent relapse, with a 1-year progression-free survival of approximately 30% among patients with CLL and 45% among those with non-Hodgkin's lymphoma.[4,6,8] In view of these outcomes, our study allowed for remission consolidation therapy with an immunomodulatory agent, anticancer drug, or hematopoietic stem-cell transplantation at the discretion of the treating physician. However, the use of postremission therapy in this study limits our assessment of the durability of response after CAR-NK therapy.

We found that allogeneic CAR-NK cells can be delivered in adoptive transfer without the serious cytokine release syndrome and neurologic toxic effects that have been associated with CAR T-cell therapy. However, we observed high-grade transient myelotoxicity, which we attributed to the lymphodepleting chemotherapy. We are unable to assess whether the CAR-NK cells contributed to the myelotoxicity. Despite the substantial HLA disparity between the CAR-NK cells and the recipient, no graft-versus-host disease was noted, a finding that was consistent with the results of both preclinical and clinical studies of allogeneic NK cells.[15,26,27] Although the preparations of CAR-NK cells contained some donor T cells, the number of these cells was apparently insufficient to initiate a graft-versus-host disease pathway. It is thought that CAR T-cell toxic effects such as cytokine release syndrome are largely mediated by interleukin-6, which is secreted by both CAR T cells and myeloid cells.[23,28] NK cells secrete less interleukin-6 than T cells. In addition, the cross-talk between NK cells and the myeloid compartment differs from that of T cells.[29] In our study, the levels of inflammatory cytokines such as interleukin-6, tumor necrosis factor α, and interferon-γ remained stable at low levels throughout.

The existing FDA-approved products containing CAR T-cells are patient-specific (autologous), which makes their manufacture complex and costly.[30,31] In our study, patients were enrolled sequentially with a minimum enrollment interval of 2 weeks to ensure safety. Thus, a fresh product of CAR-NK cells was manufactured for each patient. However, we have shown that it is possible to produce more than 100 doses of CAR-NK cells from a single cord-blood unit.[18] This capability, together with the apparently minimal HLA-matching requirements between the donor of CAR-NK cells and the patient, may pave the way for a truly off-the-shelf product that could increase treatment accessibility for many more patients. This possibility has yet to be verified, since no patient has received cryopreserved and thawed CAR-NK cells and been shown to have antitumor responses. The fact that we cannot ascertain the durability of the response is another factor that limits conclusions about the efficacy of these cells. However, our preliminary results show that CAR-NK cells can induce responses in patients with high-risk CD19-positive cancers with relatively few adverse events aside from transient myelotoxicity.

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

Supported by a grant from the M.D. Anderson Cancer Center CLL and Lymphoma Moonshot, by grants (1 R01 CA211044-01, 5 P01CA148600-03, and P50CA100632-16) from the National Institutes of Health (NIH), and by a grant (CA016672) to the M.D. Anderson Cancer Center from the NIH.

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

We thank the patients and their caregivers for participating in this study; Dr. Gianpietro Dotti for providing the CAR construct used in the clinical trial; and Dr. Richard Skinner for his comments on an earlier version of the manuscript.

REFERENCES

1. Maude SL, Laetsch TW, Buechner J, et al. Tisagenlecleucel in children and young adults with B-cell lymphoblastic leukemia. N Engl J Med 2018;378:439-48.
2. Park JH, Rivière I, Gonen M, et al. Long-term follow-up of CD19 CAR therapy in acute lymphoblastic leukemia. N Engl J Med 2018;378:449-59.
3. Porter DL, Levine BL, Kalos M, Bagg A, June CH. Chimeric antigen receptor–modified T cells in chronic lymphoid leukemia. N Engl J Med 2011;365:725-33.
4. Porter DL, Hwang WT, Frey NV, et al. Chimeric antigen receptor T cells persist and induce sustained remissions in relapsed refractory chronic lymphocytic leukemia. Sci Transl Med 2015;7(303):303ra139.
5. Turtle CJ, Hay KA, Hanafi LA, et al. Durable molecular remissions in chronic lymphocytic leukemia treated with CD19-specific chimeric antigen receptor-modified T cells after failure of ibrutinib. J Clin Oncol 2017;35:3010-20.
6. Neelapu SS, Locke FL, Bartlett NL, et al. Axicabtagene ciloleucel CAR T-cell therapy in refractory large B-cell lymphoma. N Engl J Med 2017;377:2531-44.
7. Schuster SJ, Svoboda J, Chong EA, et al. Chimeric antigen receptor T cells in re-

fractory B-cell lymphomas. N Engl J Med 2017;377:2545-54.
8. Schuster SJ. Tisagenlecleucel in diffuse large B-cell lymphoma. N Engl J Med 2019;380:1586.
9. Lee DW, Santomasso BD, Locke FL, et al. ASTCT consensus grading for cytokine release syndrome and neurologic toxicity associated with immune effector cells. Biol Blood Marrow Transplant 2019;25:625-38.
10. Davila ML, Riviere I, Wang X, et al. Efficacy and toxicity management of 19-28z CAR T cell therapy in B cell acute lymphoblastic leukemia. Sci Transl Med 2014;6(224):224ra25.
11. Neelapu SS, Tummala S, Kebriaei P, et al. Toxicity management after chimeric antigen receptor T cell therapy: one size does not fit 'ALL.' Nat Rev Clin Oncol 2018;15:218.
12. Lanier LL. Up on the tightrope: natural killer cell activation and inhibition. Nat Immunol 2008;9:495-502.
13. Wu J, Lanier LL. Natural killer cells and cancer. Adv Cancer Res 2003;90:127-56.
14. Shah N, Li L, McCarty J, et al. Phase I study of cord blood-derived natural killer cells combined with autologous stem cell transplantation in multiple myeloma. Br J Haematol 2017;177:457-66.
15. Miller JS, Soignier Y, Panoskaltsis-Mortari A, et al. Successful adoptive transfer and in vivo expansion of human haploidentical NK cells in patients with cancer. Blood 2005;105:3051-7.
16. Rubnitz JE, Inaba H, Ribeiro RC, et al. NKAML: a pilot study to determine the safety and feasibility of haploidentical natural killer cell transplantation in childhood acute myeloid leukemia. J Clin Oncol 2010;28:955-9.
17. Tagaya Y, Bamford RN, DeFilippis AP, Waldmann TA. IL-15: a pleiotropic cytokine with diverse receptor/signaling pathways whose expression is controlled at multiple levels. Immunity 1996;4:329-36.
18. Liu E, Tong Y, Dotti G, et al. Cord blood NK cells engineered to express IL-15 and a CD19-targeted CAR show long-term persistence and potent antitumor activity. Leukemia 2018;32:520-31.
19. Mehta RS, Rezvani K. Can we make a better match or mismatch with KIR genotyping? Hematology Am Soc Hematol Educ Program 2016;2016:106-18.
20. Hallek M, Cheson BD, Catovsky D, et al. iwCLL Guidelines for diagnosis, indications for treatment, response assessment, and supportive management of CLL. Blood 2018;131:2745-60.
21. Cheson BD, Fisher RI, Barrington SF, et al. Recommendations for initial evaluation, staging, and response assessment of Hodgkin and non-Hodgkin lymphoma: the Lugano classification. J Clin Oncol 2014;32:3059-68.
22. Hoyos V, Savoldo B, Quintarelli C, et al. Engineering CD19-specific T lymphocytes with interleukin-15 and a suicide gene to enhance their anti-lymphoma/leukemia effects and safety. Leukemia 2010;24:1160-70.
23. Maude SL, Frey N, Shaw PA, et al. Chimeric antigen receptor T cells for sustained remissions in leukemia. N Engl J Med 2014;371:1507-17.
24. Muftuoglu M, Olson A, Marin D, et al. Allogeneic BK virus–specific T cells for progressive multifocal leukoencephalopathy. N Engl J Med 2018;379:1443-51.
25. Felices M, Lenvik AJ, McElmurry R, et al. Continuous treatment with IL-15 exhausts human NK cells via a metabolic defect. JCI Insight 2018;3(3):e96219.
26. Olson JA, Leveson-Gower DB, Gill S, Baker J, Beilhack A, Negrin RS. NK cells mediate reduction of GVHD by inhibiting activated, alloreactive T cells while retaining GVT effects. Blood 2010;115:4293-301.
27. Ruggeri L, Mancusi A, Burchielli E, et al. NK cell alloreactivity and allogeneic hematopoietic stem cell transplantation. Blood Cells Mol Dis 2008;40:84-90.
28. Giavridis T, van der Stegen SJC, Eyquem J, Hamieh M, Piersigilli A, Sadelain M. CAR T cell-induced cytokine release syndrome is mediated by macrophages and abated by IL-1 blockade. Nat Med 2018;24:731-8.
29. Rezvani K, Rouce R, Liu E, Shpall E. Engineering natural killer cells for cancer immunotherapy. Mol Ther 2017;25:1769-81.
30. Lin JK, Muffly LS, Spinner MA, Barnes JI, Owens DK, Goldhaber-Fiebert JD. Cost effectiveness of chimeric antigen receptor T-cell therapy in multiply relapsed or refractory adult large B-cell lymphoma. J Clin Oncol 2019;37:2105-19.
31. Campbell JD, Whittington MD. Paying for CAR-T therapy amidst limited health system resources. J Clin Oncol 2019;37:2095-7.