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Myeloid cells in peripheral blood mononuclear cell concentrates inhibit the expansion of chimeric antigen receptor T cells

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Abstract

Background aims—Autologous chimeric antigen receptor (CAR) T-cell therapies have shown promising clinical outcomes, but T-cell yields have been variable. CD19- and GD2-CAR T-cell manufacturing records were reviewed to identify sources of variability.

Methods—CD19-CAR T cells were used to treat 43 patients with acute lymphocytic leukemia or lymphoma and GD2-CAR T cells to treat eight patients with osteosarcoma and three with neuroblastoma. Both types of CAR T cells were manufactured using autologous peripheral blood mononuclear cells (PBMC) concentrates and anti-CD3/CD28 beads for T-cell enrichment and simulation.

Results—A comparison of the first 6 GD2- and the first 22 CD19-CAR T-cell products manufactured revealed that GD2-CAR T-cell products contained fewer transduced cells than CD19-CAR T-cell products ($147 \pm 102 \times 10^6$ vs $1502 \pm 1066 \times 10^6$; $P = 0.0059$), and their PBMC concentrates contained more monocytes ($31.4 \pm 12.4\%$ vs $18.5 \pm 13.7\%$; $P = 0.019$). Among the first 28 CD19-CAR T-cell products manufactured, four had poor expansion yielding less than 1×10^6 transduced T cells per kilogram. When PBMC concentrates from these four patients were compared with the 24 others, PBMC concentrates of poorly expanding products contained greater quantities of monocytes ($39.8 \pm 12.9\%$ vs. $15.3 \pm 10.8\%$, $P = 0.0014$). Among the patients whose CD19-CAR T cells expanded poorly, manufacturing for two patients was repeated using cryopreserved PBMC concentrates but incorporating a monocyte depleting plastic adherence step, and an adequate dose of CAR T cells was produced for both patients.

Conclusions—Variability in CAR T-cell expansion is due, at least in part, to the contamination of the starting PBMC concentrates with monocytes.

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Keywords

acute lymphocytic leukemia; adoptive cellular therapy; chimeric antigen receptor T cells; CD19; GD2; osteosarcoma

Introduction

T cells engineered to express high-affinity T-cell receptors or chimeric antigen receptors (CAR) are promising adoptive cancer immunotherapies. Autologous CD19-CART cells have shown impressive activity in clinical trials for the treatment of chemotherapy-refractory B-cell lymphoma, acute lymphocytic leukemia (ALL) and chronic lymphocytic leukemia (CLL) [1–7]. A preliminary report suggests CD19-CAR T cells may also be useful in treating multiple myeloma [8]. A number of other CART cells are being evaluated in clinical trials including CD22-CART cells for acute lymphocytic leukemia, GD2-CART for GD2 expressing cancers and B-cell maturation antigen (BCMA)-CART cells for multiple myeloma. Few clinical trials of CAR T-cell therapy have been reported using an intent-to-treat design, and therefore, it remains unclear what fraction of patients who undergo apheresis for CAR product generation are unable to receive the therapy due to “product failure” and what factors increase the risk of such failures.

We have been manufacturing CD19-CAR T cells to treat children and young adults with B-cell acute leukemia and GD2-CAR T cells to treat children and young adults with osteosarcoma and neuroblastoma. Although clinical studies involving the use of CD19-CAR T cells manufactured in our facility have been promising [4], the manufacturing process has not yielded sufficient quantities of CD19-CAR or GD2-CAR T cells for some patients. Manufacturing protocols for both CAR T-cell products use autologous leukocytes collected by apheresis as the starting material, anti-CD3/CD28 beads for T-cell enrichment and expansion and retroviral vector supernatants for gene transfer. We speculated that the starting material used to manufacture CART cells may contribute to poor manufacturing outcome for some patients. Both leukemia and solid malignancies can affect the nature and mix of leukocytes in the peripheral blood, and many patients have been treated with several cycles of chemotherapy, which can affect circulating leukocytes. We evaluated the CD19- and GD2-CART manufacturing results to assess the consistency of the final CART-cell products and to attempt to identify factors that contribute to the poor expansion of CAR T cells observed in some patients.

Methods

Study participants

Patients in this study were enrolled in an open-label phase 1 dose-escalation study of CD19-CAR T cells in children and young adults with ALL or non-Hodgkin lymphoma or an open-label phase 1 dose-escalation study of GD2-CAR T cells in children and young adults with GD2 expressing osteosarcoma or neuroblastoma. CAR T-cell manufacturing was evaluated for 43 patients receiving CD19-CART-cell therapy and 11 patients receiving GD2-CAR T-cell therapy. Clinical results of the first 21 of the patients receiving CD19-CAR T-cell

therapy have previously been reported [4]. Forty-two patients treated with CD19-CART cells had ALL, and one had B-cell lymphoma. Among the 11 patients treated with GD2-CART cells, eight had osteosarcoma and 3 had neuroblastoma. All subjects were enrolled in protocols approved by the NCI Institutional Review Board.

Manufacturing CAR T cells

Peripheral blood mononuclear cell (PBMC) concentrates were collected using a blood cell separator (Cobe Spectra, Terumo BCT), and 10 to 15 L of blood were processed. CD19-CART cells were manufactured from the PBMC concentrates as previously described [9]. Briefly, on day 0, a fresh or cryopreserved PBMC concentrate fraction containing 600×10^6 CD3⁺ cells was enriched for CD3⁺ cells using anti-CD3/CD28 antibodies bound to paramagnetic beads (Dynabeads ClinExVivo CD3/CD28, Invitrogen) at a ratio of 3:1 (beads:cells). The cells and beads were co-incubated for 2 h at room temperature, and CD3⁺ cell enrichment was performed using a Dynal ClinExVIVO MPC magnet (Invitrogen). A total of 100×10^6 cells in the CD3⁺ fraction were resuspended at a concentration of 1×10^6 cells/mL in PermaLife bags (OriGen Biomedical) at 37°C in 5% CO₂ in AIM V medium (Gibco), supplemented with 5% heat-inactivated human AB Serum (Valley Biomedical), 1% Gluta-Max (Gibco), 40 IU/mL interleukin-2 (Novartis Vaccines and Diagnostics).

The cells were transduced twice with clinical grade MSGV-FMC63-28Z recombinant retroviral vector supernatant, once on day 2 and once on day 3, in retronectin-coated bags. The cells were maintained in culture for 7 to 11 days. The cell concentration was maintained at 0.4×10^6 cells/mL by adding fresh medium every other day. On the day of harvest, the anti-CD3/CD28 paramagnetic beads were removed using the Dynal ClinExVIVO MPC magnet (Invitrogen), washed and concentrated, and quality control assessment was performed.

A similar process was used to manufacture GD2-CART cells. Either fresh or frozen PBMCs were used; fresh cells were used for 10 patients and frozen for one patient. Cells from the first six patients were transduced twice, once on day 2 and once on day 3, with anti-GD2.28.z.OX40.ICD9 retroviral vector supernatant and cells from the remaining patients were transduced once on day 2. The change was made in an attempt to reduce transduction efficiency as a means of diminishing tonic signaling that we have reported with the scFV incorporated into this CAR receptor [10]. The cells were harvested after 10 or 11 days in culture.

For some CD19- and GD2-CAR T cell manufacturing procedures a step to deplete monocytes by plastic adherence was used by incubating anti-CD3/CD28 magnetic beads with PBMCs in T flasks rather than in bags. After 2 h the non-adherent cells were collected, and the cells were processed as described.

Statistical analysis

The values shown are mean $\pm$ 1 SD unless otherwise indicated. *P* values were determined using two-tailed *t*-tests using Excel (Microsoft). Regression analysis was also performed with Excel.

Results

Consistency of CD19- and GD2-CAR T cells

The first 22 CD19- and 6 GD2-CAR T cells were manufactured using similar methods, and the results of manufacturing these products were compared. The mean quantity of T cells generated from the first 22 CD19-CAR T-cell products and the first six GD2 products differed: $2287 \pm 1806 \times 10^6$ for CD19 CAR T-cell products versus $183 \pm 106 \times 10^6$ for GD2-CAR T-cell products ($P = 0.0107$; Figure 1A). The mean quantity of transduced T cells also differed: $1502 \pm 1066 \times 10^6$ for CD19 CAR T cells compared with $147 \pm 102 \times 10^6$ for GD2-CAR T cells ($P = 0.0059$) (Figure 1B). For CD19-CAR T cells, the quantity of total T cells in the final products was highly variable ranging from 0 to 6359×10^6 as was the quantity of transduced T cells (0 to 3803×10^6). The proportion of CD3⁺ cells in the final product was high for both CD19- and GD2-CAR T cells but slightly less in the anti-GD2-CAR T cell products ($99.3 \pm 0.70\%$ versus $97.5 \pm 1.03\%$; $P = 0.00020$; Figure 1C). No differences in the proportion of CD3 + CD8 + cells in the final product ($41.2 \pm 16.8\%$ versus $48.4 \pm 17.7\%$; $P = 0.370$) nor the proportion of CAR transduced T cells ($66.0 \pm 23.2\%$ versus $73.0 \pm 15.0\%$; $P = 0.85$) was observed between CD19-and GD2-CAR T cell products (Figure 1D).

Poorly expanding products

The manufacturing process for CD19-CART cells was changed after the first 22 products were generated. For the first 22 patients fresh PBMC concentrates were used as starting material and the culture period was 10–11 days. For patients 23 to 28, either fresh or cryopreserved PBMCs were used and the culture period was shortened to 7 days. Among the CD19-CAR T-cell products manufactured for these 28 patients, four products did not contain enough transduced T cells to meet the required dose of 1.0×10^6 or 3.0×10^6 transduced T cells per kilogram (Table I). The culture of cells from one patient, patient 22, was stopped after 8 of the scheduled 11 days of culture due to very low and falling quantities of cells, and cell transduction was not measured due to inadequate cell number. Transduction efficiency was measured for the other three patients and was found to be lower than that of the products for the other 24 patients ($20.9 \pm 1.9\%$ versus $74.2 \pm 18.0\%$; $P < 0.0001$). A review of the composition of the PBMC concentrates used to manufacture these four poorly expanding CD19-CAR T-cell products revealed that each of the four contained large quantities of either monocytes or granulocytes (Table II), and when compared with 24 PBMC concentrates that yielded sufficient quantities of transduced T cells, they contained a larger proportion of monocytes and fewer lymphocytes (Table III).

For two of the four poorly expanding products, manufacturing was repeated using a cryopreserved aliquot of the same PBMC concentrate used to manufacture the first product but using a modified procedure that incorporated a plastic adherence step to enhance monocyte depletion. Briefly, at the beginning of the culture process after anti-CD3/CD28 beads were added, the cells were incubated in flasks rather than bags for 2 h. After the incubation, non-adherent cells were transferred to bags for the remaining duration of the culture period. For both patients, the modified method resulted in sufficient quantities of cells to meet dose criteria of 1×10^6 transduced T cells per kilogram. For patient 26, the

yield of total T cells increased from 19.8×10^6 to 171×10^6 and transduced T cells from 2.4×10^6 to 160×10^6 . For patient 22, the first culture was stopped after 8 days and yielded no cells, and the second culture yielded 186×10^6 T cells and 147×10^6 transduced T cells.

Myeloid cells in PBMCs concentrates and CD19-CAR T cell yield

We evaluated the relationship between the composition of PBMC concentrates collected from the CD19-CAR T patients and the quantity of T cells and transduced T cells in the final product. Among the first 22 CD19-CAR T-cell products, we found that there was a significant inverse association between the proportion of granulocytes in the PBMC concentrate and expansion of T cells ($r = -0.362$, $P = 0.097$; Figure 2C) and transduced T cells ($r = -0.440$; $P = 0.040$; Figure 2D). There was also an inverse association between monocytes and expansion of T cells and transduced T cells, but the difference was not significant ($r = -0.219$; $P = 0.331$ and $r = -0.284$; $P = 0.200$, respectively; Figure 2A,B). Stronger associations were observed when evaluating with the sum of the proportion of monocytes and granulocytes in the PBMC concentrates and the quantity of T cells ($r = -0.417$; $P = 0.054$) and transduced T cells in the final product ($r = -0.527$; $P = 0.012$; Figure 3). There was no significant relationship between the percent of leukocytes in the PBMC concentrates and the quantity of T cells expressing CD19 CAR produced ($r = 0.198$, $P = 0.378$).

Because higher proportions of myeloid cells in the PBMC concentrates were associated with poorer T-cell expansion, we changed our manufacturing to include a plastic adherence step to improve the depletion of monocytes from the PBMC concentrates. We compared 15 CD19-CAR T cells products that were manufactured over 7 days using this new method (patients 29–43) with six CD19-CART-cell products that were manufactured over 7 days in bags without a plastic adherence step (patients 23–28). We found that one of the six products prepared without plastic adherence did not meet dose criteria, but all of the 15 products that included a plastic adherence step met dose criteria. When the six products manufactured without a plastic adherence step were compared to the 15 manufactured with a plastic adherence step, there was no difference in the number of T cells in the final product ($305 \pm 280 \times 10^6$ versus $562 \pm 287 \times 10^6$; $P = 0.081$; Figure 4A) or transduced T cells ($253 \pm 227 \times 10^6$ versus $393 \pm 167 \times 10^6$; $P = 0.168$; Figure 4B).

Monocyte depletion and expansion of GD2-CAR T cells

The final CD19-CART-cell products contained greater quantities of T cells and transduced T cells than the GD2-CAR T-cell products. To determine whether the composition of the PBMC concentrates collected from patients treated with GD2-CAR T cells may have influenced the expansion of CAR T cells, we compared PBMC concentrates collected from patients treated with CD19 and GD2-CAR T cells. The proportion of monocytes in PBMC concentrates collected from patients enrolled on the GD2-CAR T cell trial was greater than those in PBMC concentrates collected from patients on the CD19-CAR T cell trial, but the proportion of lymphocytes and CD3⁺ cells was similar (Table IV). These results provided additional evidence that monocytes may be detrimental to T-cell expansion.

Among the first six GD2-CAR T-cell products manufactured, the expansion of T cells was generally poor, but all of the products contained sufficient quantities of transduced T cells to meet dose criteria for these patients of 1×10^5 or 1×10^6 transduced T cells per kilogram. However, because of the low quantities of transduced T cells in the final product and high proportion of monocytes in the PBMC concentrates in these patients, we elected to change the GD2-CAR T-cell manufacturing protocol to incorporate a plastic adherence monocyte depletion step. A comparison of five products manufactured using the modified process that included the plastic adherence step with six products manufactured using a process without plastic adherence found that there was a trend toward the presence of greater quantities of T cells ($1404 \pm 1136 \times 10^6$ versus $183 \pm 106 \times 10^6$; $P = 0.0419$; Figure 4C) and transduced T cells ($576 \pm 437 \times 10^6$ vs $147 \pm 102 \times 10^6$; $P = 0.0645$) in the final product (Figure 4D). Interestingly, despite incorporation of plastic adherence, one of five products GD2-CAR products failed to meet dose criteria. This product was generated from fresh PBMCs harvested from a patient with neuroblastoma and the final CAR product contained only 4.8×10^6 transduced T cells. A second manufacture of GD2-CART cells for this patient used a thawed aliquot of the PBMC concentrate and a more aggressive T-cell enrichment process that included density gradient separation and plastic adherence to remove granulocytes and monocytes before initiating T-cell culture yielded 1986×10^6 transduced T cells.

Discussion

We found that the CD19- and GD2-CART-cell products manufactured at our institution contained a high proportion of CD3⁺ cells and typically 50–90% of the cells expressed either the CD19- or GD2-CAR. Slightly less than half of the CAR T cells were CD8⁺. Among CD19-CART products, we detected few CD19⁺ cells, suggesting the final product contained few, if any, leukemic cells.

Although the composition of the CART-cell products was consistent, the quantity of CD19-CART cells produced was highly variable. We investigated the effects of the composition of the PBMC concentrates that were used as the starting material for the manufacturing process on the quantity of T cells and transduced T cells in the final product. The proportion of monocytes in the PBMC concentrates, at least in part, contributed to the variability in the quantities of CD19- and GD2-CAR T cells produced. PBMC concentrates that yielded few total T cells expressing CD19-CAR contained greater quantities of monocytes than those yielding greater quantities of transduced T cells. PBMC concentrates collected from patients receiving GD2-CAR T-cell therapy contained greater proportions of monocytes than those collected from patients receiving CD19-CAR T-cell therapy, and despite using similar methods to manufacture these cells, far fewer GD2-CAR T cells were obtained. There was also an association between granulocytes in the PBMC concentrates and the expansion of CD19-CAR T cells; higher percentages of granulocytes were associated with less expansion.

Our initial manufacturing process made use of anti-CD3/CD28 beads to enrich cellular material used to start the culture for T cells. The presence of these beads in the starting material made the assessment of cell numbers and composition difficult, but we suspect that some of the monocytes and granulocytes carried over into the cells used to start the manufacturing process and these contaminating myeloid inhibited T cell expansion.

Cells of myeloid origin with unique immunosuppressive properties have been found in the blood, spleen, lymphoid tissue and tumor microenvironment in patients with cancer [11–13]. These myeloid-derived suppressor cells (MDSCs) are a heterogeneous population of immature myeloid cells that can inhibit T-cell proliferation, cytokine secretion and the recruitment of regulatory T cells. MDSCs can have a monocyte (CD14⁺) or granulocyte (CD15⁺) phenotype and most commonly have been described as CD11b⁺, CD33⁺ and HLA-DR⁻. Many of the patients treated with GD2-CAR T cells had osteosarcomas, and MDSCs have been described in the blood of patients with metastatic pediatric sarcomas [14]; however, the MDSCs in pediatric sarcoma patients are unique in that they have a monocyte morphology but are CD11b⁺, HLA-DR⁺ and do not express CD33 or CD14. We cannot exclude the possibility that T regulatory cells were present in the apheresis concentrates and that they were enriched by anti-CD3/CD28 bead selection and inhibited T-cell expansion.

Although a greater proportion of monocytes and granulocytes in PBMC concentrates was associated with less CD19-CAR T cell expansion, no such relationship was seen with PBMC concentrates used to manufacture GD2-CAR T cells. The reason for lack of association among the proportion of monocytes and granulocytes and GD2-CAR T-cell expansion is not certain, but it may be that the osteosarcoma and neuroblastoma patients treated had potent circulating MDSCs and few of the MDSCs were required to suppress T-cell expansion; thus, consequently, all patients had some degree of inhibition of T-cell proliferation.

MDSCs have been identified in patients with hematologic malignancies including non-Hodgkin lymphoma, chronic myeloid leukemia and multiple myeloma [15]. The results of this study suggest MDSCs are also present in some patients with ALL.

We attempted to improve the expansion of CD19- and GD2-CAR T cells by depleting monocytes from PMBC concentrates. Because monocytes, but not lymphocytes, adhere to plastic surfaces, we used plastic adherence to deplete the starting PBMC concentrates of monocytes. Although this improved the yield of GD2-CAR T cells, because of concerns about the efficiency of the removal of monocytes by adherence, we have changed our manufacturing process to include a more robust lymphocyte enrichment step. We are now using counter-flow elutriation to deplete PMBC concentrates of monocytes and granulocytes [16]. Anti-CD3/CD28 beads are still used to promote T cell expansion, but they are no longer used for lymphocyte enrichment. The positive selection of CD4⁺ and CD8⁺ cells using monoclonal antibodies conjugated to magnetic particles is another technique that would likely be effective for enriching the T-cell content of PBMC concentrates. T cells isolated by both elutriation and antibody selection have been used for adoptive cell therapy [16–18]. For some patients, the expansion of T cells may, however, be limited by other factors such as prior chemotherapy, the patients' disease or patients' genetics.

We cannot exclude the possibility that some of the improvement in expansion of GD2-CAR T cells in the second group of patients was due in part to the change from 2 to 1 transduction days. The tonic CAR CD3ζ phosphorylation caused by antigen-independent clustering of the GD2 CAR single-chain variable fragment of this vector results in a phenotype consistent with T-cell exhaustion, and these cells expand less efficiently *ex vivo* [19]. The reduction in the number of transductions may have reduced the tonic

phosphorylation and contributed to better expansion. However, for the one patient whose cell manufacturing was repeated, both events use a single transduction, but more aggressive lymphocyte selection resulted in better cell expansion. This unique property of the GD2-CAR vector may also account for some of the difference in expansion among CD19- and GD2-CAR T cells.

A weakness of the study was that the method used to analyze CD19-CAR expression was changed during the study. Initially, protein L was used to detect the expression of the CD19 scFV [20], but after an anti-idiotypic antibody became available [21], it was used to measure CD19-CAR expression. A comparison of the two methods found that the transduction levels measured using the two methods was similar, but the anti-idiotypic antibody resulted in less nonspecific staining.

In conclusion, we found that the expansion of CAR T cells was highly variable, and this variability was due in part to monocytes and granulocytes in the PBMC concentrates. Robust depletion of monocytes and granulocytes from the PBMC concentrates used for CAR T-cell manufacturing will likely result in higher yields, more consistent products and fewer manufacturing failures.

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Figure 1.

Composition of CD19- and GD2-CAR T-cell final products. Analysis of the first 22 CD19-CAR and first 6 GD2-CAR T-cell products are shown. (A) The quantity of T cells in the final CAR T-cell products. (B) The quantity of transduced T cells. (C) Proportion of mononuclear cells expressing CD3. (D) Proportion of CD3⁺ cells that expressed the CD19-CAR T and GD2-CAR T cell vectors. The CD19-CAR T cells are shown on the left side of each panel (triangles), and the GD2-CAR T cells on the right (diamonds). Only 21 CD19-CAR T-cell products were analyzed for CD3 and CD19-CAR vector expression because the culture of one poorly expanding product was stopped before the culture was complete.

Figure 2.

Relationship between the proportion of monocytes or granulocytes in the PBMC concentrates used as starting material for CD19-CAR T-cell production and the quantity of T cells and transduced T cells in the final product. For each of the 22 patients, the relationship among the percentage of monocytes in the PBMC concentrates and T cells in each CD19-CAR T cell product (A), the percent of monocytes and transduced T cells (B), the percent of granulocytes and T cells (C) and the percent of granulocytes and transduced T cells (D) are shown.

Figure 3.

Relationship between the proportion of monocytes plus granulocytes in the PBMC concentrates used as starting material for CD19-CAR T cell production and the quantity of T cells and transduced T cells in the final products. For each of the 22 patients, the relationship among the percent of monocytes plus granulocytes in the PBMC concentrates and T cells in the CAR T cell products (A) and the percent of monocytes plus granulocytes in the PBMC concentrates and transduced T cells in the CAR T cell products (B) are shown.

Figure 4.

Quantity of T cells and transduced T cells in CD19- and GD2-CAR T-cell products manufactured from PBMC concentrates depleted of monocytes by anti-CD3/CD28 beads or by anti-CD3/CD28 beads plus plastic adherence. (A) The quantity of T cells in 6 CD19-CAR T products that were manufactured from PBMC concentrates enriched for lymphocytes using anti-CD3/CD28 bead selection and cultured for 7 days (diamonds) and 15 CD19-CAR T-cell products that were manufactured from PBMC concentrates enriched for lymphocytes using anti-CD3/CD28 bead selection and depleted of monocytes by plastic adherence and cultured for 7 days (triangles). (B) The quantity of transduced T cells in the same CD19-CAR T cell products. (C) The quantity of T cells in 6 GD2-CAR T-cell products manufactured using anti-CD3/CD28 bead selection (diamonds) and 5 manufactured using anti-CD3/CD28 bead selection and depleted of monocytes by plastic adherence products (triangles). (D) The quantity of transduced T cells in the same GD2-CAR T cell products.

Table 1

Characteristics of CD19-CAR T-cell products that did not contain enough transduced cells to meet dose criteria.

Patient	Dose level (transduced cells/Kg)	Days in culture ^a	CD3%	CD19%	TNC ($\times 10^6$)	Transduced cells (%) ^b	Transduced cells ($\times 10^6$)	Transduced cells (per kg)
2	1×10^6	11	97.6	0.1	13.2	21.5	2.8	0.046×10^6
5	3×10^6	11	97.5	0.1	108	18.3	19.3	0.406×10^6
22 ^c	1×10^6	8	NT	NT	38.1	NT	NT	0
26	1×10^6	7	52.3	0.0	19.8	22.8	2.4	0.141×10^6

NT, not tested.

^aFor patients 1–22, cells were to be cultured for 11 days; for patients 23–26, cells were cultured for 7 days.

^bExpression of anti-CD19 CAR was measured using protein L for patients 2 and 5 and with anti-idiotypic antibody in patient 26.

^cCulture of cells from patient 22 was stopped after 8 days because of low and falling number of cells.

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Composition of each of the four PBMC concentrates that did not yield sufficient quantities of CD19-CAR T cells to meet dose criteria.

Table II

Patient	Lymphocytes (%)	Monocytes (%)	Granulocytes (%)	CD3 ⁺ Cells (%)	CD19 ⁺ cells (%)
2	54	41	4	15.3	0.6
5	45	18	35	44.5	0.1
22	31	54	7	26.1	0.1
26	39	38	19	23.9	2.1

Table III

Comparison of the composition of PBMC concentrates that resulted in successful manufacture of CD19-CAR T cell products with those that resulted in low yields.

	Met dose criteria (n = 24)	Did not meet dose criteria ^a (n = 4)	<i>P</i>
Lymphocytes (%)	75.3 ± 14.1	42.3 ± 8.4	0.00018
Monocytes (%)	15.3 ± 10.8	39.8 ± 12.9	0.0014
Granulocytes (%)	6.9 ± 8.6	16.3 ± 12.2	0.083
CD3 ⁺ (%)	57.9 ± 19.5	27.5 ± 10.6	0.005
CD19 ⁺ (%)	10.9 ± 19.0	0.7 ± 0.8	0.308

^a All products contained less than 1×10^6 transduced T cells per kilogram.

Table IV

Composition of PBMC concentrates collected from CD19- and GD2-CAR T-cell patients.

	CD19-CAR T-cell patients (n = 22)	GD2-CAR T-cell patients (n = 6)	<i>P</i>
Lymphocytes (%)	70.8 ± 16.4	61.8 ± 9.9	0.229
Monocytes (%)	18.0 ± 13.3	31.3 ± 12.4	0.043
Granulocytes (%)	8.4 ± 10.2	3.5 ± 4.4	0.277
CD3 ⁺ (%)	57.2 ± 19.9	48.8 ± 10.2	0.345