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Autologous Lymphapheresis for the Production of Chimeric Antigen Receptor (CAR) T Cells

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Abstract

Background—Chimeric antigen receptor (CAR) T cells are a promising new immunotherapy. The first step in manufacturing is to collect autologous CD3+ lymphocytes by apheresis. Patients, however, are often leukopenic or have other disease-related complications. We evaluated the feasibility of collecting adequate numbers of CD3+ cells, risk factors for inadequate collections, and the rate of adverse events.

Study Design—Apheresis lymphocyte collections from patients participating in 3 CAR T cell clinical trials were reviewed. Collections were performed on the COBE Spectra by experienced nurses, with the goal of obtaining a minimum of 0.6×10^9 and a target of 2×10^9 CD3+ cells. Pre-apheresis peripheral blood counts, apheresis parameters, and product cell counts were analyzed.

Results—Of the 71 collections, 69 (97%) achieved the minimum and 55 (77%) achieved the target. Before apheresis, the 16 patients with yields below the target had significantly lower proportions and absolute numbers of circulating lymphocytes and CD3+ lymphocytes, and higher proportions of circulating blasts and NK cells than those who achieved the target (470 vs. 1340 lymphocytes/μL, p=0.008; 349 vs. 914 CD3+ cells/μL, p=0.001; 17.6% vs. 4.55% blasts, p=0.029). Enrichment of blasts in the product compared to the peripheral blood occurred in 4 patients, including the 2 patients whose collections did not yield the minimum number of CD3+ cells. Apheresis complications occurred in 11 patients (15%), and with one exception, were easily managed in the apheresis clinic.

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Conflict of Interest Disclosure: The authors have no competing interests relevant to this article

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Web Resources

Fisher's Exact Test (http://www.langsrud.com/fisher.htm)

VassarStats: Statistical Computation Web Site (www.vassarstats.net)

Supporting Information

Additional Supporting Information may be found in the online version of this article:

Conclusions—In most patients undergoing CAR T cell therapy, leukapheresis is well-tolerated and adequate numbers of CD3+ lymphocytes are collected.

Introduction

Chimeric antigen receptor (CAR) T cells are engineered to bind to tumor-specific cell surface targets, while the adjacent signaling molecules cause T cell activation, proliferation, and tumor cell lysis.[1,2] Clinical trials have evaluated the safety and efficacy of CAR T cells for the treatment of acute lymphoblastic leukemia (ALL),[2-5] chronic lymphocytic leukemia,[3,6-8] diffuse large B cell lymphoma,[8,9] multiple myeloma,[10] neuroblastoma,[11] and sarcomas.[12] Results have been so favorable that over 120 clinical trials are currently open[13] and at least 6 companies have formed partnerships to produce and study CAR T cell products;[14] 4 companies have had initial public offerings worth over $130 million each.[14] Thus, the therapy appears poised to move from small-scale clinical trials to large-scale commercial production, and could become standard treatment for some malignancies in the future.

The complex manufacturing process involves obtaining T cells, transducing those cells with a viral vector containing the CAR cassette, and subsequently expanding them in culture.[1] Numerous variables affect the manufacturing process, including the number of starting cells, transduction efficiency, and the ability of the cells to multiply, which in turn may be affected by cell quality, cell purity,[15] culture conditions, and other factors that are as yet unknown (J. Jin, personal communication, 2016). Here, we focused on the essential first step: collection of T cells via apheresis.

Although at least one trial has examined the use of allogeneic T cells,[16] the majority have used autologous T cells. Apheresis collection of T cells from patients represents a new clinical scenario for practitioners. Historically, peripheral blood mononuclear cell (PBMC) collections have been performed in 2 settings: (1) in healthy donors to collect lymphocytes for donor lymphocyte infusions (DLI) after hematopoietic stem cell transplantation and (2) in leukemic patients with high blast concentrations, to reduce the white blood cell concentration and prevent or treat leukostasis.[17,18] In the former, individuals usually have normal cell counts and the necessary cells are easily obtained; in the latter, the cells being collected—the blasts—are abundant, and the goal of the procedure is their removal rather than their collection. And while autologous peripheral blood stem cell (PBSC) collection in patients with malignancies has been studied,[19] little is known about PBMC collections for obtaining lymphocytes.[20]

Lymphapheresis for CAR T cell manufacturing involves collecting specific numbers of CD3+ lymphocytes from patients. Unlike a DLI collection from a healthy donor, patients often have low white blood cell (WBC) counts, making it difficult to establish the red blood cell-WBC interface in the apheresis centrifuge.[20] Furthermore, underlying disease or prior treatment could affect the properties of the lymphocytes, and typical collection parameters may not be optimal.[20] Finally, patients could have complications such as gastric upset or infection that make them less stable to undergo the procedure.

The challenges of collecting CD3+ lymphocytes in a population of patients with leukemia and other malignancies have not been well-characterized. We evaluated patients undergoing lymphapheresis for the production of CAR T cells to determine the proportion who meet CD3+ cell targets, risk factors for inadequate collections, and the rate of adverse events.

Materials and Methods

Patients

Inclusion criteria for this study required enrollment in NCT01593696, NCT02315612, or NCT02107963, and lymphocyte collection by apheresis between July 1, 2012 and January 31, 2016. All 3 trials were single-center, phase 1/2, dose-escalation studies of CAR T cell therapy for relapsed or treatment-refractory pediatric malignancies. NCT01593696 and NCT02315612 included children with B cell leukemias and lymphomas that expressed CD19 and CD22, respectively. NCT02107963 included children with solid tumors that expressed GD2. The current retrospective analysis was within the scope of all 3 trials, which were approved by the Institutional Review Board of the National Cancer Institute. Data was available for all 71 patients who met the inclusion criteria.

Apheresis

Lymphapheresis procedures were performed on the COBE Spectra (TerumoBCT, Lakewood, CO) by experienced apheresis nurses collecting at a hematocrit of 2 to 3% and a collection flow rate of 1.0 mL/min. Patients under 18 years old all had central venous catheters (CVC) placed before the procedure by an experienced vascular access team; those over 18 years were assessed to determine whether their peripheral veins were adequate or whether a CVC was needed. During the procedure, all patients received intravenous calcium chloride, prepared and infused according to standard procedures that have been previously described.[21] Patients weighing less than 40 kg also received intravenous magnesium sulfate by methods previously described.[21] The instrument was primed with 1 unit of irradiated, leukocyte-reduced red blood cells for all patients weighing less than 20 kg from July 2012 through January 2014, and subsequently for all patients weighing less than 25 kg, due to a change in institution practice. If the patient experienced symptoms of hypocalcemia during the procedure, the calcium infusion rate was increased by 10-20%.

Patients were required to have stable vital signs, hemoglobin concentration greater than 8.0 g/dL, and platelets greater than $50\times10^3/\mu L$ before apheresis. Limited exceptions were made on a case-by-case basis. As a result, 3 patients had hemoglobin concentrations of 7 to 8 g/dL and 5 patients had platelets of 15 to 50 $\times10^3/\mu L$ at the time of apheresis.

Laboratory Testing

Before apheresis, a complete blood count (CBC) with automated differential was performed using a hematology analyzer (Sysmex XN-3000, Sysmex America, Lincolnshire, IL). Manual differentials were performed whenever blasts were detected. Lymphocyte phenotyping was performed by flow cytometric analysis (BD FACSCanto, BD Biosciences, San Jose, CA); the fraction of lymphocytes that expressed CD3, CD19, or CD16/56 was

multiplied by the absolute lymphocyte count to obtain the absolute T, B, or NK cell count, respectively.

After apheresis, a CBC performed on the product using a hematology analyzer (Cell-Dyn 3700, Abbott Diagnostics, Abbott Park, IL) enabled the determination of the total nucleated cells (TNC) in the PBMC concentrate. The Sysmex and Cell-Dyn analyzers were both validated against a standard and used for clinical testing. Cellular phenotypes were determined by flow cytometric analysis using a flow cytometer (BD FACSCanto or BD FACSCanto II, BD Biosciences).

CD3+ cell targets

The collections aimed for a minimum of 0.6×10^9 and a target of 2×10^9 CD3+ cells. Although CAR T cells can be manufactured using fewer cells, we have found that a small percentage of cell cultures demonstrate low transduction efficiency or expansion; in our experience, 0.6×10^9 CD3+ cells often suffices even if such difficulties are encountered, and the target of 2×10^9 enables us to cryopreserve extra cells in case a second culture is necessary.[15] The final product was infused using weight-based dosing, and patient weight varied considerably (range 16 to 140 kg). However, we elected to use a standardized manufacturing method that was independent of the dose required; consequently we targeted a fixed quantity of CD3+ cells.

Data analysis

The collection efficiency was calculated using the equation: efficiency = CD3+ cell yield / (Pre-apheresis absolute CD3+ cells/μL × 10^6 × liters processed). The mean of the absolute CD3+ cell counts before and after the procedure was not used because the post-apheresis count was not available. Associations between categorical variables were tested using the 2-sided Fisher's exact test. Associations between quantitative variables were tested using the 2-sided student's t-test. A p less than 0.05 was considered statistically significant.

Results

We reviewed 71 autologous apheresis lymphocyte collections for patients who were preparing to receive adoptive immunotherapy therapy with CD19-CAR, CD22-CAR, or GD2-CAR T cells. The majority of the patients were male, carried a diagnosis of ALL, and weighed greater than 25 kg (Table 1).

Pre-apheresis laboratory testing

On average, patients with solid tumors had lower proportions of lymphocytes compared to patients with ALL (p=0.02, Table S1). However, the absolute numbers of lymphocytes were not significantly different between the 2 groups. Among the 58 patients with ALL, 15 (26%) had detectable circulating blasts on the day of collection (Table S1).

Apheresis parameters

The 71 procedures were performed by 12 different apheresis nurses. The majority of patients required CVCs for vascular access (94%), and did not require a blood prime (83%, Table 2).

The number of total blood volumes processed varied widely. Apheresis complications occurred in 11 patients (15%, Table 2), and did not correlate with younger age or lower weight (not shown). One patient with progressive ALL involving the central nervous system developed fever, nausea, vomiting, hallucinations, seizures, and oxygen desaturation. He showed no signs of hypocalcemia during or immediately after the procedure, and his complications were thought to be related to his underlying disease rather than apheresis. He was intubated and transferred to the intensive care unit where he was treated for 19 days before returning to a lower level of care. All other complications were managed in the apheresis clinic, including all paresthesias, which resolved after adjusting the calcium infusion rate.

Collection yields and manufacturing

Only 2 collections (3%) yielded fewer than 0.6×10^9 CD3+ cells, the minimum requested for manufacturing. An additional 14 collections (20%) achieved the minimum, but not the target of 2×10^9 CD3+ cells (Figure 1A). Even among 21 patients with low absolute lymphocyte counts ($\leq 0.5\times10^3/\mu L$) or CD3+ cell counts ($\leq 300/\mu L$), 20 collections reached the minimum yield (95%, Table S2). TNC, TNC per kg, differential counts, and number of CD3+ cells obtained were highly variable among patients (Table 3). Peripheral blood CD3+ counts were acquired 0 to 11 days before apheresis and correlated with the yield of CD3+ cells per liter of blood processed (Figure 1B). After the first 48 patients had undergone apheresis, the line of best fit from Figure 1B was used to prospectively guide the number of liters processed for lymphocyte collections in the next 23 patients undergoing CAR T cell therapy.

Initial manufacturing for 8 of the 71 patients (11%) did not yield sufficient quantities of transduced CD3+ cells to meet protocol dose criteria (0.3 to 3.0×10^6 transduced CD3+ cells). Lower apheresis yields were significantly associated with manufacturing failures, which occurred in 5 of 16 patients (31%) whose PBMC concentrates contained fewer than the target number of CD3+ cells, and 3 of 55 patients (5%) who met the target (p=0.01, not shown).

Risk factors for low CD3+ cell collections

A total of 16 PBMC concentrates contained fewer than the target of 2×10^9 CD3+ cells. Characteristics such as gender, diagnosis, and clinical trial were not significantly associated with below-target yields (Table S3). Several laboratory variables, however, showed statistically significant differences when comparing the below-target and above-target groups (Table 4). Notably, all patients with below-target yields had absolute lymphocyte counts less than $1.5\times10^3/\mu L$ (Figure 2A), and all patients with NK cell fractions above 40% yielded fewer than the targeted number of CD3+ cells (Figure 2B). There was no significant difference in the CD3+ cell collection efficiency between the 2 groups (Table 4).

To determine whether the low-yield collections were due to processing inadequate volumes of blood, we reviewed the volume processed for all 16 patients whose collections yielded fewer than 2×10^9 CD3+ cells (Figure 3). Less than 3 total blood volumes were processed for 8 patients (50%), 6 of whom had fewer than 10 liters processed. Furthermore, 5 of the 8 had CD3+ cell counts below 500 cells/μL. These results suggest that collection targets may have

been reached for 8 additional patients if the duration of collection had been longer, and they emphasize the importance of customizing the amount of blood processed based on the patient's cell counts.

To determine if enrichment of blasts in the PBMC concentrate was reducing CD3+ cell yields, the proportion of blasts in the circulation and the collected product were compared. Among the 15 patients with circulating blasts, 8 had similar proportions in the blood and in the concentrate, differing by less than 10% (Figure 4). Decreased proportions of blasts were seen in the concentrate from 3 patients. Increased proportions were seen in 4 patients, 2 of whom had the lowest CD3+ cell yields in this study. Those patients are described as case studies below.

Case studies

Patient 029-03 was a 22 year-old, 81 kg male with ALL, who had previously received CD19-CAR T cells and presented for CD22-CAR T cells. On the day of apheresis, his WBC count was $9.88\times10^3/\mu L$, with 14% lymphocytes and 53% blasts. During the collection, a mid-procedure count of TNCs in the concentrate suggested adequate numbers of cells were collected, and the procedure was stopped after 10.6 liters of blood was processed. However, the collection bag was later found to contain over 90% blasts, and only 0.494×10^9 CD3+ cells. Despite the low yield, culture was initiated; high transduction efficiency and good expansion enabled production of an adequate CD22-CAR T cell product.

Patient 029-09 was a 6 year-old, 21 kg male with ALL, who had previously received CD19-CAR T cells and presented for CD22-CAR T cells. On the day of apheresis, his WBC was $2.79\times10^3/\mu L$, with 20% lymphocytes and 26% blasts. Initial flow cytometry testing of the pre-apheresis peripheral blood showed 63% of the lymphocytes were CD3+. During the collection, a mid-procedure count of the concentrate was found to contain 61% blasts, 18% NK cells, and only 2% CD3+ cells. Despite processing nearly 7 total blood volumes, only 0.288×10^9 CD3+ cells were obtained. Subsequent investigation led to re-gating of the pre-apheresis peripheral blood flow cytometry. The new analysis showed that only 15% of lymphocytes were CD3+, and the remainder bore NK cell markers. Fortunately, additional lymphocytes from the patient were stored at another institution. After shipping the cells to our laboratory, CAR T cell culture was initiated and manufacturing was successful.

Discussion

We evaluated apheresis lymphocyte collections from 71 patients who were preparing for CAR T cell immunotherapy. Despite varying levels of leukopenia, the minimum number of CD3+ lymphocytes was collected in 97% of patients, and the targeted number in 77% of patients. Apheresis complications occurred in 15% of patients, and with one exception, they were easily managed in the apheresis clinic.

Risk factors for low CD3+ cell collections included low proportions or absolute numbers of lymphocytes or CD3+ cells, high proportions of NK cells, and high proportions of circulating blasts. Patients with these characteristics should be considered at risk of

producing an inadequate yield, and in some cases, processing more blood may enable an adequate collection.

The association between high proportions of NK cells and lower CD3+ cell collection yields in most cases is likely due to the inverse relationship between NK and CD3+ cells as fractions of lymphocytes. Both should separate into the lymphocyte layer in the centrifuge, and more NK cells typically implies fewer CD3+ cells.

The association between high proportions of blasts and lower CD3+ cell collection yields may be due to lower numbers of circulating CD3+ cells, or to enrichment of blasts in the PBMC concentrate. Although the proportion of blasts in the apheresis product was often concordant with the peripheral blood, some patients showed discrepancies. Increasing fractions of lymphoblasts in the apheresis product may have been due to the physical properties of the cells causing them to accumulate in the lymphocyte layer during centrifugation. Alternatively, it could be the result of real increases in circulating blasts during the procedure due to mobilization from the bone marrow, liver, spleen, or lymph nodes.

The peripheral blood CD3+ cell count can be used to predict the yield per liter of blood processed (Figure 1B), which can then assist in tailoring the length of the apheresis procedure to the patient. These findings are consistent with previous studies that have shown correlations between pre-apheresis CD34+ cell counts and CD34+ cell collection yields in pediatric patients and donors.[21] Careful gating during flow cytometry analysis is essential to accurate determination of the CD3+ cell concentration. When the default lymphocyte gating is used, the fraction of CD3+ cells can appear falsely elevated.

While obtaining adequate quantities of CD3+ cells is important to producing sufficient quantities of CAR T cells, it is not the only factor. Early in manufacturing, we observed that CAR T cells from some patients failed to expand in culture, and this phenomenon was associated with PBMC concentrates containing greater quantities of myeloid cells.[15] Our initial manufacturing protocol, which included a step to enrich PBMC concentrates for T cells by selection with anti-CD3/CD28 beads,[22] was modified to include a step that depleted myeloid cells by plastic adherence. We are also evaluating more rigorous methods of lymphocyte enrichment including counter-flow elutriation and selection with anti-CD4 and anti-CD8 paramagnetic particles. Better enrichment of PBMC concentrates for T cells may reduce the incidence of manufacturing failures particularly for PBMC concentrates with low quantities of CD3+ cells and large quantities of contaminating cells.

As CAR T cells become more widely utilized, commercial groups will likely provide the product throughout the U.S. In order to minimize patient travel, regional apheresis sites will be required, and will need standardized yet flexible collection parameters that can be personalized for each patient. Based on the data described here, we recommend the cell processing facility specify the targeted yield of CD3+ cells. Using the pre-apheresis CD3+ cell count and graphical data similar to those presented in Figure 1B, the number of liters to be processed can be extrapolated. Care must be taken to obtain accurate CD3+ cell counts. Patients weighing over 40 kg should generally have a minimum of 10 liters and a maximum

of 24 liters processed. Patients with smaller body mass should have a minimum of 3 and a maximum of 6 total blood volumes processed. Patients with circulating blasts should be considered high-risk for inadequate collections; processing additional blood may be advisable and mid-procedure counts of the PBMC concentrate should be utilized.

Additional research will provide greater understanding regarding the collection and manufacturing processes for CAR T cells. In this study, all procedures were performed on the COBE Spectra, which will not be supported after December 31, 2017. Future collections will likely be performed on the Spectra Optia (TerumoBCT, Lakewood, CO) or Amicus (Fenwal, Lake Zurich, IL) instruments, and comparisons of the different instruments will be important. Furthermore, this study addresses only quantity of CD3+ cells for subsequent manufacturing; quality of the CD3+ cells, purity of the apheresis collection,[15] and other variables that affect the manufacturing process will require extensive evaluation.

Collection of adequate numbers of CD3+ cells for CAR T cell manufacturing is safe and feasible in the majority of patients. Longer collection procedures may facilitate increased CD3+ cell yields in patients who are at risk of low yields. As CAR T cells become more widely used, understanding different variables that affect the lymphocyte collection will aid in ensuring that all patients can have sufficient cells for manufacturing. A personalized approach to the collection process is the essential first step for CAR T cell immunotherapy.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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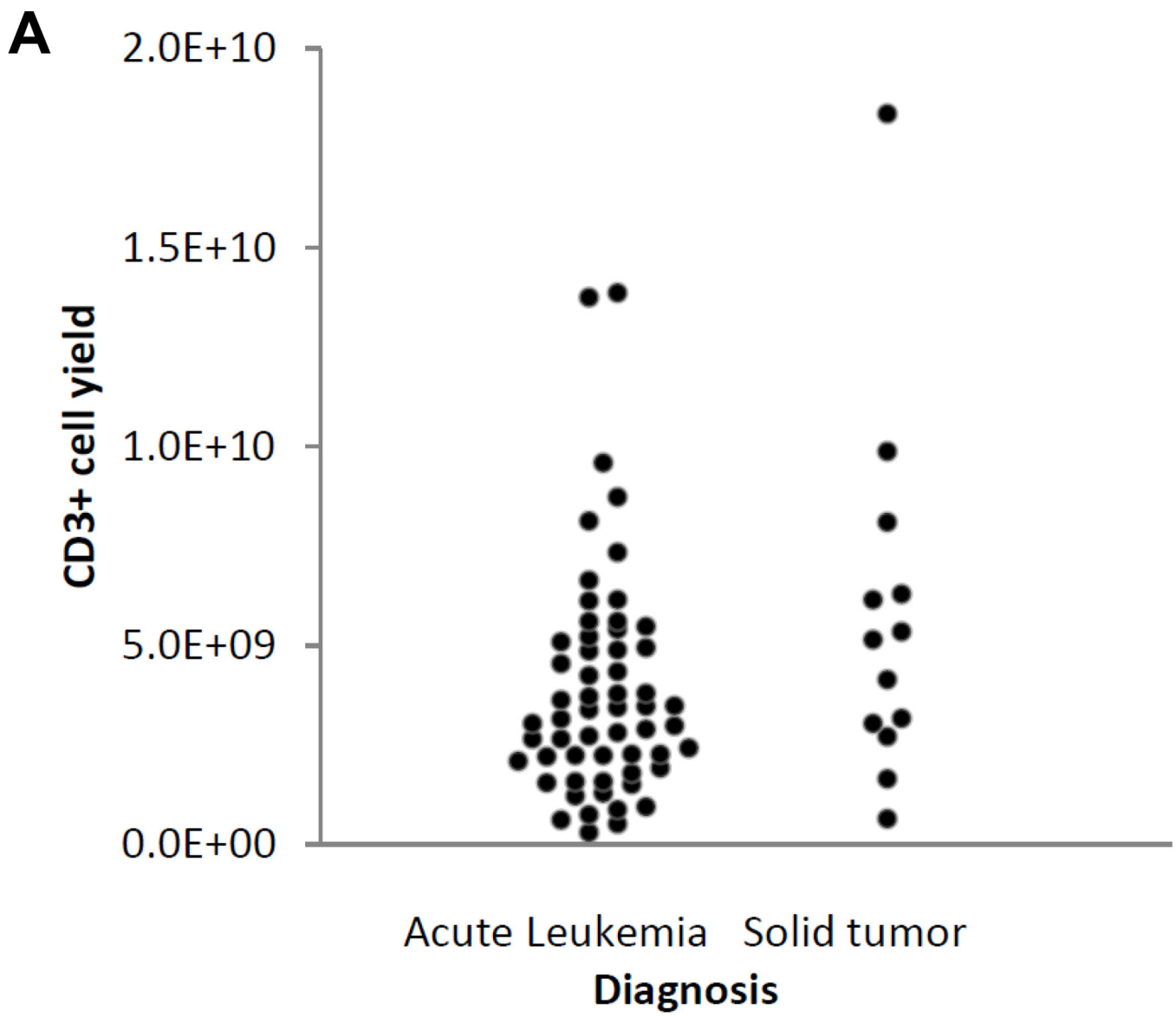


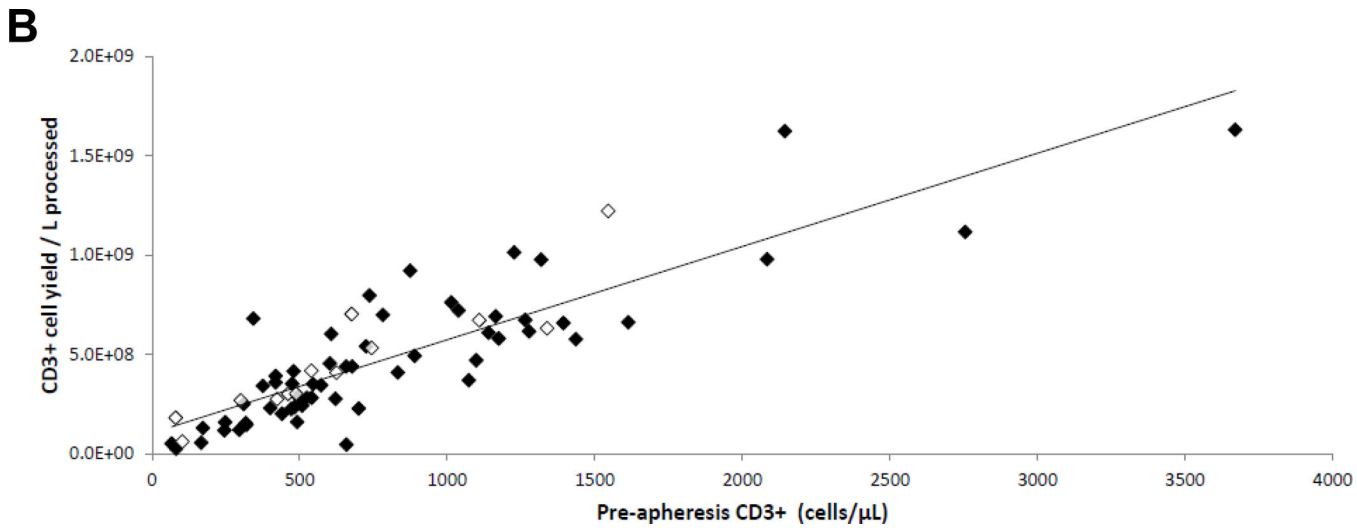
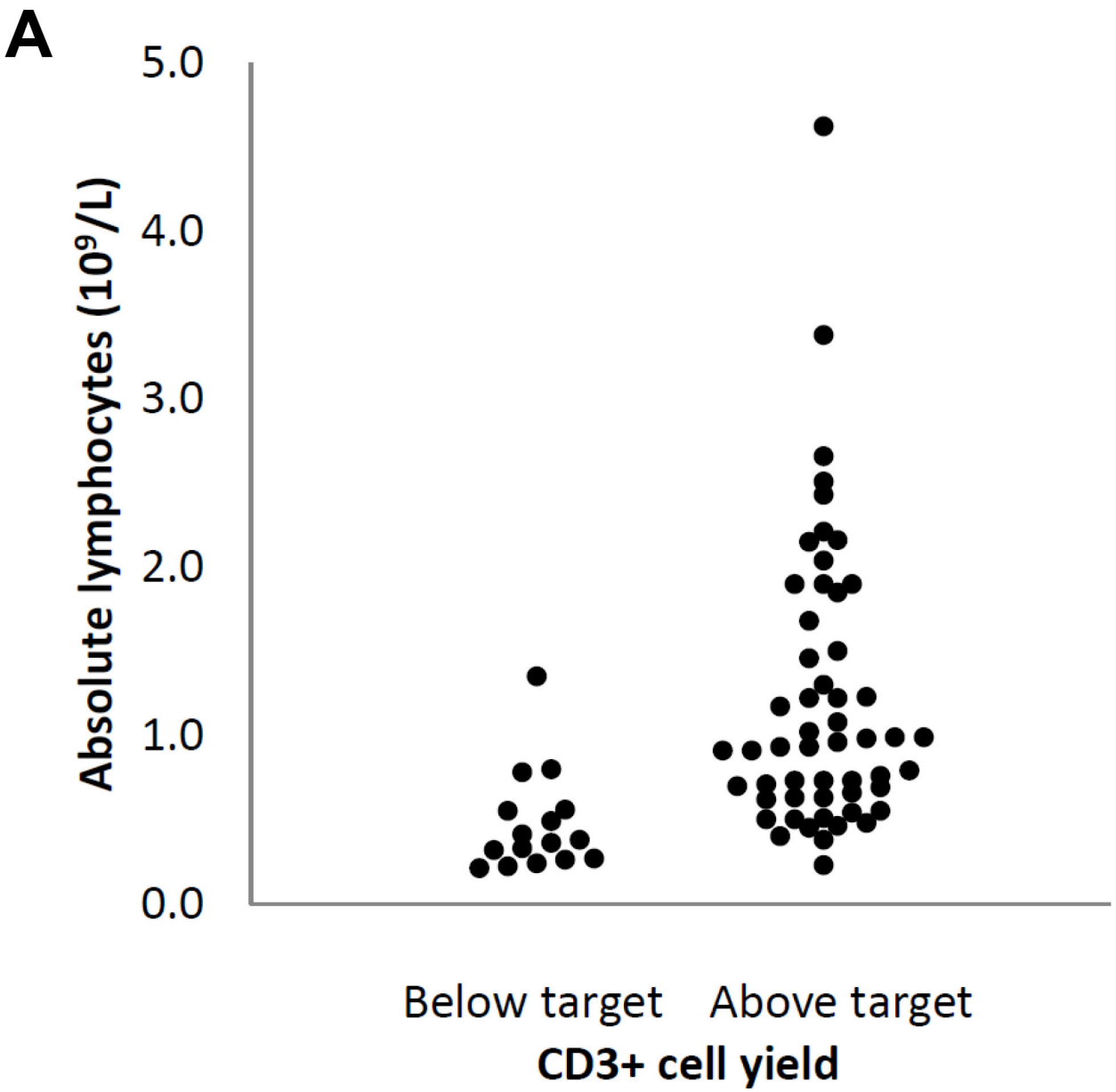


Figure 1. Apheresis CD3+ cell collection yield

Panel A. Total CD3+ cell yield by diagnosis. Each circle represents 1 patient. Panel B. CD3+ cell yield per liter of blood processed versus pre-apheresis CD3+ count. Using the pre-procedure CD3+ count (cells/μL) as the independent variable, x, the collection yield (CD3+ cells per L processed) can be estimated by the equation $y=469394x + 10^8$ ($R=0.8613$; $R^2=0.7419$). Patients with acute leukemia are represented by closed diamonds, patients with solid tumors by open diamonds.



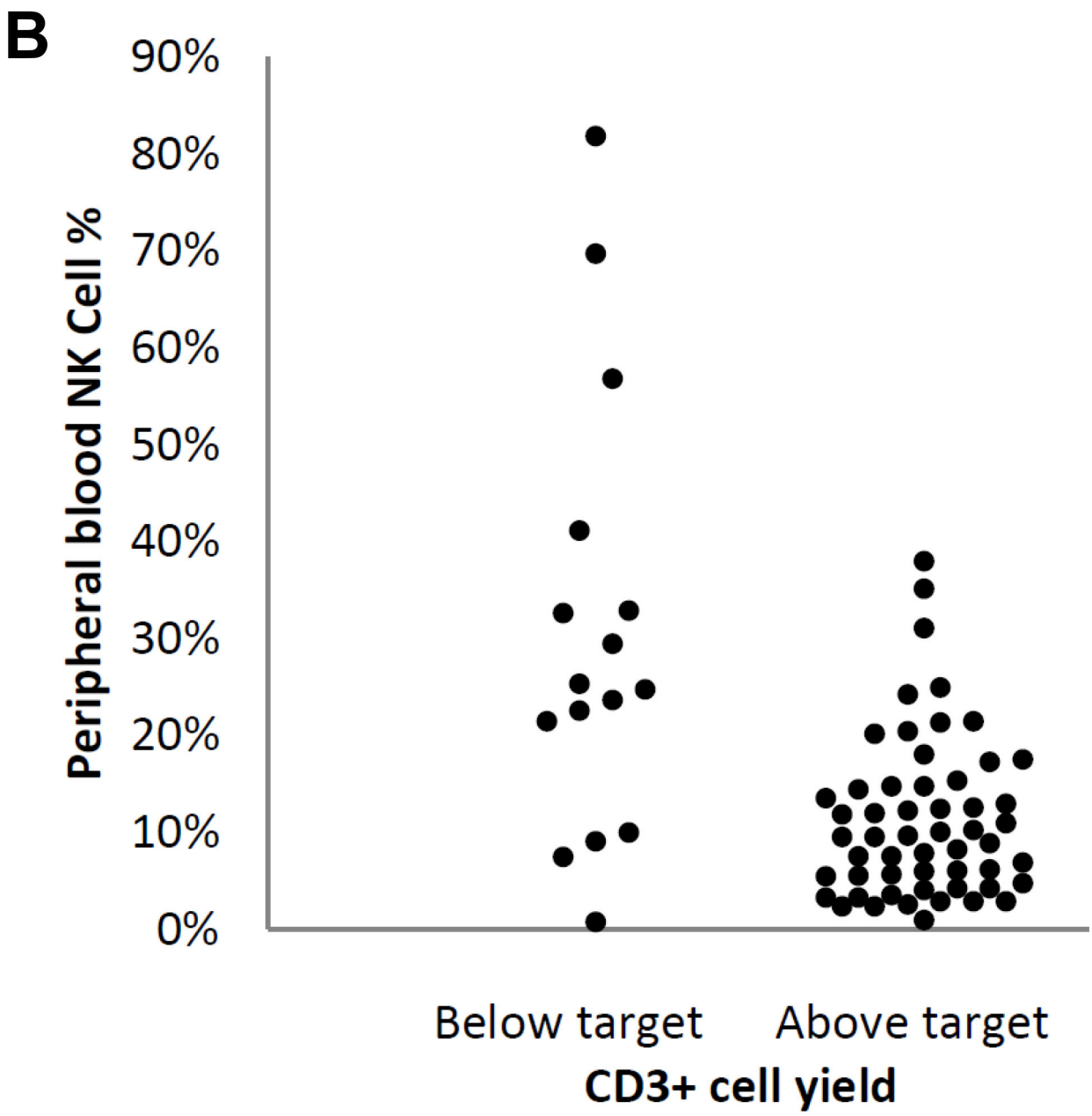


Figure 2. Comparison of absolute lymphocyte counts and peripheral blood NK cell (%) before apheresis in patients with below- and above-target CD3+ cell yields

Each circle represents 1 patient. The targeted yield was 2×10^9 CD3+ cells. Panel A. Absolute lymphocyte count in patients with below- and above-target CD3+ cell yields. A single patient with a yield above the target whose absolute lymphocyte count was 8.29×10^3/μL was not included. Panel B. Peripheral blood NK cells (%) in patients with below- and above-target CD3+ cell yields.

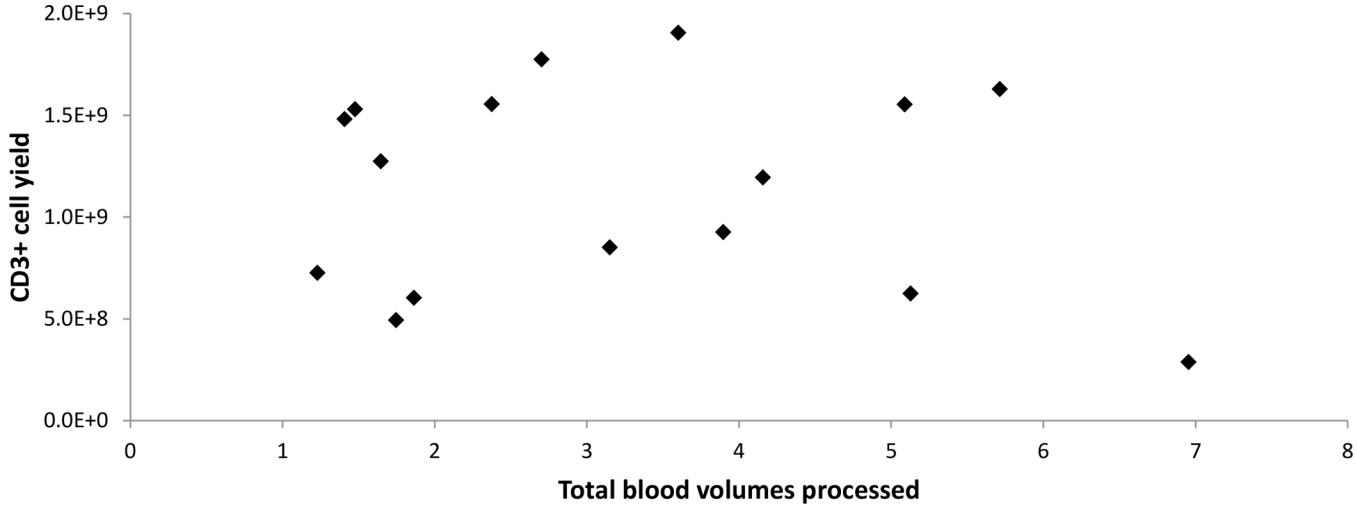


Figure 3. CD3+ cell collection yield vs total blood volumes processed

Each diamond represents 1 patient. Only the 16 patients whose collections yielded fewer than 2×10^9 CD3+ cells are depicted.

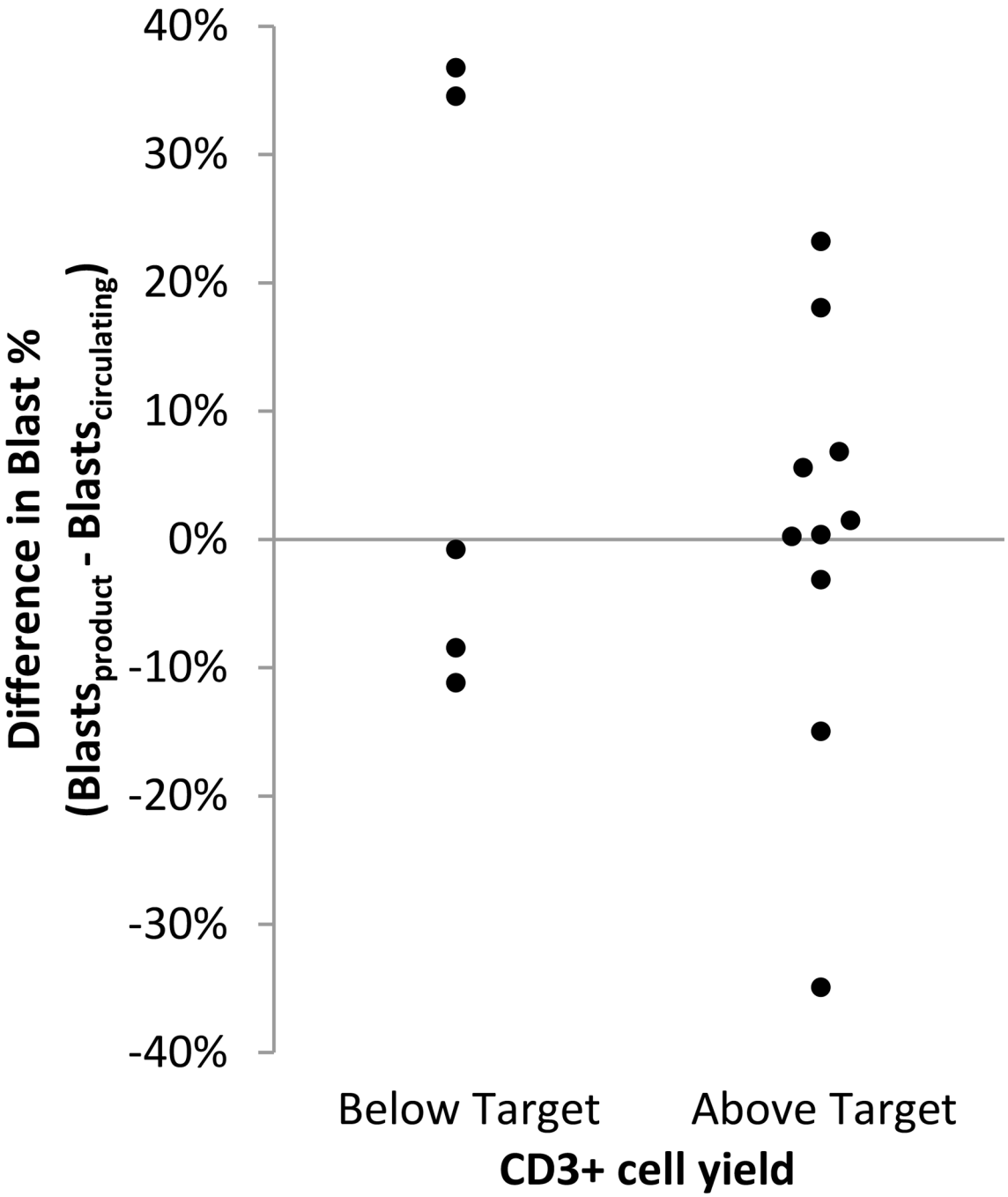


Figure 4. Difference in blast percentages in the apheresis product versus peripheral blood
Each circle represents 1 patient. Only those with circulating blasts on the day of collection are depicted.

Table 1

Patient characteristics

Parameter	Diagnosis		
	ALL n=58	Solid Tumor* n=13	Total n =71
Age (years)			
Mean	14.9	16.6	15.2
Median	14.8	16.2	14.9
Range	4.2 – 30.3	8.1 – 25.9	4.2 – 30.3
Male: Female (patients)	44:14	10:3	54:17
Clinical Trial (patients)			
CD19-CAR	49	1	50
CD22-CAR	9	0	9
GD2-CAR	0	12	12
Weight (kg)			
Mean	49.5	59.9	51.4
Median	44.2	62.7	48.0
Range	16.0 – 140.0	21.0 – 127.0	16.0 – 140.0
< 25 kg (patients)	12	2	14
≥ 25 kg (patients)	46	11	57

*Solid tumors included osteosarcoma (9 patients), neuroblastoma (3 patients), and diffuse large B cell lymphoma (1 patient).

ALL – acute lymphoblastic leukemia; CAR – chimeric antigen receptor

Table 2

Apheresis collection parameters and complications

Parameter	Diagnosis		Total	p*
	ALL n = 58	Solid Tumor n = 13	Total n =71	
Access (patients)				
Peripheral veins	2	2	4	0.15
Central venous catheter	56	11	67	
Liters processed				
Mean	9.10	12.08	9.65	0.01
Median	8.00	12.02	8.42	
Range	4.00 – 18.00	5.00 – 17.00	4.00 – 18.00	
Total blood volumes processed†				
Mean	2.86	3.13	2.91	0.50
Median	2.92	2.94	2.94	
Range	0.83 – 6.95	1.35 – 5.71	0.83 – 6.95	
Blood prime (patients)				
No	47	12	59	0.44
Yes	11	1	12	
Apheresis Complications (patients)				
No	48	12	60	0.68
Yes	10	1	11	
Tingling/paresthesias	3	0	3	
Nausea/vomiting	2	0	2	
Headache	1	0	1	
Musculoskeletal pain	1	1	2	
Bleeding	2	0	2	
Other‡	3	0	3	

*Calculated using the Fisher's exact test (categorical data) or the 2-sided student's t test (continuous data).

†Total blood volumes processed = liters processed / patient weight × 0.075.

‡Altered mental status, seizure, and respiratory distress requiring intubation and ICU admission (1 patient); oxygen desaturation to 87%, resolved with supplemental oxygen (1 patient); line clotted and procedure ended early (1 patient).

ALL – acute lymphoblastic leukemia

Table 3

Apheresis collection yields

Parameter	Diagnosis			
	ALL n = 58	Solid Tumor n = 13	Total n = 71	*p**
TNC ($\times 10^9$)				
Mean	8.83	11.9	9.39	0.19
Median	6.71	9.80	7.08	
Range	0.875 – 41.3	2.66 – 26.9	0.875 – 41.3	
TNC per kg ($\times 10^8$)				
Mean	2.31	2.31	2.31	0.99
Median	1.68	2.12	1.69	
Range	0.161 – 14.3	0.858 – 8.00	0.161 – 14.3	
Granulocytes (%)				
Mean	8	8	8	0.98
Median	4	2	3	
Range	0 – 58	0 – 55	0 – 58	
Lymphocytes (%)				
Mean	71	57	69	0.02
Median	72	60	70	
Range	7 – 99	8 – 82	7 – 99	
Monocytes (%)				
Mean	18	32	21	0.26
Median	19	34	20	
Range	0 – 54	13 – 49	0 – 54	
Percent CD3+ of CD45+				
Mean	52	47	51	0.44
Median	52	53	52	
Range	2 – 97	10 – 68	2 – 97	
Number CD3+ ($\times 10^9$)				
Mean	3.85	5.73	4.19	0.06
Median	3.25	5.13	3.43	
Range	0.288 – 13.8	0.625 – 18.3	0.288 – 18.3	
CD3+ cells /L processed ($\times 10^8$)				
Mean	4.78	4.60	4.74	0.87
Median	4.01	4.09	4.09	
Range	0.259 – 16.3	0.625 – 12.2	0.259 – 16.3	
CD3+ Yield (patients)				
$< 0.6\times 10^9$	2	0	2	1.00
$> 0.6\times 10^9$ and $< 2.0\times 10^9$	12	2	14	
$> 2.0\times 10^9$	44	11	55	

*Calculated using the Fisher's exact test (categorical data) or the 2-sided student's t test (continuous data).

ALL – acute lymphoblastic leukemia; TNC – total nucleated cells

Table 4

Lab values and demographics in patients undergoing lymphapheresis by CD3 yield

	Target yield > 2×10^9 CD3+ cells			
Parameter	**Below n = 16 (23%)**	**Above n = 55 (77%)**	**Reference Range**	***p****
Age, mean (years)	14.08	15.52	n.a.	0.452
Weight, mean (kg)	43.71	53.60	n.a.	0.184
Hemoglobin, mean (g/dL)	9.93	10.78	10.7-13.4	0.064
Platelets, mean ($\times10^3$/μL)	140.63	179.96	206-369	0.195
WBC, mean ($\times10^3$/μL)	4.08	4.04	4.31-11.0	0.964
Blasts, mean (%)	17.60	4.55	0	0.029
Blasts abs, mean ($\times10^3$/μL)	0.83	0.22	0	0.093
Neutrophil, mean (%)	53.84	50.01	34.0-71.1%	0.537
Monocyte, mean (%)	7.59	8.18	4.7-12.5%	0.735
Lymphocytes, mean (%)	19.40	34.32	15.5-56.6%	0.008
Lymphocytes abs, mean ($\times10^3$/μL)	0.47	1.34	0.97-3.96	0.008
CD3+ lymphocytes, mean (%)	63.44	79.68	60.0-83.7%	0.001
CD3+ abs, mean (cells/μL)	348.94	914.11	714-2266	0.001
NK, mean (%)	30.54	11.26	6.2-34.6%	1.3×10^{-6}
NK abs, mean (cells/μL)	171.50	123.27	126-729	0.216
Liters processed, mean	9.02	9.83	n.a.	0.482
TBV processed, mean	3.26	2.81	n.a.	0.230
Collection efficiency,† mean (%)	58.97	67.97	n.a.	0.331

*calculated using the 2-sided student's t test.

†Collection efficiency = CD3+ cell yield / (Pre-apheresis absolute CD3+ cells/μL × 10^6 × liters processed).

WBC – white blood cells; Abs – Absolute; NK – natural killer; TBV – total blood volumes; n.a. – not applicable