Safety and persistence of adoptively transferred autologous CD19-targeted T cells in patients with relapsed or chemotherapy refractory B-cell leukemias

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We report the findings from the first 10 patients with chemotherapy-refractory chronic lymphocytic leukemia (CLL) or relapsed B-cell acute lymphoblastic leukemia (ALL) we have enrolled for treatment with autologous T cells modified to express 19-28z, a second-generation chimeric antigen (Ag) receptor specific to the B-cell lineage Ag CD19. Eight of the 9 treated patients tolerated 19-28z$^+$ T-cell infusions well. Three of 4 evaluable patients with bulky CLL who received prior conditioning with cyclophosphamide exhibited either a significant reduction or a mixed response in lymphadenopathy without concomitant development of B-cell aplasia. In contrast, one patient with relapsed ALL who was treated in remission with a similar T-cell dose developed a predicted B-cell aplasia. The short-term persistence of infused T cells was enhanced by prior cyclophosphamide administration and inversely proportional to the peripheral blood tumor burden. Further analyses showed rapid trafficking of modified T cells to tumor and retained ex vivo cytotoxic potential of CD19-targeted T cells retrieved 8 days after infusion. We conclude that this adoptive T-cell approach is promising and more likely to show clinical benefit in the setting of prior conditioning chemotherapy and low tumor burden or minimal residual disease. These studies are registered at www.clinicaltrials.org as #NCT00466531 (CLL protocol) and #NCT01044069 (B-ALL protocol). (*Blood*. 2011;118(18):4817-4828)

Introduction

Despite currently available therapies, most patients with B-cell leukemias, including chronic lymphocytic leukemia (CLL) and B-cell acute lymphoblastic leukemia (B-ALL), are incurable.[1,2] For this reason, novel therapeutic strategies are needed. The adoptive transfer of genetically engineered immune effector cells aims to rapidly establish T cell–mediated tumor immunity.[3,4] In this approach, the patient's own T cells are targeted to tumor cells through a transgene-encoded Ag receptor consisting of either TCR chains or a chimeric Ag receptor (CAR). CARs are composed of an extracellular Ag recognition domain, most commonly a single chain fragment variable derived from a mAb, fused to a transmembrane domain, and a cytoplasmic signaling domain, most commonly including that of the CD3ζ chain.[3-10] When expressed in T cells, CARs efficiently redirect T-cell specificity and cytotoxicity to cells expressing the targeted Ag in HLA-independent manner.[11-18]

We have previously generated a series of CARs specific for the CD19 Ag,[11,12] a member of the Ig superfamily and component of a B-cell surface signal transduction complex.[19] Expression of CD19 is restricted to B-lineage cells and possibly follicular dendritic cells and is found in most B-cell malignancies, including CLL and B-ALL.[19-23] Significantly, CD19 is not expressed in hematopoietic stem cells. The immunologic targeting of CD19 therefore carries a minimal risk of autoimmunity or BM toxicity other than the potential induction of B-cell aplasias.

In preclinical studies, human T cells expressing CD19-specific CARs efficiently lysed a wide panel of human CD19$^+$ tumor cell lines as well as freshly isolated patient B-cell tumors.[11] Significantly, intravenously administered CD19-targeted human peripheral blood T cells eradicated systemic CD19$^+$ tumors established in SCID-Beige mice.[11,12] Our in vivo studies further showed enhanced antitumor efficacy by providing costimulatory signals to adoptively transferred T cells. Because most B-cell leukemias fail to express ligands for activating costimulatory receptors,[24,25] we overcame this limitation by replacing the inert CD8 transmembrane domain with the transmembrane and cytoplasmic signaling domains of the T-cell costimulatory CD28 receptor,[26] resulting in the 19-28z CAR, which enhances antitumor efficacy in SCID-Beige mice bearing CD19$^+$ leukemias.[12] On the basis of these preclinical data we chose to translate this approach to the clinical setting with the use of the 19-28z CAR.

After the validation of a robust process for large-scale human T-cell transduction and expansion,[27] we enrolled 10 patients with either chemotherapy refractory CLL or relapsed B-ALL on 2 phase 1 dose escalation clinical trials. The primary objective of these

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trials is to assess the safety of infusing 19-28z$^+$ T cells with or without prior cyclophosphamide-conditioning chemotherapy. Secondary objectives include assessment of clinical responses to therapy and the effect of cyclophosphamide conditioning on disease response, T-cell persistence, and T-cell function. Herein, we report our findings on the first 10 patients, 9 of which were infused with the manufactured T cells. We were able to generate sufficient CAR-transduced T cells from leukapheresis products derived from all enrolled patients. Either a marked reduction in tumor burden stable disease or B-cell aplasia was observed in 4 of the 5 evaluable patients given cyclophosphamide before T-cell infusion. We demonstrate rapid trafficking of 19-28z$^+$ T cells to sites of tumor involvement and report on the short-term persistence and function of the adoptively transferred T cells. Collectively, these data show the promise of CD19-targeted T cells for the treatment of B-cell malignancies and provide insights into how to optimally apply this strategy in the clinical setting.

Methods

Clinical protocols

CLL protocol (NCT00466531). Patients with relapsed purine analog-refractory CLL are eligible for enrollment. This is a phase 1 dose escalation trial. Patients initially undergo leukapheresis for T-cell collection. In the first step, one cohort of 3-6 patients is treated with the lowest initial dose of T cells, level 1 (1.2-3.0 $\times$ 10^7 19-28z$^+$ T cells/kg), without prior cyclophosphamide administration. In the second step with 2 cohorts, patients are treated with dose-escalating cyclophosphamide chemotherapy (1.5 and 3.0 g/m^2) followed 2 days later by infusion of modified T cells at dose level 1. After the death on study of the first patient treated in the second cohort,[28] the subsequent 3 patients were treated at the -1 T-cell dose level (0.4-1.0 $\times$ 10^7 19-28z$^+$ T cells/kg) with T-cell infusions split over 2 days to enhance safety. Enrollment on this second cohort has been completed.

B-ALL protocol (NCT01044069). Adult patients with CD19$^+$ B-ALL are eligible for enrollment. Patients can be either enrolled in first complete remission (CR1) after $\geq$ 1 round of consolidation therapy or on presentation with relapsed or refractory disease, defined as no CR after $\geq$ 2 induction regimens. Patients enrolled in CR1 underwent leukapheresis but are treated with T cells only after relapse. Patients enrolled with relapsed or refractory B-ALL leukapheresed, treated with a salvage reinduction regimen, and regardless of remission status, receive cyclophosphamide (3.0 g/m^2) followed 2 days later by split dose infusion of autologous 19-28z$^+$ T cells. This is a standard phase 1 dose-escalation trial with 3 planned T-cell doses, 3 $\times$ 10^6, 1 $\times$ 10^7, and 3 $\times$ 10^7 19-28z$^+$ T cells/kg. Patients enrolled on this protocol are not precluded, if eligible, to undergo additional therapy with allogeneic stem cell transplantation after the modified T-cell infusion.

Both trials were approved by the Memorial Sloan-Kettering Cancer Center Institutional Review Board, the Recombinant DNA Advisory Committee of the National Institutes of Health, and are supported by BB-IND 13266 approved by the US Food and Drug Administration. In both trials, informed consent is obtained from eligible patients in accordance with the Declaration of Helsinki. Patients enrolled on these trials do not receive IL-2. Adverse events during and after therapy were assessed according to the National Institutes of Health Common Terminology Criteria for Adverse Events Version 3.0 (http://ctep.cancer.gov/). The Memorial Sloan-Kettering Cancer Center Data and Safety Monitoring Board review all safety data every 6 months.

Generation and expansion of genetically modified T cells

19-28z–transduced T cells were generated as described.[27] Briefly, T cells were isolated from leukapheresis product and activated with Dynabeads ClinExVivo CD3/CD28. Release criteria include 19-28z CAR expression by PCR and marker rescue cell culture assay; residual Dynabeads ClinExVivo CD3/CD28; negative bacterial, fungal, and *Mycoplasma* cultures; endotoxin level no $>$ 5 EU/kg; Gram stain–negative on day of infusion; $>$ 80% cell viability; and CD3$^+$, CD8$^+$, CD5$^+$ (patients with CLL), CD10$^+$ (patients with ALL), and CD19$^+$ phenotype by flow cytometry and CD19-specific cytotoxicity. The assays were performed as described in Hollyman et al[27] and Taylor et al.[29]

Restimulation of T cells from postinfusion PBMCs with Dynabeads ClinExVivo CD3/CD28

PBMCs collected in cell preparation tubes (BD Biosciences) were purified from whole blood according to the manufacturer's recommendations. Patient CD3$^+$ T cells were subsequently selected and activated with Dynabeads ClinExVivo CD3/CD28 (Invitrogen) at ratio of 3 beads to 1 CD3$^+$ T cell. CD3$^+$ T cells were cultured in X-VIVO 15 medium (Lonza) supplemented with 100 U/mL IL-2. Cell samples were taken at different time points to determine the average vector copy number by quantitative RT-PCR and the expression of 19-28z CAR by flow cytometry.

Restimulation of T cells from postinfusion PBMCs with CD19$^+$CD80$^+$ artificial APCs

Human CD19 and CD80 expressing fibroblasts (3T3-CD19-CD80)[11] were irradiated at 30 Gy. Postinfusion PBMCs were thawed and plated on the 3T3-CD19-CD80 cells in X-VIVO 15 media (Lonza) supplemented with 5% human AB serum (GEMINI) and 100 U/mL IL-2. Lysis of 3T3 cells was monitored, and PBMCs were fed on day 3. Cells were counted 7 days after restimulation and stained with anti–human CD3-FITC, CD8-eFluor 450, CD4-PE-Cy7, biotinylated goat anti–mouse IgG F(ab)$_2$ followed by APC-labeled streptavidin, and 7-amino-actinomycin D (7-AAD) with the use of standard staining procedures. Data acquisition was performed on a LSRII flow cytometer, and data analysis was performed with FlowJo software (TreeStar Inc).

Characterization of cytokine profiles secreted by EOP 19-28z–transduced T cells

Samples of patient T cells transduced with 19-28z CAR were taken at the end of production (EOP). T cells (1.0 $\times$ 10^6) were plated on 3T3 artificial APCs (AAPCs) expressing CD19 or untransduced 3T3 fibroblasts as controls, in complete X-Vivo culture media in 5% human AB serum and in the absence of ILs. After 48 hours supernatants were collected and assayed for human cytokines on a Luminex IS100 instrument as per manufacturer's instructions (Millipore Corp). The cytokine levels were normalized to transduction efficiency. In addition, background levels measured on untransduced 3T3 fibroblasts were subtracted.

Other methods

Other methods, including manufacture of clinical grade PG13-19-28z vector stocks, serum cytokine analyses, cytotoxic T-lymphocyte assays, quantitative real-time PCR, Abs and reagents, flow cytometry, statistics, and IHC can be found in supplemental Methods (available on the *Blood* Web site; see the Supplemental Materials link at the top of the online article).

Results

Patient characteristics

Eight patients with relapsed purine analog-refractory CLL have been enrolled and treated (Table 1). The median age of patients was 68 years with 7 of 8 being men. All patients were previously treated with 1-4 different chemotherapy regimens, with 7 of 8 patients having received $\geq$ 2 different regimens before enrollment. Chromo-

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Table 1. Patient characteristics

Patient ID	Age at diagnosis, y	Age at treatment, y	Sex	Indication for treatment	Prior therapies	Genetic abnormalities/IgV_H mutation status	WBC count, $\times 10^3/\mu L$	ALC, $\times 10^3/\mu L$	Hgb level, g/dL	PLT count, $\times 10^3/\mu L$
CLL-1	44	51	M	Bulky LAD	FCR, PCRM	del11q	200.6	196.6	7.1	26
CLL-2	66	72	M	Bulky LAD	FR, RCVP, PCRM	Unmutated IgV_H	4.2	3.4	9.9	60
CLL-3	62	73	F	Bulky LAD	Chlorambucil, PCR, PCRM	Normal karyotype	136.4	132.3	8.9	100
CLL-4	63	69	M	Bulky LAD	R, PCRM	del11q	187.1	174	9.9	189
CLL-5	65	68	M	Bulky LAD	PCR	del11q, trisomy 12	76.3	66.4	10	162
CLL-6	56	68	M	Bulky LAD	RCVP, PCR, Bendamustine	del11q, inv1, unmutated IgV_H	97.1	92.2	8.9	174
CLL-7	52	62	M	Bulky LAD	CVP, RC, PCR, PCRM	del17p, unmutated IgV_H	1.9	1	10	61
CLL-8	58	61	M	Bulky LAD	RCVP, Alemtuzumab	del17p, monosomy 14, monosomy 15	5.4	3.3	11.6	41
ALL-1	66	67	M	Relapsed disease	C, Mitoxantrone, vincristine, etoposide	Normal karyotype	2.9	0.7	8.6	126
ALL-2	45	48	F	Relapsed disease	HyperCVAD, mitoxantrone, cytarabine, vincristine	Normal karyotype	*	*	*	*

IgV_H indicates immunoglobulin heavy chain; WBC, white blood cell; ALC, absolute lymphocyte count; Hgb, hemoglobin; PLT, platelet; LAD, lymphadenopathy; FCR, fludarabine, cyclophosphamide, rituximab; PCRM, pentostatin, cyclophosphamide, rituximab, mitoxantrone; FR, fludarabine, rituximab; RCVP, rituximab, cyclophosphamide, vincristine, prednisone; PCR, pentostatin, cyclophosphamide, rituximab; R, rituximab; CVP, cyclophosphamide, vincristine, prednisone; RC, rituximab, cyclophosphamide; C, cyclophosphamide; and HyperCVAD, cyclophosphamide, vincristine, doxorubicin, dexamethasone.

*This patient is yet to be treated with modified T cells.

deletion, del11q, and/or an unmutated Ig variable heavy chain domain. All patients exhibited advanced disease and tumor burden as evidenced by marked bulky lymphadenopathy.

Two patients with relapsed B-ALL have been enrolled and 1 patient has been treated (Table 1). The latter achieved an initial remission after induction therapy but subsequently relapsed after the third cycle of consolidation therapy at which point the patient was enrolled and T cells were obtained by leukapheresis. The patient achieved a second remission after reinduction chemotherapy and was treated on protocol. The second patient had a relapse of disease after initial induction therapy but became ineligible for treatment with modified T cells because of intervening complications.

Generation of patient-derived 19-28z–transduced T cells

Despite all patients having been heavily pretreated, we were able to obtain adequate numbers of T cells from the leukapheresis products in every case (Table 2). The mean time in culture to achieve the 19-28z$^+$ T-cell dose was 16 days (range, 11-19 days) with a mean 120-fold expansion of T cells (range, 24- to 385-fold). The transduction efficiency in CD3$^+$ T cells, as assessed by flow cytometry, ranged from 23% to 70% in CLL patient cells and from 4% to 8.6% in ALL patient cells. The average vector copy number per cell in these cell products ranged from 0.06 to 1.5 with a mean of 0.67.

Characterization of final 19-28z–transduced T cells

The final T-cell products exhibited a predominant CD4$^+$ phenotype, particularly pronounced in the CLL EOP cells (mean CD4$^+$ T-cell fraction of 88%, CD4/CD8 ratio of 10.5; Tables 3 and 4). The patients with B-ALL showed a mean CD4$^+$ T-cell fraction of 63% (Table 3). Despite a marked prevalence of CD4$^+$ T cells, all EOP T cells displayed robust in vitro cytotoxic activity against both autologous CLL tumor cells and the CD19$^+$ Raji Burkitt lymphoma cell line (Table 2). Further analyses of final T-cell products showed minimal numbers of CD4$^+$ FoxP3$^+$ T regulatory cells and a significant retention of cell surface markers, including CD27, CD28, and CD62L. Although the percentage of these markers showed some variability (Table 3), the absolute numbers of infused T cells were overall similar in all patients (supplemental Figure 1) as well as their distribution in CAR$^-$ versus CAR$^+$ T cells (Table 3; supplemental Figure 1). To further assess the infused cell products, all EOP cells were activated in Ag-specific fashion in vitro under the same conditions with the use of CD19$^+$ AAPCs.[11,30] The cytokine signatures induced by exposure to CD19 were overall

Table 2. Characteristics of the infused 19-28z–transduced T cells

Test	CLL-1	CLL-2	CLL-3	CLL-4	CLL-5	CLL-6	CLL-7	CLL-8	ALL-1	ALL-2
CD3$^+$ T cells, %										
Apheresis product	5.4	45	7.2	5.1	15.9	6.2	25	22.5	45	67
EOP cells	98	100	100	99	98	99	85	100	99	100
Tumor cells, %										
Apheresis product	94	4	90	94	50	94	28	69	3.1	0
EOP cells	0.1	0	0	0	1	0	1.2	0	0	0.2
CD3$^+$/19-28z CAR$^+$, %	23	31	53	70	32	39	25	39.3	8.6	4
Cytotoxicity (25:1)										
Auto-B tumor cells	58.8	ND	52.3	48.7	33.7	59.5	35.7	28.3	ND	ND
Raji tumor cells	51.1	47.1	52.3	46.5	43.3	73.8	72.9	24.8	29.2	8.6
Fold expansion after transduction (days in culture)	53 (17)	184 (19)	23.6 (18)	112 (15)	191 (18)	51 (15)	62.4 (15)	385 (16)	49.4 (d11)	88.5 (d11)
Total 1928z$^+$/CD3$^+$ cells infused	2.5×10^9	1.2×10^9	1.1×10^9	3.2×10^9	4×10^8	4×10^8	7.6×10^8	1.4×10^9	1.8×10^8	NA

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Table 3. Phenotype of the infused 19-28z–transduced T cells

Patient ID	CD4$^+$	CD8$^+$	CD28$^+$CD27$^-$	CD28$^+$CD27$^+$	CD62L$^+$	CCR7$^+$	CD25$^+$CD4$^+$Foxp3$^+$	Days in culture
CLL-1	94	5	83.2	11.5	9.1	ND	1.2	17
CLL-2	96	5	86.1	6.1	15.5	ND	0.61	19
CLL-3	93	8	40.6	2	17.7	ND	2.4	18
CLL-4	99	0.7	86.9	5.9	34.4	ND	0.9	15
CLL-5	87	12	56.6	20.8	40	7.7	0.7	18
CLL-5 (CAR$^+$)	87.4	11.9	62.9	16.6	40.4	10.1	ND	ND
CLL-6	79	21	45.8	30.8	51.5	3.4	1.3	15
CLL-6 (CAR$^+$)	78.6	20.8	57	19.3	52	3.7	ND	ND
CLL-7	58	27	41.3	13.5	63.4	1.1	1.2	15
CLL-7 (CAR$^+$)	66.6	21.5	49.1	13.3	54.3	1.4	ND	ND
CLL-8	91.5	8.5	81.7	9.1	33.1	19.6	ND	16
CLL-8 (CAR$^+$)	90.3	9.7	82.4	7.8	28.1	16.6	ND	ND
ALL-1	74	26	43.4	16.5	64.3	1.3	1.7	11
ALL-1 (CAR$^+$)	62.8	25.6	52.3	15.9	78.1	1.3	ND	ND
ALL-2	52	48	5.2	84.8	94.8	47.4	ND	13
ALL-2 (CAR$^+$)	47.9	51.8	28.5	64.1	86.1	36.5	ND	ND

For patients CLL-1, CLL-2, CLL-3, and CLL-4, the CD4, CD8, and memory phenotypes were analyzed in 7-AAD$^-$ populations. For patients CLL-5, CLL-6, CLL-7, ALL-1, and ALL-2, the CD4, CD8, and memory phenotypes were analyzed in both 7-AAD$^-$ and CAR$^+$ populations. All numbers are expressed in percentage of 7-AAD$^-$ or CAR$^+$ populations as indicated.

similar, showing in particular robust secretion of GM-CSF, IFN-γ, TNF-α, MIP-1α, MIP-1β, and little or no IL-2, IL-4, and IL-10 in most patients (Figure 1).

Infusion of 19-28z–transduced T cells is well tolerated

Nine patients have received intravenous infusion of 19-28z–transduced autologous T cells. Overall, patients tolerated therapy well with most patients experiencing rigors, chills, and transient fevers within 24 hours of modified T-cell infusions. In all cases, patient blood and urine cultures were obtained, and intravenous antibiotic therapy was initiated. However, with the exception of patient CLL-4, all patients readily recovered from these symptoms with negative blood and urine cultures and were released after an additional 48 hours of in-patient observation (Table 5). Patient CLL-4 experienced persistent fevers, developed a sepsis-like syndrome with hypotension and renal failure, and died within 48 hours of T-cell infusion. Previously published extensive analyses of this case point to an infectious cause as evidenced by marked serum cytokine abnormalities that preceded T-cell infusion, arguing against the 19-28z$^+$ T cells being the primary cause of this adverse outcome.[28] The subsequent cohort of 4 patients with CLL was treated at a 3-fold lower T-cell dose than CLL-4 and administered in a split-dose manner over 2 consecutive days. In addition, serum cytokine levels were assessed before both cyclophosphamide and T-cell infusions. Cytokine elevations, as noted in CLL-4, were not observed in the subsequently treated patients before or after cyclophosphamide therapy or after T-cell infusion. Significantly, serial serum analyses in these patients did not show evidence of cyclophosphamide-induced alterations in cytokine profiles compared with patients treated with T cells without cyclophosphamide conditioning (Figure 2).

Clinical responses to 19-28z$^+$ T-cell infusion

Eight patients with CLL have been treated. The first cohort of 3 patients was treated without cyclophosphamide conditioning at the lowest planned T-cell dose of 1.2-3.0 $\times$ 10^7 19-28z$^+$ T cells/kg with no objective disease responses (Table 6). Collectively, all 3 patients on this cohort exhibited further progressive disease and soon required additional salvage chemotherapy. All patients on this cohort have died of their disease. Patient CLL-4 died soon after

Table 4. CD4/CD8 ratio in apheresis, EOP 19-28z transduced T cells, and blood samples after infusion

	Apheresis		EOP 19-28z T cells	19-28z T cells after infusion
Patient ID	CD4/CD8	CD4$^+$CD28$^+$/CD8$^+$CD28$^+$	CD4/CD8	CD4/CD8 (time after infusion)
CLL-1	2.4	2.4	18.8	NA
CLL-2	0.3	0.3	19.2	NA
CLL-3	3.5	6.8	11.6	NA
CLL-4	0.5	4.0	141.4	70.4 (1 h); 33.5 (24 h)
CLL-5	0.8	1.0	7.3	NA
CLL-6	1.5	2.3	3.8	NA
CLL-7	2.5	7.0	2.5	NA
CLL-8	4.5	6.0	10.1	19 (2 wk); 8.7 (4 wk); 8.8 (5 wk)
Mean ± SD	2.0 ± 1.5	3.7 ± 2.6	10.5 ± 6.6*	NA
ALL-1	1.0	2.1	2.5	1.1 (8 d)
ALL-2	1.8	1.8	0.9	NA

All phenotypes are analyzed by flow cytometry in 7-AAD$^-$ and CD45$^+$ populations. All numbers are expressed in ratio of percentages of CD4/CD8 or

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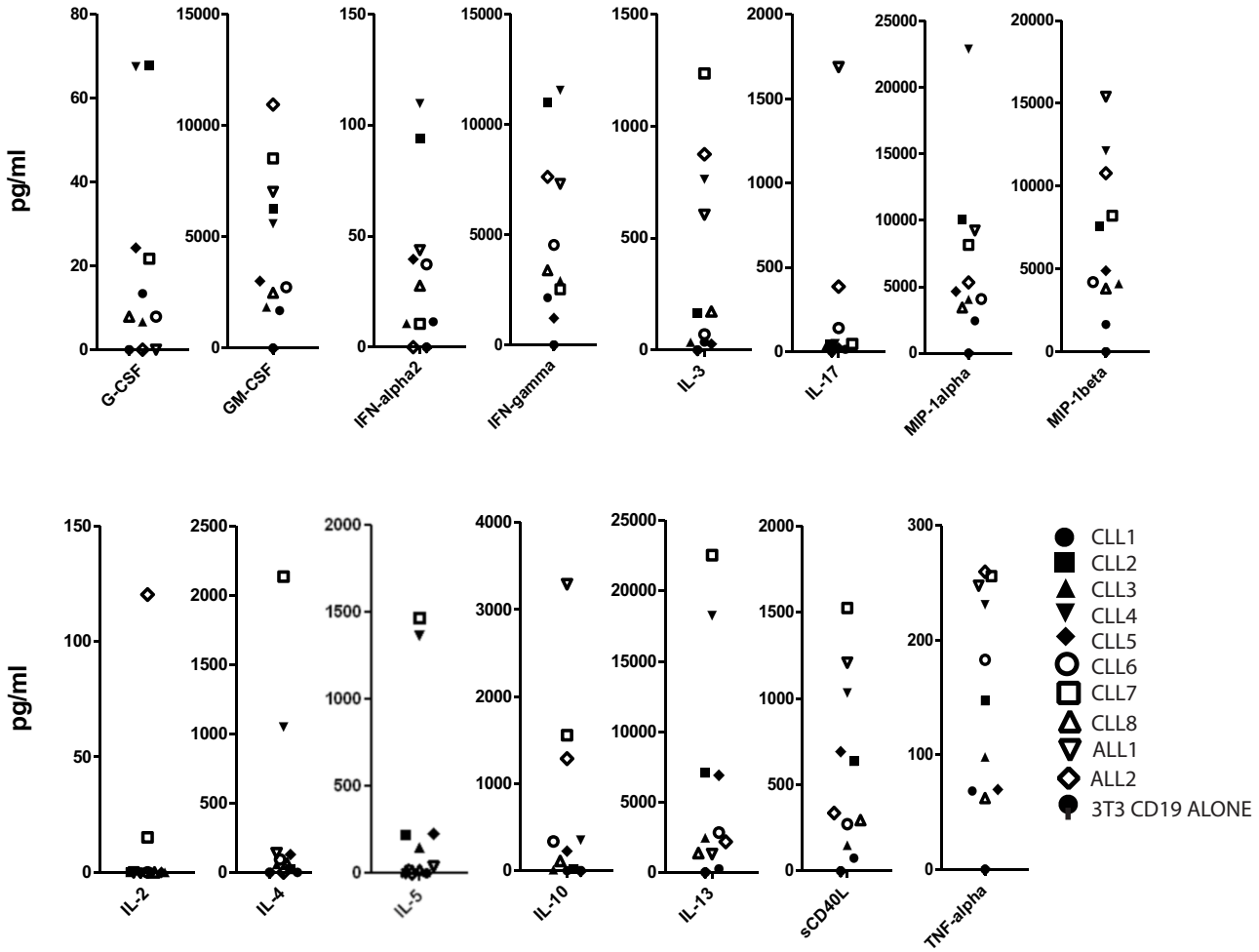

Figure 1. Cytokine analysis of EOP 19-28z–transduced T cells stimulated on CD19⁺ AAPCs. EOP 19-28z–transduced T cells were plated on 3T3 fibroblast AAPCs expressing CD19. After 48 hours in culture, cell supernatants were assayed for cytokine levels. The cytokine levels were normalized to transduction efficiency. In addition, background cytokine levels measured on 3T3 fibroblasts that did not express CD19 were subtracted.

T-cell infusion and therefore was not evaluable for clinical response.[28]

Table 5. Adverse events

Diagnosis-Patient	Adverse events	Grade	Related
CLL-1	Febrile neutropenia	3	Probable
	Rigors, chills	2	Probable
CLL-2	Fever, rigors, chills	2	Probable
	Chest pain	2	Probable
CLL-3	Fever, rigors, chills	1	Probable
CLL-4	Fever	2	Probable
	Rigors, chills, dyspnea	1	Probable
	Hypotension, renal failure	5	Possible
CLL-5	Fever	2	Probable
	Rigors, chills	1	Probable
	Hyponatremia	1	Possible
CLL-6	Fever	1	Probable
	Hypotension	2	Possible
	Febrile neutropenia	3	Possible
CLL-7	Febrile neutropenia	3	Probable
CLL-8	Fever	2	Probable
ALL-1	Neutropenia	4	Possible
	Diarrhea	2	Possible
	Hypotension	3	Possible

The next 4 patients (CLL-5 to -8) were treated with cyclophosphamide-conditioning chemotherapy, followed by infusion of $0.4\text{-}1.0 \times 10^7$ 19-28z⁺ T cells/kg. Patient CLL-5 exhibited stable to progressive disease at 1 month after treatment as assessed by computed tomography scan but subsequently developed marked objective reduction of peripheral lymphadenopathy in the absence of any further therapeutic interventions, as assessed by physical examination and computed tomography scans over the subsequent 2 months (Figure 3). This marked reduction of lymphadenopathy remained stable over the subsequent 6 months, and thereafter the patient developed disease progression in the abdomen with associated ascites and worsening cytopenias, which required further chemotherapy. The patient died 15 months after T-cell therapy with progressive chemotherapy refractory disease. Patient CLL-6 exhibited progressive disease >1 month after therapy requiring salvage chemotherapy and died of infectious complications 2 months later. Patients CLL-7 and CLL-8, both treated in the setting of rapidly progressive disease with increasing lymphadenopathy and cytopenias, exhibited stable disease with respect to lymphadenopathy over a 4- and >2-month period of expectant management, respectively, after cyclophosphamide and T-cell infusions (data not shown).

Patient ALL-1 was a 67-year-old male who relapsed during

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