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Cancer Regression in Patients After Transfer of Genetically Engineered Lymphocytes

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Abstract

Through the adoptive transfer of lymphocytes after host immunodepletion, it is possible to mediate objective cancer regression in human patients with metastatic melanoma. However, the generation of tumor-specific T cells in this mode of immunotherapy is often limiting. Here we report the ability to specifically confer tumor recognition by autologous lymphocytes from peripheral blood by using a retrovirus that encodes a T cell receptor. Adoptive transfer of these transduced cells in 15 patients resulted in durable engraftment at levels exceeding 10% of peripheral blood lymphocytes for at least 2 months after the infusion. We observed high sustained levels of circulating, engineered cells at 1 year after infusion in two patients who both demonstrated objective regression of metastatic melanoma lesions. This study suggests the therapeutic potential of genetically engineered cells for the biologic therapy of cancer.

In the past two decades, fundamental advances in immunology have introduced opportunities for the development of cellular-based therapies for the treatment of cancer (1,2). After ex vivo expansion, transfer, and clonal repopulation in patients who have received lymphodepleting conditioning, autologous tumor-infiltrating lymphocytes (TILs) have been found to mediate objective cancer regression in a measurable proportion of patients with metastatic melanoma (3–5). A limitation of this approach is the requirement that patients have preexisting tumor-reactive cells that can be expanded ex vivo. In addition, in many cancer patients, especially those with cancers other than melanoma, it is difficult to identify these tumor-reactive lymphocytes. To overcome this limitation, we set out to develop an approach to cancer immunotherapy based on the genetic modification of normal peripheral blood lymphocytes (PBLs).

Tumor-associated antigens (TAAs) are recognized by the T cell receptor (TCR) on the T lymphocyte surface, which is composed of the TCR alpha and beta chains (6). The genes encoding the TCR that are specific for a variety of TAA have now been cloned, including the TCR-recognizing MART-1 and gp100 melanoma/melanocyte differentiation antigens, the NY-ESO-1 cancer-testis antigen that is present on many common epithelial cancers, and an epitope from the p53 molecule, which is expressed on the surface of approximately 50% of cancers of common epithelial origin (7–12). In each case, these antigens were detected by the TCR when they were presented as peptides by molecules encoded by the major histocompatibility complex protein human lymphocyte antigen (HLA)–A2. In vitro transcribed

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Supporting Online Material

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RNA from four TAA-reactive TCRs (recognizing MART-1: 27–35, gp100: 209–217, NY-ESO-1: 157–165, and p53: 264–272) were electroporated into CD8⁺ PBLs, which were then cocultured with peptide-pulsed T2 cells. These transfected cells produced large amounts of interferon- γ (IFN- γ) upon stimulation with their respective peptides (Fig. 1A) and were able to recognize HLA-A2–matched tumors, including melanoma, lung cancer, and breast cancer (table S1). Furthermore, transduction with these TCR-encoding retro-viral vectors converted normal PBLs into cells capable of specifically recognizing and destroying both fresh and cultured cells from multiple common cancers (such as sarcoma and breast, lung, esophagus, and liver cancers) in vitro (9–12).

To investigate the ability of genetically engineered PBLs to recognize and destroy tumor cells in vivo, we transduced PBLs derived from patients with melanoma with the genes encoding the alpha and beta chains of the anti-MART-1 TCR. These genes were cloned from a TIL clone obtained from a cancer patient who demonstrated a near complete regression of metastatic melanoma after adoptive cell transfer (ACT) of TILs (5). A retroviral vector was constructed and optimized to express the MART-1 TCR alpha and beta chains (Fig. 1B) (13). Gene transfer efficiency, assessed by staining for the specific $\text{V}\beta 12$ protein in this TCR, resulted in expression in 30% of the transduced CD8⁺ cells (Fig. 1C), as compared with ~1% of untransduced control cell cultures (gene transfer was about equally divided between CD4 and CD8 cells). Fifteen percent of the transduced CD8⁺ cells bound the MART-1 peptide-specific HLA-A*0201 tetramer (Fig. 1C and table S2). The TCR-transduced cells were biologically active, as demonstrated by the specific secretion of IFN- γ after coculture with both MART-1 peptide-pulsed cells and HLA-A2 positive melanoma cell lines (Fig. 1D).

To investigate the in vivo efficacy of these MART-1 TCR-engineered T cells, we selected 17 HLA-A*0201 patients with progressive metastatic melanoma (Table 1) for treatment. Cancers in all patients were refractory to previous therapy with interleukin-2 (IL-2). T cell cultures from all 17 patients were biologically reactive, with specific secretion of IFN- γ after coculture with either MART-1 peptide-pulsed T2 cells and/or melanoma cell lines expressing the MART-1 antigen (Fig. 1E). Gene transfer efficiencies measured by staining for $\text{V}\beta 12$ expression in these lymphocytes ranged from 17 to 67% (42%, mean value) (Table 1 and table S2).

Patients received ACT treatments with MART-1 TCR-transduced autologous PBLs at a time of maximum lymphodepletion (13). Three patients in an initial cohort were treated with cells after an extended culture period of 19 days and had cell doubling times ranging from 8.7 to 11.9 days (Table 1, cohort 1, patients 1, 2a, and 3). In these patients, <10% of the transduced cells persisted across the time points tested during the first 30 days after infusion, and $\leq 2\%$ of the cells persisted beyond 50 days (Fig. 2A). These first three patients showed no delay in the progression of disease.

In an effort to administer gene-modified lymphocytes that were in their active growth phase, the culture conditions were modified (13) to limit the ex vivo culture period to between 6 and 9 days after stimulation of cells with antibody to CD3 (Table 1, cohort 2, cell doubling times of ≤ 2 days). In another cohort, larger numbers of actively dividing cells for ACT were generated by performing a second rapid expansion protocol (14) after 8 to 9 days (Table 1, cohort 3, cell doubling times from 0.9 to 3.3 days). In contrast to the lack of cell persistence seen in cohort 1 patients (Fig. 2A), patients in cohorts 2 and 3 (Fig. 2, B to D) all exhibited persistence of the transduced cells at >9% at 1 and 4 weeks after treatment (range, 9 to 56%). All eight patients who provided samples >50 days after treatment exhibited cell persistence of >17%, and this level of persistence was durable in seven patients during a >90-day monitoring period. In one patient (patient 14), >60% of circulating lymphocytes were positive for the gene-marked cells (Fig. 2C).

In 14 patient samples tested at one month after ACT, quantitative reverse transcription polymerase chain reaction (RT-PCR) assays revealed the presence of vector-derived RNA, confirming that gene expression continued (table S3). All but one of 15 patients analyzed had increased levels of CD8⁺/Vβ12 cells at 1 week after treatment, and the levels of 11 of the 15 patients were higher at 1 month as compared to pretreatment levels (Fig. 2E). Of 13 patients that were examined, all had increased MART-1 tetramer-binding cells after treatment (Fig. 2F), and 11 of 14 had increased numbers of enzyme-linked immunosorbent spot-positive cells (table S4).

There was, however, a discordance between the mean persistence of transduced cells at 1 month in cohorts 2 and 3 measured by PCR (26%) as compared to the measurement of Vβ12-expressing cells (8.1%) and of MART-1 tetramer-binding cells (0.8%). This discordance may in part be due to mispairing of the introduced TCR chains with the endogenous chain, as well as to the different sensitivities of the assays. The reduced expression of the transgene in the persisting cells at ≥1 month may also be a function of the described decrease (15) in the transcription of retrovirally inserted transgenes and the decline in metabolic activity during the conversion of activated cells to memory cells. This decrease in expression of the retroviral transgene would be expected to affect the measurement of tetramers, which relies on the aggregation of multiple receptors, more heavily than the detection of Vβ12 cells directly by antibody staining.

Most important, two patients demonstrated a sustained objective regression of their metastatic melanoma assessed by standard criteria [response evaluation criteria in solid tumors (RECIST)] (16). Patient 4, a 52-year-old male, had previously received treatment with interferon-α (IFN-α), a lymph node dissection, an experimental vaccine, and high-dose IL-2. The patient then developed progressive disease in the liver (4.4- by 3.3-cm mass) and axilla (1.3- by 1.2-cm mass). After treatment with the ACT protocol described above, he experienced complete regression of the axillary mass and an 89% reduction of the liver mass (Fig. 3, A and B), at which time it was removed. He remains clinically disease-free at 21 months after treatment. Patient 14, a 30-year-old male, previously received treatment consisting of a lymph node dissection, IFN-α, and high-dose IL-2. He developed an enlarging 4.0- by 2.5-cm mass in the lung hilum. After ACT treatment, he underwent regression of the hilar mass and is now clinically disease-free 20 months later (Fig. 3, C and D). Thus, two patients with rapidly progressive metastatic melanoma showed full clinical regression of disease after the transfer of genetically engineered autologous PBLs.

In responding patients 4 and 14, the number of gene-marked cells in the circulation (assumed to be 1% of total body lymphocytes) increased by factors of 1400 and 30, respectively, as compared to the number of infusion cells. At 1 year after infusion, both responding patients had sustained high levels (between 20 and 70%) of circulating gene-transduced cells (Fig. 3E). This high level of gene-marked cells was confirmed in patient 4 by limiting dilution T cell cloning of circulating lymphocytes at 1 year after treatment, which revealed that 42% (33 out of 79) of T cell clones contained the transgene as assessed by the PCR assay. These two patients also displayed Vβ12 cells that were detectable by antibody staining between 12 and 16% for >300 days after treatment (Fig. 3F). Patients 4 and 14 were also two of four patients who had >1% of circulating tetramer positive cells detectable for >15 days after cell infusion (Fig. 2F), and these two patients demonstrated anti-TAA reactivity in ex vivo coculture assays (table S5). No toxicities in any patient were attributed to the gene-marked cells. Although the genetically modified transferred cells exhibited decreased expression of the transgene with time in vivo, the functional activity was apparently sustained at a level sufficient to mediate the tumor regression that was seen.

Approaches to increase the expression and function of the transgene are being studied, including the possible use of lentiviral vectors, the use of more powerful promoters specific to T cells, the use of higher-affinity TCRs that can mediate CD8 independent antitumor reactivity in CD4 cells, the further optimization of T cell transduction methods, and the production of higher titer clinical-grade viruses. Approaches to prevent chain mispairing may include modification of the TCR constant regions, the insertion of single-chain receptors (17), or the genetic modification of hematopoietic stem cells (18). Because tumor specificity can be conferred on bulk PBL populations with high efficiency, it may be possible to select sub-populations of PBLs that have distinct anti-tumor qualities. Further genetic modification of PBLs to insert cytokine or tissue-homing molecules may be beneficial. Mouse models predict that increased lymphodepletion, either by the addition of total body irradiation to the preparative regimen or by the administration of a vaccine containing the antigen recognized by the transduced TCR, can also enhance treatment effectiveness (19,20), and these modifications are currently being explored in clinical trials.

In human subjects, normal autologous T lymphocytes, transduced *ex vivo* with anti-TAA-TCR genes and reinfused in cancer patients, can persist and express the transgene for a prolonged time *in vivo* and mediate the durable regression of large established tumors. Although the response rate (2 out of 15 patients or 13%) seen in cohorts 2 and 3 is lower than that achieved by the infusion of autologous TILs (50%), this method has potential for use in patients for whom TILs are not available. Engineering PBLs to express high-affinity TCRs recognizing the NY-ESO-1 or p53 antigens (Fig. 1A and table S1) enables the *in vitro* recognition of TAAs expressed on a variety of common cancers, and the use of these genetically engineered cells for the treatment of patients with common epithelial cancers deserves evaluation.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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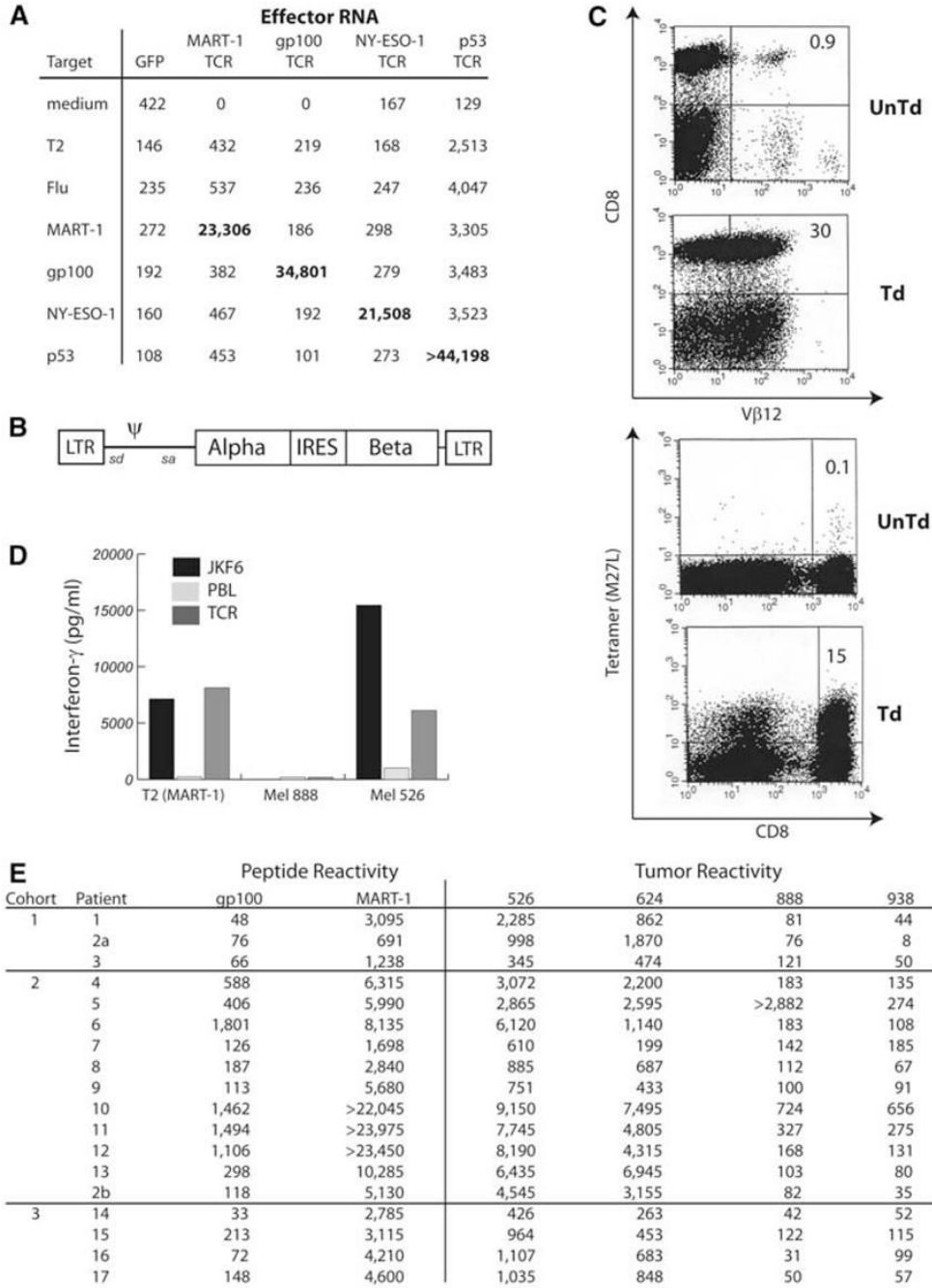


Fig. 1. Transduction and analysis of TCR-engineered cells. **(A)** CD8⁺human lymphocytes were electroporated with RNA encoding control [green fluorescent protein (GFP)] or cloned TCRs reactive with HLA-A2 restricted epitopes from the human TAAs MART-1, gp100, NY-ESO-1, and p53. Effector T cells were cocultured with T2 cells pulsed with 1 μM of the indicated peptide (values are expressed as IFN-γ in pg/ml). Values demonstrating the specific release of cytokine are in bold. **(B)** Diagram of the recombinant retroviral vector MSGV1AIB used to engineer human lymphocytes. LTR, long terminal repeat; Ψ, extended packaging signal; sd, splice donor; sa, splice acceptor; Alpha, alpha chain; IRES, internal ribosomal entry site; Beta, beta chain. **(C)** Transduced (Td) lymphocytes were analyzed 5 days after transduction for the

expression of V β 12 and MART-1 tetramer [Ala²⁷ \rightarrow Leu²⁷ (A27L)] in CD8⁺ cells in comparison with untransduced (UnTd) cells. Numbers in the upper-right corners indicate the percentage of positive cells in that quadrant. **(D)** TCR vector-engineered cells from patient 6 (TCR) were cocultured with MART-1 peptide-pulsed T2 cells, HLA-A2⁻ melanoma line (Mel 888), or HLA-A2⁺ melanoma line (Mel 526), and the amount of IFN- γ produced was determined. Control effectors were untransduced cells (PBL) and the MART-1-reactive TIL JKF6 (JKF6). **(E)** Anti-melanoma properties of genetically engineered lymphocytes were determined for all patients before infusion. The production of IFN- γ (pg/ml) after coculture with peptide-pulsed T2 cells (Peptide Reactivity) and anti-melanoma activity (Tumor Reactivity) for HLA-A2⁺ lines (526 and 624) and HLA-A2⁻ lines (888 and 938).

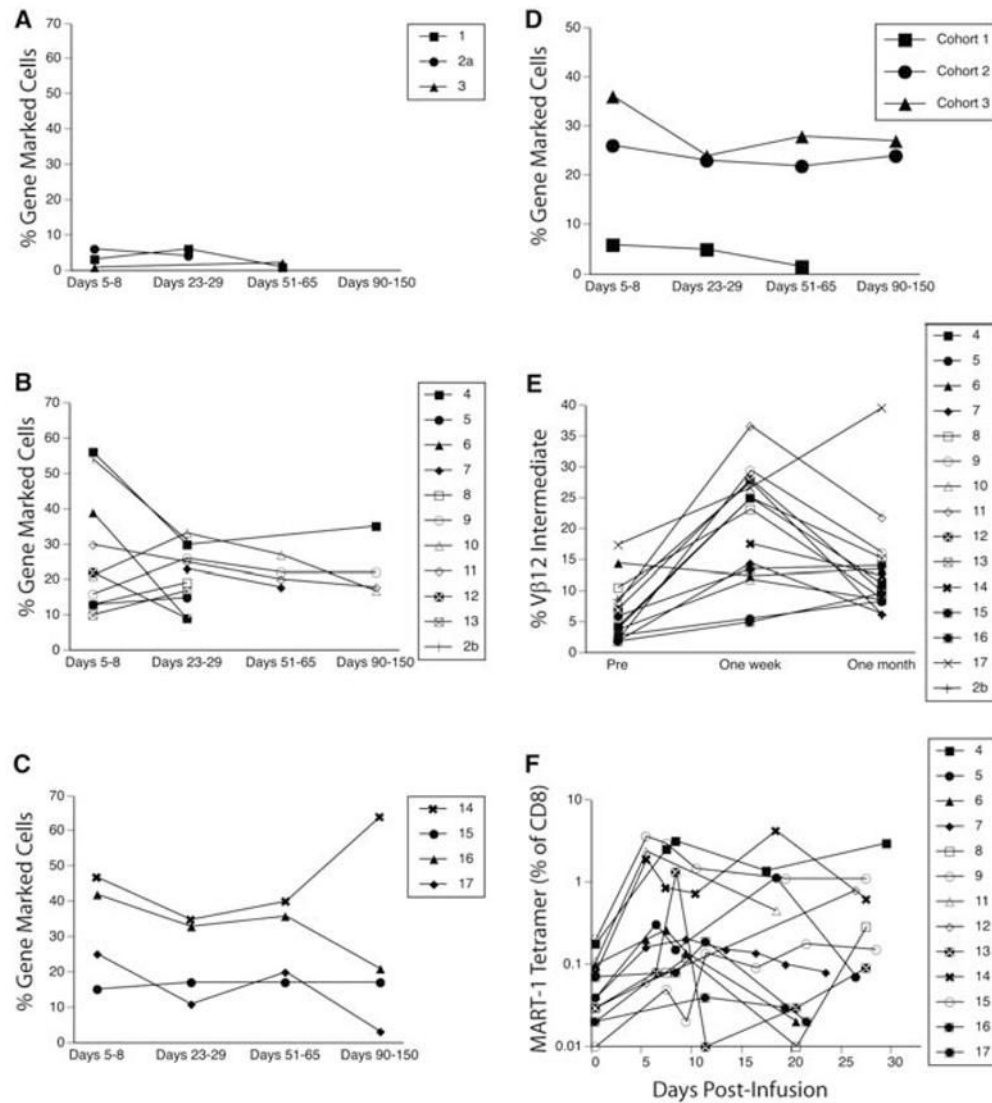


Fig. 2. Persistence of gene-marked cells. DNA extracted from peripheral blood mononuclear cells (PBMCs) was subjected to real-time quantitative PCR to determine the percentage of vector-transduced cells in patient circulation at various times after infusion. Each line represents data from a separate patient. (A) Cohort 1; (B) Cohort 2; (C) Cohort 3. (D) Mean value of the percentage of gene-marked cells for all patients in each cohort at the given time interval after treatment. (E) The percentage of CD8⁺/Vβ12⁺ cells in the intermediate gate (13) for patients in cohorts 2 and 3 is shown. (F) The percentage of CD8⁺/MART-1⁺ tetramer cells was determined for patients in cohorts 2 and 3 at the times shown. Pretreatment values for each patient are plotted as day 0 after infusion.

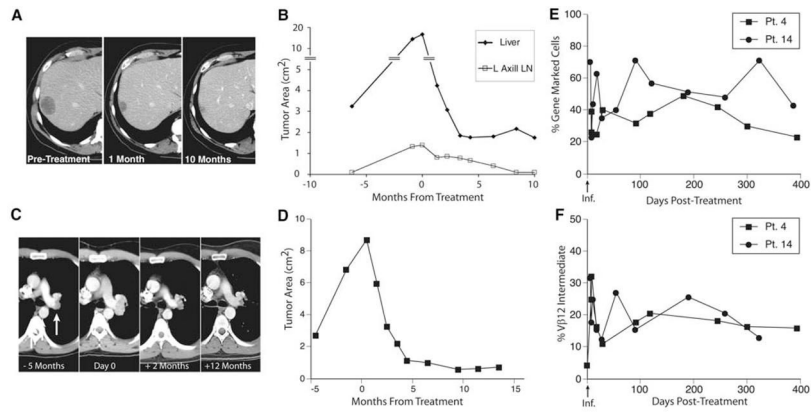


Fig. 3. Cancer regression in two patients. **(A)** Computed tomography (CT) images of liver metastasis in patient 4 taken at pretreatment, 1 month, and 10 months after treatment with TCR-engineered T cells. **(B)** Size of liver and axillary tumors and tempo of regression of tumor sites in patient 4. Day 0, beginning of treatment. L Axill LN, left axillary lymph node. **(C)** CT images of hilar lymph node metastasis in patient 14; pretreatment, day 0, and 2 months and 12 months after treatment. The white arrow indicates the mass in the lung hilum. **(D)** Size of tumor and tempo of regression in patient 14. **(E)** Quantitation of gene-marked cells in the PBMCs of patients 4 and 14 was determined by real-time quantitative PCR. Pt, patient. Day of infusion (Inf.) indicated by arrow. **(F)** The percentage of CD8⁺/Vβ12⁺ cells in the intermediate gate (13) in the circulation of patients 4 and 14.

Table 1

Patient demographics, treatments received, and clinical outcome. Ln, lymph node; Cu, cutaneous; Sub, subcutaneous; Li, liver; Lu, lung; Ad, adrenal; Pa, pancreas; Br, brain; Hi, hilum. NR, no response; PR, partial response; MR, minor or mixed response.

Cohort	Patient	Age/ sex	Total cells infused ($\times 10^{-9}$)	CD4/ CD8 (%)	VB12 (%)	MART-1 cells infused ($\times 10^{-9}$) [‡]	Days in culture	Doubling time (days) [†]	IL-2 doses [§]	Sites of evaluable disease	Response (duration in months)//
1	1	28/M	11.0	27/73	67	7.4	19	8.7	7	Ln, Cu	NR
	2a	44/F	13.0	3/95	64	8.3	19	11.9	10	Ln, Cu	NR
2	3	58/M	14.0	17/82	35	4.9	19	10.0	11	Cu, Sub	NR
	4	52/M	1.0	50/50	42	0.5	8	1.4	9	Li, Sub	PR(21)
	5	50/M	12.0	18/82	17	2.2	8	1.0	7	Lu, Ln, Sub	NR
	6	55/F	7.0	37/72	51	3.6	7	1.3	8	Lu, Ln	NR
	7	56/M	9.0	75/21	40	3.6	7	1.0	5	Lu, Ln	NR
	8	37/M	6.1	68/40	32	1.9	7	1.3	12	Lu, Ln	NR
	9	55/M	4.2	72/24	41	1.7	7	2.0	9	Ln, Ad, Sub	MR
	10	45/M	8.6	53/30	34	2.9	6	0.6	5	Ln, Sub	NR
	11	45/M	6.3	7/92	45	2.8	6	0.8	5	Lu, Pa, Ln	NR
	12	32/F	4.7	30/60	61	2.9	6	0.7	5	Br, Sub	NR
3	13	41/M	7.7	40/67	42	3.2	6	0.9	7	Lu, Sub	NR
	2b	44/F	2.1	30/59	53	1.1	6	1.9	14	Ln, Cu	NR
	14	30/M	86	11/60	40	34.4	18+9	0.9	5	Hi	PR(20)
	15	51/M	38	16/82	45	17.1	18+9	3.3	8	Lu	NR
	16	25/F	33	13/76	21	6.9	18+9	1.2	2	Lu, Li, Sub	NR
	17	20/F	23	17/78	30	6.9	17+8	1.1	3	Lu, Ln, Sub	NR

* This patient was treated twice; treatments were separated by 7 months.

[†] Determined based on cell counts in the 2 days before infusion.

[‡] Total cells infused multiplied by % VB12.

[§] 720,000 international units/kg every 8 hours. All patients were previously refractory to treatment with IL-2 alone.

// Based on RECIST criteria.