VOLUME 24 · NUMBER 31 · NOVEMBER 1 2006

JOURNAL OF CLINICAL ONCOLOGY

ORIGINAL REPORT

Phase I Study of Adoptive T-Cell Therapy Using Antigen-Specific CD8$^+$ T Cells for the Treatment of Patients With Metastatic Melanoma

Andreas Mackensen, Norbert Meidenbauer, Sandra Vogl, Monika Laumer, Jana Berger, and Reinhard Andreesen

From the Department of Hematology/Oncology, University of Regensburg, Regensburg, Germany.

Submitted May 5, 2006; accepted August 29, 2006.

Supported in part by the German José Carreras Leukemia Foundation (DJCLS H99-066) and Deutsche Krebshilfe (70-3069).

Authors' disclosures of potential conflicts of interest and author contributions are found at the end of this article.

Address reprint requests to Andreas Mackensen, MD, Department of Hematology/Oncology, University of Regensburg, Franz-Josef-Strauss-Allee 11, D-93042 Regensburg, Germany; e-mail: andreas.mackensen@klinik.uni-regensburg.de.

0732-183X/06/2431-5060/$20.00

DOI: 10.1200/JCO.2006.07.1100

ABSTRACT

Purpose
The adoptive transfer of in vitro generated tumor antigen-specific cytotoxic T lymphocytes (CTL) provides a promising approach to the immunotherapy of cancer. A phase I study was conducted to test the feasibility, safety, and survival of adoptively transferred Melan-A–specific CTL lines in melanoma patients.

Patients and Methods
Eleven HLA-A2$^+$ patients with metastatic melanoma received at least three intravenous infusions of Melan-A–specific CTL at 2-week intervals. CTL were generated by four rounds of in vitro stimulation of purified CD8$^+$ peripheral blood lymphocytes with autologous dendritic cells pulsed with an HLA-A2 binding Melan-A peptide. Each T-cell infusion was accompanied by a 6-day course of low-dose interleukin-2.

Results
A total of 52 T-cell infusions were administered, averaging 2.1 $\times$ 10^8 Melan-A–specific CTL per infusion. Clinical adverse effects were mild and consisted of chills and low-grade fever in seven of 11 patients. Clinical and immunologic responses revealed an antitumor response in three of 11 patients (one complete regression, one partial regression, one mixed response), an elevated frequency of circulating Melan-A tetramer$^+$ T cells up to 2 weeks in all the patients with a maximal frequency of 2% of total CD8$^+$ T cells, an increase in eosinophils to up to 50% in seven of 11 patients, and a selective loss of Melan-A expression in lymph node metastases in two evaluated patients after T-cell transfer.

Conclusion
Our data indicate that the adoptive transfer of antigen-specific T cells in melanoma patients can induce clinical tumor-specific immune responses without major adverse effects.

J Clin Oncol 24:5060-5069. © 2006 by American Society of Clinical Oncology

INTRODUCTION

The identification of tumor-associated antigens (TAAs) recognized by T cells has led to the development of clinical studies, which target malignant cells by augmenting the specific cellular immune response.[1] These approaches include vaccination strategies, in which the TAAs are presented in a potentially immunogenic context to induce antigen-specific T-cell responses in vivo and adoptive cell transfer strategies, in which TAA-reactive T cells are generated ex vivo and then infused to the patient to increase the number of tumor-reactive effector cells in vivo. Successful tumor eradication by adoptively transferred T cells has been demonstrated in murine models,[2] but the translation into the clinical practice has been cumbersome. The proof of principle for adoptive T-cell therapy was its use to control relapse of hematologic diseases and viral infections after allogeneic bone marrow transplantation.[3-5] Recent success of adoptive T-cell therapy using ex vivo expanded autologous tumor-reactive T cells has fuelled optimism that this concept may find a place as a specific therapy for some malignant diseases.[6-8]

In this study of adoptive T-cell therapy we target the melanoma antigen Melan-A,[9,10] which belongs to the group of melanocyte differentiation antigens. Melan-A is an ideal target antigen because of its high immunogenicity and preferential expression on melanoma cells.

We have developed improved methods for the generation of Melan-A specific cytotoxic T lymphocyte (CTL) lines from patients peripheral blood lymphocytes (PBL) and have administered these CTL to patients with melanoma demonstrating that ex vivo generated Melan-A specific CTL can be detected in

Table 1. Patient Demographics and Treatment Characteristics

Patient No.	Age (years)	Sex	KPS (%)	Prior Therapy	Disease Sites	Melan-A Expression*	No. of T-Cell Infusions	Adverse Effects	Eosinophilia (%)†	Clinical Course	Duration of Clinical Course (months)
1	50	F	80	Chemo/immuno	Sk/Lu	3+	3	Fever I°	13	PD	
2	50	M	80	Chemo/immuno	Lu/Li/B/Sk	2+	3	Fever I°	6	PD	
3	39	F	100	Chemo/immuno	Lu/Li/B	3+	3	Fever I°	12	PD	
4	35	M	100	Chemo/immuno	Lu/LN	3+	3	No	No	PD	
5	62	M	100	Chemo/immuno	LN	2+	10	Fever II°, chills	51	PR	11
6	60	M	90	Chemo/immuno	LN	3+	5	Fever II°, chills	23	SD	3
7	58	F	90	Chemo/immuno	LN/Sk	2+	3	No	No	PD	
8	63	M	80	IFN/chemo	Sk	3+	7	Fever II°, chills	26	MR	14
9	41	F	100	IFN/chemo	Li/LN	3+	3	No	No	PD	
10	68	F	90	Chemo/immuno	LN/Sk	3+	3	No	No	PD	
11	59	F	100	IFN/chemo	LN	3+	9	Fever II°, chills	14	CR	24+

Abbreviations: KPS, Karnofsky performance status; F, female; Chemo, chemotherapy; Immuno, immunotherapy; Sk, skin; Lu, lung; Fever I°, WHO grade I, < 38°C; PD, progressive disease; M, male; Li, liver; B, bone; LN, lymph node; Fever II°, WHO grade II, 38-40°C; PR, partial regression; SD, stable disease; MR, mixed response; IFN, interferon; CR, complete regression.

*Staining of tumor specimens was performed with an anti-Melan-A (A103; Novocastra, Newcastle, United Kingdom) monoclonal antibody; 2+, 50-75% of cells reactive; 3+ > 75% of cells reactive.

†Maximum peak eosinophil levels after T-cell transfer; eosinophils % of total leukocytes.

the patients blood after adoptive transfer and localize preferentially to tumor.[11] We now report on the results from a phase I clinical trial of adoptively transferred Melan-A specific CTL in patients with advanced melanoma.

PATIENTS AND METHODS

Patients and Treatment Schedule

Thirteen HLA-A2$^+$ patients with stage IV melanoma, who were refractory to standard therapeutic regimens, were eligible for treatment. The trial was designed and conducted in accordance with the Declaration of Helsinki. The therapy protocol was approved by the institutional ethics committee and registered with the regulatory state authority. All patients gave written informed consent before enrolling onto the study. HLA typing of peripheral blood mononuclear cells (PBMC) was performed at the University of Regensburg (Regensburg, Germany) HLA typing laboratory. Eosinophil levels were determined using established standards at the University of Regensburg clinical laboratory.

Eligible HLA-A2$^+$ patients received at least three intravenous infusions of Melan-A–specific CTL suspended in 100 mL of 5% human serum albumin at 2-week intervals. Each T-cell infusion was accompanied by a 6-day course of subcutaneous interleukin-2 (IL-2; 3×10^6 U daily).

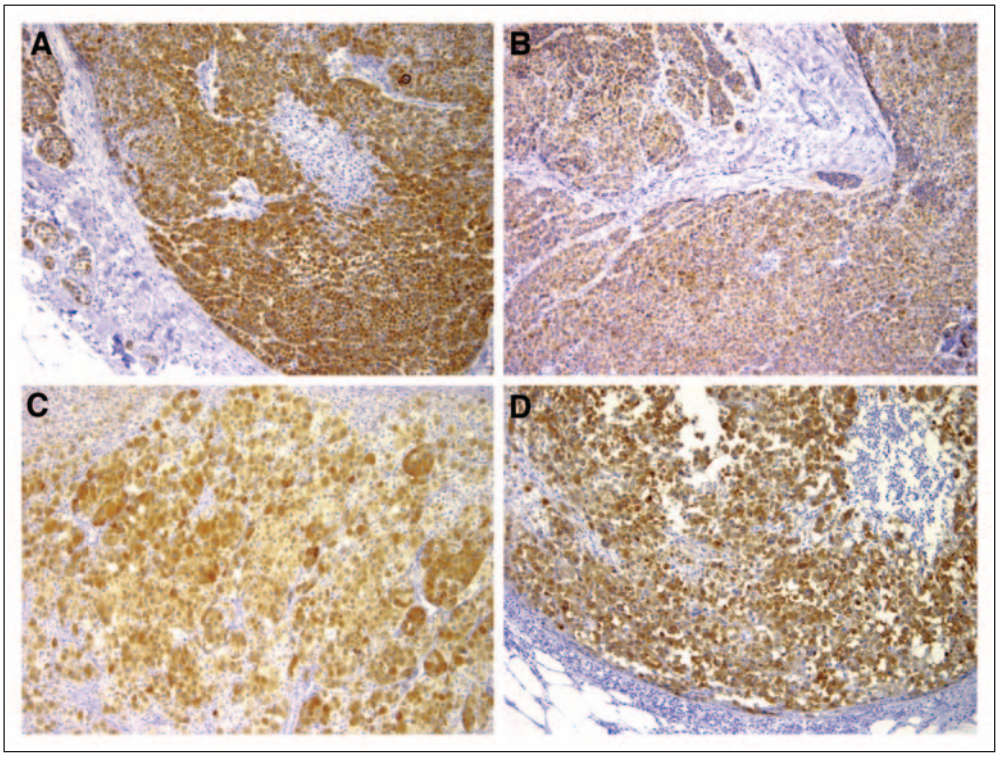

Fig 1. Immunohistochemic analysis of Melan-A expression in metastases from melanoma patients 1 (A), 3 (B), 4 (C), and 6 (D). Staining of formalin-embedded tumor specimens was performed with an anti-Melan-A (A103 Novocastra, Newcastle, United Kingdom) monoclonal antibody. Representative fields of the tissue sections are shown.

Table 2. Numbers of Transferred Melan-A-Specific T Cells and Pre- and Postlevels of Melan-A Tetramer$^+$ T Cells Within Total PBL

Patient No./ No. of T-Cell Transfer	No. of Transferred Ag-Specific T Cells ($\times 10^8$)*	Melan-A TM$^+$ T Cells Within Total CD8$^+$ PBL (%)†	
		Pre	Post
1			
1	0.15	0.01	0.07
2	0.25	0.16	0.58
3	0.35	0.21	0.27
2			
1	7.96	0.04	0.74
2	13.10	0.17	1.77
3	11.87	0.13	1.59
3			
1	1.93	0.07	0.44
2	1.34	0.07	0.13
3	1.26	0.08	0.11
4			
1	1.26	0.03	0.12
2	5.53	0.04	0.43
3	4.44	0.06	0.39
5			
1	1.29	0.03	0.38
2	1.40	0.08	0.50
3	0.92	0.04	0.28
4	0.34	ND	ND
5	0.28	0.04	ND
6	1.20	0.04	0.40
7	0.43	0.07	ND
8	0.94	0.10	ND
9	0.70	0.08	ND
10	0.64	0.10	0.38
6			
1	1.03	0.10	0.40
2	1.22	0.19	0.44
3	0.56	0.15	0.22
4	0.78	ND	ND
5	0.87	ND	ND
7			
1	0.11	0.04	0.08
2	0.13	0.09	0.12
3	0.21	ND	ND
8			
1	4.98	0.01	0.04
2	1.75	0.16	ND
3	2.05	0.19	0.24
4	1.91	0.18	ND
5	1.88	0.20	ND
6	1.82	0.19	ND
7	1.73	0.24	ND
9			
1	3.92	0.15	0.34
2	7.15	0.30	0.64
3	4.54	0.79	ND
10			
1	0.79	ND	ND
2	0.52	ND	ND
3	0.91	ND	ND
11			
1	1.32	0.03	0.13
2	1.36	0.08	0.25
3	0.79	0.15	0.31
4	1.30	0.26	ND
5	0.93	0.28	ND
6	1.94	0.30	ND
7	1.45	0.31	ND
8	1.66	ND	ND
9	2.45	ND	ND

Abbreviations: PBL, peripheral blood lymphocytes; ND, not determined; TM, tetramer.

*Numbers express the number of total CD8$^+$ Melan-A tetramer$^+$ T cells infused.

†Numbers represent percentages of Melan-A tetramer$^+$CD8$^+$ cells within total CD8$^+$ PBL before (pre) or maximal levels after (post) T-cell transfer. Maximal levels were observed between 24 hours and 6 days after T-cell infusion.

Tumor response was defined according to WHO criteria. Patient response was assessed using computed tomography (CT) or positron emission tomography (PET) scan studies at 2 weeks after the third T-cell transfer and at regular intervals thereafter. Adoptive T-cell therapy was continued at 4-week intervals in those patients who had at least stable disease or objective response after therapy. For subcutaneous melanoma, a mixed response (ie, complete regression of one target lesion while one other progresses), although formally classified as stable or progressive disease in the WHO classification, was documented as well.

Immune monitoring was performed before T-cell transfer and at least at 1, 6, 24, and 48 hours after T-cell infusion. In some patients additional blood samples were obtained at various time points. PBMC were isolated by Ficoll density gradient centrifugation.

Generation of Melan-A–Specific CTL Lines From PBL

Melan-A–specific CTL lines were generated as previously described.[11,12] PBMC were obtained by leukapheresis and CD8$^+$ T cells, purified by magnetic separation, stimulated with mature dendritic cells (DC) pulsed with the HLA-A2-restricted modified Melan-A peptide (Melan-A$_{26\text{-}35L}$; ELAGIGILTV; Clinalfa AG, Laeufelingen, Switzerland) and human β_2-microglobulin (10 μg/mL) at a responder to stimulator ratio of 5:1 to 10:1. CTL lines were cultured in complete medium, containing 10% human AB serum and 1% to 2% T-cell growth factor.[12,13] After four cycles of stimulation, phenotypic and functional analysis of T cells was performed.

Immunophenotyping and Peptide Major Histocompatibility Complex Tetramer Staining

Surface marker analysis of CTL lines and DC was performed using a FACScalibur (Becton Dickinson, San Jose, CA) and the CellQuest software (Becton Dickinson). Fluorescence conjugated monoclonal antibodies (mAbs) were purchased from Becton Dickinson. To detect Melan-A–specific T cells in the CTL lines and PBMC, phycoerythrin (PE) -labeled HLA-A2-tetramer (TM) that had been folded around ELAGIGILTV (Melan-A$_{26\text{-}35L}$) or SLYNTVATL (HIVgag) were prepared by Beckman Coulter (Fullerton, CA). For combined TM and annexin-V staining, annexin-V-FITC (PharMingen, San Diego, CA) was used.

Chromium Release Assay

The cytotoxic activity of T cells was measured by a 4 hours ^{51}chrom (Cr) release assay as described.[11] Effector-target ratios were calculated based on the number of Melan-A-TM$^+$ T cells in the effector population in order to accurately compare different T-cell populations. ^{51}Cr-labeled target cells were either T2 cells (HLA-A2$^+$/TAP$-$/$-$) pulsed with the natural Melan-A$_{26\text{-}35}$ or

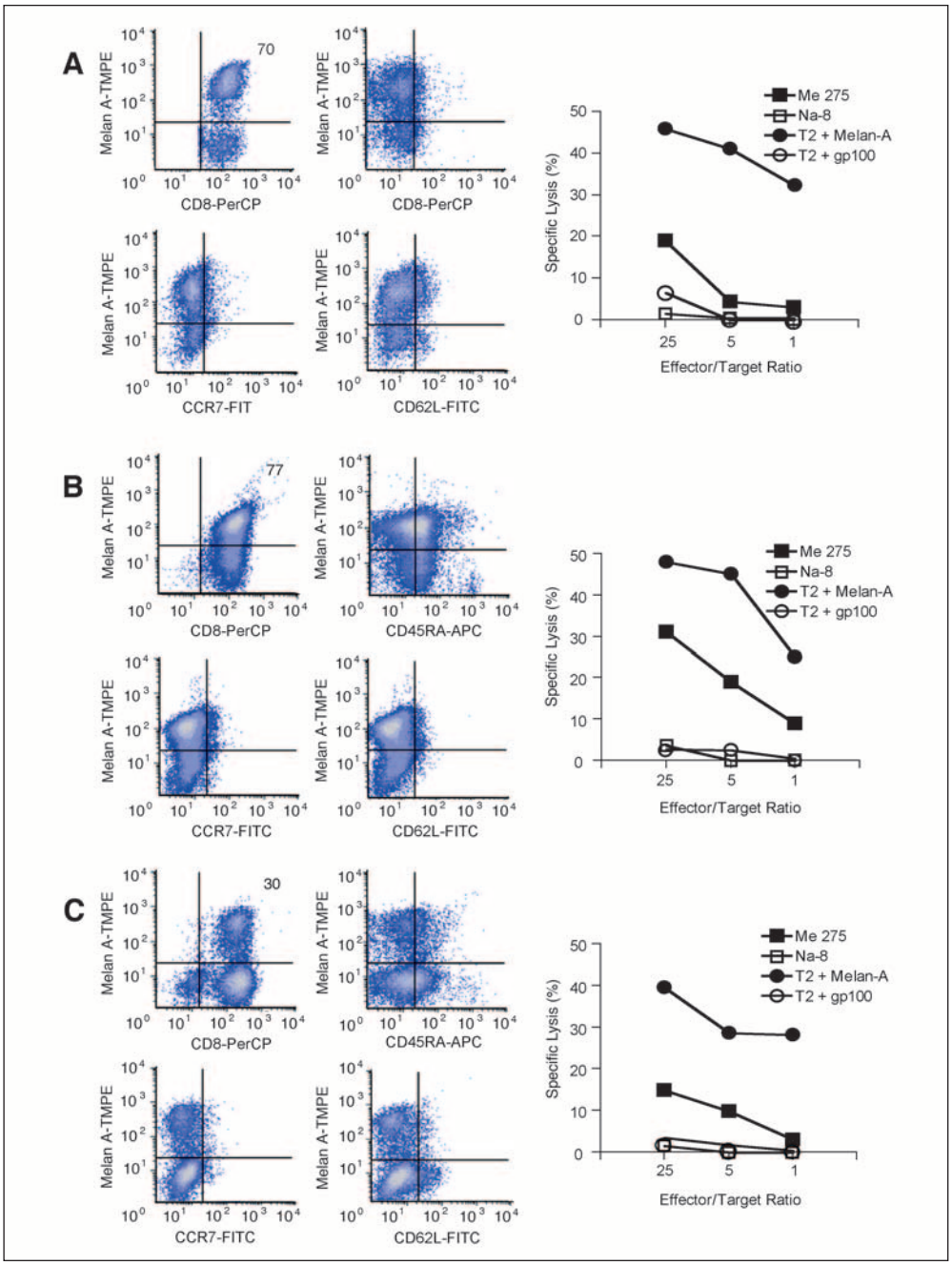

Fig 2. Functional analyses of Melan-A-specific cytotoxic T lymphocytes (CTL). (Left panels) Cells were stained with Melan-A-TM and different T-cell monoclonal antibodies. Dot plots show gated $CD3^+$ lymphocytes. Numbers in the upper right quadrants represent percent Melan-A-$TM^+/CD8^+$ cells within total $CD8^+$ CTL. (Right panels) Cytotoxic activity against Melan-A_{26-35} or $gp100_{280-288}$ peptide-pulsed T2 cells and allogeneic (Me275, HLA-$A2^+$/Melan-A^+; Na-8, HLA-$A2^+$/Melan-A^-) melanoma cells. (A) Patient 8; (B) patient 9; and (C) patient 11.

control $gp100_{280-288}$ peptide (Clinalfa), or the allogeneic melanoma cell lines Na-8-MEL (HLA-$A2^+$, Melan-A^-) and Me275 (HLA-$A2^+$, Melan-A^+).

Immunohistochemical Staining

For detection of Melan-A and anti-gp100 expression in formalin-fixed melanoma tissue embedded in paraffin, the anti-Melan-A (A103; Novocastra, Newcastle, United Kingdom) and antigp100 (HMB45; Dako, Hamburg, Germany) mAbs were used. Bound mAb was detected using the diaminobenzidine method and counterstained with hematoxylin.

Statistical Analysis

Correlation between the total number of transferred Melan-A-TM^+ CTL and the frequency of circulating Melan-A-TM^+ T cells within $CD8^+$ PBL was done using Spearman nonparametric rank correlation. Only correlations such that r_s more than 0.70 would be interpreted as being strong correlations.

RESULTS

Patient Characteristics

Eleven of 13 patients with refractory malignant melanoma completed at least three adoptive T-cell infusions and were assessable. One patient was withdrawn because of rapid disease progression, in one patient (number 12) we were unable to generate sufficient amounts of Melan-A specific CTL. The demographic and clinical characteristics of this patient population is shown in Table 1. All patients had extensive metastatic disease with lung and/or liver (patients 1 to 4 and 8), bone (patients 2 and 3), lymph node (LN; patients 1, 4 to 6, and 8), and skin metastases (patients 1, 2, and 7);

the majority had undergone multiple previous therapies including surgery, chemotherapy, chemoimmunotherapy, or high-dose interferon. Autologous Melan-A–specific CTL lines, generated in vitro, were injected intravenously through a peripheral venous catheter. Patients received at least three but up to 10 T-cell infusions.

Immunohistochemical analysis of Melan-A expression in tumor biopsies obtained before CTL transfer demonstrated abundant expression of Melan-A in all examined samples (Fig 1 and Table 1; patients 1, 3, 4, and 6).

Characteristics of Melan-A–Specific CTL Adoptively Transferred to Patients

A total of 52 T-cell infusions were administered, and on average 2.11 $\times 10^8$ Melan-A-TM$^+$ T cells (range, 0.11 to 13.1 $\times 10^8$ cells) per infusion. Transferred T cells comprised three to 10 independently expanded CTL cultures per patient. Data on the total number of Melan-A-TM$^+$ T cells infused at each time point are summarized in Table 2.

The frequency of Melan-A–specific T cells as determined by Melan-A-TM staining was 38.3% ± 17.3% (mean ± standard deviation) within total CD8$^+$ lymphocytes. As shown in Figure 2 (for CTL lines from patients 8, 9, and 11), Melan-A–specific T cells consisted of Ag-primed CTL, showing either CD45RA^{low}/CCR7$^-$ terminally differentiated effector cells or CD45RA$^-$/CCR7$^-$ effector memory cells.[14] Melan-A–specific CTL expressed activation markers such as CD95, HLA-DR, and CD69 (data not shown) but were negative for CD62L (Fig 2). Functional analysis of the CTL lines revealed a strong cytotoxic activity against Melan-A-pulsed T2 cells and allogeneic HLA-A2$^+$ Melan-A-expressing melanoma cells (Fig 2).

Adverse Events and Selective Loss of Melan-A Expression in the Tumor After Adoptive CTL Transfer

Clinical adverse effects were usually mild and consisted of chills and fever WHO grade 1 to 2 in seven of 11 patients that typically occurred within 6 to 8 hours post-CTL infusion (Table 1). Patients were monitored for signs and symptoms for autoimmunity because Melan-A is also expressed on melanocytes. No physical signs of autoimmunity were observed in any of the patients. One patient (number 3) developed a local inflammatory response with erythema at the tumor site 6 days after the second T-cell transfer.

The laboratory value with the greatest change from baseline was the peripheral blood eosinophil count. An increase in eosinophils up to 51% was observed in seven of 11 patients (Table 1). Figure 3A shows the sharp increase in the proportion of eosinophils for patient 5 at 24 hours post-CTL transfer. Eosinophil counts were still about two to three times higher 2 weeks after T-cell therapy as compared with the preinfusion value. Eosinophilia has been previously reported with IL-2 treatment regimens[15,16] and is linked primarily to production of IL-5. Significant amounts of IL-5 could be also detected in the supernatant of the transferred CTL product (data not shown) suggesting that the T cells itself could be in part responsible for the eosinophilia.

To analyze whether the T-cell transfer combined with IL-2 administration alters the distribution of lymphocyte subpopulations in patients PBL, we performed flow cytometric analysis in four melanoma patients before each transfer. No changes in total lymphocyte counts could be observed after therapy. In two of four patients, the percentage of CD3$^-$/CD16$^+$/CD56$^+$ natural killer (NK) cells increased from 9% to 24% and from 5% to 19% of total

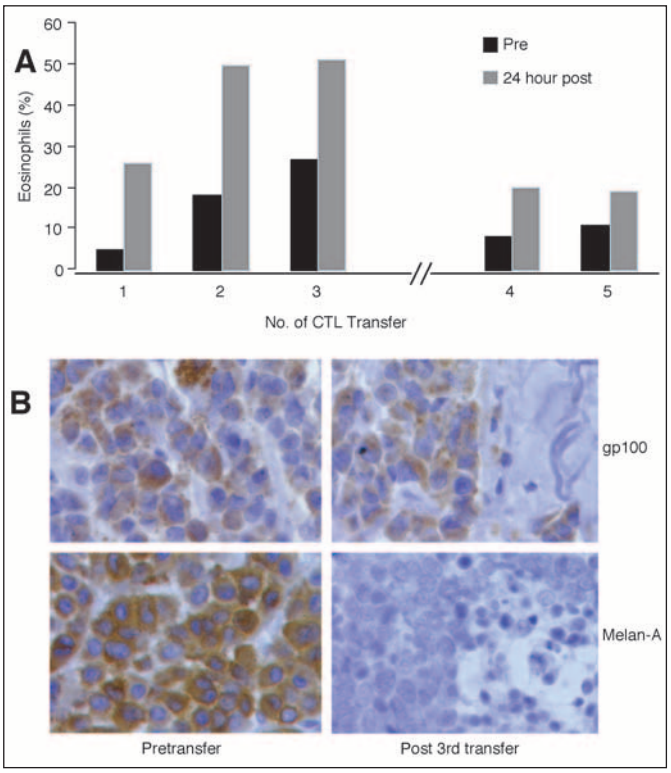

Fig 3. Eosinophila and loss of Melan-A expression in the tumor after T-cell therapy. (A) Percent eosinophils pre- and 24 hours post-T-cell infusion. Between the third and fourth transfer the therapy was discontinued for 4 weeks. (B) Melan-A and gp100 expression in a metastatic lymph node obtained from the same site before first and after third T-cell transfer.

lymphocytes, respectively (data not shown), and a slight increase of CD3$^+$/CD16$^+$/CD56$^+$ NK-T cells was observed. The T-cell therapy did not influence the percentage of circulating CD4$^+$CD25^{high} T regulatory cells.

For two patients, Melan-A expression by tumor cells was evaluated in LN metastases obtained before and after third T-cell transfer. In both patients the preinfusion tumor revealed a high expression of Melan-A, while the postinfusion metastases showed a selective loss of the Melan-A protein (Fig 3B). Expression of the gp100 protein was not affected by Melan-A–specific CTL.

In Vivo Frequency and Persistence of Transfused Melan-A–Specific CTL

The frequency and persistence of transferred Melan-A–specific CTL was determined in serial PBL samples from 10 treated patients using Melan-A TM. All patients showed an increase of circulating Melan-A-TM$^+$ CD8$^+$ T cells (mean, 10.8-fold; range, two- to 43-fold) and a peak Melan-A–specific T-cell frequency 48 hours after transfer, comprising on average 0.67% of total peripheral blood CD8$^+$ cells (range, 0.03% to 1.77%). The pre- and peak postinfusion levels of circulating Melan-A TM$^+$ CD8$^+$ T cells after each T-cell transfer are summarized in Table 2.

In two patients, additional blood samples were collected at 6 days post-T-cell infusion and revealed the highest frequency of circulating Melan-A–specific CTL (Fig 4A). Since the frequency had decreased at 14 days after transfer, these findings suggest the disappearance of the transfused T cells from the peripheral blood mainly in the second week.

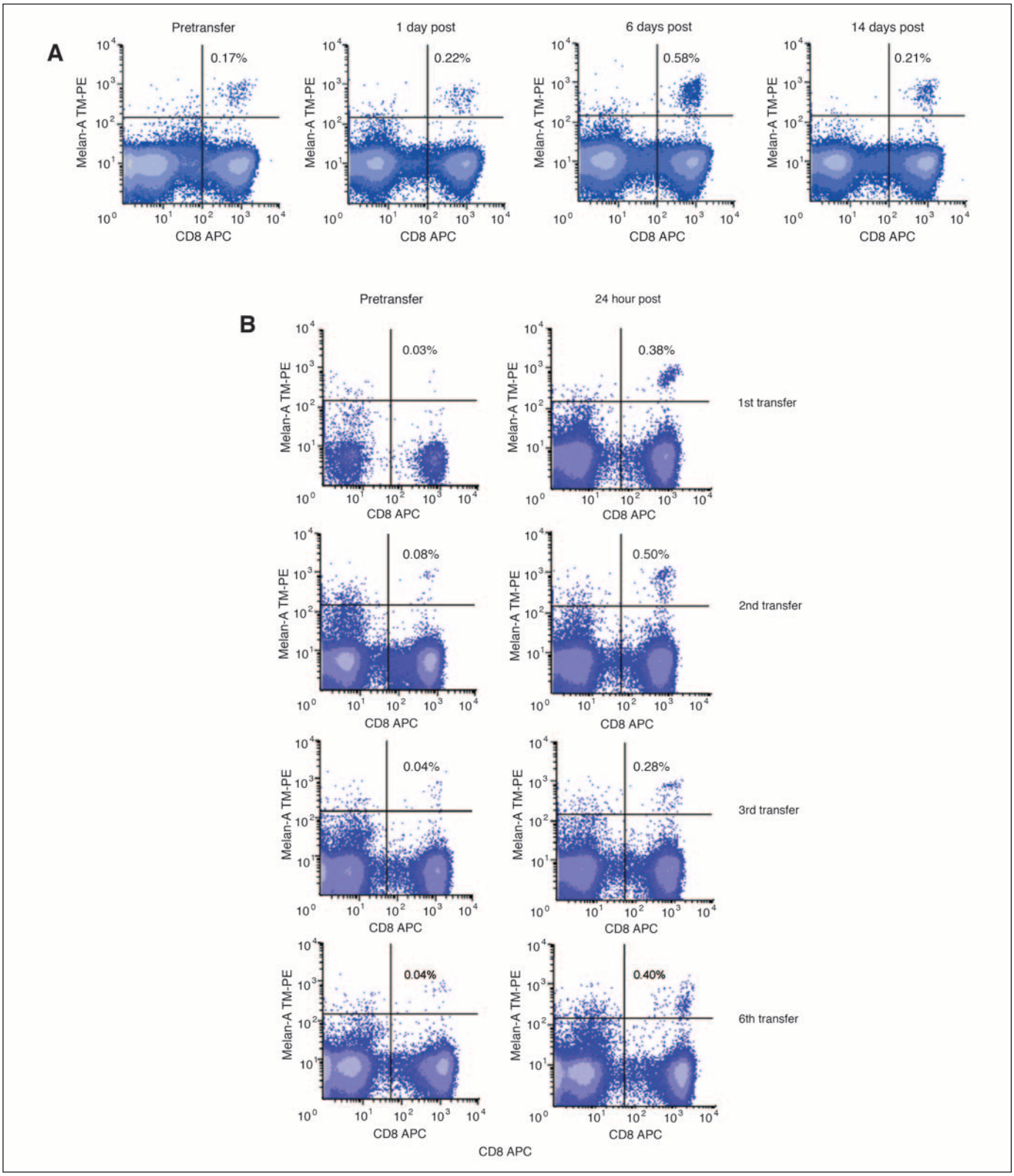

Fig 4. Long-term survival of transferred Melan-A-specific cytotoxic T lymphocytes (CTL). (A, B, and C) Peripheral blood mononuclear cells were stained with Melan-A-TM/anti-CD8 plus Annexin-V/anti-CD3 (C). Numbers in the upper right quadrants represent (A and B) percent Melan-A-TM$^+$/CD8$^+$ cells within CD8$^+$ PBL or (C) percent annexin-V$^-$/CD3$^+$ cells within CD8$^+$/Melan-A-TM$^+$ peripheral blood lymphocytes. (D) Correlation between numbers of transferred Melan-A-TM$^+$ CTL and the frequency of circulating Melan-A-TM$^+$ cells. (continued on following page)

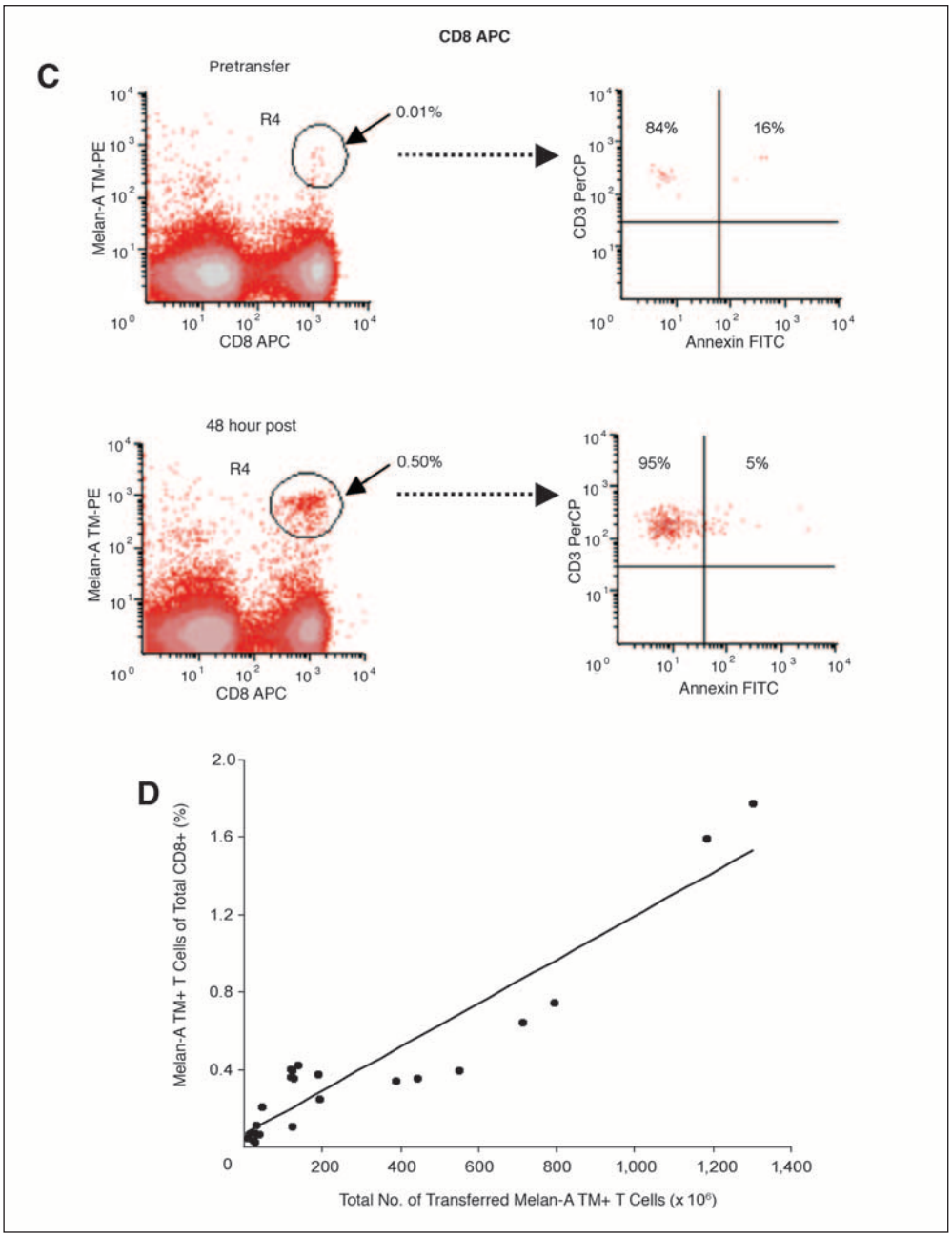

Fig 4. (continued)

There was a strong correlation between the total number of transferred Melan-A TM^+ CTL and the peak frequency of circulating Melan-A TM^+ T cells (Fig 4D; $r_s = 0.857$; $P < .0001$). Repetitive CTL infusions revealed an identical pattern in the frequencies of Melan-A TM^+ T cells at 24 hours post-T-cell infusion as demonstrated for patient 5 up to the sixth transfer (Fig 4B).

Annexin-V staining of Melan-A TM/CD8-gated T cells obtained from PBMC of four patients before, 6 hours, and 48 hours post-T-cell infusion demonstrated that the transferred T cells do not undergo apoptosis within 48 hours post-transfer (Fig 4C).

Clinical Response

All patients had progressive disease before inclusion onto the study. Three of 11 patients experienced objective clinical responses to T-cell transfusion (one complete response [24 months+], one partial response [11 months], one mixed response with complete regression of one metastatic lesion [14 months] but progressive disease of one other metastasis; Table 1). Regression of melanoma lesions after T-cell transfer was seen exclusively in subcutaneous and lymphatic, but not in visceral metastases. The three responders are documented in Figure 5 either by PET or CT scan, showing a complete regression of abdominal LN metastases in patient 11 (Fig 5A), a mixed response of subcutaneous metastases in patient 8 (Fig 5B), and a partial response of a paratracheal LN metastasis in patient 5 (Fig 5C). PET results could be confirmed either by CT (online only Appendix Fig A1 for patient 11) or by physical examination (patient 8).

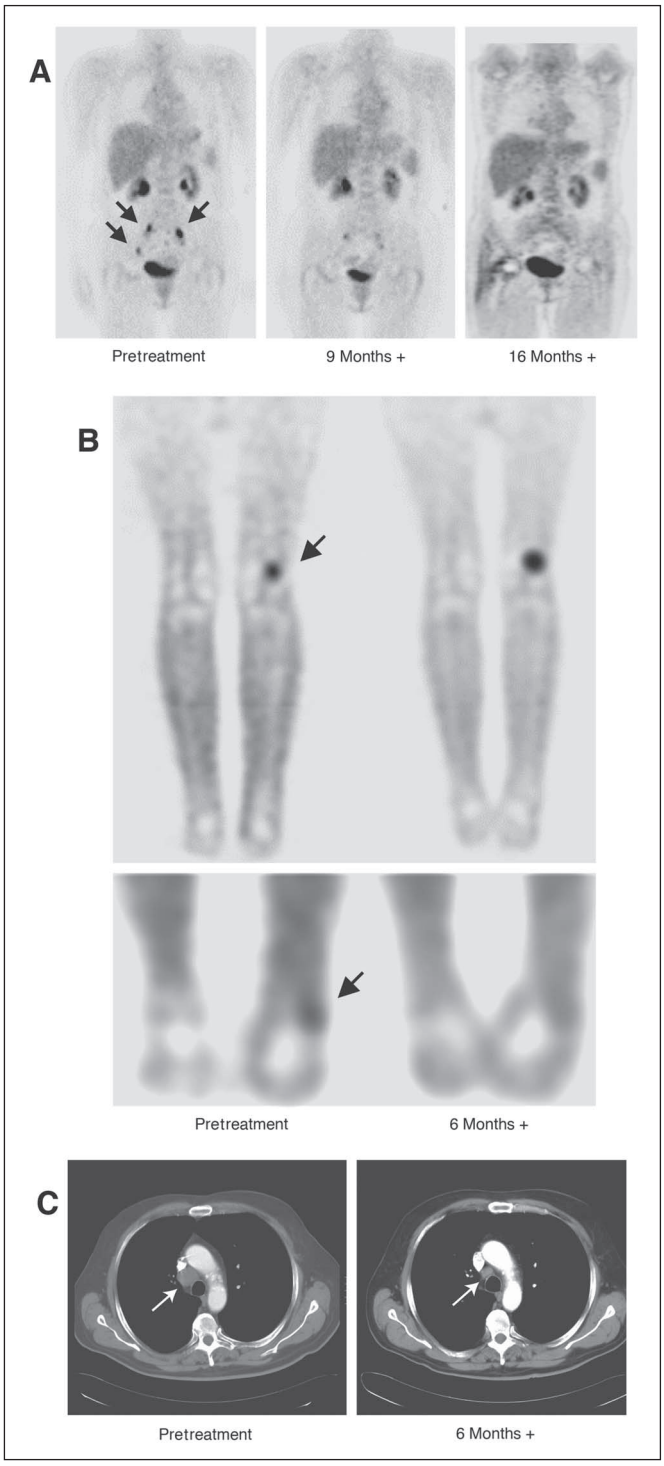

Fig 5. T-cell transfer-induced regression of metastatic disease. Positron emission tomography/computed tomography images showing (A) complete regression (CR) of abdominal lymph node (LN) metastases in patient 11; (B) CR of one subcutaneous metastasis at the left foot but progressive lesion at the left poplitea in patient 8; and (C) partial regression of a paratracheal LN metastasis in patient 5 after T-cell therapy.

Defective DC Differentiation in a Melanoma Patient Prevents In Vitro Induction of TAA Specific CTL

In one patient (patient 12) we were unable to generate high amounts of Melan-A–specific CTL (frequency between 0.15% to 2.5% Melan-A-TM$^+$ T cells after four rounds of in vitro stimulation). We therefore asked whether the antigen presenting cells in this patient may be functionally defective and studied the phenotype and function of mature DC derived from monocytes of this particular HLA-A2$^+$ melanoma patient and two HLA-A2$^+$ healthy donors. These data, summarized in the online only Appendix, revealed a defective DC function caused by abnormal differentiation of the cells.

DISCUSSION

The observation that activated T cells are responsible for the rejection of tumors in rodent models has led to the development of cellular immunotherapies for the treatment of cancer patients based on stimulating T-cell reactivity against TAAs. The adoptive transfer of ex vivo expanded immune effector cells has several advantages compared with active immunization and can overcome many of the tumor immune escape mechanisms such as production of immunosuppressive factors[17] or expression of coinhibitory molecules by the tumor.[18]

This phase I study demonstrates, that adoptively transferred antigen-specific CTL lines, recognizing the Melan-A antigen, do survive in vivo for several weeks, traffic to tumor sites, and can induce a clinical response in metastatic melanoma patients. The following conclusions emerge from this study, which included 52 T-cell infusions in 11 melanoma patients: sufficient numbers of antigen-specific CTL can be generated from melanoma patients by four rounds of in vitro stimulation of purified CD8$^+$ PBL with peptide-pulsed DC; adoptive Melan-A–specific T-cell therapy is possible without major adverse effects or signs of autoimmune disease; and clinical antitumor responses were observed in three of 11 patients, with regression of LN and subcutaneous metastases.

One of the greatest challenges for adoptive T-cell immunotherapy is currently the generation of large numbers of antigen-specific CTL after short-term culture. Our study is different from most other adoptive T-cell therapies with respect to the T-cell product: the T cells used in the National Institutes of Health study presented by Dudley and colleagues, [8] were derived from tumor-infiltrating lymphocytes and were rapidly expanded in vitro with CD3-specific mAb and high doses of IL-2 in the presence of allogeneic PBMCs. In three other clinical studies tumor-reactive T-cell clones, derived either from patient's PBL or tumor-infiltrating lymphocytes, were transferred.[6,19,20] In contrast, we used antigen-specific T-cell lines isolated from patient's PBL by short-term in vitro stimulation with autologous antigen-loaded DC. These independently stimulated CTL cultures, that can be expanded up to 400-fold in a 3-week period, were freshly prepared for each individual T-cell transfer without any freezing of the T-cell product. Cytokines, such as IL-2, acting together with signals through the T-cell receptor and costimulatory signals, can function as accelerators or brakes for T-cell proliferation and differentiation.[21] Recent studies have demonstrated that IL-2 and other common gamma-chain receptor cytokines, including IL-7 and IL-15, act at different stages of the immune response to promote proliferation and survival.[22] We used a T-cell growth factor for in vitro stimulation of antigen-specific T-cell lines, that was isolated from stimulated PBMCs, and mainly consists of IL-2, IL-4, tumor necrosis factor-α, and interferon-γ (A. Mackensen, unpublished observations). Further studies are warranted to demonstrate that this cytokine combination is more effective in generating large numbers of antigen-specific CTL than IL-2, IL-7, and/or IL-15.

The toxicities observed in our T-cell study included the expected adverse effects of low-dose IL-2 such as fever and chills, but no serious toxicities associated with high-dose IL-2, such as vascular leakage syndrome.[8] Despite the high frequencies of circulating Melan-A–specific CTL in up to 2% of total $CD8^+$ T cells, no autoimmunity directed against normal melanocytes, such as vitiligo, was observed in our patients.

The persistence of the transferred CTL and their migration to the tumor is crucial for the success of adoptive immunotherapy. Similar to the study of Yee et al,[6] low-dose IL-2 was administered in order to prolong survival of the transferred CTL. Maximal frequencies of circulating Melan-A-TM^+ T cells could be observed at day 6 post-T-cell infusion followed by a decrease at day 14, suggesting a coincidence of prolonged T-cell survival and IL-2 treatment after CTL transfer. Recent studies have shown that the administration of a lymphodepleting chemotherapy can augment the survival and in vivo expansion of adoptively transferred CTL.[8,23] Studies in mice suggested that lymphodepletion before adoptive T-cell transfer works in two ways: the depletion of host cells creates empty space, which the transferred cells fill by homeostatic proliferation. The depletion also rids the host of regulatory T cells, which would otherwise dampen the function of transferred tumor-specific T cells. Recently, Gattinoni et al[24] have shown that lymphodepletion removes endogenous cellular elements that act as sinks for IL-7 and IL-15 required for augmenting T-cell functionality.

Recent studies also suggest that the telomere length of the transferred T cells correlates with in vivo T-cell persistence after adoptive transfer.[25] Senescence of long-term expanded cells might be a specific problem for T-cell therapy because the polyclonal expansion of adult T cells in vitro is limited to 30 to 40 cell divisions due to loss of telomeres. This might explain the shorter survival of transferred T-cell clones[19] compared with T-cell lines used in our study.

Specific migration of PBL-derived TAA-specific CTLs to tumor sites has been shown previously in our ^{111}In labeling studies.[11] These data are supported by the selective downregulation of Melan-A protein in the tumor after repetitive T-cell transfer, suggesting a strong pressure of the transferred Melan-A–specific CTL on the tumor cells after entering the tumor.

In summary, we demonstrate that the transfer of antigen-specific CTL directed against Melan-A is safe, and that the CTL lines persist in vivo. They migrate to tumor sites and mediate tumor-specific immunity in some patients. Although the results show that our adoptively transferred T cells can induce even complete tumor responses, they also reveal limitations of this approach, as it is limited to patients expressing HLA alleles for which TAA-encoded peptides are available. The selective loss of the target antigen Melan-A after repetitive T-cell transfer illustrates that multiple TAAs should be targeted in future clinical trials. Adoptive transfer of antigen-specific CTL after in vitro stimulation with antigen-pulsed DC is a promising approach for a targeted therapy of patients with malignant diseases.

REFERENCES

1. Van den Eynde BJ, van der BP: T cell defined tumor antigens. Curr Opin Immunol 9:684-693, 1997
2. Eberlein TJ, Rosenstein M, Rosenberg SA: Regression of a disseminated syngeneic solid tumor by systemic transfer of lymphoid cells expanded in interleukin 2. J Exp Med 156:385-397, 1982
3. Kolb HJ, Mittermuller J, Clemm C, et al: Donor leukocyte transfusions for treatment of recurrent chronic myelogenous leukemia in marrow transplant patients. Blood 76:2462-2465, 1990
4. Walter EA, Greenberg PD, Gilbert MJ, et al: Reconstitution of cellular immunity against cytomegalovirus in recipients of allogeneic bone marrow by transfer of T-cell clones from the donor. N Engl J Med 333:1038-1044, 1995
5. Papadopoulos EB, Ladanyi M, Emanuel D, et al: Infusions of donor leukocytes to treat Epstein-Barr virus-associated lymphoproliferative disorders after allogeneic bone marrow transplantation. N Engl J Med 330:1185-1191, 1994
6. Yee C, Thompson JA, Byrd D, et al: Adoptive T cell therapy using antigen-specific CD8+ T cell clones for the treatment of patients with metastatic melanoma: In vivo persistence, migration, and antitumor effect of transferred T cells. Proc Natl Acad Sci U S A 99:16168-16173, 2002
7. Bollard CM, Aguilar L, Straathof KC, et al: Cytotoxic T lymphocyte therapy for Epstein-Barr virus+ Hodgkin's disease. J Exp Med 200:1623-1633, 2004
8. Dudley ME, Wunderlich JR, Yang JC, et al: Adoptive cell transfer therapy following nonmyeloablative but lymphodepleting chemotherapy for the treatment of patients with refractory metastatic melanoma. J Clin Oncol 23:2346-2357, 2005
9. Coulie PG, Brichard V, Van Pel A, et al: A new gene coding for a differentiation antigen recognized by autologous cytolytic T lymphocytes on HLA-A2 melanomas. J Exp Med 180:35-42, 1994
10. Kawakami Y, Eliyahu S, Delgado CH, et al: Cloning of the gene coding for a shared human melanoma antigen recognized by autologous T cells infiltrating into tumor. Proc Natl Acad Sci U S A 91:3515-3519, 1994
11. Meidenbauer N, Marienhagen J, Laumer M, et al: Survival and tumor localization of adoptively transferred Melan-A- specific T cells in melanoma patients. J Immunol 170:2161-2169, 2003
12. Oelke M, Moehrle U, Chen JL, et al: Generation and purification of CD8+ Melan-A-specific cytotoxic T lymphocytes for adoptive transfer in tumor immunotherapy. Clin Cancer Res 6:1997-2005, 2000
13. Mackensen A, Carcelain G, Viel S, et al: Direct evidence to support the immunosurveillance concept in a human regressive melanoma. J Clin Invest 93:1397-1402, 1994
14. Sallusto F, Lenig D, Forster R, et al: Two subsets of memory T lymphocytes with distinct homing potentials and effector functions. Nature 401:708-712, 1999
15. Farace F, Angevin E, Dietrich PY, et al: Low-dose IL-2 treatment: Activation of discrete T- and NK-cell sub-populations in vivo. Int J Cancer 62:523-528, 1995
16. Woodson EM, Chianese-Bullock KA, Wiernasz CJ, et al: Assessment of the toxicities of systemic low-dose interleukin-2 administered in conjunction with a melanoma peptide vaccine. J Immunother 27:380-388, 2004
17. Dudley ME, Rosenberg SA: Adoptive-cell-transfer therapy for the treatment of patients with cancer. Nat Rev Cancer 3:666-675, 2003
18. Blank C, Gajewski TF, Mackensen A: Interaction of PD-L1 on tumor cells with PD-1 on tumor-specific T cells as a mechanism of immune evasion: Implications for tumor immunotherapy. Cancer Immunol Immunother 54:307-314, 2005
19. Dudley ME, Wunderlich J, Nishimura MI, et al: Adoptive transfer of cloned melanoma-reactive T lymphocytes for the treatment of patients with metastatic melanoma. J Immunother 24:363-373, 2001
20. Vignard V, Lemercier B, Lim A, et al: Adoptive transfer of tumor-reactive Melan-A-specific CTL clones in melanoma patients is followed by increased frequencies of additional Melan-A-specific T cells. J Immunol 175:4797-4805, 2005
21. Gattinoni L, Powell DJ Jr, Rosenberg SA, et al: Adoptive immunotherapy of cancer: Building on success. Nat Rev Immunol 6:383-393, 2006
22. Schluns KS, Lefrancois L: Cytokine control of memory T-cell development and survival. Nat Rev Immunol 3:269-279, 2003
23. Robbins PF, Dudley ME, Wunderlich J, et al: Cutting edge: Persistence of transferred lymphocyte clonotypes correlates with cancer regression in patients receiving cell transfer therapy. J Immunol 173:7125-7130, 2004
24. Gattinoni L, Finkelstein SE, Klebanoff CA, et al: Removal of homeostatic cytokine sinks by lymphodepletion enhances the efficacy of adoptively transferred tumor-specific CD8+ T cells. J Exp Med 202:907-912, 2005
25. Zhou J, Shen X, Huang J, et al: Telomere length of transferred lymphocytes correlates with in vivo persistence and tumor regression in melanoma patients receiving cell transfer therapy. J Immunol 175:7046-7052, 2005
26. Oelke M, Maus MV, Didiano D, et al: Ex vivo induction and expansion of antigen-specific cytotoxic T cells by HLA-Ig-coated artificial antigen-presenting cells. Nat Med 9:619-625, 2003

Acknowledgment

We are grateful to all the patients who took part in this study.

Appendix

The Appendix is included in the full-text version of this article, available online at www.jco.org. It is not included in the PDF version (via Adobe® Reader®).

Authors' Disclosures of Potential Conflicts of Interest

The authors indicated no potential conflicts of interest.

Author Contributions

Conception and design: Andreas Mackensen, Norbert Meidenbauer, Reinhard Andreesen
Financial support: Reinhard Andreesen
Administrative support: Reinhard Andreesen
Collection and assembly of data: Andreas Mackensen, Norbert Meidenbauer, Sandra Vogl, Monika Laumer, Jana Berger
Data analysis and interpretation: Andreas Mackensen, Norbert Meidenbauer, Sandra Vogl, Monika Laumer, Jana Berger
Manuscript writing: Andreas Mackensen
Final approval of manuscript: Andreas Mackensen, Norbert Meidenbauer, Sandra Vogl, Monika Laumer, Jana Berger, Reinhard Andreesen