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See commentary by Heslop, p. 4189

Durable Complete Responses in Heavily Pretreated Patients with Metastatic Melanoma Using T-Cell Transfer Immunotherapy

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Abstract

Purpose: Most treatments for patients with metastatic melanoma have a low rate of complete regression and thus overall survival in these patients is poor. We investigated the ability of adoptive cell transfer utilizing autologous tumor-infiltrating lymphocytes (TIL) to mediate durable complete regressions in heavily pretreated patients with metastatic melanoma.

Experimental Design: Ninety-three patients with measurable metastatic melanoma were treated with the adoptive transfer of autologous TILs administered in conjunction with interleukin-2 following a lymphodepleting preparative regimen on three sequential clinical trials. Ninety-five percent of these patients had progressive disease following a prior systemic treatment. Median potential follow-up was 62 months.

Results: Objective response rates by Response Evaluation Criteria in Solid Tumors (RECIST) in the 3 trials using lymphodepleting preparative regimens (chemotherapy alone or with 2 or 12 Gy irradiation) were 49%, 52%, and 72%, respectively. Twenty of the 93 patients (22%) achieved a complete tumor regression, and 19 have ongoing complete regressions beyond 3 years. The actuarial 3- and 5-year survival rates for the entire group were 36% and 29%, respectively, but for the 20 complete responders were 100% and 93%. The likelihood of achieving a complete response was similar regardless of prior therapy. Factors associated with objective response included longer telomeres of the infused cells, the number of $CD8^+CD27^+$ cells infused, and the persistence of the infused cells in the circulation at 1 month (all $P_2 < 0.001$).

Conclusions: Cell transfer therapy with autologous TILs can mediate durable complete responses in patients with metastatic melanoma and has similar efficacy irrespective of prior treatment. *Clin Cancer Res; 17(13); 4550–7. ©2011 AACR.*

Introduction

Patients with metastatic melanoma have a poor prognosis with a 5-year survival rate of about 5% (1). There are 2 Food and Drug Administration (FDA)-approved treatments for these patients. Dacarbazine has an objective response rate of approximately 12%, with complete response rates of 2% to 3% that are often transient (2). Interleukin-2 (IL-2) has an objective response rate of approximately 15% with 4% to 5% durable complete response rates (3). Results of 2 new experimental agents have recently been reported for the treatment of patients with this disease. Ipilimumab, an antibody against the inhibitory lymphocyte receptor CTLA4, mediated a 3.6-month improvement in median survival with an objective response rate of 7% in 540 patients, but only 3 patients (0.6%) achieved a complete regression (4). PLX4032, an inhibitor of mutated *BRAF*, had an objective response rate of 77% in 48 patients, with 3 (6%) complete regressions (5). The very small number of durable complete responses makes it unlikely that many patients with metastatic melanoma will be cured utilizing any of these approaches.

There are several advantages to the use of lymphocyte transfer as an immune-based approach to treat cancer (6). Large numbers of lymphocytes can be selected *in vitro* for high reactivity against tumor antigens and grown under conditions that overcome the tolerizing influences that exist *in vivo*. Perhaps, most importantly, it is possible to modify the host prior to the cell transfer to eliminate immunoregulatory cells and provide an optimal microenvironment for the transferred cells. Previous studies have shown that transfer of cultured lymphocytes with antiviral

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Translational Relevance

This article shows that the adoptive transfer of tumor-infiltrating lymphocytes plus interleukin-2 (IL-2) following a preparative lymphodepleting regimen can lead to durable complete regressions in up to 40% of patients with metastatic melanoma. Of 20 patients, who experienced complete regressions, 19 are ongoing at more than 3 to 7 years. Eighty-six percent of patients had visceral metastases and 95% had been previously recurred after other systemic treatments. Thus, the application of this experimental approach seems capable of providing durable remissions and possible cures in patients with metastatic melanoma.

activity can prevent cytomegalovirus (7) and Epstein–Barr virus (EBV) infections and the subsequent development of posttransplant lymphoproliferative diseases (8). Fresh and cultured lymphocytes have been used to treat relapsed leukemias and lymphomas after allogeneic bone marrow transplantation as well as established EBV-induced lymphomas and nasopharyngeal tumors (8–14). A patient with metastatic melanoma responding to the transfer of cloned CD4 lymphocytes has been reported (15).

We have previously reported early results in patients with metastatic melanoma treated with the adoptive transfer of autologous tumor-infiltrating lymphocytes (TIL) selected for antitumor activity, expanded *in vitro* and reinfused into patients along with IL-2 following a lymphodepleting preparative regimen (16, 17). We now report the definitive analysis of this series with a median potential follow-up of 62 months in 93 patients with metastatic melanoma. These patients all had progressive disease and were heavily pretreated with standard and experimental regimens. Of the 93 treated patients, 52 (56%) had an objective response. Twenty patients (22%) experienced a complete regression, 19 of whom having ongoing complete responses beyond 3 years. It thus seems that for this group of heavily pretreated patients for whom a lesion can be resected to obtain TILs and who received the infusion, a high rate of complete and possibly curative regressions can be achieved.

Materials and Methods

Patients

Patients were eligible for these trials if they were 18 years or older, with measurable metastatic melanoma and Eastern Cooperative Oncology Group (ECOG) performance status of 0 or 1, life expectancy of greater than 3 months, and no evidence of active systemic infections, coagulation disorders, or other active major medical or cardiovascular or immunodeficiency diseases. Patients had progressive metastatic melanoma at entrance into the protocol and at least 4 weeks had elapsed after any prior treatment. All patients had a metastatic lesion of greater than 2-cm diameter that could be resected for growth of TILs and had cells that grew in culture to sufficient quantities with *in vitro* antitumor activity as described in the following text. Patients with 1 or 2 brain metastases less than 1 cm in diameter were eligible for this trial. All patients signed an informed consent approved by the Institutional Review Board of the National Cancer Institute.

Clinical trial design

Before receiving TIL infusion, all patients received a nonmyeloblative lymphodepleting regimen consisting of cyclophosphamide at 60 mg/kg/d for 2 days and fludarabine at 25 mg/m^2/d for 5 days. In 3 sequential trials, patients received this nonmyeloblative preparative regimen alone (43 patients) or in conjunction with total body irradiation (TBI) either 2 Gy (25 patients) or 12 Gy (2 Gy twice a day for 3 days; 25 patients). TBI was delivered at 2 Gy fractions with 15-MV photons delivered at a distance of 6 m to the patient's midline at a dose rate of 0.11 Gy/min as previously described (17). Within a day following the completion of the preparative regimen, patients received an intravenous infusion of TILs and were started on high-dose IL-2 at 720,000 IU/kg intravenously every 8 hours to tolerance. Within 1 or 2 days after TIL infusion, patients who received TBI also received a minimum of 2×10^6/kg of autologous, purified, cryopreserved CD34$^+$ hematopoietic stem cells from a granulocyte colony–stimulating factor–mobilized pheresis. Patient response to treatment was assessed utilizing Response Evaluation Criteria in Solid Tumors (RECIST) guidelines starting at approximately 4 weeks after cell administration and at regular intervals thereafter. Data in this report are updated as of August 1, 2010, with a median potential follow-up of 89.8, 58.5, and 41.5 months in the cohorts with preparative chemotherapy alone or with 2 or 12 Gy, respectively (overall median potential follow-up 62.0 months; range 35.1–118.6 months).

Preparation of TILs for infusion

Lymphocytes were grown from resected metastatic melanoma lesions in high-dose IL-2 as previously described (16–18). In brief, individual cultures in 24-well culture plates were established from either single-cell suspensions or 1- to 2-mm^3 fragments. The wells were individually grown and expanded and tested for the presence of antigen specificity, utilizing overnight cytokine release coculture assays against either autologous tumor- or human leukocyte antigen (HLA)-matched melanoma cell lines. Cultures with evidence of specific reactivity compared with allogeneic non-MHC–matched controls that exceeded 200 pg/mL of IFNγ and were at least twice control values were selected for rapid expansion as previously described (16, 17).

The average length of telomere repeats in chromosomes from the infused cells was measured by quantitative flow-FISH as previously described (19). The number and percentage of CD8$^+$CD27$^+$ cells in the infused TILs was measured after withdrawal of IL-2 for 2 days (20). The persistence of the infused cells in the circulation was measured by amplifying T-cell receptor beta variable region sequences, using the SMART RACE cDNA Amplification kit

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(Clontech Laboratories, Inc.), and comparing clonotypes in the infused TILs with those in the circulation at 1 month, using techniques previously described (21, 22).

Statistical analysis

Continuous parameters were compared between response groups [CR (complete response) vs. < CR; PR (partial response) + CR vs. NR (no response)], using an exact Wilcoxon rank-sum test, as the majority of parameters were not normally distributed in one or both of the groups to be compared. An exact Cochran–Armitage test was used to compare ordered categorical parameters (degree of TBI: 0, 200, and 1,200) and stage (M1a, M1b, and M1c) with response group (23). Dichotomous parameters (sex, HLA 02 vs. non-02) were compared with response group using Fisher's exact test. All *P* values are 2-tailed and presented without adjustment for multiple comparisons. In view of the number of parameters initially explored, (12 for each response group comparison) *P* values less than 0.01 would likely indicate significant associations whereas those for which 0.01 greater than *P* less than 0.05 would indicate strong trends.

Results

There were no significant differences in sex, age, ECOG status, or the number of cells administered among the 3 treatment cohorts (chemotherapy preparative regimen alone or plus 2 or 12 Gy). The incidence and duration of responses in each of the 3 treatment cohorts are presented in Table 1 and patient survival in Figure 1. The objective response rates in the 3 cohorts were 49%, 52%, and 72%, respectively, with complete response rates of 12%, 20%, and 40%. There was no significant difference in overall response rate among the cohorts ($P_2 = 0.08$), though there was an increased rate of complete responses associated with increasing dose of TBI ($P_2 = 0.007$; Table 2; results are descriptive and not corrected for multiple comparisons). These cohorts were accrued sequentially and thus should be compared with each other with caution. The actuarial 3- and 5-year survival rates for the entire 93 patients were 36% and 29%, respectively. For the 20 complete responders, the 3- and 5-year survival rates were 100% and 93%, for the 32 partial responders 31% and 21%, and for the 41 nonresponders 7% and 5%, respectively.

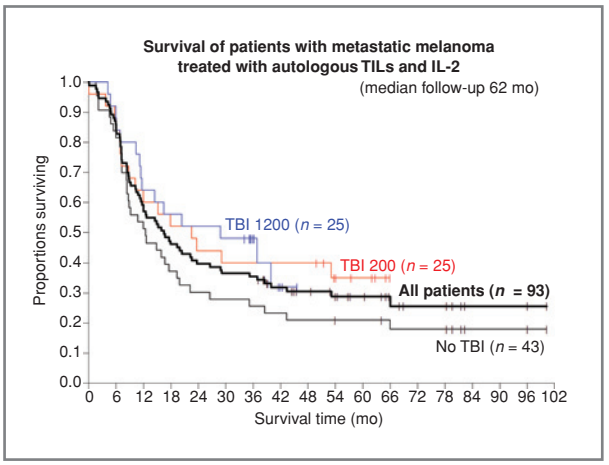

Figure 1. Overall survival of patients receiving TILs with the chemotherapy preparative regimen alone (no TBI) or plus 2 or 12 Gy TBI.

Of particular interest was the high incidence of durable complete responses seen in 20 of the 93 patients (22%), with complete regressions ongoing in 19 of these patients at 37 to 82 months. All but 2 of the completely responding patients received a single treatment; 2 received a second treatment. Eighty of the 93 patients (86%) had visceral metastases (stage M1b or M1c), including 17 of the 20 (85%) complete responders and 28 of 32 (88%) partial responders. Complete responses were seen in patients with a median of 3 different organ sites of metastases including lung, liver, adrenal, muscle, lymph nodes, and skin. The exact sites of disease in patients in the 3 cohorts are shown in Supplementary Table S1 and the stage of metastatic disease in each cohort with their response is shown in

Table 1. Cell transfer therapy

Treatment	*n* (%) of patients (duration in mo)			OR (%)
	Total	PR	CR	
No TBI	43	16 (37) 84, 36, 29, 28, 14, 12, 11, 7, 7, 7, 7, 4, 4, 2, 2, 2	5 (12) 82+, 81+, 79+, 78+, 64+	21 (49)
200 TBI	25	8 (32) 14, 9, 6, 6, 5, 4, 3, 3	5 (20) 68+, 64+, 60+, 57+, 54+	13 (52)
1,200 TBI	25	8 (32) 21, 13, 7, 6, 6, 5, 3, 2	10 (40) 48+, 45+, 44+, 44+, 39+, 38+, 38+, 38+, 37+, 19	18 (72)
Total	93	32 (34)	20 (22)	52 (56)

NOTE: Data updated as of August 1, 2010.

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Table 2. Patient and treatment characteristics

	Number of patients				P_2	
	CR	PR	NR	Total	CR vs. (PR + NR)	(CR + PR) vs. NR
Total	20	32	41	93		
Patients						
Sex						
Male	13	20	29	62	1.0	<0.51
Female	7	12	12	31		
Age						
16–30	3	5	4	12	0.84	0.79
31–45	7	11	14	32		
46–60	10	16	21	47		
61–75	0	0	2	2		
HLA						
A2	15	28	32	75	0.53	0.61
Non-A2	5	4	9	18		
Stage						
M1a	3	4	6	13	0.55	0.56
M1b	3	4	1	8		
M1c	14	24	34	72		
Treatments						
TBI, Gy						
0	5	16	22	43	0.007	0.08
2	5	8	12	25		
12	10	8	7	25		
Cells ($\times 10^{-10}$)						
<3	4	5	10	19		
3.1–5.0	3	4	12	19		
5.1–7.0	7	12	7	26		
7.1–9.0	2	4	4	10		
>9	4	7	8	19		
Mean ± SEM	6.5 ± 0.7	6.1 ± 0.5	5.5 ± 0.6		0.25	0.07
% $CD3CD8^+$	86 ± 2	79 ± 4	74 ± 4		0.59	0.14
% $CD3CD4^+$	10 ± 2	20 ± 4	24 ± 4		0.30	0.15
Telomere length, kb	6.7 ± 0.3	6.4 ± 0.2	5.1 ± 0.2		0.006	<0.001
$CD8^+CD27^+$ cells ($\times 10^{-10}$)	2.0 ± 0.3	1.5 ± 0.2	0.8 ± 0.1		0.001	<0.001
Persistence at 1 mo (%)	30.2 ± 5.5	28 ± 5.9	10.5 ± 3.5		0.003	<0.001
IL-2 doses						
<5	1	3	1	5		
5–8	15	22	19	56		
9–11	4	7	21	32		
Mean ± SEM	7.1 ± 0.4	7.7 ± 0.5	8.8 ± 0.4		0.11	0.003

Abbreviations: M1a, skin, subcutaneous, or nodal metastases; M1b, lung; M1c all other visceral sites or elevated LDH levels.

Supplementary Table S2. Representative scans and photographs of the 20 patients who experienced a complete response are shown in Supplementary Figure S1. Although 19 of the patients who experienced a complete response are ongoing long-term survivors, 8 additional patients also are alive beyond 3 years, 6 of whom had prolonged partial responses (4 had resection of all residual disease), 1 had a mixed response, and the other responded to subsequent chemotherapy.

This was a heavily pretreated group of patients as shown in Table 3. Seventy-seven of the 93 patients (83%) had progressed after receiving IL-2, either at high dose or as part of a biochemotherapy regimen, 40 (43%) had prior chemotherapy, and 37 (40%) had received both IL-2 and chemotherapy. Of the 20 complete responders, 70% had received prior IL-2 treatment, 35% prior chemotherapy, 30% both IL-2 and chemotherapy, 55% prior IFN treatment, and 25% prior anti-CTLA4 treatment. The median

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Table 3. Impact of prior treatment on response to cell transfer therapy using selected TILs

	n (%)[a]			OR[b]
	Total	CR	PR	
All patients	93	20 (22)	32 (34)	52 (56)
Prior treatment				
None	5 (5)	2 (40)	1 (20)	3 (60)
IL-2	77 (83)	14 (18)	28 (36)	42 (54)
Chemotherapy	40 (43)	7 (18)	16 (40)	23 (58)
IFN	52 (56)	11 (21)	17 (33)	28 (54)
Anti-CTLA4	11 (12)	5 (45)	2 (18)	7 (64)
IL-2$^+$ chemotherapy	37 (40)	6 (16)	16 (43)	22 (59)
IL-2$^+$ anti-CTLA4	8 (9)	3 (38)	1 (13)	4 (50)
IL-2$^+$ anti-CTLA4$^+$ chemotherapy	6 (7)	2 (33)	1 (17)	3 (50)

[a]The percentage of patients with a CR, PR, or OR in each group who had received the prior treatment.
[b]No group is statistically different from any other.

time from first diagnosis of metastatic disease to the administration of cell therapy was 18 months (mean ± SEM = 25.9 ± 2.7 months). The median number of prior systemic treatments administered was 2 (mean ± SEM = 2.0 ± 0.2). The 5-year survival rate of 29% for all 93 patients was similar regardless of prior treatment, with the possible exception of the 5-year survival rate of 44% for the 11 patients who progressed after receiving prior anti-CTLA4 treatment (Fig. 2). These latter 11 patients had received anti-CTLA4 an average of 13.7 months before starting the cell transfer therapy and all had progressive disease.

There were no significant differences in patients who experienced a complete response or any objective response compared with nonresponders in sex, age, HLA type, metastatic stage, numbers of cells administered, or lactate dehydrogenase (LDH) levels (Table 2). However, patients experiencing an objective response received fewer IL-2 doses than nonresponders (7.2 ± 0.3 vs. 8.8 ± 0.4; $P_2 = 0.003$),

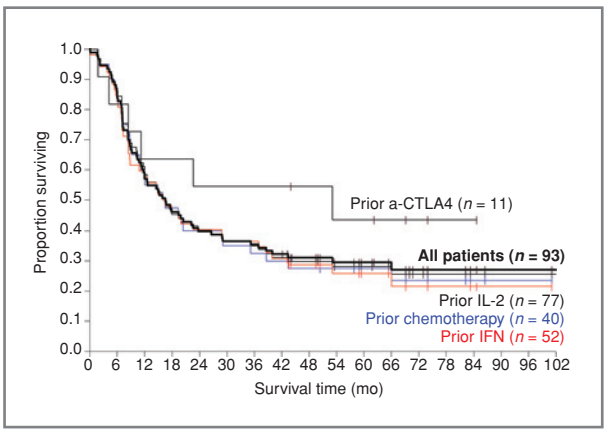

Figure 2. Overall survival of patients receiving cell transfer based on prior treatment received.

likely due to increased side effects from IL-2 resulting from increased activity of the transferred cells in responders that limited IL-2 dosing *in vivo*. As shown in Table 2 and Figure 3, objective responders differed significantly from nonresponders in receiving TILs with longer average telomeres and a larger number of $CD8^+CD27^+$ cells and in the *in vivo* persistence (of the infused cells) in the circulation at 1 month (all P_2 values <0.001). There was considerable overlap between responders and nonresponders for each of these 3 factors and thus none absolutely predicted the occurrence of an objective response. There was no difference in response rates in patients with tumors that did or did not contain *BRAF* or *NRAS* mutations.

It should be emphasized that the patients in this trial were a select group who had a resectable lesion (estimated to be about 85% of all patients). In our entire experience, viable TILs could be grown to a minimum of 5×10^6 cells (and the majority to more than 2×10^7 cells) from 75% to 85% of patients depending on whether the cultures were set up as enzymatic digests, fragments, or both. Active specific TILs were identified in 67% of all resected patients (24).

The toxicities of treatment have been previously reported and were largely due to the lymphodepleting preparative regimen or IL-2 (17, 25). Most patients tolerated the treatment well and returned to baseline. There was 1 treatment-related death in the 93 patients; a 49-year-old male who received 2 Gy TBI and died of sepsis 4 days after cell infusion from an undetected diverticular abscess present prior to treatment. One patient with a partial response who received 2 Gy TBI developed prolonged pulmonary hypertension. Five patients who received 12 Gy TBI, all of whom experienced a complete regression, developed a microangiopathic nephropathy with creatinine elevations in the range of 1.5 to 2.5 mg/dL. Their renal function has not worsened over time, and these patients are living normally.

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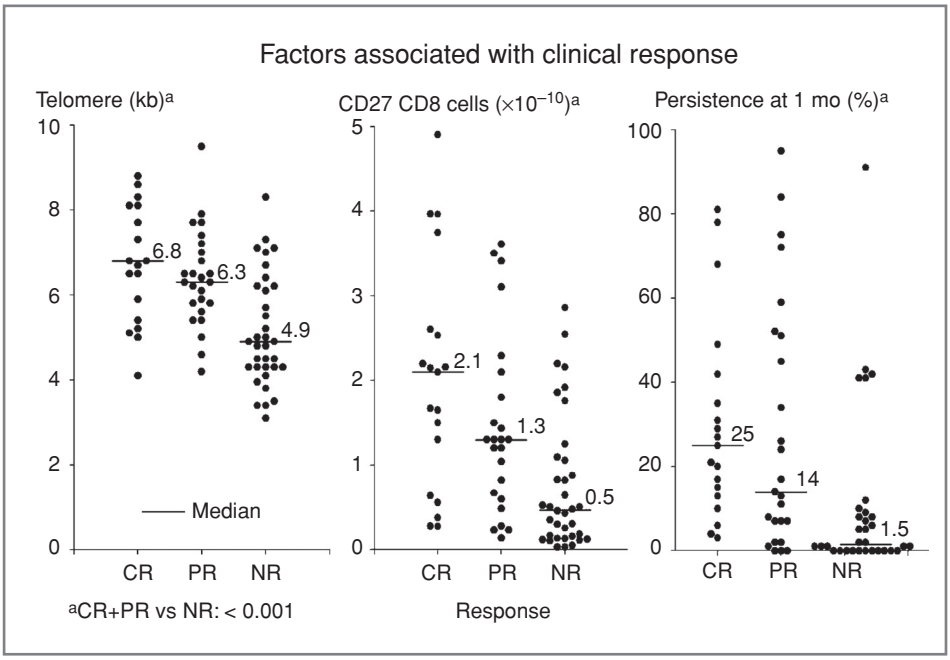

Figure 3. Mean telomere length, the number of $CD27^+CD8^+$ cells, and the percentage persistence of the infused cells in peripheral blood at 1 month after cell infusion are significantly different in objective responders (CR + PR) compared with nonresponders (all $P_2 < 0.001$). anti-CTLA4, a-CTLA4. Data updated as of January 1, 2011.

Discussion

Although many systemic treatment options for patients with metastatic solid cancers can mediate modest improvements in survival, the long-term cure of patients requires the induction of durable complete regressions. With few exceptions (such as men with germ cell tumors or women with choriocarcinoma), durable complete regressions of metastatic solid cancers are very rare. In patients with metastatic melanoma, the administration of high-dose IL-2 can mediate durable complete apparently curative responses in 4% to 5% of patients and this led to the FDA approval of IL-2 for patients with melanoma in 1998 (3, 26, 27). Durable complete regressions using dacarbazine, the only other FDA-approved treatment for melanoma, is seen in less than 1% of patients (1, 2). Recent reports of the use of ipilimumab, a molecule reactive with CTLA4 on the cell surface in 540 patients (4), and PLX4032, a mutated BRAF inhibitor in 48 patients (5), have shown complete responses in 0.6% and 6% of patients with metastatic melanoma, respectively, though both treatments seem capable of prolonging the survival of patients.

In the current report, durable complete responses were seen in 22% of patients who received this cell transfer therapy and 95% of these complete responses are ongoing beyond 3 years. It thus seems to be an effective and possibly curative treatment option for many patients with metastatic melanoma capable of receiving it.

Patients with melanoma have been the subject of many attempts at immunotherapy (6). Early reports of a low level of objective responses of melanoma to immunologic modulation with IL-2 (28) and the demonstration that lymphocytes infiltrating into melanomas (TILs) specifically recognized tumor-associated antigens (29) suggested that this disease stimulated an endogenous immune response that could be further manipulated to improve antitumor effects. Although the immunization of patients with cancer antigens (cancer vaccines) has thus far yielded only modest results (30), early studies of the isolation, growth in IL-2, and infusion of autologous TILs showed that this adoptive immunotherapy approach could mediate transient tumor regression in patients, though the lack of persistence of the transferred cells may have hampered their effectiveness (31, 32). An improvement of this treatment reported in 2002 showed that chemotherapy-induced lymphodepletion prior to adoptive cell infusion could lead to the dramatic enhancement of the persistence of the transferred cells and improved anticancer effects (16). Studies in tumor-bearing mice showed that the antitumor effects of adoptive cell transfer were a direct function of the magnitude of lymphodepletion (33), and these findings led to studies of the addition of TBI to the nonmyeloablative chemotherapy preparative regimen that had been used in earlier trials. Earlier reports of these pilot trials showed that 10 of 93 patients achieved a complete regression, though follow-up in the trials using maximum lymphodepletion at the time of that report was only 10 months (17). We now report the definitive long-term follow-up of these trials utilizing adoptive transfer of autologous TILs following preparative lymphodepleting treatment with an overall median potential follow-up of 62 months and a median potential follow-up of 41.5 months in the latest cohort adding 12 Gy irradiation. Ten of the partial responders have now converted to complete durable regressions without any further treatment. This is likely due to the resolution of scarring after tumor destruction or to the ongoing antitumor impact of persisting T lymphocytes for months after cell transfer.

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Of the 93 patients, 20 (22%) have achieved a complete regression of metastatic disease and 19 of these 20 patients have ongoing complete responses beyond 3 years. Regressions have been seen at all visceral sites. The average patient had a median of 3 different metastatic anatomic sites.

Of particular importance is the induction of durable complete responses in patients who have received and progressed through multiple prior treatment regimens. Seventy percent of the complete responders had progressed through IL-2, 35% through chemotherapy, and 30% had both IL-2 and chemotherapy. Of the 11 patients who had previously progressed after receiving anti-CTLA4 monoclonal antibody, 5 experienced a complete regression in addition to 2 partial regressions. There was no influence of any prior therapy on the likelihood of achieving a complete regression or on overall survival in these 93 patients, suggesting that this treatment approach can be useful as an upfront treatment option or as a salvage regimen for patients with progression after other therapies.

Although complete regressions were seen in patients receiving each of the preparative regimens, there is a strong suggestion that increasing the lymphodepletion by adding TBI enhanced the antitumor effects. Studies in murine models suggested that lymphodepletion prior to adoptive cell transfer reduced the competition for homeostatic cytokines such as IL-7 and IL-15 (34) that promote lymphocyte growth, and indeed we measured an increase in serum IL-15 levels in all patients on the day after the lymphodepleting regimen was completed (17). Other impacts of the lymphodepletion are likely due to transient elimination of regulatory T cells and enhancement of the activity of antigen-presenting cells (35, 36). In preliminary studies, we have seen an inverse correlation between the likelihood of response and the return of $CD4^+Foxp3^+$ cells in the circulation after treatment and this is now being further studied. In concert with results from murine models indicating that infused cells that were less differentiated and with a higher proliferative potential had increased antitumor activity (37), we noted a highly significant association between the likelihood of having a complete response and the infusion of TILs with longer telomeres, TILs with more $CD8^+CD27^+$ cells, and increased persistence in the circulation of the infused cells at 1 month after transfer. There is an inverse correlation of telomere length with time in culture (38), though there was no maximum time in culture that precluded administration of cells. These findings point the way toward increasing the therapeutic effectiveness of TILs by choosing cultures with higher average telomere lengths or cultures containing cells with a higher absolute number of $CD27^+$ cells, a marker of less differentiated cells (37). Generation of less differentiated cells might also be accomplished by minimizing time in culture and use of alternative cytokines.

It should be emphasized that not all patients with metastatic melanoma can receive this treatment approach. A metastatic nodule of at least 2-cm diameter must be present and suitable for resection. Most resections have been from soft tissue lesions, but peripheral lung and liver lesions have also been used (24). Metastatic lesions resected for symptomatic relief in the course of disease progression or lymph nodes resected for stage III disease can be cryopreserved and are a suitable source for later growth of TILs. Because about 85% of patients have lesions capable of being resected and about 55% grow cells suitable for infusion, we estimate that about 45% of all patients with metastatic melanoma can receive this treatment. About 5% of patients develop complications of tumor growth during the 4 to 6 weeks of cell preparation that preclude treatment. An important advantage of this cell transfer approach is its ability to mediate complete regressions regardless of the failure of prior treatments.

Current efforts are devoted to developing simpler and faster methods both to grow TILs with increased antitumor efficacy and to develop alternative preparative regimens. TILs grown by a simplified method that eliminates *in vitro* testing for antitumor activity (38) have mediated tumor regressions in patients with melanoma, though follow-up in these patients is short (39, 40). The ability to transduce genes encoding antitumor T-cell receptors into normal circulating lymphocytes and the use of these genetically engineered autologous cells for adoptive transfer are being explored in ongoing clinical trials (41, 42).

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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