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ORIGINAL REPORT

Randomized Selection Design Trial Evaluating CD8$^+$-Enriched Versus Unselected Tumor-Infiltrating Lymphocytes for Adoptive Cell Therapy for Patients With Melanoma

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ABSTRACT

Purpose
Adoptive cell therapy (ACT) with autologous tumor-infiltrating lymphocytes (TILs) and high-dose interleukin-2 (IL-2) administered to lymphodepleted patients with melanoma can cause durable tumor regressions. The optimal TIL product for ACT is unknown.

Patients and Methods
Patients with metastatic melanoma were prospectively assigned to receive unselected young TILs versus CD8$^+$-enriched TILs. All patients received lymphodepleting chemotherapy and high-dose IL-2 therapy and were assessed for response, toxicity, survival, and immunologic end points.

Results
Thirty-four patients received unselected young TILs with a median of 8.0% CD4$^+$ lymphocytes, and 35 patients received CD8$^+$-enriched TILs with a median of 0.3% CD4$^+$ lymphocytes. One month after TIL infusion, patients who received CD8$^+$-enriched TILs had significantly fewer CD4$^+$ peripheral blood lymphocytes ($P = .01$). Twelve patients responded to therapy with unselected young TILs (according to Response Evaluation Criteria in Solid Tumors [RECIST]), and seven patients responded to CD8$^+$-enriched TILs (35% *v* 20%; not significant). Retrospective studies showed a significant association between response to treatment and interferon gamma secretion by the infused TILs in response to autologous tumor ($P = .04$), and in the subgroup of patients who received TILs from subcutaneous tumors, eight of 15 patients receiving unselected young TILs responded but none of eight patients receiving CD8$^+$-enriched TILs responded.

Conclusion
A randomized selection design trial was feasible for improving individualized TIL therapy. Since the evidence indicates that CD8$^+$-enriched TILs are not more potent therapeutically and they are more laborious to prepare, future studies should focus on unselected young TILs.

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INTRODUCTION

Tumor-infiltrating lymphocytes (TILs) can mediate the durable regression of metastatic melanoma when administrated with interleukin-2 (IL-2) therapy to autologous patients following a lymphodepleting preparative regimen.[1] TILs were selected in vitro for tumor recognition and administered in three sequential trials to patients with refractory melanoma with nonmyeloablative lymphodepleting chemotherapy (NMA) and either no additional radiation or 2 Gy or 12 Gy of total-body irradiation. The overall response rates were 48%, 52%, and 72%, and the complete response rates were 13%, 20%, and 40%.[2] Nineteen of 20 complete responders remained free of disease after more than 5 years of follow-up. Prior therapies including chemotherapy, and ipilimumab had no impact on the likelihood of response. The generation of autologous selected TILs used in these clinical trials involved a complex process, and only 27% of patients who underwent resection for TILs generation were treated.[3] These studies demonstrated the value of adoptive cell therapy (ACT) as a salvage therapy for patients with melanoma but defined a need for simpler, more reliable TIL production methods.

Tran et al[4] established a "young TILs" method that minimized the time in culture and eliminated

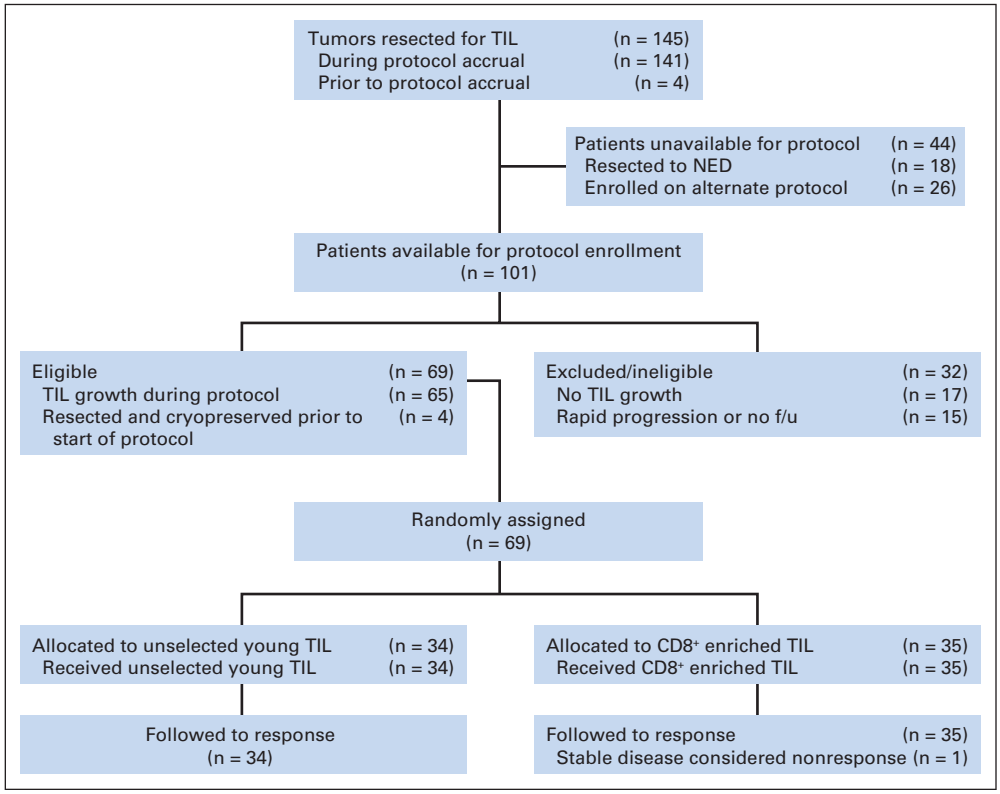

Fig 1. CONSORT diagram for this randomized selection design protocol. The success rate for patient accrual from tumor resection to protocol enrollment is estimated in the upper portion of the figure, although this represents a retrospective analysis and was not part of the protocol design. Patients were eligible for enrollment onto this protocol when young tumor-infiltrating lymphocytes (TIL) suitable for therapy were determined to be available, and 69 patients were randomly assigned and treated. f/u, follow-up; NED, no evaluable disease.

screening for tumor recognition. Itzhaki et al[5] corroborated that unselected young TILs were simpler and more reliable than selected TILs and reported the first use of young TILs for patient treatment, resulting in 15 objective responders (48%) of 31 treated patients. There has also been a rapid development of bioreactors allowing high-density T-cell expansion.[6] The perfusion-fed, suspension culture–based WAVE bioreactor (General Electric, Piscataway, NJ) and gas-permeable rapid expansion (GRex; Wolf Manufacturing, New Brighton, MN) flasks were recently introduced for clinical use with individualized T-cell therapies,[7,8] and were optimized for use in TIL therapy.[9,10]

TILs are composed of a mixture of lymphocytes with multiple functions and phenotypes, and notably, the role of CD4 lymphocytes in the infused TILs is controversial. $CD4^+$ lymphocytes with Th1-type autologous tumor reactivity can be identified in 20% of TIL cultures, and anecdotal examples of clinical tumor regression associated with $CD4^+$ lymphocytes have been reported.[11,12] Conversely, $CD4^+$ TILs with a regulatory T-cell phenotype have been isolated and expanded from melanoma tumors[13,14] and $CD4^+FoxP3^+$ regulatory T-cell reconstitution of patient peripheral blood after ACT was inversely correlated with response to $CD8^+$ TIL therapy.[15] $CD8^+$ TILs are generally associated with tumor regression. In a recent clinical trial evaluating the safety and efficacy of $CD8^+$-enriched TILs and IL-2 following NMA lymphodepletion for the treatment of patients with refractory melanoma,[16] 18 (55%) of 33 patients exhibited an objective response.[16] This trial demonstrated that $CD8^+$ TILs without $CD4^+$ lymphocytes were sufficient to mediate tumor regression. Other groups found that a high number[5] or percent[17] of infused $CD8^+$ TILs was associated with objective response, supporting a primary role for $CD8^+$ cells in TIL ACT.

In this study, we took advantage of improved production methods for individualized TIL therapies to implement a selection design clinical trial with ACT. To determine whether $CD8^+$-enriched TILs or unselected young TILs containing $CD4^+$ cells represented a better product for ACT, we designed a randomized, single-institution phase II clinical trial. Sixty-nine patients who had TILs available were randomly assigned and treated on this protocol. Thirty-four patients received unselected young TILs containing $CD4^+$ and $CD8^+$ TILs, and 35 patients received $CD8^+$-enriched young TILs. All patients received NMA before cell infusion and high-dose IL-2 therapy. The results and associated immunologic findings are reported here.

PATIENTS AND METHODS

Patients, Trial Design, and Clinical Samples

This trial was designed as a prospective selection design trial for patients who had a TIL culture available before random assignment (Fig 1) to be able to conclude at the end of the study whether one arm could be favored over the other for further study. We planned to enroll and randomly assign a total of 70 patients (35 per arm; a total of 69 patients were enrolled) to have 91% probability of correctly selecting the superior arm if the true probability of a clinical response was 25% in the arm with lower true probability of response and 40% in the arm with greater probability of response. Patients were stratified between arms based on M1a, M1b, or M1c staging (American Joint Committee on Cancer). Other eligibility criteria included the following: age 18 years or older with measurable metastatic melanoma, good performance status, normal laboratory tests, absence of active systemic infections, and eligible to receive high-dose IL-2 therapy. All patients signed an informed consent document approved by the institutional review board of the National Cancer Institute. Patients received NMA conditioning, autologous TILs by bolus infusion, and IL-2 therapy as previously described.[18] CBCs were obtained once

per day while patients were in the hospital, and differential counts were obtained when CBC was above 200 cells/μL. Patient response was assessed by using standard radiographic studies and physical examination at 4 to 6 weeks following TIL administration and at regular intervals thereafter. The Response Evaluation Criteria in Solid Tumors (RECIST) 1.0 guidelines were followed, and clinical responses were sorted into complete, partial, or nonresponding categories.

Generation and Analysis of TILs for Infusion

Patients underwent resection of a metastatic melanoma lesion, and young TILs were generated as previously described.[18] Briefly, TIL cultures were initiated from enzymatically generated single-cell digests or 1- to 3-μL tumor fragments, and cultures were established by serial passage in media containing 6,000 IU/mL IL-2. Patients with sufficient TILs were randomly assigned to receive either unselected young TILs or $CD8^+$-enriched TILs. $CD8^+$ enrichment was accomplished with CD8 nanobeads (CliniMACS, Miltenyi Biotec, Auburn, CA) as previously described.[19] Unselected young TILs were not subjected to any control manipulations with nanobeads or CliniMACS reagents. Lymphocytes were rapidly expanded once by using irradiated peripheral blood mononuclear cells (feeder cells), anti-CD3 antibody, and IL-2 as previously described[9,10,20,21] and tested for identity, potency, and sterility before patient infusion. Infused samples were evaluated by flow cytometry for CD4 and CD8 lymphocyte composition and by interferon gamma (IFN-γ) release for tumor reactivity as previously described.[18]

Statistical Analysis

The statistical significance of the difference in continuous parameters between two groups of patients was determined by using a Wilcoxon rank sum test. Survival was determined from the date of TIL infusion until the date of death or last follow-up, the probability of survival as a function of time was determined by using the Kaplan-Meier method, and significance between curves was calculated by using a log-rank test. All *P* values were two-tailed and were reported without adjustment for multiple comparisons. Because the primary objective was to select the arm with the greater number of responses, and because the study was not designed to have high statistical power to

Table 1. Patient Demographics and Treatments Administered

	Unselected Young TILs (arm 1)		$CD8^+$ Young TILs (arm 2)		Total	
Characteristic	No.	%	No.	%	No.	%
No. of patients	34	100	35	100	69	100
Sex						
Male	23	68	17	49	40	58
Female	11	32	18	51	29	42
Age, years						
11-20	0	0	1	3	1	1
21-30	5	15	4	11	9	13
31-40	7	21	8	23	15	22
41-50	12	35	10	29	22	32
51-60	6	18	9	26	15	22
61-70	4	12	3	9	7	10
ECOG PS						
0	28	82	30	86	58	84
1	6	18	5	14	11	16
Prior therapy						
Surgery	34	100	35	100	69	100
Chemotherapy	9	26	10	29	19	28
Radiotherapy	8	24	7	20	15	22
Immunotherapy	25	74	21	60	46	67
Interleukin-2	19	56	16	46	35	51
Ipilimumab	1	3	3	9	4	6
Any two or more	26	76	24	69	50	72
Any three or more	12	35	11	31	23	33
Grade						
M1a	2	6	5	14	7	10
M1b	4	12	6	17	10	14
M1c	28	82	24	69	52	75
Site of TIL						
Lymph node	13	38	20	57	33	48
Subcutaneous	15	44	8	23	23	33
Other*	6	18	7	20	13	19
Cells ($\times 10^{10}$)						
0.1-2.0	2	6	8	23	10	14
2.1-4.0	13	38	11	31	24	35
4.1-6.0	14	41	8	23	22	32
6.1-8.0	4	12	4	11	8	12
> 8.0	1	3	4	11	5	7

Abbreviations: ECOG PS, Eastern Cooperative Oncology Group performance status; TIL, tumor-infiltrating lymphocyte.
*Other anatomic sites of tumor resections for TIL harvest included small bowel (3), lung (2), liver (2), spleen (2), intramuscular (2), chest wall (1), and mesenteric mass (1).

formally identify a difference between the arms with respect to response, the *P* value reported for this difference is to be interpreted as exploratory.

RESULTS

Patient Characteristics and Treatments Administered

Melanoma tumors were accessioned from 141 patients during the 16 months that this protocol was open for accrual. Eighteen patients (13%) had no evaluable disease after resection, and 26 patients (18%) were enrolled onto alternate experimental protocols (Fig 1; Data Supplement). Thus, 97 patients had a resection and were suitable for protocol consideration. Thirty-two of these patients (33%) were not eligible for therapy (17 patients failed to grow TILs, and 15 patients had rapidly progressive disease or were lost to follow-up). Sixty-five patients (67% of eligible patients) generated TIL cultures that were suitable for therapy, and an additional four patients had cryopreserved TILs from a prior resection. These 69 patients were enrolled onto the protocol, underwent random assignment, and completed a course of treatment.

The study arms were well balanced for patient demographic characteristics (Table 1). Most patients enrolled onto this trial had advanced refractory disease: 75% with stage M1c, including 17% with brain disease at random assignment; 67% with prior immunotherapy; and 72% having at least two prior treatments, including surgery, immunotherapy, radiotherapy, or chemotherapy (Table 1). The study arms were well balanced for treatment attributes, including the total TILs infused ($P = .54$), the $CD8^+$ TILs infused ($P = .16$), and the age of the TILs infused ($P = .68$; Table 2). By design, patients with $CD8^+$-enriched cells received few $CD4^+$ cells (0.3×10^9 cells; range, 0.0 to 7.9×10^9 cells) versus unselected young TILs (8.0×10^9 cells; range, 0.8 to 31.5×10^9 cells; $P < .001$; Table 2; Data Supplement). A few patients had substantial $CD4^+CD8^+$ double-positive cells that were enriched with the $CD4^-CD8^+$ conventional $CD8^+$ TILs (Data Supplement).

The site of tumor procurement, method of TIL expansion, age of TILs at infusion, number of cells infused, percentage of $CD4^+$ lymphocytes, and tumor recognition varied substantially among patients (Data Supplement), emphasizing the challenges of developing a unique lymphocyte culture for treatment of each patient. There were few significant associations among any of these attributes. Of interest, eight of 15 patients with unselected young TILs from subcutaneous metastases responded, although none of eight patients with $CD8^+$-enriched TILs from subcutaneous metastases responded ($P = .02$; Data Supplement). For 59 patients, the cellular composition of the initial digest was determined. Lymph node resections (n = 29) started with a significantly lower percentage of tumor cells (and higher percentage of lymphocytes) than other sites of resection (n = 30; Wilcoxon rank sum $P = .038$) but the percentage of lymphocytes was not significantly associated with response to ACT. Response was also not associated with TIL age, total cell number infused, or $CD8^+$ cell number infused (Table 2).

Responses and Toxicities of Treatment

Twelve patients (35%) who received unselected young TILs exhibited an objective response, including two complete responders (both ongoing). One patient with progressive disease at enrollment now has stable disease more than 2 years after treatment and is considered a nonresponder for this analysis. Seven patients (20%) achieved an objective response on the $CD8^+$-enriched TIL arm, including three complete responders (two ongoing). Characteristics of patients who responded to treatment are summarized in the Data Supplement. There was no significant difference between the clinical

Table 2. Treatment Attributes Administered by Protocol Arm or by Patient Response

Treatment	Unselected Young TILs			$CD8^+$ Young TILs			*P**
	Median	Range	Total	Median	Range	Total	
Treatment administered by protocol arm							
No. of patients			34			35	
Age of infused TILs, days	35.5	26-51		35.0	24-43		.68
Total cells infused ($\times 10^9$)	40.9	9.8-84.9		39.0	4.5-147.0		.54
$CD8^+$ TILs infused ($\times 10^9$)	30.3	5.9-62.7		38.4	3.7-138.8		.16
$CD4^+$ TILs infused ($\times 10^9$)	8.0	0.8-31.5		0.3	0.0-7.9		< .001
IL-2 doses administered	7.0	1-10		7.0	3-10		.04
	Nonresponder			Objective responder†			
Treatment administered by protocol response							
No. of patients			50			19	
Total cells infused ($\times 10^9$)	37.7	4.5-93.4		44.2	23.9-147.0		.09
$CD8^+$ TILs infused ($\times 10^9$)	30.8	3.7-87.4		36.9	9.5-138.8		.25
$CD4^+$ TILs infused ($\times 10^9$)	0.8	0.0-31.5		6.3	0.1-28.9		.12
Age of infused TILs, days	35.0	24-51		36.0	26-48		.64
IL-2 doses administered	7.0	1-10		7.0	3-9		.91
Site of TIL origin (lymph node *v* all other)			27 *v* 23			6 *v* 13	.11
Autologous tumor recognition (no *v* yes)‡			12 *v* 22			0 *v* 12	.02

Abbreviations: IL-2, interleukin-2; TIL, tumor-infiltrating lymphocyte.
*Wilcoxon rank sum or Fisher's exact two-tailed *P* values.
†Complete or partial responder.
‡Interferon gamma release > 200 pg/mL.

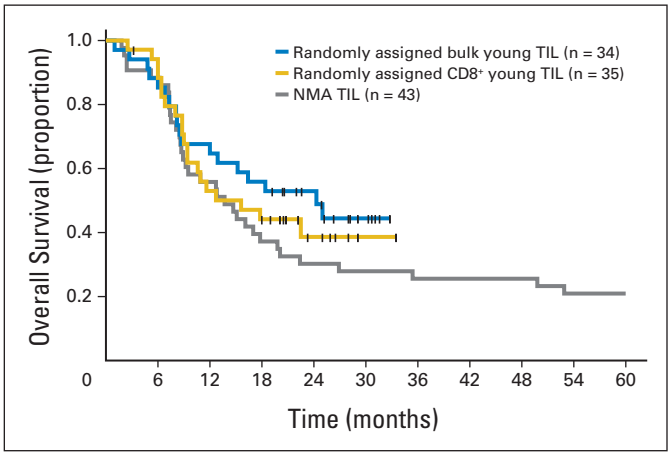

Fig 2. Kaplan-Meier survival curve for patients treated in this clinical trial by arm and for a historical control group receiving similar tumor-infiltrating lymphocyte (TIL) therapy. Patients randomly assigned to receive unselected young TILs or $CD8^+$-enriched young TILs are shown, with tick marks indicating patients who are still alive and available for follow-up. Nonmyeloablative lymphodepleting chemotherapy (NMA) TIL represents survival from a historical control group of patients who received TILs selected for tumor reactivity.[6]

responses in the two arms (Fisher's exact test $P = .19$), although this was not the primary aim of the trial, which was not powered to detect a small difference in response rates. Overall survival of patients treated with unselected young TILs and $CD8^+$-enriched TILs is shown in Figure 2 and compared with a historical control group of patients treated with ACT, which consisted of NMA conditioning and TILs selected for tumor recognition.[22] There is currently no significant difference in survival between the unselected young TILs and the $CD8^+$-enriched TIL arms ($P = .41$), with an overall median potential follow-up of 27.8 months as of October 1, 2012.

Patients treated with unselected young TILs or $CD8^+$-enriched TILs experienced toxicities typical of those reported on prior ACT clinical trials (Table 3). All patients experienced transient toxicities from the lymphodepleting chemotherapy as well as toxicities typical of high-dose IL-2 therapy that generally resolved by 8 to 10 days after cell infusion. Patients on the unselected young TILs arm received marginally fewer IL-2 doses (Wilcoxon rank sum $P = .039$), possibly reflecting a $CD4^+$ cell response to IL-2 administration. Common grade 3 or 4 nonhematologic toxicities not related to IL-2 were similar in both arms and are summarized in Table 3. There were no treatment-related mortalities in this clinical trial.

CD4 Counts in TILs Were Associated With CD4 Levels in Peripheral Blood Lymphocytes 1 Week and 1 Month After Transfer but Were Not Associated With Response

The impact of $CD8^+$ enrichment on the number and composition of patients' reconstituting lymphocytes was quantified at 1 week and 1 month after TIL transfer. The average absolute lymphocyte count was similar in both arms immediately after TIL infusion (Fig 3A) and throughout follow-up. Absolute $CD4^+$ cell counts were similar in the two protocol arms before therapy; however, after ACT, there was a significant difference in the absolute $CD4^+$ T-cell number in peripheral blood (Fig 3B). Approximately 1 week after TIL transfer, patients who received $CD8^+$-enriched TILs had fewer $CD3^+CD4^+$ lymphocytes than patients who received unselected young TILs (60 *v* 279 $CD4^+$ cells/μL; Wilcoxon rank sum $P < .001$). At approximately 1 month after TIL infusion, the significantly decreased $CD4^+$ cell counts were still observed in patients with $CD8^+$-enriched TILs (136 *v* 219 $CD4^+$ cells/μL; Wilcoxon rank sum $P = .01$). There was no significant difference between responders and nonresponders in peripheral blood $CD4^+$ or $CD8^+$ lymphocyte subsets at any time.

Table 3. Responses to Treatment and Toxicities (as of October 1, 2012)

Variable	Grade	Unselected Young TILs		$CD8^+$ Young TILs		Total	
		No.	%	No.	%	No.	%
Patients		34		35		69	
Median follow-up, months (as of October 1, 2012)		20.8		20.7			
Responses							
No. of PRs		10		4		14	20.3
Length of PR, months		32+, 31+, 30+, 26+, 19+, 16, 10, 7, 5, 3		24+, 12, 4, 4			
No. of CRs		2		3		5	7.2
Length of CR, months		30+, 29+		33+, 22+, 11			
Total		12	35	7	20	19	28
Toxicities*							
Death		0		0		0	0
Febrile neutropenia	3	13		15		28	41
Sepsis	3	1		0		1	1
Sepsis	4	4		9		13	19
Catheter-related infection	3	2		1		3	4
Upper respiratory infection	3	1		0		1	1
Hypoxia or dyspnea	3	2		0		2	3
Dyspnea	4	1		0		1	1
Intubated for somnolence		1		1		2	3

Abbreviations: CR, complete response; PR, partial response; TIL, tumor-infiltrating lymphocyte.
*All nonhematologic grade 3 and 4 toxicities not attributable to interleukin-2 were reported once for each patient at the highest grade.

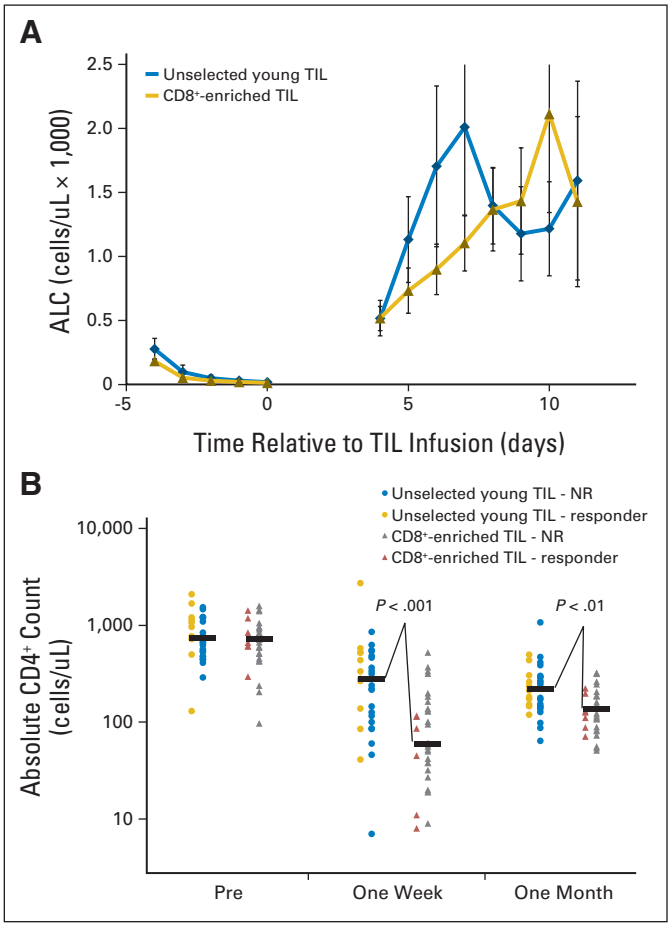

Fig 3. CD8+-enriched tumor-infiltrating lymphocytes (TILs) have an impact on peripheral blood lymphocyte recovery compared with unselected young TILs. (A) The average absolute lymphocyte count of patients on each arm is plotted over time; day 0 is the day of TIL infusion (all patients were not measured every day). Days without symbols represent fewer than 10 patients sampled. SEs of the means are shown by vertical bars. There was no significant difference in absolute lymphocyte count on any day between the randomly assigned arms. The reconstitution of neutrophils, platelets, and RBCs was also not different between the protocol arms (not shown). (B) After therapy, the absolute CD4+ lymphocyte counts in peripheral blood were significantly lower for patients receiving CD8+-enriched TILs than for patients receiving unselected young TILs. Absolute CD4+ counts for patients are shown before the start of lymphodepleting chemotherapy (Pre), at a median of 6 days (1 week) after TIL infusion (range, 5 to 14 days), and at a median of 34 days (1 month) after TIL infusion (range, 23 to 52 days). Data are not available for each patient in each category. The responding patients are jittered slightly to the left of nonresponders (NRs) to illustrate that no difference was observed between responding and nonresponding populations. Median values are indicated by a horizontal bar. Wilcoxon rank sum *P* values are shown. *P* value for Pre was not significant.

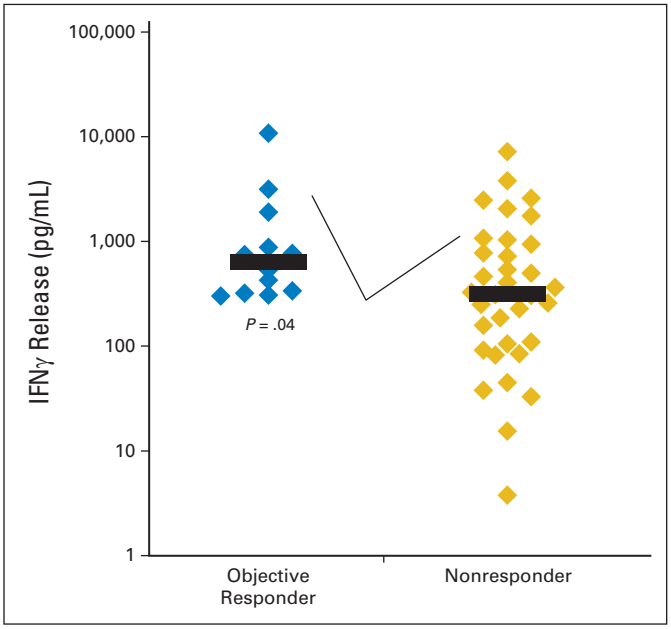

Fig 4. Tumor-infiltrating lymphocyte (TIL) release of interferon gamma (IFN-γ) in response to autologous fresh tumor is correlated with response. TILs from the infusion bag of each patient were incubated overnight with enzymatically digested fresh tumor single-cell suspension (46 patients had samples for evaluation). IFN-γ was quantified by enzyme-linked immunosorbent assay and is plotted according to the patient's clinical outcome (objective responder [partial or complete responder] *v* nonresponder). The median value for each population is plotted as a solid bar. Wilcoxon rank sum $P = .038$.

IFN-γ Secretion by TILs in Response to Autologous Fresh Tumor Was Associated With Clinical Response

Forty-six patients had fresh autologous tumor cryopreserved as a single-cell suspension, including 12 responding patients and 34 nonresponders. TILs from these 46 patients were evaluated for tumor recognition by coculture with the autologous tumor, and IFN-γ release was quantified by enzyme-linked immunosorbent assays in the supernatants. The magnitude of IFN-γ release was significantly higher in responders than in nonresponders (Wilcoxon rank sum test $P = .038$; Fig 4). We have routinely used a value of 200 pg/mL IFN release as the lower limit for specific antigen recognition of TIL cultures in past studies.[16,22] In addition, 200 pg/mL IFN release is the value set as the lower limit for lot release for TIL potency assays. Thus, we also evaluated whether clinical response was associated with secretion of greater than 200 pg/mL IFN-γ by TILs when stimulated with autologous tumor (Table 2). TILs from all 12 responders produced ≥ 200 pg/mL IFN compared with only 22 of the 34 nonresponders (Fisher's exact test $P = .02$).

TILs were also tested for recognition of HLA-A–matched melanoma cell lines (Data Supplement). TILs from 59 patients were evaluated for recognition of shared melanoma antigens expressed on tumor cell lines matched at one or more of the HLA-A*01 (n = 22), HLA-A*02 (n = 34), HLA-A*03 (n = 23), or HLA-A*24 (n = 4) loci. TILs from 30 nonresponders and 12 responders specifically recognized HLA-A locus-matched tumor cell lines, but TILS from 14 nonresponders and three responders failed to specifically recognize HLA-A locus-matched melanoma cell lines (Fisher's exact test $P = .52$). These results suggest that TILs commonly recognize HLA-restricted shared melanoma antigens such as melanocyte differentiation antigens. However, recognition of shared antigens was not associated with tumor regression in this study.

DISCUSSION

The primary goal of this study was to select the optimal TIL product for use in future ACT studies on the basis of the arm with the most responses. Patients with advanced melanoma were evaluated for clinical outcome following ACT after being randomly assigned to receive

unselected young TILs that contained $CD4^+$ lymphocytes or $CD8^+$-enriched TILs. The arms were well balanced for patient demographic factors and treatments administered. This selection design trial was not powered to detect a small impact of $CD4^+$ cells on clinical outcome, but patients who received unselected young TILs received significantly more $CD4^+$ cells compared with patients in the $CD8^+$-enriched TILs arm, and they had an increased percentage of circulating $CD3^+CD4^+$ lymphocytes in their blood for a month following TILs infusion. Despite this measurable impact on patients' immune systems, there were no significant differences between the two protocol arms in objective response, overall survival, or toxicity (except slightly less IL-2 in patients who received unselected young TILs). Interestingly, eight (53%) of 15 patients who received unselected young TILs from subcutaneous lesions responded to treatment, and more studies are warranted to investigate tumor-reactive $CD4^+$ cells, especially from this anatomic location. Overall, there were more responses in the arm that received unselected young TILs than in the arm that received $CD8^+$-enriched TILs (35% *v* 20%); furthermore, unselected young TILs are simpler and less expensive to manufacture than $CD8^+$-enriched young TILs. In addition, a prior study found no evidence of regulatory T cells in the highly activated and in vitro expanded $CD8^+$-enriched young TILs that were infused into patients.[15] Thus, this randomized trial supports the use of unselected young TILs containing $CD4^+$ cells for future ACT trials.

Objective clinical response to ACT was significantly associated only with recognition of autologous tumor ($P = .02$) and the magnitude of IFN release to autologous tumor ($P = .04$), and not with other cell infusion characteristics, patient demographics, or any other attribute we measured. In agreement with one prior study,[23] but in contrast to another,[24] this study shows that clinical response was associated only with recognition of autologous tumor and not with recognition of shared melanoma antigens. These studies may suggest that successful ACT requires an immune reaction directed at unique antigens present only on the autologous tumor and that TIL therapy could be improved through the use of an appropriate vaccine expressed by the autologous tumor,[25] or selection for autologous tumor-reactive cells.

The optimal treatment strategy for patients with melanoma is unknown,[26] and clinical trials are underway to identify the best combinations of or sequence of agents that are already approved by the US Food and Drug Administration, including vemurafenib (Genentech/Roche), ipilimumab (Bristol-Myers Squibb) or IL-2 (Hoffmann-La Roche). Will ACT with TILs ever become a standard of care?[27] Improved TIL production strategies have made individualized T-cell therapies simpler, cheaper, and faster. Here we report the first use of TILs in a randomized selection design trial. In this 16-month study, an autologous TIL product was successfully developed for 67% of the 97 eligible patients who had a tumor harvest. The use of efficient, high-density bioreactors contributed to rapid accrual, and all 69 patients who underwent random assignment received TIL treatment. Several institutions have established TIL production and explored the efficacy of ACT for patients with melanoma in clinical trials, with reported response rates for young TILs and $CD8^+$-enriched TILs of 38% to 55%.[5,7,16,17,28,29] This study is a phase II randomized study, and the overall combined response rate was 28%. The reason(s) for the lower response rate in this trial are not readily identifiable but could include lower cell numbers administered, lack of tumor-reactive lymphocytes, less effective bulk TIL culturing methods, different patient populations, or random chance. It is interesting that despite lower objective response rates, the overall survival for patients in both young TIL arms is similar to that of patients with selected TILs (Fig 2). Further randomized testing is warranted to identify the true underlying response rates for TIL therapies, and multicenter trials are a reasonable next step.

AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

The author(s) indicated no potential conflicts of interest.

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