Published OnlineFirst June 1, 2011; DOI: 10.1158/1078-0432.CCR-10-2769

Clinical Cancer Research

Impact of Clinical and Pathologic Features on Tumor-Infiltrating Lymphocyte Expansion from Surgically Excised Melanoma Metastases for Adoptive T-cell Therapy

Richard W. Joseph[1], Vijay R. Peddareddigari[1], Ping Liu[2], Priscilla W. Miller[1], Willem W. Overwijk[1], Nebiyou B. Bekele[2], Merrick I. Ross[3], Jeffrey E. Lee[3], Jeffrey E. Gershenwald[3], Anthony Lucci[3], Victor G. Prieto[4], John D. McMannis[5], Nicholas Papadopoulos[1], Kevin Kim[1], Jade Homsi[1], Agop Bedikian[1], Wen-Jen Hwu[1], Patrick Hwu[1], and Laszlo G. Radvanyi[1]

Abstract

Purpose: Clinical trials on adoptive T-cell therapy (ACT) using expanded tumor-infiltrating lymphocytes (TIL) have shown response rates of over 50% in refractory melanoma. However, little is known how clinical and pathologic features impact TIL outgrowth isolated from metastatic melanoma tumors.

Experimental Design: We analyzed the impact of clinical and pathologic features on initial TIL outgrowth in 226 consecutive patients undergoing tumor resection. Successful initial TIL outgrowth was defined as $\geq$40 million viable lymphocytes harvested from all tumor fragments in a 5-week culture. To normalize for the different size of resected tumors and thus available tumor fragments, we divided the number of expanded TIL by the starting number of tumor fragments (TIL/fragment).

Results: Overall, initial TIL outgrowth was successful in 62% of patients, with patients $\leq$30 years of age (94%; $P = 0.01$) and female patients (71% vs. 57% for males; $P = 0.04$) having the highest rate of success. Systemic therapy 30 days before tumor harvest negatively impacted initial TIL outgrowth compared to patients who never received systemic therapy (47% vs. 71%, $P = 0.02$). Biochemotherapy within 0 to 60 days of tumor harvest negatively impacted the initial TIL outgrowth with a success rate of only 16% ($P < 0.0001$).

Conclusion: Parameters such as age, sex, and the type and timing of prior systemic therapy significantly affect the success rate of the initial TIL outgrowth from tumor fragments for ACT; these parameters may be helpful in selecting patients for melanoma ACT. *Clin Cancer Res; 17(14); 4882–91. ©2011 AACR.*

Introduction

Metastatic melanoma is one of the most immunogenic cancers, and adoptive T-cell therapy (ACT) using expanded tumor-infiltrating lymphocytes (TIL) has shown great promise as an effective therapy. Since the introduction of ACT in 1988 (1), changes in the preparation regimens and expansion of T cells have produced clinical response rates as high as 51% to 72% (2–6).

One of the major limitations of ACT is the ability to generate TIL from surgically resected tumor fragments with a success rate ranging from 31% to 94% (4, 7–10). A critical first step in generating TIL is the initial outgrowth of lymphocytes from the first 5 weeks in culture. Although there is no definitive cut-off that defines a successful initial outgrowth, we have found that at least 40×10^6 TIL are necessary to move forward with the subsequent large-scale expansion. Given the time- and resource-intensive nature of this therapy, its associated morbidity and cost, and the availability of alternative treatment options for patients with metastatic melanoma, parameters that would predict a successful initial TIL outgrowth could be used as a tool for patient selection and treatment prioritization.

Here, we have determined what patient's clinical characteristics, primary tumor characteristics, and types of prior systemic therapy affect the rate of TIL expansion or TIL antitumor reactivity.

Patients and Methods

Patient selection

Patients with stage IV melanoma, stage III in-transit disease, or recurrent regional nodal disease were enrolled following informed consent. Some patients underwent

Authors' Affiliations: Departments of [1]Melanoma Medical Oncology, [2]Biostatistics, [3]Surgical Oncology, [4]Pathology, and [5]Stem Cell Transplant and Cellular Therapy, The University of Texas, M.D. Anderson Cancer Center, Houston, Texas

Corresponding Authors: Laszlo G. Radvanyi, Department of Melanoma Medical Oncology, University of Texas, M.D. Anderson Cancer Center, Houston, TX 77030. Phone: 713-563-9489; Fax: 713-563-3424; E-mail: lradvanyi@mdanderson.org and Patrick Hwu, Department of Melanoma Medical Oncology, University of Texas, M.D. Anderson Cancer Center, Houston, TX 77030. Phone: 713-563-1727; Fax: 713-563-3424; E-mail: phwu@mdanderson.org

doi: 10.1158/1078-0432.CCR-10-2769

©2011 American Association for Cancer Research.

Downloaded from clincancerres.aacrjournals.org on June 12, 2021. © 2011 American Association for Cancer Research.

Published OnlineFirst June 1, 2011; DOI: 10.1158/1078-0432.CCR-10-2769

Translational Relevance

At present, the treatment of metastatic melanoma is limited to only three FDA approved regimens: bolus high-dose IL-2, dacarbazine, and ipilimumab. Although still experimental, adoptive cell therapy (ACT) using tumor-infiltrating lymphocytes (TIL) derived from metastatic tumor tissue has produced response rates greater than 50%. However, one of the major limitations in ACT is that TIL cannot always be expanded to adequate numbers from all patients for therapeutic use. Our study addressed this issue by determining how clinical characteristics of the patient, including the type of prior systemic therapy, impacts the rate of successful initial TIL outgrowth. We found that some of these parameters, especially prior systemic therapy other than high-dose IL-2, negatively affected successful initial TIL outgrowth. Our data should help guide clinicians on choosing when to refer a metastatic melanoma patient for a tumor resection for the purpose of TIL expansion for ACT.

multiple tumor resections to generate TIL, but to most accurately define the success rate of the initial TIL outgrowth, we only included the first tumor harvest. Tissue from metastatic surgical resections was used to expand TIL under an IRB-approved protocol (LAB06-0755) approved by the Institutional Review Board of University of Texas MD Anderson Cancer Center.

Defining successful initial TIL outgrowth, TIL/fragment, and antitumor reactivity

Tumor fragments were processed and TIL expanded according to previously published methods (3). Briefly, 3 to 5 mm^2 tumor fragments (range of 4–48 tumor fragments) were placed into 24-well plates in 2 mL of culture medium containing 6,000 IU/mL of IL-2 (Proleukin), and after 5 weeks the viable TIL were counted. The cells cultured from this initial outgrowth were then used to do a large-scale cell expansion using a rapid expansion protocol (REP) to generate the final TIL infusion product. After multiple preclinical studies, we determined 40×10^6 TIL to be the minimal number necessary for the subsequent REP to expand an adequate number of T cells (ranging from 20 to 150 billion) for the final GMP-grade TIL infusion product for an ongoing ACT clinical trial at M.D. Anderson Cancer Center (NCT00338377). The size of resected tumor and number of tumor fragments used to generate TIL varied, and to normalize for this difference we divided the total viable TIL by the number of tumor fragments to create a ratio of TIL/tumor fragment (TIL/fragment). Values of TIL/fragment are shown as a power of 10^6, unless otherwise indicated. Antitumor reactivity of TIL against melanoma was determined in samples with successful TIL expansion by measuring IFN-γ (≥400 pg/mL by ELISA) in culture supernatants collected from triplicate 24-hour cocultures of 1×10^5 TIL with 1×10^5 autologous or histocompatibility leukocyte antigen (HLA) class I-matched allogeneic melanoma lines in 96-well plates.

Assessment of clinical and pathologic characteristics

Clinical and pathologic data were retrospectively obtained from the patient record, including age, gender, biopsy site, melanoma histological type, primary tumor location, serum lactate dehydrogenase (LDH) at the time of tumor resection, and prior treatment history. LDH was determined before tumor resection using a CLIA-certified assay as part of the routine clinical monitoring of patients in the Department of Laboratory Medicine at MD Anderson Cancer Center. A level of ≥618 IU/L was considered as elevated, and values below this were considered as normal.

Prior therapy assessment

We categorized patients based on the last systemic therapy before tumor harvest. We categorized prior systemic therapy into five groups: IL-2, other immunotherapy, chemotherapy, biochemotherapy, and targeted therapy. Prior radiation was not included as a prior systemic therapy. "Other immunotherapy" was defined as IFN-α, anti-CTLA-4, vaccine, or GM-CSF including if given in the adjuvant setting. Chemotherapy included all cytotoxic agents including isolated limb perfusion with melphalan. Biochemotherapy was defined as the combination of cytotoxic chemotherapy with either IL-2 or IFN-α. Targeted agents included mTOR and tyrosine kinase inhibitors when given not in combination with chemotherapy. Finally, we included patients who received adjuvant therapy, including interferon, in the previous treated groups.

To assess how the timing of the last systemic therapy affected initial TIL outgrowth and TIL/fragment we categorized patients based on the time from the last systemic therapy to tumor harvest into the following four time periods: 0–30 days, 31–60 days, 61–90 days, and >90 days.

Pathology review and mutational analysis

Pathologic information on the primary tumor was available on a subset of patients. We used the pathologist review in determining ulceration, mitotic figures, Breslow depth, presence of TIL, and histology of the primary tumor. A subset of our patients underwent testing for mutational analysis in B-RAF, N-RAS, or C-KIT. Mutations were evaluated by CLIA-certified pyrosequencing of B-RAF (exon 15), N-RAS (codons 12, 13, and 61), and C-KIT (exons 11, 13, and 17) as previously reported (11). Patients were considered to be WT/WT if they did not harbor a B-RAF or a N-RAS mutation.

Statistical analysis

The success rate of the initial TIL outgrowth was correlated with clinical and pathologic parameters using the

Downloaded from clincancerres.aacrjournals.org on June 12, 2021. © 2011 American Association for Cancer Research.

Published OnlineFirst June 1, 2011; DOI: 10.1158/1078-0432.CCR-10-2769

Table 1. Impact of patient characteristics on the success rate of the initial TIL outgrowth and TIL/fragment

Category	All samples	Samples with >40 × 10^6 TIL	P^a	Mean TIL/fragment (×10^6)	P^b
All patients	226	139 (62)[c]	–	12.84	–
Age			**0.01**		0.19
14–30	16	15 (94)		21.23	
31–45	55	34 (62)		13.11	
46–60	116	72 (62)		12.82	
61–75	39	18 (46)		9.07	
Gender			**0.04**		0.20
Female	76	54 (71)		15.09	
Male	150	85 (57)		11.69	
Stage			0.89		0.21
III	76	46 (61)		15.06	
IV	150	93 (62)		11.71	
LDH			0.35		0.70
Normal	150	93 (62)		13.46	
Elevated	59	32 (54)		12.30	

[a]Fisher's exact test was used. *P* values below 0.05 are in bold.
[b]Wilcoxon rank-sum test or Kruskal–Wallis test was used.
[c]Numbers in parentheses indicate percentage successful TIL growers based on total number in that group shown in the All samples column.

Fisher's exact test. Wilcoxon rank-sum test or Kruskal–Wallis test were used to compare TIL/fragment values between or among clinical and pathologic groups. When correlating LDH to TIL/fragment we used Pearson's correlation coefficient. A *P* value less than 0.05 was considered as statistically significant. All tests were done using SAS 9.2 by SAS Institute, Inc.

Results

Influence of patient characteristics on initial TIL outgrowth and TIL/fragment

In total, 226 patients (76 female and 150 male) with a median age of 51 years (range 14–70 years) were enrolled in the study. The overall success rate of the initial TIL outgrowth was 62% ($n = 139$). As shown in Table 1, successful outgrowth was associated with a younger age (49.5 vs. 52.7 years; $P = 0.03$) with patients in the youngest subgroup (14–30 years) having the highest success rate (94%). Females were more likely to have a successful TIL outgrowth than males (71% vs. 57%; $P = 0.04$). Neither disease stage nor LDH (normal or elevated) at the time of resection impacted the success rate of the initial TIL outgrowth. The median number of tumor fragments used to generate TIL was 12 (mean 13.5; range 4–48), and patients with a successful outgrowth actually had a lower mean number of tumor fragments (12.97 vs. 14.38; $P = 0.03$).

Table 1 also shows the mean number of TIL per fragment according to age, gender, stage, and LDH status. The mean number of TIL/fragment was 12.84 (median 5.5 × 10^6; range 0–123.7 × 10^6) with none of the indicated parameters affecting TIL/fragment yield. However, when analyzed in a linear fashion elevated LDH significantly inversely correlated with TIL/fragment ($P = 0.03$; data not shown).

In some cases, the TIL cryopreserved after expansion from tumor fragments were thawed and further expanded using the REP. TIL from 22 of the 139 patients for whom initial TIL outgrowth was successful had their TIL further expanded using the REP (3). Among these, all TIL successfully underwent secondary expansion in the REP with an average fold-expansion of 1,665-fold ±677 (range of 359- to 2,660-fold; median of 1,656-fold).

Influence of primary tumor characteristics and resection site on successful TIL generation and TIL/fragment

Information on the primary tumor was available for a subset of our patients, and we correlated the features of the primary tumor with the success rate of initial TIL outgrowth and TIL/fragment (Table 2). The pathologic features of the primary, the location, the subtype, and the mutation status did not impact the rate of successful initial TIL outgrowth (Table 2). Patients with brisk TIL in the primary tumor ($n = 2$) had a 100% success rate in generating TIL, and conversely none of the patients with absent TIL in primary ($n = 3$) had successful TIL expansion. Patients with a mucosal melanoma primary ($n = 7$) had the highest percentage (86%) of successful TIL outgrowth;

Downloaded from clincancerres.aacrjournals.org on June 12, 2021. © 2011 American Association for Cancer Research.

Published OnlineFirst June 1, 2011; DOI: 10.1158/1078-0432.CCR-10-2769

Table 2. Impact of primary tumor and resection site on the success rate of the initial TIL outgrowth and TIL/fragment

Category	All samples	Samples with >40 × 10^6 TIL	P^a	Mean TIL/fragment (×10^6)	P^b
Mitotic figures			0.51		**0.02**
<5.0	45	30 (67)[c]		18.93	
>5.0	45	26 (58)		8.22	
Ulceration			0.56		0.34
Yes	58	36 (62)		11.43	
No	57	39 (68)		15.11	
Breslow			0.77		0.14
<1.0 mm	33	21 (64)		18.06	
1.01–2.0 mm	35	21 (60)		12.70	
2.01–4.0 mm	43	27 (63)		10.60	
>4.0 mm	29	15 (52)		6.89	
TIL presence			**0.04**		0.28
Non-brisk	89	57 (64)		13.5	
Absent	3	0		0	
Brisk	2	2 (100)		31.2	
Primary site			0.10		0.52
Skin	172	103 (60)		12.70	
Unknown	31	23 (74)		15.98	
Acral	11	6 (55)		13.96	
Mucosal	7	6 (86)		9.04	
Choroid	5	1 (20)		0.73	
Skin			0.30		0.09
Torso	66	43 (65)		10.01	
Lower limbs	42	20 (48)		12.22	
Face/neck/scalp	38	23 (61)		12.11	
Upper limbs	26	17 (65)		21.20	
Skin histology			0.08		0.46
Superficial spreading	52	34 (65)		13.74	
Nodular	34	19 (56)		11.56	
Unclassified	16	13 (81)		19.49	
Lentigo maligna	5	1 (20)		1.27	
Desmoplastic	2	2 (100)		17.63	
Mutation tested			0.17		0.59
B-RAF	62	43 (69)		16.63	
N-RAS	21	10 (48)		19.50	
WT/WT	15	9 (60)		12.58	
C-KIT	3	3 (100)		30.50	
TIL biopsy site			0.05		0.39
Skin/subcutaneous	86	45 (52)		10.22	
Lymph node	75	48 (64)		14.85	
Viscera	56	41 (73)		13.34	
More than 1 site	5	4 (80)		23.25	
Brain	4	1 (25)		11.08	

[a]Fisher's exact test was used. *P* values below 0.05 are in bold.
[b]Wilcoxon rank-sum test or Kruskal–Wallis test was used.
[c]Numbers in parentheses indicate percentage successful TIL growers based on total number in that group shown in the All samples column.

Downloaded from clincancerres.aacrjournals.org on June 12, 2021. © 2011 American Association for Cancer Research.

Published OnlineFirst June 1, 2011; DOI: 10.1158/1078-0432.CCR-10-2769

however, the numbers were too small to be statistically significant. Patients who had a visceral tumor resected ($n = 56$) had the highest rate of successful initial TIL outgrowth (73% vs. 52%, $P = 0.05$).

The only aspect of the primary tumor that correlated with TIL/fragment was mitotic figures. Information on the mitotic figures of the primary was available for 90 patients with the median number of mitotic figures per mm^2 being 5 (Table 2). Patients whose primary tumor had greater than the median number of mitotic figures correlated with less TIL/fragment when compared with patients whose primary tumor had less than the median mitotic figures (8.2×10^6 vs. 18.9×10^6, respectively; $P = 0.02$).

Impact of last systemic therapy and the timing of last systemic therapy before TIL harvest on TIL generation and TIL/fragment

Table 3 summarizes our analysis of all 226 TIL expansion attempts based on prior systemic therapy before the tumor harvest. The majority of patients (69%) received systemic therapy before TIL harvest, and when grouped as a whole, patients who received prior systemic therapy (Table 3, Prior systemic therapy) had a statistically insignificant lower rate of initial TIL outgrowth (71% vs. 57%, $P = 0.06$), but a significant decrease in TIL/fragment (11.53 vs. 15.82×10^6, $P = 0.02$) when compared to patients who did not receive prior systemic therapy. We then analyzed the impact of the type of systemic therapy anytime before tumor harvest (Table 3, Last systemic therapy before resection) and found that biochemotherapy negatively impacted both the success rate of the initial TIL outgrowth and yield of TIL/fragment ($P = 0.003$ in both cases). Targeted therapy seemed to have a negative impact on TIL/fragment but not the success rate of the initial TIL outgrowth. However, the number of samples was too small to make any definitive conclusions. Figure 1 further illustrates this negative effect of prior biochemotherapy on the yield of TIL per tumor fragment of each patient. We found no difference in the mean or median number of tumor fragments placed in culture between the different pretreatment groups.

The timing of prior systemic therapy also significantly impacted the success rate of the initial outgrowth and TIL/fragment (Table 3, Impact of timing of last systemic therapy). Patients who received systemic therapy within 30 days of tumor harvest had the lowest rate of successful initial TIL outgrowth and the least TIL/fragment when compared to patients who did not receive prior systemic therapy (47% success rate, $P = 0.02$; 7.48×10^6 TIL/fragment, $P = 0.004$). Further analysis of the specific type

Table 3. Effect of the last systemic therapy on the success rate of the initial TL outgrowth and TIL/fragment

Category	Number of samples	Samples with $>40 \times 10^6$ TIL	P^a (versus no previous therapy)	Mean TIL/fragment ($\times 10^6$)	P^b (versus no previous therapy)
Prior systemic therapy					
No	69	49 (71)[c]	–	15.82	–
Yes	157	90 (57)	0.06	11.53	**0.02**
Last systemic therapy anytime before resection					
IL-2	26	17 (65)	0.62	12.49	0.58
Other Immunotherapy	47	30 (63)	0.43	17.80	0.55
Biochemotherapy	32	13 (41)	**0.003**	5.96	**0.003**
Chemotherapy	48	29 (60)	0.24	9.43	0.05
Targeted	4	1 (25)	0.08	1.17	**0.04**
Impact of timing of last systemic therapy					
Day 0–30	43	20 (47)	**0.02**	7.48	**0.004**
Day 31–60	38	21 (55)	0.14	11.62	0.06
Day 61–90	20	12 (60)	0.42	9.12	0.15
>90 days	56	37 (66)	0.57	15.44	0.60
Therapy within 0–30 days					
IL-2	7	4 (57)	0.43	7.36	0.30
Other Immunotherapy	5	2 (40)	0.17	3.08	0.08
Biochemotherapy	9	1 (11)	**0.0009**	3.27	**0.006**
Chemotherapy	19	13 (68)	1.0	11.8	0.40
Targeted	3	0 (0)	**0.03**	0	**0.03**

[a]Fisher's exact test was used. *P* values below 0.05 are in bold.

[b]Wilcoxon rank-sum test or Kruskal–Wallis test was used.

[c]Numbers in parentheses indicate percentage successful TIL growers based on total number in that group shown in the Number of samples column.

Downloaded from clincancerres.aacrjournals.org on June 12, 2021. © 2011 American Association for Cancer Research.

Published OnlineFirst June 1, 2011; DOI: 10.1158/1078-0432.CCR-10-2769

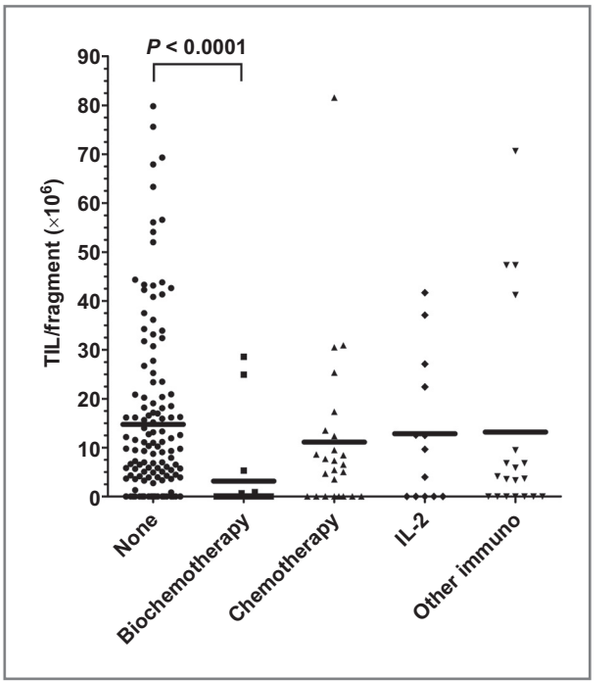

Figure 1. Systemic biochemotherapy within 60 days of tumor harvest significantly impacts the growth of TIL. Tumors were harvested from metastatic melanoma patients who had received the indicated forms of prior therapy within 60 days of surgery. The tumors were cut up into 3 to 5 mm^2 pieces (fragments) and cultured with IL-2 for 5 weeks in 24-well plates as described in Patients and Methods. Patients who received biochemotherapy within 60 days had a lower total yield of TIL/fragment as compared to those who had no prior therapy. IL-2, other immunotherapies, or chemotherapy did not affect TIL/fragment.

of systemic therapy within 30 days before tumor harvest (Table 3, Therapy within 0–30 days) revealed that biochemotherapy and targeted therapy negatively impacted the initial TIL outgrowth and TIL/fragment. It must be noted however that only a few samples ($n = 3$) were in the "Targeted" category. For this reason, this patient subgroup was omitted from further analysis.

Table 4 individually summarizes the effect of the timing of the last systemic therapy given before tumor resection. When compared to patients who received no priory systemic therapy, biochemotherapy given 0 to 30 days or 31 to 60 days before tumor harvest negatively impacted the success rate of the initial outgrowth (11% and 20%, respectively) and TIL/fragment. However, when given beyond 60 days, and especially beyond 90 days, biochemotherapy no longer negatively affected the success rate of the initial outgrowth or TIL/fragment. There were no time points when IL-2, other immunotherapy, and chemotherapy negatively impacted the success rate of the initial TIL outgrowth or TIL/fragment.

Reactivity of TIL

We also tested the antitumor reactivity of TIL in 128 of the 139 patients successfully generating the minimal 40×10^6 TIL (92% of successful growers). The TIL were tested for reactivity against either autologous (when available) or HLA class I–matched melanoma tumor cell lines using IFN-γ release assays. Overall, 77/128 (60%) of the patients tested for TIL reactivity had reactive TIL (see Patients and Methods for reactivity criteria). Of note, this could be an underestimate of reactivity, because assays done in the absence of an available autologous tumor line could miss unique antigens as well as antigens restricted by alternative MHC alleles. We did not find any correlations between the clinical characteristics, primary tumor characteristics, or previous treatment with TIL reactivity (data not shown).

Success rate of TIL expansion from second tumor harvest

The success rate of expanding TIL from tumor fragments from a first resection ($\geq 40 \times 10^6$ TIL yield after 5 weeks) was 62%. Fourteen patients whose initial TIL outgrowth was unsuccessful underwent a second tumor harvest. Five (36%) of these 14 patients successfully generated TIL on the second harvest and none of these patients had received systemic therapies within 60 days before the second harvest. Five of the 9 patients (55%) who did not have a successful initial TIL outgrowth on the second harvest received systemic therapy within 60 days of the second tumor harvest.

Discussion

The purpose of this analysis was to understand what clinical or pathologic characteristics impact successful TIL generation when anticipating treating a metastatic melanoma patient with ACT. An understanding of how parameters affect successful TIL expansion could be used to better select patients who would generate enough TIL for ACT. Our key findings were that younger and female patients were more likely to successfully generate TIL, and that certain types of prior therapy (biochemotherapy in particular) could negatively impact the ability to expand enough TIL for ACT. We also found that the timing of the type of systemic therapy significantly impacted the success of TIL growth, with the negative effects of biochemotherapy waning when given later than 60 days before tumor harvest for TIL expansion.

Our institution was able to successfully generate TIL in 62% of all patients on the first tumor harvest, in line with other published reports ranging from 34% to 94% (7–10). All attempts at secondary expansion were successful, with an average of 1,665-fold expansion of the cells after the REP. A number of other recent studies have reported somewhat higher success rates for expanding TIL from resected metastatic melanomas. For example, Goff and colleagues (8) reported a 94% success rate, Nguyen and colleagues (9) reported a 72% success rate, and Besser and colleagues (10) reported a 97% success rate. There are a number of caveats in comparing the TIL expansion success rates across different institutions. First, the cut-off for successful initial TIL outgrowth

Downloaded from clincancerres.aacrjournals.org on June 12, 2021. © 2011 American Association for Cancer Research.

Published OnlineFirst June 1, 2011; DOI: 10.1158/1078-0432.CCR-10-2769

Table 4. Effect of the timing of the last systemic therapy before tumor harvest on the success rate of the initial TIL outgrowth and TL/fragment

Category	Number of samples	Samples with >40 × 10^6 TIL	P^a (versus no previous therapy)	Mean TIL/fragment (×10^6)	P^b (versus no previous therapy)
Biochemotherapy					
Day 0–30	9	1 (11)[c]	**0.0009**	3.28	**0.006**
Day 31–60	10	2 (20)	**0.003**	3.10	**0.006**
Day 61–90	3	2 (67)	1.0	5.94	0.48
>90 days	10	8 (80)	0.72	11.26	0.77
IL-2					
Day 0–30	7	4 (57)	0.43	7.36	0.30
Day 31–60	6	4 (67)	1.0	19.25	0.77
Day 61–90	4	3 (75)	1.0	7.99	0.59
>90 days	9	6 (67)	1.0	13.96	0.97
Other immunotherapy					
Day 0–30	5	2 (40)	0.17	3.08	0.08
Day 31–60	8	6 (75)	1.0	26.79	0.51
Day 61–90	5	3 (60)	0.63	18.47	0.90
>90 days	29	19 (66)	0.64	17.75	0.64
Chemotherapy					
Day 0–30	19	13 (68)	1.0	11.85	0.40
Day 31–60	13	8 (62)	0.52	5.84	0.06
Day 61–90	8	4 (50)	0.25	5.02	0.12
>90 days	8	4 (50)	0.25	13.94	0.71

[a]Fisher's exact test was used. *P* values below 0.05 are in bold.

[b]Wilcoxon rank-sum test or Kruskal–Wallis test was used.

[c]Numbers in parentheses indicate percentage successful TIL growers based on total number in that group shown in the Number of samples column.

differs between institutions. We defined a successful initial outgrowth as 40 × 10^6 TIL while others used 5 × 10^6 (8) and 30 × 10^6 (9, 10). Using different thresholds to define successful initial TIL outgrowth will obviously impact the success rate. Second, the methods to isolate and grow TIL differ between centers, with some studies expanding TIL from single cell suspensions from enzymatic tumor digests, tumor fragments, or a mixture of both tumor fragments and cell suspensions from tumor enzymatic digests (8–10). Third, the prior therapy before tumor resection, as we have shown here, can affect the outcome, and it is likely that the type and timing for prior therapy before tumor harvest differed among the institutions. Finally, in the study by Goff and colleagues (8) from the NCI (Bethesda, MD), the reported success rate of initial TIL expansion from the tumor included a significant number of patients that had more than one attempt (two–three) to grow TIL after surgery. These issues point to the need to further optimize and then standardize TIL expansion methodologies across centers to more accurately gauge the rate of successful TIL expansion and the effects of patient clinical and pathological characteristics on TIL growth across different centers.

Until this analysis, age and gender had not been identified as a factor in TIL expansion success. The reason why younger patients were more successful at growing TIL ($P = 0.01$) is unclear, and one of the following could play a role: higher telomere lengths in lymphocytes from younger people (12, 13), the higher proportion of naive T cells at the onset of disease (14), or age-associated decrease in T-cell diversity and decline in immune function with older patients harboring more differentiated effector-memory and effector cells with a shorter lifespan. A younger patient age has also been associated with longer TIL persistence after infusion into patients undergoing ACT (15, 16). The reason why female patients were more successful in generating TIL is also unknown, but it could be related to estrogen's inhibitory effect on T-suppressor cells and stimulatory effect on T-helper cells (17), or the increased incidence of autoimmune disease such as lupus (18) or rheumatoid arthritis (19) in females. Lower androgen levels in females may also play a role in regulating stronger T-cell responses, as androgen ablation has been found to have positive immunoregulatory effects (20). The ability to expand adequate TIL from tumor biopsies could also be a functional biomarker indicating the relative strength of a patient's immune system against their disease

Downloaded from clincancerres.aacrjournals.org on June 12, 2021. © 2011 American Association for Cancer Research.

Published OnlineFirst June 1, 2011; DOI: 10.1158/1078-0432.CCR-10-2769

or the directing of an immune response to a larger array of self antigens in females and perhaps explain why female melanoma patients overall have longer survival than males (21). It is also possible that a host of genetic factors, such as the extent and polymorphism in immune system-related factors (e.g., HLA subtypes, lymphocyte signaling molecules, innate and adaptive immune system cytokines, and immunosuppressive factors) play a role in modulating the parameters measured here.

Characteristics of the primary tumor are well known to predict outcomes in patients with melanoma (22, 23). Ulceration, high mitotic rate, and greater Breslow thickness are associated with a higher stage and worse survival (22, 23). Although the tumor specimens taken for TIL harvest were not from the primary tumor, we hypothesized that the pathologic factors of the primary tumor that lead to a poorer prognosis might also influence the ability to successfully generate TIL or the number of TIL per tumor fragment later on during the disease course. Although none of the factors of the primary tumor were associated with the success rate of growing TIL (getting at least 40×10^6 TIL), high mitotic rate was associated with an expansion of fewer TIL/fragment. The etiology of these findings is unclear, but one hypothesis is that metastatic tumors that originate from a primary with a higher mitotic rate might lead to a higher proliferating, more aggressive metastatic tumor that is better able to create an immunosuppressive environment and thus decrease the ability of T cells to proliferate and/or persist in the tumor microenvironment (24, 25). Tumors expressing higher T-cell inhibitory ligands, such as B7H1 (PD-1 ligand) for example, have been shown be more aggressive and suppress T-cell activation and T-cell function (26, 27). It is also possible that more aggressive, faster dividing tumors attract higher numbers of $CD4^+$ $Foxp3^+$ regulatory T cells that suppress T cell growth and survival in the tumor microenvironment.

The location of the primary tumor is well known to be associated with different mutations suggesting a different biology of the tumors (28). Although the tumor used to harvest TIL came from a metastatic and not the primary site, we examined if the location of the primary tumor influenced either successful TIL generation or the total number of TIL per fragment generated. However, we did not find any statistically significant association between the site of the primary tumor and the success rate of expanding TIL as well as TIL per fragment. Of note, acral and mucosal melanomas are known to have the highest frequency of C-KIT mutations (11), and all 3 patients in our study who had a C-KIT mutation successfully generated TIL and had the highest mean and median TIL/fragment. However, our numbers of C-KIT mutant patients were low and this needs to be confirmed with a larger sample size. Another parameter we examined is whether the location of the metastatic tumor used to grow TIL from was associated with the quality and quantity of TIL expansion. Again, as found by other investigators such as Goff and colleagues (8), we did not find any effect of the tumor location for TIL growth and these parameters. This is actually good news for ACT because conceivably patients with metastases in any location in the body would be eligible for TIL expansion and therapy. One location that may be problematic however is brain metastases. We were successful in only one out of four attempts to expand TIL from brain metastases (Table 2). Most of our tumors have been from lymph node, subcutaneous, and other visceral sites. It is noteworthy that we have successfully expanded TIL in 3 out of 5 small bowel metastases after carefully dissecting out the non-lumenal portion of the tumor, suggesting that this is also a viable option if bacterial or fungal contamination can be avoided (the two cases that did not grow were contaminated).

One of the most evident parameters affecting the success rate of TIL generation and TIL/fragment was the type and timing of systemic therapy before tumor resection. Patients, who received either no prior therapy, or no therapy within 60 days of TIL harvest, had the highest rate of TIL generation with 71% and 64%, respectively. Patients who received either IL-2 or other immunotherapy as their last systemic therapy before tumor harvest at any time point before tumor harvest had similar rates of TIL success and TIL/fragment as patients who had no prior systemic therapy. Interestingly, in our cohort of patients, immunotherapy did not significantly increase the yield of TIL per tumor fragment relative to no prior therapy even when given within 30 days of the tumor harvest. Although this data needs to be followed up by monitoring future patients receiving immunotherapies such as IL-2, anti-CTLA4, vaccines, and other immunopotentiators, these results suggest prior immunotherapy with IL-2, for example, is not necessary to successfully expand TIL to large numbers for ACT; again, good news for centers contemplating doing ACT, but who do not do these types of immunotherapy.

Perhaps one of the most interesting findings of our study was that biochemotherapy was the only form of prior therapy that had deleterious effects on successful generation of TIL and the yield of TIL per tumor fragment, whereas other forms of chemotherapy without IL-2 and IFN-α (including limb perfusion with melphalan, and systemic therapy with Abraxane, dacarbazine, paclitaxel, vinblastine, and cisplatin) had very little impact. Studies have shown that the recovery of T cells after cytotoxic chemotherapy takes weeks to months (29), and we would have expected that patients who received both biochemotherapy or chemotherapy within 0 to 60 days to have a decreased rate of successful expansion. Little is known about the effects of chemotherapy and biochemotherapy on TIL and whether any particular chemotherapy selects or spares TIL in comparison to other lymphocytes. In addition, at present it is unclear why biochemotherapy in particular was especially deleterious. It is possible that the immune activation induced by IL-2 and IFN-α, may drive T cells into cell cycle where they become susceptible to the chemotherapy agents of the biochemotherapy regimen (cisplatin, vinblastine, and either dacarbazine or temozolomide). Although biochemotherapy was

Downloaded from clincancerres.aacrjournals.org on June 12, 2021. © 2011 American Association for Cancer Research.

Published OnlineFirst June 1, 2011; DOI: 10.1158/1078-0432.CCR-10-2769

deleterious to TIL expansion, this effect seemed to disappear when the therapy was given over 60 days before tumor harvest for TIL growth. Thus, we would recommend that patients do not receive biochemotherapy within 60 days before tumor harvest if this is clinically feasible. Unexpectedly, patients pretreated with targeted therapies had a significantly lower rate of successful TIL generation and TIL/fragment. However, the number of patients treated with targeted therapy 2 months before tumor harvest ($n = 4$) was small making it difficult to draw conclusions about the effect of any one specific targeted agent on TIL growth.

Clinically, there remains a question of whether a second attempt at TIL generation will be successful if an initial attempt fails. Fourteen patients whose first tumor resection did not successfully expand TIL underwent a second tumor resection, and 5/14 (36%) were able to generate an adequate number of TIL from the second harvest. All 5 patients who generated TIL from the second harvest did not receive systemic therapy within two months before the second harvest, whereas 5/9 (55%) of the patients who did not generate TIL on the second harvest underwent systemic therapy 60 days before their second tumor harvest. This further suggests that systemic therapy within 60 days of tumor resection is a powerful inhibiting factor in the generation of TIL. Although there is likely an intrinsic yet unknown biology of who is capable of growing TIL or not, the absence of systemic treatment 60 days before resection is the most powerful predictor of successful TIL generation, and if possible we recommend TIL harvest either occur before receiving systemic therapy or waiting at least 2 months after receiving therapy.

In summary, our results have shown that TIL can be successfully expanded from isolated tumor fragments in culture in 62% of cases in an unselected group of metastatic melanoma patients. The most important conclusion was that systemic therapy can adversely impact TIL expansion, especially when given within 60 days before tumor harvest, and therefore, we would recommend surgery or biopsies for TIL expansion be done 60 days after systemic therapy is given. It appears that younger patients and female patients have the best chances of generating TIL, and patients who receive either biochemotherapy 2 months before resection have the worst chances of expanding TIL to adequate numbers for ACT. If validated in a test set of patients, a combination of these parameters may be a powerful selection tool to decide which patients will likely have a successful TIL expansion for ACT.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Acknowledgments

We thank all participating patients, nurses, laboratory scientists (especially Chantale Bernatchez), and data coordinators for help on this project. We especially like to extend our deepest gratitude to all of our TIL cell therapy specialists who culture the cells day-to-day: Rahmatu Mansaray, Orenthial J. Fulbright, Chris Toth, Renjith Ramachandran, Chris Toth, and Seth Wardell.

Grant Support

This work was funded by NIH/NCI grants (1RO1 CA111999-01A2). We are also grateful for support from the Dr. Miriam and Sheldon Adelson Medical Research Foundation (AMRF). In addition, we thank the American Society of Clinical Oncology who in partly funded this work through the ASCO Young Investigator Award.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked *advertisement* in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

Received October 19, 2010; revised April 12, 2011; accepted April 29, 2011; published OnlineFirst June 1, 2011.

References

1. Rosenberg SAPB, Aebersold PM, Solomon D, Topalian SL, Toy ST, Simon P, et al. Use of tumor infiltrating lymphocytes and interleukin-2 in the immunotherapy of patients with metastatic melanoma. N Engl J Med 1988;319:1676–80.
2. Dudleg JC, Wunderlich JR, Yang JC, Hwu P, Schwartzentruber DJ, Topalian SL, et al. A phase I study of nonmyeloablative chemotherapy and adoptive transfer of autologous tumor antigen-specific T lymphocytes in patients with metastatic melanoma. J Immunother 2002;25:243–51.
3. Dudley ME, Wunderlich JR, Shelton TE, Even J, Rosenberg SA. Generation of tumor-infiltrating lymphocyte cultures for use in adoptive transfer therapy for melanoma patients. J Immunother 2003; 26:332–42.
4. Rosenberg SA, Dudley ME. Cancer regression in patients with metastatic melanoma after the transfer of autologous antitumor lymphocytes. Proc Natl Acad Sci U S A 2004;101(Suppl 2):14639–45.
5. Dudley ME, Wunderlich JR, Yang JC, Sherry RM, Topalian SL, Restifo NP, et al. Adoptive cell transfer therapy following non-myeloablative but lymphodepleting chemotherapy for the treatment of patients with refractory metastatic melanoma. J Clin Oncol 2005;23:2346–57.
6. Dudley ME, Yang JC, Sherry R, Hughes MS, Royal R, Kammula U, et al. Adoptive cell therapy for patients with metastatic melanoma: evaluation of intensive myeloablative chemoradiation preparative regimens. J Clin Oncol 2008;26:5233–9.
7. Schiltz PM, Beutel LD, Nayak SK, Dillman RO. Characterization of tumor-infiltrating lymphocytes derived from human tumors for use as adoptive immunotherapy of cancer. J Immunother 1997;20:377–86.
8. Goff SL, Smith FO, Klapper JA, Sherry R, Wunderlich JR, Steinberg SM, et al. Tumor infiltrating lymphocyte therapy for metastatic melanoma: analysis of tumors resected for TIL. J Immunother 2010;33:840–7.
9. Nguyen LT, Yen PH, Nie J, Liadis N, Ghazarian D, Al-Habeeb A, et al. Expansion and characterization of human melanoma tumor-infiltrating lymphocytes (TILs). PLoS One 2010;5:e13940.
10. Besser MJ, Shapira-Frommer R, Treves AJ, Zippel D, Itzhaki O, Schallmach E, et al. Minimally cultured or selected autologous tumor-infiltrating lymphocytes after a lympho-depleting chemotherapy regimen in metastatic melanoma patients. J Immunother 2009;32:415–23.
11. Curtin JA, Busam K, Pinkel D, Bastian BC. Somatic activation of KIT in distinct subtypes of melanoma. J Clin Oncol 2006;24:4340–6.
12. Baird DM, Kipling D. The extent and significance of telomere loss with age. Ann N Y Acad Sci 2004;1019:265–8.
13. Iancu EM, Speiser DE, Rufer N. Assessing ageing of individual T lymphocytes: mission impossible? Mech Ageing Dev 2008;129:67–78.
14. Pittet MJ, Valmori D, Dunbar PR, Speiser DE, Liénard D, Lejeune F, et al. High frequencies of naive Melan-A/MART-1-specific CD8(+) T cells in a large proportion of human histocompatibility leukocyte antigen (HLA)-A2 individuals. J Exp Med 1999;190:705–15.

Downloaded from clincancerres.aacrjournals.org on June 12, 2021. © 2011 American Association for Cancer Research.

Published OnlineFirst June 1, 2011; DOI: 10.1158/1078-0432.CCR-10-2769

15. Zhou J, Dudley ME, Rosenberg SA, Robbins PF. Persistence of multiple tumor-specific T-cell clones is associated with complete tumor regression in a melanoma patient receiving adoptive cell transfer therapy. J Immunother 2005;28:53–62.
16. Shen X, Zhou J, Hathcock KS, Robbins P, Powell DJ, Rosenberg SA, et al. Persistence of tumor infiltrating lymphocytes in adoptive immunotherapy correlates with telomere length. J Immunother 2007;30:123–9.
17. Ahmed SA, Talal N. Sex hormones and the immune system—Part 2. Animal data. Baillieres Clin Rheumatol 1990;4:13–31.
18. Cooper GS, Dooley MA, Treadwell EL, St Clair EW, Parks CG, Gilkeson GS. Hormonal, environmental, and infectious risk factors for developing systemic lupus erythematosus. Arthritis Rheum 1998;41:1714–24.
19. Dugowson CE, Koepsell TD, Voigt LF, Bley L, Nelson JL, Daling JR. Rheumatoid arthritis in women. Incidence rates in group health cooperative, Seattle, Washington, 1987–1989. Arthritis Rheum 1991;34:1502–7.
20. Lynch HE, Goldberg GL, Chidgey A, Van den Brink MR, Boyd R, Sempowski GD. Thymic involution and immune reconstitution. Trends Immunol 2009;30:366–73.
21. Schuchter L, Schultz DJ, Synnestvedt M, Trock BJ, Guerry D, Elder DE, et al. A prognostic model for predicting 10-year survival in patients with primary melanoma. The Pigmented Lesion Group. Ann Intern Med 1996;125:369–75.
22. Balch CM, Gershenwald JE, Soong SJ, Thompson JF, Ding S, Byrd DR, et al. Multivariate analysis of prognostic factors among 2,313 patients with stage III melanoma: comparison of nodal micrometastases versus macrometastases. J Clin Oncol 2010;28:2452–9.
23. Balch CM, Gershenwald JE, Soong SJ, Thompson JF, Atkins MB, Byrd DR, et al. Final version of 2009 AJCC melanoma staging and classification. J Clin Oncol 2009;27:6199–206.
24. Lizee G, Radvanyi LG, Overwijk WW, Hwu P. Improving antitumor immune responses by circumventing immunoregulatory cells and mechanisms. Clin Cancer Res 2006;12:4794–803.
25. Lizee G, Radvanyi LG, Overwijk WW, Hwu P. Immunosuppression in melanoma immunotherapy: potential opportunities for intervention. Clin Cancer Res 2006;12:2359s–65s.
26. Hamanishi J, Mandai M, Iwasaki M, Okazaki T, Tanaka Y, Yamaguchi K, et al. Programmed cell death 1 ligand 1 and tumor-infiltrating CD8+ T lymphocytes are prognostic factors of human ovarian cancer. Proc Natl Acad Sci U S A 2007;104:3360–5.
27. Thompson RH, Gillett MD, Cheville JC, Lohse CM, Dong H, Webster WS, et al. Costimulatory B7-H1 in renal cell carcinoma patients: indicator of tumor aggressiveness and potential therapeutic target. Proc Natl Acad Sci U S A 2004;101:17174–9.
28. Curtin JA, Fridlyand J, Kageshita T, Patel HN, Busam KJ, Kutzner H, et al. Distinct sets of genetic alterations in melanoma. N Engl J Med 2005;353:2135–47.
29. Williams KM, Hakim FT, Gress RE. T cell immune reconstitution following lymphodepletion. Semin Immunol 2007;19:318–30.

Downloaded from clincancerres.aacrjournals.org on June 12, 2021. © 2011 American Association for Cancer Research.

Published OnlineFirst June 1, 2011; DOI: 10.1158/1078-0432.CCR-10-2769

AACR American Association for Cancer Research

Clinical Cancer Research

Impact of Clinical and Pathologic Features on Tumor-Infiltrating Lymphocyte Expansion from Surgically Excised Melanoma Metastases for Adoptive T-cell Therapy

Richard W. Joseph, Vijay R. Peddareddigari, Ping Liu, et al.

Clin Cancer Res 2011;17:4882-4891. Published OnlineFirst June 1, 2011.

Updated version	Access the most recent version of this article at: doi:10.1158/1078-0432.CCR-10-2769

Cited articles	This article cites 29 articles, 11 of which you can access for free at: http://clincancerres.aacrjournals.org/content/17/14/4882.full#ref-list-1
Citing articles	This article has been cited by 4 HighWire-hosted articles. Access the articles at: http://clincancerres.aacrjournals.org/content/17/14/4882.full#related-urls

E-mail alerts	Sign up to receive free email-alerts related to this article or journal.
Reprints and Subscriptions	To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.
Permissions	To request permission to re-use all or part of this article, use this link http://clincancerres.aacrjournals.org/content/17/14/4882. Click on "Request Permissions" which will take you to the Copyright Clearance Center's (CCC) Rightslink site.

Downloaded from clincancerres.aacrjournals.org on June 12, 2021. © 2011 American Association for Cancer Research.