Impact of Prior Treatment on the Efficacy of Adoptive Transfer of Tumor-Infiltrating Lymphocytes in Patients with Metastatic Melanoma

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ABSTRACT

Purpose: Adoptive cell transfer (ACT) of autologous tumor-infiltrating lymphocytes (TIL) can mediate durable responses in patients with metastatic melanoma. This retrospective analysis provides long-term follow-up and describes the effect of prior therapy on outcomes after ACT-TIL.

Patients and Methods: Patients with metastatic melanoma underwent surgical resection of a tumor for generation of TILs and were treated with a lymphodepleting preparative regimen followed by adoptive transfer of TILs and intravenous IL2. Clinical characteristics of enrolled patients and treatment characteristics of TIL infusion products over two decades of ACT were analyzed to identify predictors of objective response.

Results: Adoptive transfer of TILs mediated an objective response rate of 56% (108/192) and median melanoma-specific survival of 28.5 months in patients naïve to anti–programmed cell death-1 (PD-1) therapy compared with 24% (8/34) and 11.6 months in patients refractory to anti–PD-1 (aPD-1). Among patients with *BRAF* V600E/K–mutated disease, prior treatment with targeted molecular therapy was also associated with a decreased response rate (21% vs. 60%) and decreased survival (9.3 vs. 50.7 months) when compared with those patients naïve to targeted therapy. With a median potential follow-up of 89 months, 46 of 48 complete responders in the aPD-1–naïve cohort have ongoing responses after a single treatment and 10-year melanoma-specific survival of 96%.

Conclusions: Patients previously treated with PD-1 or MAPK inhibition are significantly less likely to develop durable objective responses to ACT-TIL. While ACT-TIL is currently being investigated for treatment-refractory patients, it should also be considered as an initial treatment option for eligible patients with metastatic melanoma.

See related commentary by Sznol, p. 5156

Introduction

Over the past thirty years, the approach to the management of metastatic melanoma has been dramatically altered. In 1998, IL2 was the first drug approved for the treatment of metastatic melanoma in over 20 years, representing a shift from the clinical paradigm of palliative resections and chemotherapy. Effective adoptive cell transfer (ACT), the passive transfer of *ex vivo* activated immune cells, was first reported in 1988 and development continued over a period of time that included the approval of two new classes of drugs for the treatment of metastatic melanoma. The identification of inhibitory pathways triggered by engagement of cytotoxic lymphocyte antigen-4 (CTLA-4) and programmed cell death-1 (PD-1) led to the clinical development and FDA approval of immune checkpoint inhibitors (ipilimumab, nivolumab, and pembrolizumab). Identification of an activating mutation of *BRAF* in nearly half of all melanoma tumors introduced a new line of targeted therapies attempting to disrupt a constitutively activated MAPK pathway.

In the midst of this evolution, ACT of tumor-infiltrating lymphocytes (TIL) continued to elicit responses in patients with metastatic melanoma at the NCI and other specialized centers with the most recently published data from Surgery Branch, NCI citing a complete response rate of 24% and a median survival of over 3 years (1–4). Long-term follow-up of landmark studies of ipilimumab/nivolumab combination therapy and pembrolizumab monotherapy have demonstrated complete response rates of 14%–22%, median survival of more than 60 and 32.7 months, and estimated 5-year survival rates of 52% and 38.7%, respectively (5, 6). Recent reports of 5-year outcomes with combination BRAF/MEK inhibition show a complete response rate of 19%, median survival of 25.9 months, and 5-year survival rates of 34% (7).

The advancements demonstrated by these clinical trials have begun to decrease the number of annual deaths from melanoma but also highlight the need for ongoing development of treatment options for patients with advanced and metastatic disease (8). While many are now studying the combination of these classes of drugs in patients naïve to treatment, few are studying the impact of these classes of drugs on each other. Here we report our analysis of the ACT-TIL experience in the Surgery Branch, NCI – a total of 226 patients spanning almost two decades, with an emphasis on the effect of prior systemic therapy on patient and treatment characteristics associated with response.

Patients and Methods

Patient eligibility

All patients had measurable metastatic melanoma and were 18 years of age or older. Further criteria included an Eastern Cooperative Oncology Group (ECOG) performance status ≤ 1, life expectancy of at least 3 months, and no evidence of active major medical,

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Note: Supplementary data for this article are available at Clinical Cancer Research Online (http://clincancerres.aacrjournals.org/).

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Translational Relevance

In this study, we demonstrate that adoptive cell transfer of autologous tumor-infiltrating lymphocytes (ACT-TIL) can mediate durable complete responses in patients with metastatic melanoma after a single treatment. We describe significantly lower objective response rates and post–cell transfer melanoma-specific survival in patients whose tumors were anti–PD-1 refractory. While the presence of a *BRAF* V600E/K mutation did not affect the likelihood of response, we also describe significantly lower objective response rates and survival in those patients with *BRAF* V600E/K tumors that were refractory to BRAF and/or MEK inhibitors. We demonstrate that there were no significant differences in known poor prognostic factors [tumor burden, lactate dehydrogenase, etc.] when comparing treatment-naïve and -refractory patients. While ACT-TIL is a strategy now pursued solely for patients with treatment-refractory metastatic melanoma, we believe these data suggest that ACT-TIL could be considered as a first-line strategy for select patients.

cardiovascular, or immunodeficient diseases. Active systemic infections and coagulation disorders were exclusion criteria. Small volume brain metastases were permitted (three or fewer deposits, less than 1 cm in diameter). All patients were greater than 4 weeks from their last systemic therapy and demonstrated progression prior to ACT. Long-term follow-up is provided for patients enrolled on multiple early-phase trials of ACT ($n = 93$; 9) and a randomized controlled trial ($n = 101$; 1) previously described in detail. Additional patients ($n = 32$) were treated as part of protocols NCT01993719 (Trial 1) and NCT02621021 (Trial 2). The CONSORT diagram of enrollment is provided in Supplementary Fig. S1. All patients signed informed consents approved by the Institutional Review Board (IRB) of the NCI following the principles of the Declaration of Helsinki.

Trial design

All patients received a lymphodepleting chemotherapy regimen prior to infusion of autologous TILs on day 0. Cyclophosphamide (60 mg/kg daily) was administered on days -7 and -6. Fludarabine (25 mg/m^2 daily) was administered for 5 consecutive days, in a concurrent (start day -7) or sequential fashion (start day -5) with respect to cyclophosphamide. Cell infusions consisted of a maximum of 2e11 lymphocytes administered on day 0, followed by aldesleukin (720,000 IU/kg) administered intravenously every 8 hours to tolerance. Although lymphodepletion was augmented with total body irradiation (TBI) in some patients, this variable was tested in a randomized trial and did not demonstrate differences in objective response rates or survival (1). Additional subjects were included in an intention-to-treat analysis of overall response if lack of TIL growth or progressive disease prevented enrollment onto protocol for intended treatment ($n = 25$).

Efficacy

Response to treatment was measured using RECIST 1.0 guidelines with the first evaluation no sooner than 4 weeks from infusion and at regular intervals thereafter. In Trial 2, response was measured using RECIST 1.1 prospectively, but the application of RECIST 1.0 calculation methods to tumor measurements for this analysis did not alter response status. The long diameter of the largest tumor on baseline tumor measurements was recorded as a surrogate for burden of disease.

Laboratory procedures

TIL subcultures were derived from freshly resected metastatic melanoma tumor deposits as previously described (10). After representative sampling for standard diagnostic pathology, the remainder of the tumor was dissected into multiple small fragments, each 2–3 mm^3, which were individually placed in a single well of a 24-well tissue culture plate and supplemented with media containing high-dose IL2 (6,000 IU/mL, Clinigen). After an initial growth phase, subcultures were assessed for phenotype (CD3, CD4, CD8, and CD56, BD Biosciences) and were either placed directly into rapid expansion (REP) or cryopreserved based on the patient's clinical need. Subcultures were selected for REP and clinical infusion based on a variety of evolving factors including rate of proliferation and higher CD8/CD4 ratios. REP consisted of stimulation with OKT3 (CD3 antibody, Miltenyi Biotec) and IL2 (3,000 IU/mL) in the presence of irradiated feeders, autologous when possible, at a 200:1 ratio.

Statistical considerations

Melanoma-specific survival, overall survival, progression-free survival (PFS), and duration of response were calculated from the date of infusion of autologous TILs. Patients who developed a second malignancy requiring systemic treatment were censored for progression and melanoma-specific survival at the time of pathologic diagnosis. Potential follow-up was calculated using known function time method with a data cutoff of December 1, 2020 (11). Kaplan–Meier survival curves were created and analyzed using the log–rank Mantel–Haenszel technique. In screening potential factors associated with response, differences between continuous variables were compared using the Wilcoxon rank–sum test. A Fisher's exact test was applied to dichotomous parameters, and ordered categorical parameters were analyzed by a Cochran–Armitage test for trend. All P values are two-tailed and unadjusted for multiple comparisons. Analyses were performed to compare patients with objective responses with those without a response. Subset analysis of prognostic factors was also performed in those patients whose tumors harbored *BRAF* V600E/K mutations. Those parameters associated with response ($P < 0.10$ by univariate analyses described above) were further examined by multivariate logistic regression.

Results

Overall response and survival following ACT-TIL

From 2000 to 2018, 226 patients were enrolled on single arm early phase or randomized later phase trials of ACT employing a standard lymphodepleting regimen and delivering TILs grown and expanded in standard conditions. The overall response rate was 51% (116/226) with a complete response rate of 22% (49/226). Over half of the cohort ($n = 133$) were treated in the context of randomized trials, allowing for the inclusion of patients who underwent surgical resection for generation of TILs but were unable to proceed to treatment ($n = 25$) in an intention to treat analysis, and the overall response rate was 41% (64/158) with a complete response rate of 18% (29/158) in this subgroup of patients.

The entire cohort of 226 patients demonstrated a median overall survival of 20.6 months (95% CI, 15.2–29.9) with estimated 3-, 5-, and 10-year survival of 41%, 35%, and 32%. The durable nature of complete responses to TILs was best illustrated by melanoma-specific survival (MSS, **Fig. 1A**). Median MSS for the entire cohort was 22.2 months [95% confidence interval (CI), 16.2–32.0]. Patients achieving a complete response have a 10-year MSS of 96%.

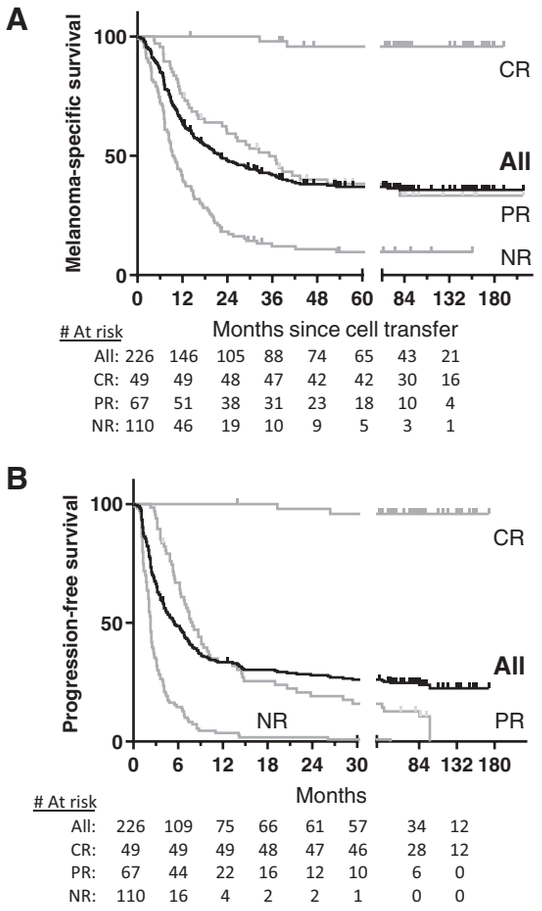

Figure 1.

Survival analysis of patients after adoptive transfer of autologous TILs (ACT) following a standard lymphodepletion. **A,** MSS and **B,** PFS of all patients with response to ACT illustrated (in gray) to demonstrate the durability of CRs. Median MSS of entire cohort was 22.2 months (95% CI, 16.2–32.4). Median PFS was 5.5 months (95% CI, 4.1–7.1).

With a potential median follow-up of 85 months in surviving patients, only two of 49 complete responders developed recurrent melanoma in the absence of medically-indicated immunosuppression. Median PFS of the entire cohort was 5.5 months (95% CI, 4.1–7.1; **Fig. 1B**). Of the 67 partial responders, 6 patients with long term ongoing responses have not required additional therapy but continue to demonstrate stable residual radiographic abnormalities. Of the 171 patients with progressive disease, 29 were still alive. Seven patients with progressive disease were managed with surgical resection and remain disease free. Pattern of progression was captured for patients treated since 2010; new sites of disease developed in 47 (49%) of progressing patients ($n = 96$) and the remaining patients progressed in existing tumors. Most patients with progressive disease returned to the care of their home oncologists, and treatment details were unavailable. Median survival after progression was 8.5 months (95% CI 6.6–11.0).

Five patients developed aggressive second solid tumor malignancies (e.g., colon, uterine, ovarian) while in surveillance of ongoing complete responses, and another developed acute undifferentiated leukemia refractory to standard chemotherapy ultimately requiring an allogeneic bone marrow transplant. With the intense immunosuppression required to establish and maintain the transplant, the patient died from rapidly progressive melanoma 11 months later.

Influence of patient characteristics on response to ACT-TIL

The effect of patient characteristics on response to ACT-TIL was compared across the entire cohort. There were no significant differences in likelihood of response based on sex, age, baseline neutrophil-to-lymphocyte ratio (NLR) or platelet count within the cohort of 226 patients (Supplementary Table S1). The presence of *BRAF* V600E/K mutation was found in 62 of the 128 patients who were tested; 98 patients were treated prior to the widespread availability of mutation testing and were classified as unknown. Among these three groups (positive, negative, unknown) there was no difference in response to ACT-TIL.

The 2018 American Joint Committee on Cancer (AJCC) Staging Classification was used to describe the extent of each patient's metastatic disease, and patients were less likely to respond with increasing stage ($P = 0.0058$). This difference is likely driven by the low response rate in patients with brain metastases (12). Baseline lactate dehydrogenase was also significantly different among response groups, with higher values associated with nonresponse ($P = 0.040$) although the response rate of patients with elevated LDH was 45% (40/88), including 15 complete responders. Response rates were not different when analyzed for the degree of LDH elevation ($P = 0.10$). Using baseline measurement of each patient's largest single tumor as another surrogate of disease, nonresponders had larger tumors than those who responded to ACT ($P = 0.008$).

Influence of prior therapy on response to ACT-TIL

After identifying patient characteristics associated with lower response rates, the influence of prior therapy on rates of objective response to ACT-TIL was considered. Most patients (188/226, 83%) underwent at least one systemic therapy prior to experimental protocol treatment with ACT-TIL (**Table 1**). One third of patients (77/226, 34%) had disease that had progressed through at least one checkpoint blockade prior to enrollment. Of the 43 patients refractory to aCTLA-4 without exposure to aPD-1, there was no difference in response rate (26/43, 60%) when compared with those naïve to any immune checkpoint therapy (82/149, 55%, $P = 0.60$).

Out of the 226 patients enrolled, 34 were refractory to PD-1 blockade (monotherapy, $n = 25$; dual blockade with ipilimumab, $n = 6$; monotherapy followed by dual blockade, $n = 3$). These patients demonstrated a decreased response rate to ACT-TIL with an objective response rate (ORR) of 24% (8/34) when compared with aPD-1–naïve patients (56%, 108/192, $P = 0.0006$). Median MSS in patients with previous aPD-1 exposure was decreased at 11.6 months compared with 28.5 months in the naïve population ($P = 0.0010$), as was median PFS at 3.2 months compared with the 6.5 months ($P < 0.0001$, **Fig. 2A**).

There was no difference in the pattern of progression in aPD-1–naïve and refractory patients; new sites of disease developed in 12 of 33 (36%) and 35 of 63 (56%), respectively ($P = 0.088$). Postprogression survival was not different between aPD-1–naïve and refractory patients (8.4 vs. 8.5 months, $P = 0.27$). However, 68 of 138 aPD-1 naïve patients succumbed to disease without access to aPD-1 therapy (prior to 2012), and there was a significant difference when this cohort was removed from the analysis (15.6 vs. 8.5 months, $P = 0.005$).

Using length of aPD-1 treatment as a surrogate for prior benefit from aPD-1, there was no difference between patients responding or not responding to ACT-TIL (median 7.1 vs. 5.3 months respectively,

Table 1. Prior therapy and likelihood of response.

		Number of patients (% of total)			ORR (95% CI)	*P*
	Total	**CR**	**PR**	**NR**	**OR = CR+PR**	**OR vs. NR**
All Patients	226	49 (22)	67 (30)	110 (49)	51% (45–58)	
Prior systemic therapy[a]						
None	38	8 (21)	9 (24)	21 (55)	45% (30–60)	0.63
One prior	77	23 (30)	24 (31)	30 (39)	61% (50–71)	
Two prior	66	11(17)	19 (29)	36 55)	45% (34–57)	
>Two prior	45	7 (16)	15 (33)	23 (51)	49% (35–63)	
Immune checkpoint mAbs						
Any immune checkpoint inhibitor	77	16 (21)	18 (31)	43 (56)	44% (33–55)	0.12
aCTLA-4 (single agent)	67	15 (22)	16 (24)	36 (54)	46% (35–58)	0.38
Only aCTLA-4 inhibitor (aPD-1 naïve)	43	15 (35)	11 (26)	17 (40)	60% (46–74)	0.24
Any PD-1/PD-L1 inhibitor (±aCTLA-4)	34	1 (3)	7 (21)	26 (76)	24% (12–40)	0.0006
aPD-1/aPD-L1 (single agent)	27	0	7 (26)	20 (74)	26% (13–45)	
aCTLA-4/aPD-1 (combination)	9	1 (11)	1 (11)	7 (78)	22% (4–55)	
Other immunotherapy						
IL2	105	25 (24)	36 (34)	44 (42)	58% (49–67)	0.063
Biochemotherapy	13	2 (15)	5 (38)	6 (46)	54% (29–78)	>0.99
Adjuvant IFNα	83	19 (23)	25 (30)	39 (47)	53% (42–63)	0.78
Vaccine[a]	61	12 (20)	24 (26)	25 (52)	59% (47–70)	0.18
Other						
Dacarbazine or temozolomide	34	5 (15)	15 (44)	14 (41)	59% (42–74)	0.36
BRAF and/or MEK inhibitor[b]	19	0	4 (21)	15 (79)	21% (9–43)	0.0057

Note: aCTLA-4 (e.g., ipilimumab, tremilimumab); aPD-1 (e.g., nivolumab, pembrolizumab).
Abbreviations: CR, complete response; NR, no response; PR, partial response.
[a]Vaccine not considered systemic therapy.
[b]Among those with known V600E/K mutations ($n = 62$).

$P = 0.43$). Similarly, there was no difference in response when grouped by duration of therapy ($P = 0.48$): ≤3 months, 2/12 (17%); 3.1–6 months, 2/8 (25%); >6 months, 4/14 (29%). The median time from last dose of aPD-1 to cell therapy was 4.6 months. One patient whose tumors progressed after 8 months of dual checkpoint blockade prior to ACT-TIL achieved the only durable complete response (84 months, ongoing) in this population (**Table 2**).

Patients whose tumors expressed *BRAF* V600E/K ($n = 62$) had been eligible for prior treatment with MAPK inhibitors; only 19 had received targeted molecular therapy (**Table 1**). In this group, there was a significant decrease in response rates to ACT with no complete responders and 4 partial responders (4/19, 21%) when compared with patients with *BRAF* V600E/K mutations but naïve to targeted therapy (26/43, 60%, $P = 0.0057$). There was no difference in duration of prior MAPK inhibition between responders and nonresponders to ACT-TIL (median 5.9 vs. 5.0 months, $P = 0.88$).

There was no difference in median MSS or PFS between those patients bearing tumors with or without a *BRAF* V600E/K mutation (22.2 vs. 35.9 months, $P = 0.28$, and 4.9 vs. 7.5 months, $P = 0.058$, **Fig. 2B**), but both MSS and PFS were decreased in those patients with a *BRAF* V600E/K mutated tumor who had progressed through BRAF/MEK inhibition prior to ACT-TIL (**Fig. 2C**), 9.3 compared with 50.7 months ($P \leq 0.0001$) and 2.5 compared with 6.6 months ($P = 0.0001$), respectively. Postprogression survival was also significantly longer in patients naïve to MAPK inhibition (16.6 vs. 7.5 months, $P = 0.001$). Among those patients for whom *BRAF* testing was not performed, median MSS was 17.6 months (95% CI, 11.7–28.9), and median PFS was 5.1 months (95% CI, 3.2–7.3).

Of the 34 patients with disease refractory to PD-1 checkpoint inhibition, 18 were eligible for BRAF/MEK inhibitors. Eleven patients were refractory to both classes of therapy, and there was no difference in response rates to ACT-TIL between those patients with BRAF/MEK naïve and refractory disease (1/7 naïve vs. 2/11 refractory, $p > 0.99$). Conversely, of the 19 patients with BRAF/MEK refractory disease, 11 patients were also refractory to PD-1 checkpoint therapy; there was no difference in response rates to ACT-TIL between those patients with PD-1–naïve (25%, 2/8) and refractory disease (18%, 2/11).

Decreased MSS was seen in patients with melanoma refractory to BRAF/MEK inhibition regardless of prior aPD-1 exposure with a median survival of 8.7 or 12.1 months (aPD-1 refractory or naïve, respectively) compared with an undefined median survival in those patients naïve to both classes of drug ($P \leq 0.0001$, P = 0.0052, respectively). Patients refractory to either class of drug experienced shorter PFS (Supplementary Fig. S2).

Influence of treatment characteristics on response in ACT-TIL

TIL were administered to 224 of the 226 patients enrolled. The characteristics of the infusion product and the ensuing clinical course were analyzed for differences between responders and nonresponders (Supplementary Table S2).

There was a small difference in response rate based on site of TIL harvest, driven by an increased likelihood of response when TIL were generated from a subcutaneous deposit. Patients who received a higher total cell number were more likely to respond to TIL therapy. There was a significant difference in the cell phenotype of the infusion product, with patients achieving a response having received a higher absolute number of CD8+ TILs (**Fig. 3**). The association of response to cell numbers (CD3 and CD8) was also seen in a prior analysis, though the differences in site of resection were not previously associated with clinical response (1).

Response did not correlate with number of doses of IL2. The addition of total body irradiation was already investigated in the

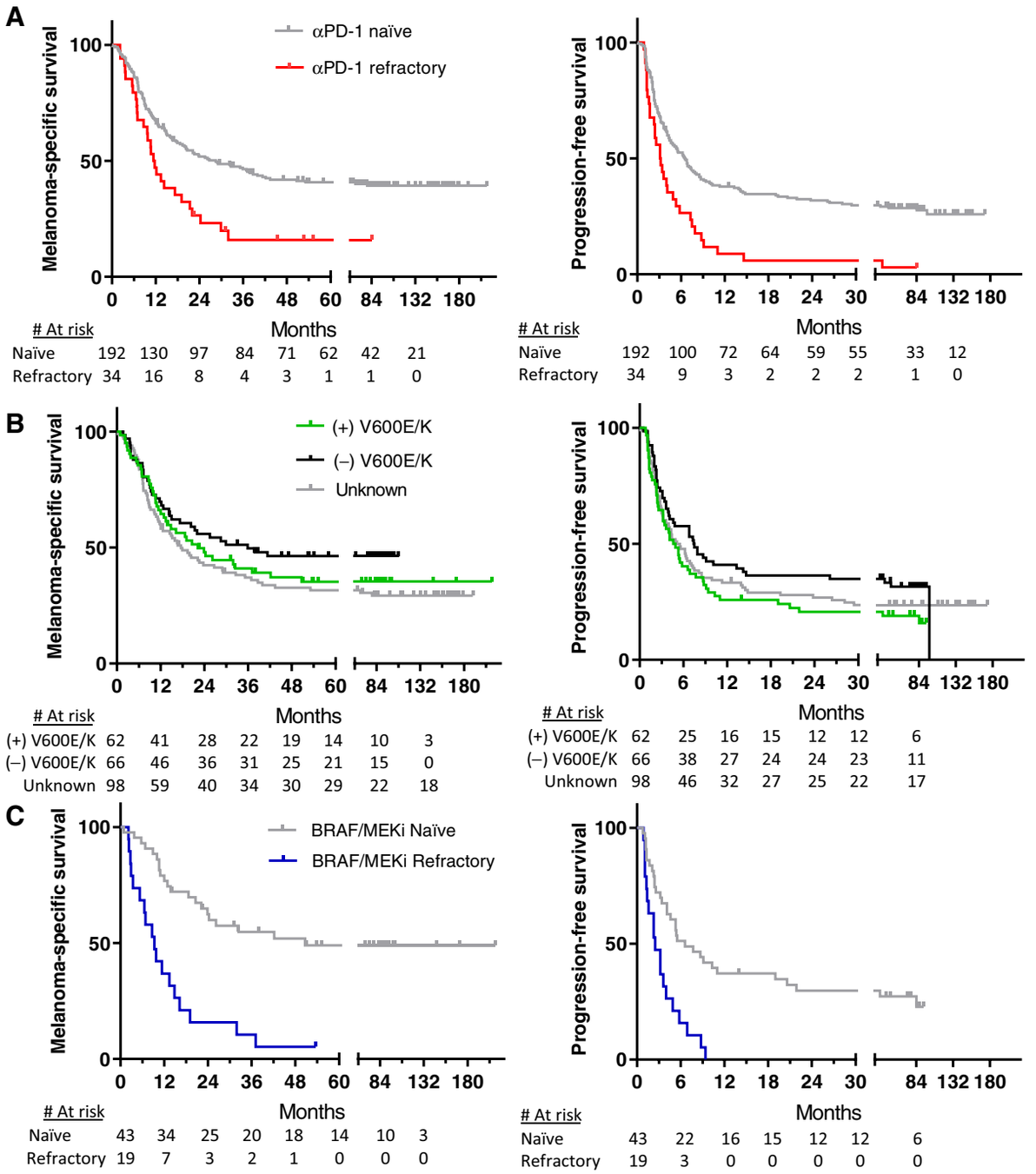

Figure 2.

Survival of patients after adoptive transfer of autologous TILs (ACT) following a standard lymphodepletion. **A,** Decreased MSS and PFS was observed in patients refractory to aPD-1 therapy. **B,** Presence or absence of *BRAF* V600E/K mutation did not significantly affect MSS or PFS after ACT. **C,** In the presence of *BRAF* V600E/K mutation, decreased MSS and PFS was observed in patients refractory to BRAF±MEK inhibition.

context of a controlled randomized trial and did not demonstrate a difference in likelihood of response and was not further interrogated in this larger cohort (1). The delivery of chemotherapy (7-day sequential vs. 5-day concurrent) also did not influence likelihood of response (Supplementary Table S2).

Influence of prior therapy on factors associated with response

Given the effect of prior therapy with aPD-1 and BRAF/MEK inhibition on response rates, analyses were performed separately on patients with and without exposure to these drugs to evaluate baseline or treatment characteristics that may have contributed to the

Table 2. Duration of response.

Group description	Median potential follow-up (25th-75th %ile)	*n* (%) of patients (duration in months, + indicates ongoing, italics indicate response in patients refractory to both agents)			
		Total	CR	PR	OR (%)
All	105 months (84–175)	226	49 (22)	67 (30)	116 (51)
aPD-1 naïve	113 months (88–180)	192	48 (25) 173+, 156+, 153+, 149+, 138+, 138+, 137+, 136+, 136+, 135+, 132+, 132+, 121+, 114+, 109+, 92+, 89+, 88+, 88+, 87+, 86+, 86+, 86+, 86+, 85+, 84+, 84+, 81+, 78+, 77+, 77+, 76+, 74+, 73+, 72+, 72+, 65+, 55+, 53+, 50+, 47+, 46+, 45+, 37+, 33+, 26, 19, 14+	60 (31) 98, 94+, 94+, 93+, 85+, 84, 79+, 61+, 37, 29, 28, 24, 22, 21, 19, 15, 15, 14, 13, 13+, 11, 10, 10, 10, 9, 9, 9, 9, 8, 8, 8, 7, 7, 7, 7, 7, 6, 6, 6, 6, 6, 5, 5, 5, 5, 5, 4, 4, 4, 4+, 4, 4, 4, 4, 3, 3, 3, 3, 3, 2	108 (56)
aPD-1 refractory	64 months (52–81)	34	1 (3) 84+	7 (21) 39, 15, 9, 7, 5, *5, 3*	8 (24)
BRAF V600E/K (+)	86 months (60–99)	62	10 (16)	20 (32)	30 (48)
MAPKi naïve	88 months (73–105)	43	10 (23) 88+, 86+, 86+, 85+, 77+, 73+, 72+, 50+, 45+, 14+	16 (37) 94+, 84, 37, 22, 21, 19, 10, 9, 9, 8, 5, 5, 5, 5, 4, 3	26 (60)
MAPKi refractory	65 months (58–92)	19	0	4 (21) 9, *5*, 4, *3*	4 (21)

differences in response. There were no significant differences in factors associated with a lower likelihood of response (distribution across stage, LDH, or largest tumor diameter) nor in patient distribution across sex, age, or *BRAF* status (**Table 3**).

Patients with prior treatment with aPD-1 received a higher number of CD4+ cells compared with aPD-1–naïve patients ($P = 0.0007$, **Fig. 3**). Patients refractory to aPD-1 treatment also received a median one less dose of IL2 post cell infusion ($P = 0.0003$), however, IL2 was not associated with response. The total cells given, CD8+ cells given and posttreatment peak absolute lymphocyte count (ALC) were not significantly different in patients based on their prior exposure to aPD-1 therapy. Among patients with tumors bearing a *BRAF* V600E/K mutation, no significant differences in treatment characteristics was observed between those with and without prior treatment with BRAF/MEK inhibition (**Fig. 3**). Specifically, there were no differences seen in factors associated with clinical response (i.e., total CD3+ cells, CD8+ cells infused, or largest baseline tumor diameter).

Safety

The adverse events associated with ACT have been discussed in detail in prior publications and are primarily associated with the lymphodepleting preparative chemotherapy and known IL2 toxicities. By deliberate design, all patients developed transient cytopenias and were managed with transfusions and marrow support (e.g., filgrastim). The change from sequential to concurrent chemotherapy did not alter the length of time of neutropenia – Grade 3 (<1 K/μL, median 8 days for both groups, $P = 0.74$) or Grade 4 (<0.5 K/μL, median 7.5 and 7.0 days, $P = 0.32$). There were also no differences in the length of thrombocytopenia – Grade 3 (<50K/μL, median 7 days for both groups, $P = 0.90$) or Grade 4 (<25K/μL, median 2.5 and 3.0 days, $P = 0.33$). Thrombotic microangiopathy was observed in 25% of patients that received TBI (1200 cGy) as part of their preparative regimen (13). That strategy has been abandoned (Supplementary Table S3).

Discussion

This study provides an analysis of 226 patients with metastatic melanoma, collected over 20 years and four trials in the Surgery Branch and demonstrates a significantly decreased likelihood of response for patients treated with anti–PD-1 (aPD-1) antibodies or MAPK inhibition. Prior to this analysis little was known about the impact of immune checkpoint or MAPK inhibition on treatment with ACT (14). One industry effort, reported in abstract form, described an objective response rate of 36.4% in patients refractory to PD-1 blockade (15). Here we have demonstrated that a single adoptive cell transfer of autologous TILs can mediate meaningful durable responses in eligible patients with metastatic melanoma. Responses can still be observed in multi-treatment refractory disease, supporting the continuing development of ACT-TIL as a later-line therapy. More controversially, the low incidence of prolonged adverse events with ACT-TIL and the lower response rates after prior treatment with aPD-1 therapy or BRAF/MEK inhibition suggest that first-line ACT-TIL should be considered.

This analysis of the largest single institutional experience with ACT for metastatic melanoma demonstrated a significant decrease in response rates in patients who are refractory to aPD-1 therapy. While the exact mechanism for this is unclear, it is possible, even likely, that there is a shared mechanism of resistance in aPD-1 and ACT-TIL refractory disease. Tumor characteristics consistent with immune evasion such as altered neoantigen presentation, decreased mutational burden, and upregulation of coinhibitory receptors may similarly degrade the ability of either ACT or PD-1 blockade to eliminate cancer cells (14, 16). The *in vivo* effects of lymphodepletion on the PD-1/PD-L1 in the tumor stroma of patients during ACT have not been well-studied, as on-treatment biopsies are limited secondary to the increased risks of thrombocytopenia. Patient selection may also play a role here, in that patients who did not receive a clinical benefit from aPD-1 therapy may possess immunoresistant tumors that may

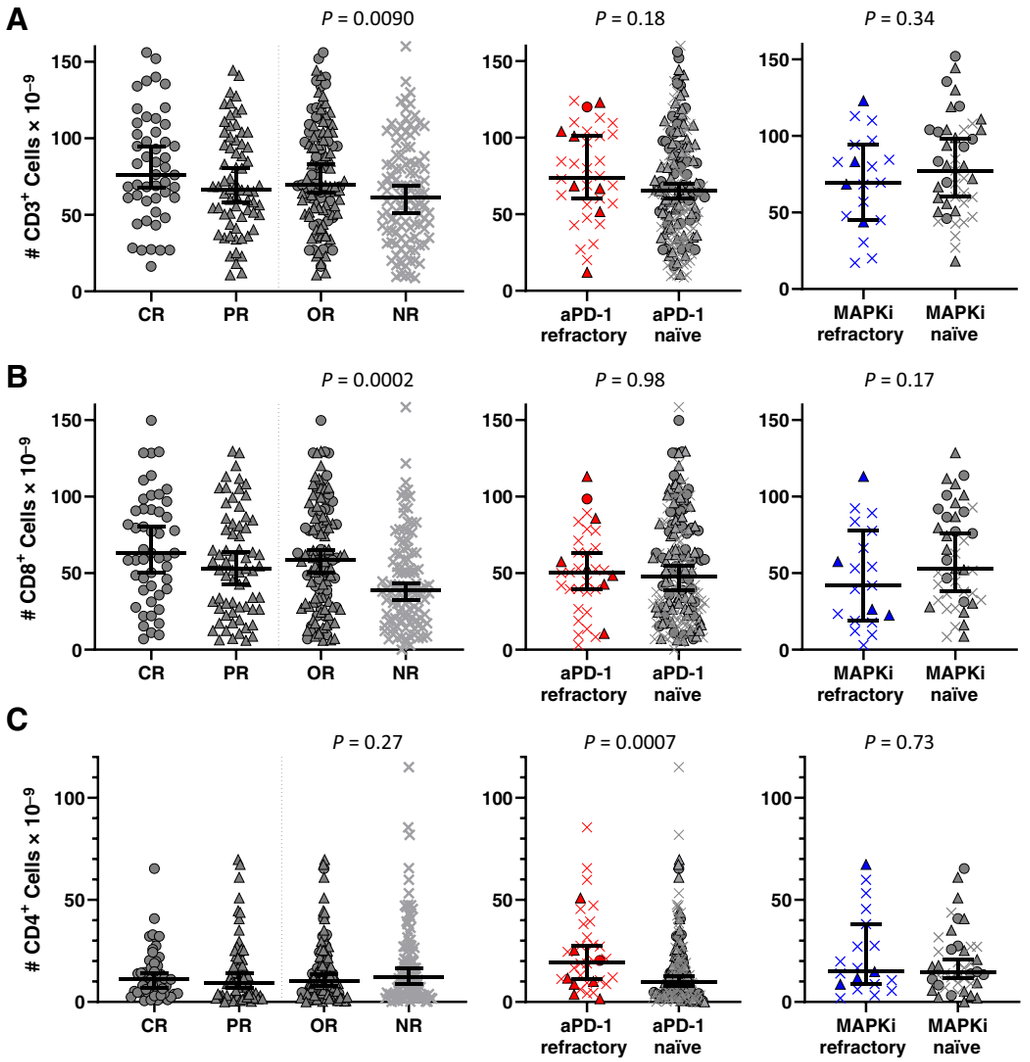

Figure 3.
Characterization of infusion products. **A,** Total CD3+ cells administered to patients with respect to (left to right) response to ACT-TIL, prior aPD-1 therapy, and BRAF mutation and inhibitor status. **B,** Total CD8+ cells. **C,** Total CD4+ cells.

not harbor tumor-specific TIL. However, patients that were refractory to aPD-1 did not present at a more advanced disease state as compared with the aPD-1–naïve patients, with similar LDH, stage, and tumor burden. Furthermore, TIL from refractory tumors can recognize fresh tumor digest (17). It is important to note that response to ACT after aPD-1 progression, while less frequent, is possible indicating that *ex vivo* stimulation and expansion may circumvent *in situ* T-cell inhibition or overwhelm tumor resistance with a numerical advantage (18).

Unexpectedly, lower response rates to ACT were also observed in patients with tumors refractory to BRAF/MEK inhibition. The driving force to this diminution is likely not due to the mutation itself, as patients with tumors harboring a *BRAF* V600E/K mutation and no prior mutation directed therapy still achieved an overall response rate of 60%. It is possible that the use of MAPK-targeted therapies (BRAF/MEK inhibitors) alter the tumor microenvironment or directly affect TIL rendering these patients resistant to ACT. Interestingly, early studies suggested that BRAF inhibition might augment immunotherapy responses by increased levels of CD4+ and CD8+ infiltration and increased antigen presentation (19, 20). In a pilot trial combining vemurafenib with ACT, objective responses were achieved, but correlative in vitro experiments demonstrated impairment of TIL and peripheral blood lymphocyte (PBL) proliferation and viability at higher serum equivalent concentrations of BRAF inhibitor (21). It has been proposed that MEK inhibition also impairs CD8+ T-cell function, with decreased proliferative response and decreased effector function (22). Many of these studies have been performed on tumor specimens obtained during treatment, with little known about the persistence of these effects after treatment. If the negative impact on the tumor microenvironment is long-lasting, tumors that are resected for derivation of TIL may be compromised. Further studies need to be performed to evaluate a direct correlation, however this potential alteration of the tumor-infiltrating CD8+ T cells may represent an explanation for the observed decrease in response rates to ACT

Table 3. Patient and treatment characteristics by prior therapy.

	Number of patients (%)				Number of patients (%)			
	Total	aPD-1 naïve	aPD-1 refractory	P^a	*BRAF* V600 E/K	BRAFi±MEKi naïve	BRAFi±MEKi refractory	P^a
Patients treated	224	190	34		61	42	19	
Patient characteristics								
Sex Female	78 (35)	67 (35)	11 (32)	0.85	19 (31)	14 (33)	5 (26)	0.77
Male	148 (66)	125 (65)	23 (68)		42 (69)	28 (67)	14 (74)	
Age (years), median	47	47	52	0.077	45	46	39	0.99
Stage (2018 AJCC)								
M1A	49 (22)	42 (22)	7 (21)	0.98	12 (20)	11 (26)	1 (5)	0.11
M1B	33 (15)	29 (15)	4 (12)		12 (20)	8 (19)	4 (21)	
M1C	107 (47)	88 (46)	19 (56)		26 (43)	16 (38)	10 (53)	
M1D	37 (16)	33 (17)	4 (12)		11 (18)	7 (17)	4 (21)	
BRAF status								
(+) V600E/K Mutation	61 (48)	43 (47)	18 (53)	0.54	61	42	19	-
(−) V600E/K Mutation	65 (52)	49 (53)	16 (47)		-	-	-	
LDH (U/L), median	191	190	195	0.90	194	175	270	0.18
(25th–75th %ile)		(147–297)	(161–276)		(154–286)	(146–238)	(158–566)	
Platelets (K/μL), median	235	237	224	0.58	230	227	240	0.79
(25th–75th %ile)		(187–281)	(200–274)		(192–289)	(190–289)	(193–290)	
Baseline tumor								
Diameter[b] (cm), median	4.2	4.1	4.2	0.29	4.0	3.8	4.4	0.19
(25th–75th %ile)	(2.7–6.1)	(2.7–6.0)	(3.4–6.4)		(2.7–5.1)	(2.5–5.0)	(3.1–5.7)	
Source of TILs								
Subcutaneous deposit	86 (38)	76 (40)	10 (29.5)	0.76	20 (33)	11 (26)	9 (47)	0.22
Lymph node	80 (36)	66 (35)	14 (41)		28 (46)	22 (52)	6 (32)	
Viscera	58 (26)	48 (25)	10 (29.5)		13 (21)	9 (21)	4 (21)	
Treatment characteristics								
Total cells $x10^{-9}$ Median	68.2	65.5	74.0	0.18	77.4	78.9	69.5	0.34
(25th–75th %ile)	(43.7–95.1)	(41.9–94.2)	(56.5–101.9)		(50.8–103.3)	(54.6–104.0)	(46.3–89.3)	
Cell phenotype								
$CD4^+$ $x10^{-9}$ Median	11.1	9.5	19.6	0.0007	14.9	14.6	14.9	0.73
(25th–75th %ile)	(3.9–21.7)	(3.2–19.8)	(10.2–30.8)		(8.7–27.3)	(8.7–27.0)	(8.7–32.8)	
$CD8^+$ $x10^{-9}$ Median	49.3	47.6	50.2	0.98	52.2	52.6	41.9	0.17
(25th–75th %ile)	(25.8–77.7)	(23.7–78.4)	(38.3–65.7)		(27.6–85.1)	(32.1–87.7)	(20.6–72.0)	
IL2 (doses), median	6	6	5	0.0003	5	5	5	0.87
(25th–75th %ile)	(5–8)	(5–8)	(3–6)		(4–7)	(4–7)	(3–7)	
Peak ALC (Day 0 to Day +9)								
Median $x10^{-3}/\mu L$	0.56	0.55	0.57	0.76	0.81	0.73	1.00	0.70
(25th–75th %ile)	(0.23–1.50)	(0.25–1.61)	(0.16–1.23)		(0.26–1.50)	(0.29–1.56)	(0.13–1.34)	

Note: BRAFi (e.g., vemurafenib, dabrafenib, encorafenib); MEKi (e.g., cobimetinib, trametinib, binimetinib).
[a]all *P* values are between naïve and refractory, uncorrected.
[b]long diameter of largest baseline tumor.

post-BRAF/MEK inhibition. A post-hoc analysis of response to pembrolizumab also identified lower objective response rates in patients refractory to MAPK inhibitors, however those patients also demonstrated differences in baseline prognostic characteristics (23). In this analysis of ACT-TIL, there were no baseline differences in stage, LDH, NLR, or tumor burden identified between patients naïve and refractory to MAPK inhibition.

Patients that were naïve to modern therapy demonstrated longer MSS after cell transfer than those whose tumors were refractory to PD-1 and/or BRAF/MEK inhibitors. While the difference in observed response rates to ACT-TIL between those groups is likely the largest driver of this finding, survival metrics also reflect any postprogression therapy. It is likely that aPD-1 and/or BRAF/MEK inhibitor therapy was proffered to naïve patients when they returned to their home oncologists after progression on these experimental protocols. Inferences about the efficacy of that strategy can be drawn from the differences in postprogression survival seen between the naïve and refractory groups.

A recent high-dimensional analysis of melanoma TIL infusion products has identified stem-like cells ($CD39^-CD69^-$) associated with development of complete response and persistence. None of the patients in that analysis were refractory to PD-1 or BRAF/MEK inhibitors, but the application of those findings to the infusion products of refractory patients may be informative (24). Prior studies also inferred an association with certain toxicities (e.g., thrombotic microangiopathy, vitiligo) with response, but the late-onset nature of these events creates responder bias (13, 25). While there has been extensive retrospective analysis, the quantity and quality of neoantigen reactivity has not been prospectively studied in patients with metastatic melanoma, a strategy that we have adopted for patients with epithelial cancer and should be explored in this treatment-refractory population (26–30). Further analysis of neoantigen reactivity is outside the clinical scope of this report.

The window to establish ACT as front-line therapy for metastatic melanoma faces many logistical obstacles, however, these data

suggest that consideration should be given to studying the effectiveness of ACT obtained from tumors resected prior to systemic therapy for delivery after progression on approved treatments. This strategy might answer whether the reduced effectiveness of TIL after exposure to other therapies is a consequence of simple patient selection, tumor immunoediting, or active compromise of the TIL repertoire.

ACT can yield durable responses in patients with metastatic melanoma after a single treatment. The benefit of this durability is not only of oncologic value but also psychosocial and economic. An analogous experience favorably compares the costs of a complex single treatment of CD19 chimeric antigen receptor (CAR)-T cells with annual costs of ongoing chemotherapy for patients with hematologic malignancies (31). Ongoing evolution of clinical management has led to fewer ICU admissions and less IL2-related toxicity, overcoming another hurdle to wider adoption of the strategy. The transient nature of almost all serious side effects requiring time-limited supportive care should be compared with longer term abnormalities that can result from checkpoint blockade. When discussing the sequential nature of treatments, patients who progress after ACT likely remain eligible for additional treatments, whereas the converse may not always be true. Grade 3/4 adverse event rates are as high as 59% with combination ipilimumab/nivolumab therapy and patients incur a risk of long-term endocrine toxicities requiring ongoing steroid therapy (14).

Conclusion

ACT remains a safe and viable option for patients with metastatic melanoma. While current approved therapies can provide tremendous clinical benefit for many patients, specialized academic centers and industry partners continue to pursue cell-based strategies for patients whose disease has not been controlled by those first-line therapies. From this analysis, waiting to utilize ACT of TIL as a later-line treatment decreases the likelihood of attaining any response, partial or complete. While ACT may have limitations in its accessibility compared with other therapies, such as aPD-1 or BRAF/MEK inhibition, it can also provide durable responses. As the field moves forward with biomarkers that more accurately predict response to checkpoint inhibition, those patients unlikely to derive benefit from first-line therapy may view adoptive transfer as a more viable option. When creating treatment plans for patients with metastatic melanoma, the response rates and durability provide a basis for ACT to be considered earlier in the disease course for eligible patients with access to the strategy.

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Authors' Contributions

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