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Treatment of metastatic uveal melanoma with adoptive transfer of tumor infiltrating lymphocytes: a single-center phase 2 study

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Abstract

Background—Uveal melanoma is a rare tumor with no established treatments once metastases develop. Although a variety of immune based therapies have demonstrated efficacy in metastatic cutaneous melanoma, their use in ocular variants has been disappointing. Recently, adoptive T cell therapy has shown salvage responses in multiple refractory solid tumors. Thus, we sought to determine if adoptive transfer of autologous tumor infiltrating lymphocytes (TIL) could mediate regression of metastatic uveal melanoma.

Methods—In this ongoing single-center, two-stage phase 2, single-arm trial, patients with histologically confirmed metastatic ocular melanoma (aged ≥ 16 years) were enrolled. Key eligibility criteria were an ECOG performance status of 0 or 1, progressive metastatic disease, and adequate hematological, renal, and hepatic function. Metastasectomy operations were performed to procure tumor tissue to generate autologous TIL cultures, which then underwent large scale ex vivo expansion. Patients were treated with lymphodepleting conditioning chemotherapy followed by intravenous infusion of autologous TIL and high-dose interleukin-2. The primary end-point was objective tumor response in evaluable patients per protocol using Response to Evaluation Criteria

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Conflicts of Interest: None

AUTHOR CONTRIBUTIONS: USK conceived and designed the study.

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in Solid Tumors (RECIST), version 1.0. An interim analysis of this ongoing trial is currently being reported. The trial is registered with ClinicalTrials.gov as NCT01814046.

Findings—From the completed first stage and ongoing expansion stage of this trial, a total of twenty-one consecutive metastatic uveal melanoma patients were enrolled and received TIL therapy. Seven of 20 evaluable patients demonstrated objective tumor regression (35%; 95% CI: 16%-59%). Among the responders, six patients achieved partial response (PR), two of which are ongoing and have not reached maximum response. One patient achieved complete response (CR) of numerous hepatic metastases currently ongoing at 21 months post therapy. Three of the responders were refractory to prior immune checkpoint blockade. Common grade 3 or more toxic effects were related to the lymphodepleting chemotherapy regimen and included lymphopenia, neutropenia, and thrombocytopenia (21 [100%] of patients for each toxicity); anemia (14 [67%] of patients); infection (6 [29%] of patients). There was one treatment-related mortality secondary to sepsis induced multi-organ failure.

Interpretation—To our knowledge, this is the first report describing the ability of adoptive transfer of autologous TIL to mediate objective tumor regression in patients with metastatic uveal melanoma. These initial results challenge the belief that metastatic uveal melanoma is immunotherapy resistant and support the further investigation of immune based therapies for this cancer. Refinement of this T cell therapy is critical to improve the frequency of clinical responses and the general applicability of this treatment modality. This research was fully supported by the Intramural Research Program of the NIH, NCI, Center for Cancer Research.

Keywords

Metastatic uveal melanoma; immunotherapy; TIL

Research in Context

Evidence before this study

Before undertaking this study, we searched PubMed for the terms “metastatic uveal melanoma” and “immunotherapy” for articles published in any language. Although a variety of immune based therapies have demonstrated efficacy in metastatic cutaneous melanoma patients, their use in uveal melanoma has been disappointing. Recent reports evaluating immune checkpoint blockade therapy via anti-CTLA-4, anti-PD-1 and anti-PD-L1 have cumulatively shown limited efficacy in metastatic uveal melanoma patients. These findings have led to speculation that melanomas arising from the uveal tract may represent an immunotherapy resistant variant. There are currently no published reports investigating the efficacy of adoptive transfer of tumor infiltrating lymphocytes (TIL) in metastatic uveal melanoma.

Added value of this study

This report describes the largest cohort of metastatic uveal melanoma patients treated with adoptive transfer of autologous TIL. An interim analysis of this ongoing trial found seven of 20 (35%) evaluable patients demonstrating objective tumor regression by RECIST criteria. One patient achieved complete tumor regression of numerous hepatic metastases, currently

ongoing at 21 months post therapy. To our knowledge, this is the first description of the ability of TIL transfer to mediate objective tumor responses in patients with metastatic uveal melanoma, including individuals whose disease was refractory to immune checkpoint blockade. The level of anti-tumor reactivity found within the infused TIL was strongly associated with clinical response.

Implications of all available evidence

Adoptive T cell therapy using autologous TIL has been reported to induce salvage responses in a variety of refractory solid tumors. This study now demonstrates that this immunotherapeutic approach can also induce clinical responses in patients with metastatic uveal melanoma, a cancer that currently has no standard effective treatments. These initial findings challenge the belief that metastatic uveal melanoma is immunotherapy resistant and support the further investigation of immune based therapies for this cancer.

Introduction

Uveal melanoma is the most common primary malignancy arising within the adult eye. Overall, however, this is a rare cancer with an annual incidence of ~6 per million in the U.S, accounting for 3.7% of all melanomas (1). These tumors originate within the pigmented uveal tract (which include the choroid, ciliary body, and iris) and are notable for characteristic cytogenetic changes (2), oncogenic mutations in *GNAQ* or *GNA11* (3, 4), and an unusual predilection to aggressively metastasize to the liver resulting in a dismal prognosis (5). Although a variety of immune based therapies have demonstrated efficacy in metastatic melanoma of cutaneous origin (6-10), their use in uveal variants has been disappointing, thus far (11-20). These findings have led to speculation that melanomas arising within the eye may represent an immunotherapy resistant variant (21).

Recently, we discovered an immunogenic subset of uveal melanoma based upon in vitro reactivity profiling of tumor infiltrating lymphocytes (TIL) isolated from freshly resected liver metastases (22). In the current study, we sought to determine if adoptive transfer of such TIL could mediate cancer regression in metastatic uveal melanoma patients, especially after prior immunotherapy failure. Adoptive T cell therapy using autologous TIL has been reported to induce salvage responses in a variety of refractory solid tumors (23, 24) with durable and complete regression in metastatic cutaneous melanoma patients (8). However, the efficacy of TIL therapy in uveal melanoma patients has not been formally investigated. Here we report, to our knowledge, the first clinical and immunologic findings after adoptive TIL transfer in metastatic uveal melanoma patients.

Patients and Methods

Study design and participants

This two-stage phase 2 clinical trial was conducted in the Surgery Branch of the National Cancer Institute (NCI) after review and approval by the NCI Institutional Review Board. Patients gave informed consent in accordance with the Declaration of Helsinki. The first stage of the study was designed to initially evaluate efficacy in 19 patients as a basis to determine whether an expansion stage was warranted (see statistical section). To satisfy the

endpoint of the first stage and account for a single non-evaluable patient, 20 uveal melanoma patients were enrolled and treated. Enrollment to the expansion stage has begun and is currently ongoing. One patient has been enrolled to the expansion cohort with the plans to accrue up to 33 total patients to the protocol. To be eligible for the trial, subjects had to be 16 years of age or older with a histologically confirmed diagnosis of metastatic ocular melanoma. Given our interest in evaluating the therapeutic response of metastatic melanoma arising specifically from the uveal tract, ophthalmologic documentation was obtained for all patients to determine whether the primary tumor had uveal origin. One enrolled patient was confirmed after treatment to have had ocular melanoma arising from the conjunctiva based upon ophthalmologic history and whole exomic mutational sequencing of a metastatic tumor which revealed the presence of a characteristic *NRAS* driver mutation. This patient was eligible for protocol therapy, but was censored from the current report of exclusively uveal melanoma patients.

There was no requirement for prior systemic therapy, given the lack of known effective systemic treatments for metastatic uveal melanoma. If patients did receive prior systemic treatment, more than four weeks must have elapsed before initiation of the current trial therapy, and patients' toxicities must have recovered to a grade 1 or less (except for toxicities such as alopecia or vitiligo). All patients were required to have progressive and measurable metastatic disease with an Eastern Cooperative Oncology Group performance status of 0 or 1 and life expectancy greater than three months at the time of enrollment. Patients were required to have adequate hematological, renal, and hepatic function with laboratory values demonstrating WBC $\geq 3000/\text{mm}^3$, platelet count $\geq 100,000/\text{mm}^3$, hemoglobin $> 8.0 \text{ g/dl}$, ALT and AST ≤ 3.5 times the upper limit of normal, creatinine $\leq 1.6 \text{ mg/dl}$, and total bilirubin $\leq 2.0 \text{ mg/dl}$. Patients were excluded if they had active systemic infections, coagulation disorders or other active major medical illnesses of the immune system. Patients could be enrolled into two cohorts depending on their expected ability to tolerate high dose interleukin-2. Patients with adequate cardiovascular and respiratory function were enrolled into the main cohort of the study to receive the preparative chemotherapy, TIL infusion, and high dose interleukin-2. This cohort is the subject of the current report. Patients who were deemed medically ineligible to receive high-dose IL-2 due to pre-treatment comorbidities were allowed enrollment upon the protocol in a separate non-comparative predefined exploratory cohort in which they could receive the therapy without IL-2 administration. Due to limited accrual thus far, this exploratory cohort is not being reported in the current analysis. Clinical responses and criteria for progression were determined according to Response Evaluation Criteria in Solid Tumor (RECIST) version 1.0. This study utilized the Common Terminology Criteria for Adverse Events (CTCAE version 3.0) for toxicity and adverse event reporting.

Procedures

Metastasectomy operations were performed upon all patients to procure tumor tissue to generate autologous TIL for therapy. After surgical procurement of a metastatic lesion, the fresh tumor underwent sterile dissection in the Surgery Branch Cell Production Facility. A representative sample of tumor was sent for formal pathologic analysis, while geographically discrete 1 to 2 mm^3 tumor fragments ($n=24$) were placed individually in

wells of a 24-well culture plate containing complete media with human AB serum and recombinant IL-2 (6000 IU/ml). Remaining fresh tumor was processed by mechanical and enzymatic digestion to provide a single cell suspension of autologous tumor targets for TIL reactivity testing. After approximately 2 weeks of growth, individual fragment T cell cultures were selected for further expansion based upon proliferative capacity and evidence of autologous tumor reactivity using a standardized IFN- γ release assay when sufficient tumor targets were available. Final large scale expansion of selected TIL cultures was performed with anti-CD3 antibody (OKT-3, 30 ng/ml; Ortho Biotech) and IL-2 (3000 IU/ml) in the presence of irradiated peripheral blood mononuclear cells (PBMC) feeder cells.

The planned patient treatment involved a single cycle of adoptive TIL transfer which began with a nonmyeloablative lymphodepleting conditioning chemotherapy regimen consisting of intravenous cyclophosphamide (60 mg/kg) daily for 2 days followed by fludarabine (25 mg/m²) daily for 5 days, as previously described (8). One day after completion of their preparative regimen, patients received a single infusion of expanded autologous TIL intravenously followed by high-dose intravenous interleukin-2 (720,000 IU/kg) every 8 hours stopping for dose limiting side effects, as previously described (8) (Schema shown in Appendix p.1).

Tumor size was evaluated pretreatment and post treatment by CT and/or MRI monthly for three months and at 2-3 month intervals thereafter for the duration of follow up. Additionally, at each follow up visit, physical examination and routine laboratory monitoring was performed. Duration of overall response was measured from the time measurement criteria were met for CR/PR (whichever was first recorded) until the first date that recurrent or progressive disease was objectively documented using RECIST version 1.0 criteria. All declarations of response were presented and confirmed at a weekly tumor board in the Surgery Branch, NCI. Duration of stable disease was measured from the start of the treatment until the criteria for progression were met. Patients experiencing sustained stable disease (>4 months), partial or complete response could receive a maximum of one additional TIL re-treatment when progression by RECIST criteria was documented after evaluation by the principal investigator. Patients were taken off study after documentation of progressive disease, unless they were eligible for re-treatment.

For immunologic correlation studies, cryopreserved samples of the TIL infusion products underwent flow cytometric phenotyping with anti-human CD3, CD8, CD4, CD45RO, and CD62L monoclonal antibodies. The specific anti-tumor reactivity of the infused TIL was assessed by independent flow cytometric and ELISA based assays. Following overnight co-culture of the TIL with their autologous source tumor, the frequency of tumor-reactive T cells was determined by flow cytometric measurement of T cell activation markers using antibodies against CD137 (anti-4-1BB; BD Biosciences) and CD 134 (anti-OX40; eBiosciences). The percentage of tumor-reactive T cells was calculated as the sum of the frequency of OX40+CD4+CD3+ cells and 4-1BB+CD8+CD3+ cells in response to autologous tumor stimulation minus the background frequency seen against autologous antigen-presenting cells (APCs). The supernatant from these respective co-cultures was assessed by ELISA to determine the tumor induced IFN- γ cytokine production.

Outcomes

The primary endpoint of this phase 2 trial was to determine the proportion of metastatic ocular melanoma patients with a complete and/or partial response by RECIST version 1.0 criteria after receiving adoptive TIL therapy. Secondary endpoints were to study the immunologic correlates associated with clinical response and to define the toxicity associated with TIL therapy in this patient population. Overall survival was not a pre-specified end point of this study, but this data is being recorded for future reporting after more mature follow-up.

Statistical Analysis

Primary endpoint analysis of therapeutic response was performed on all patients who underwent at least one scheduled follow up assessment after TIL therapy per protocol. For assessment of treatment related toxicity, all enrolled patients were included. The study was conducted as an optimal two-stage phase 2 trial in order to rule out an unacceptably low 15% clinical response rate (PR+CR; $p_0=0.15$) in favor of a higher response rate of 35% ($p_1=0.35$). With an $\alpha=0.10$ (probability of accepting an ineffective treatment=0.10) and $\beta=0.10$ (probability of rejecting an effective treatment=0.10), the study was designed to initially enroll 19 patients and if 4 or more of the first 19 had a clinical response, the trial would open to accrual for the second stage to enroll an additional 14 patients. Cumulatively, if 8 or more responses were observed in 33 patients, these findings would be considered promising to warrant further study in later trials. The efficacy endpoint of the first stage of this trial has been met and enrollment is currently being expanded to 33 total patients. The interim reporting of this ongoing trial was not pre-specified in the protocol, but is being presented because of encouraging clinical activity in a disease with no standard therapeutic options. The cut-off date for clinical data acquisition was January 30, 2017. The trial is registered with ClinicalTrials.gov as NCT01814046.

For exploratory analyses comparing attributes of responding and non-responding patients (Table 4), the Wilcoxon rank-sum test was utilized for the comparison of continuous variables which were not normally distributed within the groups. Dichotomous variables were compared using Fisher's exact test. The association between the in vitro flow cytometric and ELISA assessment of TIL reactivity was determined by calculating the correlation coefficient and presented as a R^2 value. To study the association between TIL characteristics and clinical response, only the initial TIL infusion was assessed if the patient had received more than one cell infusion. All P values are 2-tailed, but have not been adjusted for multiple comparisons, given the exploratory nature of the association studies. $P<0.05$ was considered statistically significant. Excel and GraphPad Prism (v6.01) were used for analyses.

Role of the Funding Source

The funder of this study had no role in the design, collection, analysis, or reporting of this trial. All authors had access to raw data for review and participated fully in reviewing and submitting the manuscript for publication. The corresponding author had full access to all the data in the study and had final responsibility for the decision to submit for publication.

Results

Twenty-seven patients with metastatic uveal melanoma were found to be eligible for the clinical trial and underwent metastasectomy operations for the purpose of generating therapeutic TIL (Appendix p.2; CONSORT diagram). Large scale expansion of TIL was achievable in 26 patients (96%). Among the patients with available TIL, five patients did not undergo TIL therapy. Two patients had subsequently become ineligible for protocol therapy and three patients had early TIL cultures with no detectable in vitro anti-tumor reactivity upon testing. Thus, overall, six patients (22%) who underwent surgical resection with the intent of TIL therapy did not undergo treatment.

Between June 7, 2013 and July 14, 2016, twenty uveal melanoma patients were enrolled and treated with adoptive TIL transfer in the first stage of this trial. The ongoing expansion stage began on September 9, 2016 with the enrollment of an additional patient. Thus, in sum, twenty-one uveal melanoma patients were treated consecutively with TIL therapy and comprise the current report. The baseline characteristics of the patients are summarized in Table 1. The median age was 54 years (range: 32-63) with 8 female and 13 male patients. Management of the primary uveal tumor prior to referral had included eye-preserving radiotherapy in 15 patients (71%) and enucleation in 6 patients (29%). The median time between primary eye tumor treatment and the diagnosis of metastatic disease was 2.6 years (Appendix p.3). At the time of enrollment, all patients had measurable and progressive metastatic disease with 18 patients (86%) having M1b or M1c disease by AJCC (7th edition) staging criteria for uveal melanoma. The median time between diagnosis of metastatic disease and TIL therapy was 1.2 years (Appendix p.3). Although liver was the predominant site of metastasis (20 patients; 95%), extrahepatic spread was also commonly identified (17 patients; 81%). Additional patient related characteristics including baseline levels of LDH, alkaline phosphatase, and tumor size are presented in Appendix p.3. Twelve (57%) of the enrolled patients had received greater than 2 prior systemic therapy regimens, with 9 patients (43%) having received immune checkpoint blockade with either single agent or combinatorial anti-CTLA-4 and anti-PD-1 antibodies. No patient had achieved an objective response to any of these prior systemic therapies.

The characteristics of the surgically procured metastases that were used to generate TIL are summarized in Table 2. The main site of tumor resection was the liver (12 patients; 57%) with the remaining metastases harvested from a variety of extrahepatic sites. Driver mutational analysis performed on the resected metastases revealed somatic mutations in either *GNAQ* or *GNAI1* in 19 of the patients (90%), consistent with the frequencies of these mutations reported in primary uveal tumors (3, 4). Mutations of the tumor suppressor gene, *BAP-1*, which have been associated with early and aggressive metastasis (25), were found in 8 of the enrolled patients (38%; Appendix p.3). Interestingly, the metastatic tumor from one patient did not express *GNAQ*, *GNAI1*, or *BAP-1* mutations, but instead harbored an *NRAS* (c.182A>G, p.Gln61Arg) mutation. Whole exomic sequencing performed upon this metastasis did confirm the additional presence of an *SF3B1* mutation (c.1874G>A, p.Arg625His), strongly supporting its uveal origin (26). The median time between the date of metastasectomy and start of systemic therapy was 5 weeks.

The characteristics of the administered TIL products are summarized in Table 2. The TIL infusions were highly heterogeneous in their frequencies of CD4+ and CD8+ T cells. Each of these subsets displayed predominantly effector memory differentiation based upon cell surface expression of CD45RO and loss of the lymph node homing molecule, CD62L (Appendix p.4). To accurately assess the personalized in vitro tumor reactivity for each TIL product, we performed a co-culture of the T cells with their respective autologous tumor which had been freshly cryopreserved at the time of surgical procurement. TIL products generated for 18 patients underwent such reactivity testing, while TIL for three patients (1, 11 and 16) could not be analyzed due to insufficient quantities of cryopreserved tumor targets. The frequency of tumor-reactive T cells within the TIL infusion products was determined by flow cytometric measurement of the T cell activation markers, OX40 (CD134) on CD4+ T cells and 4-1BB (CD137) on CD8+ T cells in response to autologous tumor stimulation (Appendix p.5). The functional anti-tumor reactivity of these T cells was assessed by ELISA based measurement of IFN- γ cytokine release. There was a strong correlation between tumor-reactive T cell frequency (by flow cytometry) and IFN- γ cytokine release (by ELISA) in the administered TIL ($R^2 = 0.91$, $P = 0.0001$) (Appendix p. 6). Based upon these respective assays, we observed significant heterogeneity in the TIL product for both the percentage of tumor-reactive T cells (range: 0.1%-48.12%) (Figure 1A) and the levels of IFN- γ release in response to autologous tumor stimulation (range: 10-12,806 pg/mL) (Figure 1B).

All patients received the complete nonmyeloablative lymphodepleting conditioning chemotherapy regimen as planned without dose or scheduling alteration. All patients were treated with a single infusion of TIL with the exception of two patients who received an additional cycle of cell therapy after demonstrating stable disease for 5 and 6 months, respectively. The median number of TIL administered during initial therapy was 85×10^9 cells (range: $17-138 \times 10^9$) (Table 2). After cell infusion, patients received varying number of IL-2 doses (median: 5, range: 0-8), as determined by clinical status. The median follow-up time was 7.3 months (IQR 4.4-17.0) from time of treatment to the date of the last protocol indicated evaluation.

All adverse events observed in all patients enrolled to the study ($n=21$) are listed in Table 3. There were no acute toxicities (occurring within 7 days) that were associated with TIL infusion. IL-2 related toxicities were generally mild and self-resolved after approximately one week. All patients ($n=21$), however, experienced transient grade 3 or more hematologic toxicities (lymphopenia, neutropenia, and thrombocytopenia) which were anticipated from the lymphodepleting chemotherapy regimen. Grade 3 anemia was seen in 14 (67%) of patients. These hematologic toxicities typically lasted ~7-10 days before immune reconstitution. During the period of neutropenia, fevers were observed in 6 (29%) of patients. Documented infections based upon clinical diagnosis or surveillance blood cultures were seen in 6 (29%) of patients. Generally, these infections were low grade and resolved with supportive care after immune reconstitution. However, there was one treatment-related death resulting from an infectious complication leading to sepsis induced multi-organ failure. This patient presented with an antecedent history of heavy smoking and emphysema and subsequently developed a severe respiratory syncytial virus pneumonia and overwhelming sepsis during the period of chemotherapy induced neutropenia. The clinical

course was further complicated by grade 3 cardiac arrhythmias and grade 4 CNS ischemia necessitating withdrawal of support on day 17. Another patient developed an unusual delayed grade 4 neurotoxicity likely related to fludarabine administration which presented as progressive lower extremity weakness and paralysis one month after cell therapy. This neurologic toxicity was self-limiting and improved with physical rehabilitation. We observed one intravenous catheter related venous thrombosis that completely resolved after thrombolytic therapy. Autoimmune related adverse events were not seen after TIL therapy with the exception of one patient who demonstrated mild patchy vitiligo several months after treatment.

In the first stage of this trial, six patients demonstrated an objective tumor response from a cohort of 19 evaluable patients (32%; 95% CI: 14%-57%). One additional patient enrolled to this cohort suffered a treatment related mortality before follow up and was not evaluable for this response analysis. In the expansion stage of the trial, one patient was enrolled who also achieved a clinical response. Thus, overall, seven of 20 evaluable patients demonstrated objective tumor regression (35%; 95% CI: 16%-59%) (Table 2; Figure 2A). Among the responders, six patients achieved partial response (PR), two of which are ongoing and have not reached maximum response. One patient achieved complete response (CR) currently ongoing at 21 months post therapy. Typically, the kinetics of tumor response was rapid after TIL transfer with measurable evidence of regression by the first month post therapy (Figure 2B). Tumor regression were seen in a variety of sites including extra-hepatic and intra-hepatic metastases. Five of the responding patients had been heavily pretreated with three or more metastatic regimens with no clinical response to these treatments; three of these patients had demonstrated progression after both CTLA-4 and PD-1 immune checkpoint blockade. One patient was notable for having significant rapid progression of numerous hepatic metastases after prior treatment with sequential CTLA-4 and PD-1 immune checkpoint blockade. However, subsequent to TIL infusion, this treatment refractory patient demonstrated complete tumor regression ongoing nearly two years after TIL therapy. In four of the partial responders, the duration of responses ranged from 3 to 9 months. Interestingly, disease progression in these patients was not typically seen at sites of prior tumor regression, but rather with development of new metastases (Figure 2B).

To aid future study design, we next performed exploratory analyses to determine if any patient or treatment-related characteristics were associated with improved clinical outcome after TIL therapy (Table 4). Interestingly, we did not observe a significant difference in the number of non-synonymous mutations harbored in tumors from responding versus non-responding patients. In both groups the mutational burden was low (median number: 29 vs. 27 respectively; $P=0.55$). We did observe an association between clinical response and the autologous tumor reactivity of the infused TIL, as assessed by the in vitro flow cytometric and ELISA assays (Table 4 and Figure 3 A-C). TIL infused into responding patients ($n=5$) versus non-responding patients ($n=12$) had a greater frequency of tumor-reactive T cells (median: 9.4% vs. 0.6%, respectively; $P=0.011$), greater absolute numbers of tumor-reactive T cells (median: 8.1×10^9 vs. 0.5×10^9 respectively; $P=0.008$), and higher levels of IFN- γ release after autologous tumor stimulation (median: 3583 vs. 18 pg/ml, respectively; $P=0.006$). From these preliminary results, we hypothesized that since TIL with higher levels of in vitro tumor reactivity were associated with clinical tumor regression, TIL with

reactivity below a critical threshold might represent a therapeutically ineffective cell product for transfer in future clinical trials. To define exploratory criteria for insufficient tumor specific T cell reactivity, we calculated a set of cutoffs based upon the upper 95% confidence limits observed with clinically ineffective TIL. We found that among ten patients whose TIL possessed either <3% tumor-reactive T cells, $<2 \times 10^9$ tumor-reactive T cells, or <100 pg/ml of tumor-induced IFN- γ release, there were no clinical responses. In contrast, among seven patients who received TIL exceeding each of these criteria, there were five objective responders. The stratification of TIL based upon these combined reactivity criteria was significantly associated with clinical response (Table 4; Fisher's exact test, $P=0.003$), with the low criteria being associated with treatment failure.

Discussion

Here, we report the largest cohort of metastatic uveal melanoma patients treated with adoptive transfer of autologous TIL. We observed that a single infusion of TIL after a nonmyeloablative lymphodepleting conditioning regimen could induce objective tumor regression in seven of 20 (35%) metastatic uveal melanoma patients, including individuals whose disease was refractory to immune checkpoint blockade. One highly pre-treated patient demonstrated durable complete regression of numerous hepatic metastases that is ongoing close to two years after TIL infusion. To our knowledge, this is the first report describing the ability of TIL transfer to mediate tumor regression in patients with this rare variant of melanoma. These results challenge the belief that uveal melanoma is an immunotherapy resistant cancer.

In clinical practice, immunotherapies have become the primary treatment modality for patients with metastatic melanoma of cutaneous origin. By clinically augmenting pre-existing anti-tumor T cell responses with either systemic cytokine (6), antibodies blocking checkpoint molecules (7, 9, 10), or adoptive transfer of autologous TIL (8), significant and potentially curative cancer regression can now be achieved in cutaneous melanoma patients. However, the role of these immune based therapies for the treatment of metastatic uveal melanoma has been unclear. In recent published reports of immune checkpoint inhibitors targeting either CTLA-4, PD-1 or PD-L1, objective regression of metastatic uveal melanoma by standardized oncologic criteria has been rare (12-20). These findings have led to hypotheses that uveal melanoma may represent a tumor variant that is poorly recognized by the host immune system, and consequently resistant to immunotherapeutic strategies. It has been speculated that since the primary tumor arises in the eye, an immune privileged site, the tumor and its metastases harbor local immunosuppressive or cellular immuno-evasive factors that render immunotherapies unsuccessful (21). Another theory proposes that since sun-shielded ocular melanomas have far fewer somatic mutations compared to sun-exposed cutaneous melanomas, there are consequentially fewer potential mutated neo-epitope targets for effective anti-tumor immunity. However, our recent identification of tumor reactive TIL from freshly resected uveal melanoma metastases (22) combined with the clinical efficacy of TIL transfer noted in the current trial serve to challenge these theories and suggest that potent immune responses can be generated against metastatic uveal melanoma.

The precise mechanism for the anti-tumor responses observed in this study is still under investigation. All patients received a single cycle of fludarabine and cyclophosphamide, not intended as a direct cytotoxic therapy, but rather as a lymphocyte depleting regimen prior to cell transfer to enhance T cell engraftment and efficacy. Although neither of these chemotherapies has demonstrated clinical activity in metastatic melanoma (27, 28), we cannot completely exclude their possible role in the tumor responses. It should be noted, however, that in reviews of single agent and combinatorial chemotherapy, response rates have been <5% in metastatic ocular melanoma (29, 30), suggesting that any contribution of the chemotherapy in this trial was likely limited. In support of an immune mediated anti-tumor mechanism is the strong association that we observed between clinical response and the level of in vitro anti-tumor reactivity and the frequency of tumor-reactive T cells within the TIL infusion. We observed that approximately 50- 60% of expanded TIL in our study had strong anti-tumor reactivity based upon our exploratory criteria. Among ten patients treated with poorly reactive TIL, there were no clinical responses. In contrast, among seven patients who received TIL with strong anti-tumor reactivity, there were five objective responders. We did, however, observe that two patients did not show clinical tumor regression despite having received TIL with potent in vitro anti-tumor reactivity. A potential explanation may stem from deficient engraftment and persistence of these reactive T cells. Prior analyses of TIL transfer in cutaneous melanoma patients have associated the ability of the transferred cells to persist in vivo with their anti-tumor efficacy (8, 31). Efforts are ongoing to assess T cell persistence in the current trial and its relationship to response.

Interestingly, we found the adoptive transfer of TIL could mediate tumor regression in highly treatment refractory uveal melanoma patients who had shown progression after both anti-CTLA-4 and anti-PD-1 checkpoint blockade. These findings might be explained by several potential advantages of adoptive T cell therapy when compared to other immune based therapies. First, T cell populations having optimal recognition of autologous tumor antigens can be preferentially isolated ex vivo for therapy. Next, these selected T cells can be expanded to large numbers utilizing in vitro conditions that overcome tolerizing factors that might exist within the tumor microenvironment. Finally, and importantly, the host can be conditioned prior to cell transfer to eliminate regulatory and immunosuppressive factors. The combinations of these actions may explain how the transfer of TIL in the current study could induce tumor regression when prior immunotherapies were ineffective. We cannot, however, exclude the possibility that prior checkpoint inhibition in our patients could have had an immunologic impact on the endogenous TIL that was procured for adoptive transfer. Although not the focus of the current study, future efforts are aimed at determining whether initial checkpoint inhibition can have a “priming” effect on the TIL repertoire and their activation status.

Similar to our previous TIL transfer studies in cutaneous melanoma (8, 32), we found nearly all of the significant toxicities observed in the current trial were associated with chemotherapy-induced bone marrow suppression. Hematologic toxicities, such as neutropenia, were typically transient and not associated with clinically significant infectious sequelae. The one notable exception was a patient who died after developing overwhelming sepsis and multi-organ failure secondary to a severe treatment-refractory respiratory syncytial virus pneumonia. Given the patient's prior history of heavy smoking and

emphysema, this treatment-related mortality emphasizes the need to screen eligible patients for their risk of developing serious infections during the neutropenic period. Interestingly, in contrast to the toxicities observed with immune checkpoint inhibitors, we did not observe significant autoimmune adverse events after TIL therapy.

Although the initial immunotherapeutic responses observed in this trial are encouraging, there are important limitations of the current study that should be considered before extrapolating these results to the general care of metastatic uveal melanoma patients. Individuals enrolled in this study were highly selected based upon strict eligibility criteria to ensure that they could tolerate the preparative chemotherapy regimen and high dose interleukin-2. As a result, the treated patients had a young median age (54 years), good performance status (ECOG 0-1), near normal hepatic reserve, and a rate of tumor growth that would allow time to generate the TIL product (~5 weeks). Thus, the subjects described in this report may have had a more favorable metastatic tumor biology and potentially an enhanced responsiveness to immune based treatments when compared to other patients with more advanced and aggressive disease. Although we observed that four of the seven clinical responders in this trial carried mutations of the *BAP-1* tumor suppressor gene, more extensive correlative studies with other poor prognostic attributes such as size and location of the primary uveal tumor, gene expression profile (GEP) class, and clinical symptoms upon initial metastatic diagnosis were not evaluated in this pilot study. Ongoing efforts are aimed at prospectively determining whether TIL therapy is effective in patients with these poor prognostic features.

Building upon the initial findings of this study, future efforts are focused on improving the frequency, completeness, and durability of the clinical responses. Biopsies from both treatment-refractory and treatment-responsive tumors are being accrued for future genomic and proteomic profiling to identify correlates of clinical response. Further, in an attempt to define the immunogenic targets responsible for the remissions seen in this trial, the source tumors are undergoing whole exomic sequencing to delineate their tumor-specific non-synonymous mutations. In prior analyses of cutaneous melanoma patients who achieved durable clinical responses, TIL were found that immunologically recognized one or more somatic mutations (33, 34). These findings provide compelling evidence that tumor specific mutations can generate neo-epitopes that elicit robust autologous immunologic responses in cutaneous melanoma patients. Interestingly, we previously reported that uveal melanoma metastases harbor far fewer non-synonymous mutations compared to cutaneous melanoma metastases (22). Ongoing studies are now being conducted in the current cohort of responding uveal melanoma patients to determine if their TIL recognize neo-epitopes derived from these few somatic mutations. Future selective administration of mutation specific T cell populations may help to enhance clinical efficacy.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Figure 1. In vitro assessment of autologous tumor reactivity found within TIL infusion
 (A.) Frequency of tumor-reactive T cells within the TIL infusion product as determined by the sum of the flow cytometric measurements of the T cell activation markers, OX40 (CD134) on CD4+ T cells and 4-1BB (CD137) on CD8+ T cells following overnight co-culture with cryopreserved autologous tumor digests. Percentages represent mean of triplicate cultures. (B.) Tumor induced IFN- γ production by TIL as determined by ELISA of the supernatant following overnight co-culture with cryopreserved autologous tumor digests minus the background seen against autologous APCs (negative control). IFN- γ values represent the mean of triplicate cultures. (*) indicates undetectable levels based upon the sensitivity of the respective assays. N/A indicates data not available due to insufficient quantities of cryopreserved tumor targets.

Figure 2. Clinical responses in metastatic uveal melanoma patients after TIL therapy
 (A.) Waterfall plot demonstrating the maximum percentage decrease in the sum of the reference diameters of the target lesions from baseline to nadir, as assessed by the investigator. Data are shown for patients who underwent at least one tumor assessment after treatment (n=20). Orange bars specify patients who had not responded after receiving prior immune checkpoint blockade; blue bars indicate patients who had not received prior immune checkpoint blockade. The horizontal dashed lines indicate a 30% reduction in the tumor burden in the target lesions (the cut-off for objective response by RECIST criteria). Each of the patients achieving 30% reduction were confirmed responders by RECIST criteria. The percentage increase was truncated at 100%. '+' indicates ongoing response at time of report. (B.) Spider plots depicting pattern of changes in cumulative target lesion size over time for each patient (n=20) after TIL therapy.

Figure 3. Association between clinical response and pre-treatment TIL reactivity

TIL administered to responding (R, n=5) and non-responding (NR, n=12) patients were compared based upon their (A.) frequency of tumor-reactive T cells, (B.) absolute numbers of infused tumor-reactive T cells, and (C.) levels of IFN- γ release after autologous tumor stimulation. Each dot represents an individual infused TIL product that underwent reactivity testing. Exploratory in vitro criteria defining TIL with sufficient (orange shading) and insufficient (blue shading) anti-tumor reactivity are shown. Statistical comparisons were performed with the Wilcoxon rank-sum test. *P* values are 2-tailed, but have not been adjusted for multiple comparisons. Bar denotes median value.

Table 1

Baseline patient characteristics

Pt No.	Age (yrs)	Sex	Primary Ocular Tumor	Primary Ocular Therapy	Metastatic Sites	AJCC M stage for Uveal Melanoma	Prior Metastatic Therapy
1	55	F	Uveal	Enuc	Liv, Bo	M1c	anti-CTLA-4, anti-PD-1, regional
2	47	F	Uveal	RT	Liv, Lu, Panc, Bo	M1a	None
3	59	M	Uveal	RT	Liv, Lu, SQ, Per, LN	M1c	anti-CTLA-4, anti-PD-1
4	56	M	Uveal	RT	Liv, Brain, Lu, Om, Per	M1c	MEKi, chemo, other
5	63	F	Uveal	RT	Liv	M1c	None
6	55	F	Uveal	Enuc	Liv	M1b	None
7	56	M	Uveal	Enuc	Liv	M1c	None
8	47	M	Uveal	RT	RP	M1b	None
9	58	M	Uveal	RT	Liv, IM	M1c	None
10	52	F	Uveal	RT	Liv, Bo, Om, Per	M1c	None
11	54	F	Uveal	RT	Liv, LN	M1a	None
12	37	M	Uveal	RT	Liv, Om, LN	M1b	anti-CTLA-4, chemo, regional
13	50	F	Uveal	Enuc	Liv, Lu, LN, SQ, Om, Per, Bo, Panc	M1b	anti-CTLA-4, anti-PD-1, MEKi, regional
14	35	F	Uveal	RT	Liv, Lu, LN, SQ, Bre, Bo, Panc	M1b	Regional, chemo, other
15	53	M	Uveal	RT	Liv, LN, Om, Per, SQ	M1c	anti-CTLA-4, anti-PD-1, MEKi, regional, other
16	53	M	Uveal	Enuc	Liv	M1b	anti-CTLA-4, anti-PD-1, other
17	32	M	Uveal	Enuc	Liv, Lu	M1b	anti-CTLA-4, anti-PD-1, other
18	57	M	Uveal	RT	Liv, Lu	M1b	anti-CTLA-4, anti-PD-1
19	61	M	Uveal	RT	Liv, Per, Bo	M1a	None
20	53	M	Uveal	RT	Liv, Per, LN, IM	M1b	Regional, other
21	62	M	Uveal	RT	Liv, Per, RP, LN, IM, SQ	M1b	Chemo, anti-CTLA-4, anti-PD-1, Regional, other

RT, Radiotherapy; Enuc, Enucleation; Liv, Liver; Lu, Lung; Panc, Pancreas; Bo, Bone; SQ, Subcutaneous; Per, Peritoneum; LN, Lymph Node; Om, Omentum; IM, Intramuscular; RP, Retroperitoneum; Bre, Breast;

Table 2

Characteristics of procured metastases, administered T cells, and outcomes

Procured Metastases Characteristics			Administered TIL Characteristics				Treatment and Outcome			
Pt No.	Tumor Harvest Site	GNAQ/11 Mutation Status	Nucleotide, Amino Acid Change	CD4+ (%)	CD8+ (%)	% Tumor Reactive Cells	Tumor Induced IFN- γ (pg/ml)	No. of Cells ($\times 10^6$)	No. of IL-2 Doses	Duration of RECIST Response (months)
1a	Liv	GNAQ	c.626A>T, p.Gln209Leu	4	95	N.D.	N.D.	114	7	SD (5)/NR
1b				51	48	N.D.	N.D.	31	6	NR
2a	Liv	Neither	--	14	85	<0.1	<10	138	5	SD (6)/NR
2b				51	44	1.6	184	30	6	NR
3	Liv	GNA11	c.626A>T, p.Gln209Leu	19	78	9.2	1492	101	2	NR
4	Om	GNA11	c.626A>T, p.Gln209Leu	14	82	0.6	108	114	3	NR
5	Liv	GNA11	c.626A>T, p.Gln209Leu	76	21	<0.1	19	85	4	NR
6	Liv	GNAQ	c.626A>C, p.Gln209Pro	62	36	0.7	<10	86	6	NR
7	Liv	GNAQ	c.626A>C, p.Gln209Pro	67	30	3.3	108	31	5	NE (TRM)
8	RP	GNAQ	c.626A>T, p.Gln209Leu	51	45	<0.1	20	79	4	NR
9	Liv	GNA11	c.626A>T, p.Gln209Leu	95	2	0.3	<10	67	3	NR
10	Liv	GNA11	c.626A>T, p.Gln209Leu	76	27	8.0	656	79	0	PR (4)
11	Liv	GNAQ	c.626A>C, p.Gln209Pro	65	29	N.D.	N.D.	92	6	PR (5)
12	Om	GNA11	c.626A>T, p.Gln209Leu	65	32	3.6	26	90	5	NR
13	Om	GNA11	c.626A>T, p.Gln209Leu	26	74	15.7	737	18	6	NR
14	Bre	GNA11	c.626A>T, p.Gln209Leu	16	78	3.7	725	74	2	PR (9)
15	SQ	Neither	--	2	98	48.1	12806	126	2	PR (3)
16	Liv	GNAQ	c.626A>C, p.Gln209Pro	60	39	N.D.	N.D.	128	8	CR (20+)
17	Lu	GNAQ	c.626A>C, p.Gln209Pro	60	38	0.5	15	75	6	NR
18	Liv	GNA11	c.626A>T, p.Gln209Leu	78	23	3.0	13	17	8	NR
19	Liv	GNA11	c.626A>T, p.Gln209Leu	63	38	<0.1	17	83	5	NR
20	LN	GNAQ	c.626A>C, p.Gln209Pro	19	82	10.6	3583	76	6	PR (4+)
21	LN	GNA11	c.626A>T, p.Gln209Leu	2	98	9.4	6550	111	3	PR (4+)

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N.D., Not done due to insufficient fresh tumor tissue; SD, Stable Disease; NR, Non Responder; PR, Partial Responder; NE, Non evaluable; TRM, Treatment Related Mortality. Initial treatment and re-treatment are denoted by a and b respectively.

Table 3

Adverse events observed in all patients during therapy (n=21 patients)

Adverse Event	Grade 1-2	Grade 3	Grade 4	Grade 5
Lymphopenia	0	0	21 (100%)	0
Neutropenia	0	0	21 (100%)	0
Thrombocytopenia	0	1 (5%)	20 (95%)	0
Anemia	0	14 (67%)	0	0
Creatinine	1 (5%)	6 (29%)	0	0
Dyspnea	4 (19%)	2 (10%)	0	0
Febrile neutropenia	0	6 (29%)	0	0
Infection	0	5 (24%)	0	1 (5%) [*]
Fatigue	4 (19%)	0	0	0
AST, SGOT (serum glutamic oxaloacetic transaminase)	0	3 (14%)	0	0
Bilirubin (hyperbilirubinemia)	0	3 (14%)	0	0
Alkaline phosphatase	0	2 (10%)	0	0
Albumin, serum-low (hypoalbuminemia)	0	2 (10%)	0	0
Phosphate, serum-low (hypophosphatemia)	0	2 (10%)	0	0
Hypotension	2 (10%)	0	0	0
Cardiac Arrhythmia	1 (5%)	1 (5%)	0	0
Hypopigmentation	1 (5%)	0	0	0
ALT, SGPT (serum glutamic pyruvic transaminase)	0	1 (5%)	0	0
CNS cerebrovascular ischemia	0	0	1 (5%)	0
Cough	0	1 (5%)	0	0
Edema	1 (5%)	0	0	0
Hypoxia	1 (5%)	0	0	0
Neuropathy: motor	0	0	1 (5%)	0
PTT (Partial Thromboplastin Time)	0	1 (5%)	0	0
Rash/desquamation	1 (5%)	0	0	0
Renal failure	0	1 (5%)	0	0
Sodium, serum-low (hyponatremia)	0	1 (5%)	0	0
Thrombosis/thrombus/embolism	0	1 (5%)	0	0
Vision-blurred vision	1 (5%)	0	0	0

Data are n (%).

* Death secondary to sepsis induced multi-organ failure

Table 4

Association between patient and treatment characteristics with clinical outcome

	Responders (R)	Non-responders (NR)	P value
Patients treated, n (% of total)	7 (35%)	13 (65%)	
Age, median (25th-75th percentile)	53 (52-54)	55 (47-59)	0.25
Sex, n (% of R or NR)			
Female	3 (43%)	5 (38%)	1
Male	4 (57%)	8 (62%)	
Primary eye therapy, n (% of R or NR)			
Radiotherapy	6 (86%)	9 (69%)	0.61
Enucleation	1 (14%)	4 (31%)	
Prior metastatic therapies, n (% of R or NR)			
None	2 (29%)	6 (46%)	0.64
Two or more regimens	5 (71%)	7 (54%)	
Prior immunotherapy, n (% of R or NR)			
None	4 (57%)	7 (54%)	1
Anti-CTLA-4 and/or anti-PD-1	3 (43%)	6 (46%)	
Source of TIL, n (% of R or NR)			
Liver metastasis	3 (43%)	8 (62%)	0.64
Extra-hepatic metastasis	4 (57%)	5 (38%)	
Number of non-synonymous tumor mutations, median (25th-75th percentile)	29 (23-78)	27 (20-81)	0.55
Doses of IL-2 administered, median (25th-75th percentile)	3 (2-6)	5 (4-6)	0.19
Number of TILs administered, median (25th-75th percentile) $\times 10^9$			
Total CD3+	92 (76-126)	85 (71-108)	0.5
CD8+	58 (27-109)	31 (16-86)	0.29
CD4+	14 (3-60)	40 (15-56)	0.66
Phenotype of TILs administered, median (25th-75th percentile)			
% CD8+	78 (29-98)	38 (28-80)	0.32
% CD4+	19 (2-65)	60 (17-71)	0.25
Frequency of tumor reactive TILs (%)[*], median (25th-75th percentile)	9.4 (5.9-29.4)	0.6 (0.1-3.5)	0.011
Number of tumor reactive TILs[*], median (25th-75th percentile) $\times 10^9$	8.1 (4.5-36)	0.5 (0.1-2.3)	0.008
Tumor induced IFN-γ release by TILs (pg/ml)[*], median (25th-75th percentile)	3583 (691-9678)	18 (11-88)	0.006
In vitro tumor reactivity criteria, n (% of tested R or NR)[*]			
3% frequency, 2×10^9 cells, <u>and</u> IFN- γ 100 pg/ml	5 (100%)	2 (17%)	0.003

	Responders (R)	Non-responders (NR)	P value
<3% frequency, <2×10 ⁹ cells, <u>or</u> IFN- γ <100 pg/ml	0 (0%)	10 (83%)	
Tumor unavailable for testing	2	1	

* Responders (n=5) vs. Non-responders (n=12)