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Immunotherapy Following Regional Chemotherapy Treatment of Advanced Extremity Melanoma

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Abstract

Purpose—Following regional chemotherapy (RC) for melanoma, approximately 75 % of patients will progress. The role of immunotherapy after RC has not been well established.

Methods—A prospective, single-institution database of 243 patients with in-transit melanoma (1995–2013) was queried for patients who had progression of disease after RC with melphalan and subsequently received systemic immunotherapy. Fifteen patients received IL-2 only, 12 received ipilimumab only, and 6 received IL-2 followed by ipilimumab. Fisher's exact test was used to determine if there was a difference in number of complete responders after immunotherapy.

Results—With IL-2 alone, all patients progressed. After ipilimumab alone, three patients had a complete response and nine had progressive disease. Six additional patients received IL-2 first then ipilimumab. All six progressed on IL-2 but three went on to have a complete response to ipilimumab while three progressed. The use of ipilimumab at any time in patients who progressed after RC was associated with higher rate of complete response compared to use of IL-2 alone (33 vs. 0 %; $p = 0.021$).

Conclusions—Patients with progression after regional therapy for melanoma may benefit from immunologic therapy. In this group of patients, immune checkpoint blockade with ipilimumab has a higher complete response rate than T cell stimulation with IL-2, with no complete responders in the IL-2 only group. Furthermore, the complete response rate for ipilimumab in our cohort is higher than reported response rates in the literature for ipilimumab alone, suggesting that the effects of immunotherapy may be bolstered by previous regional treatment.

In-transit melanoma is defined as locally recurrent deposits of tumor in the cutaneous and subcutaneous tissue located between the primary site and regional lymph node basin. Unresectable disease, typically defined as greater than three lesions or one lesion >5 cm, can be treated effectively with regional chemotherapy (RC), where high concentrations of drug [usually l-phenylalanine mustard, (LPAM)] are circulated in the affected limb in the form of isolated limb infusion (ILI) or hyperthermic isolated limb perfusion (HILP). Complete response (CR) rates for RC have ranged from 23 to 82 %, although in the long term, approximately 65–85 % of these patients will experience a recurrence.^{1–4} Systemic

treatment is considered if recurrent extremity disease is not amenable to wide excision or repeat regional therapy or if metastatic disease is present.

Recently, immune-targeted systemic therapies for metastatic melanoma have shown considerable promise. Ipilimumab is an anti-CTLA-4 antibody with an overall response rate of approximately 11 %.^{5,6} Other approved immunotherapies for advanced melanoma include IL-2 and interferon- α 2b, while GM-CSF, various melanoma vaccines, and the anti-PD-1 and anti-PD-L1/PD-L2 monoclonal antibodies are currently in clinical trial development. IL-2 is a well-established systemic option with a complete response rate of approximately 6 %; however, extended durability among complete responders ranges from 1.5 to 148 months.^{7,8} Fifty-nine percent of complete responders remained disease-free after 7 years.⁹ Patients with only cutaneous or subcutaneous metastases have a higher response rate (53.6 %) to IL-2 compared with those with disease at other sites.¹⁰

Adjuvant systemic therapy following failure of RC is poorly described in the literature. Cytotoxic chemotherapy agents for a variety of cancers have been shown to have off-target effects in generating antitumor immune responses, whose effects can be augmented further by immune-modulating agents.^{11–13} Thus, there also may be a role for the combined use of cytotoxic (i.e., LPAM) and immunologic agents for the treatment of advanced extremity melanoma. The purpose of this study was to describe response rate and survival of patients who had been treated with immune-based therapies after RC progression.

PATIENTS AND METHODS

A prospective, single-institution database (1995–2013) was reviewed for patients with intransit melanoma who had progression of disease after RC and subsequently received systemic immunotherapy (Fig. 1). The Institutional Review Board at Duke University approved this study, and informed consent was obtained for all subjects. All patients had in-transit extremity melanoma (stage IIIB, IIIC, or IV) melanoma by American Joint Commission on Cancer classification.¹⁴ Any patient with stage IV disease had distant disease resected before RC. High disease burden was defined as the presence of any one lesion greater than 3 cm in diameter or 10 or more lesions.

Regional chemotherapies included were: HILP with melphalan, ILI with melphalan, ILI with melphalan in conjunction with ADH-1, ILI with melphalan in conjunction with sorafenib, and ILI with temozolomide. These procedures were performed as previously described in the literature.^{2,15} Patients were evaluated at 2, 6 weeks, and 3 months following RC. Response to RC was determined at 3 months post-RC by Response Evaluation Criteria in Solid Tumors modified for cutaneous disease and PET/CT scan.² Follow-up clinical exam and PET/CT imaging was obtained every 3 months for the first year, every 6 months for the next 4 years, and every 12 months thereafter.

To be included in this study, patients must have received at least one dose of ipilimumab and/or IL-2 after their last RC treatment. We define immunotherapy for the purpose of this study as either ipilimumab or IL-2. Ipilimumab was given on a standard dosing regimen at 3 mg/kg unless unknown due to a blinded clinical trial. IL-2 was given either as a single agent

(600,000 IU/kg per dose, delivered intravenously) or as part of a combination therapy (dacarbazine, cisplatin, vinblastine, interferon, and/or temozolomide). Response to immunotherapy was indicated in the patient chart from clinical data and radiography imaging reports.

Fisher's exact test, Chi squared test, and Student's *t* test were used to compare baseline patient characteristics of age, sex, site, disease burden, number and type, or previous regional therapies, and response to previous RC. A Fisher's exact test was used to determine if there was a difference in number of complete responders after immunotherapy with IL-2 versus ipilimumab (either alone or following IL-2). Median months from immunotherapy to progression or duration of complete response to immunotherapy also were calculated. For those patients who progressed, difference between immunotherapy groups in the time from immunotherapy to progression was compared using Student's *t* test.

Median months of survival were calculated. A Kaplan–Meier survival curve was used to visualize those patients who received either Ipilimumab or IL-2 and those with progressive disease after RC but who did not receive immunotherapy. Death status was queried against the Social Security Death Index to capture any patients whose deaths were not documented in the patient chart. Patients were assumed to have had died due to progressive disease unless otherwise documented. Differences in survival probabilities between groups were tested with the log-rank test. Statistical significance for all tests was set at $p < 0.05$. All statistical analysis was performed using R version 0.96.331.

RESULTS

A total of 243 patients were identified who underwent RC between 1995 and 2013. Of these, 33 patients received immunotherapy following RC due to persistent or recurrent disease (5 patients), progression within the limb (4 patients), or progression outside the limb (24 patients). Fifteen patients received IL-2 alone (defined as IL-2 as a single agent or part of a combination therapy not, including ipilimumab), 12 received ipilimumab only, and 6 received IL-2 then ipilimumab. The remaining 208 patients in the RC database had complete follow-up information and had received neither of these immunotherapies. Of these, 150 had extremity progressive disease.

There were no significant differences in tumor burden, response to RC, age at the time of RC, sex, and treatment site between immunotherapy groups. Type of RC and number of prior regional treatments were not significantly different between groups. Although there were seven complete responders at 3 months post-RC, all patients eventually progressed and required systemic chemotherapy. A few patients received other systemic chemotherapies following RC but before immunotherapy (Table 1).

Systemic immune treatment was initiated a median of 1.7 months after progression on RC for IL-2 group and 1.9 months for the ipilimumab group. Those patients who received both immunotherapies received IL-2 after 1 month and ipilimumab after 9.3 months (Table 2).

Using IL-2 alone, 15 of 15 patients (100 %) progressed. Using ipilimumab alone, three patients (25 %) had a complete response, whereas nine (75 %) had progressive disease. Six

additional patients received IL-2 first then ipilimumab. In this group, four had progressive disease after IL-2, whereas two had stable disease. They were all transitioned to ipilimumab, to which three patients (50 %) had a complete response and three (50 %) progressed.

Complete tumor response with systemic therapy only occurred with ipilimumab (6/18, 33 % CR). All patients treated with IL-2 alone progressed. This difference in complete response was statistically significant ($p = 0.021$). In addition, complete response among ipilimumab-treated patients was durable, with disease-free survival of at least 16.2 months among those treated with ipilimumab only and 15.3 months among those treated with IL-2 and then ipilimumab. For those patients who did progress after immunotherapy, patients treated with ipilimumab alone had a longer time to disease progression than those treated with IL-2 alone (median 4.2 vs. 2.5 months), which may reflect the difference in time clinicians wait before reimaging after each type of treatment. This difference was not significant ($p = 0.36$).

Median survival from the time of regional therapy for those who received any immune treatment was 30 months compared with 24 months for patients who did not receive immunotherapy following RC. This survival difference was not significant ($p = 0.18$). The median survival from the time of immunotherapy for those who received any ipilimumab was 13.4 months, whereas the median survival for those who received IL-2 only was 10.1 months; this difference was not significant ($p = 0.22$; Figs. 2 and 3).

DISCUSSION

RC is an effective treatment strategy for locally advanced melanoma of the extremity, with complete response rates ranging from 23 to 82 %.^{1–3} However, 55 % of patients will progress rapidly in the short term, and 65–85 % of complete responders will eventually progress.⁴ When disease is no longer amenable to regional treatment, patients have a variety of systemic treatments from which to choose.

Immunological agents have demonstrated efficacy in melanoma with generation of immunologic memory as the key to durable tumor control. In this study, we demonstrate that patients with advanced extremity melanoma who progress after regional therapy may be salvaged with immunologic therapy. Immune checkpoint blockade with ipilimumab had a higher complete response rate than T-cell stimulation with IL-2 in our patient population. Patients treated with ipilimumab, either alone or following IL-2, had a superior CR rate of 33 % compared with 0 % for the IL-2 only group. This CR rate for ipilimumab is in fact much higher than reported overall response rates for ipilimumab as a single agent. The established overall response rate (complete and partial response) for ipilimumab as a single agent is between 4.2 and 10.9 % at a 3-mg/kg dose level and 11.1 % at the 10-mg/kg dose level.^{5,6} This raises the question as to whether patients who undergo RC, including those who have disease progression after RC, have an augmented antitumor response related to cytotoxic chemotherapy-induced tumor cell death that can be exploited by immunologic agents. At minimum, patients who receive immunologic treatment after RC are not disadvantaged by their previous regional treatment. Furthermore, progression after RC does not identify a group of patients resistant to ipilimumab. In contrast, it is surprising that in regionally treated patients, partial and complete response rates are not higher for patients

who received IL-2. Established overall response rates to IL-2 as a single agent are approximately 6 %, but range from 5 to 27 %, and response to IL-2 as part of a biochemotherapy regimen range from 0 to 32.2 %.^{7,8,16} A large review of IL-2 therapy suggests that patients with isolated soft-tissue metastases have a higher response rate (53.6 %) than those with metastases elsewhere.¹⁰ However, our six patients with isolated soft tissue disease at the time of IL-2 treatment all progressed. Among patients who progress after RC, T-cell stimulation with IL-2 does not appear to be an effective rescue therapy.

In the cohort of patients who received both IL-2 and ipilimumab, the CR rate after ipilimumab (50 %) also is greater than established overall response rates to ipilimumab as a single agent. Previous studies have shown that prior clinical response and progression-free survival with single-agent IL-2 did not predict response to ipilimumab and that response to ipilimumab following IL-2 are similar to ipilimumab alone.¹⁷ Our data demonstrate that prior RC did not alter this relationship between these two immunotherapies and that patients who fail IL-2 are not disadvantaged by this course of therapy and could still benefit from subsequent ipilimumab treatment.

We also looked at all patients in our database with disease progression following RC and compared survival outcomes between those who did and did not receive immunotherapy. Median survival for patients who received immunotherapy was 29.8 months and for patients with disease progression who did not receive immunotherapy was 24.2 months. The difference in survival was not significant ($p = 0.22$). Limited sample size and only early follow-up data for immune-treated patients may explain the absence of a difference in survival between groups.

A number of ongoing and upcoming clinical trials combining RC and immunotherapy will help better elucidate the combined effect of these treatments on response and overall survival. Memorial Sloan-Kettering Cancer Center is currently conducting a phase II study of adjuvant ipilimumab after ILI (clinicaltrials.gov: NCT01323517). In this trial, patients receive ipilimumab 1–3 weeks after a melphalan-based ILI. Our institution will soon begin a phase II trial of neoadjuvant ipilimumab followed by standard-of-care ILI. Neoadjuvant use of ipilimumab may alter the tumor microenvironment by changing the ratio of T-regulatory cells to CD8 + T cells in tumor-infiltrating lymphocyte populations.¹⁸ What effect these changes may have on subsequent RC response remains to be determined.

Our interest in the interplay of RC and immunotherapy stems from data emerging on the role of the immune response, both systemically and in the tumor microenvironment, in affecting disease outcomes. Our group has recently shown that melanoma downregulation of the type III TFG-beta receptor (TbetaRIII) suppresses the local immune microenvironment and that advanced melanoma patients with higher plasma levels of soluble TbetaRIII exhibit superior response rates and improved overall survival following ILI regional therapy.¹⁹ Patients with intransit melanomas have been demonstrated to be globally immunosuppressed, suggesting a role for immunotherapy to stimulate immune response to more normal levels.²⁰ Within the tumor microenvironment, melphalan-based RC has been shown to upregulate the costimulatory molecules CD80 and CD86 to produce T-cell stimulation in response to tumor antigen.²¹ Melphalan-based RC also has been reported to alter T-cell stimulation, NK cell

activity, and sICAM-2 levels in the peripheral blood.^{22,23} Furthermore, our group has demonstrated variable tissue hypoxia for in-transit melanoma tumors using optical spectroscopy.²⁴ Altered vasculature in the tumor, driven by HIF-1 and VEGF, affect drug delivery and HIF-1- α expression in hypoxic tumor cells may also inhibit tumor-specific T cells, thus protecting the tumor from immune destruction.^{25–29} Immune modulators, such as ipilimumab, may be able to augment the immune-activating properties of RC to alter dynamics in favor of tumor destruction and systemic immune response.^{30–33}

This current study on adjuvant immunotherapy following progression after RC has several limitations. This was a retrospective review of patients who had undergone RC and were found to have received IL-2 or ipilimumab. In addition, our IL-2 group consisted of patients who had both been treated with high-dose, single-agent IL-2 and those who had been treated with IL-2 as part of a biochemotherapy regimen. These two components of our IL-2 group may not be comparable. Lastly, response to immune treatment was not measured against the specific immune-related response criteria but rather was determined by clinical exam and radiography, with response designated by the clinician in the patient chart.

Despite these limitations, we conclude that patients with progression after regional therapy for melanoma may be salvaged with immunologic therapy. In this group of patients, immune checkpoint blockade with ipilimumab has a higher complete response rate than T-cell stimulation with IL-2. In addition, ipilimumab response rates after RC appear to be higher than response rates reported in the literature for ipilimumab alone for systemic disease, which suggest a synergism between these two treatment strategies. Our findings in regionally treated patients are consistent with the promising data currently emerging on immunotherapy for the treatment of metastatic melanoma. Based on our data, we recommend that systemic ipilimumab be considered for patients who progress after RC of their in-transit melanoma. Further study of the combined use of RC and systemic immunotherapy may allow us to define appropriate treatment algorithms in the neoadjuvant or pre-progression posttreatment settings in this unique population of melanoma patients.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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FIGURE 1.

Selection of patients included in this study: 125 patients progressed and did not receive IL-2 and/or Ipilimumab; 6 patients received IL-2 then Ipilimumab; 12 patients received Ipilimumab only; 15 patients received IL-2 only; 33 patients progressed and received IL-2 and/or Ipilimumab; 158 patients progressed after ILI; 83 patients with no evidence of disease after ILI; 241 patients with follow-up data; 243 patients who had undergone ILI.

FIGURE 2.

Comparing survival from the time of regional therapy among patients with disease progression after regional chemotherapy, by whether or not they received any form (IL-2 or Ipilimumab) of adjuvant immunotherapy: 150 patients did not receive immunotherapy, whereas 33 patients did receive immunotherapy. There was no difference in survival between groups ($p = 0.18$).

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FIGURE 3. Comparing survival between patients who received ipilimumab alone, IL-2 alone, or both therapies, measured from the time of most recent immunotherapy initiation. There was no difference in survival between groups ($p = 0.47$). One patient did not have an IL-2 start date

TABLE 1

Patient and regional chemotherapy characteristics

	IL-2only	Ipilimumab only	IL-2 then ipilimumab
<i>N</i>	15	12	6
Age at regional treatment (mean, SD)	54 ± 13	65 ± 9	47 ± 13
Sex (M:F)	7:8	7:5	2:4
Site (upper:lower extremity)	0:15	4:8	3:3
Last regional therapy type			
HILP	8	3	1
LPAM	4	5	2
Sorafenib	2	0	0
ADH-1	1	1	1
TMZ	0	3	2
Number of regional therapies prior to immunotherapy			
1	12	5	4
2	3	6	0
3	0	1	1
4	0	0	1
Response to last regional chemotherapy (3 months Post-RC by RECIST criteria)			
CR	3	3	1
PR	4	0	0
SD	0	5	1
PD	8	4	4
Other systemic treatments			
Vemurafenib	0	1	0
Vaccine	1	0	0
GM-CSF	0	0	2
Temozolomide	0	0	1

HILP hyperthermic isolated limb infusion with melphalan; *LPAM* isolated limb infusion with melphalan and dactinomycin; *Sorafenib* LPAM-based ILI in combination with sorafenib; *ADH-1* LPAM-based ILI in combination with ADH-1; *TMZ* temozolomide-based ILI; CR complete response; *PR* partial response; *SD* stable disease; *PD* progressive disease

TABLE 2

Immunotherapy characteristics

	IL-2only	Ipilimumab only	IL-2 then ipilimumab
<i>N</i>	15	12	6
Median time from progression on RC to start of immunotherapy (mo)	1.7	1.9	IL-2: 1.0 Ipi:9.3
Response immunotherapy			
CR	0	3 (25 %)	3 ^a (50 %)
PR	0	0	0
SD	0	0	0
PD	15 (100 %)	9 (75 %)	3 ^a (50 %)
Median time from immunotherapy to progression (mo)	2.5	4.2	4.9 ^b
Median duration of complete response, at least (mo)	NA	16.2	15.3 ^b

CR complete response; *PR* partial response; *SD* stable disease; *PD* progressive disease

^aResponse to ipilimumab. After IL-2, 4 patients had PD and 2 had SD before starting ipilimumab

^bFrom start of ipilimumab