Active Immunotherapy of Metastatic Melanoma with Allogeneic Melanoma Lysates and Interferon α

Ulka Vaishampayan, Judith Abrams, Denise Darrah, Vicky Jones, and Malcolm S. Mitchell

Karmanos Cancer Institute, and Division of Hematology/Oncology, Department of Medicine, Wayne State University, Detroit, Michigan 48201 [U. V., M. S. M.]; Karmanos Cancer Institute, and Department of Biostatistics, Wayne State University, Detroit, Michigan 48201 [J. A.]; and University of California, San Diego Cancer Center, La Jolla California 92039 [D. D., V. J.]

ABSTRACT

Purpose: A therapeutic lyophilized melanoma vaccine consisting of two mechanically disrupted allogeneic melanoma cell lines and the immunological adjuvant Detox-PC (Melacine) has demonstrated encouraging activity in metastatic malignant melanoma, often in regimens containing pretreatment with low-dose cyclophosphamide. In addition, IFN-α2b (INTRON A; Schering-Plough Corporation, Kenilworth, NJ) has shown efficacy in melanoma refractory to IFN. Pretreatment with low-dose cyclophosphamide and the high proportion of prolonged durations of remission and the high proportion of prolonged durations of remission in their original form stored frozen at −80°C (“frozen lysates”) and also of the lyophilized Melacine preparation have been performed in stage IV melanoma (2, 3). The Phase I study of 19 patients proved the feasibility of immunization against melanoma-associated antigens in 50% of the patients and elicited a 29% objective clinical response (2). The Phase II trial of the frozen lysates in 25 patients demonstrated a response in 5 patients (20%), objective regressions and one long-term stability, which was associated with an increase in precursors of cytolytic T cells against melanoma cells with clinical benefit (3). In Phase II trials of the lyophilized preparation, Melacine, at a single institution an objective response rate of 15.5% was observed (4).

INTRODUCTION

Melanoma has had a rising incidence, with 51,400 estimated cases in the United States in the year 2001 (1). It is the most lethal skin malignancy with an estimated 7,800 deaths attributable to it in 2001 (1). The majority of the deaths are caused by metastatic melanoma, which is usually resistant to standard cytotoxic therapy, including highly toxic combinations. Hence, immunotherapy has become the mainstay of treatment in advanced malignant melanoma. Active specific immunotherapy (“vaccination”) is a strategy that uses tumor-associated antigens to induce an immune response against the tumor. Although both autologous and allogeneic vaccines have been studied, we have favored an allogeneic vaccine that we devised, prepared from mechanically lysed melanoma cell lines, expressing the range of melanoma-associated antigens as well as alloantigens. A preparation called Melacine (Corixa-Montana) consists of lyophilized lysates of two melanoma cell lines, MSM-M-1 and MSM-M-2, admixed with the immunological adjuvant Detox-PC (Corixa-Montana) immediately before use.

Phase I and Phase II studies of these melanoma cell lysates, in their original form stored frozen at −80°C (“frozen lysates”) and also of the lyophilized Melacine preparation have been performed in stage IV melanoma (2, 3). The Phase I study of 19 patients proved the feasibility of immunization against melanoma-associated antigens in 50% of the patients and elicited a 29% objective clinical response (2). The Phase II trial of the frozen lysates in 25 patients demonstrated a response in 5 patients (20%), objective regressions and one long-term stability, which was associated with an increase in precursors of cytolytic T cells against melanoma cells with clinical benefit (3). In Phase II trials of the lyophilized preparation, Melacine, at a single institution an objective response rate of 15.5% was observed (4).

IFN-α2b (INTRON-A; Schering-Plough Corporation, Kenilworth, NJ) has been tested in metastatic and adjuvant settings in melanoma (5–7). One large randomized trial demonstrated a persistent 11% overall survival benefit with adjuvant IFN therapy in resected high-risk melanoma (7). A study was conducted with the administration of IFN-α in metastatic melanoma patients who had demonstrated refractoriness to Melacine. This trial had a very promising 44% objective response rate. The
median survival of responders was 36 months with a median duration of response of 11 months (8). Responses were elicited in visceral metastatic sites as well as s.c. tissue and lung. On the basis of these encouraging results, a Phase II trial of Melacine and IFN-α in combination was performed in metastatic melanoma. The results of this study are the subject of this report.

PATIENTS AND METHODS

Eligibility Criteria. Eligible patients were required to have a histologically proven diagnosis of melanoma and measurable or evaluable metastatic disease. If patients had been previously treated, they were not eligible for enrollment until 4 weeks had elapsed from the time of previous treatment, such as radiation therapy, chemotherapy, or immunotherapy. Good performance status (Karnofsky scale, ≥70%) was required for enrollment, with adequate bone marrow, hepatic, and renal function (WBC count, ≥3000/mm²; platelet count, ≥100,000/mm²; serum bilirubin, ≤2 mg/dl; and serum creatinine, ≤2 mg/dl). Patients with brain metastases were allowed to enroll if the metastases were controlled for at least 6 weeks by surgery or radiotherapy. Exclusion criteria included allergy to eggs (because the adjuvant was cultured in chicken egg extract), concurrent treatment with corticosteroids or antiestrogens, and the absence of a delayed hypersensitivity reaction to at least one of a standard battery of microbial skin test antigens. These comprised Strepokinase/Streptodornase, Candida, Mumps, Trichophyton (Hollister-Stier Laboratories), and intermediate strength purified protein derivative. Patients with a prior malignancy other than nonmelanoma skin cancers were accepted, but had to have been disease free for a minimum of 5 years. A signed informed consent was obtained from every individual enrolled on the protocol.

Preparation of Lysates and Melacine. The lysates were derived from two distinct melanoma cell lines cultured from the s.c. nodules of two female patients. Both cell lines have been deposited with the American Type Culture Collection. MSM-M-1 (abbreviated M-1) was obtained in 1980, is amelanotic, nearly tetraploid, grows slowly and expresses HLA-A2, HLA-B12, HLA-B62, and HLA-C3 and HLA class II antigens DR4, DR10, DRw53, and DQ8. It also expresses ganglioside GD3 but not GD2. The second cell line (MSM-M-2 or M-2) is diploid with a small percentage of hypodiploid cells and trisomy of chromosome 7. It is highly pigmented, rapidly growing and expresses HLA-A28 (now called HLA-A*6802), -A31, -B51, -B60, -C2, and -C6 and is devoid of HLA Class II antigens by serological assays. However, the genes coding for HLA Class II antigens are present in this cell line.

Mechanical disruption of the melanoma cells for this study was performed at Corixa-Montana with a Polytron stainless steel high-speed tissue homogenizer (Tekmar, Cincinnati, OH) and three cycles of freeze thawing. By serological assays, this preparation contains the common melanoma antigens tyrosinase, gp100, and MART-1/Melan A. Melacine (Corixa-Montana), the lyophilized version of this lysate preparation, stored at 4°C, was used in this study. The lyophilized lysates were mixed with the immunological adjuvant Detox-PC (Corixa-Montana) within 30 min before injection.

Treatment Plan. Cyclophosphamide was administered at a dose of 300 mg/m² i.v. 3 days before the first dose of Melacine. This low dose of cyclophosphamide was intended to down-regulate suppressor T-cell activity, which has been demonstrated experimentally (9–11). Melacine was administered at a dose of 2 × 10⁹ tumor cell equivalents per dose admixed with 0.25 ml of Detox-PC s.c., divided between two sites once a week on weeks 1–4 and week 6. This course was repeated if there was no evidence of disease progression at week 8. Maintenance therapy with Melacine was given monthly thereafter, until progression or excessive toxicity occurred. IFN-α was started in the evening after the fourth Melacine injection at a dose of 5,000,000 units/m² s.c. three times a week until progression occurred. This dose level was based on previous immunological and therapeutic studies with this preparation (8, 12). The dose of IFN-α was reduced to 2,500,000 units/m² three times/week if any grade 3 or 4 toxicity occurred. No dose re-escalation was permitted. If IFN-α was not tolerated despite dose adjustment, it was discontinued. Detox-PC was omitted at the first sign of severe granulomas, but the melanoma lysate portion of Melacine was continued.

Statistical Methods. The primary end point originally selected for this Phase II trial was objective response rate. Response to therapy was assessed every 8 weeks. Patients were taken off the protocol if there was disease progression, a treatment delay of more than 4 weeks, or administration of any other antitumor therapy, or if the patient refused further treatment. Patients were evaluable for response if they completed the first course of therapy, i.e., 8 weeks of treatment. Disease response was assessed using standard criteria for solid tumors (13). Time-to-disease progression was calculated from the date of registration to the date of progressive disease or death. Secondary end points included assessment of toxicity, time to treatment failure, and overall survival. Exact binomial methods were used to calculate CIs around response rates. Kaplan-Meier product limit methods were used to estimate median survival and cumulative survival proportions.

RESULTS

Characteristics of the Patients. Between October 1994 and May 1996 a total of 57 individuals were screened for this study, 10 of whom were found to be ineligible after registration but before treatment. Ineligibility was attributable to incorrect diagnosis, patient’s changing his/her mind about enrolling, rapid progression of the disease before the protocol could be started, or refusal of the insurance company to pay for the study. Reasons for inevaluability included failure to complete 8 weeks of treatment (eight patients) because of rapid progression of disease before the first evaluation point in six patients and failure to comply with the protocol and request to be removed from the study in one patient each (Fig. 1). The median age of enrolled patients was 61 years (range, 23–84 years); 16 participants were female and 31 were male (Table 1). Approximately 25% of the patients had lung metastases only, whereas 57% of the group had metastatic disease in visceral sites and bone.

Treatment. Forty-seven patients were treated per protocol, and the median duration of therapy was 17 weeks. Thirty-
nine patients (83%) completed the first 8 weeks of therapy, and 31 patients completed 16 weeks of therapy; 24 of these received subsequent monthly maintenance treatment. One patient completed 8 weeks, three completed 9 weeks, and one each completed 11, 12, 14, and 15 weeks of therapy. The median duration of maintenance therapy was 19 weeks, (range, 1–288 weeks).

Two patients had a sustained remission and, thus, received treatment beyond 2 years.

**Toxicity.** Melacine was extremely well tolerated. The predominant toxicity noted was local pain at the site of injection for no longer than 24 h after the injection. No severe granulomas or sterile abscesses occurred, because the Detox portion of the vaccine, which we have found to be responsible for these delayed hypersensitivity reactions, was discontinued at the first sign of a significant granuloma. The IFN-related toxicities were mainly constitutional, such as an influenza-like syndrome. There were no treatment-related hospitalizations or mortalities.

**Response and Survival.** Of 39 evaluable patients, CRs2 and PRs were observed in two patients each (10.2%); four (10%) had a minimal response (measurable shrinkage of tumor masses but less than PR or CR) (Table 2). On the basis of these data, we estimate, with 95% confidence, that the objective response rate of patients receiving this therapy is between 10 and 35%. Moreover, ~74% of patients had either an objective remission or a stabilization of their disease. With 95% confidence, we estimate that at least 56% and at most 87% of patients receiving this therapy might expect response or stabilization of their disease. Twenty-one patients (53.8%) demonstrated disease stabilization for at least 4 months (Table 2).

Follow-up was complete as of February 2001. The median survival for all of the 47 patients was 12.5 months (95% CI, 8.3, 15.0); 1- and 2-year overall survival rates were 53 and 17%, respectively (Fig. 2). When analyses were restricted to evaluable patients, median survival was 14.3 months (95% CI, 9.8, 19.5) with 1- and 2-year survival rates of 64 and 21%, respectively (Fig. 3). In the evaluable patients, the median time to failure was 6 months (95% CI, 3, 8), with 1- and 2-year progression-free survival rates of 28 and 10%. The median duration of disease response or stabilization was 8 months (95% CI, 6, 13 months). Median time to treatment failure for all of the 47 patients was 4.0 months (95% CI, 3, 7) with 1- and 2-year progression-free survival rates of 23 and 9%, respectively (Fig. 2). All four of the patients with an objective response, and four with disease stabilization beyond 1 year, survived more than 2 years. Cumulative disease stabilization rates were 38% at 1 year and 14% at 2 years.

**DISCUSSION**

The previously observed objective response rate of 44% with IFN-α administered after Melacine failure was not duplicated in this study of concomitant therapy (Table 3). Concomitant rather than sequential treatment was chosen here to reduce the time required to administer the treatment regimen and to avoid the risk of having the patient not respond to Melacine before affording him or her the potential benefit of the IFN-α. Although the combination of Melacine and IFN resulted in few objective clinical responses, meaningful disease stabilization was achieved in more than one-half of the patients. The possible explanations for this phenomenon are several. It has been shown that the HLA phenotype of the tumor is a significant predictor of

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2 The abbreviations used are: CR, complete remission; PR, partial remission; S.D., stable disease; CI, confidence interval.
response to Melacine in patients with metastatic melanoma (14) and, more recently, with prolonged disease-free response in Stage II melanoma (15). Several HLA Class I phenotypes such as HLA-A2, -B12 and -C3 were associated with a higher response rate to treatment with Melacine alone, perhaps because those alleles presented important immunodominant melanoma epitopes. This could explain the variation in the response rates seen among the three trials (2, 3, 8) of Melacine therapy. Patient selection can also play a large role in explaining the differences in response rates across Phase II trials. Some prognostic factors such as serum lactic dehydrogenase levels, performance status, and sites of metastatic disease influencing survival have been reported (16). There are also likely to be a number of other characteristics of the patients that are yet unknown but that may be important in determining the response to vaccine treatment.

The most striking feature in our studies of Melacine in combination with IFN-α2b was that there was a distinct clinical benefit and prolonged survival despite the lack of an overt clinical response. This may reflect the distinct mode of action of biological therapies such as vaccines, which may permit a stalemate with the tumor, whereby the growth of the latter is controlled but the tumor mass is not significantly reduced. Although the results of this Phase II trial and those of biochemotherapy are not directly comparable, the median survival achieved with our minimally toxic regimen of melanoma vaccine and IFN-α2b was little different from that achieved by biochemotherapy regimens (17–21). Melacine-based regimens had no reported treatment-related mortalities or hospitalizations. The low incidence of systemic toxicity and local toxicity, if careful attention is paid to reducing or eliminating the adjuvant before severe granulomas occur, makes this therapy widely acceptable to patients.

The advent of biological therapy in the oncology therapeutics arena necessitates an appreciation of the concept of tumor containment without size reduction. When that translates into a likelihood of extending survival, the regimen may effect a therapeutic benefit often exceeding that of cytotoxic therapies. This may be analogous to the control of a disease such as hypertension by medication without the elimination of the underlying condition. Stabilization or nonprogression of disease in a substantial proportion of patients has been observed throughout Phase I-III clinical trials with Melacine (22), and other vaccines such as Theratope in breast cancer. In a Phase II multicenter study of 139 patients, Elliott et al. (23) observed an improved survival (median, 22.3 months) for all patients who had stabilization of disease for at least 6 months. In that trial, the objective response rate was 8%, but 23% of patients with S.D. derived therapeutic benefit also. Unlike cytotoxic chemotherapy, vaccines may permit the host to reach a state of balance with the tumor, in which the net result of tumor growth and destruction is zero. That might lead to a more useful increase in survival than rapid destruction and rapid regrowth of the tumor.

![Figure 2](image1.png)

**Fig. 2** Survival curves (Kaplan-Meier) of all of the 47 patients entered into the study.

![Figure 3](image2.png)

**Fig. 3** Survival curves (Kaplan-Meier) of the 39 evaluable patients entered on study.

<table>
<thead>
<tr>
<th>Study (Ref.)</th>
<th>No. of patients</th>
<th>CR</th>
<th>PR</th>
<th>S.D.</th>
<th>Clinical benefit</th>
<th>MS</th>
<th>Med duration of CR/PR/S.D.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phase I (2)</td>
<td>17</td>
<td>2</td>
<td>3</td>
<td>3</td>
<td>8 (47%)</td>
<td>NR</td>
<td>NR</td>
</tr>
<tr>
<td>Phase II (3)</td>
<td>25</td>
<td>1</td>
<td>3</td>
<td>1</td>
<td>5 (20%)</td>
<td>NR</td>
<td>17 mo</td>
</tr>
<tr>
<td>Melacine–IFN (8)</td>
<td>18</td>
<td>5</td>
<td>3</td>
<td>0</td>
<td>8 (44%)</td>
<td>10.1 mo</td>
<td>11 mo</td>
</tr>
<tr>
<td>Melacine + IFN (current study)</td>
<td>39</td>
<td>2</td>
<td>2</td>
<td>25</td>
<td>29 (61.7%)</td>
<td>12.5 mo</td>
<td>4 mo</td>
</tr>
<tr>
<td>All patients</td>
<td>47</td>
<td>2</td>
<td>2</td>
<td>25</td>
<td>29 (74.3%)</td>
<td>14 mo</td>
<td>8 mo</td>
</tr>
<tr>
<td>Evaluable</td>
<td>39</td>
<td>2</td>
<td>2</td>
<td>25</td>
<td>29 (61.7%)</td>
<td>12.5 mo</td>
<td>4 mo</td>
</tr>
</tbody>
</table>

a MS, median survival; Med, median progression-free survival; NR, not reported.
b Low-dose cyclophosphamide pretreatment given to six patients.
c Number of evaluable patients.
after cytotoxic therapy. Clinical benefit (CR + PR + S.D. or nonprogression) and the durations thereof are likely to emerge as valid end points in future Phase II trials involving biological therapy. In conclusion, there is adequate clinical evidence for further investigation of the combination of Melacine and low-dose IFN-α2b as a well-tolerated and efficacious regimen in metastatic melanoma. An ongoing Phase III trial (Fig. 4) is testing the efficacy of this therapy after resection of Stage III melanoma, comparing it with standard single-agent high-dose IFN therapy.

REFERENCES


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