

Adjuvant Therapy of Stage III and IV Malignant Melanoma Using Granulocyte-Macrophage Colony-Stimulating Factor

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Purpose: To evaluate granulocyte-macrophage colony-stimulating factor (GM-CSF) as surgical adjuvant therapy in patients with malignant melanoma who are at high risk of recurrence.

Patients and Methods: Forty-eight assessable patients with stage III or IV melanoma were treated in a phase II trial with long-term, chronic, intermittent GM-CSF after surgical resection of disease. Patients with stage III disease were required to have more than four positive nodes or a more than 3-cm mass. All patients were rendered clinically disease-free by surgery before enrollment. The GM-CSF was administered subcutaneously in 28-day cycles, such that a dose of 125 $\mu\text{g}/\text{m}^2$ was delivered daily for 14 days followed by 14 days of rest. Treatment cycles continued for 1 year or until disease recurrence. Patients were evaluated for toxicity and disease-free and overall survival.

Results: Overall and disease-free survival were significantly prolonged in patients who received GM-CSF compared with matched historical controls. The median survival duration was 37.5 months in the study patients versus 12.2 months in the matched controls ($P < .001$). GM-CSF was well tolerated; only one subject discontinued drug due to an adverse event (grade 2 injection site reaction).

Conclusion: GM-CSF may provide an antitumor effect that prolongs survival and disease-free survival in patients with stage III and IV melanoma who are clinically disease-free. These results support institution of a prospective, randomized clinical trial to definitively determine the value of surgical adjuvant therapy with GM-CSF in such patients.

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PATIENTS WITH metastatic melanoma have a dismal prognosis and respond poorly to currently available chemotherapies and biologic agents, which result in median survival durations of between 6 and 9 months.¹⁻³ In contrast, patients who undergo complete surgical resection of metastatic disease (stage IV) enjoy prolonged survival durations of between 11 and 19 months.^{1,4-7} It has been suggested that adjuvant treatment with immune modulators such as vaccines and cytokines after complete surgical resection may further prolong median survival rates.^{3,8,9} Such agents are believed to have their greatest potential in the setting of minimal residual disease. Therefore, complete surgical resection of metastatic melanoma offers an ideal setting in which to evaluate immune modulatory agents for efficacy.

Granulocyte-macrophage colony-stimulating factor (GM-CSF) is a multifunctional molecule and plays a vital role in

various functions of the immune system. One of the most important activities relative to cancer therapy is the ability of GM-CSF to activate macrophages. GM-CSF stimulates peripheral-blood monocytes in vitro to become cytotoxic for human melanoma cells.^{10,11} The in vivo administration of GM-CSF results in an increase in the functional capacity of monocytes, as determined by the cytotoxicity of monocytes against antibody-coated xenogeneic cells¹² and HT29 colon carcinoma cells.¹³ GM-CSF also serves as the principal mediator of proliferation, maturation, and migration of dendritic cells,¹⁴⁻¹⁶ which are antigen-presenting cells that play a major role in the induction of primary and secondary T-cell immune responses. Finally, GM-CSF causes increased production of matrix metalloelastase elastase by tumor infiltrating macrophages,¹⁷ which results in the production of angiostatin by the macrophages.¹⁸ The angiostatin inhibits angiogenesis and suppresses the growth of pulmonary metastases.

Here, we report the results of an open-label, multicenter, phase II trial evaluating long-term intermittent GM-CSF therapy in patients with advanced melanoma after surgical resection. These results suggest that GM-CSF may have a role in the surgical adjuvant treatment of patients with melanoma.

PATIENTS AND METHODS

Patient Population

Eligible patients were those with stage III or IV malignant melanoma as defined by the American Joint Committee on Cancer (AJCC). Patients with stage III (node-positive) disease were required to have

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more than four positive nodes and/or a nodal mass greater than 3 cm in diameter. Patients were clinically disease-free at the time of study entry, having undergone surgical resection of nodal or metastatic disease. Patients in whom residual disease was suspected postoperatively may have received adjuvant radiotherapy. Some patients were given prophylactic whole-brain irradiation. Patients were not excluded if they had received prior chemotherapy, radiation therapy, or immunotherapy, although they must have completed therapy at least 1 month before study entry. The institutional review boards at the participating institutions approved the protocol, and all patients provided written informed consent.

Treatment Regimen

The study drug, GM-CSF (sargramostim), was supplied by Immunex Corporation, Seattle, WA, as a lyophilized powder that was reconstituted with bacteriostatic water for injection (United States Pharmacopeia). Patients were required to begin the study drug within 60 days of the surgical resection or completion of radiation therapy. GM-CSF was administered in multiple cycles, at a dose of 125 $\mu\text{g}/\text{m}^2$ daily subcutaneously for 14 consecutive days followed by 14 days of rest. These 28-day cycles were repeated for at least 1 year or until disease recurrence or significant toxicity occurred. Concurrent chemotherapy or biologic therapy was not allowed. Patients receiving GM-CSF who had a localized recurrence that was treated surgically or who completed 12 months of therapy without recurrence were allowed to continue or discontinue GM-CSF therapy. Patients who required systemic therapy such as chemotherapy were discontinued from the study drug. Adverse event data were collected and coded according to National Cancer Institute common toxicity criteria. The GM-CSF dose was reduced by 50% in subsequent courses if the absolute neutrophil count (ANC) exceeded 20,000 cells/ μL in prior cycles.

Historical Controls

Matched historical controls were obtained from the University of Alabama at Birmingham (UAB) Melanoma Registry. This database consists of all 1,477 patients who were treated at UAB between 1960 and 1988. There were 601 stage III/IV patients in the database, of whom 192 had been rendered tumor-free by surgical resection and were available for matching. In addition to survival information, the database includes demographic data, clinical and pathologic characteristics of the primary lesion, the clinical and pathologic stage at presentation, surgical and adjuvant treatments, and number and times of recurrences. Patients with stage III disease were matched on the basis of number of positive nodes and patients with stage IV melanoma were matched to controls based on the presence of visceral or nonvisceral metastases. The matchings were done at UAB in a ratio of 1:1, without knowledge of survival information or any other outcome data. If more than one match was found in the database for any subject, then age and sex were used to select the closest match for stage III patients, and actual site of metastasis was used to find the closest match for patients with stage IV disease. These matching criteria represent the most important prognostic factors for stage III and stage IV malignant melanoma, respectively.^{19,20}

Statistical Methods

This study is a phase II, open-label trial of GM-CSF as adjuvant therapy in patients with stage III/IV malignant melanoma. The primary aims of this trial were to gain preliminary information regarding disease recurrence and survival. Survival and disease-free survival

Table 1. Characteristics of the Study Population

Characteristic	GM-CSF (n = 48)		Control (n = 48)	
	No. of Patients	%	No. of Patients	%
Stage of disease				
III	14	29	14	29
IV	34	71	34	71
Age*				
> 65 years	14	29	8	17
Median, years	55		48.5	
Range, years	23-81		18-84	
Sex				
Male	30	62	30	63
Female	18	38	18	37
Location of primary tumor				
Extremity	15	31	11	23
Head and neck	7	15	10	21
Trunk	17	35	19	39
Other†	0	0	8	17
Unknown	9	19	0	0
Thickness of primary tumor				
< 1.5 mm	16	33	9	10
1.5-4.0 mm	11	23	18	37
> 4.0 mm	4	8	11	23
Not known	13	27	10	21
NA	4	8	0	0

Abbreviation: NA, not applicable.

* Age at time of GM-CSF therapy (treated group) or at diagnosis of metastatic disease (control group).

† Location known but not stated.

were measured from the date of surgical resection or the last day of radiation treatment in patients receiving adjuvant radiotherapy after surgical resection. For the UAB historical controls, survival and disease-free survival were measured from the date of first surgical resection of metastatic disease. Survival and disease-free survival were compared using time-to-event methodology. In these analyses, patients who did not experience an outcome were censored at the time of last follow-up. The Kaplan-Meier method was used to obtain estimates of median survival and disease-free survival times and to generate survival and disease-free survival curves. The survival curves were compared using log-rank tests. Comparisons to the matched controls were made for the overall patient population and also by AJCC stage of disease.

RESULTS

Characteristics of the Study Population

Fifty-one patients were entered onto the study and 48 patients were assessable, having received at least one dose of GM-CSF. The other three patients were found to be ineligible due to the presence of gross disease (two patients) or inappropriate disease stage (one patient). The 48 patients treated with GM-CSF were matched with 48 control patients (Table 1). The patient populations were identical in terms of stage of disease (14 patients with stage III disease

Table 2. Baseline Disease Characteristics

Characteristic	GM-CSF		Control	
	No. of Patients	%	No. of Patients	%
Stage III disease, n = 14				
No. of positive nodes				
< 5	8	57*	8	57
5-10	4	29	4	29
> 10	2	14	2	14
Time from primary tumor to study entry, months†				
Median	12.4		7.2	
Range	2.1-389.1		2.4-50.4	
Stage IV disease, n = 34				
Site of metastases				
Visceral	23	68	23	68
Subcutaneous	11	32	11	32
No. of anatomic sites				
1	25	74	24	71
> 1	9	26	10	29
Time from primary tumor to study entry, months				
Median	21.0		7.2	
Range	0.5-170.3		0-79.2	
Prior therapy				
None	15	44	NA	
Radiation therapy	15	44		
Surgery‡	10	29		
Interferon	5	15		
Levamisole	3	9		
Vaccine	3	9		
Chemotherapy	1	3		
Heated limb perfusion	1	3		

Abbreviation: NA, not available.

* All patients with stage III disease in the treatment group with fewer than five nodes had a bulky lymph node mass.

† GM-CSF, n = 12; control, n = 14.

‡ All patients had surgical excision of metastatic disease immediately before study entry. This listing reflects prior surgery for metastatic disease.

and 34 patients with stage IV disease in the treatment and control groups) and were comparable in terms of age and sex. There were more patients in whom the location of the primary tumor was unknown in the treated patients and more in whom the location of the primary tumor was classified as "other" in the control patients. There were more patients with "thin" primary tumors (< 1.5-mm thickness) in the treated than in the control population, but neither the histopathology or location of the primary tumor is a prognostic factor in this population of patients who have already developed metastatic disease.¹⁹

For the patients with stage III melanoma, there was complete agreement in the study and control populations with regard to the number of nodes involved (Table 2). The number of nodes involved with melanoma is well accepted

as the most important prognostic indicator for patients with stage III disease.²⁰ Patients with stage IV disease were perfectly matched for site of metastases (visceral v subcutaneous) and closely matched for the number of anatomic sites (Table 2). These are recognized as the most important prognostic indicators for this patient population.¹⁹ The time from diagnosis of the primary tumor to study entry was comparable in the stage III patients but quite different in the stage IV patients, largely because of the wide differences in the range. Other variables could not be matched. For example, there were eight patients with fewer than five nodes among the patients in the experimental group with stage III disease, and those patients were required to have bulky lymph node disease with a mass of a diameter greater than 3 cm. The control patients could not be matched for this characteristic because the information was not collected in the database. Also, three of the 14 patients with stage III melanoma in the study group were known to have had one resected lymph node metastasis before the second resected lymph node metastasis that qualified them for entry onto the study; those in the matched controls did not. Ten of the patients with stage IV disease in the study group had prior visceral metastases; those in the control group did not. The patients with stage IV disease in the study group had undergone a number of prior therapies; 44% had undergone prior radiation therapy, 29% had undergone prior surgical procedures for metastatic disease, and some patients had received biotherapy or chemotherapy. These data were not available for the patients in the control group.

GM-CSF Therapy

Patients received GM-CSF for a median duration of 11.5 cycles (range, two to 49 cycles). Twenty-four received fewer than 12 cycles; twenty-three discontinued therapy because of disease progression after a median of six cycles, and one patient discontinued therapy because of an adverse event (grade 2 injection site reaction) after three cycles. Seven patients received 12 cycles, and seventeen (35%) received more than 12 cycles, with a median of 24 cycles. Three patients interrupted the dosing schedule for more than 2 months.

Side Effects

Therapy was generally well tolerated. Forty-four patients (92%) suffered at least one mild adverse event, including the most common side effects that are associated with the administration of GM-CSF: transient myalgias, weakness, and mild fatigue (27 patients, 56%); rashes (five patients, 10%); and mild erythema at the site of injection (28 patients, 58%). Dose adjustment for ANC of greater than 20,000 cells/ μ L was not required for any patient; however, one

patient had a 50% decrease for grade 3 asthenia. No treatment-related serious adverse events were observed. Two patients experienced grade 4 neutropenia, one experienced grade 4 thrombocytopenia, one experienced grade 4 hyperglycemia, and one experienced grade 4 hyperbilirubinemia; all of these events were unrelated to the study drug.

Hematologic Response

The median ANC increased from 4,212 cells/ μ L at baseline to 8,557 cells/ μ L at 14 days in cycle 1. ANC returned to baseline by the next cycle. Similar ANC levels were observed during treatment in subsequent cycles. No difference in hemoglobin or platelets was observed over a course of treatment or cumulative doses of GM-CSF.

Survival

The median survival increased three-fold in patients who received GM-CSF as compared with that of the matched historical controls, from 12.2 months to 37.5 months, with observed survival rates of 89% at 1 year and 64% at 2 years (Table 3). The median survival and survival rates at 1 and 2 years for patients in the subgroups with stage III or stage IV disease were similarly increased (Table 3). Compared with that of matched historical controls, survival was significantly better for the overall patient population ($P < .001$; Fig 1), patients with stage III disease ($P = .04$; Fig 2), and patients with stage IV disease ($P < .001$; Fig 3). The disease-free survival of the patients who were treated with GM-CSF was prolonged as compared with that of the matched historical controls ($P = .03$; Fig 4), but not for patients with stage III or stage IV disease (data not shown).

Recurrences

Thirty-five patients had at least one disease recurrence on study (Table 4). In 18 patients, the disease recurrence was localized and could be excised completely. In five of these 18 patients, there were further localized recurrences, which also could be excised. The remainder had multiple lesions that required systemic therapy. Combination chemotherapy was the most common type of treatment (11 patients), and in most patients, this consisted of the combination of dacarbazine, carmustine, cisplatin, and tamoxifen. Only four patients received biochemotherapy.

DISCUSSION

These results indicate that the administration of GM-CSF to patients with metastatic malignant melanoma who are at high risk for recurrence after surgical resection of metastatic disease results in a statistically significant prolongation of overall and disease-free survival compared with matched

historical controls. Furthermore, GM-CSF is well tolerated and results in minimal toxicity.

Because of the potential impact of prognostic variables on the clinical outcome, we used a statistical analysis in which each patient treated with GM-CSF was paired with a control subject. Pairs were matched for number of nodes (patients with stage III disease)²⁰ or location and number of metastatic sites (patients with stage IV disease)^{1,4,5,19,21}; these are the two most important prognostic indicators for these patient populations. The groups were generally well matched demographically. The stage III patients were well matched for the time between diagnosis of the primary tumor and the first lymph node recurrence, but the stage IV patients had a substantially longer time between diagnosis of the primary tumor and the first distant metastasis. There have been several reports indicating that remission time before metastasis is not a significant prognostic indicator of survival,^{5,20-22} and one report claims that it is.⁷

Despite these matching characteristics, there were a number of features that would indicate that the population treated with GM-CSF might have a worse prognosis than the control patients. For example, in the population of patients with stage III melanoma, there were eight patients with fewer than five nodes in both the treatment and control groups. In the treatment group, these patients all had bulky nodal disease, which placed them in the N2 category according to the AJCC staging system.

The patients included in the study reported herein are not typical of the overall population of patients with stage IV disease; they represent a selected subpopulation of patients who present with metastatic disease that can be surgically excised. This is demonstrated by the fact that in the database used here as historical controls, only 32% of the patients were in this group. Patients in this subpopulation who undergo complete resection of all gross disease have a longer median survival than patients who are treated by other means, such as chemotherapy. Median survival after complete resection of metastatic melanoma has been reported by various centers to range from 11 to 19 months when the patients were treated with surgery alone (Table 5). The reason for this wide range in median survival may rest in the distribution of the location of the metastatic sites and whether or not the metastases were solitary or multiple in the patient population under investigation, because it is recognized that the outcome is dependent on these prognostic indicators.

Because the treatment options that are available for therapy of metastatic melanoma are different now from those that were available at the time when the historical controls were treated, one must consider whether the improved survival might be due to improved therapeutic

Table 3. Survival

Patient Population	1-Year Survival Rate (%)	2-Year Survival Rate (%)	Median Survival (months)
Overall*			
GM-CSF	89	64	37.5
Control	45	15	12.2
Stage III†			
GM-CSF	92	66	35.8
Control	70	23	15.2
Stage IV‡			
GM-CSF	88	64	37.5
Control	35	12	8.1

* $P < .001$.

† $P = .04$.

‡ $P < .001$.

options. Combination chemotherapy regimens have had little impact on survival,²³ and attention has recently focused on combination biochemotherapy regimens.²⁴⁻²⁷ Because only four (8%) of the 48 patients in the GM-CSF treatment group were given biochemotherapy, we think it is unlikely that this could be the sole explanation for the improved survival of the study patients.

The effect of surgical adjuvant therapy with GM-CSF seemed to have a greater impact on survival than on disease-free survival. The same seems to be the case in the study with the polyvalent melanoma cell vaccine, although the authors didn't comment on that aspect of their therapy.^{8,28} We postulate that therapy with GM-CSF may be changing the biology of the behavior of metastatic melanoma. Recurrences in the treated population were often localized and could be treated with localized means rather than requiring systemic therapy. Data regarding this aspect of the nature of recurrences were not in the database of the matched controls or in any other database of which we are

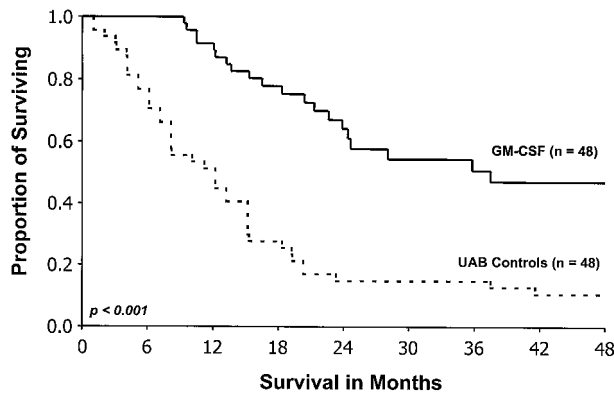


Fig 1. Life-table analysis of survival of patients with high-risk malignant melanoma treated with GM-CSF as surgical adjuvant therapy as compared with survival of matched historical controls.

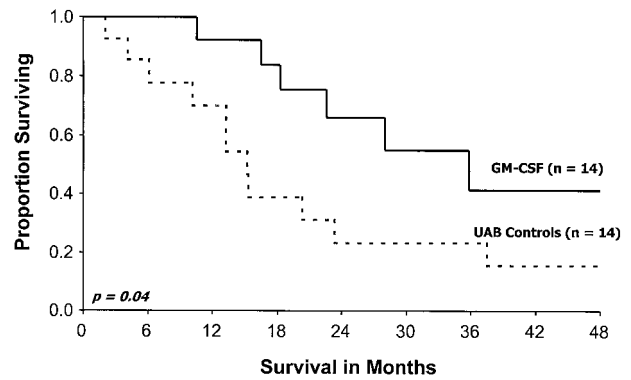


Fig 2. Life-table analysis of survival of patients with stage III high-risk malignant melanoma treated with GM-CSF as surgical adjuvant therapy as compared with survival of matched historical controls.

aware, so we could not conduct a comparative analysis. Nonetheless, it is reasonable to postulate that the macrophages activated by the GM-CSF could eradicate small metastatic tumor nodules that would have appeared as systemic metastases, although they could not overcome a nodule that was larger at the time of treatment and would subsequently appear as a localized metastasis. This postulate is supported by the observation that small tumors are infiltrated with a relatively large number of macrophages, whereas large tumors are not.²⁹⁻³¹

Physicians caring for patients with melanoma face a dilemma in determining which adjuvant therapy to recommend. The only agent currently approved in the United States for adjuvant therapy of melanoma is interferon alfa-2b, and it is recommended for use in a high-dose regimen. The efficacy of this regimen is modest⁹ and the treatment is associated with considerable toxicity, especially in older patients. Moreover, a follow-up study confirmed disease-free survival benefit but failed to confirm

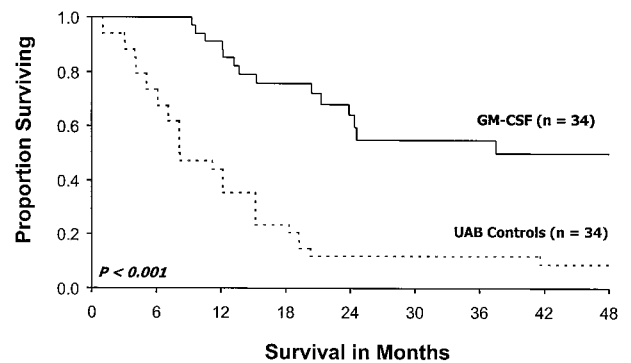


Fig 3. Life-table analysis of survival of patients with stage IV high-risk malignant melanoma treated with GM-CSF as surgical adjuvant therapy as compared with survival of matched historical controls.

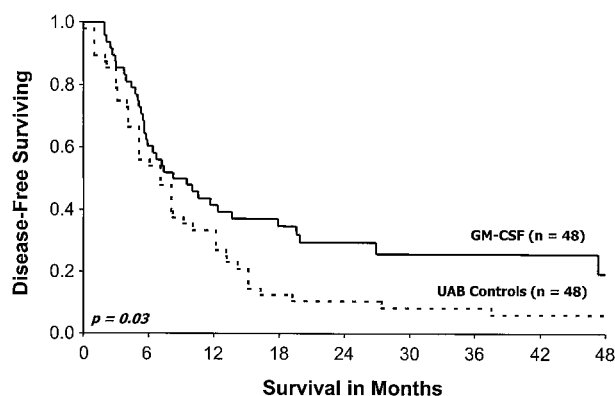


Fig 4. Life-table analysis of disease-free survival of patients with high-risk malignant melanoma treated with GM-CSF as surgical adjuvant therapy as compared with survival of matched historical controls.

overall survival benefit of this regimen or of a low-dose interferon regimen in prolonging survival in patients with high-risk melanoma.³² Low-dose interferon alfa has also been evaluated in a large, randomized study in adjuvant therapy of patients with stage II melanoma and results have been promising, although the low-dose regimen does not seem effective in higher-risk patients.³³ In a large, prospective, randomized trial, levamisole was reported to be effective in the adjuvant therapy of patients with high-risk melanoma,³⁴ and on the basis of that study, levamisole has been approved for this purpose in Canada, but not in the United States. A similar prospective, randomized, placebo-controlled trial failed to show benefit of levamisole in the adjuvant treatment of melanoma, but the dose used was slightly different.³⁵

Table 4. Recurrences in Patients Given GM-CSF as Surgical Adjuvant Therapy for High-Risk Malignant Melanoma

Characteristic	No. of Patients
Patient population	48
Disease recurrence	35
Localized disease, surgically excised	18
Further localized disease, excised	5
Systemic therapy	
None	25
Chemotherapy, n = 12	
Combination	11
Single drug	1
Biotherapy, n = 5	
Vaccine	2
Interferon	2
Vaccine + interferon	1
Biochemotherapy	4
Other	2

Table 5. Median Survival After Complete Resection of Metastatic Melanoma

No. of Patients	Median Survival (months)	First Author
69	18	Overett TK ⁴
61	11.4	Hena MH ⁵
22	11	Karp, NS ⁶
114	19	Karakousis C ⁷
NS	11.7	Brand CU ¹
63	25*	Tafra L ³
75	37.2*	Hsueh EC ⁸

Abbreviation: NS, not stated.

* Patients were treated with surgery followed by immunotherapy with a polyvalent melanoma cell vaccine.

There is current enthusiasm for the potential of vaccines for adjuvant therapy of melanoma, but the only large-scale, prospective, randomized, placebo-controlled study reported to date has been negative.³⁶ There have been a number of positive reports of efficacy of vaccines in phase II trials, but none of these have yet been established to be effective in properly designed phase III trials. These include a GM2 ganglioside-keyhole limpet hemocyanin conjugate vaccine,³⁷ a polyvalent melanoma vaccine administered with Bacillus Calmette-Guérin,²¹ a polyvalent melanoma antigen vaccine consisting of shed antigens,³⁸ a hapten-modified autologous vaccine,³⁹ and a synthetic peptide vaccine administered with interleukin-2.⁴⁰ A number of other vaccines for adjuvant therapy of melanoma are in earlier stages of testing. Although results are encouraging, none of these vaccines is approved for marketing and they are generally not available outside of clinical trials. Because of the success of biochemotherapy in the treatment of patients with advanced melanoma,²⁵ this regimen is now being tested in the adjuvant setting, but results are not yet available.

Toxicities demonstrated in this study were substantially less severe than those reported for other biologic agents (interleukin-2 and interferon alfa). In fact, only one patient prematurely discontinued treatment because of an adverse event (grade 2 injection site reaction). This safety profile allows long-term maintenance therapy to be delivered in the majority of patients. The results reported herein suggest that GM-CSF may have efficacy as surgical adjuvant treatment of melanoma, and a properly designed, prospectively randomized clinical trial is warranted.

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