

Recombinant Human Granulocyte-macrophage Colony-stimulating Factor (GM-CSF, Sargramostim) Administered for 3 Years as Adjuvant Therapy of Stages II(T4), III, and IV Melanoma

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Summary: A hypothesis generating study was conducted to evaluate the safety and efficacy of prolonged (3 y) administration of granulocyte-macrophage colony-stimulating factor (GM-CSF, sargramostim) as surgical adjuvant therapy in patients with melanoma at high risk of recurrence. Ninety-eight evaluable patients with stages II(T4), III, or IV melanoma were given prolonged treatment with GM-CSF after surgical resection of disease. The GM-CSF was administered subcutaneously in 28-day cycles, such that a dose of 125 µg/m² was delivered daily for 14 days followed by 14 days rest. Treatment cycles continued for 3 years or until disease recurrence, which could not be surgically excised. Patients were evaluated for toxicity, disease-free survival, and melanoma-specific survival. Prolonged administration of GM-CSF was well tolerated; grade 1 or 2 side effects occurred in 82% of the patients. There were no grade 3 or 4 treatment-related side effects. Two patients developed acute myelogenous leukemia after completion of 3 years of GM-CSF administration. With a median follow-up of 5.3 years, the median melanoma-specific survival has not yet been reached. The 5-year melanoma-specific survival rate was 60%. The current study has expanded the preliminary evidence on GM-CSF as adjuvant therapy of patients with melanoma who are at high risk for recurrence.

Key Words: adjuvant, GM-CSF, melanoma, prolonged therapy, AML

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The only agent currently approved in the United States for adjuvant therapy of melanoma is high-dose interferon alpha (IFN- α) for patients with stage IIB and stage III melanoma. It is an imperfect solution and the search continues to build on this early success and identify agents for surgical adjuvant therapy of melanoma that are more effective and less toxic than high-dose interferon.

Granulocyte-macrophage colony-stimulating factor (GM-CSF) (sargramostim, Leukine, Bayer Healthcare Pharmaceuticals Inc, Wayne, NJ) is a hematopoietic growth factor, which like granulocyte colony stimulating factor (filgrastim, Neupogen, Amgen, Thousand Oaks, CA) stimulates proliferation and differentiation of hematopoietic progenitor cells, and both are approved for this purpose.^{1,2} In addition, GM-CSF has immunologic activities that granulocyte colony stimulating factor does not have and plays a vital role in various diverse functions of the immune system. These include its ability to activate macrophages, which distinguish tumor cells from normal cells and kill only the tumor cells,³ stimulation of peripheral blood monocytes in vitro to become cytotoxic for human melanoma cells,^{4,5} production of monocyte activation and tumoricidal activity after in vivo administration,⁴ and stimulation of production of an angiogenesis inhibitor by macrophages.⁶ GM-CSF also serves as the principal mediator of proliferation, maturation and migration of dendritic cells,^{7–9} antigen presenting cells that play a major role in the induction of primary and secondary T-cell immune responses.

We previously conducted an open-label, multicenter, phase 2 trial evaluating intermittent GM-CSF therapy for 1 year in patients with stages IIIC and IV melanoma after surgical resection and compared progression-free and survival outcomes with those in matched historic controls.¹⁰ The results suggested that GM-CSF may have a role in the surgical adjuvant treatment of melanoma and led to a phase 3 randomized trial to determine the efficacy of GM-CSF as adjuvant therapy, which is currently being conducted (protocol E4697).¹¹

The dosing regimen used in our original study was arbitrary and was based on the safety data available at the time we initiated the study (1993). The randomized phase 3 study led by the Eastern Cooperative Group (ECOG) used this same regimen, as our study had suggested efficacy and exploration of the clinical outcome of different dosing regimens in the adjuvant setting would be time-consuming.

Review of the results of our previous study, in which patients were treated with adjuvant GM-CSF therapy for

1 year,¹⁰ indicated that 6 of the 48 (12%) patients experienced recurrences of their melanoma within 1 to 2 years of discontinuing GM-CSF therapy (unpublished data). This suggested the possibility that the treatment was not killing residual melanoma cells, but merely suppressing their growth. We postulated that more prolonged therapy would be needed. Therefore, we conducted the current trial to evaluate safety and efficacy of adjuvant therapy with GM-CSF administered for 3 years, rather than 1 year in patients with melanoma at high risk for recurrence. In the course of this study, we noted the development of acute myelogenous leukemia (AML) in 2 of the study patients who received GM-CSF treatment for 3 years.

PATIENTS AND METHODS

Patient Population

Eligible patients were those with stages II(T4), III, or IV melanoma as defined by the current (2002) American Joint Committee on Cancer (AJCC). Patients were clinically disease-free at the time of study entry having undergone surgical resection of all known disease. Patients were required to begin treatment, within 90 days of the last surgery, in which patient melanoma was present. Adjuvant radiotherapy was allowed in patients in whom residual disease was suspected postoperatively. Patients were not excluded if they had received prior chemotherapy, radiation therapy, or immunotherapy; they must have completed therapy at least one month before the study entry. The Institutional Review Board at the participating institution approved the protocol and all patients provided written informed consent.

Treatment Regimen

The study drug, GM-CSF, was supplied by Bayer Healthcare Pharmaceuticals Inc, Wayne, NJ as a lyophilized powder that was reconstituted with the Bacteriostatic Water for Injection USP. GM-CSF was administered in multiple cycles, at a dose of 125 µg/m² daily subcutaneously for 14 consecutive days followed by 14 days rest. These 28-day cycles were repeated for at least 3 years or until disease recurrence that could not be surgically excised or significant toxicity occurred. Concurrent chemotherapy or biologic therapy was not allowed. Patients requiring systemic therapy such as chemotherapy were required to discontinue taking the study drug.

Statistical Methods

This study is an open-label trial of administration of GM-CSF for 3 years as adjuvant therapy in patients with malignant melanoma at high risk for recurrence. The aims of this trial were to gain preliminary information regarding safety of this regimen and efficacy as defined by disease recurrence and survival. Adverse events were evaluated using the National Cancer Institute Common Terminology Criteria for Adverse Events, Version 3.0. Melanoma-specific survival and disease-free survival were measured from the date of surgical resection for the primary tumor in patients with stage II (T4) disease or the date of resection of metastases for patients with stages III and IV disease. The median follow-up time for each survival outcome was calculated using the Kaplan-Meier estimate of potential follow-up, also called the "reverse Kaplan-Meier" method.¹² Kaplan-Meier techniques were used to obtain estimates

of median melanoma-specific survival and disease-free survival times and to generate corresponding survival curves.

Epidemiologic Methods

We conducted several investigations in an effort to determine whether or not the prolonged therapy with GM-CSF was related to the development of AML. We conducted a review of the Bayer Healthcare Pharmaceuticals Inc, Global Pharmacovigilance—USA adverse event database. Under the Freedom of Information Act, we conducted a similar review of the Food and Drug Administration (FDA) database for cases reported directly to the FDA. We reviewed the published literature, American Society of Clinical Oncology (ASCO) abstracts, and contacted investigators to locate information on patients who had received GM-CSF therapy for 6 months or more and contacted the statistician responsible for data on patients in the ECOG trial (E4697) in which melanoma patients in 3 of the 6 arms are randomized to receive GM-CSF for 1 year in the adjuvant setting.

Exploratory analyses were also undertaken in an effort to provide additional information about the natural course of malignant melanoma with respect to the risk of development of AML. These analyses used large databases to quantify the crude risk of AML in patients with melanoma. One database used was the United Kingdom's General Practice Research Database (GPRD), the world's largest computerized database on anonymized longitudinal medical records from primary care.¹³ The other was the Surveillance Epidemiology and End Results (SEER) database with registries that operate and maintain a high quality population-based cancer reporting system in the USA.¹⁴ We calculated the incidence rate of AML in melanoma patients in our study and the background incidence rate in the GPRD and SEER.

Case Reports

1. M.H, male, aged 47 years had a melanoma classified as T3aN1aM0; stage IIIA. The patient had no prior treatment for melanoma and did not receive postoperative radiation therapy. He took GM-CSF in the dosage described above and was just completing 3 years of therapy when the diagnosis of AML was established. The French-American-British (FAB) classification was M0 and he had a chromosomal t(9,11) translocation and tetrasomy 21q. The patient underwent induction chemotherapy but died in February 2005, 5 weeks after the diagnosis of AML. Family history included cancer, but no AML.
2. TdV, male, aged 57 years had a melanoma and classified as TXN3M0; stage IIIC. The patient had no prior treatment for melanoma and did not receive postoperative radiation therapy. He took GM-CSF in the dosage described above, completed 3 years of therapy, and then discontinued the study medication per the study protocol. The diagnosis of AML was established 2 years later. The FAB classification was M0 and chromosomal analysis showed no abnormality. He died of AML 9 months after diagnosis. Additional information is that he worked as a sheet metal worker and had heavy industrial exposure to carcinogens.

RESULTS

Characteristics of the Study Population

One hundred and two patients entered the study and 98 patients were evaluable having received at least one dose of GM-CSF. The other 4 patients were found to be ineligible due to the presence of gross disease (3 patients) or unacceptable comorbidity (myelofibrosis, 1 patient). Baseline characteristics of the overall study population are shown in Table 1. The largest population consisted of patients with stage III disease (n = 69). Forty-four percent of the study patients had received prior therapy, including prior biologic therapy and/or surgical procedures in addition to excision of the primary tumor, wide reexcision of the primary and regional node dissection (Table 2). Patients who had received prior therapy were only eligible if they developed recurrent disease after treatment, had that disease surgically excised, and began GM-CSF therapy within 90 days of the last surgery, in which the presence of melanoma was demonstrated.

Melanoma-specific and Disease-free Survival

The melanoma-specific and disease-free survival outcomes are illustrated in Figure 1. With a median duration of follow-up of 5.3 years, the median duration of melanoma-specific survival has not been reached yet. The corresponding 5-year survival rate is 60% [95% confidence interval (CI) = 49%, 70%]. With a median duration of follow-up for disease-free survival of 5.8 years, the median disease-free survival is 1.4 years (95% CI = 1.0, 4.2). The corresponding 5-year disease-free survival rate is 36% (95% CI = 27%, 46%). For patients with stage III disease, 5-year melanoma-specific survival rate 67% (95% CI = 56%, 79%). For patients with stage IV disease, the 5-year melanoma-specific survival rate is 40% (95% CI = 19%, 60%).

Types of Recurrence

Of the 98 patients included in this study, 62 had recurrence of their disease. Of these, 43 had localized disease, which was treated with surgery, and these patients continued on trial per the protocol. Eighteen of the 43 patients treated with surgery for recurrent disease remains alive and without evidence of disease. In 19 of

TABLE 2. Melanoma Treatment History (n = 98)

Prior Therapy	No. Patients	Percent
Prior therapy or procedure (all types)	43	44
Surgery (excluding excision and reexcision of primary and regionallymph node dissection)	25	26
Biologic therapy*	25	26
High-dose interferon	13	13
Vaccine	10	10
Levamisole	1	1
Other	3	3
Chemotherapy single agent regimen	3	3
Chemobiotherapy	2	2
Radiotherapy	1	1
Other	1	1

*Two subjects received more than one biologic therapy (both received high-dose interferon and vaccine).

the 62 patients with disease recurrence, the first recurrence was systemic disease not amenable to surgery. Eighteen of these 19 patients have died and 1 patient is alive with disease. Of the 24 patients with stage IV disease included in this study, 22 have had recurrences, treated surgically, and 9 remain alive without evidence of disease.

Side Effects

Adjuvant GM-CSF therapy was well tolerated. Eighty patients (82%) had at least one grade 1 (74%) or grade 2 (8%) treatment-related adverse event. The most common side effects were mild injection site reactions (68%), erythema at the injection site (57%), and flu-like symptoms (54%), (Table 3). One patient had a treatment-related serious adverse event. This patient developed chest pain in the middle of his 14-day cycle on GM-CSF and was admitted to a local hospital for evaluation. No abnormalities were found and the patient was discharged the day after with a diagnosis of sternal pain, grade 2, thought related to GM-CSF administration. There were 10 additional patients who had serious adverse events, who were not thought to be related to the study medication. There were grade 3 or 4 adverse events in 6 patients, all thought unrelated to the study drug. Beside the side effects observed during therapy, 2 patients, who never experienced recurrence of melanoma, developed AML after having completed 3 years of treatment with GM-CSF.

Epidemiologic Analysis

On the basis of the consumption data from Bayer Healthcare Pharmaceuticals Inc, as GM-CSF was launched in the United States in 1991, the number of patients who have been exposed to therapy is estimated to be greater than 3,00,000 patients. Search of this database did not reveal any additional cases of secondary myeloid malignancy or myelodysplasia (MDS). The review of the FDA database also did not reveal any additional cases of AML or MDS in patients without a preexisting hematologic malignancy. The review of the published literature, ASCO abstracts, and contacts with investigators revealed 651 patients who had received 6 months or more of GM-CSF therapy. The majority of patients received GM-CSF for less than 3 years. There were no other occurrences of AML or MDS in these patients. In the 800 patients enrolled in the ECOG trial (E4697) with intent to treat with GM-CSF for

TABLE 1. Baseline Characteristics of the Study Population (n = 98)

Characteristic	No. Patients	Percent
AJCC Stage		
IIB	2	2.0
IIC	3	3.1
IIIA	13	13.3
IIIB	27	27.5
IIIC	29	29.6
IVM1a	6	6.1
IVM1b	8	8.2
IVM1c	10	10.2
Sex		
Male	68	69.4
Female	30	30.6
Age (y)		
Mean (± SD)	53.1 (± 12.41)	
Median (min, max)	53.5 (15, 84)	

AJCC indicates American Joint Committee on Cancer.

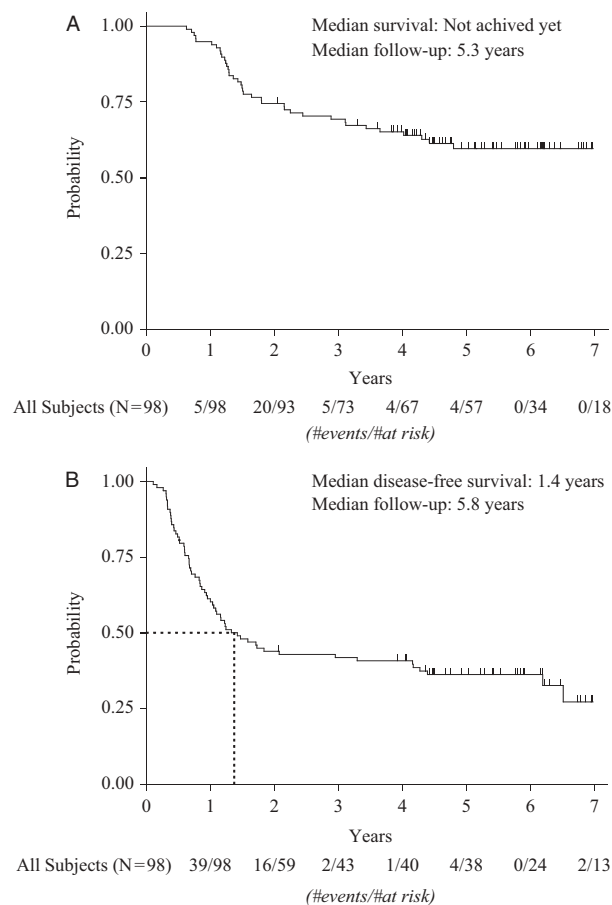


FIGURE 1. A, Melanoma-specific survival of study population. B, Disease-free survival of study population.

1 year, all have completed treatment and are undergoing follow-up. No cases of AML or MDS have been found.¹⁵ Analysis of the 2 large databases indicated that the crude risk for melanoma patients to develop AML is 0.02% in the GPRD and 0.05% in the SEER database¹⁶ versus 2.04% in our study of 98 patients with melanoma. We performed an additional analysis, which took the duration of exposure into account. In our study, patients were treated for 370 person-years. The rate of incidence of AML is therefore 541/100,000 person-years (95% CI: 6.8/100,000-1500/100,000 person-years). The incidence rate of AML in melanoma patients calculated from the GPRD database is 3.2/100,000 person-years (95% CI: 0.26/100,000-7.8/100,000 person years) (Table 4).

DISCUSSION

The patients included in this study were all at high risk for recurrence according to the current AJCC staging system. Moreover, 44% of them had failed previous adjuvant therapy, thus making the prognosis worse than one would expect for previously untreated patients. Nonetheless, with a median follow-up of 5.3 years, the median duration of melanoma-specific survival has not been achieved yet and the corresponding 5-year survival rate was 60%. The median duration of disease-free survival was only 1.4 years and the 5-year disease-free survival was only

TABLE 3. Subject Incidence of Adverse Events (n = 98)

Characteristic	No. Patients	Percent
Skin	79	80.6
Injection site reaction	67	68.4
Erythema	56	57.1
Pruritus	31	31.6
Urticaria	10	10.2
Rash	4	4.1
Desquamation	1	1.0
Flu-like symptoms	53	54.1
Fatigue	47	48.0
Myalgia	10	10.2
Sweats	5	5.1
Chills/rigors	4	4.1
Fever	3	3.1
Arthralgia	2	2.0
Gastrointestinal	8	8.2
Nausea	5	5.1
Abdominal pain	2	2.0
Loose stools	1	1.0
Gastric discomfort	1	1.0
Vomiting	1	1.0
Neurologic	6	6.1
Headache	6	6.1
Cardiac	7	7.1
Chest pain	6	6.1
Congestive heart failure	1	1.0
Respiratory	5	5.1
Dyspnea	5	5.1
Wheezing	1	1.0
Pain	5	5.1
Bone	3	3.1
Joint	2	2.0
Sternal	1	1.0
Circulatory	1	1.0
Edema	1	1.0
Other*	17	17.3

*Other: facial flushing (2), hot flashes (2), 1 each: bladder infection, decreased platelets, diffuse rash (West Nile Virus), impotency, increased sensitivity to sunlight, numbness and twitching feet, pain in back and legs, paresthesia neck, seizure, shingles, skin cellulitis, tiny clear blister on legs, yeast infection.

36%. These figures seem discordant to those for melanoma-specific survival. Further examination of the data indicates that the majority of patients who had disease recurrence had limited disease that could be surgically excised and these patients enjoyed a survival benefit. Only 19 (31%) of the patients had distant metastases as the first recurrence. This suggests that GM-CSF therapy may change the biologic behavior of melanoma such that it recurs in a fashion amenable to surgery rather than as widespread disease, in which the only option is systemic therapy, which is not very effective.

We considered several options regarding different dosing regimens that might build on the observations in our previous study and provide an even better survival outcome. These considerations included increasing the daily dose of drug, dosing more frequently, or increasing the duration of treatment. Given the length of time and numbers of patients necessary to evaluate outcomes in adjuvant studies in patients with melanoma who are at high risk for recurrence, we could investigate only one of these variables and chose increasing the duration of treatment from 1 to 3 years because of the observation of disease recurrences when patients stopped treatment after 1 year in

TABLE 4. Summary of Results of Epidemiologic Analysis

	Melanoma Patients	AML Cases*	Crude Risk %	95% CI (%)	Incidence Rate	95% CI
Clinical study	98	2	2.04	0.25-7.4	541/100,000 person-years	6.8/100,000-1500/100,000 person-years
GRPD	13,291	3	0.02	0.005-0.066	3.2/100,000 person-years	0.26/100,000-7.8/100,000 person-years
SEER	93,396	48	0.05	0.037-0.068	Data for calculation not available	Data for calculation not available

*Cases of AML observed in patients with a previous diagnosis of melanoma.

AML indicates acute myelogenous leukemia; CI, confidence interval; GRPD, general practice research database; SEER, surveillance epidemiology and end results.

our previous study. A trial that was subsequently published using a different biologic (intermediate dose IFN- α 2b) as postsurgery adjuvant therapy in patients with melanoma suggested that duration of treatment seemed more important than dose.¹⁷ This observation led to the largest adjuvant trial ever conducted in patients with stage III melanoma. Patients were randomized to treatment with 5 years of pegylated IFN- α 2b (PEG-IFN) or observation.¹⁸ The results showed benefit in terms of relapse-free survival but not in overall survival. There was improvement in distant metastasis-free survival only in the subgroup of patients with microscopic nodal involvement.

Toxicities demonstrated in this study were common, but substantially less severe than that reported for other biologics used in the treatment of melanoma (interleukin-2 and IFN- α); however, the occurrence of 2 cases of AML after 3 years of treatment with GM-CSF is worrisome. Owing to the small number of patients known to have received GM-CSF treatment for 3 or more years, there is not enough information available to determine whether the 2 cases of AML reported here represent more than a chance finding. To our knowledge, AML has not been reported in any patient receiving 1 year or less of GM-CSF therapy for melanoma.

The prescribing information for GM-CSF states that the possibility that GM-CSF can act as a growth factor for any tumor type, particularly myeloid malignancies cannot be excluded. There is some data, in which MDS patients treated with GM-CSF converted to AML after treatment,¹⁹⁻²¹ but the rates are similar to the rate of transformation seen in patients with MDS without GM-CSF treatment.²² Retsas et al²³ reported 2 cases of MDS and 1 case of AML in a population of 2076 evaluable patients with melanoma registered over a period of 37 years. All of these patients had received prior chemotherapy and none had received GM-CSF.

In the 2 patients described herein both had AML classified as FAB M0, but there were some notable dissimilarities between them. One had extensive occupational exposure to industrial solvents. The other had a family history of cancer. Cytogenetic analyses indicated a normal karyotype for 1 patient, and a complex karyotype with balanced translocation and tetrasomy 21q in the other. One might have supposed that if there were a common etiology for the development of AML (exposure to prolonged administration of GM-CSF), the characteristics of the AML in these patients might have been similar, which it was not. Moreover, the extensive occupational exposure to industrial solvents in one of the patients makes it unclear whether or not development of AML might have

been related to this exposure to carcinogens rather than GM-CSF.

In our study, patients with surgically excised stage IV disease treated with adjuvant GM-CSF had a 40% 5-year melanoma-specific survival. An international trial of a different immunotherapeutic, a vaccine named Canvaxin, was conducted in which patients with resected stage IV disease were randomized to Canvaxin or placebo. The study was stopped prematurely by the Data Safety Monitoring Board for futility. At the time the trial was stopped the 5-year survival for the patients receiving Canvaxin was 40%, similar to the results reported herein, but the 5-year survival was 45% for patients randomized to placebo.²⁴ The studies are not comparable because the patient populations may be different; nonetheless, the results of both studies emphasize the potential benefit of surgery in patients with stage IV disease. By comparison, the 5-year survival for patients with stage IV disease treated with modalities other than surgery (chemotherapy, biologics, combinations, clinical trials) is virtually nil.

The outcomes reported herein have potential implications for the phase III randomized trial of adjuvant vaccine therapy and/or GM-CSF in patients with melanoma (protocol E4697),¹¹ which has completed accrual of 800 patients who are still being treated and/or followed. The study design of E4697 is different in that patients who have disease recurrence may be removed from the study and offered other therapeutic modalities. Our data suggest that patients who have a limited recurrence may benefit from surgery and this may translate into a prolongation of the overall survival. Such a survival benefit may be missed if patients in E4697 are taken off study before they reach the requirement for systemic therapy.

As the treatment options available for therapy of metastatic melanoma are different now from those that were available at the time the historic controls were treated, one must consider whether or not the improved survival might be due to improved therapeutic options. Combination chemotherapy²⁵ or chemiotherapy regimens²⁶⁻²⁸ have had little impact on survival. There is currently no data to support efficacy of any systemic therapy for metastatic disease and median survival remains 6 to 9 months, the same as it has for the last 4 decades.

This study has expanded the preliminary evidence on GM-CSF as adjuvant therapy of patients with melanoma who are at high risk for recurrence. The results reported herein suggest that prolonged GM-CSF may have efficacy in prolonging survival when administered as surgical adjuvant treatment of patients with melanoma who are at high risk for recurrence. The occurrence of 2 cases of AML

in patients participating in this study is worrisome, but determination as to whether or not it was caused by the prolonged administration of GM-CSF is limited by the fact that only a small number of patients were included in our study. We hope that this report will encourage others who have observed similar cases to report them. A definitive conclusion regarding efficacy of adjuvant GM-CSF therapy in patients with melanoma will await the results of the ongoing randomized phase 3 trial (protocol E4697).

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