# Perilesional Injection of r-GM-CSF in Patients with Cutaneous Melanoma Metastases

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**Based on evidence that granulocyte-macrophage colony stimulating factor (GM-CSF) induces a potent systemic antitumor immunity, we tested recombinant GM-CSF in advanced melanoma. Seven patients with histologically confirmed cutaneous melanoma metastases were treated with perilesional intracutaneous injections of recombinant GM-CSF and observed for a follow-up time of 5 y. All but two patients had a decrease in the total number of metastases. At the end of the 5 y follow-up three of the seven patients are still alive with only one patient receiving other than surgical therapy, and one patient died tumor free at the age of 93. The remaining three patients died from progressive melanoma. Perilesional intradermal GM-CSF therapy resulted in a mean survival time of 33 mo. The treatment was well tolerated and no side-effects other than local erythema at the injection sites and mild drowsiness were seen. Immunohistochemical analysis with staining for CD14 and GM-CSF receptor demonstrated an increased infiltration of monocytes into both injected and noninjected cutaneous melanoma metastases compared with lesions excised prior to the initiation of therapy. The same was true for CD4- and CD8-positive lymphocytes. This phenomenon, together with GM-CSF-induced leukocyte counts of more than 20,000 during therapy, support the possible impact of a systemic over a locally induced reaction by GM-CSF. To our knowledge this is the first report that intracutaneously injected GM-CSF results in long-lasting reduction of melanoma metastases.** ***Key words: granulocyte-macrophage colony stimulating factors/melanoma/metastases/recombinant/therapy. J Invest Dermatol 117:371–374, 2001***

Malignant melanoma is one of the most aggressive human neoplasms. At present no effective treatment exists for melanoma metastases and the advanced disease is basically incurable and associated with a mean survival of only 6 mo (Itoh *et al*, 1992). Despite the grim prognosis of advanced melanoma, spontaneous regression of primary and particularly of secondary melanoma is a rare but well-known phenomenon pointing towards immunologic host defense mechanisms (Avril *et al*, 1992; Balch *et al*, 1992; Blessing and McLaren, 1992). These defense mechanisms practically never result in long-term clinical benefit. Besides the classical tumor infiltrating lymphocytes (Rosenberg *et al*, 1986), cells of the monocyte-macrophage lineage are the predominant cell type infiltrating melanoma. Tumor-associated macrophages have been shown both to promote and to inhibit tumor growth, depending on their state of activation and on the model system employed (Mantovani *et al*, 1992). GM-CSF was shown to be the most effective molecule in inducing antitumor reactivity (Dranoff *et al*, 1993). In this pilot study we therefore tested the potential role of recombinant (r)-GM-CSF as a therapeutic approach for patients with cutaneous melanoma metastases.

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## MATERIALS AND METHODS

**Eligibility** Patients were required to have histologic proof of cutaneous melanoma metastases, a Karnofsky scale greater than 60%, and a life expectancy of more than 12 wk. Patients with brain metastases and patients with a history of allergies or intolerance to exogenous protein were excluded from the study. Seven melanoma patients with cutaneous metastases met the eligibility criteria and were entered into the study. A full medical history and physical examination, a cranial CT scan, a chest X-ray, sonography of the lymph nodes, and a thorough evaluation of hematologic and blood chemistry parameters were performed prior to the initiation of treatment. Written informed consent was obtained from all patients and the study was approved by the institutional ethics committee. Standard WHO criteria were used for the evaluation of response and toxicity (WHO, 1979).

**Treatment** A daily dose of 400 μg of r-GM-CSF (Leukomax, Novartis) reconstituted with 1 ml of sterile water was administered intradermally over 5 d at eight to ten sites around three to six cutaneous metastases chosen as indicator lesions. After 21 d this treatment cycle was repeated around the same lesions. Patients were monitored daily during the injection periods, weekly until the end of the study (day 42), and were routinely followed thereafter to a mean time of 59.7 ± 1.6 mo.

**Histologic and immunohistochemical analysis** Metastatic melanoma lesions were evaluated prior to the initiation of treatment and after r-GM-CSF administration (injected lesions and noninjected lesions) by standard hematoxylin and eosin histology and by immunohistochemistry on paraffin-embedded tissue for HMB 45 and on frozen sections for GM-CSF receptor, CD4, CD8, and CD14. The antibodies used were anti-HMB 45 (Dako, Carpinteria, CA) at a dilution of 1:50, anti-GM-CSF receptor (Upstate Biotechnology, Lake Placid, NY) at a

dilution of 1:200, anti-CD4 (Becton Dickinson, Mountain View, CA, clone SK3/4) at a dilution of 1:100, anti-CD8 (Becton Dickinson, clone SK2) at a dilution of 1:100, and anti-CD14 (Becton Dickinson, clone Mo-P9) at a dilution of 1:100. Appropriate isotype controls were used. Sections were incubated with the primary antibodies at the respective dilutions for 1 h at room temperature and thereafter with biotinylated horse antimouse antibody at a dilution of 1:200 for 1 h. Endogenous peroxidase activity was blocked with 0.015% $H_2O_2$. The signal was detected with the streptavidin-biotin-peroxidase method, and 3-amino-9-ethylcarbazole was used as a chromogen. Slides were counterstained with hematoxylin. The immunostaining intensity was rated as follows: –, none; +, weak; ++, moderate; +++, intense.

**Follow-up proceedings** After the end of the initial treatment period (day 42) patients' follow-up consisted of routine blood screening and radiologic restaging (either CT or chest X-ray plus abdominal sonography) every 6 mo. No patient failed to turn up for the control examinations during follow-up. No patient was lost to follow-up.

## RESULTS

**Patients' characteristics** The patient characteristics of seven melanoma patients with cutaneous metastases who met the eligibility criteria are given in **Table I**. All had cutaneous/subcutaneous metastases, none had visceral metastases, and three had had lymph node metastases that had been removed by lymphadenectomy.

**Table I. Patients' characteristics**

| | | |
|---|---|---|
| No. of patients entered | | 7 |
| Male:Female | | 5 : 2 |
| Mean age in years (range) | | 76.4 (45-90) |
| Primary melanoma | NMM | 4/7 |
| | ALM | 1/7 |
| | occult | 2/7 |
| Cutaneous metastases | | 7/7 |
| Lymph node metastases | | 3/7 |
| Visceral metastases | | 0/7 |
| Previous treatment | Immunotherapy (IL-2, alpha IFN) | 3/7 |
| | Chemotherapy | 2/7 |
| | Surgery | 7/7 |
| | None | 0/7 |

**Clinical response** Each patient received two treatment cycles with perilesionally, intracutaneously administered r-GM-CSF. This treatment resulted in an initial size reduction of the injected indicator lesion in six of seven patients. The total number of cutaneous metastases was reduced in all but two patients and, notably, there was a reduction of the number of metastases even if the indicator lesion was not reduced in size, demonstrating a therapeutic effect beyond the injection site. Treatment responses as seen on day 42 are summarized in **Table II**.

Three patients were still alive after the end of a 5 y follow-up period. One patient (#6) was free of all melanoma metastases at 5 y follow-up and had then reached complete remission. Of the other two patients, one received only surgical therapy (excision of cutaneous metastases) and one (patient #5) received additional treatment with interferon-$\alpha_2$ ($3 \times 10^6$ units three times a week for 1 y). Both are tumor free to date as shown by routinely performed CT scans. Of the remaining four patients, one died free of melanoma metastases at the age of 93 after receiving only surgical treatment of localized metastases. The other three patients died due to progressive melanoma. Taken together, this results in a mean long-term survival of 33 mo after initiation of treatment. In our small group of patients no relation of long-term survival to the histologic type or the depth of invasion of the primary tumor could be observed. There was also no relationship between lymph node metastases excised prior to treatment and long-term survival (see **Table III**).

r-GM-CSF treatment was well tolerated by all seven patients leading only to mild drowsiness and local erythema at the injection sites. White blood count was more than doubled in all patients after intradermal injection of the first dose of 400 μg r-GM-CSF and rose to more than 20,000 during each 5 d treatment period (22,700 ± 8600; data not shown). A rise in eosinophils was also observed with a mean peak level of 11.6% ± 4.4% in the relative blood count.

**Table II. Clinical response after two cycles of r-GM-CSF treatment**

| Patient number | Percent size change of injected lesion | Total number of cutaneous metastases prior to treatment | Total number of cutaneous metastases after treatment |
|---|---|---|---|
| 1 | +69.3 ↑ | 91 | 62 ↓ |
| 2 | –19.0 ↓ | 3 | 7 ↑ |
| 3 | –100.0 ↓ | 4 | 1 ↓ |
| 4 | –61.0 ↓ | 33 | 14 ↓ |
| 5 | –84.4 ↓ | 5 | 7 ↑ |
| 6 | –100.0 ↓ | 4 | 0 ↓ |
| 7 | –54.4 ↓ | 16 | 12 ↓ |

**Table III. Outcome after 5 y of follow-up and relation to prior lymph node metastases[a]**

| Patient number | Survival time (mo) | Tumor related death Yes/No | Tumor free at time of death or end of follow-up | Lymph node metastases prior to treatment Y/N |
|---|---|---|---|---|
| 1 | 38 | N | Y | N |
| 2 | 62 | - | Y | N |
| 3 | 4 | Y | N | Y |
| 4 | 6 | Y | N | N |
| 5 | 58 | - | Y | Y |
| 6 | 58 | - | Y | Y |
| 7 | 5 | Y | N | N |

[a]Y, yes; N, no.

**Table IV. Immunohistochemical changes caused by r-GM-CSF treatment[a]**

| | Lesions prior to treatment | Lesions after treatment | |
|---|---|---|---|
| | | Injected | Non-Injected |
| *Melanoma cells* | | | |
| HMB45 | ++ | ++ | ++ |
| GM-CSF-R | - | - | - |
| *Monocytes/Macrophages* | | | |
| CD14 | + | +++ | +++ |
| GM-CSF-R | + | +++ | +++ |
| *Lymphocytes* | | | |
| CD4 | + | +++ | +++ |
| CD8 | + | ++ | ++ |

[a]Immunostaining intensity: -, none; +, weak; ++, moderate; +++, intense.

**Immunohistochemical analysis** Treatment with r-GM-CSF did not alter the expression of the GM-CSF receptor (GM-CSF-R) on HMB-45-positive melanoma cells in any of the seven patients investigated (**Table IV**). The number of CD14-positive monocytes/macrophages within the tumor was increased about 3-fold by treatment with GM-CSF and the same was true for GM-CSF-R in the investigated samples. This increase in GM-CSF-R is probably due to the increased number of CD14-positive cells of the monocyte/macrophage lineage, but we cannot exclude an increase in receptor density per CD14-positive cell as the reason for this increased staining. In addition, a high increase of CD4-positive lymphocytes and a moderate increase of CD8-positive lymphocytes in the tumor lesions were observed. We want to stress that no immunohistochemical differences between injected indicator lesions and reactions in distant metastases exposed only systemically to r-GM-CSF were observed (**Table IV**). All stainings with isotype controls remained negative.

## DISCUSSION

Improved antitumor strategies have altered the prognosis of a variety of human malignancies. Metastatic melanoma remained one of the notable exceptions, however, despite the known immunogenicity of this tumor (Avril *et al*, 1992; Balch *et al*, 1992; Blessing and McLaren, 1992). A number of studies have demonstrated that the host response to tumor challenge can be decisively influenced by the inoculation of tumor cells genetically engineered to express cytokines, in particular GM-CSF (Dranoff *et al*, 1993; Pardoll, 1993; Armstrong *et al*, 1996). Dranoff *et al* found GM-CSF, most often associated with growth and differentiation of hematopoietic progenitors, to be the most powerful of 10 molecules tested.

In this study we describe the effect of intracutaneous GM-CSF on stage IV patients with cutaneous melanoma metastases. One complete remission was obtained and three of seven patients are still alive at 5 y without any other further treatment than surgical removement of metastases and, in one patient, 1 y of interferon $\alpha_2$. One patient died tumor free due to cardiovascular disease after also receiving only surgical treatment of residual metastases. Thus GM-CSF administration resulted in a mean survival of 33 mo after initiation of treatment; survival is still 28.8 mo if one excludes from evaluation patient #5 for receiving an additional therapy with interferon-$\alpha$. In advanced melanoma surgical treatment alone results in a median survival of 15.7 mo (Brand *et al*, 1997).

If one compares the response of the individual patients on day 42 (end of the second treatment period) with the long-term results (**Table II** *versus* **Table III**), there is no uniformity of response. For example, patient #2 initially even had an increase in the number of metastases with a slight decrease in the size of the indicator lesion but is one of three patients still alive with only surgical treatment after GM-CSF therapy. This implies that a systemic effect induced by locally injected GM-CSF seems to be more relevant for long-term survival than the initial local response. The nature of a possible predictive factor for therapeutic susceptibility to GM-CSF therapy remains unclear as already discussed elsewhere (Si *et al*, 1996).

In our study, intracutaneous GM-CSF treatment caused an infiltration of all the investigated cutaneous melanoma lesions by cells of the monocytic and lymphoid lineage leading to a decrease in the total number of metastases in all but two patients, and a decrease in the size of the indicator lesion in all but one patient. This differs from the results of other studies, but these were short term and doses of GM-CSF employed were different. In a study by Si *et al* on 13 patients with at least three subcutaneous melanoma metastases injected intralesionally with GM-CSF only three patients showed a partial regression of the lesions and only four of 11 evaluable patients showed an increase of T cells and macrophages around the metastases. Si *et al*, however, used a dose of 15-50 μg per d delivered intralesionally in contrast to the 400 μg delivered perilesionally in our study. Robinson *et al* (1998) applied GM-CSF continuously intralesionally by using a pump system with a dose of 10 μg per 24 h and observed a partial response in one of eight patients. GM-CSF was also used as an aerosol in patients with lung metastases including one patient suffering from melanoma metastases who showed a partial regression of his lesions (Anderson *et al*, 1999). All these data come from short-term studies, however, and to date no comparable 5 y follow-up data exist.

As Dranoff *et al* (1993) showed that irradiated tumor cells expressing GM-CSF were capable of protecting mice more effectively against subsequent tumor inoculation than tumor cells alone, efforts have been undertaken to develop therapeutic strategies using GM-CSF together with peptide vaccines or tumor cells in various combinations. Leong *et al* (1999) applied r-GM-CSF together with an autologuos melanoma vaccine and observed regression of all tumor lesions in 10% of their patients and a partial response in another 10%. Soiffer *et al* (1998) used GM-CSF-expressing irradiated melanoma cells for vaccination and observed a high degree of infiltration with T cells and destruction of metastases by up to 80% of the initial lesional volumes. A recent study by Mastrangelo *et al* (1998) employed a vaccinia virus system to transfect GM-CSF encoding c-DNA into melanoma metastases. In their study they found regression of the injected lesions and also regression of distant metastases comparable to the reaction pattern seen in our patients. One can argue that these vaccination strategies have a higher potential in inducing antitumor immunity but a major problem with the strategies is that they are technically difficult to apply, need a specialized center, and therefore are available only for a limited number of patients. On the other hand intradermal GM-CSF administration is easy to perform.

Additionally a recent study in melanoma patients using GM-CSF in an adjuvant setting showed prolongation of overall and disease-free survival (Spitler *et al*, 2000) and further supports the potentials of GM-CSF in melanoma therapy.

Taken together, the data provided in this work show that GM-CSF applied intracutaneously and perilesionally appears as a promising, easily applicable, and well tolerated therapeutic strategy for patients with skin metastases of malignant melanoma. GM-CSF seems to augment host anti-melanoma immunity and to provide favorable long-term results. Follow-up studies with larger groups of patients will clearly be needed to learn more about optimal dosage, treatment schedules, and potential combinations with other therapeutic strategies to optimize treatment outcome.

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