

REVIEW

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Current status of granulocyte–macrophage colony-stimulating factor in the immunotherapy of melanoma

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Abstract

In 2012, it was estimated that 9180 people in the United States would die from melanoma and that more than 76,000 new cases would be diagnosed. Surgical resection is effective for early-stage melanoma, but outcomes are poor for patients with advanced disease. Expression of tumor-associated antigens by melanoma cells makes the disease a promising candidate for immunotherapy. The hematopoietic cytokine granulocyte–macrophage colony-stimulating factor (GM-CSF) has a variety of effects on the immune system including activation of T cells and maturation of dendritic cells, as well as an ability to promote humoral and cell-mediated responses. Given its immunobiology, there has been interest in strategies incorporating GM-CSF in the treatment of melanoma. Preclinical studies with GM-CSF have suggested that it has antitumor activity against melanoma and can enhance the activity of anti-melanoma vaccines. Numerous clinical studies have evaluated recombinant GM-CSF as a monotherapy, as adjuvant with or without cancer vaccines, or in combination with chemotherapy. Although there have been suggestions of clinical benefit in some studies, results have been inconsistent. More recently, novel approaches incorporating GM-CSF in the treatment of melanoma have been evaluated. These have included oncolytic immunotherapy with the GM-CSF–expressing engineered herpes simplex virus talimogene laherparepvec and administration of GM-CSF in combination with ipilimumab, both of which have improved patient outcomes in phase 3 studies. This review describes the diverse body of preclinical and clinical evidence regarding use of GM-CSF in the treatment of melanoma.

Keywords: Granulocyte macrophage-colony stimulating factor, GM-CSF, Melanoma, Cellular immunotherapy

Introduction

In 2012, it was estimated that 9180 people in the United States would die from melanoma and more than 76,000 new cases would be diagnosed [1]. The primary treatment for melanoma, excision of the malignancy, is highly effective in early-stage disease but is not a meaningful option for metastatic disease except in patients with solitary metastases or limited-volume disease [2]. For patients with advanced disease, cytotoxic chemotherapy has a limited role and curative potential in <1% of patients. For BRAF-mutant patients, treatment with vemurafenib, dabrafenib, and/or trametinib have high rates of objective response,

but median duration of response is typically ~6–8 months, after which rapid disease progression is common [2]. However, some patients with melanoma respond to immunotherapy with durable responses, and thus immunotherapy shows substantial promise for further improving durable control of advanced melanoma.

Expression of tumor-associated antigens by melanoma cells makes the disease a promising candidate for immunotherapy [3]. The potential for immunotherapy in melanoma has been demonstrated by improved outcomes among patients with stage III melanoma receiving interferon- α 2b [4] and patients with metastatic melanoma receiving the anti-cytotoxic T-lymphocyte antigen 4 (CTLA-4) antibody ipilimumab alone or in combination

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with a gp100 peptide vaccine or dacarbazine [5,6], as well as by durable complete responses with high-dose interleukin-2 [7] and high response rates after adoptive T cell transfer therapies [8].

The hematopoietic cytokine granulocyte–macrophage colony-stimulating factor (GM-CSF) has been investigated as a monotherapy, and as a component of combination therapies for melanoma. Preclinical evidence has suggested that GM-CSF may have antitumor effects, but results from clinical trials evaluating GM-CSF present a complex picture. This review evaluates evidence regarding use of GM-CSF in melanoma and potential future strategies in this setting.

Review

Immunobiology of GM-CSF

GM-CSF was identified as a factor responsible for expansion and activation of granulocytes and macrophages, but has since been found to have many direct and indirect effects on multiple cell types, including cell proliferation, maturation, and survival (Figure 1A) [9]. GM-CSF plays a critical role in development and maturation of dendritic cells (DCs) and proliferation and activation of T cells, linking the innate and acquired immune response [10]. In mice, increased numbers of eosinophils, monocytes, macrophages, and lymphocytes were observed in the draining lymph node in response to treatment with irradiated melanoma cells expressing GM-CSF, resulting in a sustained antitumor response [11]. GM-CSF has also been shown to favor expansion of DC1 populations [12,13] and to increase DC-mediated responses to tumor cells (Figure 1B) [14]. In vitro studies using human myeloid leukemia cells suggest that, in addition to promoting antigen presentation, GM-CSF directs these cells toward a DC phenotype [15,16]. The role of GM-CSF in neutrophil proliferation and survival led to its use in amelioration of neutropenia following induction chemotherapy in elderly patients with acute myeloid leukemia [17].

Studies evaluating GM-CSF in preclinical models of melanoma

The immune adjuvant properties of GM-CSF led to numerous preclinical studies assessing the ability of GM-CSF to inhibit tumor growth and/or mediate tumor regression. In a seminal study, a panel of recombinant retroviral vectors expressing various cytokines, co-stimulatory molecules, or adhesion molecules were used to infect murine B16 melanoma cells. Infected cells were then irradiated and injected subcutaneously into immune-competent hosts, followed by a subsequent challenge with wild-type B16 cells [11]. The GM-CSF–secreting tumor vaccines conveyed 90% protection, whereas vaccines expressing interleukin-2 and interferon- γ failed to mediate antitumor protection [11]. Additionally, analysis of the vaccination site revealed an

influx of dividing monocytes and granulocytes, with a coincident increase in lymphocytes in tumor-draining lymph nodes, suggesting direct augmentation of antigen presentation and T-cell priming against the tumor [11].

Exogenously administered GM-CSF has been shown to augment antitumor immunity. Mice immunized with an HIV envelope peptide vaccine plus GM-CSF exhibited increased cytotoxic T lymphocyte (CTL) activity compared with vaccine alone or vaccine with interleukin-2 [18]. Studies using a tumor-associated antigen vaccine (neu), in combination with GM-CSF, produced increased neu-specific antibodies alongside an enhanced CTL response [19,20]. However, tumor protection was ultimately dependent on a cell-mediated response since depletion of CD8⁺ T cells abrogated tumor regression.

Genetically modified vaccines can generate protective anti-melanoma immune responses in animal models [21]. B16 tumor lines expressing bioactive levels of murine GM-CSF have been generated and assessed [11,21,22]. Levels of GM-CSF at the site of GM-CSF–expressing tumor transplantation remain elevated for days, whereas GM-CSF dissipated rapidly after injection of irradiated tumor with recombinant GM-CSF [22]. The pharmacokinetic longevity associated with the vaccine correlated with both increased DC infiltration and tumor protection against wild-type tumors [22].

Oncolytic immunotherapy with modified herpes simplex viruses (HSV) and vaccinia viruses expressing GM-CSF have also shown promise. These vectors have elicited tumor responses in mice when injected directly into tumor lesions [23–25]. In a murine melanoma model, a temperature-sensitive strain of HSV encoding murine GM-CSF significantly reduced Harding-Passey melanoma tumor growth and improved survival of tumor-bearing mice [25]. Similarly, preclinical studies demonstrated a potent lytic effect against lesions injected with the GM-CSF–expressing oncolytic HSV talimogene laherparepvec (T-VEC; formerly Oncovex^{GM-CSF}) [26]. Notably, when virus expressing GM-CSF was employed, regression of uninjected, distant lesions was enhanced compared with a regression observed with a control virus not expressing GM-CSF.

The combination of GM-CSF–secreting tumor vaccines with other immunotherapies is another potentially promising approach. Antibodies that block the co-inhibitory T-cell molecules CTLA-4 and PD-1 plus GM-CSF have boosted immune responses against melanomas [5,27–31]. Additionally, activation of co-stimulatory molecules (CD80, CD86, CD137), exogenous cytokine administration (interleukin-2, interferon- γ), and blockade of tumor angiogenesis can inhibit melanoma progression when combined with GM-CSF–expressing vaccines [32–36]. These preclinical studies strongly suggest that the combination of GM-CSF–based tumor vaccines with immunomodulatory agents has potential for clinical use.

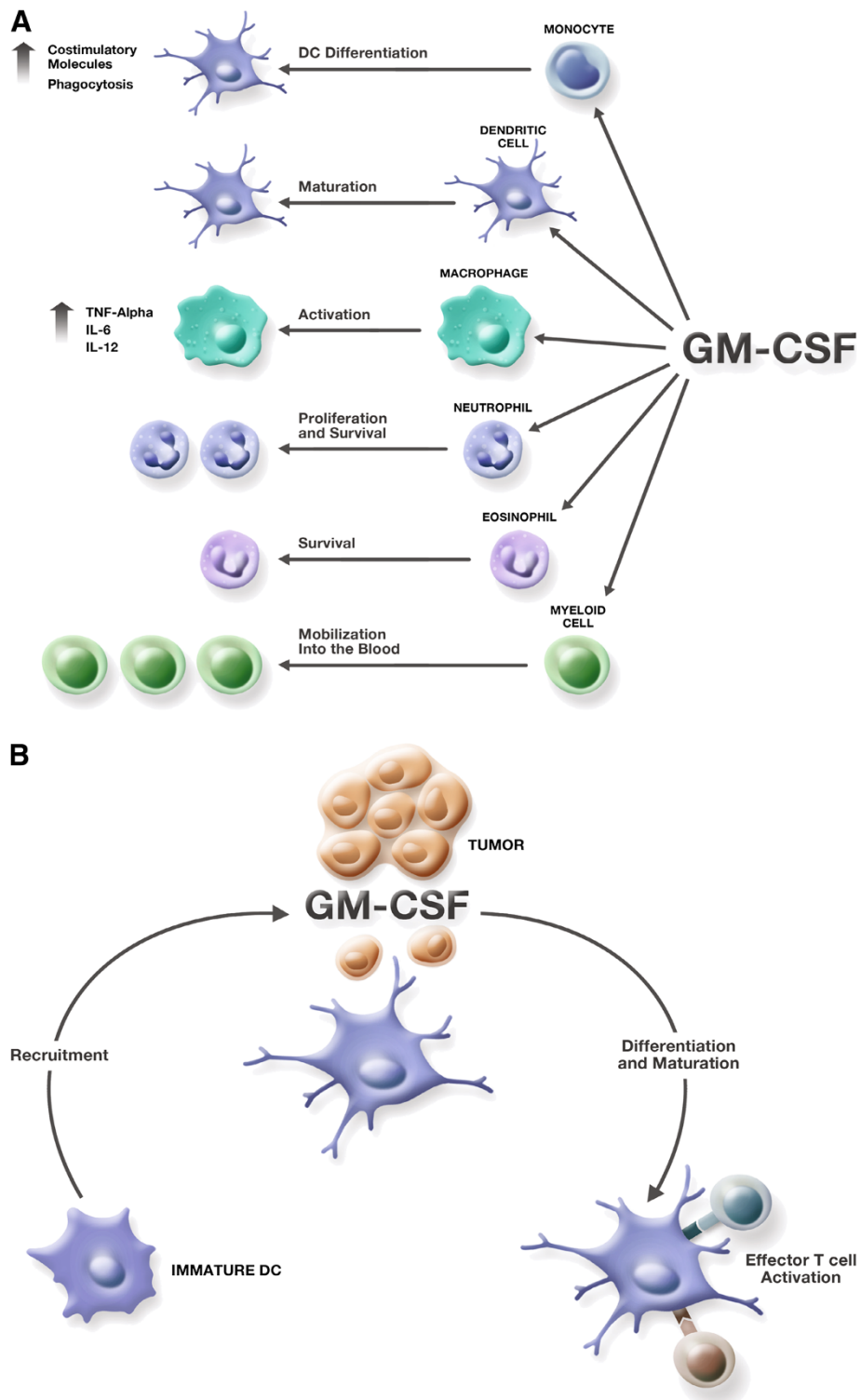


Figure 1 Immunobiologic effects of GM-CSF. **(A)** Effects of GM-CSF on cells of the immune system. **(B)** Effects of GM-CSF on dendritic cells and T cells in the tumor microenvironment.

Clinical trials evaluating exogenously administered GM-CSF in patients with melanoma

Given the evidence of antitumor activity in preclinical models of melanoma, there has been interest in using GM-CSF to improve outcomes in the clinical setting. Numerous studies have evaluated use of recombinant GM-CSF in completely resected stage III/IV melanoma patients. Data from these studies, however, have been inconsistent.

GM-CSF as an adjuvant therapy

GM-CSF has been evaluated as an adjuvant, systemic monotherapy following regional lymphadenectomy, to prevent or to delay recurrence in high-risk stage III patients (Table 1). In a phase 2 study, there were statistically significant improvements in survival among patients with stage III/IV disease ($P = 0.04$ and $P < 0.001$, respectively) receiving GM-CSF ($125 \mu\text{g}/\text{m}^2$ daily for 14 consecutive days in 28-day cycles up to 1 year) compared with historical controls [37]. A subsequent single-arm study using the same dosing regimen evaluated treatment with GM-CSF over 3 years and reported a 5-year survival rate of 60%, with 5-year disease-free survival (DFS) of 67% and 40% for patients with stage III and IV disease, respectively [38]. The effect of GM-CSF on DC has been proposed as a mechanism for supporting antitumor immunity; consistent with this hypothesis, treatment with recombinant GM-CSF has increased mature DCs in melanoma patients [39]. A randomized study of recombinant GM-CSF ($125 \mu\text{g}/\text{m}^2$ daily for 14 consecutive days in 28-day cycles) versus placebo in patients with completely resected stage IIIB/IIIC/IV or mucosal melanoma, there was a trend toward improvement in DFS although it did not reach statistical significance (11.5 vs 9.2 months; HR, 0.88; $P = 0.14$), and no improvement in overall survival (OS; 69.6 vs 62.4 months; HR, 0.96; $P = 0.78$) [40]. However, an improvement in DFS (HR, 0.74; $P = 0.04$) and a trend toward improved OS (HR, 0.72; $P = 0.07$) was observed in stage IV patients ($n = 258$) [40].

GM-CSF as intratumoral monotherapy

Several small studies have evaluated GM-CSF administered as a monotherapy by direct injection into metastatic lesions (Table 2). Clinical responses in two of these studies were modest, with only one partial response (PR) and no complete response (CR) reported [44,45]. In contrast, a study using perilesional injection of GM-CSF ($400 \mu\text{g}/\text{d}$ over 5 days) to treat metastatic melanoma described reduced lesion size in 6/7 patients and a reduction in cutaneous metastases in 5/7 patients [46]. Three patients were still alive at 5-years follow-up; a fourth died tumor-free at age 93. In these studies, there was evidence of increases in DC and T-cell counts and infiltration at injected sites, and in some cases at uninjected sites, suggesting an immunologic effect of GM-CSF injection [44-46]. Other studies using novel methods (such as aerosolization and immunoembolization) to delivering GM-CSF to melanoma metastases at sites that are not readily injectable have met with mixed results (Table 2) [47-50].

GM-CSF in combination with chemotherapy

A number of early-phase clinical studies have evaluated GM-CSF in conjunction with chemotherapy (Table 3). These studies were typically small with a single-arm design and used a variety of different drug regimens and dosing schedules; thus, as a whole, they are difficult to interpret. Clinical efficacy in studies varied widely, with response rates ranging from no response to >40% [53-58]. It is worth noting that several of the studies reporting high overall response rates also reported significant increases in T-cell, DC, macrophage, or natural killer-cell populations following treatment [53,55,58]. One study evaluating a chemotherapy regimen of dacarbazine, interferon- $\alpha 2\text{b}$, interleukin-2, and tamoxifen with three doses of GM-CSF reported a dose-response effect with increasing exposure to GM-CSF via administration over a greater number of days ($P = 0.016$) [59].

Table 1 Clinical studies evaluating adjuvant GM-CSF in patients with surgically resected melanoma

Citation	Evaluable patients	GM-CSF dose schedule	Control	Clinical response
Spitler et al. [37]	48	$125 \mu\text{g}/\text{m}^2$ for 14 d, 28-d cycles, for 1 y	Historical	OS: 37.5 mo
Markovic et al. [41]	70 (Stage IV) 149 (Stage III)	$125 \mu\text{g}/\text{m}^2$ for 14 d, 28-d cycles, for 1 y $125 \mu\text{g}/\text{m}^2$ for 14 d, 28-d cycles, for 1 y	Observation	OS: 6.6 y (GM-CSF) vs 6.8 y (control) OS: 8.6 (GM-CSF) vs 5.2 y (control)
Isla et al. [42]	24	150 mg/d for 2 y	None	DFS at 1 y: 88.8%
Elias et al. [43]	45	$125 \mu\text{g}/\text{m}^2$ for 14 d, then IL-2 9×10^6 IU/ m^2 for 4 d/28-d cycle, \pm autologous vaccine	None	DFS at 15.9 mo: 60% OS at 21 mo: 64% (21 mo follow-up)
Spitler et al. [38]	98	$125 \mu\text{g}/\text{m}^2$ for 14 d, 28-d cycles, for 3 y	None	DFS: 1.4 y 5-y survival: 60%
Lawson et al. [40]	743	$250 \mu\text{g}/\text{m}^2$ for 14 d, 28-d cycles, for 1 y	Placebo	OS: 62.4 mo for placebo vs. 69.6 mo for GM-CSF (HR, 0.96) DFS: 9.2 mo for placebo vs. 11.5 mo for GM-CSF (HR, 0.88)

DFS = disease-free survival; GM-CSF = granulocyte-macrophage colony-stimulating factor; HR = hazard ratio; IL-2 = interleukin-2; OS = overall survival.

Table 2 Studies evaluating GM-CSF as a monotherapy in patients with advanced melanoma

Citation	Evaluable patients	GM-CSF dose schedule	Route of administration	Clinical response	Observations
Si et al. [44]	13	15–50 µg/lesion at 2 sites per patient Site 1: 5 times daily Site 2: 5 times daily then once weekly for 6 mo	Intralesional	1 PR, 8 SD	Responding patients had increased T-cell and Langerhans cell infiltration of the tumor
Nasi et al. [45]	16	10, 20, 40, or 80 µg/injection for 10 d	Intralesional	3 SD	Significant increase in DCs and T cells at injection sites
Vaquerano et al. [51]	1	500 µg/d for 4 d, monthly	Intralesional	1 PR	Regression of melanoma cells
Hoeller et al. [46]	7	400 µg/d for 5 d, 21-d cycle	Perilesional	6 with reduced lesion size	Increased infiltration of monocytes and lymphocytes was observed in injected and systemic sites
Ridolfi et al. [52]	14	150 µg/lesion plus IL-2 3 × 10 ⁶ IU for 5 d, 21-day cycle	Intralesional (GM-CSF) Perilesional (IL-2)	2 PR, 2 MR, 7 SD	Some evidence of systemic immune activation
Rao et al. [47]	14	250 µg twice daily for 7 d on alternating weeks	Aerosol delivery for lung metastases	6 SD	Upregulation of cytotoxic T lymphocytes was observed in peripheral blood
Markovic et al. [48]	35	500–2000 µg (250-µg/dose increments) twice daily on days 1–7 and 15–21, over 28 d	Aerosol delivery for lung metastases	1 PR, 5 SD	A trend toward increased immune response was observed with higher doses; MTD was not reached
Sato et al. [49]	31	25–2000 µg every 4 wk	Hepatic artery immunoembolization	2 CR, 8 PR, 10 SD	Prolonged PFS correlated with higher GM-CSF doses
Eschelman et al. [50]	52	2000 µg every 4 wk	Hepatic artery immunoembolization	5 PR, 12 SD	Trend toward increased OS with GM-CSF; prolonged OS with GM-CSF in patients with bulky metastases

CR = complete response; DC = dendritic cell; GM-CSF = granulocyte-macrophage colony-stimulating factor; IL-2 = interleukin-2; MTD = maximum tolerated dose; MR = mixed response; OS = overall survival; PD = progressive disease; PFS = progression free survival; PR = partial response; SD = stable disease.

Table 3 Studies evaluating GM-CSF in combination with chemotherapy in patients with advanced melanoma

Citation	Evaluable patients	GM-CSF dose schedule	Other agents	Clinical response
Schacter et al. [53]	40	20 µg/m ² once daily for 7 d every 3 wk	BCNU, CDDP, DTIC, tamoxifen, IFN-α	9 CR, 11 PR, 2 SD OS:14 mo
Gajewski et al. [60]	7	5 µg/kg for 6 d	DTIC, CDDP, IL-2, IFN-α	1 CR, 1 PR, 2 MR
Gibbs et al. [61]	60	250 µg/m ² for 20 d, 28-d cycle	TMZ, CDDP, IL-2, IFN-α	1 CR, 11 PR Median OS: 11 mo
Vaughan et al. [59]	19	Arm 1: 450 µg/m ² on days 4, 5, 15, and 16 Arm 2: 450 µg/m ² on days 4, 5, 15, 16; 225 µg/m ² on days 6–10 and 17–21, 28-d cycle Arm 3: 450 µg/m ² on days 4–10 and 15–21, 28-d cycle	DTIC, CDDP, IL-2, IFN-α, TAM	2 CR, 4 PR OS: 6.2 mo Trend toward increasing response with higher GM-CSF doses
Gong et al. [62]	30	5 µg/kg (first 25 patients) or 450 µg/m ² (last 8 patients) for 6 d	DTIC, CDDP, IL-2, IFN-α	3 CR, 4 PR, 6 MR, 7 SD Median OS: 15 mo
Groenewegen et al. [63]	31	2.5 µg/kg for 10 d	DTIC, IL-2, IFN-α	4 CR, 6 PR Median OS: 8 mo 1-y survival: 22%
De Gast et al. [64]	74	2.5 µg/kg for 12 d	TMZ, IL-2, IFN-α	4 CR, 19 PR, 13 SD OS: 8.3 mo 1-y survival: 41%
Smith et al. [65]	8	125 and 250 µg/m ² /d for 7 d every 2 wk, 28-d cycle	IL-2	0 CR, 0 PR
Fruehauf et al. [54]	10	250 µg/m ² for 11 d	DOX, VIN	0 CR, 5 PR Median time to progression: 8 mo
Lewis et al. [66]	71	250 µg/m ² for 20 d, 28-d cycle	TMZ, CDDP, IFN-α, IL-2	0 CR, 10 PR Median OS: 8.6 mo
Weber et al. [67]	31	125 µg/m ² for 12 d, 28-d cycle	TMZ, IL-2, IFN-α	4 CR, 4 PR, 7 SD OS: 13.1 mo
Jin et al. [55]	18	175 µg/m ² for 4 d, 21-d cycle	DTIC, IL-2	4 CR, 8 PR
O'Day [56]	131	Induction: 500 µg/d for 10 d or once daily until ANC >5000/µL Maintenance: 250 µg/d for 14 d	Induction: VBL, CDDP, DTIC, IL-2, IFN-α Maintenance: IL-2	10 CR, 47 PR, 38 SD Median OS: 13.5 mo 1-y survival: 57%
Gunturu et al. [68]	18	250 µg/m ² from day 8 until AGC >5000 cells/µL on 2 consecutive days	CTX, FLU, MESNA, IL-2	1 CR, 3 PR
Locke et al. [57]	14	250 µg/m ² until WBC >30000/µL or for 10 d, 21-d cycle	OX, DOX	0 CR, 0 PR, 5 SD
Lutzky et al. [69]	30	125 µg/m ² for 35 d	IL-2	0 CR, 4 PR, 8 SD Median OS: 10.7 mo 1-y survival: 32.5%

AGC = absolute granulocyte count; ANC = absolute neutrophil count; BCNU = carmustine; CDDP = cisplatin; CR = complete response; CTX = cyclophosphamide; DOX = docetaxel; DTIC = dacarbazine; FLU = fludarabine; GM-CSF = granulocyte-macrophage colony-stimulating factor; IFN-α = interferon-α; IL-2 = interleukin-2; MESNA = sodium 2-mercaptoethanesulfonate; MR = mixed response; OS = overall survival; OX = oxaliplatin; PFS = progression-free survival; PR = partial response; SD = stable disease; TAM = tamoxifen; TMZ = temozolomide; VBL = vinblastine; VIN = vinorelbine.

GM-CSF as an adjuvant with cancer vaccines

Given the significant body of evidence from preclinical studies [11,18,20,21,70], GM-CSF has been evaluated as an adjuvant to cancer vaccines in a number of clinical studies. Several approaches to administering GM-CSF as an adjuvant have been employed, including coadministration with the vaccine [71-74], injection at the vaccination site [75,76] systemic administration [77,78], and administration of a plasmid/viral vector encoding GM-CSF [79,80]. The dose of GM-CSF administered as an adjuvant is typically less than the recommended overall weekly dose of 250 $\mu\text{g}/\text{m}^2/\text{day}$ for 21 days for use in myeloid reconstitution after autologous bone marrow transplantation [17].

In contrast to data from murine studies, the adjuvant effect of GM-CSF in human trials is inconsistent. In a study that evaluated coadministration of GM-CSF with multipeptide (including gp100 and tyrosinase peptides) melanoma vaccines incorporating GM-CSF and incomplete Freund's adjuvant (IFA) in patients with advanced melanoma, there was a high T-cell response rate and a correlation between T-cell reactivity to the melanoma peptides and clinical outcome [72]. GM-CSF combined with IFA as an adjuvant for a vaccine containing 12 melanoma peptides resulted in a similar immunologic response in patients with resected stage III/IIIB/IV melanoma [74]. Similarly, systemic administration of GM-CSF following a peptide vaccination augmented T-cell response in three patients with advanced melanoma [77]. However, in a study that evaluated intradermal vaccination with tyrosinase peptides followed by intradermal GM-CSF, detectable T-cell responses were observed in only 4/15 (27%) evaluable patients [76].

Although results from these studies have suggested that administration of adjuvant GM-CSF might improve immune responses, none included control groups. Recently, clinical trials including controls have evaluated effects of GM-CSF administered locally at the vaccination site (Table 4). In a phase 1 study comparing different adjuvant strategies, vaccination with tyrosinase peptide plus GM-CSF or keyhole limpet hemocyanin (KLH) did not induce greater immune responses compared with vaccination with peptide alone, although combination with GM-CSF plus KLH had a moderate adjuvant effect [75]. Two recent randomized prospective trials suggested that addition of GM-CSF to melanoma vaccines did not improve cellular immune responses and, indeed, may have had negative effects [71,81]. Notably, both studies combined GM-CSF with another adjuvant (IFA or BCG) which may have influenced the immune response. The inconsistent effect of GM-CSF on immune responses to vaccines may be due in part to competing effects inducing dendritic cell maturation on one hand, and inducing myeloid suppressor cells on the other. A recent phase 2, randomized controlled

trial (E1696) evaluated treatment of advanced melanoma with multipeptide vaccine alone or with subcutaneous interferon- α , GM-CSF, or interferon- α plus GM-CSF [78,82]. Consistent with results of studies evaluating administration of GM-CSF at the vaccine site, no significant improvement in T-cell or clinical response was observed with interferon- α and/or GM-CSF administration with vaccination [78,82].

Results from clinical studies evaluating GM-CSF as an adjuvant to melanoma vaccines suggest the biologic effects of GM-CSF are complex and can be influenced by numerous factors. GM-CSF administered with a heat shock protein vaccine has been implicated in the induction of myeloid-derived suppressor cells (MDSC) in melanoma patients [83]. On the other hand, daily subcutaneous administration of GM-CSF (125 $\mu\text{g}/\text{m}^2$ for 14 days in 28-day cycles) increased circulating mature DC but did not increase MDSC in melanoma patients [39]. It has been suggested that negative immunologic effects of GM-CSF may be associated primarily with high doses of GM-CSF (doses of 225 $\mu\text{g}/\text{d}$ or higher in melanoma patients) [84]. In a trial of a multipeptide melanoma vaccine, immune responses were lower with GM-CSF plus IFA than with IFA alone (the dose used in that trial was arguably less than 20 $\mu\text{g}/\text{day}$) [81]. Thus, even low doses of GM-CSF administered with a multipeptide vaccine may have negative immunologic effects. Additional studies are also needed to determine if GM-CSF alters the function of vaccine-induced T cells and whether inclusion of GM-CSF with the vaccine may affect clinical outcome.

Novel strategies incorporating GM-CSF

GM-CSF-expressing oncolytic immunotherapy

Preclinical studies have indicated an important role for GM-CSF in the tumor microenvironment and have suggested that increased expression of GM-CSF can inhibit tumor growth. However, administration of exogenous recombinant GM-CSF appears insufficient to mediate clinically meaningful improvements in outcomes in most instances. Consequently, there has been significant interest in novel treatment approaches incorporating GM-CSF.

Among the most extensively evaluated agents is the attenuated, oncolytic, herpes simplex virus, type 1 (HSV-1)-encoding human GM-CSF talimogene laherparepvec (T-VEC; formerly Oncovex^{GM-CSF}) [85-88]. The vector was generated from the HSV JS1 strain and was attenuated by functional deletion of the *ICP34.5* and *ICP47* viral genes, which renders the virus nonpathogenic in normal eukaryotic cells, promotes selective replication in tumor cells, and enhances immunogenicity [26]. T-VEC has also been engineered to encode human GM-CSF, which further enhances the antitumor immune response. T-VEC is proposed to have a dual mechanism of action resulting in local tumor destruction by

Table 4 Cancer vaccines testing the adjuvant effect of GM-CSF administered locally at the site of vaccination

Citation	Design (Enrollment)	Ag (Route)	GM-CSF form (Route)	Coadmin-istration?	Study design	Effect of GM-CSF	Summary effect of GM-CSF
Scheibenbogen et al. [75]	Sequential cohorts (n = 43)	Tyrosinase peptides (ID/SC)	Protein (ID/SC) 75 µg/d x 4 d/vaccine	Yes	Sequential: 1. Peptides alone 2. Peptides + GM-CSF 3. Peptides + KLH 4. Peptides + GM-CSF + KLH	Minimal adjuvant effect Sequential trial cohorts	Minimal adjuvant effect
Slingluff et al. [81]	Randomized (n = 121)	Melanoma peptides (ID/SC)	Protein 110 µg (ID/SC)	Yes	Randomized: 1. Peptides + IFA 2. Peptides + IFA + GM-CSF	Negative on CD4 and CD8 T cells; too few events to differences in survival between groups	Diminished, compared with IFA
Faries et al. [71]	Randomized (n = 97)	Whole melanoma cell vaccine (ID)	Protein 200 µg/m ² /d x 5 days (ID)	Yes	Randomized: 1. Whole cell vaccine + BCG + GM-CSF 2. Whole cell vaccine + BCG	Better Ab, worse DTH; more Eos, Dec monocytes; more deaths	Diminished compared with BCG
Kirkwood et al. [78]	2 x 2 (n = 120)	MART-1, gp100, and tyrosinase peptides (SC)	250 µg/d x 14 out of 28 days	Yes	2 x 2: Arm A: Peptide Vaccine Alone Arm B: GM-CSF (250 µg/d x 14 out of 28 d) + vaccine Arm C: IFN-α + vaccine Arm D: GM-CSF + IFN-α + vaccine	No effect across treatment arms on best overall response	Minimal adjuvant effect

ID = intradermal; SC = subcutaneous; IFA = incomplete Freund's adjuvant; BCG = Bacille Calmette-Guerin.

introduction of an oncolytic virus into tumor cells and induction of a systemic antitumor immune response.

In a phase 1 study, patients with subcutaneous or cutaneous metastases from breast, gastrointestinal, head and neck, or melanoma malignancies were treated with T-VEC. Clinically stable disease was noted in 3/26 patients administered intralesional T-VEC, with no patients having CR or PR during the study period [86]. However, follow-up biopsies in 14/19 patients showed necrosis, infiltration of T cells, and the presence of replicating virus [86]. Notably, there was evidence of expression of GM-CSF in injected lesions. In a subsequent phase 2 study among 50 patients with unresectable stage III/IV melanoma administered intralesional T-VEC, the overall response rate was 26% (8 CR, 5 PR) [88]. Two additional patients were rendered disease-free by surgical resection following treatment. One-year survival was 58% for all patients and 40% among patients with stage IVM1c disease. Immunologic analysis of a subset of patients enrolled in the phase 2 study confirmed the presence of both local and distant antitumor immune responses following T-VEC administration. Biopsies performed on 11 patients after their sixth intratumoral injection were compared to nonstudy patients with metastatic melanoma [87]. In general, postvaccination tumors demonstrated extensive lymphocyte infiltration. Evaluation of both injected and noninjected lesions in T-VEC-treated patients revealed a significant increase in MART-1-specific T-cell response in both tumor and distant disease sites compared with unvaccinated controls. Regulatory CD4⁺FoxP3⁺ and suppressor CD8⁺FoxP3⁺ T cells and myeloid-derived suppressor cells were evaluated in injected tumor biopsies and found to be decreased when compared to unvaccinated control patients. When primary injected lesions were compared to distant noninjected lesions, the same general phenotypic pattern of effector T-cell and Treg infiltrates was seen; a greater number of CD4⁺FoxP3⁺ and CD8⁺FoxP3⁺ cells were present at the nontarget site than at the target sites. A randomized, phase 3 study, OPTiM, comparing intralesional T-VEC to subcutaneous GM-CSF demonstrated an improvement in durable response rate (16% vs 2%, respectively) and a trend toward improved OS at interim analysis (HR, 0.79 [95% CI, 0.61–1.02]) favoring the T-VEC arm [89]. The most frequently occurring adverse events were fatigue, chills, and pyrexia [89].

Systemic combination immunotherapy

GM-CSF has also been combined with ipilimumab, an anti-CTLA-4 monoclonal antibody. In a multicenter, phase 2 study, patients with metastatic melanoma were randomized to treatment with ipilimumab plus GM-CSF (250 µg/d subcutaneously for 14 days in 21-day cycles) or ipilimumab alone [90]. Patients receiving combination

therapy experienced a significant improvement in 1-year OS. Although there were no significant differences in overall toxicity between the treatment arms, patients receiving combination therapy had a lower rate of serious adverse events.

Several other approaches utilizing GM-CSF are being developed. These include an oncolytic vaccinia virus encoding GM-CSF [91], autologous dendritic cells and allogeneic whole tumor cells encoding GM-CSF [92–94], adjuvant GM-CSF following vaccination with peptide or RNA encoding melanoma peptides [95,96] and autologous dendritic cell or whole tumor cell vaccines [97], and GM-CSF DNA vaccines [98].

Conclusions

GM-CSF has been studied extensively in murine models and in human clinical trials, alone and as adjuvant therapy for melanoma. There is evidence from numerous studies that GM-CSF can induce antitumor immunity when administered by a variety of different routes. Although there was initial enthusiasm for recombinant GM-CSF based on uncontrolled clinical trials in stage III/IV melanoma, therapeutic benefit has not been confirmed in larger, prospective, randomized trials. The adjuvant or combination use of GM-CSF has been more promising although results have been inconsistent and may depend on the potency of the immunotherapy regimen, GM-CSF dose, route and schedule of administration, and stage of disease. There is emerging evidence that GM-CSF may be a regulatory cytokine with the ability to promote both effector and regulatory/suppressor T cell populations. Thus, strategies that block suppressor T cell and myeloid-derived suppressor cell elements may enhance the antitumor activity of GM-CSF. GM-CSF has been particularly effective in studies of oncolytic HSV therapy of melanoma and in combination with ipilimumab. Further research into the basic biology of GM-CSF on effector and suppressor immune cells and expanded clinical studies of combination treatments will help define the full therapeutic potential of GM-CSF in treatment of melanoma.

Abbreviations

CTLA-4: Cytotoxic T-lymphocyte antigen 4; CR: Complete response; DC: Dendritic cells; DFS: Disease-free survival; GM-CSF: Granulocyte-macrophage colony-stimulating factor; HR: Hazard ratio; HSV: Herpes simplex viruses; IFA: Incomplete Freund's adjuvant; KLH: Keyhole limpet hemocyanin; MDSC: Myeloid-derived suppressor cells; OS: Overall survival; PD-1: Programmed death 1; PR: Partial response; T-VEC: Talimogene laherparepvec.

Competing interests

HLK serves as a paid consultant and advisory board member for Amgen Inc. CLS is a scientific advisory board member for Immatics Biotechnologies and Polynoma LLC and a funded investigator for GlaxoSmithKline in the field of cancer vaccines; all funds from these activities are paid to the University of Virginia, not to Dr. Slingluff personally. Dr. Slingluff is an inventor on several patents for peptides used in cancer vaccines; the patents are held by the

University of Virginia Licensing and Ventures Group. CER and TH have no conflicts to declare.

Authors' contributions

All authors participated in critical analysis of the literature and drafting of the manuscript. All authors read and approved the final manuscript.

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