Review

For reprint orders, please contact: reprints@futuremedicine.com

Melanoma Management

An update on GM-CSF and its potential role in melanoma management

Robert O Dillman*[1,2]

[1]Chief Medical Officer, AIVITA Biomedical, Inc. Irvine, CA 92612, USA

[2]Clinical Professor Medicine, University of California Irvine, Irvine, CA 92697, USA

*Author for correspondence: bob@aivitabiomedical.com

Practice points

- The regulatory approval for GM-CSF is to stimulate recovery of granulocytes and monocytes in neutropenic patients who have received chemotherapy, especially in the setting of hematopoietic stem cell transplantation.
- Randomized trials failed to confirm significant anticancer activity for monotherapy GM-CSF as an immunotherapy in the treatment of surgically resected melanoma, nor in patients with regional and distant soft-tissue melanoma.
- Intratumor injections of GM-CSF monotherapy have been used for many years, but no large trials of that approach have been reported.
- There is a good rationale for including GM-CSF as an adjuvant in vaccines, but systemic administration of GM-CSF does not augment the effects of antimelanoma peptide or allogeneic tumor cell vaccines. However, local GM-CSF appears to provide immune enhancing effects and survival benefit when it is admixed with DC loaded *ex vivo* with autologous tumor antigens, or when cytolytic virus that secretes GM-CSF is injected locally into tumors.
- Talimogene laherparepvec, herpes simplex virus that secretes GM-CSF, is commercially available for the treatment of metastatic melanoma.

GM-CSF drives the differentiation of granulocytes and monocyte/macrophages from hematopoietic stem cell progenitors. It is required for differentiating monocytes into dendritic cells (DC). Although approved for recovery of granulocytes/monocytes in patients receiving chemotherapy, G-CSF is preferred. Enthusiasm for GM-CSF monotherapy as a melanoma treatment was dampened by two large randomized trials. Although GM-CSF has been injected into tumors for many years, the efficacy of this has not been tested. There is a strong rationale for GM-CSF as a vaccine adjuvant, but it appears of benefit only for strategies that directly involve DCs, such as intratumor talimogene laherparepvec and vaccines in which DCs are loaded with antigen *ex vivo* and injected admixed with GM-CSF.

First draft submitted: 25 May 2020; Accepted for publication: 13 July 2020; Published online: 29 July 2020

Keywords: dendritic cells • GM-CSF • immunotherapy • intratumor injections • melanoma • vaccines

Despite recent therapeutic advances that include monoclonal antibodies that inhibit immune checkpoints, and oral-targeted therapies that interfere with the proliferative effects that result from *B-RAF* mutations, metastatic melanoma remains a highly lethal disease. Furthermore, these therapies are associated with significant toxicities that have limited their widespread adoption and use. For this reason, there is a continuing interest in additional agents, especially immunotherapies, that may contribute to the management of this disease. GM-CSF has been considered a promising immunotherapy for the treatment of melanoma for more than 30 years. This review focuses on its historical development and clinical applications as a hematopoietic growth factor, on its investigational uses as an anti-melanoma monotherapy and as an adjuvant for anti-melanoma vaccines. The review does not cover combinations of GM-CSF with chemotherapy or with immunotherapies other than vaccines.

Historical development of GM-CSF

Identification & biological function

GM-CSF is a 127-amino-acid glycoprotein originally isolated from medium conditioned with factors secreted by pneumocytes from lipopolysaccharide-injected mice [1]. The GM-CSF designation derived from its ability to stimulate mouse bone marrow cell proliferation *in vitro*, and to generate colonies of granulocytes and macrophages

Future Medicine

Table 1. GM-CSF entities.

Generic name	Sargramostim	Molgramostim	Regramostim
Original developer	Immunex (WA., USA)	Genetics Institute (MA., USA)	Sandoz (Switzerland)
Cells for recombinant DNA manufacturing	Yeast	Bacteria	Mammalian
Species	*Saccharomyces cerevisiae*	*Escherichia coli*	Chinese hamster ovary
Glycosylation	Somewhat	None	Moderate
Commercial approval	USA	None	None
Commercial name	Leukine®	Malgradex®	None
Listings on clinicaltrials.gov	1396	30	0
Current manufacturer	Partner Therapeutics MA, USA	Savara TX, USA	Unknown
Manufacturing location	WA, USA	TX, USA	Unknown

ex vivo. GM-CSF can be produced by macrophages, fibroblasts, activated T-lymphocytes, natural killer (NK), mast, endothelial cells and some malignant cells [2]. It is a powerful hematopoietic stimulating factor for the expansion and maturation of monocyte-macrophages, dendritic cells (DC) and granulocytes from hematopoietic progenitor cells [3,4], and promotes proliferation of erythroid and megakaryocytic progenitors when combined with other hematopoietic factors [5].

The genes for GM-CSF are located on chromosome 5q31 in a cluster encoding for cytokines associated with T-helper type 2 immune responses, including IL-3, IL-4, IL-5 and IL-13 [6]. Potent inducers of GM-CSF include bacterial endotoxins and inflammatory cytokines such as TNF-α, IL-1, and IL-6. [7]. Its expression and secretion are inhibited by IFN-γ, IL-4 and IL-10 [8,9], and immunosuppressive agents such as corticosteroids [10] and cyclosporine A [11]. GM-CSF blood levels are typically very low (less than 1.0 pg/ml) or undetectable, except in patients with certain hematopoietic disorders, but can reach very high levels at local inflammatory sites [12].

Mice with homozygous deletion of the *GM-CSF* gene develop normally with normal hematopoiesis, healthy appearance, immune competency and good fertility [13,14]. However, GM-CSF appears to be essential for normal pulmonary function and resistance to infection. GM-CSF-deficient mice develop lung abnormalities including peribronchovascular infiltration by antibody-producing B cells [13]. Neutralizing anti-GM-CSF autoantibodies are associated with pulmonary alveolar proteinosis [15], a rare lung disease characterized by macrophages that are incapable of clearing surfactant from alveolar lung spaces [16]. There is interest in using aerosolized GM-CSF as a treatment for pulmonary alveolar proteinosis [17].

Manufacturing

By 1985, GM-CSF had been cloned [18] with its DNA expressed in bacteria, yeast and mammalian cells, thus enabling mass production of recombinant GM-CSF [19]. These different GM-CSF entities are summarized in Table 1. The amino acid sequence of sargramostim differs from natural human GM-CSF only by substitution of leucine at position 23. Molgramostim had six fewer amino acids and substitution of methionine at position 1. In 1987, Immunex, Inc. (WA, USA) initiated clinical trials with sargramostim, the recombinant GM-CSF manufactured in yeast.

Routes of administration, safety & toxicity

As summarized in Table 2, the pharmacokinetics, safety and dosing of GM-CSF in humans was established in a series of trials conducted during 1987–1992 [20–26]. Intravenous (IV) bolus injections were associated with peak levels and systemic release of toxic cytokines that limited dosing. Bolus injections were less effective in inducing leukocytosis, probably because of the lack of continuously detectable GM-CSF blood levels. Lower-dose continuous IV infusions and daily subcutaneous (SC) injections were well-tolerated over 7–14 days; single daily SC injections were preferred because of convenience. Single SC injections were associated with gradually increasing blood levels that peaked at about 12 h and became undetectable by 24 h. Daily administration beyond 14 days was associated with increasing bone discomfort, leukocytosis and flu-like symptoms. A single SC dose was associated with minimal toxicity other than local injection site reactions. Adverse events common to both IV and SC administration included bone discomfort from intramedullary expansion of hematpoietic cells, and flu-like symptoms because of the secondary immune inflammatory effects. These were easily managed with antihistamines and nonsteroidal anti-inflammatory

Table 2. Phase I trials of GM-CSF in cancer patients.

Author (year)	Source	Patients, n	Dose range	Route	Beneficial effects on leukocytes	Common adverse events	Comments	Ref.
Thompson (1989)	Yeast	16 MDS	0.3, 1.0, 3.0, 10 mcg/kg	SC daily x10 days	Yes, at all doses, dose-dependent	Dose dependent Fever and flu-like syndrome	MTD of 10.0 mcg/kg	[20]
Hermann (1991)	Yeast	27 MDS	15–1000 mcg/m^2	SC or IV daily x14 days	Yes, at all doses	Well tolerated all doses	Increased WBC prefered 250 mcg/day SC x14 days	[23]
Edmonson (1992)	Yeast	57 Various cancers	3 doses and 1-day CTX, CBP	IV and SC three doses five schedules	Yes, at all doses	Fever, arthralgia, pulmonary serositis, skin rash	5 mcg/kg SC q12h x14 days 1 day postchemo considered best	[24]
Bukowski (1993)	Yeast	17 Lung cancer	60, 125, 250, 500 mcg/m^2	IV continuous infusion x14 days	Yes, at all doses	Well tolerated at lower doses. Fever, fatigue	250 mcg/m^2 MTD, pulmonary toxicity	[26]
Berthaud (1993)	*E. coli*	14 various solid cancers	250, 500, 750, 1000 mcg//m^2	SC daily for 10 days	Yes, at all doses	Fever, local irritation, lethargy, arthalgia	No MTD	[25]
Steward (1989)	*E. coli*	20 various solid tumors	0.3–60 mcg/kg	IV 30 min infusions x10 days	Yes at 10 mcg/kg or higher	Bone pain, fever and pruritus. DLT included pericarditis and capillary-leak syndrome.	Severe toxicity was noted in 80% of patients at the dose of 60 mcg/kg	[21]
Lieschke (1990)	*E. coli*	21 various malig-nancies	0.3–3 mcg/kg 0.3–20 mcg/kg	IV bolus x10 days IV 2-h infusion x10 days	Yes	Bone pain, fever, rash, lethargy, weight gain. Hypoxia and hypotension frequent during first infusion of doses ≥1 mcg/kg	MTD 15 mcg/kg 2-h infusion	[22]

CBP: Carboplatin; CTX: Cyclophosphamide; DLT: Dose-limiting toxicity; MDS: Myelodysplastic syndrome; MTD: Maximum tolerated dose.

Table 3. FDA-approved marketing indications for sargramostim GM-CSF†.

	Indication	Dosing
1	To shorten time to neutrophil recovery and to reduce the incidence of severe and life-threatening infections and infections resulting in death following induction chemotherapy in adult patients 55 years and older with acute myeloid leukemia (AML) (1991)	250 mcg/m^2/day infused IV over 4 h until recovery
2	For mobilization of hematopoietic progenitor cells into peripheral blood for collection by leukapheresis and autologous transplantation in adult patients (1991)	Single 250 mcg/m^2 injected SC or 250 mcg/m^2 infused IV over 24 h
3	To accelerate myeloid reconstitution following autologous bone marrow or peripheral blood progenitor cell transplantation in adult and pediatric patients ≥2 years of age (1991)	Single 250 mcg/m^2 injected SC or 250 mcg/m^2 infused IV over 24 h
4	To accelerate myeloid reconstitution following allogeneic bone marrow transplantation in adult and pediatric patients ≥2 years of age (1991)	250 mcg/m^2 infused IV over 2 h
5	For treatment of delayed neutrophil recovery or graft failure after autologous or allogeneic bone marrow transplantation in adult and pediatric patients ≥2 years of age (1991)	250 mcg/m^2 infused IV over 2 h, daily for 14 days
6	To accelerate myeloid reconstitution and function in adults and children acutely exposed to myelosuppressive doses of radiation (2018)	Once daily SC injection: • 7 mcg/kg for adults and pediatric patients weighing >40 kg • 10 mcg/kg for pediatric patients 15–40 kg • 12 mcg/kg for pediatric patients <15 kg

†summarized from Leukine® package insert.

agents, but oral glucocorticoids were required in occasional patients who experienced serositis, lung infiltrates or persistent dermatologic toxicity. The frequency of adverse events was somewhat higher for GM-CSF manufactured in *E. coli* compared with GM-CSF manufactured in yeast.

Regulatory approval, marketing indications & formulation

In addition to stimulating proliferation and differentiation of myelomonocytic cells, GM-CSF also enhances their production of cytokines and ability to perform phagocytosis. This reduces the duration of febrile neutropenia and the risk of infection in neutropenic patients. GM-CSF accelerates the recovery of granulocytes and monocytes in patients whose bone marrow production has been suppressed by chemotherapy, and this was the basis for its approval by the US FDA [27]. In February 1991, the yeast-derived recombinant GM-CSF sargramostim (Leukine®) gained FDA approval as a leukocyte growth factor for SC or IV administration. The current marketing indications are summarized in Table 3. In 2018, the label was expanded to include adults and children acutely exposed to myelosuppressive doses of radiation. Sargramostim is provided as a 250 mcg white, preservative-free, lyophilized powder in single dose vials for reconstitution with 1 ml sterile water or sterile saline for injection. Reconstituted liquid sargramostim is a clear, colorless liquid with pH range of 6.7–7.7.

In February 1991, another hematopoietic growth factor, granulocyte colony stimulating factor (G-CSF) (filgastrim, Neupogen®) was also approved to enhance leukocyte recovery after chemotherapy. In 2002, a long-acting G-CSF (peg-filgastrim, Neulasta®) also acquired regulatory approval. These two G-CSF formulations, manufactured by Amgen (CA, USA) have dominated the neutropenia clinical marketplace, so much so that sargramostim was never granted approval internationally for this purpose. In 2002, when Amgen acquired Immunex, the manufacturers of sargramostim, they had to divest themselves of the product which was sold to Berlex. In 2007, Berlex became part of Bayer HealthCare; in 2009 Genzyme (MA, USA) acquired sargramostim; in 2011 Genzyme was acquired by Sanofi (Paris, France); February 2018 the global rights for sargramostim were transferred to Partner Therapeutics (MA, USA) with a manufacturing facility in WA, USA. Sargramostim is the only GM-CSF approved for human use in the US, and has been used primarily as an alternative to G-CSF to prevent neutropenia in oncology patients treated with myelosuppressive chemotherapy. Sargramostim has not been approved for clinical use in Australia, Canada, Europe or Japan.

GM-CSF as anticancer monotherapy

Over the years, there has been interest in GM-CSF as an anticancer immunotherapy because of its protean effects on the immune system [28–31]. For instance, in addition to its proliferative and differentiation effects on cells of myelomonocytic lineage, GM-CSF increases NK activity, increases expansion of DC populations, increases IL-12 production by DC, attracts DC to vaccination injection sites, increases DC-mediated cellular responses to

tumor cells, activates and enhances monocyte cytotoxicity and secretion of IFN-γ and TNF-α in cancer patients, increases defense against infection by various pathogens, promotes sustained increase in CCL17/TARC (thymus and activation regulated chemokine), and exacerbates autoimmune diseases such as rheumatoid arthritis, autoimmune encephalitis and myocarditis, but appears to be beneficial in myasthenia gravis, thyroiditis, colitis and Type 1 diabetes mellitus. GM-CF had no proliferative or antitumor effects *in vitro* on cancer cells from solid tumors [32], but injections of GM-CSF inhibited tumor growth in some murine cancer models [33,34], although in one study systemic GM-CSF monotherapy facilitated growth of B16 melanoma and S180 sarcoma cancers in mice [35]. As summarized in Table 4, in humans with metastatic melanoma, there have been four moderate-size single arm Phase II trials [28,36–38], one small randomized Phase II trial [39] and two large Phase III randomized trials [40,41] that each had a study arm testing GM-CSF as an anticancer immunotherapy. The rationale for GM-CSF monotherapy was the presumption of a suppressed ongoing antitumor response that might be overcome by nonspecific immune stimulation by GM-CSF. In all but one of these trials GM-CSF was injected SC at a dose of 125 mcg/m2/dose or 250 mcg/dose, daily for 14 consecutive days of each 28-day treatment cycle. GM-CSF was well-tolerated in all four trials that used the 28-day cycle, but more than 33% of patients experienced grade 3 or 4 pyrexia with the 21-day cycle. The frequency of myalgias and arthralgia was much higher when GM-CSF was administered for several months. Based on the two randomized trials, GM-CSF is not considered effective as a monotherapy for treating melanoma. Most likely the general nonspecific immune stimulating effects induced by systemic administration of GM-CSF are not sufficient to enhance existing local immune responses. When GM-CSF is administered systemically, it is likely that insufficient drug reaches tumor sites or dermal sites of vaccination for a beneficial effect, but sufficient levels may be achieved in association with intratumor injections of GM-CSF (or intratumor secretion of GM-CSF), or local injection of antigen-loaded DCs admixed in GM-CSF.

GM-CSF as an adjuvant for vaccines

Preclinical studies

The most important aspect of vaccines is their antigens. An adjuvant is a substance that facilitates induction and/or enhancement of the immune response to the antigen. Vaccines are often administered with an adjuvant, or formulated with an adjuvant, in an effort to obtain stronger humoral and cellular immune responses. Most adjuvants are antigenic in their own right and/or induce inflammation. Because of its pro-inflammatory cytokine effects, it was proposed that GM-CSF might be a potent adjuvant for anticancer vaccines [42,43]. Preclinical studies showed that addition of GM-CSF enhanced both humoral and cellular immune responses by inducing proliferation, differentiation and activation of macrophages, antigen-presenting cells (APC) including DC, neutrophils, and indirectly, T cells [44,45]. Thus, GM-CSF provides a link between innate and adaptive immunity, especially interactions between T lymphocytes and APC that are critical for antitumor immunity. The effects of GM-CSF on T cells appear to be induced indirectly via APCs, especially DC. GM-CSF is essential for DC development and maturation [46]. GM-CSF not only has the capacity to increase antigen-induced immune responses, but it also can alter the Th1/Th2 cytokine balance. It appears that GM-CSF can stimulate both Th1 and Th2 type responses depending on immune cells and cytokines in the immediate local environment.

Comparative studies in animal models using tumor cells transfected with genes for various cytokines suggested that GM-CSF was the most effective adjuvant for vaccine immunotherapy [47]. Tumor models included B16-F1O melanoma, CT-26 colon carcinoma, Lewis lung carcinoma, RENCA renal cell carcinoma and CMS-5 fibrosarcoma. Proliferating tumor cells were transfected with retroviruses expressing murine GM-CSF, IFN-γ, IL-2, IL-4, IL-5, IL-6, ICAM-1, CD2, IL-1 receptor antagonist and human TNF-α. GM-CSF was the most powerful immunostimulant of the ten molecules tested. This was considered especially noteworthy given the emerging data showing that GM-CSF played an important role in the maturation and/or function of DCs [46,48]. GM-CSF-secreting tumor cells mobilize and activate DCs and NK-T cells and increase production of cytokines such as IL-12 that are required for the activation of $CD4^+$ T lymphocytes [49] GM-CSF may induce a subset of DCs that are rich in costimulatory molecules and more efficient in the phagocytosis of dead and dying cancer cells. In a B16F10 melanoma comparative mouse study, DCs loaded with MAGE-1 antigen and modified to secrete GM-CSF were superior to tumor cells modified to secrete GM-CSF for preventing lung metastases and were associated with better survival [50].

Clinical trials of GM-CSF as a vaccine adjuvant

Clinical trials employing GM-CSF as an adjuvant have been conducted for more than 20 years with inconsistent results [30,51]. Adjuvant GM-CSF has been administered in various ways including coadministration (admixing)

Table 4. Trials of GM-CSF monotherapy as an immunotherapy to treat melanoma.

Author (year)	GM-CSF dosing	Clinical setting	Trial design	Patients, n	Toxicity	Efficacy	Ref.
Spitler (2000)	125 mcg/m^2 SC daily x14 of each 28-day cycle for up to 1 year	Resected stage IIIB, IIIC and IV	Phase II single arm	48	92% at least one mild AE. 58% injection site erythema; 56% transient myalgias and mild fatigue 10% skin rash	38 mos median survival vs 12 mos historical control	[28]
Daud (2008)	125 mcg/m^2 SC daily x14 of each 28-day cycle	Resected stage IIIB, IIIC, and IV	Phase II single arm	39	88% injection site reaction, 61% fatigue, 61% athralgia/myalgia, 39% flu-like symptoms	>65 mos median survival, transient increased DC but not MDSC	[36]
Spitler (2009)	125 mcg/m^2 SC daily x14 of each 28-day cycle for up to 3 years	Resected stage II, III and IV	Phase II single arm	98	Grade 1 or 2 in 82%; mostly injection site, no grade 3 or 4 AE	5-year survival 60%	[37]
Grotz (2014)	250 mcg SC daily x14 of each 28-day cycle for up to 3 years	Resected stage III	Phase II single arm	152	Toxicity not reported	Trend toward better survival compared with contemporary cohort of 165 untreated patients	[38]
Ravaud (2001)	5 mcg/kg bid x14 days of each 21-day cycle	Metastatic melanoma	Phase II random GM-CSF ± DTIC	32	Grade 3 or 4 fever in 12/32 (6 each arm)	No objective responses in either arm	[39]
Lawson (2015)	250 mcg SC daily x14 of each 28-day cycle for up to 1 year	HLA-2 negative, resected stage 3 or 4	Phase III random 1:1 GM-CSF vs placebo	378	More AEs if GM-CSF, 27 vs 17% (p = 0.022), grade 3–4 12 vs 7% (p = 0.149) Injection site reactions, headache	No difference overall or relapse free survival	[40]
Andtbacka (2015, 2019)	125 mcg/m^2 SC daily x14 of each 28-day cycle	Unresected stage 3 or 4 with one or more potentially injectable lesions	Phase III random 2:1 intratumor herpes simplex-secreting GM-CSF vs SC GM-CSF	436	AEs more frequent with intratumor injections: fatigue (50 vs 36%); chills (49 vs 9%), fever (43 vs 9%), nausea (36 vs 20%), syndrome (30 vs 15%), injection-site pain (28 vs 6%), influenza-like	SC GM-CSF inferior response rate (6 vs 26% p < 0.001) and overall survival (19 vs 23 mos) p = 0.051	[41,78]

AE: Adverse event; DTIC: Dacarbazine; MDSC: Myeloid-derived suppressor cell; Random: Randomized; SC: Subcutaneous.

with vaccine [52–57], secretion by transfected autologous or allogeneic cells injected SC and/or intradermal (ID) [58–61]; injection at or near the vaccination site [57,58,62–64], and repeated injections at distant SC sites [40,65–69]. As shown in Table 5, most of the cancer clinical trials utilizing adjuvant GM-CSF have been conducted in patients with regional or distant metastatic melanoma. These trials have not consistently shown a benefit for adjuvant GM-CSF in terms of specific immune responses or clinical outcome. The largest randomized trial testing GM-CSF as an adjuvant was E9647, which enrolled stage 3 and 4 melanoma patients who had been rendered clinically free of cancer by surgery [40]. In this trial, 435 HLA-A2-positive patients were randomized to one of four different treatment arms: GM-CSF + peptide vaccine, placebo + peptide vaccine, GM-CSF + placebo vaccine or double placebo. The well-characterized melanoma lineage peptide antigens included in the vaccine were gp100, tyrosinase and MART-1, which were emulsified in Montanide ISA-51, a blend of mannide monooleate surfactant and mineral oil. GM-CSF or placebo were injected SC daily for 2 weeks of each 4-week period; therefore this trial could be considered to be comparing combination immunotherapy (GM-CSF plus peptide vaccine vs peptide alone and vs GM-CSF alone), or to be comparing vaccine with or without GM-CSF as an adjuvant. There was no difference in survival between any two of the arms. Samples of peripheral blood were assayed for immune effects and prognostic biomarkers [69]. Most patients treated with GM-CSF-developed neutralizing antibodies, which were associated with improved survival, perhaps because those patients were more immune competent. GM-CSF did not increase the rate of antigen-specific responses. Of note, a $CD8^+$ response was associated with worse rather than a better outcome, raising the possibility that immunization to these specific antigens, which probably were irrelevant antigens for many of the patients, may have distracted or diverted the immune system from ongoing immune responses to patient-specific neoantigens.

It may be significant that GM-CSF effects appeared more favorable in the trials in which peptide antigens were admixed with GM-CSF [52–54], or antigen-loaded DC were admixed with GM-CSF [55–57], or GM-CSF was secreted by autologous or allogeneic melanoma cells [58–61]. This suggests that local effects of GM-CSF, even if relatively brief, are more important than systemic effects in terms of vaccine objectives. In a randomized trial in patients with metastatic melanoma, DC loaded with autologous tumor antigens from irradiated self-renewing cancer cells were associated with a 50% 5-year survival rate in a single arm-trial trial [61], and a more than doubling of median survival and rate of 3-year survival, and a 70% reduction in the risk of death in a randomized trial [57]. Admixing GM-CSF with DCs that are preloaded with tumor antigens may increase the immune enhancing effects by direct effects on the DC and indirect effects from the local inflammatory response induced by GM-CSF. The allogeneic cell line component of Melanoma GVAX expresses the common melanoma antigens tyrosinase, gp100 and MART-1/Melan-A, and MAGE-A3 [70]. This approach was discontinued in melanoma after a single-arm trial (49), and a randomized clinical trial in pancreatic cancer yielded disappointing results [71] The disappointing clinical activity observed with peptide vaccines may be due to limitations in approaches that do not include patient-specific neoantigens [72].

Intratumor GM-CSF as a vaccine adjuvant

Intra-tumor injection of GM-CSF could be beneficial because the tumor is a reservoir of antigens and local DC; therefore, stimulation by GM-CSF in the presence of increased tumor antigen release could induce a favorable immune response. Local injection of GM-CSF is associated with a local increase in the number of DCs whether the injection is into the skin, or directly into tumor [73]. However, SC injections of GM-CSF do not reliably induce an increase in DCs in tumor lesions. Animal studies showed that addition of intratumor injection of GM-CSF resulted in more *in situ* DCs and tumor infiltrating lymphocytes compared with combination immunotherapy alone [74]. There are two approaches for utilizing intratumor GM-CSF: injection of GM-CSF into the tumor, or injection of a virus that encodes the *GM-CSF* gene for secreting GM-CSF. If the virus is cytolytic, then there are also direct antitumor effects that increase the release of tumor-associated antigens in the tumor microenvironment. Table 6 summarizes data from selected studies involving direct injection of GM-CSF into melanoma metastases [75,76], or secretion of GM-CSF by cytolytic virus directly injected into melanoma metastases [40,77,78]. In addition, complete regressions of in-transit and satellite melanoma lesions have been reported following intratumor injection of GM-CSF [79]. Talimogene laherepvec, which consists of a cytolytic Herpes simplex virus transfected with the *GM-CSF* gene, was granted regulatory approval for the treatment of metastatic melanoma based on a randomized trial in patients with at least one injectable metastatic lesion [41]. The design of this trial has been criticized for not using intralesional GM-CSF as the control arm in a double-blinded manner rather comparing with the same schedule of daily SC GM-CSF that yielded disappointing results in patients with surgically resected stage 3 or 4

Table 5. GM-CSF as an adjuvant in antimelanoma vaccine trials.

Author (year)	GM-CSF Route	GM-CSF Per Dose	Vaccine	Stage	Trial design	Patients, n	Results	Ref.
Slingluff (2003)	Admixed SC and ID	225 mcg SC	Four peptides weekly x6	III, IV	Phase IB–II random in GM-CSF or on DC	26	Minimal toxicity. Immune response greater in GM-CSF than in DC-loaded ($p < .02$)	[52]
Chianese-Bullock (2005)	Admixed SC and ID	110 mcg SC	12 peptides weekly x6	IIB, III, IV	Phase II single arm	25	Immune responses after three weekly injections	[53]
Slingluff (2009)	Admixed SC and ID	110 mcg SC	12 peptides weekly x6 and months 3, 6, 9, 12	IIB, IIC, III, IV	Phase II random 1:1 ± GM-CSF	121	Minimal toxicity. $CD8^+$ response 34% w/ GM- CSF, 73% w/o GM-CSF, $p < 0.001$, no difference in survival	[54]
Dillman (2009)	Admixed SC	500 mcg SC	Auto DC-ITC weekly x3 + months 4 through 8	IV recurrent III	Phase II single arm	54	Minimal toxicity. 50% 5-year overall survival in stage IV and recurrent stage III	[55]
Dillman (2012, 2018)	Admixed SC	500 mcg SC	Auto DC-ITC weekly x3 + months 4 through 8	IV recurrent III	Phase II random 1:1 DC-ITC vs ITC	42	Minimal toxicity. DC-ITC >ITC survival and immune response	[56,57]
Soiffer (1998)	SC and ID	Encoded in ITC	Secreted	III, IV	Phase IB	21	Immune infliltration of tumors	[58]
Soiffer (2003)	SC and ID	Encoded in ITC	Secreted	III, IV	Phase II	34	1 CR, 1 PR Infiltration with leukocytes including DC	[59]
Luiten (2005)	SC and ID	Secreted by tumor cells	Autologous tumor cells	IV	Phase I/II single arm	38	Minimal toxicity for three tri-weekly injections; increase in T cells to tumor peptide antigens; rapid progression in 10	[60]
Lipson (2015)	ID	Encoded in ITC	Secreted	Resected IIB–IV	Phase IB	20	Safe, increased PBM. No increase in antigen-specific T cells	[61]
Scheiben-bogen (2000)	Nearby SC	75 or 150 mcg SC x 4 days	1 peptide biweekly x4, bimonthly x2	IV	Phase II	16	limited clinical and immune response to HLA class 1 peptide	[62]
Scheiben-bogen (2003)	Nearby SC	75 mcg SC x 4 days	Peptides biweekly x4, bimonthly x2	III, IV	Phase II random IB 1:1:1	43	Antigen-specific T cells in 3/9 no adjuvant, 4/9 GM-CSF, 0/10 KLH	[63]
Dillman (2007)	Nearby SC	500 mcg SC	Auto ITC weekly x3 + months 4 through 8	IV recurrent III	Phase II	74	Minimal toxicity. Median survival 20.5 months, 5-year 28%	[64]
Dillman (2012, 2018)	Nearby SC	500 mcg SC	Auto ITC weekly x3 + months 4 through 8	IV, recurrent III	Phase II random 1:1 DC-ITC vs ITC	42	Minimal toxicity. ITC <DC-ITC survival and immune response	[56,57]
Weber (2003)	Distant SC	250 mcg SC days 1–5	Peptides in IFA biweekly x4, q 4 weeks x3	Resected II	Phase II random 1:2 ± GM-CSF	48	Mild toxicity, local pain, fever fatigue, one severe neutropenia. More immune response with GM-CSF	[65]
Atzpodien (2007)	Distant SC	62.5 mcg SC x 4 days q 4 weeks	4 peptides and KLH ID and SC q 4 weeks x6 months	Resected recurrent III and 4	Phase II	24	Well-tolerated, 92% 2-year OS	[66]
Faries (2009)	Distant SC	200 mcg/m^2 SC days 1–5	Allo TC + BCG every 2 weeks x5, then monthly x4	Resected II–IV	Phase II random 1:1 ± GM-CSF	97	Grade 1 or 2 fatigue and injection site reactions more common in GM-CSF arm. No immune response advantage for addition of GM-CSF.	[67]
Kirkwood (2009)	Distant SC	250 mcg/m^2 SC daily x 14 of 28 days	Peptides SC biweekly for 1 year	Resected III or IV	Phase II random 1:1:1 IFN-α or GM-CSF or both	120	No difference GM-CSF, IFN-α or both (stage IV)	[68]
Lawson (2015) Butterfield (2017)	Distant SC	125 mcg/m^2 daily for 14 of 28 days	Peptides SC biweekly for 1 year	Resected III or IV	Phase III random 1:1:1:1 four vaccine arms	435	No difference GM-CSF vs placebo Counterintuitive immune changes with GM-CSF	[40] [69]

Auto: Autologous; allo: Allogeneic; BCG: Bacille Calmette-Guerin; DC: Dendritic cell; ID: Intradermal; IFA: Incomplete Freund's adjuvant; IFN-α: Interferon alpha; ITC: Irradiated tumor cell; KLH: Keyhole limpet hemocyanin; met: Metastasis; PBL: Peripheral blood lymphocyte; PBM: Peripheral blood monocyte; SC: Subcutaneous; rand: Randomized; TC: Tumor cell; w: With, w/o: Without.

Table 6. Intratumor GM-CSF in antimelanoma vaccine trials.

Author (year)	Vaccine	GM-CSF Dose	Stage	Trial design	# pts	Results	Ref.
Si (1996)	Injected	15–50 mg injected into two SCmets	III, IV	Phase IB	13	3 PR	[75]
Ridolfi (2002)	Injected	150 mcg per lesion followed by peritumor IL-2	III, IV	Phase IB	14	2 PR, 2 MR Including distant lesions	[76]
Senzer (2009)	Injected, encoded with Herpes virus	Secreted	IIIc & IV	Phase II	50	26% ORR, some responses in noninjected lesions	[77]
Andtbacka (2015, 2019)	Injected, encoded with Herpes virus	Secreted	III & IV	Phase III random 2:1 vs SC GM-CSF	436	DRR 19 vs 1% ORR 32 vs 6% OS 23 vs 19 mos	[41,78]

DRR: Durable response rate; ORR: Objective response rate; OS: Overall survival; PR: Partial response; MR: Minimal response.

melanoma [40]. Furthermore, most of the objective responses took place only in injected lesions, which could have resulted from the cytolytic effects of the virus, rather than in non-injected lesions that ideally would have benefited from the abscopal effect of systemic immunization resulting from tumor antigen loading of intratumor DC. In the final analysis, the virus-GM-CSF complex was associated with a higher durable response rate ($p < 0.001$, higher objective response rate ($p < 0.001$) and longer overall survival ($p = 0.049$) [78]. The viral construct produced complete responses in 17% and partial responses in 15% of the 295 patients in that treatment arm. Based on these results talimogene laherparpevec is being extensively studied in combination with other antimelanoma treatments as reviewed elsewhere [80]. It is of note that both SC daily GM-CSF and intratumor virus secreting GM-CSF improved response rates when combined with the cytotoxic T lymphocyte antigen-4 inhibitor ipilimumab compared with ipilimumab alone [81,82].

Summary of observations on GM-CSF as a vaccine adjuvant

As a vaccine adjuvant, the preponderance of evidence suggests that systemic GM-CSF does not enhance the immune response to allogeneic tumor cells or common HLA-restricted peptide antigens. However, there are at least two approaches in which increased local levels of GM-CSF in proximity to DC may facilitate induction of antitumor immune responses: admixing GM-CSF with antigen-loaded DC, and intratumor injection of a cytolytic virus that secretes GM-CSF.

Conclusion

- The current regulatory-approved indication for GM-CSF is to benefit neutropenic patients, but G-CSF products dominate this market;
- Randomized trials have shown that GM-CSF is not effective as an anticancer monotherapy for the treatment of metastatic melanoma, including patients whose cancers have been resected;
- Systemic administration of GM-CSF does not augment the effects of antimelanoma peptide or allogeneic tumor cell vaccines, but GM-CSF appears to provide immune enhancing effects when it is admixed with DC loaded *ex vivo* with autologous tumor antigens, or when cytolytic virus that secretes GM-CSF is injected indirectly into tumors.

Future perspective

During the next few years the results from multiple trials of intratumor talimogene laherparepvec will become available, especially in combination with other immunotherapies. There may be formal trials of intralesional GM-CSF injections, and other intratumor vectors designed to secrete GM-CSF likely will be tested as well. Because of the interest in *ex vivo* antigen-loaded DCs, and the apparent importance of GM-CSF with that approach, one can expect additional studies to better clarify the role of high local concentrations of GM-CSF in such approaches.

Financial & competing interests disclosure

The author is an employee of AIVITA Biomedical, Inc. and owns stock in that company. The author has no other relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript apart from those disclosed.

No writing assistance was utilized in the production of this manuscript.

References

Papers of special note have been highlighted as: • of interest; •• of considerable interest

1. Burgess AW, Camakaris J, Metcalf D. Purification and properties of colony-stimulating factor from mouse lung-conditioned medium. *J. Biol. Chem.* 252(6), 1998–2003 (1977).
2. Griffin JD, Cannistra SA, Sullivan R *et al.* The biology of GM-CSF: regulation of production and interaction with its receptor. *Int. J. Cell. Cloning* 8(Suppl.), 35–44; discussion 44–45 (1990).
3. Burgess AW, Metcalf D. The nature and action of granulocyte macrophage colony stimulating factors. *Blood* 56(6), 947–958 (1980).
4. Metcalf D. The granulocyte-macrophage colony-stimulating factors. *Science* 229(4708), 16–22 (1985).
5. Gasson JC. Molecular physiology of granulocyte-macrophage colony-stimulating factor. *Blood* 77(6), 1131–1145 (1991).
6. Van Leeuwen BH, Matinson ME, Webb GC, Young IG. Molecular organization of the cytokine gene cluster, involving the human IL-3, IL-4, IL-5, and GM-CSF genes, on human chromosome 5. *Blood* 73(5), 1142–1148 (1989).
7. Schwager I, Jungi TW. Effect of human recombinant cytokines on the induction of macrophage procoagulant activity. *Blood* 83(1), 152–160 (1994).
8. Jansen JH, Wientjens GJ, Fibbe WE *et al.* Inhibition of human macrophage colony formation by interleukin 4. *J. Exp. Med.* 170(2), 577–582 (1989).
9. Ozawa H, Aiba S, Nakagawa, Tagami H. Interferon-gamma and interleukin-10 inhibit antigen presentation by Langerhans cells for T helper type 1 cells by suppressing their CD80 (B7-1) expression. *Eur. J. Immunol.* 26(3), 648–652 (1996).
10. Adcock IM, Caramori G. Cross-talk between pro-inflammatory transcription factors and glucocorticoids. *Immunol. Cell Biol.* 79(4), 376–384 (2001).
11. Hatfield SM, Roehm NW. Cyclosporine and FK506 inhibition of murine mast cell cytokine production. *J. Pharmacol. Exp. Ther.* 260(2), 680–688 (1992).
12. Metcalf D, Nicola NA, Mifsud S, Di Rago L. Receptor clearance obscures the magnitude of granulocyte-macrophage colony stimulating factor responses in mice to endotoxin or local infections. *Blood* 93(5), 1579–1585 (1999).
13. Stanley E, Lieschke GJ, Grail D *et al.* Granulocyte/macrophage colony-stimulating factor-deficient mice show no major perturbation of hematopoiesis but develop a characteristic pulmonary pathology. *Proc. Natl Acad. Sci. USA* 91(12), 5592–5596 (1994).
14. Wada H, Noguchi Y, Marino MW *et al.* T cell functions in granulocyte macrophage colony-stimulating factor deficient mice. *Proc. Natl Acad. Sci. USA* 94(23), 12557–12561 (1997).
15. Trapnell BC, Whitsett JA, Nakata K. Pulmonary alveolar proteinosis. *N. Engl. J. Med.* 349(26), 2527–2539 (2003).
16. Kitamura T, Tanaka N, Watanabe J *et al.* Idiopathic pulmonary alveolar proteinosis as an autoimmune disease with neutralizing antibody against granulocyte/macrophage colony-stimulating factor. *J. Exp. Med.* 190(6), 875–880 (1999).
17. Tazawa R, Ueda T, Abe M *et al.* Inhaled GM-CSF for pulmonary alveolar proteinosis. *N. Engl. J. Med.* 381(10), 923–932 (2019).
18. Cantrell MA, Anderson D, Cerretti DP *et al.* Cloning, sequence, and expression of a human granulocyte/macrophage colony-stimulating factor. *Proc. Natl Acad. Sci. USA.* 82(18), 6250–6254 (1985).
19. Hussein AM, Ross M, Vredenburgh J *et al.* Effects of granulocyte-macrophage colony stimulating factor produced in Chinese hamster ovary cells (regramostim), *Escherichia coli* (molgramostim) and yeast (sargramostim) on priming peripheral blood progenitor cells for use with autologous bone marrow after high-dose chemotherapy. *Eur. J. Haematol.* 55(5), 348–356 (1995).
• **Comparison of the three different GM-CSFs that have been mass-produced using recombinant DNA technology.**
20. Thompson JA, Lee DJ, Kidd P *et al.* Subcutaneous granulocyte-macrophage colony-stimulating factor in patients with myelodysplastic syndrome: toxicity, pharmacokinetics, and hematological effects. *J. Clin. Oncol.* 7(5), 629–637 (1989).
21. Steward WP, Scarffe JH, Austin R *et al.* Recombinant human granulocyte macrophage colony stimulating factor (rhGM-CSF) given as daily short infusions–a phase I dose-toxicity study. *Br. J. Cancer* 59(1), 142–145 (1989).
22. Lieschke GJ, Maher D, O'Connor M *et al.* Phase I study of intravenously administered bacterially synthesized granulocyte-macrophage colony-stimulating factor and comparison with subcutaneous administration. *Cancer Res.* 50(3), 606–614 (1990).
23. Herrmann F, Mertelsmann R, Lindemann A *et al.* Clinical use of recombinant human hematopoietic growth factors (GM-CSF, IL-3, EPO) in patients with myelodysplastic syndrome. *Biotechnol. Ther.* 2(3–4), 299–311 (1991).
24. Edmonson JH, Hartmann LC, Long HJ *et al.* Granulocyte-macrophage colony stimulating factor. Preliminary observations on the influences of dose, schedule, and route of administration in patients receiving cyclophosphamide and carboplatin. *Cancer* 70(10), 2529–2536 (1992).

25. Berthaud P, Eugene-Joichine I, Spielmann M *et al.* Phase I trial of recombinant human granulocyte-macrophage colony stimulating factor. Results in patients with advanced tumors. *Bull. Cancer* 80(5), 418–430 (1993).
26. Bukowski RM, Murthy S, McLaine D *et al.* Phase I trial of recombinant granulocyte-macrophage colony stimulating factor in patients with lung cancer: clinical and immunologic effects. *J. Immunother. Emphasis Tumor Immunol.* 13(4), 267–274 (1993).
27. Demetri GD, Antman KHS. Granulocyte-macrophage colony-stimulating factor (GM-CSF): preclinical and clinical investigations. *Semin. Oncol.* 19(4), 362–385 (1992).
28. Spitler LE, Grossbard ML, Ernstoff MS *et al.* Adjuvant therapy of stage III and IV malignant melanoma using granulocyte-macrophage colony-stimulating factor. *J. Clin. Oncol.* 18(8), 1614–1621 (2000).
29. Kaufman HL, Ruby CE, Hughes T *et al.* Current status of granulocyte-macrophage stimulating factor in the immunotherapy of melanoma. *J. Immunother. Cancer* 2, 11 (2014).
30. Hoeller C, Michielin O, Ascierto PA *et al.* Systematic review of the use of granulocyte-macrophage colony stimulating factor in patients with advanced melanoma. *Cancer Immunol. Immunother.* 65(9), 1015–1034 (2016).
31. Zhan Y, Lew AM, Chopin M. The pleiotropic effects of the GM-CSF rheostat on myeloid cell differentiation and function: more than a numbers game. *Front. Immunol.* 10, 2679 (2019).
32. Berdel WE, Danhauser-Reidl S, Oberberg D, Zafferani M. Effects of hematopoietic growth factors on malignant nonhematopoietic cells. *Semin. Oncol.* 19(4 Suppl. 2), 41–45 (1992).
33. Hill AD, Redomon HP, Austin OM *et al.* Granulocyte-macrophage colony-stimulating factor inhibits tumour growth. *Br. J. Surg.* 80(12), 1543–1546 (1993).
34. Noviello E, Cosimi A, Casartelli CL *et al.* Effect of granulocyte -macrophage colony-stimulating factor on growth of a xenotraslpanted human ovarian cancer cell line IGROV-1 in nude mice. *In Vivo* 8(2), 207–213 (1994).
35. Zheng Q, Li X, Cheng X *et al.* Granulocyte-macrophage colony-stimulating factor increases tumor growth angiogenesis directly by promoting endothelial cell function and indirectly by enhancing mobilization and recruitment of proangiogenic granulocytes. *Tumour Biol.* 39(2), 1010428317692232 (2017).
36. Daud A, Mirza N, Lenox B *et al.* Phenotypic and functional analysis of dendritic cells and clinical outcome in patients with high-risk melanoma treated with adjuvant granulocyte macrophage colony-stimulating factor. *J. Clin. Oncol.* 26(19), 3235–3241 (2008).
37. Spitler LE, Weber RW, Allen RE *et al.* 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. *J. Immunother.* 32(6), 632–637 (2009).
38. Grotz TE, Kottschade L, Pavey ES *et al.* Adjuvant GM-CSF improves survival in high-risk stage IIIC melanoma: a single center study. *Am. J. Clin. Oncol.* 37(5), 467–472 (2014).
39. Ravaud A, Delaunay M, Chevreau C *et al.* Granulocyte-macrophage colony-stimulating factor alone or with dacarbazine in metastatic melanoma: a randomized Phase II trial. *Br. J. Cancer* 85(10), 1467–1471 (2001).
40. Lawson DH, Lee S, Zhao F *et al.* Randomized, placebo-controlled Phase III trial of yeast-derived granulocyte-macrophage colony-stimulating factor (GM-CSF) versus peptide vaccination versus GM-CSF plus peptide vaccination versus placebo in patients with no evidence of disease after complete surgical resection of locally advanced and/or stage IV melanoma: a trial of the Eastern Cooperative Oncology Group-American College of Radiology Imaging Network Cancer Research Group (E4697). *J. Clin. Oncol.* 33(34), 4066–4074 (2015).

•• **Large placebo-controlled multicenter randomized trial that failed to show benefit of repeated daily GM-CSF as a monotherapy vs placebo, nor as a vaccine adjuvant (or combination immunotherapy) in HLA-A2-positive patients receiving a multipeptide vaccine.**

41. Andtbacka RHI, Kaufman HL, Collichio F *et al.* Talimogene laherparepvec improves durable response rate in patients with advanced melanoma. *J. Clin. Oncol.* 33(25), 2780–2788 (2015).

•• **Randomized trial that showed inferiority of daily subcutaneous injections of GM-CSF compared with intratumor injections of a GM-CSF-secreting Herpes virus construct.**

42. Schrader JW. Peptide regulatory factors and optimization of vaccines. *Mol. Immunol.* 28(3), 295–299 (1991).
43. Armitage JO. Emerging applications of recombinant granulocyte-macrophage colony- stimulating factor. *Blood* 92(12), 4491–4508 (1998).
44. Disis ML, Bernhard H, Shiota FM *et al.* Granulocyte-macrophage colony-stimulating factor: an effective adjuvant for protein and peptide-based vaccines. *Blood* 88(1) 202–210 (1996).
45. Shi Y, Liu CH, Roberts AI *et al.* Granulocyte-macrophage colony-stimulating factor (GM-CSF) and T-cell responses: what we do and don't know. *Cell Res.* 16(2), 126–133 (2006).
46. Van de Laar L, Coffer PJ, Woltman AM. Regulation of dendritic cell development by GM-CSF: molecular control and implications for immune homeostasis and therapy. *Blood* 119(15), 3383–3393 (2012).
47. Dranoff G, Jaffee E, Lazenby A *et al.* Vaccination with irradiated tumor cells engineered to secrete murine granulocyte-macrophage colony-stimulating factor stimulates potent, specific, and long-lasting anti-tumor immunity. *Proc. Nat. Acad. Sci. USA* 90(8), 3539–3543 (1993).

- **Influential murine tumor model study that unutilized irradiated tumor cells that secreted various cytokines with potential as vaccine adjuvants, It was determined that GM-CSF was the best of the ten molecules that were tested in terms of immune and antitumor effects.**

48. Steinman RM. The dendritic cell and its role in immunogenicity. *Annu. Rev. Immunol.* 9, 271–296 (1991).
49. Simmons AD, Li B, Gonzalez-Edick M *et al.* GM-CSF secreting cancer immunotherapies: preclinical analysis of the mechanism of action. *Cancer Immunol. Immunother.* 56(10), 1653–1665 (2007).
50. Klein C, Bueler H, Mulligan RC. Comparative analysis of genetically modified dendritic cells and tumor cells as therapeutic cancer vaccines. *J. Exp. Med.* 191(10), 1699–1708 (2000).
51. Parmiani G, Castelli C, Pilla L *et al.* Opposite immune functions of GM-CSF administered as vaccine adjuvant in cancer patients. *Annal. Oncol.* 18, 226–232 (2007).
52. Slingluff CL Jr, Petroni GR, Yamshchikov GV *et al.* Clinical and immunologic results of a randomized Phase II trial of vaccination using four melanoma peptides either administered in granulocyte-macrophage colony-stimulating factor in adjuvant or pulsed on dendritic cells. *J. Clin. Oncol.* 21(21), 4016–4026 (2003).
53. Chianese-Bullock KA, Pressley J, Garbee C *et al.* MAGE-A1, MAGE-A10-, and gp100-derived peptides are immunogenic when combined with granulocyte-macrophage colony-stimulating factor and montanide ISA-51 adjuvant and administered as part of a multipeptide vaccine for melanoma. *J. Immunol.* 174(5), 3080–3086 (2005).
54. Slingluff CL Jr, Petroni GR, Olson WC *et al.* Effect of granulocyte/macrophage colony-stimulating factor on circulating CD8+ and CD4+ T-cell responses to a multipeptide vaccine: outcome of a multicenter randomized trial. *Clin. Cancer Res.* 15(22), 7036–7044 (2009).
55. Dillman RO, Selvan SR, Schiltz PM *et al.* Phase II trial of dendritic cells loaded with antigens from self-renewing, proliferating autologous tumor cells as patient-specific anti-tumor vaccines in patients with metastatic melanoma: final report. *Cancer Biother. Radiopharm.* 24(3), 311–319 (2009).

- **Single arm trial with a 50% 5-year survival rate in 54 patients with stage IV or recurrent stage III metastatic melanoma, who were treated with subcutaneous injections of dendritic cells loaded with antigens from irradiated, self-renewing, autologous melanoma cells admixed with GM-CSF**

56. Dillman RO, Cornforth AN, Depriest C *et al.* Tumor stem cell antigens as consolidative active specific immunotherapy: a randomized Phase II trial of dendritic cells versus tumor cells in patients with metastatic melanoma. *J. Immunother.* 35(8), 641–649 (2012).
57. Dillman RO, Cornforth AN, McClay EF *et al.* Randomized Phase II trial of autologous dendritic cell vaccines versus autologous tumor cell vaccines in patients with metastatic melanoma: 5-year follow up and additional analyses. *J. Immunother. Cancer* 6(1), 19 (2018).

•• **Randomized trial in which patients were treated with either a patient-specific autologous dendritic cell (DC) vaccine (autologous DCs loaded *ex vivo* with antigens from self-renewing autologous melanoma cells) or a patient-specific tumor cell vaccine (irradiated self-renewing autologous tumor cells). Both cell vaccines were admixed in GM-CSF and then injected SC, as opposed to SC injections of vaccine and repeated daily SC injections of GM-CSF. Survival and immune effects were better in the DC arm.**

58. Soiffer R, Lynch T, Mihm M *et al.* Vaccination with irradiated autologous melanoma cells engineered to secrete human granulocyte-macrophage colony-stimulating factor generates potent antitumor immunity in patients with metastatic melanoma. *Proc. Natl Acad. Sci. USA* 95(22), 13141–13146 (1998).
59. Soiffer R, Hodi FS, Haluska F *et al.* Vaccination with irradiated, autologous melanoma cells engineered to secrete granulocyte-macrophage colony-stimulating factor by adenoviral-mediated gene transfer augments antitumor immunity in patients with metastatic melanoma. *J. Clin. Oncol.* 21(17), 3343–3350 (2003).
60. Luiten RM, Kueter EWM, Mooi W *et al.* Immunogenicity, including vitiligo, and feasibility of vaccination with autologous GM-CSF–transduced tumor cells in metastatic melanoma patients. *J. Clin. Oncol.* 23(35), 8978–8991 (2005).
61. Lipson EJ, Sharfman WH, Chen S *et al.* Safety and immunologic correlates of melanoma GVAX, a GM-CSF secreting allogeneic melanoma vaccine administered in the adjuvant setting. *J. Transl. Med.* 13, 2014 (2015).
62. Scheibenbogen C, Schmittel A, Keilholz U *et al.* Phase II trial of vaccination with tyrosinase peptides and granulocyte-macrophage colony-stimulating factor in patients with metastatic melanoma. *J. Immunother.* 23(2), 275–281 (2000).
63. Scheibenbogen C, Schadendorf D, Bechrakis NE *et al.* Effects of granulocyte-macrophage colony-stimulating factor and foreign helper protein as immunologic adjuvants on the T-cell response to vaccination with tyrosinase peptides. *Int. J. Cancer* 104(2), 188–194 (2003).
64. Dillman RO, Depriest C, DeLeon C *et al.* Patient-specific vaccines derived from autologous tumor cell lines as active specific immunotherapy: results of exploratory Phase I/II trials in patients with metastatic melanoma. *Cancer Biother. Radiopharm.* 22(3), 309–321 (2007).
65. Weber J, Sondak VK, Scotland R *et al.* Granulocyte-macrophage-colony-stimulating factor added to a multipeptide vaccine for resected Stage II melanoma. *Cancer* 97(1), 186–200 (2003).
66. Atzpodien J, Reitz M. GM-CSF plus antigen peptide vaccination in locally advanced melanoma patients. *Cancer Biother. Radiopharm.* 22(4), 551–555 (2007).

67. Faries MB, Hsueh EC, Ye X *et al.* Effect of granulocyte/macrophage colony stimulating factor on vaccination with an allogeneic whole-cell melanoma vaccine. *Clin. Cancer Res.* 15(22), 7029–7035 (2009).

68. Kirkwood JM, Lee S, Moschos SJ *et al.* Immunogenicity and antitumor effects of vaccination with peptide vaccine+/-granulocyte-monocyte colony-stimulating factor and/or IFN-alpha2b in advanced metastatic melanoma: Eastern Cooperative Oncology Group Phase II Trial E1696. *Clin. Cancer Res.* 15(4), 1443–1451 (2009).

69. Butterfield LH, Zhao F, Lee S, Tarhini AA *et al.* Immune correlates of GM-CSF and melanoma peptide vaccination in a randomized trial for the adjuvant therapy of resected high-risk melanoma (E4697). *Clin. Cancer Res.* 23(17), 5034–5043 (2017).

70. Nemunaitis J. Vaccines in cancer: GVAX®, a GM-CSF gene vaccine. *Expert Rev. Vaccines* 4(3), 259–274 (2005).

71. Le DT, Picozzi VJ, Ko AH *et al.* Results from a Phase IIb, randomized multicenter study of GVAX pancreas and CRS-207 compared with chemotherapy in adults with previously treated metastatic pancreatic adenocarcinoma (ECLiPSE Study). *Clin. Cancer Res.* 25(18), 5493–5502 (2019).

72. Dillman RO, Cornforth AN, Nistor GI. Dendritic cell vaccines for melanoma: past, present, and future. *Melanoma Manag.* 3(4), 273–289 (2016).

73. Nasi ML, Lieberman P, Busam KJ *et al.* Intradermal injection of granulocyte-macrophage colony-stimulating factor (GM-CSF) in patients with metastatic melanoma recruits dendritic cells. *Cytokines Cell Mol. Ther.* 5(3), 139–144 (1999).

74. Pan PY, Li Y, Li Q *et al. In situ* recruitment of antigen-presenting cells by intratumoral GM-CSF gene delivery. *Cancer Immunol. Immunother.* 53, 7–25 (2004).

75. Si Z, Hersey P, Coates AS. Clinical responses and lymphoid infiltrates in metastatic melanoma following treatment with intralesional GM-CSF. *Melanoma Res.* 6(3), 247–255 (1996).

76. Ridolfi L, Ridolfi R. Preliminary experiences of intralesional immunotherapy in cutaneous metastatic melanoma. *Hepatogastroenterology* 49(44), 335–359 (2002).

77. Senzer NN, Kaufman HL, Amatruda T *et al.* Phase II clinical trial of a granulocyte-macrophage colony-stimulating factor-encoding, second-generation oncolytic herpesvirus in patients with unresectable metastatic melanoma. *J. Clin. Oncol.* 27(34), 5763–5771 (2009).

78. Andtbacka RHI, Collichio F, Harrington KJ *et al.* Final analyses of OPTiM: a randomized Phase III trial of talimogene laherparepvec versus granulocyte-macrophage colony-stimulating factor in unresectable stage III-IV melanoma. *J. Immunother. Cancer* 7(1), 145 (2019).

•• **Final report of the randomized trial that resulted in approval of talimogene laherparepvec, GM-CSF-secreting Herpes simplex virus, as an intratumor injection, even though it was not compared with daily subcutaneous GM-CSF in an open-label trial, rather than to intratumor injections of GM-CSF in a double-blinded trial.**

79. Feeney KJ, Mastrangelo MJ. Intralesional therapy for in-transit and satellite metastases in melanoma. *Surg. Oncol. Clin. N. Am.* 24(2), 299–308 (2015).

80. Conry RM, Westbrook B, McKee S, Norwood TG. Talimogene laherparepvec: first in class oncolytic virotherapy. *Hum. Vaccin. Immunother.* 14(4), 839–846 (2018).

81. Hodi FS, Lee S, McDermott DF *et al.* Ipilimumab plus sargramostim vs ipilimumab alone for treatment of metastatic melanoma: a randomized clinical trial. *JAMA* 312(17), 1744–1753 (2014).

82. Chesney J, Puzanov I, Collichio F *et al.* Randomized, open-label Phase II study evaluating the efficacy and safety of talimogene laherparepvec in combination with ipilimumab versus ipilimumab alone in patients with advanced, unresectable melanoma. *J. Clin. Oncol.* 36(17), 1658–1667 (2018).