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

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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.

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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 immunotherapeutic 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

References

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**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 |

**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 hematopoietic 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 x 10 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/m² | SC or IV daily x 14 days | Yes, at all doses | Well tolerated all doses | Increased WBC, prefered 250 mcg/day SC x 14 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 q 12 h x 14 days 1 day postchemo considered best | [24] |
| Bukowski (1993) | Yeast | 17 Lung cancer | 60, 125, 250, 500 mcg/m² | IV continuous infusion x 14 days | Yes, at all doses | Well tolerated at lower doses, fever, fatigue | 250 mcg/m² MTD, pulmonary toxicity | [26] |
| Berthaud (1993) | E. coli | 14 various solid cancers | 250, 500, 750, 1000 mcg/m² | SC daily for 10 days | Yes, at all doses | Fever, local irritation, lethargy, arthralgia | No MTD | [25] |
| Steward (1989) | E. coli | 20 various solid tumors | 0.3–60 mcg/kg | IV 30 min infusions x 10 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 malignancies | 0.3–3 mcg/kg 0.3–20 mcg/kg | IV bolus x 10 days IV 2-h infusion x 10 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    | 250 mcg/m²/day infused IV over 4 h until recovery                       |
| 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)                                       |                                                                        |
| 2. For mobilization of hematopoietic progenitor cells into peripheral blood | Single 250 mcg/m² injected SC or 250 mcg/m² infused IV over 24 h       |
| for collection by leukapheresis and autologous transplantation in adult     |                                                                        |
| patients (1991)                                                           |                                                                        |
| 3. To accelerate myeloid reconstitution following autologous bone marrow  | Single 250 mcg/m² injected SC or 250 mcg/m² infused IV over 24 h       |
| or peripheral blood progenitor cell transplantation in adult and pediatric   |                                                                        |
| patients ≥2 years of age (1991)                                          |                                                                        |
| 4. To accelerate myeloid reconstitution following allogeneic bone marrow   | 250 mcg/m² infused IV over 2 h                                        |
| transplantation in adult and pediatric patients ≥2 years of age (1991)    |                                                                        |
| 5. For treatment of delayed neutrophil recovery or graft failure after     | 250 mcg/m² infused IV over 2 h, daily for 14 days                     |
| autologous or allogeneic bone marrow transplantation in adult and pediatric |                                                                        |
| patients ≥2 years of age (1991)                                          |                                                                        |
| 6. To accelerate myeloid reconstitution and function in adults and         | Once daily SC injection:                                              |
| children acutely exposed to myelosuppressive doses of radiation (2018)     | • 7 mcg/kg for adults and pediatric patients weighing                   |
|                                                                          | • 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/m² 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. 38% 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/m² 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% arthralgia/myalgia, 39% flu-like symptoms | ≥65 mos median survival, transient increased DC but not MDSC | [36] |
| Spitler (2009) | 125 mcg/m² 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, ressected 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/m² SC daily x14 of each 28-day cycle | Unressted 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 5%), 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 [46,65–69]. 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 mannnide 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
| Author (year) | GM-CSF Route | GM-CSF Per Dose | Vaccine | Stage | Trial design | Patients, n | Results |
|--------------|--------------|-----------------|---------|-------|-------------|-------------|---------|
| Slingluff (2003) | Admixed SC and ID | 225 mcg SC | Four peptides weekly x6 | III, IV | Phase II–III random in GM-CSF or on DC | 26 | Minimal toxicity; immune response greater in GM-CSF than in DC-loaded (p < .02) |
| 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 |
| 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; CD8³ response 34% w/ GM-CSF, 73% w/o GM-CSF; p < 0.001, no difference in survival |
| 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 |
| 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 |
| Soiffer (1998) | SC and ID | Encoded in ITC Secreted | 12 peptides weekly x6 | IIB, III, IV | Phase II | 21 | Immune infiltration of tumors |
| Soiffer (2003) | SC and ID | Encoded in ITC Secreted | Auto ITC weekly x3 ± months 4 through 8 | IV recurrent III | Phase II | 34 | 1 CR, 1 PR, infiltration with leukocytes including DC |
| 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 |
| Lipson (2015) | ID | Encoded in ITC Secreted | Resected III–IV | Phase II | 20 | Safe, increased PBM. No increase in antigen-specific T cells |
| 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 |
| Scheiben-bogen (2003) | Nearby SC | 75 mcg SC x 4 days | Peptides biweekly x4, bimonthly x2 | IIB, III, IV | Phase II random 1:1:1 | 43 | Antigen-specific T cells in 3/9 no adjuvant, 4/9 GM-CSF, 0/10 KLH |
| 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% |
| 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 |
| 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 |
| Atzpodien (2007) | Distant SC | 62.5 mcg SC x 4 days q 4 weeks | 4 peptides and KLH II D and SC q 4 weeks x6 months | Resected recurrent III and 4 | Phase II | 24 | Well-tolerated, 92% 2-year OS |
| Faries (2009) | Distant SC | 200 mcg/m² 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. |
| Kirkwood (2009) | Distant SC | 250 mcg/m² SC daily x 14 of 28 days | Peptides SC biweekly for 1 year | Resected III or IV | Phase II random 1:1 IFN-α or GM-CSF or both | 120 | No difference GM-CSF, IFN-α or both (stage IV) |
| Lawson (2015) | Distant SC | 125 mcg/m² daily for 14 of 28 days | Peptides SC biweekly for 1 year | Resected III or IV | Phase III random 1:1:1 four vaccine arms | 435 | No difference GM-CSF vs placebo |

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 | Illc & 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.

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 laherparepvec 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 intraläsional 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

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