Clinical uses of GM-CSF, a critical appraisal and update

Martha Arellano
Sagar Lonial

Emory University, Winship Cancer Institute, Atlanta, GA, USA

Abstract: The role of granulocyte-macrophage-colony-stimulating factor (GM-CSF) in the supportive care of cancer patients has been evaluated with promising results. More recently, GM-CSF has been added to regimens for the mobilization of hematopoietic progenitor cells. An expanding role for GM-CSF in regulating immune responses has been recognized based upon its activity on the development and maturation of antigen presenting cells and its capability for skewing the immune system toward Th1-type responses. GM-CSF has been shown to preferentially enhance both the numbers and activity of type 1 dendritic cells (DC1), the subsets of dendritic cells responsible for initiating cytotoxic immune responses. The increase in DC1 content and activity following local and systemic GM-CSF administration support a role for GM-CSF as an immune stimulant and vaccine adjuvant in cancer patients. GM-CSF has shown clinical activity as an immune stimulant in tumor cell and dendritic cell vaccines, and may increase antibody-dependent cellular cytotoxicity. The successful use of myeloid acting cytokines to enhance anti-tumor responses will likely require the utilization of GM-CSF in combination with cytotoxic or other targeted therapies.

Keywords: GM-CSF, G-CSF, cancer, hematopoietic cytokines

Introduction/background

Granulocyte-macrophage-colony-stimulating factor (GM-CSF) and granulocyte-colony-stimulating factor (G-CSF) belong to the family of hematopoietic cytokines. Their activities include stimulating the proliferation of granulocyte and/or macrophage progenitor cells, influencing differentiation, inducing maturation, and stimulating the functional activity of mature hematopoietic cells (Inaba et al 1992; Metcalf and Nicola 1995; Metcalf 1998). Colony stimulating factors (CSF) are not only important as mediators of the cellular response to immunologic or infectious insults but are also essential for maintaining basal hematopoiesis (Dranoff et al 1994; Fantuzzi 2003). G-CSF-deficient mice manifest a chronic neutropenia and an impaired response to infectious challenge leading to premature death (Lieschke et al 1994). GM-CSF-deficient mice, on the other hand, have normal levels of steady-state blood cell production, but exhibit defective phagocytosis and decreased oxygen radical production by granulocytes and macrophages; responses which are essential for the anti-bacterial defense (Stanley et al 1994; Zhan et al 1998; LeVine et al 1999). These mice also exhibit decreased tumor necrosis factor-alpha and leukotriene secretion, abnormal para-bronchial accumulations of B and T lymphocytes, and decreased catabolism of alveolar surfactant lipids and proteins leading to a syndrome reminiscent of pulmonary alveolar proteinosis (Stanley et al 1994; Paine et al 2001). Double G-CSF and GM-CSF knockout mice exhibited a greater degree of neutropenia, and had an increased mortality rate in the early post-natal period compared to mice deficient in G-CSF alone (Seymour et al 1997). Enzler et al (2003) found that mice deficient in GM-CSF and gamma interferon (IFN-γ)

Correspondence: Martha Arellano
Emory University, Winship Cancer Institute, 1365C Clifton Road, Suite C2068, Atlanta, Georgia, USA 30322
Email martha.arellano@emoryhealthcare.org

acquired lymphoproliferative disorders and solid tumors in a background of chronic inflammation, supporting the relationship between inflammation and carcinogenesis.

Recombinant GM-CSF has made significant contributions in the supportive care of cancer patients, owing to enhanced myeloid recovery after cytotoxic chemotherapy. Recently GM-CSF has been successfully included in mobilization regimens for hematopoietic progenitor cell transplantation (Cashen et al 2004; Lonial et al 2004). Recent data on the effects of GM-CSF on dendritic cells has led to growing interest in its use as primary immunotherapy. The ability of GM-CSF to generate of type 1 dendritic cells (DC1), which can skew T-cells toward a Th-1 phenotype has been demonstrated and is an attractive approach toward generating anti-tumor effects (Ferlazzo et al 2000). Peripheral blood mononuclear cells (PBMC), T-cells and antigen presenting cells (APC) cultured with GM-CSF exhibited increased production of type 1 cytokines (interleukin-12, interferon-alpha, tumor necrosis factor-alpha) and decreased production of type 2 cytokines (interleukin-4 and interleukin-10) compared to cells treated with control media or G-CSF (Eksioglu et al 2007). In addition, APC treated with GM-CSF induced higher proliferation of allogeneic T-cells compared to APC treated with G-CSF or control media (Eksioglu et al 2007). The capacity of GM-CSF to skew the immune system toward Th1 effects in vitro suggests a role for GM-CSF in cell-mediated immune therapy and is currently being tested in-vivo. Dendritic cells (DC) have come to be recognized as the sensors of tissue injury, infection, or malignant transformation and as the agents responsible for the initial activation of the immune response (Matzinger 1994). DC are the antigen presenting cells of the immune system, have the capacity to express both HLA class I and HLA class II restricted peptides, and express the co-stimulatory molecules needed for T-cell activation (Banchereau et al 2000). A number of reports have shown that administration of GM-CSF can induce anti-tumor immune responses and tumor regressions. These immune activities are attributed to the action of GM-CSF on DC (Dranoff et al 1993; Wos et al 1996; Davidson et al 1998). The limitations of GM-CSF as an immune adjuvant, and its modest clinical activity, have been attributed to discordance between the observed immune response measured in the laboratory and the clinical correlates of anti-tumor activity. The need to generate the co-stimulatory signals required to break immune tolerance, the proper dosing and timing of cytokines, the state of disease at time of treatment, and the role of concomitant chemotherapy are the topics of current investigation. In this review, we will discuss the role of GM-CSF as primary treatment and as an immune adjunct to the therapy of cancer.

Relationship between cancer and inflammation

The connection between inflammation and malignant transformation has been recognized for over a century. In 1863, Dr. Rudolf Virchow recognized a possible relationship between chronic inflammation and the development of cancer, based on observations of inflammatory infiltrates and spontaneous regression of malignant tumors (Balkwill and Mantovani 2001; Schreiber 2003). In the late 1890s, William Coley, a surgeon in New York, observed tumor regressions in patients with cancer who recovered from acute skin infections. He then developed a vaccine composed of extracts of inactivated bacteria, which he administered to cancer patients with variable results (Nauts et al 1953). He observed that tumor regressions were more common among patients who developed both a local and a systemic inflammatory response (Hoption Cann et al 2002). The premise was that non-specific activation of the immune system could lead to (specific) cytokine-mediated anti-tumor effects. While cytokines are credited with potent anti-tumor effects, a counteracting effect of tumor-secreted cytokines, and tumor-associated tolerizing T-cells, or immature DC has also been documented (Perrot et al 2007; Wang and Wang 2007). Some solid tumors, for example, are capable of inducing immune tolerance via down-regulation of antigen-specific T-cell responses by tolerigenic APC (Cuenca et al 2003). Immature tumor-infiltrating DC are capable of compromising the tumor-specific immune response in draining lymph nodes (Perrot et al 2007). The premise that the immune system can be manipulated in vivo supports a role for the manipulation of the cytokine/co-stimulatory signal milieu in the treatment of cancer using recombinant cytokines, such as GM-CSF.

Approaches will need to account for the level of maturation of tumor-associated DC, and the number of tumor-associated regulatory T-cells

GM-CSF may enhance tumor-specific antigen presentation leading to better recognition of tumors by the immune system via effects on DC. However, its benefit has been limited to patients with minimal residual disease, and dose-escalation has been limited by significant systemic toxicities. The optimal use of cytokines may be directly in the tumor micro-environment. Furthermore, better quantitative

measures of antigen-specific immune responses are needed (Keilholz et al 2002).

Role of GM-CSF in supportive therapy

Numerous clinical trials have established the role of CSF in the prevention and treatment of febrile neutropenia (Garcia-Carbonero et al 2001; Mizutani et al 2003; Repetto et al 2003). The majority of clinical trials investigated the role of G-CSF in the supportive care of cancer patients; therefore, the current recommendations do not address the specific use of GM-CSF in this setting. The American Society of Clinical Oncology (ASCO) has provided guidelines for the use of CSF (either G-CSF or GM-CSF) in patients receiving chemotherapy for solid and hematological malignancies (Smith et al 2006). No recommendation was made regarding the equivalency of G-CSF and GM-CSF. Rowe et al reported a significant reduction of infectious complications in patients with acute myeloid leukemia (AML) who received GM-CSF after induction chemotherapy, compared to placebo (Rowe et al 1995). However, Zittoun et al (2006) reported a decreased complete response (CR) rate in patients with AML who received GM-CSF with induction chemotherapy, indicating that the routine use of cytokines for acceleration of hematopoietic recovery may not always be indicated; and the use of CSF for priming of leukemic cells is not recommended.

Dendritic cells as regulators of immune responses

DC play a central role in the initiation of innate and adaptive immune responses. Pattern recognition receptors, known as toll-like receptors (TLR), on the surface of DC are bound by proteins, lipids, and nucleic acids resulting in DC activation (Kadowaki et al 2001; Dillon et al 2004). Antigen-specific T-cell immune responses are initiated by DC when these bound antigens are internalized, degraded, and presented as processed peptides on the surface of HLA molecules (Hart 1997; Bancereau and Steinman 1998; Banchereau et al 2000). Two main categories of peripheral blood and bone marrow derived DC have been described in humans, type 1, myeloid dendritic cells (DC1), and type 2 plasmacytoid dendritic cells (DC2) (Grouard et al 1997). DC1 and DC2 differ in the type of cell differentiation markers and TLR expressed on their surface, their cytokine milieu, and their effect on polarizing T-cell immune responses (Rissoan et al 1999; Amsen et al 2004; Dillon et al 2004). The maturation status of DC is an important determinant of the type of immune response generated upon DC activation. For example, antigen presentation by immature DC leads to generation of anergic CD4+ T-cells (Kuwana et al 2001), and immuno-suppressive CD8+ T-cells with antigen-specificity (Gilliet and Liu 2002). Immature DC2 progenitors play a crucial role in the response to viral infection by releasing large amounts of alpha and beta interferon (Siegal et al 1999; Fonteneau et al 2003; Larsson et al 2003; Coccia et al 2004). The targeting of DC by synthetic TLR ligands is a topic of current clinical and pre-clinical research.

Generation of dendritic cells by GM-CSF

Colony-stimulating factors can differentiate hematopoietic progenitor cells into specific DC lineages (Santiago-Schwarz et al 1992; Grouard et al 1997; Olweus et al 1997; Rissoan et al 1999; Siegal et al 1999; Berthier et al 2000; Ferlazzo et al 2000). Hematopoietic stem cells cultured in GM-CSF and Flt3 can differentiate along a myelo-monocytic lineage into DC1 (Ferlazzo et al 2000). CD14+ progenitor cells cultured in GM-CSF and IL-4 can also differentiate into immature DC1 (Ferlazzo et al 2000; Basak et al 2002). In contrast, treatment of hematopoietic progenitors with G-CSF and IL-3 can mobilize large numbers of (plasmacytoid) DC2 (Arpinati et al 2000). Thus, while GM-CSF and G-CSF have similar effects on the mobilization of neutrophils, they have significantly different effects on the mobilization and differentiation of DC1 and DC2, with culture in GM-CSF leading to differentiation of progenitors into DC1, and culture in G-CSF leading to differentiation of progenitors into DC2 (Arpinati et al 2000). Due to their effects on DC1 and DC2, these 2 cytokines are optimal agents for cellular immune therapy.

Cytokines and peripheral blood hematopoietic progenitor cell transplantation

Peripheral blood as a source of stem cells for clinical stem cell transplantation was introduced by Korbling et al and Kessinger et al in the 1980s (Korbling et al 1981; Kessinger et al 1986). CSF are now widely used for the mobilization of hematopoietic progenitor cells into the peripheral circulation, allowing collection of CD34+ cells for autologous and allogeneic hematopoietic progenitor cell transplantation. Essential differences have been noted between bone marrow (BMT) and peripheral blood grafts, owing to differences in the ratio of early pluripotent, self-renewing stem cells to lineage-committed, late progenitor cells, and the accessory cells in the grafts (Korbling and Anderlini 2001). These differences may account for the observed differences in clinical outcomes after

transplantation. A study by the European working group for blood and marrow transplant reported similar rates of overall survival, leukemia-free survival, and similar incidence of graft versus host disease (GVHD) when comparing bone marrow with cytokine-mobilized peripheral blood grafts. They did, however, observe improved platelet recovery with cytokine-mobilized peripheral graft compared to BMT (Schmitz et al 1998). A randomized, multi-center trial of cytokine-mobilized peripheral blood progenitor cell grafts versus BMT reported by Schmitz et al (2002), found an increased risk of acute and chronic GVHD among recipients of cytokine-mobilized peripheral blood grafts, but no difference in survival compared to recipients of bone marrow transplants. In contrast, in another randomized trial comparing peripheral blood hematopoietic progenitor cell and bone marrow transplantation, Bensinger et al found more rapid neutrophil and platelet recovery and similar rates of acute and chronic GVHD. In that trial, the duration of chronic GVDH was longer among recipients of blood progenitor cell grafts. Furthermore, recipients of blood progenitor cell grafts had a higher estimated probability of overall survival and 2-year disease-free survival (Bensinger et al 2001). Interestingly, Urbini and colleagues found a higher number of CD34+ cells in peripheral blood grafts mobilized with G-CSF compared to bone marrow, and the dose of CD34+ cells infused correlated with the number of DC1 in peripheral blood grafts and DC2 in bone marrow allografts. In addition, among recipients of cytokine mobilized grafts, a significantly shorter overall survival and a trend toward lower disease free survival was noted among recipients of larger numbers of CD34+ cells (Urbini et al 2003).

The fact that the incidence of acute GVHD in recipients of peripheral blood progenitor cell grafts was similar to, or only slightly higher than in BMT recipients, despite the higher content of T cells in the peripheral blood grafts, suggests that there may be quantitative differences in the other immune cellular components of peripheral blood grafts; such as, the presence of increased numbers of immunosuppressive DC2 in G-CSF mobilized peripheral blood grafts compared to bone marrow grafts (Arpinati et al 2000).

Clinical outcomes after hematopoietic progenitor cell transplantation: contribution of dendritic cells

Recent growing interest in the role of accessory cells in hematopoietic progenitor cell grafts has led to further studies of the immune-modulatory effects of CSF on the constituents of the peripheral blood progenitor cell graft. Flt3 and GM-CSF administration led to mobilization of increased numbers of DC1 in the cellular apheresis product (Gasparetto et al 2002). In contrast, administration of G-CSF lead to mobilization of increased numbers of DC2 cells in the grafts (Arpinati et al 2000). The clinical consequences of mobilizing more DC1 with GM-CSF and more DC2 with G-CSF remain unknown, and are the subject of current investigation.

Waller et al tested the hypothesis that the cellular constituents of the graft could affect clinical outcomes after bone marrow transplantation (Waller et al 2001). They performed a retrospective study of 113 patients with hematological malignancies who received non-T cell-depleted bone marrow grafts from HLA-matched siblings. After evaluating patient and disease-specific characteristics, characteristics of the graft constituents, and clinical outcomes, they reported that patients who received larger numbers of donor DC2 had significantly worse clinical outcomes, with lower event-free survival, less chronic GVHD, and an increased incidence of relapse than their counterparts who received fewer numbers of DC2 cells (Waller et al 2001).

The significance of absolute numbers of DC in post-transplant outcomes was also evaluated in a clinical study by Reddy and colleagues (Reddy et al 2004). Fifty patients undergoing allogeneic transplantation for hematological disorders were evaluated. After evaluating the constituents of the grafts, as well as the type and number of DC in the peripheral blood early after engraftment, they noted that patients with lower absolute numbers of DC ($<$4.97 cells/μL) at engraftment had worse clinical outcomes compared to patients with higher numbers of DC at engraftment, with lower overall survival (p = 0.002), increased incidence of relapse (p = 0.002), and a higher incidence of acute GVHD (p = 0.0005). Multivariable analysis confirmed that low DC count was independently associated with death (hazard ratio [HR], 3.8; p = 0.02), time to relapse (HR, 11.6; p = 0.001), and acute GVHD (HR, 3.3; p = 0.04). The effect was similar when DC1 were analyzed separately. However, when DC2 were analyzed separately, the effect was only significant for increased incidence of acute GVHD among patients with lower numbers of DC2 at engraftment. The independent effect of DC1 and DC2 was not confirmed in the multivariate analysis (Reddy et al 2004).

Subsequently, Lonial and colleagues hypothesized that the combination of G-CSF and GM-CSF administered for the mobilization of stem cells after chemotherapy would reduce the content of DC2 in the autologous blood hematopoietic

progenitor cell grafts compared with administration of G-CSF alone after chemotherapy (Lonial et al 2004). They randomized 35 patients with lymphoma and multiple myeloma to receive either G-CSF or the combination of G-CSF plus GM-CSF after chemotherapy. Blood hematopoietic progenitor cell grafts were collected by large volume apheresis. They found a similar incidence of cytokine-related adverse events, and similar numbers of stem cells mobilized between the 2 treatment groups. There were minor differences with respect to the content of T cells in the apheresis products. However, grafts mobilized with the combination of GM-CSF plus G-CSF had significantly fewer DC2 and similar numbers of DC1 compared with grafts mobilized with G-CSF alone. A third cohort of patients received G-CSF and the sequential administration of GM-CSF 6 days later. Grafts from these patients had a markedly decreased DC2 content compared with grafts mobilized from patients treated with G-CSF alone or with the simultaneous administration of both cytokines. This preliminary trial formed the foundation for a randomized clinical trial where normal volunteer donors were randomized to receive either G-CSF or G-CSF plus GM-CSF in order to evaluate the impact of these cytokines on DC content, T-cell polarization, and immune function after allogeneic transplantation. Fifty patients were enrolled in the trial with 25 in the GM+G-CSF and 25 in the G-CSF alone arm. All patients were successfully mobilized. Among donors mobilized with G-CSF alone, the mean number of collections was 1.48 compared with 1.08 in the group receiving the combination of GM+G-CSF (p = 0.01). There was a trend toward a higher total cell dose in the G-CSF arm (p = 0.09). Two of the 25 donors in the G/GM group required more than 1 apheresis, and 8 of 25 donors in the G-CSF alone group required more than 1 collection to achieve an adequate number of CD34+ cells. Analysis of the T-cell and T-cell subset data revealed that in the group receiving G-CSF alone, there was a significantly higher percent and total T-cell, CD4+ and CD8+ cell content of the grafts when compared with the group receiving the GM+G-CSF combination. Among dendritic cell subsets in the grafts, there was a significantly lower percentage and fewer absolute numbers of DC2, as well as a lower delivered DC2 dose/kg for the group randomized to receive GM-CSF plus G-CSF compared with the group randomized to receive G-CSF alone (p < 0.001). There was no significant difference in the DC1 content or the content of CD34+ cells between the 2 treatment arms. Proliferation of the graft in response to T and B-cell mitogens was measured on the graft itself. Cells were exposed to mitogens or control for 72 hours, and then thymidine incorporation was measured. So far, there

are available data on mitogen stimulation for 32 patients, showing a trend toward more IL-12 secretion for G+GM-CSF mobilized grafts, and more IL-2 secretion for G-CSF mobilized grafts. There have been no differences in the incidence of GvHD, relapse or survival between the 2 cytokine arms to date (Lonial et al 2004; Lonial et al 2006).

These data, and data indicating that cross-presentation of antigen by DC2 may induce antigen-specific tolerance by T cells, suggest that the addition of GM-CSF to regimens during mobilization of peripheral blood progenitor cell grafts may be a clinically applicable strategy to enhance innate and acquired immunity after peripheral blood progenitor cell transplantation (Kuwana et al 2001; Gilliet and Liu 2002). Larger clinical trials are needed to determine the exact consequences of altering the DC1 and DC2 content of peripheral blood hematopoietic progenitor cell grafts. Those effects may include: incidence of acute and chronic GVHD, engraftment, graft rejection, graft versus leukemia effect and response to infection.

Role of GM-CSF in post transplant immune reconstitution

Recent data on the differential effects of GM-CSF and G-CSF on the DC subsets in the graft, has inspired clinical studies to investigate whether the administration of these cytokines following autologous hematopoietic stem cell transplantation may influence the post-transplant reconstitution of cellular immunity. Fattorossi and colleagues conducted a randomized, prospective clinical trial to test for differences in immune recovery among 39 patients with ovarian and breast cancer who received either G-CSF or GM-CSF after high dose myeloablative chemotherapy and autologous transplantation. At day 12, GM-CSF was more efficient at up-regulating membrane molecules on phagocytic cells important for antibody-dependent cytotoxicity and for the uptake of immune complexes compared to treatment with G-CSF; and at day 80, a significantly higher proportion of mitogen-stimulated T cells from GM-CSF-treated patients expressed interleukin-2 receptor, and a higher proportion of these T cells were actively proliferating (Fattorossi et al 2001) . Recently, Gazitt and colleagues showed that among 29 non-Hodgkin's lymphoma (NHL) patients receiving cyclophosphamide plus GM-CSF, G-CSF or GM-CSF followed by G-CSF for stem cell collection, patients mobilized with the GM-CSF containing regimens mobilized higher numbers of DC, and had a higher probability of survival compared to patients receiving G-CSF alone (median of 55 months versus 15 months; p = 0.02). Of note, there was no

difference in the ratio of DC1:DC2 between CSF regimens. This finding supports the hypothesis that higher numbers of DCs in the graft might be associated with prolonged survival of NHL patients after autologous transplantation (Gazitt et al 2006). Further studies in larger populations of patients are merited. More is known about the effects of G-CSF than the effects of GM-CSF on post-transplant immune reconstitution. Volpi and colleagues reported on the effects of G-CSF administration in the post-transplant setting among 43 patients with acute leukemia who received T-cell depleted peripheral blood progenitor cell transplants from HLA haplo-identical related donors, compared to a cohort of 36 patients with acute leukemia who did not receive G-CSF after transplantation. They found significantly delayed recovery of Th1-type CD4+ T-cells (low IL-4 and IL-10, and high IL-12 receptor expression), a higher proportion of CD4+ T-cells with a Th2 phenotype (high levels of IL-4 and IL-10, and low levels of IL-12 receptor expression), and impaired production of IL-12 by dendritic cells, compared to patients who did not receive post-transplant G-CSF. T-cells from recipients of post-transplant G-CSF had significantly decreased in-vitro reactivity to fungal pathogens compared to T-cells from patients who did not receive post-transplant G-CSF. This finding suggests an increased susceptibility to opportunistic infections in the G-CSF treated cohort, given that Th1-responses are necessary for anti-fungal protection (Volpi et al 2001). The effect of G-CSF on post-transplant immune reconstitution appeared to be dependent on G-CSF's influence on DC maturation and differentiation; given that administration of G-CSF following transplantation favored the appearance of IL-12-deficient DC which polarize T-cells toward Th2 responses. Fagnoni reported a similar effect of post-transplant G-CSF in children (Fagnoni et al 2004). Ringden et al performed a retrospective analysis to determine the role of post-transplant treatment with G-CSF in patients with AML and acute lymphocytic leukemia (ALL) who received allogeneic BMT or peripheral blood grafts. They found that prophylactic, post-transplant treatment with G-CSF led to a higher risk of acute and chronic GVHD, higher transplant related mortality, and decreased overall survival and leukemia-free survival rates in patients who received BMT only. Post-transplant G-CSF led to faster engraftment of absolute neutrophil count but slower engraftment of platelets in transplant recipients irrespective of the type of graft (Ringden et al 2004). These findings suggest that post-transplant administration of G-CSF may cause an imbalance in dendritic cell content or function, resulting in impaired cellular immunity in the early post-transplant period. This may lead to an increase in the incidence of GVHD (Fagnoni et al 2004). Current guidelines support the use of colony stimulating factors for mobilization of autologous and allogeneic grafts and after peripheral blood progenitor cell transplantation in the autologous setting only (Smith et al 2006). Further studies are needed to support the addition of GM-CSF after allogeneic transplantation.

GM-CSF may improve antibody-dependent cell-mediated cytotoxicity

The anti-CD20 antibody, rituximab, used alone or in combination with chemotherapy, is an established treatment for non-Hodgkin's lymphoma (NHL) (Cvetkovic and Perry 2006). Augmenting the expression of CD20 antigen on the tumor cells may increase the cell kill and therefore increase the effectiveness of the antibody (Venugopal et al 2000). Preliminary data suggest that GM-CSF can up-regulate the CD20 expression on lymphoid B cells in vitro, but these results have not been reproducible in vitro nor in vivo (Venugopal et al 2000; Chow et al 2001; Yagci et al 2005).

Venugopal and colleagues performed experiments on cells from patients with chronic lymphocytic leukemia (CLL) where CLL cells were cultured with cytokines and the expression of CD 20 on the surface of the CLL cells was measured before and after cytokine exposure. They found a statistically significant up-regulation of CD20 antigen expression on CLL cells after culture with GM-CSF, IL-4, or TNF-alpha. Flow cytometry evaluation revealed an increase in fluorescence intensity as well in the percentage of cells expressing the antigen (Venugopal et al 2000). This led to further studies which have revealed promising, but inconsistent results. Olivieri and his group showed the feasibility of rescuing patients with NHL relapsing after autologous transplantation with a regimen containing rituximab, CHOP chemotherapy and GM-CSF. They reported a 75% overall response rate (60% complete remission, and 15% partial response) among 20 patients with aggressive NHL who relapsed after autologous transplantation (Olivieri et al 2005). Rapoport and colleagues reported promising results utilizing post-transplant rituximab and GM-CSF after autologous transplantation among a group of patients with advanced NHL and Hodgkin's disease (Rapoport et al 2002). However neither up-regulation of CD20 antigen, nor a change of the proportion of CD20 positive cells was observed after culture with GM-CSF in a study by Yagci et al (2005) on cells from 18 patients with CLL.

GM-CSF may augment the graft versus leukemia effect of allogeneic transplantation

Relapse of acute leukemia after allogeneic transplantation remains a significant therapeutic challenge, affecting approximately one third of all patients with acute leukemia who receive allogeneic transplantation as a curative therapy. Salvage post-transplant maneuvers have focused on utilization of second transplants, but these are limited to a minority (10% in most series) of fit patients. Clinical and pre-clinical data has suggested a role for cytokine therapy in the induction of graft versus leukemia effects in the setting of post-transplant relapse (Slavin et al 1996; Cortes et al 1998; Boyer et al 2000; Mohty et al 2002; Kolb et al 2004; Li and Waller 2004). Improving the antigen-presenting capacity of leukemic blasts may lead to clinically-significant anti-leukemic effects. The feasibility of generating DC-like leukemic antigen presenting cells upon treatment with cytokines, including GM-CSF has been demonstrated (Santiago-Schwarz et al 1994; Mohty et al 2002). The level of co-stimulatory molecule expression on leukemic blasts has been hypothesized to play a role in the capacity of leukemic blasts to present antigen to effector cells (Vereecque et al 2000; Whiteway et al 2003). A retrospective study at our institution reviewing the treatment of acute leukemia relapsed after allogeneic transplantation revealed promising results among a minority of patients treated with GM-CSF and interferon-alpha-2b (Arellano et al 2007). A prospective clinical trial at our center is currently investigating the feasibility and activity of a regimen utilizing the combination of GM-CSF and interferon-alpha-2b after cytoreduction to treat acute leukemia relapsed after allogeneic transplantation. Correlative studies will test the hypothesis that GM-CSF and interferon-alpha-2b act by up-regulating co-stimulatory molecules on leukemic blasts, and down-regulating regulatory T-cells leading to improved antigen presentation and durable graft versus leukemia effects (Figure 1).

GM-CSF for the treatment of solid tumors

Role of GM-CSF in the management of renal cell carcinoma

Renal cell carcinoma (RCC) is known to be an immunogenic tumor. Interferon-alpha (IFN-alpha) has been established as the standard treatment for metastatic RCC with response rates ranging between 10 and 20%. High dose interleukin-2 has yielded similar results, but its use has been limited by significant toxicities (Coppin et al 2005). Previous
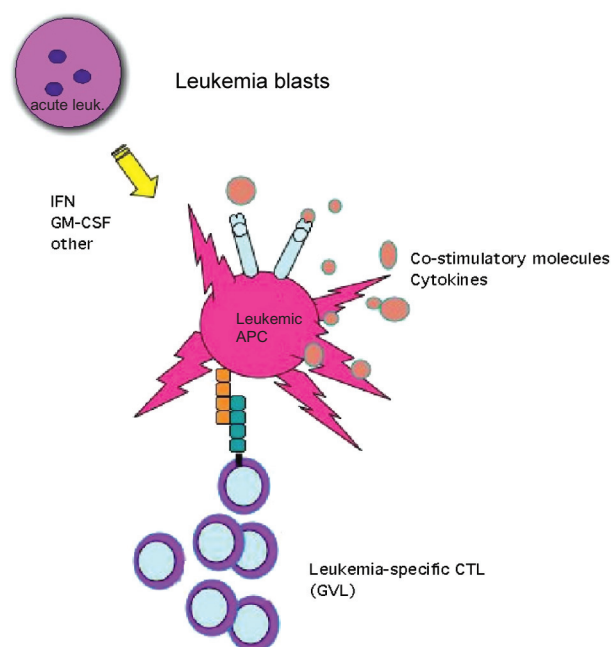

Figure 1 GM-CSF and IFN may upregulate co-stimulatory molecule expression on leukemic blasts leading to stimulation of cytotoxic T-cells and a GVL effect.
Abbreviations: APC, antigen presenting cells; GVL, graft versus leukemia; GM-CSF, granulocyte-macrophage-colony-stimulating factor; IFN, interferon; CTL, cytotoxic T lymphocyte.

observations have indicated that GM-CSF can potentiate the effect of IL-2 on T-cell activation (Masucci et al 1990; Groenewegen and de Gast 1999). Subsequently multiple trials have tested the activity of GM-CSF combinations for RCC with modest results. Table 1 summarizes activity and toxicities of GM-CSF in the treatment of renal cell carcinoma. Verra and colleagues studied the effect of simultaneous administration of low dose IL-2, IFN-alpha and GM-CSF in metastatic RCC in a phase I study and, subsequently in a multicenter phase II study, showing tolerability and promising efficacy with 19% overall responses (9% CR, 10% PR) (De Gast et al 2000; Verra et al 2003). Recently, the same group conducted a phase I study of peri-operative low-dose IL-2, IFN-alpha, and GM-CSF in resectable RCC. In addition to determining the maximum tolerated dose of the cytokine combination in the peri-operative setting, the investigators studied the effects of the cytokines in the peripheral blood and at the tumor site. They found higher numbers of tumor-infiltrating T-cells and mature DC1 in tumors resected from patients who received peri-operative cytokines, compared to a control group of tumors resected from non-cytokine treated patients (Verra et al 2005). The advent of targeted therapy for RCC has marked a new paradigm in the treatment of this tumor (Motzer and Bukowski 2006), and further studies may find a role for combining cytokines with the newer targeted agents.

Table 1 Recent trials using GM-CSF in patients with renal cell carcinoma

Author	Population	Immune therapy	Dose of GM-CSF	Outcomes	Toxicities (most common)
Correale et al 2005	19 Metastatic RCC	IL-2 0.5 MIU BID SQ D 6–15	150 μg /day SQ D 1–5	PR: 4/19 SD: 11/19 mTTP = 9 months 2 year OS 42% 3 year OS 26%	Bone pain, asthenia, and fever
Verra et al 2003	59 Progressive RCC	IL-2: 4 MIU/m² SQ INF-α :5MIU SQ × 12 days every 3 wks	2.5 μg/kg SQ D 1–12	CR: 5/59 PR: 6/59 mOS: 9.5 mos.	Flu-like symptoms, transient LFT elevations
Lissoni et al 2003	25 Metastatic RCC	IL-2: 6 MIU/day SQ 6 D/wk × 4 wks 2 cycles (21-day intervals) Maintenance: 6 days/ month until progression	13 patients also received GM-CSF 0.3 μg /kg D 1–3 each week of Il-2	IL-2 alone PR: 3/13 SD: 8/13 IL-2 + GM PR: 3/12 SD: 6/12	More asthenia occurred in the IL-2 + GM group
Smith et al 2003	21 (13 with metastatic RCC)	IL-2: 72,000 IU/kg TID × on D 2–6 and D16–20 ± GM-CSF	125 or 250 μg /m²/day SQ D 1–7 and D 15–21	No PR or CR SD: 4/13 with RCC	Grade 3 confusion in 4 pts (3 on IL-2 alone)
Schmidinger et al 2001	55 Metastatic RCC	IL-2: 4.5 MU day 1–4 wks. 3 and 6 INF-γ: 100 μg TIW wks 1 and 4	400 μg SQ D 1–5 weeks 2 and 5	CR: 1/53,. PR: 4/53 SD: 14/53 mOS: 12 mos	No toxicities greater than grade 2
Westermann et al 2001	10 Stage IV RCC Pilot study	IL-2: 4 MU/m² SQ and INF-α: 5 MIU/m² SQ multiple dosing schedules	5 μg /kg SQ D 1 + 4 week 1 D 1, 3, 5 week 2 D 1, 3, 5 weeks 3–5 D 1, 3, 5 weeks 7–9	PR 2/10 1/10 mixed response	One grade 3 fever No other grade 3–4 toxicities
Tate et al 2001	13 Metastatic RCC, Phase I	IL-6 1, 5, or 10 μg/kg/day D 1–14	3 μg /kg/day D1–14	No responses	DLT: thrombocytosis and hyperbilirubinemia
De Gast et al 2000	18 11 RCC 7 melanoma Phase I	IL-2: 1, 4, or 8 MIU/m², and INF-α: 5 MIU SQ × 12 days every 3 wks	2.5 or 5 μg /kg/day SQ	CR: 3/11 SD: 5/11	DLT: fever with chills, hypotension, fluid retention
Ryan et al 2000	20 Metastatic RCC	IL-2: 11 MIU SQ D1–4 weekly INF-α: 10 MIU SQ 2 days/week c-RA 1 mg/kg daily orally for 4 weeks	1.25 μg /day SQ D 1–14	PR: 1 SD: 3 1-year survival rate: 48%	Grade 3 fever, fatigue, anorexia mucositis, and dermatitis One on-study death
Hotton et al 2000	16 pts with RCC and pulmonary metastases	IL-2: 1.5, 2.25, or 4.5 MIU/m²/day 96 h CIV D 1–4, 8–11, and 15–18	1.25, 2.25, or 2.5 g/kg/day SQ D 8–19	14 evaluable 0/14 had ≥50% shrinkage of total tumor burden nor reduction in pulmonary metastases	Grade 3–4 toxicities: lymphopenia, thrombocytopenia, elevated PT, thrombosis, hypotension, hypocalcemia, hyperglycemia, pain, constipation Grade 5: neurologic

Abbreviations: c-RA, cis-retinoic acid; CR, complete response; D, days; INF-α, interferon alpha; INF-γ, interferon gamma; OR, overall response; PR, partial response; pt, patient; SQ, subcutaneous; IL-2, interleukin 2; PR, partial response; mOS, median overall survival; LFT, liver function test; wks, weeks; mTTP, median time to progression; CIV, continuous intra-venous infusion; PT, prothrombin time.

Role of GM-CSF in the management of malignant melanoma

Unresectable melanoma carries a poor prognosis with limited options for treatment (Parmiani et al 2007). GM-CSF has been shown to induce cytotoxic T-cells and activated DC at tumor sites and draining lymph nodes (Parmiani et al 2007). The use of GM-CSF in combination with IL-2 or IFN-alpha has yielded promising results, but is associated with significant systemic toxicity. Table 2 summarizes activity and toxicities of GM-CSF-containing regimens in the treatment of malignant melanoma. Delivery of therapy into local sites of disease may circumvent systemic toxicity and is the subject of current investigation.

GM-CSF and cancer vaccine development

Addressing questions pertaining to the choice of vector, the specificity of the antigen, and the choice of co-stimulatory molecules is crucial to the optimal development of cancer vaccines. Tumor-associated antigens from tumor cells (both autologous and allogeneic), proteins, peptides, and nucleic acid have been used as immunogens. Genetically modified allogeneic tumor cells as well as recombinant viruses or bacterial genes have been utilized as vectors. Pre-existing immunity to the vector itself has limited the use of vaccines based on viral vectors (Rosenberg et al 1998). Vaccination in the absence of the co-stimulatory signals necessary to break tolerance can lead to anergy. Therefore, some vectors have been designed to express not only tumor-associated antigens, but also, co-stimulatory molecules and cytokines. Dranoff et al (1993) introduced GM-CSF as an important adjuvant in cancer vaccine trials, based on his observations that irradiated tumor cells expressing murine GM-CSF could stimulate potent, long-lasting, and tumor-specific immunity. In order to circumvent systemic toxicity and to increase immune responses, injection into the local tumor environment has been proposed. Hersch and colleagues used intratumor injection of HLA-B7/beta2-microglobulin genes as plasmid DNA in lipid into patients with malignant melanoma. In a phase I trial setting, they reported a 36% response at the locally injected tumor and a 19% systemic anti-tumor response (Hersh and Stopeck 1997).Vaccine trials utilizing GM-CSF or engineering tumor cells to secrete GM-CSF showed encouraging results in the treatment of solid tumors including: malignant melanoma, breast carcinoma, pancreatic cancer, renal cell carcinoma, non-small cell carcinoma of the lung and prostate cancer (Schmidt et al 1997; Simons et al 1997;

Hung et al 1998; Soiffer et al 1998; Disis et al 1999; Gaudernack and Gjertsen 1999; Leong et al 1999). Cassaday and colleagues performed a phase I study of immunization using particle-mediated epidermal delivery (PMED) of genes for gp100 and GM-CSF into uninvolved skin of melanoma patients. Two groups of 6 patients each were treated; group 1 received PMED with cDNA for gp100, and group 2 received PMED with cDNA for GM-CSF followed by PMED for gp100 at the same site. Biopsies were obtained and evaluated to assess transgene expression, gold-bead penetration, and dendritic cell infiltration. Exploratory studies included flow cytometric analyses of peripheral blood lymphocytes and evaluation of delayed-type hypersensitivity to gp100 peptide in HLA-A2 + patients. Local toxicity in both groups was mild and resolved within 2 weeks. No vaccine-related systemic toxicity was reported, including no induction of pathologic auto-antibodies. GM-CSF transgene expression in vaccinated skin sites was detected. GM-CSF and gp100 PMED yielded a greater infiltration of DC into vaccine sites than did gp100 PMED alone. Immunologic monitoring suggested modest activation of an anti-melanoma response (Cassaday et al 2007). This study demonstrated tolerability and induction of anti-melanoma immune responses with a local approach. Additional investigation utilizing this technique is warranted. Bendandi and colleagues tested the hypothesis that immune therapy is more effective in the setting of minimal residual disease (MRD). They documented clearance of the t(14,18) translocation by PCR from the peripheral circulation in 8 of 11 patients with lymphoma and MRD, after administration of a GM-CSF containing vaccine (Bendandi et al 1999). Currently our center participates in a multi-institutional trial of vaccine therapy for AML after remission induction. GM-CSF may enhance antigen presentation by recruiting DC to the (vaccine) site where antigen is taken up, processed, and presented to T-cells in draining lymph nodes, generating systemic tumor-specific responses (Borrello and Pardoll 2002).

Cell-mediated vaccines: role of dendritic cells

DC are ideal candidates for use in vaccination, owing to their role in antigen presentation. DC can be isolated from the peripheral circulation by FACS sorting or magnetic bead isolation, or can be generated in large quantities ex-vivo from peripheral blood progenitors in media supplemented with cytokines, including GM-CSF (Berthier et al 2000). These DC may then be matured with cytokine culture prior to loading with antigen. They have yielded promising

Table 2 GM-CSF-containing regimens in patients with malignant melanoma

Author	Population	Concurrent therapy	Dose of GM-CSF	Outcomes	Toxicities (most common)
Lutzky et al 2003	11	Thalidomide: intra-patient dose escalation 50–400 µg PO daily	125 µg/m² SQ daily × 14 days	3 alive without recurrent disease	Most common: fatigue, dizziness, somnolence, constipation
Weber et al 2005	31	Temozolamide: days 1–5 INFα-2b: days 6–17 IL-2: days 6–17	125 µg/m² SQ days 6–17	CR: 4 PR: 4 SD: 7 mOS: 15.9 mos	SAEs in 7 patients Most common toxicity: flu-like symptoms
De Gast et al 2003	74	Temozolamide: days 1–5 INFα-2b: days 6–17 IL-2: days 6–17 Repeated every 22 days in stable/ responding pts	2.5 µg/kg SQ days 6–17	CR: 4 s PR: 19 SD: 13 mOS: 252 day	DLT: thrombocytopenia Grade 3–4 lymphopenia was observed All patients developed flu-like syndrome
Groenewegen and de Gast 1999; Groenewegen et al 2002	32	DTIC: day 1 IL-2: days 6–17 INFα-2b: days 6–17	2.5 µg/kg SQ days 2–12	CR: 4 PR: 6 mOS: 8 mos	Treatment was well tolerated
Janik et al 2001	28	Topotecan: 1.5 mg/m² daily x 5 days Cycle repeated every 21 days	250 µg/m² SQ QD post CHT 250 µg/m² SQ BID × 5 D prior to CHT; or none	CR: 0 PR: 1	Treatment was well tolerated
Ravaud et al 2001	32	A: None B: DTIC 800 mg/m² D 1 21-day cycles Cross-over from A to B allowed for non-response/progression	A: 5 µg/kg BID SQ × 14 D B: 5 µg/kg BID SQ D2-19, 21-day cycles	Best response was SD: A: 0 B: 3 OS A: 6.3 months B: 7.3 months	Dose alteration due to toxicity in 20% A and 4.7% B Grade 3 or 4 toxicity occurred in 40% A and 76.5% B
Gajewski and Flickinger 2000	7	Cisplatin, DTIC: D1 IL-2: D 8–14 INF-α: D 8,10,12,14 28 day cycles	5 µg/kg SQ D 2–7	CR: 1 PR: 1 MR: 2	Treatment was well tolerated
Gibbs et al 2000	72	Temozolamide: D1–5 Cisplatin: D 1–3 IL-2: D 1–4 INF-α: D 1–5 28-day cycles	250 µg SQ days 6–25	CR: 1 PR: 11 mOS: 11 mos	Significant toxicity with grade 2 and 4 thrombocytopenia and renal impairment
Vaughan et al 2000	19	Cisplatin: D 1–3 DTIC: D 1–3 Tamoxifen: daily IL-2: D 6–10, 17–21 INFα-2b: days 6–10 and 17–21 28-day cycles	450 µg/m² SQ D 4,5,15,16 or 450 µg /m² SQ D 4,5,15,16 and 225 µg/m² SQ D 6–10, 17–21 or 450 µg /m² SQ D 4–10, 15–21	OR: 6 CR: 2 mOS: 6.2 mos.	Grade 3–4 toxicities: bone marrow suppression, hypotension, pulmonary edema, confusion, and increased serum creatinine
Schachter et al 1998	40	INF-α: days 1,3,5 Carmustine: day 8 Cisplatin: days 8–10 DTIC: days 8–10 Tamoxifen: daily 21-day cycles	20 µg/m²/day SQ × 7 days following chemotherapy	CR: 9 PR: 11 Survival benefit in responders: 22 mos. vs 8 mos.(p = 0.0001)	Treatment was well tolerated

Abbreviations: bid, twice daily; TIW, 3 times weekly; DTIC, dacarbazine; INF-α, interferon alpha; IL-2, interleukin 2; PD, progressive disease; PO, orally; PR, partial response; pt, patient; OR, overall response; mOS, median overall survival; SQ, subcutaneously; LFT, liver function test; wks, weeks; CHT, chemotherapy; D, days; DLT, dose-limiting toxicity; SAEs, serious adverse events.

Table 3 Clinical trials using GM-CSF transduced tumor cells as vaccines

Authors	Tumor type	Clinical results
Slingluff et al 2003 .	Melanoma Randomized comparison of multipeptide vaccine either in emulsion with GM-CSF or pulsed on monocytoid DCs	– Overall immune responses, including T-cell responses were superior in the GM-CSF arm, compared to the DC arm – Helper T-cell responses were detected and correlated with T-cell reactivity to the melanoma peptides – 2 PR in the GM-CSF arm, 1 in the DC arm. -2 SD in the GM-CSF arm and 1 in the DC arm – mOS:14.8 months for patients in the GM-CSF arm and 6.2 months for the DC arm.
Nelson et al 2000	Renal cell carcinoma	– Increase in DTH response against autologous tumor cells – 1 PR
Simons et al 1999; Simons and Sacks 2006	Prostate cancer	– Increase in DTH response – Induction of tumor specific T-cell and B-cell responses
Kusumoto et al 2001	Melanoma	– Increase in DTH response – Increase in melanoma specific CTLs in most patients
Soiffer et al 1998; Soiffer et al 2003	Melanoma	– Increase in DTH response – Eosinophilia – TILs highly cytotoxic – 1 CR, 1PR, 1MR, 3 minor responses
Salgia et al 2003	Non-small cell lung Cancer	– Increase in DTH response – T-cell and plasma cell infiltration of metastatic sites – 5 SD, 1 mixed response
Jaffee et al 2001	Pancreatic cancer	– Increase in DTH response – Increased systemic GM-CSF levels – 2/5 patients receiving highest cell dose >25 months in CR

Abbreviations: DTH, delayed type hypersensitivity; PR, partial response; CTLs, cytotoxic T lymphocytes; TILs, tumor infiltrating lymphocytes; MR, minor response; SD, stable disease; mOS, median overall survival; CR, complete response.

results and continue to be tested in the treatment of solid and hematological malignancies. Results from phase 1 and 2 clinical trials indicate that tumor-peptide loaded DC can induce clinically significant immune responses in patients with lymphoma and melanoma (Hsu et al 1996; Hersey et al 2004). Antigen-loaded DC as cancer vaccines have been limited by uncertainty regarding the best DC subtype to use, the optimal maturation status of the DC, the best site of administration (sub-cutaneous, intra-venous, or intra-nodal) and the optimal schedule of administration. More research is needed in order to answer these questions and define the optimal use of GM-CSF as an adjuvant in cell-mediated vaccines.

Future directions

Pre-clinical and clinical data support the role of GM-CSF as an immune adjuvant in the treatment of malignant solid and liquid tumors, but the challenge remains to devise combinations of cytotoxic and cytokine therapy which are synergistic in breaking immune tolerance, enhancing antigen presentation and up-regulating anti-tumor T-cell responses. Local production of GM-CSF by tumors in the setting of tumor-specific vaccination has shown promise in the induction of anti-tumor immune responses. However, the laboratory correlates of response; such as, the lymphocytic and inflammatory infiltrates that develop at the site of vaccination and cytokine injection, do not reproducibly correlate with improved clinical outcomes, and well designed translational studies are needed to better define the anti-tumor activity of GM-CSF and other cytokines. Vaccines and cytokine therapies are attractive for use in patients who cannot tolerate further cytotoxic chemotherapy, owing to their relatively low toxicity, and in patients whose tumors are in a minimal residual disease state.

The success of cytokine therapy will likely depend upon defining the most favorable combination of cytokines, the optimal site and route of administration, reaching a MRD status prior to cytokine therapy, and development of surrogate endpoints of anti-tumor activity that can be used to design subsequent clinical trials.

Disclosures

MA receives research support from Bayer Healthcare. SL has served as a consultant for, and received research support from, Berlex (now known as Bayer Healthcare).

References

Amsen D, Blander JM, Lee GR, et al. 2004. Instruction of distinct CD4 T helper cell fates by different notch ligands on antigen-presenting cells. *Cell,* 117:515–26.

Arellano ML, Langston A, Winton E, et al. 2007. Treatment of relapsed acute leukemia after allogeneic transplantation: a single center experience. *Biol Blood Marrow Transplant,* 13 116–23.

Arpinati M, Green CL, Heimfeld S, et al. 2000. Granulocyte-colony stimulating factor mobilizes T helper 2-inducing dendritic cells. *Blood,* 95:2484–90.

Balkwill F, Mantovani A. 2001. Inflammation and cancer: back to Virchow? *Lancet,* 357:539–45.

Bancereau J, Steinman RM. 1998. Dendritic cells and the control of immunity. *Nature,* 392:245–52.

Banchereau J, Briere F, Caux C, et al. 2000. Immunobiology of dendritic cells. *Ann Rev Immunol,* 18:767–811.

Basak Saroj K, Harui A, Stolina M, et al. 2002. Increased dendritic cell number and function following continuous in vivo infusion of granulocyte macrophage-colony-stimulating factor and interleukin-4. *Blood,* 99:2869–79.

Bendandi M, Gocke CD, Kobrin CB, et al. 1999. Complete molecular remissions induced by patient-specific vaccination plus granulocyte-monocyte colony-stimulating factor against lymphoma. *Nat Med,* 5:1171–7.

Bensinger WI, Martin PJ, Storer B, et al. 2001. Transplantation of bone marrow as compared with peripheral-blood cells from HLA-identical relatives in patients with hematologic cancers. [see comments]. *N Engl J Med,* 344:175–81.

Berthier R, Martinon-Ego C, Laharie AM, et al. 2000. A two-step culture method starting with early growth factors permits enhanced production of functional dendritic cells from murine splenocytes. *J Immunol Methods,* 239:95–107.

Borrello I and Pardoll D. 2002. GM-CSF-based cellular vaccines: a review of the clinical experience. *Cytokine Growth Factor Rev,* 13:185–93.

Boyer MW, Waller EK, Bray RA, et al. 2000. Cytokine upregulation of the antigen presenting function of acute myeloid leukemia cells. *Leukemia,* 14:412–8.

Cashen AF, Link D, Devine S, et al. 2004. Cytokines and stem cell mobilization for autologous and allogeneic transplantation. *Curr Hematol Rep,* 3:406–12.

Cassaday RD, Sondel PM, King DM, et al. 2007. A phase I study of immunization using particle-mediated epidermal delivery of genes for gp100 and GM-CSF into uninvolved skin of melanoma patients. *Clin Cancer Res,* 13:540–9.

Chow KU, Schneider B, Mitrou PS, et al. 2001. Influence of various cytokines on the expression of CD20 on the surface of CLL-cells in vitro. *Leuk Res,* 25:99–100.

Coccia EM, Severa M, Giacomini E, et al. 2004. Viral infection and Toll-like receptor agonists induce a differential expression of type I and lambda interferons in human plasmacytoid and monocyte-derived dendritic cells. *Eur J Immunol,* 34:796–805.

Coppin C, Porzsolt F, Awa A, et al. 2005. Immunotherapy for advanced renal cell cancer. *Cochrane Database Syst Rev,* CD001425.

Correale P, Marsili S, Sabatino M, et al. 2005. Immunotherapy of renal cell carcinoma with granulocyte macrophage colony stimulating factor and very low dose interleukin-2. *Oncol Rep,* 13:751–6.

Cortes J, Kantarjian H, O'brien S, et al. 1998. GM-CSF can improve the cytogenetic response obtained with interferon-alpha therapy in patients with chronic myelogenous leukemia. *Leukemia,* 12:860–4.

Cuenca A, Cheng F, Wang H, et al. 2003. Extra-lymphatic solid tumor growth is not immunologically ignored and results in early induction of antigen-specific t-cell anergy: dominant role of cross-tolerance to tumor antigens. *Cancer Res,* 63:9007–9015.

Cvetkovic RS, Perry CM. 2006. Rituximab: a review of its use in non-Hodgkin's lymphoma and chronic lymphocytic leukaemia. *Drugs,* 66:791–820.

Davidson JA, Musk AW, Wood BR, et al. 1998. Intralesional cytokine therapy in cancer: a pilot study of GM-CSF infusion in mesothelioma. *J Immunother,* 21:389–98.

De Gast GC, Batchelor D, Kersten MJ, et al. 2003. Temozolomide followed by combined immunotherapy with GM-CSF, low-dose IL2 and IFN alpha in patients with metastatic melanoma. *British Journal of Cancer,* 88:175–80.

De Gast GC, Klumpen HJ, Vyth-Dreese FA, et al. 2000. Phase I trial of combined immunotherapy with subcutaneous granulocyte macrophage colony-stimulating factor, low-dose interleukin 2, and interferon alpha in progressive metastatic melanoma and renal cell carcinoma. *Clin Cancer Res,* 6:1267–72.

Dillon S, Agrawal A, Van Dyke T, et al. 2004. A toll-like receptor 2 ligand stimulates Th2 responses in vivo, via induction of extracellular signal-regulated kinase mitogen-activated protein kinase and c-Fos in dendritic cells. *J Immunol,* 172:4733–43.

Disis ML, Grabstein KH, Sleath PR, et al. 1999. Generation of immunity to the HER-2/neu oncogenic protein in patients with breast and ovarian cancer using a peptide-based vaccine. *Clin Cancer Res,* 5:1289–1297.

Dranoff G, Crawford AD, Sadelain M, et al. 1994. Involvement of granulocyte-macrophage colony stimulating factor in pulmonary homeostasis. *Science,* 264:713–6.

Dranoff G, Jaffee E, Lazenby A, et al. 1993. Vaccination with irradiated tumor cells engineered to secrete murine granulocyte-macrophage colony-stimulating factor stimulates potent, specific, and long-lasting anti-tumor immunity. *Proc Natl Acad Sci,* USA, 90:3539–43.

Eksioglu EA, Mahmood SS, Chang M, et al. 2007. GM-CSF promotes differentiation of human dendritic cells and T lymphocytes toward a predominantly type 1 proinflammatory response. *Exp Hematol,* 35:1163–71.

Enzler T, Gillessen S, Manis JP, et al. 2003. Deficiencies of GM-CSF and interferon gamma link inflammation and cancer. *J Exp Med,* 197:1213–9.

Fagnoni FF, Oliviero B, Giorgiani G, et al. 2004. Reconstitution dynamics of plasmacytoid and myeloid dendritic cell precursors after allogeneic myeloablative hematopoietic stem cell transplantation. *Blood,* 104:281–9.

Fantuzzi G. 2003. Cytokine Knockouts. New Jersey: Humana Press.

Fattorossi A, Battaglia A , Pierelli L, et al. 2001. Effects of granulocyte-colony-stimulating factor and granulocyte/macrophage-colony-stimulating factor administration on T-cell proliferation and phagocyte cell-surface molecules during hematopoietic reconstitution after autologous peripheral blood progenitor cell transplantation. *Cancer Immunol Immunother,* 49:641–8.

Ferlazzo G, Klein J, Paliard X, et al. 2000. Dendritic cells generated from CD34+ progenitor cells with flt3 ligand, c-kit ligand, GM-CSF, IL4, and TNF-alpha are functionalantigen-presneting cells resembling monocyte-derived dendritic cells. *Journal of Immunotherapy,* 23:48–58.

Fonteneau JF, Gilliet M, Larsson M, et al. 2003. Activation of influenza virus-specific CD4+ and CD8+ T-cells: a new role for plasmacytoid dendritic cells in adaptive immunity. *Blood,* 101:3520–3526.

Gajewski T, Flickinger S. 2000. A Phase II study of outpatient chemoimmunotherapy using cisplatin and DTIC followed by GM-CSF, IL-2, and IFN-alpha-2b in patients with metastatic melanoma. *Proc Am Soc Clin Oncol,* 19:(abstr 2271).

Garcia-Carbonero R, Mayordomo JI, Tornamira MV, et al. 2001. Granulocyte colony-stimulating factor in the treatment of high-risk febrile neutropenia: a multicenter randomized trial. *J Natl Cancer Inst,* 93:31–38.

Gasparetto C, Gasparetto M, Morse M, et al. 2002. Mobilization of dendritic cells from patients with breast cancer into peripheral blood stem cell leukapheresis samples using Flt-3-ligand and G-CSF or GM-CSF. *Cytokine,* 18:8–19.

Gaudernack G, Gjertsen MK. 1999. Combination of GM-CSF with antitumour vaccine strategies. *Eur J Cancer,* 35:S33–5.

Gazitt Y, Akay C, Thomas C 3rd. 2006. No polarization of type 1 or type 2 precursor dendritic cells in peripheral blood stem cell collections of non-hodgkin's lymphoma patients mobilized with cyclophosphamide plus G-CSF, GM-CSF, or GM-CSF followed by G-CSF. *Stem Cells Dev,* 15:269–77.

Gibbs P, O`Day S, Richards J, et al. 2000. A multicenter phase ii study of modified biochemotherapy (BCT) for stage IV melanoma incorporating temozolomide, decrescendo interleukin-2 (IL-2) and GM-CSF. *Proc Am Soc Clin Oncol (abstr 2255),* 19.

Gilliet M, Liu YJ. 2002. Generation of human CD8 T regulatory cells by CD40 ligand-activated plasmacytoid dendritic cells. *J Exp Med,* 195:695–704.

Groenewegen G, Bloem A, De Gast GC. 2002. Phase I/II study of sequential chemoimmunotherapy (SCIT) for metastatic melanoma: outpatient treatment with dacarbazine, granulocyte-macrophage colony-stimulating factor, low-dose interleukin-2, and interferon-alpha. *Cancer Immunol Immunother,* 51:630–6.

Groenewegen G, De Gast GC. 1999. GM-CSF can cause T cell activation; results of sequential chemo-immunotherapy. *Eur J Cancer,* 35(Suppl 3):S23–4.

Grouard G, Rissoan MC, Filgueira L, et al. 1997. The enigmatic plasmacytoid T cells develop into dendritic cells with interleukin (IL)-3 and CD40-ligand. *J Exp Med,* 185:1101–11.

Hart DN. 1997. Dendritic cells: unique leukocyte populations which control the primary immune response. *Blood,* 90:3245–87.

Hersey P, Menzies SW, Halliday GM, et al. 2004. Phase I/II study of treatment with dendritic cell vaccines in patients with disseminated melanoma. *Cancer Immunol Immunother,* 53:125–34.

Hersh EM, Stopeck AT. 1997. Advances in the biological therapy and gene therapy of malignant disease. *Clin Cancer Res,* 3:2623–9.

Hoption Cann SA, Van Netten JP, Van Netten C, et al. 2002. Spontaneous regression: a hidden treasure buried in time. *Med Hypotheses,* 58:115–19.

Hotton KM, Khorsand M, Hank JA, et al. 2000. A phase Ib/II trial of granulocyte-macrophage-colony stimulating factor and interleukin-2 for renal cell carcinoma patients with pulmonary metastases: a case of fatal central nervous system thrombosis. *Cancer,* 88:1892–901.

Hsu FJ, Benike C, Fagnoni F, et al. 1996. Vaccination of patients with B-cell lymphoma using autologous antigen-pulsed dendritic cells. *Nat Med,* 2:52–8.

Hung K, Hayashi R, Lafond-Walker A, et al. 1998. The central role of CD4(+) T cells in the antitumor immune response. *J Exp Med,* 188:2357–68.

Inaba K, Inaba M, Romani N, et al. 1992. Generation of large numbers of dendritic cells from mouse bone marrow cultures supplemented with granulocyte/macrophage colony-stimulating factor. *J Exp Med,* 176:1693–702.

Jaffee EM, Hruban RH, Biedrzycki B, et al. 2001. Novel allogeneic granulocyte-macrophage colony-stimulating factor-secreting tumor vaccine for pancreatic cancer: a phase I trial of safety and immune activation. *J Clin Oncol,* 19:145–56.

Janik JE, Miller LL, Korn EL, et al. 2001. A prospective randomized phase II trial of GM-CSF priming to prevent topotecan-induced neutropenia in chemotherapy-naive patients with malignant melanoma or renal cell carcinoma. *Blood,* 97:1942–6.

Kadowaki N, Ho S, Antonenko S, et al. 2001. Subsets of human dendritic cell precursors express different toll-like receptors and respond to different microbial antigens. *J Exp Med,* 194:863–9.

Keilholz U, Weber J, Finke JH, et al. 2002. Immunologic monitoring of cancer vaccine therapy: results of a workshop sponsored by the Society for Biological Therapy. *J Immunother,* 25:97–138.

Kessinger A, Armitage JO, Landmark JD, et al. 1986. Reconstitution of human hematopoietic function with autologous cryopreserved circulating stem cells. *Exp Hematol,* 14:192–6.

Kolb HJ, Rank A, Chen X, et al. 2004. In-vivo generation of leukaemia-derived dendritic cells. *Best Pract Res Clin Haematol,* 17:439–51.

Korbling M, Anderlini P. 2001. Peripheral blood stem cell versus bone marrow allotransplantation: does the source of hematopoietic stem cells matter? *Blood,* 98:2900–8.

Korbling M, Burke P, Braine H, et al. 1981. Successful engraftment of blood derived normal hemopoietic stem cells in chronic myelogenous leukemia. *Exp Hematol,* 9:684–90.

Kusumoto M, Umeda S, Ikubo A, et al. 2001. Phase 1 clinical trial of irradiated autologous melanoma cells adenovirally transduced with human GM-CSF gene. *Cancer Immunol Immunother,* 50:373–81.

Kuwana M, Kaburaki J, Wright TM, et al. 2001. Induction of antigen-specific human CD4(+) T cell anergy by peripheral blood DC2 precursors. *Eur J Immunol,* 31:2547–57.

Larsson M, Fonteneau JF, Bhardwaj N. 2003. Cross-presentation of cell-associated antigens by dendritic cells. *Curr Top Microbiol Immunol,* 276:261–75.

Leong SP, Enders-Zohr P, Zhou YM, et al. 1999. Recombinant human granulocyte macrophage-colony stimulating factor (rhGM-CSF) and autologous melanoma vaccine mediate tumor regression in patients with metastatic melanoma. *J Immunother,* 22:166–74.

Levine AM, Reed JA, Kurak KE, et al. 1999. GM-CSF-deficient mice are susceptible to pulmonary group B streptococcal infection. *J Clin Invest,* 103:563–9.

Li JM, Waller EK. 2004. Donor antigen-presenting cells regulate T-cell expansion and antitumor activity after allogeneic bone marrow transplantation. *Biol Blood Marrow Transplant,* 10:540–51.

Lieschke GJ, Grail D, Hodgson G, et al. 1994. Mice lacking granulocyte colony-stimulating factor have chronic neutropenia, granulocyte and macrophage progenitor cell deficiency, and impaired neutrophil mobilization. *Blood,* 84:1737–46.

Lissoni P, Mengo S, Bucovec R, et al. 2003. Clinical and biological effects of interleukin-2 with or without a concomitant administration of granulocyte-macrophage colony-stimulating factor in metastatic cancer patients. *In Vivo,* 17:73–5.

Lonial S, Hicks M, Rosenthal H, et al. 2004. A randomized trial comparing the combination of granulocyte-macrophage colony-stimulating factor plus granulocyte colony-stimulating factor versus granulocyte colony-stimulating factor for mobilization of dendritic cell subsets in hematopoietic progenitor cell products. *Biol Blood Marrow Transplant,* 10:848–57.

Lonial S, Mcmillan S, Torre C, et al. 2006. A randomized trial to evaluate the impact of cytokines (G-CSF or GM + G-CSF) on dendritic cell and T-cell content and function when mobilizing normal donors for allogeneic progenitor cell transplant. *Blood,* 108:(abstr 3377).

Lutzky J, Blaustein A, Weingrad D, et al. 2003. Phase I/II study of GM-CSF and thalidomide in patients with high-risk malignant melanoma. *Proc Am Soc Clin Oncol,* 22:(abstr 2916).

Masucci G, Ragnhammar P, Wersall P, et al. 1990. Granulocyte-monocyte colony-stimulating-factor augments the interleukin-2-induced cytotoxic activity of human lymphocytes in the absence and presence of mouse or chimeric monoclonal antibodies (mAb 17-1A). *Cancer Immunol Immunother,* 31:231–5.

Matzinger P. 1994. Tolerance, Danger, and the Extended Family. *Ann Rev Immunol,* 12:991–1045.

Metcalf D. 1998. Cell-cell signalling in the regulation of blood cell formation and function. *Immunol Cell Biol,* 76:441–7.

Metcalf D, Nicola NA. 1995. The hematopoietic colony stimulating factors. New York, NY: Cambridge University Press.

Mizutani K, Takeuchi S, Ohashi Y, et al. 2003. Clinical usefulness of macrophage colony-stimulating factor for ovarian cancers: Long-term prognosis after five years. *Oncol Rep,* 10:127–31.

Mohty M, Olive D and Gaugler B. 2002. Leukemic dendritic cells: potential for therapy and insights towards immune escape by leukemic blasts. *Leukemia,* 16:2197–204.

Motzer RJ, Bukowski RM. 2006. Targeted therapy for metastatic renal cell carcinoma. *J Clin Oncol,* 24:5601–8.

Nauts HC, Fowler GA, Bogatko FH. 1953. A review of the influence of bacterial infection and of bacterial products (Coley's toxins) on malignant tumors in man; a critical analysis of 30 inoperable cases treated by Coley's mixed toxins, in which diagnosis was confirmed by microscopic examination selected for special study. *Acta Med Scand Suppl,* 44:1–103.

Nelson WG, Simons JW, Mikhak B, et al. 2000. Cancer cells engineered to secrete granulocyte-macrophage colony-stimulating factor using ex vivo gene transfer as vaccines for the treatment of genitourinary malignancies. *Cancer Chemother Pharmacol,* 46(Suppl):S67–72.

Olivieri A, Lucesole M, Capelli D, et al. 2005. A new schedule of CHOP/rituximab plus granulocyte-macrophage colony-stimulating factor is an effective rescue for patients with aggressive lymphoma failing autologous stem cell transplantation. *Biol Blood Marrow Transplant,* 11:627–36.

Olweus J, Bitmansour A, Warnke R, et al. 1997. Dendritic cell ontogeny: a human dendritic cell lineage of myeloid origin. *Proc Natl Acad Sci,* USA, 94:12551–6.

Paine R 3rd, Morris SB, Jin H, et al. 2001. Impaired functional activity of alveolar macrophages from GM-CSF-deficient mice. *Am J Physiol Lung Cell Mol Physiol,* 281:L1210–8.

Parmiani G, Castelli C, Santinami M, et al. 2007. Melanoma immunology: past, present and future. *Curr Opin Oncol,* 19:121–7.

Perrot I, Blanchard D, Freymond N, et al. 2007. Dendritic cells infiltrating human non-small cell lung cancer are blocked at immature stage. *J Immunol,* 178:2763–9.

Rapoport AP, Meisenberg B, Sarkodee-Adoo C, et al. 2002. Autotransplantation for advanced lymphoma and Hodgkin's disease followed by post-transplant rituxan/GM-CSF or radiotherapy and consolidation chemotherapy. *Bone Marrow Transplant,* 29:303–12.

Ravaud A, Delaunay M, Chevreau C, et al. 2001. Granulocyte-macrophage colony-stimulating factor alone or with dacarbazine in metastatic melanoma: a randomized phase II trial. *Br J Cancer,* 85:1467–71.

Reddy V, Iturraspe JA, Tzolas AC, et al. 2004. Low dendritic cell count after allogeneic hematopoietic stem cell transplantation predicts relapse, death, and acute graft-versus-host disease. *Blood,* 103:4330–5.

Repetto L, Biganzoli L, Koehne CH, et al. 2003. EORTC Cancer in the Elderly Task Force guidelines for the use of colony-stimulating factors in elderly patients with cancer. *European Journal of Cancer,* 39:2264–72.

Ringden O, Labopin M, Gorin NC, et al. 2004. Treatment with granulocyte colony-stimulating factor after allogeneic bone marrow transplantation for acute leukemia increases the risk of graft-versus-host disease and death: a study from the acute leukemia working party of the European Group for Blood and Marrow Transplantation. *J Clin Oncol,* 22:416–23.

Rissoan MC, Soumelis V, Kadowaki N, et al. 1999. Reciprocal control of T helper cell and dendritic cell differentiation. *Science,* 283:1183–6.

Rosenberg SA, Zhai Y, Yang JC, et al. 1998. Immunizing patients with metastatic melanoma using recombinant adenoviruses encoding MART-1 or gp100 melanoma antigens. *J Natl Cancer Inst,* 90:1894–1900.

Rowe JM, Andersen JW, Mazza JJ, et al. 1995. A randomized placebo-controlled phase III study of granulocyte-macrophage colony-stimulating factor in adult patients (>55 to 70 years of age) with acute myelogenous leukemia: a study of the Eastern Cooperative Oncology Group (E1490). *Blood,* 86:457–62.

Ryan CW, Vogelzang NJ, Dumas MC, et al. 2000. Granulocyte-macrophage-colony stimulating factor in combination immunotherapy for patients with metastatic renal cell carcinoma: results of two phase II clinical trials. *Cancer,* 88:1317–24.

Salgia R, Lynch T, Skarin A, et al. 2003. Vaccination with irradiated autologous tumor cells engineered to secrete granulocyte-macrophage colony-stimulating factor augments antitumor immunity in some patients with metastatic non-small-cell lung carcinoma. *J Clin Oncol,* 21:624–30.

Santiago-Schwarz F, Belilos E, Diamond B, et al. 1992. TNF in combination with GM-CSF enhances the differentiation of neonatal cord blood stem cells into dendritic cells and macrophages. *J Leukoc Biol,* 52:274–81.

Santiago-Schwarz F, Coppock DL, Hindenburg AA , et al. 1994. Identification of a malignant counterpart of the monocyte-dendritic cell progenitor in an acute myeloid leukemia. *Blood,* 84:3054–62.

Schachter J, Rakowsky E, Sulkes A, et al. 1998. A sequential four-drug chemotherapy and biotherapy with interferon alpha and GM-CSF-an innovative protocol for the treatment of metastatic melanoma. *Cancer Biother Radiopharm,* 13:155–64.

Schmidinger M, Steger G, Wenzel C, et al. 2001. Sequential administration of interferon-gamma, GM-CSF, and interleukin-2 in patients with metastatic renal cell carcinoma: results of a phase II trial. *J Immunother,* 24:257–62.

Schmidt W, Maass G, Buschle M, et al. 1997. Generation of effective cancer vaccines genetically engineered to secrete cytokines using adenovirus-enhanced transferrinfection (AVET). *Gene,* 190:211–16.

Schmitz N, Bacigalupo A, Hasenclever D, et al. 1998. Allogeneic bone marrow transplantation vs filgrastim-mobilised peripheral blood progenitor cell transplantation in patients with early leukaemia: first results of a randomised multicentre trial of the European Group for Blood and Marrow Transplantation [see comments]. *Bone Marrow Transplant,* 21:995–1003.

Schmitz N, Beksac M, Hasenclever D, et al. 2002. Transplantation of mobilized peripheral blood cells to HLA-identical siblings with standard-risk leukemia. *Blood,* 100:761–7.

Schreiber H. 2003. Tumor Immunology. In Paul W (Ed.) Fundamental Immunology. 5th ed. Lippincott, Williams, and Wilkins.

Seymour JF, Lieschke GJ, Grail D, et al. 1997. Mice lacking both granulocyte colony-stimulating factor (CSF) and granulocyte-macrophage CSF have impaired reproductive capacity, perturbed neonatal granulopoiesis, lung disease, amyloidosis, and reduced long-term survival. *Blood,* 90:3037–49.

Siegal FP, Kadowaki N , Shodell M, et al. 1999. The nature of the principal type 1 interferon-producing cells in human blood. *Science,* 284:1835–7.

Simons JW, Jaffee EM, Weber CE, et al. 1997. Bioactivity of autologous irradiated renal cell carcinoma vaccines generated by ex vivo granulocyte-macrophage colony-stimulating factor gene transfer. *Cancer Res,* 57:1537–46.

Simons JW, Mikhak B, Chang JF, et al. 1999. Induction of immunity to prostate cancer antigens: results of a clinical trial of vaccination with irradiated autologous prostate tumor cells engineered to secrete granulocyte-macrophage colony-stimulating factor using ex vivo gene transfer. *Cancer Res,* 59:5160–8.

Simons JW, Sacks N. 2006. Granulocyte-macrophage colony-stimulating factor-transduced allogeneic cancer cellular immunotherapy: the GVAX vaccine for prostate cancer. *Urol Oncol,* 24:419–24.

Slavin S, Naparstek E, Nagler A, et al. 1996. Allogeneic cell therapy with donor peripheral blood cells and recombinant human interleukin-2 to treat leukemia relapse after allogeneic bone marrow transplantation. *Blood,* 87:2195–204.

Slingluff CL Jr, Petroni GR, Yamshchikov GV, et al. 2003. 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,* 21:4016–26.

Smith Ii JW, Kurt RA, Baher AG, et al. 2003. Immune effects of escalating doses of granulocyte-macrophage colony-stimulating factor added to a fixed, low-dose, inpatient interleukin-2 regimen: a randomized phase I trial in patients with metastatic melanoma and renal cell carcinoma. *J Immunother,* 26:130–8.

Smith TJ, Khatcheressian J, Lyman GH, et al. 2006. 2006 update of recommendations for the use of white blood cell growth factors: an evidence-based clinical practice guideline. *J Clin Oncol,* 24:3187–205.

Soiffer R, Hodi FS, Haluska F, et al. 2003. 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,* 21:3343–50.

Soiffer R, Lynch T, Mihm M, et al. 1998. 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, 95:13141–6.

Stanley E, Lieschke GJ, Grail D, et al. 1994. Granulocyte/macrophage colony-stimulating factor-deficient mice show no major perturbation of hematopoiesis but develop a characteristic pulmonary pathology. *Proc Natl Acad Sci,* USA, 91:5592–6.

Tate J, Olencki T, Finke J, et al. 2001. Phase I trial of simultaneously administered GM-CSF and IL-6 in patients with renal-cell carcinoma: clinical and laboratory effects. *Ann Oncol,* 12:655–9.

Urbini B, Arpinati M, Bonifazi F, et al. 2003. Allogeneic graft CD34(+) cell dose correlates with dendritic cell dose and clinical outcome, but not with dendritic cell reconstitution after transplant. *Exp Hematol,* 31:959–65.

Vaughan MM, Moore J, Riches PG, et al. 2000. GM-CSF with biochemotherapy (cisplatin, DTIC, tamoxifen, IL-2 and interferon-alpha): a phase I trial in melanoma. *Ann Oncol,* 11:1183–9.

Venugopal P, Sivaraman S, Huang XK, et al. 2000. Effects of cytokines on CD20 antigen expression on tumor cells from patients with chronic lymphocytic leukemia. *Leuk Res,* 24:411–5.

Vereecque R, Buffenoir G, Preudhomme C, et al. 2000. Gene transfer of GM-CSF, CD80 and CD154 cDNA enhances survival in a murine model of acute leukemia with persistence of a minimal residual disease. *Gene Therapy,* 7:1312–16.

Verra N, De Jong D, Bex A, et al. 2005. Infiltration of activated dendritic cells and T cells in renal cell carcinoma following combined cytokine immunotherapy. *Eur Urol,* 48:527–33.

Verra N, Jansen R, Groenewegen G, et al. 2003. Immunotherapy with concurrent subcutaneous GM-CSF, low-dose IL-2 and IFN-alpha in patients with progressive metastatic renal cell carcinoma. *Br J Cancer,* 88:1346–51.

Volpi I, Perruccio K, Tosti A, et al. 2001. Postgrafting administration of granulocyte colony-stimulating factor impairs functional immune recovery in recipients of human leukocyte antigen haplotype-mismatched hematopoietic transplants. *Blood,* 97:2514–21.

Waller EK, Rosenthal H, Jones TW, et al. 2001. Larger numbers of CD4(bright) dendritic cells in donor bone marrow are associated with increased relapse after allogeneic bone marrow transplantation. *Blood,* 97:2948–56.

Wang HY, Wang RF. 2007. Regulatory T cells and cancer. *Current Opinion in Immunology,* 19:217–23.

Weber RW, O'Day S, Rose M, et al. 2005. Low-dose outpatient chemobiotherapy with temozolomide, granulocyte-macrophage colony stimulating factor, interferon-{alpha}2b, and recombinant interleukin-2 for the treatment of metastatic melanoma. *J Clin Oncol,* 23:8992–9000.

Westermann J, Reich G, Kopp J, et al. 2001. Granulocyte/macrophage-colony-stimulating-factor plus interleukin-2 plus interferon alpha in the treatment of metastatic renal cell carcinoma:a pilot study. *Cancer Immunol Immunother,* 49:613–20.

Whiteway A, Corbett T, Anderson R, et al. 2003. Expression of co-stimulatory molecules on acute myeloid leukaemia blasts may effect duration of first remission. *Br J Haematol,* 120:442–51.

Wos E, Olencki T, Tuason L, et al. 1996. Phase II trial of subcutaneously administered granulocyte-macrophage colony-stimulating factor in patients with metastatic renal cell carcinoma. *Cancer,* 77:1149–53.

Yagci M, Akar I, Sucak GT, et al. 2005. GM-CSF does not increase CD20 antigen expression on chronic lymphocytic leukemia lymphocytes. *Leuk Res,* 29:735–8.

Zhan Y, Lieschke GJ, Grail D, et al. 1998. Essential roles for granulocyte-macrophage colony-stimulating factor (GM-CSF) and G-CSF in the sustained hematopoietic response of Listeria monocytogenes-infected mice. *Blood,* 91:863–9.

Zittoun R , Suciu S , Mandelli F, et al. 1996. Granulocyte-macrophage colony-stimulating factor associated with induction treatment of acute myelogenous leukemia:a randomized trial by the European Organization for Research and Treatment of Cancer Leukemia Cooperative Group. *J Clin Oncol,* 14:2150–9.