reported relative risks 2–3 times higher among smokers of black tobacco than of blond tobacco cigarettes. The greater effect of the black variety was not explained by potential confounders. It is also interesting to note that the relative risk for blond tobacco smokers was around 2.0, i.e. similar to the estimates found for smoking in countries where black tobacco has not been used in the last decades [7].

Some effect modifications were noted for other smoking characteristics. Increasing age at start seemed to be associated with decreasing relative risks in a few investigations [5, 6, 8], irrespective of type of tobacoo. In the Italian study, this pattern was evident for smokers of black tobacco but not for smokers of blond tobacco, after allowing for time since quitting. In addition, switching from black to blond tobacco did not change the risk substantially in comparison with smoking black tobacco throughout life, indicating a more important effect of the black variety early in life.

Quitting smoking is associated with a dramatic drop in the risks of bladder cancer [7], irrespective of the type of tobacco. In the two studies which considered the effect of quitting separately by type, the relative risk remained well above the level of non-smokers only for smokers of black tobacco.

The study of time-related variables is complex, since they are strictly correlated. Only if a large proportion of the subjects stopped smoking and then started again several times, a distinction between duration, age started and time since quitting can be made on statistical grounds; usually, however, such a distinction cannot be made [9]. The overall picture concerning the type of tobacco and bladder cancer, as far as time-related variables are concerned, seems to be the following: in general, tobacco smoking mainly exerts a "late stage" action, as is clearly indicated by the rapid drop in risks after discontinuation. Black tobacco, however, seems to have also an "early stage" action, as suggested by the stronger association with age at start, the fact that the relative risks remain high after 10 or 15 years since quitting and the fact that switching to blond tobacco does not seem to be different from continuing to smoke black tobacco.

Other observations are interesting, including the lack of protection from the use of filters among black tobacco smokers. This seems to indicate that bladder carcinogens belong to the highly volatile fraction of smoke, possibly aromatic amines. In addition, such carcinogens seem to be affected by the level of inhalation, as the French study suggests. Finally, the dose-response relation between the number of cigarettes smoked and the risk of bladder cancer (Table 4) is worth mentioning. In most of the studies there is a less than linear relation, with a steep increase at low doses and a plateau at high doses. This observation might indicate that metabolic pathways of activation/deactivation of bladder carcinogens are of importance in the carcinogenic process. All these hypotheses have to be verified in biochemical epidemiology investigations.

1. Elson LA, Betts TE. Death rates from cancer of the respiratory and oral tracts in different countries, in relation to the types of tobacco smoked. *Eur J Cancer Clin Oncol* 1981, **17**, 109–113.
2. Vineis P, Estève J, Terracini B. Bladder cancer and smoking in males: type of cigarettes, age at start, effect of stopping, and interaction with occupation. *Int J Cancer* 1984, **34**, 165–170.
3. Vineis P, Estève J, Hartge P, Hoover R, Silverman DT, Terracini B. Effects of timing and type of tobacco in cigarette-induced bladder cancer. *Cancer Res* 1988, **48**, 3849–3852.
4. Iscovich J, Castelletto R, Estève J, *et al.* Tobacco smoking, occupational exposure and bladder cancer in Argentina. *Int J Cancer* 1987, **40**, 734–740.
5. Clavel J, Cordier S, Boccon-Gibod L, Hemon D. Tobacco and bladder cancer in males: increased risk for inhalers and black tobacco smokers. *Int J Cancer* 1989, **44**, 605–610.
6. De Stefani E, Correa P, Fierro L, Fontham E, Chen V, Zavala D. Black tobacco, mate, and bladder cancer. A case-control study from Uruguay. *Cancer* 1991, **67**, 536–540.
7. IARC. *Tobacco Smoking*. Monographs on the Evaluation of the Carcinogenic Risk of Chemicals to Humans, vol. 38. Lyon, IARC, 1986.
8. Hartge P, Silverman D, Hoover R, *et al.* Changing cigarette habits and bladder cancer risk: a case control study. *J Natl Cancer Inst* 1987, **78**, 1119–1125.
9. Hayes R, Vineis P. Time dependence in human cancer. *Tumori* 1989, **75**, 189–195.

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Emerging Clinical Uses for GM-CSF

J. Howard Scarffe

INTRODUCTION

GRANULOCYTE–MACROPHAGE colony-stimulating factor (GM-CSF), like interleukin-3 (IL-3), displays a broader range of haematopoietic activities than the more lineage-restricted factors such as granulocyte colony-stimulating factor (G-CSF), macrophage stimulating factor (M-CSF) and erythropoietin. GM-CSF interacts with early, multipotent progenitor cells, promoting the growth and differentiation of burst and colony-forming units. GM-CSF is not as effective as IL-3 at supporting primitive blast cells, erythroid and megakaryocyte colony formation [1] although the effects of both these factors are enhanced by other CSFs such as IL-1 and IL-6 [2–6].

The actions of GM-CSF further down the cascade are more restricted, with predominant effects on the maturation of monocytes and granulocytes. Evidence from both murine and human systems suggests GM-CSF can promote the growth of megakaryocytes and eosinophil colonies and assist the growth of erythrocyte progenitors [7–15]. These effects are primarily seen when acting in concert with the later-acting growth factors such as erythropoietin, M-CSF and G-CSF. Both GM-CSF and M-CSF

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seem to be necessary for the optimal growth of macrophages [1], while GM-CSF may interact with other growth factors to promote the production of megakaryocytes [8–11]. This strongly suggests the utility of exploring combinations of the CSFs.

Physiologically, the local production of GM-CSF in tissues probably promotes recruitment and proliferation of neutrophils and less differentiated macrophages, acting to enhance the isolated inflammatory response [16]. The secretion of GM-CSF by extra-medullary cells may be important in the local modulation of host defence.

FUNCTIONAL EFFECTS

Neutrophils

GM-CSF seems to have a central role in regulating the function of mature blood cells. It promotes the priming of oxidative metabolism by neutrophils so that there is a pronounced release of superoxide ions in response to second messengers of the immune response such as leukotriene B_4 [17]. This has often been demonstrated with human neutrophils *in vitro* [17–21] and has been confirmed *in vivo* [22–24] and represents one of a number of biochemical changes [25]. GM-CSF enhances the phagocytosis of bacteria [26] and yeast [27, 28]. Antibody-dependent cell cytotoxicity (ADCC) is also promoted by GM-CSF [29, 30].

Inhibition of neutrophil migration by GM-CSF was originally demonstrated *in vitro* [31]. This may be related to changes in the expression of adhesion proteins on the surface of neutrophils [32–35]. This may reflect part of the normal role of GM-CSF. Inhibition of neutrophil migration may be a normal function of GM-CSF causing immobilisation of neutrophils at the site of infection [33]. Effects on neutrophil migration *in vivo* have been reported by Peters using a skin window technique [35] in patients receiving GM-CSF after bone marrow transplantation (BMT) and has subsequently been confirmed in other patients [36–38]. One report recognised that effects on migration could be related to the dose of GM-CSF administered, and proposed that doses lower than 360 μg/m^2 per day (i.e. within the normal dose range) may avoid this problem while maintaining beneficial myelopoietic effects [37]. However, the skin window technique measures the effect of GM-CSF on migration to a sterile site. During infection chemotactic factors induce the migration of neutrophils from the vasculature and GM-CSF enhances the responses of neutrophils to chemotactic stimuli [25, 39]. Initial fears that this action could affect the ability of neutrophils to attend sites of infection may prove irrelevant as lower rates of infection in patients receiving GM-CSF become apparent from clinical trials.

Eosinophils, monocytes and macrophages

GM-CSF has extensive functional effects on other myeloid cells. It promotes the cytotoxicity of human eosinophils *in vitro* [40] including ADCC [27], and increases the release of histamine from basophils [41]. Human mononuclear phagocytes are also highly responsive to GM-CSF *in vitro* with increases in oxidative metabolism, phagocytosis and cytotoxicity [16], and enhanced tumour kill activity [1, 42], although concomitant administration with other factors (for example IL-1) may be required to demonstrate a clinical effect [43]. Recent *in vitro* studies suggest that GM-CSF stimulates enhanced fungicidal [44] and ADCC activity by monocytes [44]. Similar effects on macrophage function have also been reported including phagocytosis and superoxide production [45], and the dramatic uptake and killing of a protozoan by macrophages [46]. In murine systems GM-CSF also enhances the responsiveness of both monocytes and macrophages to M-CSF [47].

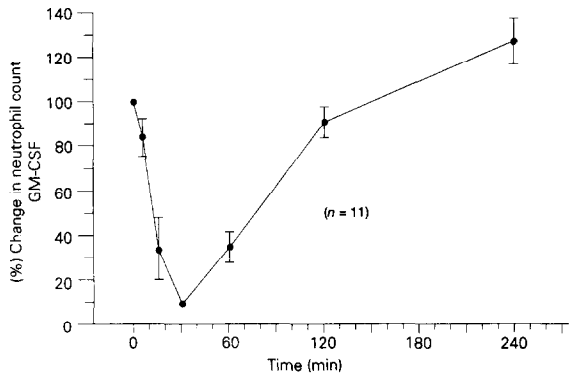

Fig. 1. Percentage changes in neutrophil count after 1 h infusion of rhGM-CSF (15 μg/m^2). Reproduced with kind permission from Devereux *et al.* [48].

CLINICAL PROFILE

The immediate effect of GM-CSF administration is to cause a transient and rapid fall in the number of circulating leucocytes (Fig. 1) which returns to normal in 4–6 hours at all dose levels and with either subcutaneous or intravenous injection [48–51]. This is followed by a sustained increase in leucocytes which has been reported to occur in a triphasic pattern [51, 52] with an early increase in the total leucocyte count over the first 4 days followed by a plateau phase, with a further rise occurring at 8–10 days (Fig. 2). The first phase has been attributed to the demargination of pre-existing mature cells, the second to cells produced by the stimulation of progenitor cells in the bone marrow. After stopping GM-CSF the white blood cells counts return to baseline within 3–5 days. The increase in leucocytes observed is mainly attributed to an increase in neutrophils, with some increases in eosinophils and monocytes although a modest increase in lymphocyte count has been observed occasionally [50]. During treatment with GM-CSF there is an increase in bone marrow cellularity with an increased myeloid:erythroid ratio. Eosinophilia may occur, and the marrow shows a left shift, with up to 10% of neutrophils at 6 hours being early band cells [49, 50]. Peripheral blood progenitor cells increase following GM-CSF treatment [49, 53]. However, little increase in bone marrow progenitors has been observed because of the dilutional effect of increased marrow cellularity.

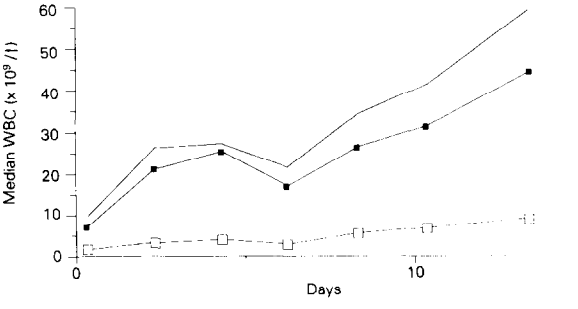

Fig. 2. Profile of median total leucocyte count (—), neutrophil count (-■-) and eosinophil count (-□-) for patients receiving continuous intravenous infusion of GM-CSF at 3 μg/kg per day for 10 days followed by escalation of the dose to 10 μg/kg per day. Reproduced with kind permission from Steward *et al.* [51].

Table 1. Efficacy of GM-CSF: comparison of route of administration

Route	Daily dose (μg/kg) to achieve endpoint†	Ref.
	Not achieved at	
Bolus*	25	49
30-min infusion*	30	54
2-h infusion*	20	55
Subcutaneous	3–10	50
Continuous infusion*	3–10	52

*Intravenous administration
†That is a four-fold increase in WBC which exceeds a total count of 20×10^9/l within 14 days of starting treatment.

Increases in white blood cell count have been shown to be dose dependent [49] and analysis of several published phase I studies indicates that route and schedule are also important (Table 1) with lower doses required by subcutaneous or continuous intravenous administration for responses similar to those achieved with short intravenous infusions of higher doses. Intravenous bolus injections would however seem to be relatively ineffective.

Cancer chemotherapy

Myelotoxicity has been the most limiting side-effect of potentially curative cancer chemotherapy. The neutropenia experienced by patients shortly after cytotoxic therapy often results in bacterial and secondary fungal infections which are a major cause of morbidity and may be fatal [56]. The management of febrile neutropenic patients is costly because of the prolonged hospitalisation and intravenous antibiotic therapy required. Such myelotoxicity restricts the dose and frequency of administration of cytotoxic agents. GM-CSF may therefore be used to reduce the period of neutropenia following chemotherapy to reduce infectious complications and allow any myelosuppressive regimens to be given at full dose on schedule. There is also suggestive evidence that increasing the dose intensity of chemotherapy may improve the tumour response [57]. This may be achieved by increasing the dose, reducing the time between cycles, or both.

Early dose-ranging trials provided firm evidence that GM-CSF can support cell counts through the nadir which follows chemotherapy [58, 59]. Some of these studies also have given preliminary indications of other benefits. Antman and colleagues achieved an improved tumour response rate of 79% compared to 52% in a previous study [58]. In a study by Herrmann and others, the duration of hospital stay and the requirement for parenteral antibiotics were also reduced [49]. Ho *et al.* reported shorter periods of thrombocytopenia (from 7 to 3 days) with reduced rates of infection and stomatitis [60]. 9 out of 14 patients with non-Hodgkin lymphoma (NHL) achieved complete remission compared to 2 out of 14 not given GM-CSF.

Using continuous infusion of GM-CSF in Manchester, UK, we have been able to attenuate the duration of thrombocytopenia and neutropenia induced by melphalan (120 mg/m^2), to times at least as short as those reported in a historical series receiving autologus BMT [51, 52].

Scheduling in cancer chemotherapy

Edmonson and others reported that they could overcome the limiting thrombocytopenia of their protocol by rescheduling the treatment programme for GM-CSF [61]. A more interesting approach attempts to protect myeloid cells from the effects of cell cycle-specific agents. This was originally proposed by Aglietta *et al.* [62, 63] from a study of the cell kinetics of the response to GM-CSF. This study suggested that a short period of administration just prior to chemotherapy would enable the cytotoxic agents to be given on schedule. If the GM-CSF was stopped 24–48 hours before the chemotherapy then the normal bone marrow cells could be put out of cycle and protected from toxicity. This has recently been confirmed in patients with inoperable metastatic sarcoma, where myeloprotection could be significantly enhanced by optimising the timing and schedule of both GM-CSF and chemotherapy [64]. Benefits in myeloid cell counts seem to derive from an increased myeloid mass and quiescent progenitors at the initiation of chemotherapy.

Bone marrow transplantation

Evidence from animal and human studies suggests that escalating the dose of cytotoxic therapy may translate into improved tumour response [57, 65]. However, the myelotoxicity of aggressive chemo/radiotherapy require support with allogeneic and autologous BMT to restore normal marrow function. Despite this procedure, recovery of neutrophil counts takes approximately 3 weeks [66] during which time the patient remains at considerable risk of bacterial and fungal infections. Thrombocytopenia may persist for long periods requiring repeated platelet transfusions. The high morbidity rates, 5–10% infective deaths [67] and high costs have limited the use of BMT.

Brandt *et al.* administered GM-CSF (2.0–32.0 μg/kg per day) intravenously to patients, with breast cancer (13) or melanoma (6), for 14 days after BMT [68]. Compared to 24 historical controls, myeloid recovery was more rapid, with steeper increases in leucocytes and granulocytes, producing significantly higher counts by the end of GM-CSF treatment. No differences in platelet counts were reported but these authors reported lower morbidity and mortality during hospitalisation compared to the control group; fewer patients presented bacteraemias (16% vs. 35%). In a separate study intravenous daily doses of 15–240 μg/m^2 were given to 15 patients with lymphoid malignancies [55]. At doses above 60 μg/m^2 per day neutrophil and platelet counts recovered earlier, with fewer days of fever and earlier discharge from hospital. There was a small reduction in the duration of dependency on platelet transfusion.

Initial studies indicated that GM-CSF can be used to promote the myeloid recovery achieved with BMT [55, 68, 69, 70]. Preliminary reports of six randomised double-blind trials have provided more convincing evidence of significantly faster recoveries in the neutrophil counts with GM-CSF from a total of 546 patients [71–76]. These studies, which enrolled patients with a variety of malignant disorders, have revealed other benefits. Compared with patients receiving placebo, those given GM-CSF had a marked improvement in experience of infections with reduction in the use of antibiotics and other hospital resources. One study estimated that the mean saving per patient could be $10 000. Experience from 10 patients indicates that the severity of graft versus host disease may be ameliorated with GM-CSF [77] and that depressed neutrophil function may be restored [78].

Gianni *et al.* have sought to optimise recovery by supplementing BMT with stem cells collected from peripheral blood (PBSCs) by leukaphoresis during the rebound in cell counts which occurs after cyclophosphamide [79]. The mononucleated

cells collected were used to complement the autologous BMT, because previous attempts employing PBSCs alone had met with some failure of engraftment [80]. Significant improvements in time to recovery of blood counts were recorded over those experienced by patients receiving BMT alone. This was thought to be related to the large dose of CFU-GM administered, confirming the correlation observed by others that recovery is related to the number of progenitors administered [81–84]. Life-threatening infections have only been seen in patients who did not receive blood-derived cells.

An interesting finding was the recovery of platelet counts in the group receiving PBSCs. There were no platelet transfusions after day 11 and 4 out of 6 patients only required one transfusion, both statistically significant changes compared to the control group. Requirements for red blood cells were also reduced and patients receiving peripheral blood leucocytes were discharged earlier from hospital (21 days vs. 31). The authors expect this procedure to substantially reduce the incidence of severe infections and render isolation unnecessary.

A report by Socinski *et al.* has suggested that GM-CSF given after chemotherapy can increase the pool of circulating progenitors suggesting that this alone might deliver sufficient PBSCs for transplantation [85]. Gianni *et al.* have now employed GM-CSF to further augment the harvest of peripheral blood stem cells from seven patients scheduled to received TBI/melphalan for lymphoma [86]. GM-CSF produced significantly higher levels of CFU-GM in peripheral blood to such an extent that fewer sessions of leukaphoresis produced a greater yield of progenitors than in 7 control patients. Patients receiving progenitors collected with GM-CSF demonstrated faster recoveries of total and neutrophil counts compared to the control group (Fig. 3). There was also a dramatic recovery in platelet counts by days 9–10 and a reduction in the severity of mucositis. Similar results were observed by Peters *et al.*, although no changes in platelet counts were reported [87]. Studies now suggest that the engraftment of peripherally collected stem cells, augmented by GM-CSF, may be so reliable that the security of concomitant BMT is no longer necessary [88–90].

Direct comparisons have been made between BMT alone and PBSCs reinfusion with [91] or without [92] bone marrow. The use of PBSCs collected with the aid of GM-CSF produced marked improvements in the rate of recovery of cell counts (ANC>500/μl 19 days after PBSC plus BMT compared with 25 days after BMT alone [91] and 14 days after PBSC alone compared with 21 days after BMT alone [92]). There were also marked savings in the number of days of hospitalisation: 27 days (PBSC+BMT) vs. 35 days (BMT) [91] and 24 days (PBSC) vs. 38 days (BMT) [92]. Attempts to correlate the myeloid recovery with the numbers of progenitor cells harvested are now ongoing and will improve the reliability of this technique.

The astute use of GM-CSF in the collection of peripheral blood stem cells promises to reduce the requirement for extensive support measures such as sterile environments, antibiotic therapy and platelet transfusions making intensive chemotherapy cheaper and safer. It also makes intensive chemotherapy available for the initial treatment of selected tumours, with the objective of curing the disease. Mumcuoglu *et al.* have shown that combination of GM-CSF with IL-3 can be used to augment the potential of harvested bone marrow *in vitro* to such an extent that after donation of this marrow to the patient administration of GM-CSF may be unnecessary [93].

One of the major complications of allogeneic BMT is the development of graft vs. host disease (GVHD) where immuno-

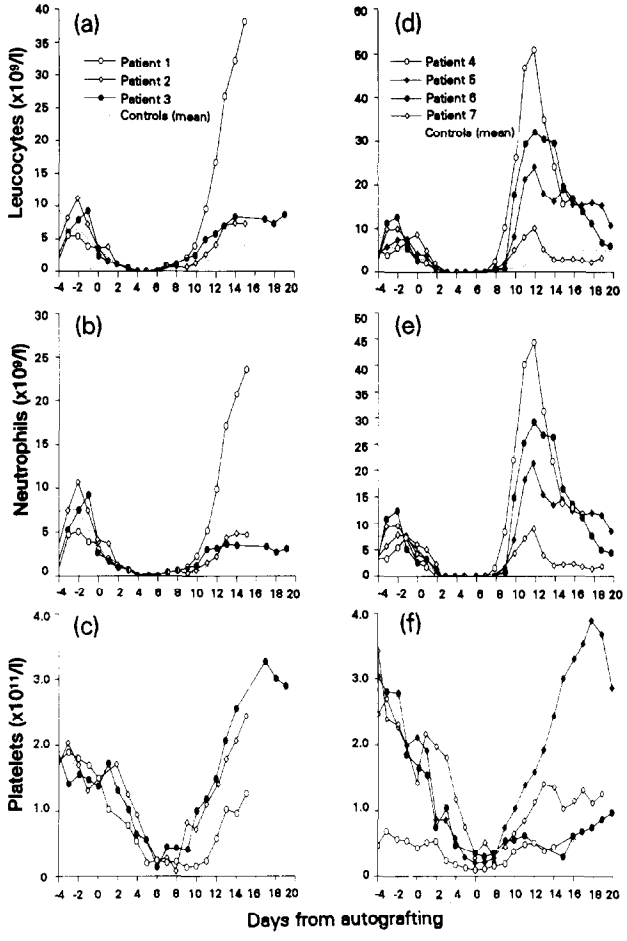

Fig. 3. Time course of leucocyte, neutrophil and platelet counts after high-dose chemoradiotherapy and autografting (day 0) of control or GM-CSF-exposed circulating progenitors. Patients 4–7 (d–f) received additional GM-CSF treatment after transplantation starting from 24 h after autografting and continuing for 10–11 days. Reproduced with kind permission from Gianni *et al.* [86].

competent T-cells from the graft react with HLA antigens belonging to the host [94]. The risk of GVHD can be significantly reduced by removing T-cells from the donor marrow. However, T-cell depletion in itself increases the risk of graft failure [95]. This may reflect a defect in endogenous GM-CSF production as T-cells obtained up to 18 months after BMT (with or without T-cell depletion) express little or no GM-CSF [96].

A number of patients will, therefore, experience graft failure, the proportion depending on the underlying disease. In such patients the prognosis will be poor and most will die of secondary bleeding complications and severe infections, despite further attempts at engraftment [97].

Two studies suggest that GM-CSF will improve the outcome for patients with graft failure. In a study of 37 patients with failed allogeneic ($n=15$), autologous ($n=21$) or syngeneic ($n=1$) bone marrow transplant, GM-CSF was administered at 60–1000 μg/m^2 per day for 14 or 21 days [96]. At doses below 500 μg/m^2 per day, GM-CSF was well tolerated and did not exacerbate the severity of GVHD in allogenic recipients. The survival rates of patients treated with GM-CSF was significantly better than those of a historical control group [98]. In another study of 9 patients with failed or delayed engraftment (6 allogenic, 3 autologous) GM-CSF was administered at 3–10 μg/kg per day over 30 minutes [99]. Here, 6 out of 7 evaluable patients

had a marked rise in neutrophil counts and there was no increase in GVHD.

These findings are in accord with a report by Brandwein *et al.* for 6 patients treated with GM-CSF for 14 days at 5 or 10 μg/kg per day [100]. 3 patients responded with at least a 7-fold increase in granulocyte counts which in 2 patients returned to pretreatment levels 4 and 7 weeks after GM-CSF. The experience of the remaining patients (1 death, 2 non-responders) led these authors to propose that the response to GM-CSF may vary and that some patients with no residual stem cells will remain unresponsive to GM-CSF alone.

Antitumour activity

The functional enhancement of macrophage and monocyte-mediated ADCC has led some investigators to suggest that GM-CSF may have the potential for antitumour activity. Supporting evidence comes from *in vitro* observations of GM-CSF-stimulated human and murine bone marrow cultures, which show significant lysis of two tumour cell lines [101]. This antitumour effect has also been observed *in vivo* in two mouse models of cyclophosphamide and TBI therapy followed by BMT [101]. Ruff *et al.* reported that GM-CSF inhibits the proliferation of small cell lung cancer (SCLC) cell lines when given at high doses [102]. However, other conflicting results have been published [103–105]. The demonstration of receptors for G-CSF and GM-CSF on SCLC cells may be relevant in this respect, although transduction to a functional intracellular signal should also be sought [106].

Morstyn and Burgess have proposed that an antitumour effect is unlikely to be achieved with systemic administration but could be feasible for isolated tumours where local administration of GM-CSF might be more effective [107]. However, this does not follow from our own observations in 20 patients undergoing a phase I trial of GM-CSF. These patients received infusions on days 1–10 and 21–30, with infusions every other day from days 31–50 at doses ranging from 0.3 to 60 μg/kg per day. Regular monitoring of evaluable sites revealed stabilisation of disease in 7 patients up to a minimum of 70 days after the study started. 1 patient with a heavily pretreated liposarcoma experienced a significant reduction (>50%) in tumour volume, a response which lasted 6 months [52].

Metcalf has suggested that the degree of stimulation and suppression in leukaemia may vary according to the leukaemic population and that *in vitro* screening assays should be developed to identify those patients in which there is antileukaemic potential for GM-CSF [108]. This may already be a pressing need as *in vitro* culture of leukaemic cell lines has suggested that combination of GM-CSF with other cytokines will inhibit their growth [109]. The development of leukaemia inhibitory factor (LIF) and its possible synergistic actions with GM-CSF may have important consequences in this area [110].

Stimulation of tumour activity and leukaemia

Some tumour cell lines have proliferated in response to GM-CSF [104, 111, 112]. However, stimulation of the growth of non-haematological tumours is not normally seen in the presence of serum and therefore may not be clinically relevant. Where growth has been observed, it is frequently modest. Furthermore, screening of GM-CSF in the human tumour clonogenic assay has provided no consistent evidence of stimulation of the growth of fresh tumour explants [113, 114]. From murine studies there is no evidence that the overproduction of GM-CSF which can be produced in transgenic mice has any effect on leukaemic transformation, even when an attempt was made to induce leukaemia by irradiation [108]. Other work suggests that where there are existing abnormalities, CSFs can facilitate transformation and that caution should be taken in patients with myelodysplastic syndrome at risk of developing myeloid leukaemia [108].

However, where leukaemia exists, the proliferative effect of GM-CSF can be used to advantage as this cytokine increases the proportion of bone marrow cells in the S phase [115, 116]. This is particularly useful for acute myeloid luekaemia treated with S phase-specific agents such as cytarabine. GM-CSF potentiates the cytotoxicity of cytarabine by increasing the number of cells in the S phase *in vitro* [117, 118]. Metabolism of cytarabine is necessary for its full cytotoxic activity. GM-CSF seems to antagonise the phosphorylation of cytarabine to a greater degree in normal cells than in leukaemic cells [118]. This *in vitro* effect might translate into improved selectivity of cytarabine for leukaemia with the use of GM-CSF.

Results from early trials with GM-CSF given after chemotherapy have been encouraging. Beuchner and colleagues administered GM-CSF to 30 high-risk patients with AML, 4 days after chemotherapy at 250 μg/m^2 per day by continuous intravenous infusion [119]. In comparison to historical controls, GM-CSF-treated patients had more complete responses (11/25, 44% vs. 12/41, 29%; NS) with significantly fewer early deaths (4/25, 16% vs. 18/41, 44%; $P<0.02$).

Myelodysplastic syndrome

Early studies established dose-related increases in leucocyte count with GM-CSF [120, 121] and that while the granulocyte count is maintained, proliferation of the blast cells may be controlled or reduced [122].

Firm evidence of GM-CSF's ability to repair myelopoiesis in MDS has emerged in three randomised trials. Schuster *et al.* randomised 133 patients to either GM-CSF (3 μg/kg per day subcutaneously) or observation, over a 90-day period [123]. The neutrophil counts of observation patients remained at the baseline values of around 0.6×10^9/l while patients receiving GM-CSF had significant increase ($P<0.01$) to around 3.8×10^9/l at the end of the 90-day period. There were also increases in monocytes ($P<0.01$), eosinophils ($P<0.001$) and lymphocytes ($P<0.01$) and fewer major infections (15 vs. 33%, $P<0.02$) in patients treated with GM-CSF.

The two other studies differentiate patients according to their risk of transformation to leukaemia. In low-risk patients, very encouraging response rates of 60–70% have been reported which were not proportional to the starting counts [124] or the dose of GM-CSF. In high-risk patients, who received low-dose cytarabine with concomittant or subsequent administration of GM-CSF, 46% patients were classified as partial responders or better [125]. Further, some improvement in bone marrow function was documented after several weeks' follow-up. Two of these studies monitored transformation and found no evidence that GM-CSF promoted progression to leukaemia.

Aplastic anaemia

Responses to cytokines may be less impressive in aplastic anaemia (AA) than in MDS, probably because there are fewer stem cells and progenitors available. Vadhan Raj and others treated 10 patients with moderate to severe AA with 1.5–13 μg/kg (60–500 μg/m^2) GM-CSF by continuous daily infusion for 2 weeks [126]. Treatment was repeated after a 2-week rest period. White cell count increased 1.6–10-fold in all

patients because of similar increases in neutrophils, eosinophils and monocytes. Despite this, the absolute cell counts achieved were limited by the severity of the disease indicating that sufficient target cells are needed for an adequate response.

Similar results have been reported by others [127] although the response to GM-CSF can be highly variable [128]. The increase in the numbers of erythrocytes reported probably reflects the burst-promoting activity of GM-CSF which might be more prevalent with the high endogenous levels of erythropoietin in patients with AA. There was no saving of platelet or red blood cell transfusions. A small study of 6 transfusion-dependent patients with Diamond–Blackfan anaemia has shown that GM-CSF and IL-3 have marked erythroid-stimulating activity in some of these individuals [129].

The need for suitable target cells in AA is emphasised by a report of four compassionate need cases of very severe and treatment-resistant disease [130]. These patients received conventional treatment for infections and were dependent on platelet and red cell transfusions. In 3 patients with no evidence of residual myelopoiesis, GM-CSF had no effect despite an increase in dose to 32 μg/kg per day in 1 patient.

AIDS

Defects in the function and numbers of lymphocytes, monocytes and neutrophils probably contribute to the high incidence of opportunistic infections and neoplasms in AIDS [131, 132]. Leucopenia is a common dose-limiting effect of the therapies currently employed to control infection, neoplasms [133, 134] and viral replication [135–137] in AIDS or AIDS-related complex, where cell counts may already be low. The first study of GM-CSF in AIDS was reported in 1987 in patients selected on the basis of leucopenia [138]. GM-CSF was given as a single intravenous dose over 60 min followed by a 14-day continuous infusion 48 h after the initial injection. Following an initial rise in WBC, the product of demargination of mature cells from the marrow, there was a period of sustained increase in leucocyte count. This second phase was attributed to increased myelopoiesis because of increased bone-marrow cellularity in 11/14 patients. However, it was clear after treatment that continued infusion of GM-CSF would be required to maintain this response. Other published studies now support the effectiveness of GM-CSF for leucopenia in HIV-related illness [134] including correction of zidovudine (AZT)-induced cytopenia and biochemical abnormalities [133] and the neutropenia associated with gancyclovir [136]. GM-CSF (1–15 μg/kg per day) has been given in a compassionate programme in conjunction with gancyclovir to 17 patients with cytomegalovirus retinitis in AIDS and bone marrow intolerance to the antiviral agent. The experience with these patients has been encouraging. While 3 out of 16 evaluable patients died, the remainder maintained normal neutrophil counts with no resistance to gancyclovir and prevention of retinitis progression in virtually all patients [136].

GM-CSF can also correct the dysfunction of myeloid cells which is associated with AIDS. In a study by Baldwin *et al.* neutrophils collected from AIDS patients were functionally enhanced by GM-CSF in an equivalent manner to neutrophils collected from normal donors, with enhanced superoxide release in response to a chemotactic agent [139]. Neutrophils collected from patients during infusion with GM-CSF functioned normally in terms of oxidative metabolism and ADCC, and in 3 patients with abnormalities of phagocytosis or intracellular killing, were corrected by GM-CSF infusion *in vivo*. Therefore, GM-CSF has a therapeutic potential in AIDS for enhancing host defence which could translate into improved control or prophylaxis of opportunistic infections and possibly mortality and survival [140].

Some studies have suggested that GM-CSF can upregulate the replication of HIV in infected cell-lines and monocytes [141]. However this was not found in an *in vitro* study of alveolar macrophages infected with HIV-1, which in fact suggested that GM-CSF may enhance the inhibitory effect of zidovudine on viral replication [142]. This is in accord with the increase in anabolic phosphorylation of zidovudine in monocytes incubated with GM-CSF [141] and that combination of these two agents synergistically inhibits HIV replication in a monocytic cell line [143].

Anti-infective properties

The ability of GM-CSF to enhance the function of neutrophil, eosinophil and the monocyte–macrophage lineages has suggested its use in promoting host defence. Two reports have provided evidence which supports a clinical role for GM-CSF in promoting the response to acute infections. The earliest of these has described how GM-CSF can reduce the proportion of cultured peritoneal macrophages collected from mice infected with *Leishmania tropica* [46]. In this study there was a continuous decrease in the percentage of infected cells reaching less than 10% on day 4 compared to 30% in controls and there was an indication of increased killing of these parasites. A later study has demonstrated that GM-CSF could inhibit the replication of *Trypanosoma cruzi* by the activation of macrophages in both human and murine cultures using the homolgous cytokine [144]. This was mirrored by increases in the ability to release hydrogen peroxide.

Increases in circulating CSFs certainly seem to be a normal component of the biological response to bacterial infection and to some degree proportional to the inoculum given and the numbers of infecting bacteria at the site of infection [145]. However, Jensen and colleagues reported that GM-CSF had no effect on the antibacterial effect of peripheral blood monocytes and pulmonary macrophages *in vitro* where an effect of gamma interferon could be demonstrated [146]. More successful results have been reported for fungal infections [147]. GM-CSF seems to stimulate the fungicidal activity of human monocytes *in vitro* and this is associated with enhanced production of superoxide [44].

Many clinical studies of GM-CSF now also assess infection and secondary endpoints related to it. The pattern emerging is that there are significant reductions in serious infection and marked savings in antibiotic use, hopsitalisation and support measures such as isolation [49, 60].

However, it may be difficult to demonstrate a prophylactic anti-infective effect in direct comparisons against the established efficacy of antibiotics. A recent example of this has come from a small study of patients receiving intensive chemotherapy for lymphoma. GM-CSF (125 μg/m^2 intravenously over 6 hours, days 6–21) was compared with prophylactic antibiotics (days 6–21). The number of documented infections was higher with GM-CSF which, coupled with higher requirements for red blood cells and platelets, led to early termination of the study [148].

Chronic and congenital neutropenia

In a study of chronic severe neutropenia, a dose of 4–26 μg/kg per day (150–1000 μg/m^2 per day) for 12–14 consecutive days produced increases in neutrophil counts from less than 0.25 to 3–19 × 10^9/l [149]. 2 patients had life-threatening infections which resolved and 2 other patients underwent major surgery

without postoperative infectious complications. However, these results are in direct contrast to those reported by Welte *et al.* in patients with severe congenital neutropenia [150]. Here GM-CSF did not elevate neutrophil counts in 4 of the 5 patients treated, although there were marked increases in eosinophils. However, small but adequate increases in neutrophils could be achieved by using G-CSF, an effect explained as a consequence of a defective G-CSF production or response. Similar results have been reported in two individual cases where an increase in the white cell count was predominantly due to increases in eosinophils [151, 152].

More heartening recoveries have been observed in two cases of drug-induced agranulocytosis [153] and two with glycogen storage disease with histories of neutropenia and neutrophil dysfunction [154]. In the later cases, increases in peripheral granulocyte counts were associated with shorter healing times for infections.

These discrepancies again underly the necessity of identifying patients able to respond to GM-CSF. Bone marrow cultures identified a depleted but responsive population of CFU-GM in a 13-year-old boy with cyclic neutropenia who was then protected from neutrophil nadir by 5 days' prophylaxis with GM-CSF (4 μg/kg per day) [155]. This implies that estimating suitable progenitors such as CFU-GM or CFU-MK is required in diseases where these populations are abnormally low.

SAFETY

The safety profile of GM-CSF is related to both the dose and route of administration. As has been seen in the efficacy section, intravenous bolus or short infusions are the least effective method of administration; therefore, in phase I studies higher doses were required to obtain an increase in white blood cell count. It is not surprising, therefore, that the most toxicity has been seen at high doses given by the intravenous bolus or short infusion route in early phase I studies. The incidence of serious side-effects has been markedly reduced in later studies when lower doses have been administered by subcutaneous injection or continuous intravenous infusion.

Pericarditis has been described in a total of 8 patients, usually after 7 or 8 days of treatment with GM-CSF. However, this is usually associated with high doses of GM-CSF and has settled on withdrawal [54, 58, 156]. Thrombotic episodes may also be related to high doses of GM-CSF. 1 patient with pancreatic cancer died from a pulmonary embolism after receiving 60 μg/kg per day of GM-CSF for 7 days by intravenous bolus [58]. 2 other patients receiving 64 μg/kg per day GM-CSF developed thrombosis around the tip of the central venous catheter, and a pulmonary embolus was documented in 1 of these [66]. A 9-year-old girl developed unexplained thromboses of the inferior vena cava while receiving a dose of 32 μg/kg per day by intravenous infusion for severe AA [130]. Although most patients had other possible causes for these episodes (underlying malignancy or chemotherapy), thromboembolism should possibly be regarded as being related to GM-CSF administration at high doses.

Australian workers [156] have reported a first-dose effect of GM-CSF. They described a syndrome of hypoxia and hypotension that followed the first but not subsequent doses of GM-CSF. 17 of 48 patients treated in phase I and II studies developed the syndrome after the first dose of 20 of 78 cycles of treatment, but none showed the reaction after any subsequent GM-CSF treatments. Oxygen saturation decreased (12 patients) during first-dose reaction by 8% (± 4%), and blood pressure dropped >20 mmHg in 9 patients, but with no significant changes in chest X-ray or lung perfusion scan studies. Factors predisposing to the syndrome were doses of >32 μg/kg per day by intravenous bolus or short infusion rather than subcutaneous injection together with a diagnosis of lung cancer. The onset of the syndrome appeared related to GM-CSF serum levels and route of administration. For example, the onset was later in patients given the drug administered by continuous subcutaneous infusion than those receiving subcutaneous bolus injections. The authors were not able to demonstrate any changes in serum levels of compliment, histamine or tumour necrosis factor α (TNF-α). The mechanism of this effect is not clear but it is suggested that it is related to a vasoactive mediator. Other investigators have not identified this syndrome in any detail, but have described a few patients with some of the characteristics. It would appear prudent to carefully observe patients with lung cancer receiving higher doses of GM-CSF.

Other less serious side-effects include fever, bone pain, myalgia, lethargy, skin rashes and dyspnoea. Fevers are transient and are easily contolled with paracetamol. Bone pain, however, can be substantial, particularly after intravenous or subcutaneous bolus. The severity is dose-related, especially when doses over 15 μg/kg are used. Dermatological side-effects are largely restricted to subcutaneous use although pruritus occasionally

Table 2. Adverse events of GM-CSF (dose less than 10 μg/kg per day) in phase I/II trials

Route	Patients	First dose reaction	Fever	Bone pain	Thrombosis	Pericarditis	Systemic symptoms
Subcutaneous [50, 122, 157, 130, 149]	34	1	18	12	0	0	10
Intravenous bolus or short infusion [49, 51, 54, 55, 81, 158]	136	2	23	16	0	0	27
Continuous intravenous infusion [49, 51, 52, 58, 68, 69, 120, 127, 126, 130, 138, 149]	125	?4	23	16	0	0	44
Total events	295	3+?4 (2%)	64 (22%)	44 (14%)	0	0	81 (27%)
WHO grade III/IV	295	3+?4 (2%)	0	5	0	0	4

? = not definite.

occurs when other routes are used. Three types of skin rashes occur: a maculopapular rash, a local erythema at the site of injection and a further immune-type response with previously unaffected injection sites being inflamed together with new sites. All rashes usually settle within a few days of discontinuation of GM-CSF treatment [156].

Since the routine dose of GM-CSF will probably be 5 μg/kg per day, depending on the disease state, the toxicities seen at higher doses are not relevant. The adverse events seen in 18 published phase I and II trials of 295 patients receiving <10 μg/kg per day or <500 μg/m^2 per day is shown in Table 2. At these dose levels only 16 patients (5%) had severe WHO grade III or IV toxicity (3 definite first pass reactions, 4 possible first pass reactions, 5 severe bone pain and 4 severe systemic symptoms). Systemic symptoms were present in 27% of patients and fever in 18%. These symptoms can be easily managed with oral paracetamol. No episodes of pericarditis or thromboembolism were reported at these doses.

CONCLUSION

The extent of GM-CSF's actions on haematopoiesis has shown it to be particularly useful in a number of disease areas, with indications for the neutropenia of chemotherapy in cancer and AIDS, and the support of bone marrow transplantation. Especially important here is GM-CSF's action on eosinophils and monocyte/macrophage cell function. Existing studies suggest this may translate into improved control of infection in larger fully-controlled, phase III trials. The pleiotropic nature of GM-CSF may provide distinctive opportunities for GM-CSF, used alone or in combination, in addition to restoring the neutrophil count.

Results from animal studies suggest that combination of CSFs with the principal regulators of stem cell activity (IL-1, IL-3) will greatly improve our ability to manipulate myelopoiesis to clinical advantage. Clinical results are now emerging to support this hypothesis for GM-CSF/IL-3 combinations where the sequential use of GM-CSF after IL-3 produces additional improvements in neutrophil count (1.6–5.6 times) while promoting the numbers of progenitor cells, but preserving the platelet effect seen with IL-3 alone [159].

In summary, the broad range of GM-CSF's actions throughout the haemopoietic process offers opportunities for significant therapeutic improvements in a number of diseases in the not too distant future.

1. Sieff CA, Emerson SG, Donahue RE, *et al.* Human recombinant granulocyte-macrophage colony-stimulating factor: a multilineage hematopoietin. *Science* 1985, **230**, 1171–1173.
2. Otsuka T, Miyajima A, Brown N, *et al.* Isolation and characterization of an expressible cDNA encoding human IL-3. Induction of IL-3 mRNA in human T cell clones. *J Immunol* 1988, **140**, 2288–2295.
3. Clark SC. Biological activities of human granulocyte-macrophage colony-stimulating factor. *Int J Cell Cloning* 1988, **6**, 365–377.
4. Caracciolo D, Clark SC, Rovera G. Human interleukin-6 supports granulocytic differentiation of hematopoietic progenitor cells and acts synergistically with GM-CSF. *Blood* 1989, **73**, 666–670.
5. Leary AG, Ikebuchi K, Hirai Y, *et al.* Synergism between interleukin-6 and interleukin-3 in supporting proliferation of human hematopoietic stem cells: comparison with interleukin-1α. *Blood* 1988, **71**, 1759–1763.
6. Zsebo KM, Wypych J, Yuschenkoff VN, *et al.* Effects of hematopoietin-1 and interleukin-1 activities on early hematopoietic cells of the bone marrow. *Blood* 1988, **71**, 962–968.
7. Leary AG, Yang YC, Clark SC, Gasson JC, Golde DW, Ogawa M. Recombinant gibbon interleukin 3 supports formation of human multilineage colonies and blast cell colonies in culture: comparison with recombinant human granulocyte-macrophage colony-stimulating factor. *Blood* 1987, **70**, 1343–1348.
8. Robinson BE, McGrath HE, Quesenberry PJ. Recombinant murine granulocyte macrophage colony-stimulating factor has megakaryocyte colony-stimulating activity and aguments megakaryocyte colony stimulation by interleukin 3. *J Clin Invest* 1987, **79**, 1648–1652.
9. Sonada Y, Yang YC, Wong GG, Clark SC, Ogawa M. Analysis in serum-free culture of the targets of recombinant human hematopoietic growth factors: interleukin 3 and granulocyte/macrophage-colonly-stimulating factor are specific for early developmental stages. *Proc Natl Acad Sci USA* 1988, **85**, 4360–4364.
10. Tomonaga M, Golde DW, Gasson JC. Biosynthetic (recombinant) human granulocyte-macrophage colony-stimulating factor: effect on normal bone marrow and leukemia cell lines. *Blood* 1986, **67**, 31–36.
11. Long MW, Hutchinson RJ, Gragowski LL, Heffner CH, Emerson SG. Synergistic regulation of human megakaryocyte development. *J Clin Invest* 1988, **82**, 1779–1786.
12. Donahue RE, Emerson SG, Wang EA, Wong GG, Clark SC, Nathan DG. Demonstration of burst-promoting activity of recombinant human GM-CSF on circulating erythroid progenitors using an assay involving the delayed addition of erythropoietin. *Blood* 1985, **66**, 1479–1481.
13. Bruno E, Miller ME, Hoffman R. Interacting cytokines regulate in vitro human megakaryocytopoiesis. *Blood* 1989, **73**, 671–677.
14. Emerson SG, Sieff CA, Wang EA, Wong GG, Clark SC, Nathan DG. Purification of fetal hematopoietic progenitors and demonstration of recombinant multipotential colony-stimulating activity. *J Clin Invest* 1985, **76**, 1286–1290.
15. Bonnem EM, Morstyn G. Granulocyte macrophage colony stimulating factor (GM-CSF) current status and future development. *Semin Oncol* 1988, **15**, 46–51.
16. Ruef C, Coleman DL. Granulocyte-macrophage colony-stimulating factor: pleiotropic cytokine with potential clinical usefulness. *Rev Infect Dis* 1990, **12**, 41–62.
17. Sullivan R, Fredette JP, Leavitt JL, Gadenne AS, Griffin JD, Simons ER. Effects of recombinant human granulocyte-macrophage colony-stimulating factor (GM-CSFrh) on transmembrane electrical potentials in granulocytes: relationship between enhancement of ligand-mediated depolarization and augmentation of superoxide anion (0–2) production. *J Cell Physiol* 1989, **139**, 361–369.
18. Sullivan R, Griffin JD, Simons ER, *et al.* Effects of recombinant human granulocyte and macrophage colony-stimulating factors on signal transduction pathways in human granulocytes. *J Immunol* 1987a, **139**, 3422–3430.
19. Weisbart RH, Golde DW, Clark SC, Wong GG, Gasson JC. Human granulocyte-macrophage colony-stimulating factor is a neutrophil activator. *Nature* 1985, **314**, 361–363.
20. Weisbart RH, Golde DW, Gasson JC. Biosynthetic human GM-CSF modulates the number and affinity of neutrophil f-met-leu-phe receptors. *J Immunol* 1986, **137**, 3584–3587.
21. English D, Broxmeyer HE, Gabig TG, Akard LP, Williams DE, Hoffman R. Temporal adaptation of neutrophil oxidative responsiveness to n-formly-methionyl-leucyl-phenylalanine. *J Immunol* 1988, **141**, 2400–2406.
22. Sullivan R, Fredette JP, Socinski M, *et al.* Enhancement of superoxide anion release by granulocytes harvested from patients receiving granulocyte-macrophage colony-stimulating factor. *Br J Haematol* 1989, **71**, 475–479.
23. Kaplan SS, Basford RE, Wing EJ, Shadduck RK. The effect of recombinant human granulocyte macrophage colony-stimulating factor on neutrophil activation in patients with refractory carcinoma. *Blood* 1989, **73**, 636–638.
24. Weisbart RH, Kwan L, Golde DW, Gasson JC. Human GM-CSF primes neutrophils for enhanced oxidative metabolism in response to the major physiological chemoattractants. *Blood* 1987, **69**, 18–21.
25. Coffey RG. Mechanism of GM-CSF stimulation of neutrophils. *Immunol Res* 1989, **8**, 236–248.
26. Fleischmann J, Golde DW, Weisbart RH, Gasson JC. Granulocyte-macrophage colony-stimulating factor enhances phagocytosis of bacteria by human neutrophils. *Blood* 1986, **68**, 708—711.
27. Lopez AF, Williamson DJ, Gamble JR, *et al.* Recombinant human granulocyte-macrophage colony-stimulating factor stimulates in

vitro mature human neutrophil and eosinophil function, surface receptor expression, and survival. *J Clin Invest* 1986, **78**, 1220–1228.
28. Metcalf D, Begely CG, Johnson GR, *et al.* Biologic properties in vitro of a recombinant human granulocyte-macrophage colony-stimulating factor. *Blood* 1986, **67**, 37–45.
29. Metcalf D, Begley CG, Johnson GR, *et al.* Biologic properties *in vitro* of a recombinant human granulocyte-macrophage colony-stimulating factor. *Blood* 1986, **67**, 37–45.
30. Vadas MA, Nicola NA, Metcalf D. Activation of antibody-dependent cell-mediated cytotoxicity of human neutrophils and eosinophils by separate colony-stimulating factors. *J Immunol* 1983, **130**, 795–799.
31. Gasson JC, Weisdorf RH, Kaufman SE, *et al.* Purified human granulocyte-macrophage colony-stimulating factor: direct action on neutrophils. *Science* 1984, **226**, 1339–1342.
32. Socinski MA, Cannistra SA, Sullivan R, *et al.* Granulocyte-macrophage colony-stimulating factor induces the expression of the CD11b surface adhesion molecule on human granulocytes *in vivo*. *Blood* 1988, **72**, 691–697.
33. Arnaout MA, Hakim RM, Todd RF, Dana N, Colten HR. Increased expression of an adhesion-promoting surface glycoprotein in the granulocytopenia of hemodialysis. *N Engl J Med* 1985, **312**, 457–462.
34. Currie MS, Padmanabhan J, Peters WP, Kurtzberg J, Cohen HJ, Rao KMK. Contrasting effects of GM- and G-CSF on human neutrophil adhesion proteins LAM1 and CR3, *in vivo* (abstr). *Blood* 1990, **76** (Suppl.).
35. Peters WP. The effect of recombinant human colony-stimulating factors on hematopietic reconstitution following autologous bone marrow transplantation. *Semin Hematol* 1989, **26**, 18–23.
36. Toner GC, Jakubowski AA, Crown JPL, Meisenberg B, Sheridan C, Gabrilove JL. Colony-stimulating factors and neutrophil migration. *Ann Intern Med* 1989, **110**, 847.
37. Addison IE, Johnson B, Devereux S, Goldstone AH, Linch DC. Granulocyte-macrophage colony-stimulating factor may inhibit neutrophil migration *in vivo*. *Clin Exp Immunol* 1989, **76**, 149–153.
38. Vadhan-Raj S, Keating M, LeMaistre A, *et al.* Effects of recombinant human granulocyte-macrophage colony-stimulating factor in patients with myelodysplastic syndromes. *N Engl J Med* 1987, **317**, 1546–1552.
39. Wang JM, Colella S, Allavena P, Mantovani A. Chemotactic activity of human recombinant granulocyte-macrophage colony-stimulating factor. *Immunology* 1987, **60**, 439–444.
40. Owen WF Jr, Rothenberg ME, Silberstein DS, *et al.* Regulation of human eosinophil viability, density, and function by granulocyte/macrophage colony-stimulating factor in the presence of 3T3 fibroblasts. *J Exp Med* 1987, **166**, 129–141.
41. Haak-Frendscho M, Arai N, Arai KI, Baeza ML, Finn A, Kaplan AP. Human-recombinant granulocyte-macrophage colony-stimulating factor and interleukin 3 cause basophil histamine release. *J Clin Invest* 1988, **82**, 17–20.
42. Kleinerman ES, Knowles RD, Lachman LB, Gutterman JU. Effect of recombinant granulocyte/macrophage colony-stimulating factor on human monocyte activity in vitro and following intravenous administration. *Cancer Res* 1988, **48**, 2604–2609.
43. Weisbart RH, Gasson JC, Golde DW. Colony-stimulating factors and host defense. *Ann Intern Med* 1989, **110**, 297–303.
44. Smith PD, Lamerson CL, Banks SM, *et al.* Granulocyte-macrophage colony-stimulating factor augments human monocyte fungicidal activity for *candida albicans*. *J Infect Dis* 1990, **161**, 999–1005.
45. Coleman DL, Chodakewitz JA, Bartiss AH, Mellors JW. Granulocyte-macrophage colony-stimulating factor enhances selective effector functions of tissue-derived macrophages. *Blood* 1988, **72**, 573–578.
46. Handman E, Burgess AW. Stimulation by granulocyte-macrophage colony-stimulating factor of *Leishmania tropica* killing by macrophages. *J Immunol* 1979, **122**, 1134–1137.
47. Chen BD, Clark CR, Chou T. Granulocyte/macrophage colony-stimulating factor stimulates monocyte and tissue macrophage proliferation and enhances their responsiveness to macrophage colony-stimulating factor. *Blood* 1988, **71**, 997–1002.
48. Devereux S, Linch DC, Campos Costa D, Spittle MF, Jelliffe AM. Transient leucopenia induced by granulocyte-macrophage colony-stimulating factor. *Lancet* 1987, **ii**, 1523–1524.
49. Herrmann F, Schulz G. Lindemann A, *et al.* Hematopoietic responses in patients with advanced malignancy treated with recombinant human granulocyte-macrophage colony-stimulating factor. *J Clin Oncol* 1989, **7**, 159–167.
50. Lieschke GJ, Maher D, Cebon J, *et al.* Effects of bacterially synthesized recombinant human granulocyte-macrophage colony-stimulating factor in patients with advanced malignancy. *Ann Intern Med* 1989, **110**, 357–364.
51. Steward WP, Scarffe JH, Bonnem E, Crowther D. Clinical studies with recombinant human granulocyte-macrophage colony-stimulating factor. *Int J Cell Cloning* 1990, **8**, 335–346.
52. Steward WP, Scarffe JH, Dirix LY, *et al.* Granulocyte-macrophage colony stimulating factor (GM-CSF) after high-dose melphalan in patients with advanced colon cancer. *Br J Cancer* 1990, **61**, 749–754.
53. Morstyn G, Stuart-Harris R, Bishop J, *et al.* Optimal scheduling of granulocyte macrophage colony stimulating factor (GM-CSF) for the abrogation of chemotherapy-induced neutropenia in small cell lung cancer. *Proc Am Soc Clin Oncol* 1989, **8**, 218.
54. Steward WP, Scarffe JH, Austin R, *et al.* Recombinant human granulocyte macrophage colony stimulating factor (rhGM-CSF) given as daily short infusions—a phase I dose-toxicity study. *Br J Cancer* 1989, **59**, 142–145.
55. Nemunaitis J, Singer JW, Buckner CD, *et al.* Use of recombinant human granulocyte-macrophage colony-stimulating factor in autologous marrow transplantation for lymphoid malignancies. *Blood* 1988, **72**, 834–836.
56. Morstyn G, Lieschke GJ, Sheridan W, Layton J, Cebon J, Fox RM. Clinical experience with recombinant human granulocyte colony-stimulating factor and granulocyte macrophage colony-stimulating factor. *Semin Hematol* 1989, **26**, 9–13.
57. Frei E III, Canellos GP. Dose: a critical factor in cancer chemotherapy. *Am J Med* 1980, **69**, 585–594.
58. Antman KS, Griffin JD, Elias A, *et al.* Effect of recombinant human granulocyte-macrophage colony-stimulating factor on chemotherapy-induced myelosuppression. *N Engl J Med* 1988, **319**, 593–598.
59. Morstyn G, Stuart-Harris R, Bishop J, *et al.* Optimal scheduling of granulocyte-macrophage colony stimulating factor for the abrogation of chemotherapy induced neutropenia in small cell lung function. *Proc Am Soc Clin Oncol* 1989, **8**, 218.
60. Ho AD, Del-Valle F, Engelhard M, *et al.* Mitoxant/high dose Ara-C and recombinant human GM-CSF in the treatment of refractory non-Hodgkin's lymphoma. A pilot study. *Cancer* 1990, **66**, 423–430.
61. Edmonson JH, Long HJ, Jeffers JA, Buckner JC, Colon-Otero G, Fitch TR. Amelioration of chemotherapy-induced thrombocytopenia by GM-CSF: apparent dose and schedule dependency. *J Natl Cancer Inst* 1989, **81**, 1510–1511.
62. Aglietta M, Piacibello W, Sanavio F, *et al.* Kinetics of human hemopoietic cells after *in vivo* administration of granulocyte-macrophage colony-stimulating factor. *Am Soc Clin Invest* 1989, **83**, 551–557.
63. Aglietta M, Piacibello W, Sanavio F, *et al.* Kinetics of human hemopoietic cells following in vivo administration of GM-CSF (abstr.). *J Cell Biochem* 1989, **48** (Suppl. 13C).
64. Vadhan-Raj S, Broxmeyer H, Hittelman W, *et al.* Two possible mechanisms for the abrogation of chemotherapy (CT-) induced myelosuppression by GM-CSF: Increased myeloid mass and non-cycling progenitors (abstr.). *Blood* 1990, **76** (Suppl.).
65. Henderson IC, Hayes DF, Gelman R. Dose-response in the treatment of breast cancer: a critical review. *J Clin Oncol* 1988, **6**, 1501–1515.
66. Klingemann HG. Clinical applications of recombinant human colony-stimulating factors. *Can Med Assoc J* 1989, **140**, 137–142.
67. Armitage JO, Jagannath S, Spitzer G, *et al.* High dose therapy and autologous marrow transplantation as salvage treatment for patients with diffuse large cell lymphoma. *Eur J Cancer Clin Oncol* 1986, **22**, 871–877.
68. Brandt SJ, Peters WP, Atwater SK, *et al.* Effect of recombinant human granulocyte-macrophage colony-stimulating factor on hematopoietic reconstitution after high-dose chemotherapy and autologous bone marrow transplantation. *N Engl J Med* 1988, **318**, 869–876.
69. Devereux S, Linch DC, Gribben JG, McMillan A, Patterson K, Goldstone AH. GM-CSF accelerates neutrophil recovery after autologous bone marrow transplantation for Hodgkin's disease. *Bone Marrow Transplant* 1989, **4**, 49–54.
70. Devereux S, Goldstone AH, Patterson K, Linch DC. The use of

recombinant human granulocyte-macrophage colony-stimulating factor to accelerate recovery of the autologous bone marrow transplantation in man. *Br J Haematol* 1988, **69**, 103.
71. Bennett CL, Greenberg P, Gulati SC, Advani R, Bonnem E. GM-CSF decreases duration of cytopenia and hospitalization, and in-hospital costs in patients with Hodgkin's disease treated with high-dose chemotherapy and autologous bone marrow transfusion (ABMT) (abstr.). *Blood* 1990, **76** (Suppl.).
72. De Witte T, Gratwohl, Van Der Lely N, *et al.* A multicentre double blind randomized trial of recombinant human granulocyte-macrophage colony stimulating factor (rhGM-CSF) in recipients of allogeneic T-cell depleted bone marrow (abstr.). *Blood* 1990, **76** (Suppl.).
73. Link H, Boogaerts M, Carella A, *et al.* Recombinant human granulocyte-macrophage colony stimulating factor (RH-GM-CSF) after autologous bone marrow transplantation for acute lymphoblastic leukemia and non-Hodgkin's-lymphoma: A randomized double blind multicenter trial in Europe (abstr.). *Blood* 1990, **76** (Suppl.).
74. Rabinowe S, Freedman A, Demetri G, *et al.* Randomized double blinded trial of rhGM-CSF in patients with B-cell non-Hodgkin's lymphoma (B-NHL) undergoing high dose chemoradiotherapy and monoclonal antibody purged autologous bone marrow transplantation (ABMT) (abstr.). *Blood* 1990, **76** (Suppl.).
75. Advani R, Greenberg P, Gulati S, Hoglund M, Bonnem EM. Randomized placebo controlled trial of granulocyte-macrophage colony stimulating factor in patients undergoing autologous bone marrow transplantation (BMT) (abstr.). *Blood* 1990, **76** (Suppl.).
76. Gorin NC, Coiffier B, Pico J, *et al.* Granulocyte-macrophage colony-stimulating factor (GM-CSF) shortens aplasia duration after autologous bone marrow transplantation (ABMT) in non-Hodgkin's lymphoma: a randomized placebo-controlled double-blind study (abstr.). *Blood* 1990, **76** (Suppl.)
77. Singer JW, Nemunaitis J, Bianco JC, *et al.* rhGM-CSF following allogeneic bone marrow transplantation from unrelated marrow donors: A phase II study (abstr.). *Blood* 1990, **76** (Suppl.).
78. Gadish M, Kletter Y, Flidel O, Nagler A, Slavin S, Fabian I. Effects of recombinant human granulocyte (G-CSF) and granulocyte/macrophage colony stimulating factor (GM-CSF) on neutrophil (PMN) function following autologous bone marrow transplantation (ABMT) (abstr.). *Blood* 1990, **76** (Suppl.).
79. Gianni AM, Bregni M, Siena S, *et al.* Rapid and complete hemopoietic reconstitution following combined transplantation of autologous blood and bone marrow cells. A changing role for high dose chemo-radiotherapy? *Hematol Oncol* 1989, **7**, 139–148.
80. Bell AJ, Hamblin TJ, Oscier DG. Peripheral blood stem cell autografting. *Hematol Oncol* 1987, **5**, 45–55.
81. Blazar BR, Kersey JH, McGlave PB, *et al.* *In vivo* administration of recombinant human granulocyte/macrophage colony-stimulating factor in acute lymphoblastic leukemia patients receiving purged autografts. *Blood* 1989, **73**, 849–857.
82. Jin NR, Hill RS, Petersen FB, *et al.* Marrow harvesting for autologous marrow transplantation. *Exp Hematol* 1985, **13**, 879–884.
83. Gorin NC. Collection, manipulation and freezing of haemopoietic stem cells. *Clin Haematol* 1986, **15**, 19–48.
84. Jagannath S, Reading CL, Dicke KA, *et al.* Clinical application of Percoll gradient-separated bone marrow. *Bone Marrow Transplant* 1987, **1**, 281–288.
85. Socinski MA, Cannistra SA, Elias A, *et al.* Granulocyte-macrophage colony stimulating factor expands the circulating haemopoietic progenitor cell compartment in man. *Lancet* 1988, **i**, 1194–1198.
86. Gianni AM, Siena S, Bregni M, *et al.* Granulocyte-macrophage colony-stimulating factor to harvest circulating haemopoietic stem cells for autotransplantation. *Lancet* 1989, **ii**, 580–585.
87. Peters WP, Kurtzberg J, Kirkpatrick G, *et al.* GM-CSF primed peripheral blood progenitor cells (PBPC) coupled with autologous bone marrow transplantation (ABMT) will eliminate absolute leukopenia following high dose chemotherapy (HDC). *Mini Plenary Session* 1989, **50a**.
88. Hester J, Wallerstein R, Champlin R, *et al.* Reconstitution with recombinant human granulocyte-macrophage stimulating factor (rhGM-CSF) derived peripheral blood progenitor cells (PBPC) following cytoxan, VP16, platinum (CVP) therapy: kinetics of granulocyte (PMN) recovery (abstr.). *Blood* 1990, **76** (Suppl.).
89. Long G, Chao N, Negrin R, Horning S, O'Connor P, Stallbaum B, Blume K. Efficacy of peripheral blood-derived stem cell (PBSC) rescue compared with bone marrow (BM) plus PBSC rescue after high dose therapy. *Blood* 1990, **76** (Suppl.).
90. Shea TC, Mason JR, Storniolo AM, *et al.* Beneficial effect from sequential harvesting and reinfusing of peripheral blood stem cells (PBSC) in conjunction with rHu GM-CSF (Schering/Sandoz) and high-dose carboplatin (CBDCA) (abstr.) *Blood* 1990, **76** (Suppl.).
91. Antman K, Elias A, Hunt M, *et al.* GM-CSF potentiated peripheral blood progenitor cell (PBPC) collection and use after high dose chemotherapy (abstr.). *Blood* 1990, **76** (Suppl.).
92. Elias A, Ayash L, Anderson K, *et al.* Hematologic support during high dose intensification for breast cancer: Recruitment of peripheral blood progenitor cells (PBPC) by GM-CSF and chemotherapy (abstr.). *Blood* 1990, **76** (Suppl.).
93. Mumcuoglu M, Naparstek E, Slavin S. The use of recombinant cytokines for enhancing immunohematopoietic reconstitution following bone marrow transplantation II. The influence of lymphokines on CFU-GM colonies from human untreated, ASTA-Z or campath-1M treated bone marrow. *Bone Marrow Transplant* 1990, **5**, 153–158.
94. Ferrara JLM, Deeg HJ. Graft-versus-host disease. *N Engl J Med* 1991, **324**, 667–674.
95. Champlin R. T-cell depletion to prevent graft-versus-host disease after bone marrow transplantation. *Hematol Oncol Clin N Am* 1990, **4**, 687–698.
96. Thomas S, Clark SC, Rappeport JM, Nathan DG, Emerson SG. Deficient T-cell granulocyte-macrophage colony stimulating factor production in allogeneic bone marrow transplant recipients. *Transplantation* 1990, **49**, 703–708.
97. Klingemann HG, Eaves AC, Barnett MJ, *et al.* Recombinant GM-CSF in patients with poor graft function after bone marrow transplantation. *Clin Invest Med* 1990, **13**, 77–81.
98. Nemunaitis J, Singer JW, Buckner CD, *et al.* Use of recombinant human granulocyte-macrophage colony-stimulating factor in graft failure after bone marrow transplantation. *Blood* 1990, **76**, 245–253.
99. Klingemann HG, Eaves AC, Barnett MJ, *et al.* Recombinant GM-CSF in patients with poor graft function after bone marrow transplantation. *Clin Invest Med* 1990, **13**, 77–81.
100. Brandwein JM, Nayar R, Baker MA, *et al.* Variable response to GM-CSF in graft failure after autologous marrow transplantation (abstr.). *Blood* 1990, **76** (Suppl.).
101. Mazumder A, Agah R, Charak B. Novel use of GMCSF as an antitumor immune potentiator after transplantation (abstr.). *Blood* 1990, **76** (Suppl.).
102. Ruff MR, Farrar WL, Pert CB. Interferon γ and granulocyte/macrophage colony-stimulating factor inhibit growth and induce antigens characteristic of myeloid differentiation in small-cell lung cancer cell lines. *Proc Natl Acad Sci USA* 1986, **83**, 6613–6617.
103. Baldwin GC, DiPersio J, Kaufmann SE, *et al.* Characterization of human GM-CSF receptors on non-hematopoietic cells. *Blood* 1987, **70**, 166–172.
104. Munker M, Munker R, Saxton RE, Koeffler HP. Effect of recombinant monokines, lymphokines, and other agents on clonal proliferation of human lung cancer cell lines. *Cancer Res* 1987, **47**, 4081–4085.
105. Pedrazzoli P, Ziberia C, Gibelli N, Bacciocchi G, Robustelli della Cuna G, Cazzola M. GM-CSF and IL-3 stimulate proliferation of small-cell lung cancer (SCLC) cells (abstr.). *Blood* 1990, **76** (Suppl.).
106. Baldwin GC, Golde DW, Economou J, Gasson JC. Identification and characterization of a low affinity GM-CSF receptor on fresh and cultured human melanoma cells (abstr.). *Blood* 1990, **76** (Suppl.).
107. Morstyn G, Burgess AW. Hemopoietic growth factors: a review. *Cancer Res* 1988, **48**, 5624–5637.
108. Metcalf D. The colony stimulating factors. Discovery, development, and clinical applications. *Cancer* 1990, **65**, 2185–2195.
109. Maekawa T, Metcalf D, Gearing DP. Enhanced suppression of human myeloid leukemic cell lines by combinations of IL-6, LIF, GM-CSF and G-CSF. *Int J Cancer* 1990, **45**, 353–358.
110. Gough NM, Williams RL, Hilton PJ, *et al.* LIF: A molecule with divergent actions on myeloid leukaemic cells and embryonic stem cells. *Reprod Fertil Dev* 1989, **1**, 281–288.
111. Dedhar S, Gaboury L, Galloway P, *et al.* Human GM-CSF is a growth factor active on a variety of cell types of non-hematopoietic origin. *Proc Natl Acad Sci USA* 1988, **85**, 9253–9257.
112. Baker WJ, Hargis JB, Danesi R, LaRocca RV. Effect of recombi-

nant human granulocyte-macrophage colony stimulating factor (RHGM-CSF) on the proliferation of osteogenic sarcoma cells (abstr.). *Blood* 1990, **76** (Suppl.).
113. Foulke RS, Marshall MH, Trotta PP, Von Hoff DD. *In vitro* assessment of the effects of granulocyte-macrophage colony-stimulating factor on primary human tumors and derived lines. *Cancer Res* 1990, **50**, 6264–6267.
114. Salmon SE, Liu R. Effects of granulocyte-macrophage colony-stimulating factor on in vitro growth of human solid tumours. *J Clin Oncol* 1989, 7, 1346–1350.
115. Socinski SA, Cannistra A, Elias A, Antman K, Schnipper L, Griffen J. *In vivo* administration of GM-CSF increases the number of peripheral blood progenitor cells and the cycling fraction of bone marrow (BM) CFU-GM. *Proc Am Soc Clin Oncol* 1988, 7, 613A.
116. Burke PJ, Wendel KA, Nicholls PD, Hall KL, Morrall L, Bonnem EM. A phase I trial of granulocyte-macrophage stimulating factor (GM-CSF) and humoral stimulating activity (HSA) as biomodulators of timed sequential therapy (TST) of leukemia (AML) (abstr.). *Blood* 1990, **76** (Suppl.)
117. Cannistra SA, Groshek P, Griffen JD. Granulocyte-macrophage colony-stimulating factor enhances the cytotoxic effects of cytosine arabinoside in acute myeloblastic leukemia and in the myeloid blast crisis phase of chronic myeloid leukemia. *Leukemia* 1989, **3**, 328–334.
118. Bhalla K, Birkhofer M, Arlin Z, Grant S, Lutzky J, Graham G. Effect of recombinant GM-CSF on the metabolism of cytosine arabinoside in normal and leukemic human bone marrow cells. *Leukemia* 1988, **2**, 810–813.
119. Buechner T, Hiddemann W, Koenigsmann M, *et al.* Recombination human GM-CSF following chemotherapy (CT) versus chemotherapy alone in patients with high-risk AML (abstr.). *Blood* 1990, **76** (Suppl.).
120. Ganser A, Völkers B, Greher J, *et al.* Recombinant human granulocyte-macrophage colony-stimulating factor in patients with myelodysplastic syndromes—a phase I/II trial. *Blood* 1989, **73**, 31–37.
121. Thompson JA, Lee DJ, Kidd P, *et al.* Subcutaneous granulocyte-macrophage colony-stimulating factor in patients with myelodysplastic syndrome: toxicity, pharmacokinetics, and hematological effects. *J Clin Oncol* 1989, 7, 629–637.
122. Ganser A, Ottmann OG, Schultz G, Hoelzer D. Recombinant human granulocyte-macrophage colony-stimulating factor and low-dose cytosine arabinoside in patients with myelodysplastic syndrome. *Onkologie* 1989, **12**, 13–15.
123. Schuster MW, Larson RA, Thompson JA, Coiffier B, Bennett JM, Israel RJ. Granulocyte-macrophage colony-stimulating factor (GM-CSF) for myelodysplastic syndrome (MDS): results of a multi-center randomized controlled trial (abstr.). *Blood* 1990, **76** (Suppl.).
124. Willemz R, Visani G, De Witte T, *et al.* A randomized phase I/II study with recombinant human GM-CSF in patients (PTS) with myelodysplastic syndromes at a relatively low risk of developing acute leukemia (abstr.). *Blood* 1990, **76** (Suppl.).
125. Gerhartz HH, Marcus R, Delmer A, *et al.* Randomized phase II study with GM-CSF and low-dose ARAC in patients with "high risk" myelodysplastic syndromes (MDS) (abstr.). *Blood* 1990, **76** (Suppl.).
126. Vadhan-Raj S, Buescher S, Broxmeyer HE, *et al.* Stimulation of myelopoiesis in patients with aplastic anemia by recombinant human granulocyte-macrophage colony-stimulating factor. *N Engl J Med* 1988, **319**, 1628–1634.
127. Champlin RE, Nimer SD, Oette, Golde DW. Granulocyte-macrophage colony-stimulating factor (GM-CSF) treatment for aplastic anemia. *Clin Res* 1988, **36**, 563A.
128. Antin JH, Smith BR, Holmes W, Rosenthal DS. Phase I/II study of recombinant human granulocyte-macrophage colony-stimulating factor in aplastic anemia and myelodysplastic syndrome. *Blood* 1988, **72**, 705–713.
129. Dunbar CE, Smith D, Kimball J, Garrison L, Nienhuis AW, Young NS. Hematopoietic growth factor treatment of Diamond-Blackfan anemia (abstr.). *Blood* 1990, **76** (Suppl.).
130. Nissen C, Tichelli A, Gratwohl A, *et al.* Failure of recombinant human granulocyte-macrophage colony-stimulating factor therapy in aplastic anemia patients with very severe neutropenia. *Blood* 1988, **72**, 2045–2047.
131. Armstrong D, Gold JWM, Dryjanski J, *et al.* Treatment of infections in patients with the acquired immunodeficiency syndrome. *Ann Intern Med* 1985, **103**, 738–743.
132. Bowen DL, Lane HC, Fauci AS. Immunopathogenesis of the acquired immunodeficiency syndrome. *Ann Intern Med* 1985, **103**, 704–709.
133. Bhalla K, Birkhofer M, Grant S, Graham G. The effect of recombinant human granulocyte-macrophage colony-stimulating factor (rGM-CSF) on 3′-azido-3′-deoxythymidine (AZT)-mediated biochemical and cytotoxic effects on normal human myeloid progenitor cells. *Exp Hematol* 1989, **17**, 17–20.
134. Mitsuyasu RT, Miles SA, Golde DW. The use of myeloid hematopoietic growth factors in patients with HIV infection. *Int J Cell Cloning* 1990, **8** (Suppl. 1), 347–354.
135. Ganser A, Greher J, Völkers B, Staszewski S, Hoelzer D. Inhibitory effect of azidothymidine, 2′-3′-dideoxyadenosine, and 2′-3′-dideoxycytidine on *in vitro* growth of hematopoietic progenitor cells from normal persons and from patients with AIDS. *Exp Hematol* 1989, **17**, 321–325.
136. Grossberg HS, Bonnem EM. GM-CSF with ganciclovir for the treatment of CMV retinitis in aids. *N Engl J Med* 1989, **320**, 1560.
137. Fischl MA, Parker CB, Pettinelli C, *et al.* A randomized controlled trial of a reduced daily dose of zidovudine in patients with the acquired immunodeficiency syndrome. *N Engl J Med* 1990, **323**, 1009–1014.
138. Groopman JE, Mitsuyasu RT, DeLeo MJ, Oette DH, Golde DW. Effect of recombinant human granulocyte-macrophage colony-stimulating factor on myelopoiesis in the acquired immunodeficiency syndrome. *N Engl J Med* 1987, **317**, 593–598.
139. Baldwin GC, Gasson JD, Quan SG, *et al.* Granulocyte-macrophage colony-stimulating factor enhances neutrophil function in acquired immunodeficiency syndrome patients. *Proc Natl Acad Sci* 1988, **85**, 2763–2766.
140. Scadden DT. Granulocyte macrophage colony stimulating factor (GM-CSF) in AIDS. *Prog Clin Biol Res* 1990, **338**, 163–176.
141. Perno CF, Yarchoan R, Coonery DA, *et al.* Replication of human immunodeficiency virus in monocytes. Granulocyte/macrophage colony-stimulating factor (GM-CSF) potentiates viral production yet enhances the antiviral effect mediated by 3′-azido-2′3′-dideoxythymidine (AZT) and other dideoxynycleoside congeners of thymidine. *J Exp Med* 1989, **169**, 933–951.
142. Hammer SM, Gillis JM, Pinkston P, Rose RM. Effect of zidovudine and granulocyte-macrophage colony-stimulating factor on human immunodeficiency virus replication in alveolar macrophages. *Blood* 1990, **75**, 1215–1219.
143. Hammer SM, Gillis JM. Synergistic activity of granulocyte-macrophage colony-stimulating factor and 3′-azido-3′-deoxythymidine against human immunodeficiency virus *in vitro*. *Antimicrob Agents Chemother* 1987, **31**, 1046–1050.
144. Reed SG, Nathan CF, Pihl DL, *et al.* Recombinant granulocyte/macrophage colony-stimulating factor activates macrophages to inhibit trypanosoma cruzi and release hydrogen peroxide. *J Exp Med* 1987, **166**, 1734–1746.
145. Cheers C, Haigh AM, Kelso A, Metcalf D, Stanley ER, Young AM. Production of colony-stimulating factors (CSFs) during infection: separate determinations of macrophage-, granulocyte-, granulocyte-macrophage-, and multi-CSFs. *Infec Immun* 1988, **56**, 247–251.
146. Jensen WA, Rose RM, Burke RH Jr, Anton K, Remold HG. Cytokine activation of antibacterial activity. *Cell Immunol* 1988, **117**, 369–377.
147. Anaissie E, Bodey GP, Obrien S, Gutterman J, Vadhan-Raj S. Effect of granulocyte-macrophage colony-stimulating factor (GM-CSF) on myelopoiesis and disseminated mycoses in neutropenic patients with hematologic malignancies (abstr.). 31st Annual Meeting of the American Society of Hematology. *Blood* 1989, **74** (Suppl. 1), 152.
148. Rodriguez M, Swan F, Hagemeister F, *et al.* High dose ESHAP with GMCSF vs prophylactic antibiotics (abstr.). *Blood* 1990, **76** (Suppl.).
149. Ganser A, Ottmann OG, Erdmann H, Schultz G, Hoelzer D. The effect of recombinant human granulocyte-macrophage colony-stimulating factor on neutropenia and related morbidity in chronic severe neutropenia. *Ann Intern Med* 1989, iii, 887–892.
150. Welte K, Zeidler C, Reiter A, *et al.* Differential effects of granulocyte-macrophage colony-stimulating factor and granulocyte-macrophage colony-stimulating factor in children with severe congenital neutropenia. *Blood* 1990, **75**, 1056–1063.

151. Migliaccio AR, Migliacco G, Dale DC, Hammond WP. Hematopoietic progenitors in cyclic neutropenia: effect of granulocyte colony-stimulating factor *in vivo*. *Blood* 1990, **75**, 1951–1959.
152. Vadhan-Raj S, Jeha SS, Buescher S, *et al.* Stimulation of myelopoiesis in a patient with congenital neutropenia biology and nature of response to recombinant human granulocyte-macrophage colony-stimulating factor. *Blood* 1990, **75**, 858–864.
153. Nand S, Bayer R, Felten W, Godwin W. Granulocyte macrophage colony stimulating factor for the treatment of drug induced agranulocytosis (abstr.). *Blood* 1990, **76** (Suppl.).
154. Hurst D, Lipani J, Becker J, Kilpatrick L, Douglas SD. rhu-GMCSF treatment of neutropenia in glycogen storage disease IB (abstr.). *Blood* 1990, **76** (Suppl.).
155. Pedrazzoli P, Locatelli F, Zecca M, Nespoli L, Cazzola M. Cyclic neutropenia treated with GM-CSF (abstr.). *Blood* 1990, **76** (Suppl.).
156. Lieschke GJ, Cebon J, Morstyn G. Characterization of the clinical effects after the first dose of bacterially synthesized recombinant human granulocyte-macrophage colony-stimulating factor. *Blood* 1989, **74**, 2634–2643.
157. Thompson JA, Douglas JL, Kidd P, *et al.* Subcutaneous granulocyte-macrophage colony-stimulating factor in patients with myelodysplastic syndrome: toxicity, pharmacokinetics, and hematological effects. *J Clin Oncol* 1989, **7**, 629–637.
158. Antin JH, Smith BR, Holmes W, Rosenthal DS. Phase I/II study of recombinant human granulocyte-macrophage colony-stimulating factor in aplastic anemia and myelodysplastic syndrome. *Blood* 1988, **72**, 705–713.
159. Ganser A, Lindemann A, Seipelt G, *et al.* Synergistic effects of sequential IL-3/GM-CSF treatment in comparison to IL-3 alone *in vivo* (abstr.). *Blood* 1990, **76** (Suppl.).

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Cancer Risks Related to Electricity Production

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The International Agency for Research on Cancer has previously evaluated the cancer risks associated with fossil fuel-based industrial processes such as coal gasification and coke production, substances and mixtures such as coal tars, coal tar pitch and mineral oils, and a number of substances emitted from fossil-fuelled plants such as benzo[a]pyrene and other polycyclic aromatic hydrocarbons, arsenic, beryllium, cadmium, chromium, nickel, lead and formaldehyde. Based on these evaluations and other evidence from the literature, the carcinogenic risks to the general population and occupational groups from the fossil fuel cycle, the nuclear fuel cycle and renewable cycles are reviewed. Cancer risks from waste disposal, accidents and misuses, and electricity distribution are also considered. No cycle appears to be totally free from cancer risk, but the quantification of the effects of such exposures (in particular of those involving potential exposure to large amounts of carcinogens, such as coal, oil and nuclear) requires the application of methods which are subject to considerable margins of error. Uncertainties due to inadequate data and unconfirmed assumptions are discussed. Cancer risks related to the operation of renewable energy sources are negligible, although there may be some risks from construction of such installations. The elements of knowledge at our disposal do not encourage any attempt toward a quantitative comparative risk assessment. However, even in the absence of an accurate quantification of risk, qualitative indication of carcinogenic hazards should lead to preventive measures.
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INTRODUCTION

ELECTRICITY PRODUCTION entails a wide range of health risks for both the workers and the general public [1]. Some of them are well known (e.g. accidental deaths among coal miners), but many other effects are still matters of debate (e.g. increased cancer risk in populations living close to nuclear power plants). Acute health effects, such as accidents, account for the majority of deaths related to electricity production, according to some researchers [2–5]. However, the burden of chronic diseases is more difficult to estimate, and low estimates may reflect lack of knowledge as well as lack of risk. In particular, cancer is more difficult to quantify than other chronic health effects, because of its long latency, the lack of specificity of most aetiological associations so far elucidated, in particular for the more common cancers (i.e. one exposure may cause several types of cancers and the same cancer can be due to several exposures), and the relative rareness of most cancer types.

A further health consequence of electricity production is psychological, mainly stress, which may lead to increased morbidity. The perception of risk from various forms of electricity production may not reflect the actual risks. In particular, nuclear power, because it is relatively new compared to coal-burning, and because radiation cannot be sensed, is *a priori* more stressful for the neighbouring populations than heavily polluting—but

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