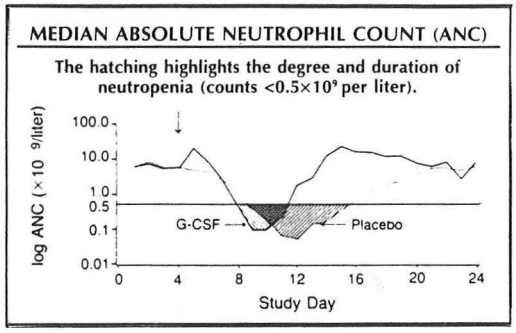
Clinical Use of Hematopoietic Growth Factors

Internal Medicine Grand Rounds

Southwestern Medical School



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I. Introduction

The subject of today's Grand Rounds is a review of the two myeloid colony-stimulating factors, G-CSF and GM-CSF, that have been released for the treatment of neutropenia. However, these molecules represent only the first of probably 20 or more cloned human factors that affect the growth and differentiation of hematopoietic cells and which are currently either in phase I trials or undergoing preclinical studies in animals. Although it is outside the scope of today's discussion, Table 1 lists many of these cytokines, their chromosome location, and size (for a review, see Reference 1). Of note, the genes for a number of these growth factors cluster on the long arm of human chromosome 5, and nearly all of the factors are relatively low molecular weight glycoproteins of 10-40 kilodaltons. In Table 2, the cellular source and major target cells of many of these cytokines are listed. As we shall see later, recent studies are beginning to define subfamilies of related molecules, bringing some order to this bewildering array of apparently unrelated cytokines.

Table 1. Cytokines involved in the regulation of hematopoiesis

Molecule	Synonym	Chromos. localisat.	mRNA kb	MW, kD
Hematopoietic growth factors				
SCF	MGF, kit-ligand	nr	nr	18
multi-CSF	IL-3	5q	1	14–18
GM-CSF	CSF-α	5q21-32	1	14–35
G-CSF	CSF-β	17q11-22	1.6	18–22
M-CSF	CSF-1	5q33	1.8–4.0	36–90
Erythropoietin		7q21	2.0	32–35
Non-CSF interleukins				
IL-1-α	hemopoietin-1	2q14	2.2	17–31
IL-1-β		2q14	1.6	17–31
IL-2	TOGF	4p	0.9	15
IL-4	BSF-1	5q	0.9	15–20
IL-5	BCGF-II, TRF	5q	1.7	12–18
IL-6	IFN-β2, BSF2	7p21	1.3–1.6	24
IL-7		8q12-13	1.8–2.4	17
IL-9	p40	nr	0.8	40
Growth factor-inducing factors				
TNF-α	cachectin	6p23	1.6	17
Lymphotoxin	TNF-β	6p23	1.7	18
IL-6	see above			
IFN-γ	type-II IFN	12	1.7	15–45
Chemotactic factors				
IL-8	NCF/NAP, TCF	nr	1.8	10
MCAF		nr	0.7	32
MIP-1		nr	nr	8–10
MIP-2		nr	nr	8
Others				
LIF	HILDA	22q12	4.0	24
Activin		nr	nr	nr
Inhibin		nr	nr	32
Oncostatin M		nr	2.0	28
TGF-β		nr	1.8	25

In addition to the cytokines themselves, the last decade has also seen the identification and cloning of the genes for the specific cell surface receptors that are expressed on the target hematopoietic cells and permit a biological response to the relevant cytokine. Although at first glance, it might seem that the receptor molecules would not have a potential for clinical use, in fact, the receptors of many of these growth factors have already been engineered into a hybrid molecule consist-

TABLE 2
Hemolymphopoietic Growth Factors: Interleukins

Growth factor	Source	Major target cell
IL-1	Monocytes, leukocytes	Monocyte, neutrophil, endothelial cell, fibroblast
IL-2	T cells	T cell
IL-4	T cells	Cofactor for myeloid proliferation
IL-5	T cells	Eosinophil
IL-6	Fibroblast, leukocytes, epithelial cells	B cell, myeloma cell, myeloid precursors, T cell, megakaryocyte
IL-7	Leukocytes	B cell, megakaryocyte
IL-8	Leukocytes	Granulocyte
IL-9	Leukemic cell line	Helper T cell, erythroid progenitor
IL-11	Stromal cell line	Megakaryocyte, B cell, blast cell

Hemolymphopoietic Growth Factors: Colony-Stimulating Factors and Erythropoietin

Growth factor	Source	Major target cell
Erythropoietin	Kidney	Erythrocyte
GM-CSF	T cells, monocytes, Endothelial cells, fibroblasts	Granulocyte, monocyte, eosinophil, erythroid, megakaryocyte
IL-3	T cells, macrophages (?)	Granulocyte, monocyte, eosinophil, erythroid, basophil, megakaryocyte
G-CSF	Monocytes, fibroblasts, endothelial cells	Granulocyte (others at high concentration)
M-CSF	Monocytes, endothelial cells, placenta, human urine	Monocyte

ing of human IgG molecules in which the two antigen binding sites are replaced by the ligand binding region of the cytokine receptor. Potentially, these constructs can be used to scavenge and block the activity of the targeted growth factors with an affinity that is generally several orders of magnitude higher than that obtained using monoclonal antibodies. Moreover, the IgG construct appears to have the same long half-life in the circulation that is characteristic of native human IgG.

Finally, the possibility also exists to create novel molecules altogether that are fusions of two known cytokines. One of these, a fusion of GM-CSF and IL-3 called PIXY321, is already in phase I clinical trials (for a review see Ref. 2). Surprisingly, this fusion molecule seems to have novel properties that are different that one would see by simply mixing the two separate cytokines (3). Of course, along these lines, many additional studies will be forthcoming that look at the clinical effects of combinations of growth factors, the most widely reported to date examining GM-CSF and IL-3 (4-6).

Today, however, our task is somewhat simpler, as we will focus on the two hematopoietic growth factors that have been released for the treatment of neutropenia, G-CSF and GM-CSF. For those of you who are interested in the history of the discovery of these factors in in vitro assays, and the approach to their cloning and initial trials in animals, I reviewed this subject in a previous Grand Rounds approximately 5 years ago, at a time when both G-CSF and GM-CSF were just being tested in humans in phase I trials. Thus, today, we have approximately 5 years of experience with the human clinical use of these hematopoietins, which have been employed world-wide since their FDA approval in February 1991.

II. Structure of G-CSF

As shown in Figure 1, G-CSF is an 18-22 kD protein synthesized from a gene located on the long arm of human chromosome 17. The genomic organization of the gene consists of 5 exons spanning 2 kilobases of DNA, which is processed into the mature G-CSF protein. Two types of G-CSF arise from splicing differences, containing either 177 amino acids (a-type) or 174 amino acids (b-type). In the molecule lacking the three amino acids at position 35, O-glycosylation occurs at a threonine at position 133 (Fig. 2). The molecular mechanisms that control the size of the G-CSF molecule produced, however, the type b protein appears to be 10 times more active in in vitro assays. For detailed reviews of human G-CSF and its receptor, see References 7 and 8.

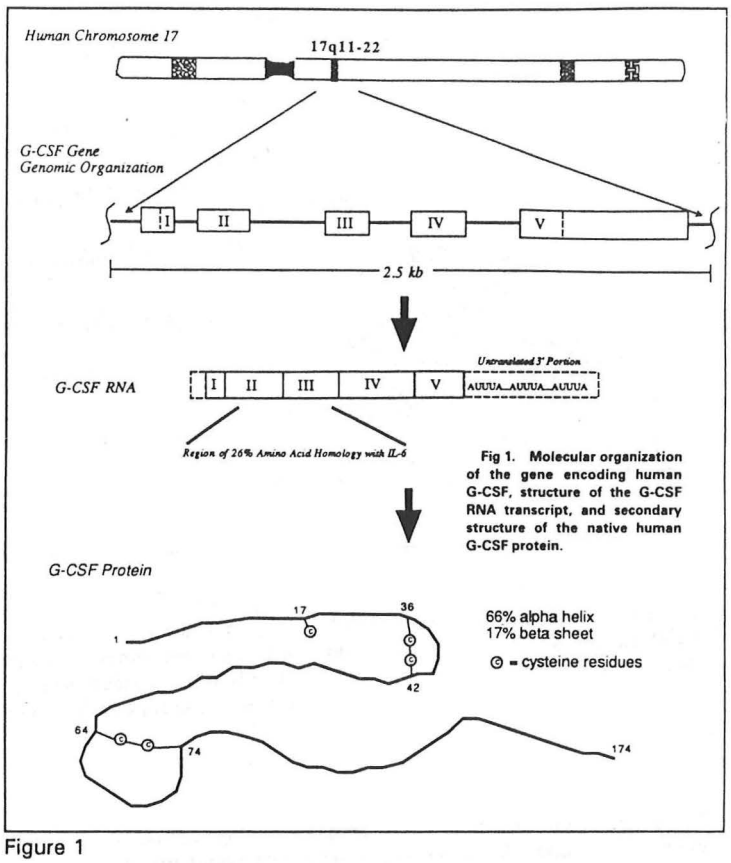

Fig 1. Molecular organization of the gene encoding human G-CSF, structure of the G-CSF RNA transcript, and secondary structure of the native human G-CSF protein.

Figure 1

Interestingly, the G-CSF gene is somewhat homologous to the IL-6 gene. IL-6 has a variety of activities in vitro, but its major effect appears to be enhancement of immunoglobulin secretion, stimulating the differentiation of late B cells, and supporting the growth of myeloma plasma cells (9). It is tempting to speculate that the homology in structure between G-CSF and IL-6 corresponds in some way to the relative similarity in lineage specificity between the two cytokines, i.e. both seem to act primarily on a single cell lineage at very late stages of development to promote terminal differentiation, albeit granulocytic cells for G-CSF and B cells for IL-6. However, there is additional data to suggest the IL-6 may have other activities on megakaryocytes and early stem cells, although most of these latter activities appear to be observed only in combination with other cytokines.

The G-CSF product available for human use from the AMGEN company is produced in E. coli by recombinant DNA technology. Because it is produced in E. coli, it lacks the glycosylation found in the native human molecule and has an added methionine at the N-terminus, but otherwise is

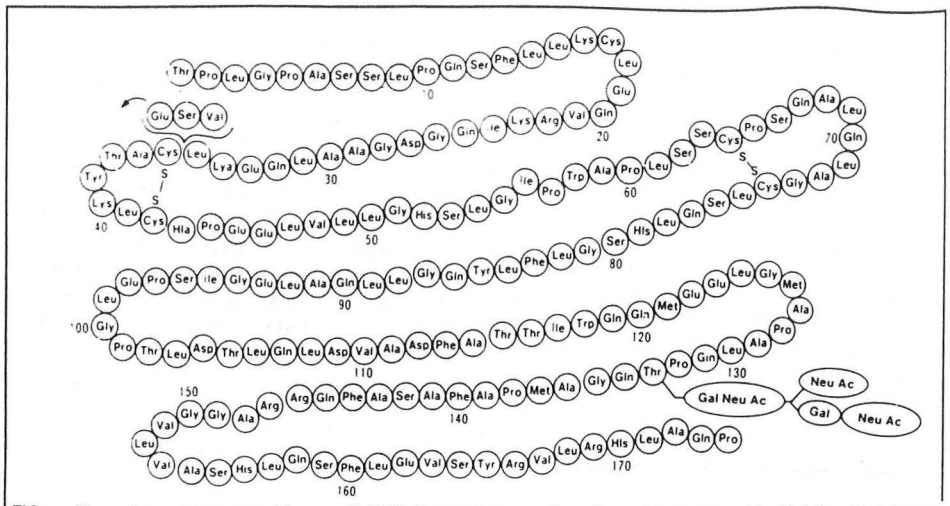

FIG. The primary structure of human G-CSF. Type "a" has an insertion of 3 amino acids (Val-Ser-Glu) between Leu 35 and Cys 36 of Type "b". Thr 133 is the site of glycosylation.

Figure 2

identical to the human type b molecule. The lack of glycosylation does not appear to have any adverse effect on biological activity. However, recent evidence suggest that the glycosylated molecule derived from chinese hamster ovary cells, and thus identical to the normal human molecule, may be more stable that the unglycosylated product currently in clinical use (10).

III. Secretion of G-CSF

Unlike erythropoietin, which is produced largely in the kidney, most cytokines, including G-CSF are expressed in a remarkable diversity of both types and locations of cells. Several reports have demonstrated that G-CSF mRNA is expressed constitutively in a variety of cell types in vitro, particularly blood monocytes, fibroblasts, and mesothelial/endothelial cells (11-13). The half-life of the mRNA is short, less than 15 minutes, however, and it is difficult to detect actual G-CSF protein produced by these unstimulated cells. In contrast, after exposure of these cells to inducers of G-CSF, such as lipopolysaccharide, the half-life of G-CSF mRNA increases transiently, resulting in an accumulation of message, and production of detectable G-CSF protein. The post-transcriptional regulation of the G-CSF mRNA has been linked to a poly-AUUUA sequence present in the 3'-untranslated region of the mRNA (14), a sequence that is also present in several other cytokines, including GM-CSF, IL-1, IL-6, and tumor necrosis factor. One suggestion created by the distribution of this 3'-mRNA instability sequence is that this may serve as a mechanism for the coordinate regulation of a variety of cytokine genes.

In addition to these post-transcriptional mechanisms of G-CSF regulation, transcriptional

mechanisms have also been described, consisting of several upstream elements in the G-CSF gene promoter that possess similarities to known classes of regulatory elements in other genes (7-8). One decanucleotide sequence (GAGATTCCCC) is highly conserved in sequence in the upstream regions of G-CSF, GM-CSF, and IL-3, and has been proposed as the critical CSF or cytokine consensus element, but a protein factor that binds to this sequence has not been isolated as yet. Finally, the production of G-CSF in vitro has been stimulated by a number of different agents, including tumor necrosis factor (15), IL-1 (16), IL-3 (17), IL-4 (18), gamma-interferon(19), and GM-CSF itself (17). Figure 3 is a recent schema that attempts to account for the known mechanisms of G-CSF production by non-hematopoietic cells and monocytes.

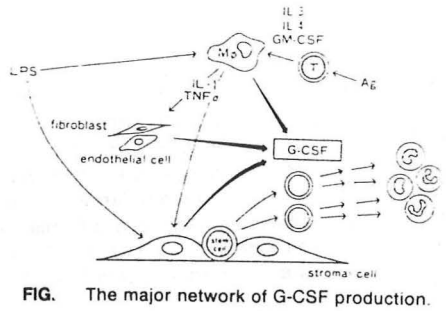

FIG. The major network of G-CSF production.

Figure 3

IV. Serum Levels and Physiologic Role of G-CSF in Humans

The physiologic role of G-CSF in vivo is still not completely understood. However, there is some evidence that G-CSF does play a role in maintaining normal steady-state hematopoiesis. First, although the levels of G-CSF in normal individuals are usually undetectable or very low as measured by either bioassays or ELISA methods (< 30 pg/mL), during infection the levels of G-CSF may rise to very high levels (> 2000 pg/mL) (20-24). In addition, there is good evidence for an inverse correlation between G-CSF levels and the peripheral neutrophil count in aplastic anemia, or following cytotoxic chemotherapy (22). Thus, as shown in Figure 4, the highest levels of G-CSF are found in the most severe cases of aplastic anemia. G-CSF levels also rise at the nadir of neutrophil counts in patients with cyclic neutropenia (22), a condition which will be discussed in more detailed subsequently.

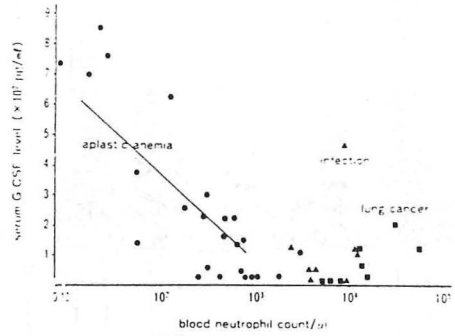

FIG. . Graph showing the relationship between serum G-CSF level and blood neutrophil count in patients with aplastic anemia, infectious diseases, and lung cancer. The G-CSF levels were estimated by enzyme immunoassay (EIA). In aplastic anemia, there was a reverse correlation between them ($r = -0.8169$, $p < 0.01$).

Figure 4

Most interestingly, in the dog there is one piece of evidence that suggests that G-CSF not only regulates granulocytes during stress, but may also be essential for the maintenance of a basal level of hematopoiesis. Normal dogs treated with human G-CSF have occasionally developed antibodies against the human molecule that nevertheless cross-react with the endogenous canine growth factor. In these dogs, a profound neutropenia has been described, and the neutropenia does not resolve

until the antibody disappears (25). In addition, infusion of the plasma from one neutropenic dog into a normal animal produced prompt neutropenia. Thus, this one piece of data suggests that G-CSF may in fact be the physiologic regulator of granulopoiesis, i.e. the molecule that is analogous to erythropoietin for red blood cells.

The effects of G-CSF on human granulopoiesis has been examined in vivo in two patients given tritiated thymidine to label the bone marrow cells induced to proliferate by the growth factor (26). The survival of technicium labeled neutrophils was also studied. The results showed that the increase in the peripheral neutrophil count resulted from increased proliferation at all stages of granulopoiesis, particularly resulting from increased entry of cells into the myeloblast compartment. Most importantly, the half-life of circulating neutrophils was not different from normal, demonstrating that the increase in neutrophils was not simply due to a longer half-life of cells in the circulation. Sequestration of the neutrophils in the spleen, liver, or lungs was not observed. Thus, the overall effect of G-CSF appears to result from increased marrow production and acceleration of granulocyte maturation.

It is also interesting to note that very long term exposure to G-CSF has been studied by introducing the G-CSF gene into murine bone marrow cells (27). Not surprisingly, the animals carrying this gene, which was under the control of a very strong viral promoter, and thus expressed very high levels of the cytokine, developed sustained granulocytosis in the peripheral blood. Although some infiltration of the liver and lungs was noted, no premature death or organ damage was noted in mice that over-expressed G-CSF for more than 6 months. As we shall see, this result is in contrast with the extensive tissue damage and premature death seen with chronic over-production of GM-CSF (28).

V. The G-CSF Receptor

The gene for the G-CSF receptor has been cloned and mapped to human chromosome 1 (29, 30). As shown in Fig. 5, the G-CSF receptor is homologous to the beta subunit of the IL-6 receptor, but appears to function as a high affinity binding site for G-CSF by homodimerization, rather than by association with a different subunit (31). In this regard, it is interesting to note the recent findings that the beta IL-6 receptor molecule, which is also termed gp130, is also involved in the high affinity receptors for two additional cytokines, Oncostatin-M and LIF (leukemia inhibitory factor) (32). As shown in Fig. 6, the gp130 chain (in black), can function by itself as a low

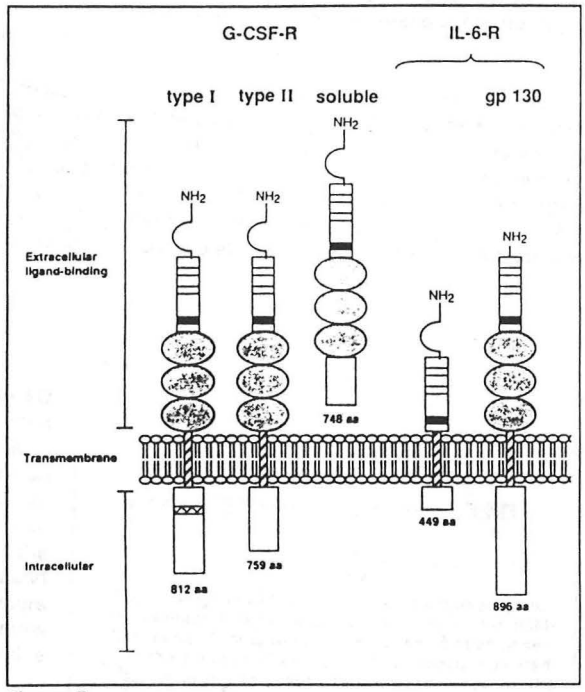

Figure 5

affinity receptor for Oncostatin, or in combination with the low affinity alpha LIF receptor, form a high affinity receptor that binds both LIF and Oncostatin. Oncostatin M has multiple effects in vitro, including the inhibition of some cancer cell lines, but it has recently been identified as the most potent mitogen known for AIDS-Kaposi's sarcoma cells (33, 34). It is tempting to speculate that the G-CSF receptor molecule, which is homologous to the gp130 component of the IL-6 receptor, might also interact with an alpha subunit, possibly to bind different cytokines which may not yet be identified.

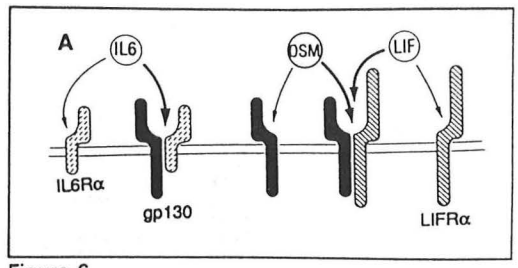

Figure 6

Also shown in Fig. 5 is the finding that alternative splicing leads to the production of apparently three different receptor species, two that are membrane bound but have different lengths of the intracytoplasmic domain, and one that lacks the transmembrane domain and appears to be a secreted form of the receptor (35). Relatively little is known yet about the regulation of receptor expression or the significance of these different forms of the receptor. In addition, the receptor cytoplasmic domain lacks any known kinase activity or domain, and thus the mechanism responsible for signal transduction after binding of G-CSF is also unclear.

As might be expected, the G-CSF receptor is found on progenitor cells committed to the granulocyte lineage, mature neutrophils, and some, but not all, myeloid leukemia cells (36, 37). Neutrophils appear to express from 50-500 receptors per cell. Numerous studies have shown that approximately half of myeloid leukemia cells have receptors for G-CSF with an affinity and number that does not seem to be significantly altered (38-44). Although the G-CSF receptor is limited largely to myeloid cells, endothelial cells (45), placenta (46), and some small cell lung cancer cell lines (47) have been shown to express the receptor, although the physiologic significance of this relatively low level of expression is unclear.

VI. Structure of GM-CSF

GM-CSF is a glycoprotein of 14-35 kD synthesized from a gene located on human chromosome 5q. This location is very close to the gene for IL-3 and also in the general region of chromosome 5 that includes the genes for IL-5, IL-4, and macrophage CSF. As shown in Fig. 7, the gene spans approximately 2.5 kB of the genomic DNA and consists of three introns and four exons. The gene product is a mature protein of 127 amino acids, but varying patterns of glycosylation account for the highly variable molecular weight (48).

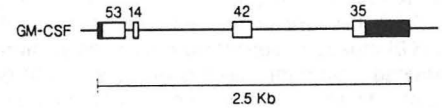

FIG. . Diagram of the structure of the human granulocyte-macrophage colony-stimulating factor (GM-CSF) gene. Coding regions are shown as open boxes, with the number of amino acids encoded indicated. Shaded areas indicate untranslated areas of the mRNA (adapted from reference 10).

Figure 7

As shown in Table 3, several different recombinant preparations of GM-CSF have been developed by the drug companies, depending on the source of the molecule, i.e. E. coli, yeast, or mammalian cells. The product available for use currently is the yeast product of Immunex which is glycosylated, but the pattern and carbohydrate moiety accounting for this glycosylation may be different from that of the native human molecule. Interestingly, the unglycosylated product obtained from E. coli has the highest specific activity (49), and the ability of GM-CSF to stimulate proliferation decreases with increasing glycosylation. Although the function of the molecule in vivo does not appear to be affected by the presence or absence of glycosylation, it is possible that the pharmacokinetics may be somewhat different and the frequency of antibody formation (50) or toxicity (51) may also be affected. Presumably, glycosylation is normally involved in the secretory process, increasing the binding to plasma proteins and thus increasing survival in the circulation.

TABLE *Available recombinant preparations of GM-CSF*

Source	Company	Specific activity, units/mg of protein
Yeast	Immunex	5×10^7
	Behringwerke	
	HRPI	
Escherichia coli	Schering-Plough	1×10^8
	Sandoz	
Cos cell	Sandoz	$4–8 \times 10^6$
CHO cell	Sandoz	5.4×10^6
	Genetics Institute	

GM-CSF, granulocyte-macrophage colony-stimulating factor; HRPI, Hoechst-Roussel Pharmaceuticals, Inc.

Table 3

VII. Secretion of GM-CSF

GM-CSF is produced by activation of a variety of cell types, including T cells, macrophages, mast cells, endothelial cells, and fibroblasts (for a review of GM-CSF, see Ref. 52). Inducers of GM-CSF expression by these cells are in part similar to those described for G-CSF, i.e. tumor necrosis factor, IL-1, and lipopolysaccharide (16, 53-55). As discussed previously for G-CSF, both transcriptional and post-transcriptional mechanisms account for the increase in GM-CSF accumulation after cell stimulation. Thus, the GM-CSF gene also has the 3' untranslated region AUUUA sequence that appears to affect mRNA stability and the decanucleotide consensus sequence in the 5' promoter region that is also found in IL-3, IL-2, and G-CSF.

VIII. Serum Levels and Physiologic Role of GM-CSF in Humans

Unlike G-CSF, GM-CSF is rarely detected in the circulation (24, 56). Therefore, GM-CSF behaves like a locally produced and acting factor, rather than as a hormone. This model of GM-CSF function is consistent with a potential physiologic role of GM-CSF in enhancing local host defenses at the site of an immune challenge. Consistent with this model is the finding that GM-CSF may produce an inhibition of neutrophil migration at inflammatory sites with prolonged exposure (57-59). Thus, there has been some concern that in the setting of an established tissue infection, parenteral administration of GM-CSF may actually impair the migration of neutrophils to the site of infection despite leading to a marked increase in numbers of circulating granulocytes. This effect on neutrophil migration has not been seen with G-CSF (60). On the other hand, the clinical significance of this effect of GM-CSF, particularly at lower doses, is unclear. One recent report suggests that co-administration of pentoxifylline, an inhibitor of tumor necrosis factor, may be useful in preventing this effect of GM-CSF (61, 62).

The effects of GM-CSF on human granulopoiesis in vivo has recently been examined by labeling bone marrow cells with tritiated thymidine both in vivo and in vitro in four patients receiving a 10 day course of GM-CSF (63). The results showed that the peripheral half-life of granulocytes during GM-CSF administration was increased approximately 6 times over normal, as shown in Table 4 in comparison to results obtained with G-CSF by the same group. Although these studies are clearly preliminary and need independent confirmation, the data also suggest that if one did not include the stimulation of eosinophilia that occurs with GM-CSF, the total stimulation of peripheral neutrophils was approximately 3-4 fold. Although there was also an acceleration of cell-cycling in the bone marrow, a finding in accord with studies by another group (64), leading to increased production by the marrow compartment, simple measurement of the peripheral granulocyte count markedly overestimated the actual stimulation of neutrophil production by GM-CSF.

TABLE - COMPARISON OF G-CSF- AND GM-CSF-STIMULATED NEUTROPHILS

Neutrophil production	Normal	GM-CSF	G-CSF
Maximum count ($\times 10^{-9}$ ml)	5.2	17.0	35.0
Appearance in peripheral blood (days)	4–7	4.5–6.5	1–2
Peripheral half-life, $t_{1/2}$ (hrs)	8	48	7.6
Amplification enhancement factor	1	1.5	9.4
Extra amplification divisions	0	0.6	3.2

From Lord *et al.* (1989). – Based on Cronkite *et al.* (1959). – Based on Athens *et al.* (1961) and Dancey *et al.* (1976).

Table 4

Also in contrast to G-CSF, prolonged exposure to GM-CSF in a transgenic animal model leads to blindness, caused by an accumulation of macrophages in the eye, and wasting and premature death associated with infiltration of macrophages into striated muscle (28). In a related model in which the GM-CSF gene was overexpressed in bone marrow cells by infection with a retrovirus construct, a fatal myeloproliferative syndrome is observed, although transplantation experiments into secondary recipients suggest that the proliferation is non-neoplastic, i.e. non-transplantable (65).

IX. The GM-CSF Receptor

Two distinct chains that together constitute a high-affinity GM-CSF receptor have been cloned. The alpha chain, which was cloned from placenta (66), by itself can bind GM-CSF with low affinity. However, because the cytoplasmic tail consists of only 54 amino acids, it is thought to lack signalling activity. The second beta subunit, which by itself cannot bind GM-CSF, has also been cloned (67), and together the alpha and beta subunits can bind GM-CSF at high affinity.

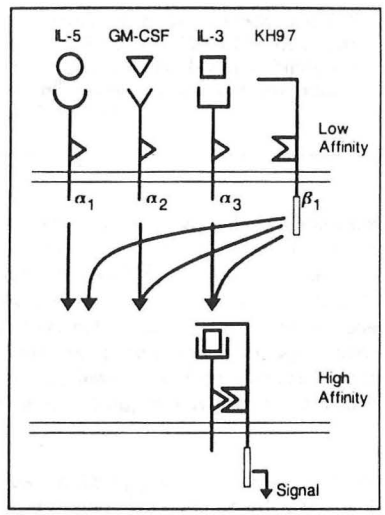

Figure 8

Interestingly, very recent experiments have shown that the IL-3 and Il-5 receptors all share the same beta chain with the GM-CSF receptor (68-70), as shown in Fig. 8. Fig. 9 compares the subunit sharing observed with the IL-3, GM-CSF, and IL-5 receptors with the IL-6, LIF, and Oncostatin M receptors. Of interest, this scheme suggests a mechanism for possible competition between IL-3, IL-5 and GM-CSF for limiting amounts of the common beta subunit. In this regard, it is interesting to note that IL-3 and GM-CSF have many of the same target cells in vitro, although IL-3 appears to stimulate an earlier progenitor population than GM-CSF, while all three cytokines are able to stimulate eosinophils. In fact, the major activity of IL-5 appears to be in the eosinophil lineage, where it is

probably the most important cytokine (71, 72).

Not surprisingly, the GM-CSF receptor appears to be widely expressed on hematopoietic cells (73, 74). In addition, receptors have been found on endothelial cells (45) and some tumor cells, including small cell lung cancer and melanoma (75). Melanoma cells, however, only express the low-affinity receptor, and do not appear capable of transducing a signal when stimulated with GM-CSF (52). Neutrophils possess the high-affinity GM-CSF receptor at about 800-1000 sites per cell. The highest receptor expression is observed on the most mature cells.

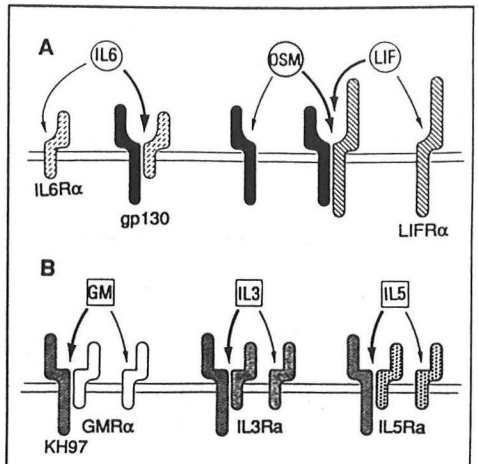

Fig. Comparison of the IL-6, OSM, and LIF receptor system (**A**) to the GM-CSF, IL-3, and IL-5 receptor system (**B**). High-affinity binding is represented by broad arrows and low-affinity binding by thin arrows.

Figure 9

X. Comparison of G-CSF and GM-CSF: Pharmacology, Effects on Myeloid Cells, and Side-Effects

GM-CSF administered to human subjects by either intravenous or subcutaneous routes produces an initial immediate and transient fall in the circulating neutrophils, monocytes, and eosinophils (76-78). This effect is thought to be due to sequestration of leukocytes in the lungs (79) and occurs with every dose of the drug, followed by recovery to baseline within several hours. Continued daily dosing of the drug, however, produces progressive leukocytosis with a marked left shift, i.e. with the appearance of immature granulocytes (76-78, 80). The response to continued administration has also been described as biphasic, with the first rise seen in the first five days, followed by another transient fall in numbers, and then a second response that continues until the patient is no longer receiving GM-CSF. This pattern of response has been attributed to an initial demargination and release from a bone marrow storage pool, followed at later times by actual increased marrow production, although recent kinetic studies described previously suggest that the second phase may also result from a much longer half-life of neutrophils in the circulation. Following cessation of the drug, the white blood count returns to normal over 3-5 days.

Although the recommended dose of GM-CSF for human use is 250 $\mu g/M^2$/day given as an IV infusion over 2 hours, some investigators have recommended extending the time of infusion to 6 hours to reduce side-effects. In various studies doses have actually ranged from 100-500 $\mu g/M^2$/day, or 2.5-12.5 μg/kg/day, with the magnitude of the leukocytosis being dose dependent. Despite the approval for IV administration, however, the optimal schedule for GM-CSF appears to be 5 μg/kg/day given subcutaneously in two divided doses. The s.c. route of administration is associated with better efficacy as compared to IV bolus schedules, and the high peak levels achieved with IV routes are more likely to result in shortness of breath at the first treatment and a higher incidence of pericarditis.

Phase I studies of recombinant G-CSF demonstrated that the drug produces a dose-dependent leukocytosis when given by either IV bolus (81, 82) , continuous IV infusion (83), or subcutaneous administration (84, 85). The recommended dose of G-CSF is 5 μg/kg/day given either subcutaneous-

ly or as a single IV injection. No upper limit for G-CSF dose has been established as patients have been treated to achieve white blood counts of 200,000 without serious side-effects. An acute, transient fall in neutrophil counts has been observed with G-CSF (81), similar to that seen for GM-CSF. With continued administration, the morphology of the circulating neutrophils changes, with a left shift to more immature forms and the appearance of Dohle bodies and toxic granulations. Recently, the appearance of giant neutrophils in the circulation in response to G-CSF has been described (86). Interestingly, neutrophils with the Pelger-Huet anomaly have been reported to respond to G-CSF with an enhancement of nuclear segmentation (87).

Table 5. Effects of CSF on mature cells

Factor	Target cells	
G-CSF	neutrophils	↑ antibody-dependent cell-mediated cytotoxicity
		↑ generation of superoxide anion
		↑ release of arachidonic acid in response to chemoattractants
GM-CSF	neutrophils	↑ priming of cells to activation by bacterial protein
		↑ antibody-dependent cell-mediated cytotoxicity
		↑ metabolic energy
		↑ phagocytosis (bacteria, parasites, yeast), inhibits migration
		↑ cell adhesiveness to endothelium
		↑ generation of superoxide
		↑ production of IL1
	monocytes	↑ chemotaxis
		↑ release of prostaglandin E, plasminogen activator, interferons, tumour necrosis factor, M-CSF
		↑ antibody-dependent cytotoxicity
		↑ cell adhesion

Table 5

Both G-CSF and GM-CSF have been shown to enhance a large number of effector cell functions seen in mature neutrophils. Thus, in addition to stimulating the growth of neutrophil progenitors, both cytokines have well documented effects on neutrophil function, including the induction of adherence proteins, stimulation of phagocytosis, and priming for chemotaxis, degranulation, and superoxide anion production. These effects are summarized in Table 5. Of note, however, GM-CSF has marked effects on macrophages, eosinophils, and basophils which are not seen with G-CSF. For reviews of these effector cell functions, see references 52 for GM-CSF and 8 for G-CSF. Both G-CSF and GM-CSF have also been reported to increase the numbers of hematopoietic progenitor cells in the blood by approximately 10 fold (88, 89). As we shall see later, this effect is being exploited to harvest cells from the peripheral blood for autologous transplantation.

Side-effects of both drugs have been surprisingly limited. For G-CSF, mild bone pain controlled by acetaminophen was the only consistently observed adverse effect, occurring in about 25% of patients. In addition, elevations of alkaline phosphatase, LDH, and serum urate have occurred and are thought to be related to the leukocytosis and increased cell turnover. Splenic enlargement has been seen in children with chronic neutropenia receiving G-CSF for prolonged periods. In addition, individual cases have been reported of Sweet's syndrome (acute febrile neutrophilic dermatosis) in a patient with pre-existing hairy cell leukemia and cutaneous vasculitis (90) and bullous pyoderma gangrenosum at the site of previous eczema in a patient receiving G-CSF for small cell lung cancer (91). One case of transient thrombocytopenia has been reported (92) and a recent case of

anaphylaxis has been described (93).

Although GM-CSF is also generally well tolerated, it appears to be more toxic than G-CSF at equally effective doses. Toxicities include bone pain, fever, malaise, myalgia, arthralgia, anorexia, mild rises in the transaminases, and a rash at the site of injection. More seriously, higher doses of GM-CSF have been associated with the capillary leak syndrome, including pleural and pericardial effusions, ascites, and renal failure. These latter problems, however, have only been frequently observed at doses of more than 30 μg/kg/day. Although some of this toxicity has been attributed to induction of TNF, it is also possible that the marked eosinophilia that occurs at high doses of GM-CSF may also play a role in certain types of tissue damage. In this regard, a recent case of epidermolysis bullosa acquisita characterized by skin bullae filled with eosinophils has recently been reported in a patient receiving GM-CSF (94). Other patients with cutaneous eruptions have also been reported.

Other toxicities attributed to GM-CSF appear to relate to its stimulation of macrophages of the reticuloendothelial system or induction of antigen-presenting cells, TNF, and possibly IL-6. Thus, two cases of rheumatoid arthritis flares (96, 97), several cases of reactivation of autoimmune thyroid disease with reversible thyroid dysfunction (98), and several cases with acceleration or reactivation of other autoimmune diseases, such as ITP (78) and autoimmune hemolytic anemia have been temporally associated with the administration of GM-CSF (99). Based on these observations, it seems reasonable to observe caution in patients with a history of autoimmune disorders or collagen vascular diseases.

Two additional effects of GM-CSF have recently been described that raise some concern for long term drug use. In human studies, GM-CSF appears to inhibit the generation of natural killer cells (100). Somewhat similarly, in mice given GM-CSF, a reversible, marked inhibition of primary B cell lymphopoiesis has recently been reported (101).

X. Overview of Clinical Trials with G-CSF and GM-CSF

Table 6 summarizes the range of clinical conditions that have been proposed as indications for GM-CSF or G-CSF. The common thread linking most of these disease states, of course, is neutropenia severe enough to cause fever and infection. As we shall see, the utility of the CSF's in many of these neutropenic states has been the subject of intense investigation in the last five years. However, for the purposes of this review, I am going to divide the causes of neutropenia into two somewhat different groups which may be more useful for analyzing the results of the available clinical trials.

In the first group are states that are associated with chronic stable neutropenia of any cause, whether it be congenital or acquired, benign or neoplastic, idiopathic or immune-mediated. The

Table · Potential applications of CSFs

Correction of insufficient hematopoiesis:
Treatment of Anemia
Prevention of chemotherapy-induced neutropenia
Possibility of dose intensification
Autologous bone marrow transplantation
Stimulation of hematopoiesis in primary bone marrow failure
Aplastic anemia
Congenital neutropenia or other idiopathic cytopenias
Use in the treatment of leukemias
Treatment of acute myeloid leukemia
Myelodysplastic syndromes
Expansion and recruitment of circulating progenitor cells
Peripheral blood stem cell transplantation
Activation of effector cell function
Infections
Leukocyte function disorders
AIDS
Tumor cytotoxicity

Table 6

important concept here is that these states are not the result of an acute event or injury, and thus the marrow is not expected to under cycling or kinetic changes in the absence of the cytokine.

In the second group, however, are acute neutropenias that involve an injury to the marrow, usually drug-induced, that is expected to be reversible, albeit with a time course and duration of neutropenia severe enough to be associated with high risk of morbidity and/or mortality. In this category, I will also include a unique congenital disease, cyclic neutropenia, which may serve as a possible model for the optimal use of cytokines in the acquired, drug-induced marrow cycling states, most commonly following chemotherapy. Finally, at the end, a third miscellaneous group of uses for CSF's, such as acute leukemia, harvesting of peripheral blood stem cells, and acceleration of recovery after bone marrow transplantation will be considered.

XI. Risk of Infection with Neutropenia

Before considering the results of trials of CSF's, it may be appropriate to briefly review the data on the risk of infection in neutropenia. Obviously, prolonged and severe neutropenia is an overwhelming risk factor for bacterial infection, particularly when the neutrophil count is less than 100 per mm^3. However, to evaluate the clinical trials of CSF's, where the endpoints are generally days of fever, days of neutropenia, and incidence of proven infection, an assessment of the relative risks inherent at various endpoints, such as 1000, 500, or 250, or 100 absolute neutrophils provides a valuable baseline for assessing claims of benefit from CSF's.

Surprisingly, data on the quantitative relationship between the severity and duration of

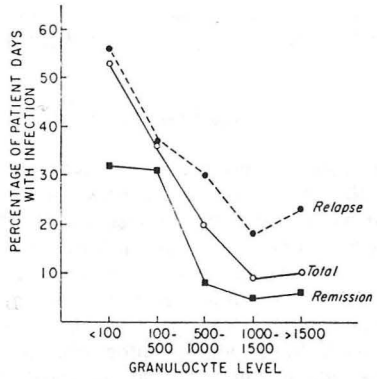

FIGURE 1. The effect of granulocyte level on the presence of identified infection. The percentage of days spent with infection is plotted at each level of circulating granulocytes. This percentage decreased with increasing granulocyte level and was always higher during relapse than during remission.

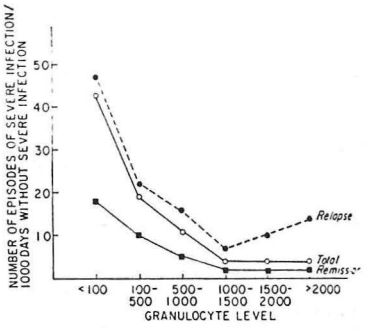

FIGURE 2. The frequency of infectious episodes related to the granulocyte level. The number of episodes of severe infection/1,000 days without severe infection is plotted for each granulocyte level. The risk of developing severe infection decreases with increasing granulocyte level. However, no further reduction occurrs above a granulocyte level of 1,500/mm^3. The risk is greater in relapse than in remission for every granulocyte level.

Figure 10

neutropenia and the risk of infection is relatively limited. The classic study carried out in 1966 on leukemic patients by Bodey and his colleagues at M.D. Anderson is summarized in Fig. 10 (102). For patients in remission, probably a better group to compare to other patients with malignancy or with qualitatively normal neutrophils, the risk of infection and the frequency of infectious episodes appears to be modestly increased for neutrophil counts in the 500-1000 range, and markedly elevated for counts between 100 and 500, with a further increase in risk occurring with counts less than 100. A subsequent study by the same investigators in patients with metastatic breast cancer suggested that the risk of infection was increased only by ANC's less than 500 (102). Other investigators have emphasized the risk for short durations of neutropenia at an ANC of 100 or less (103).

The implications of these findings are that studies which only report, for example, a decrease in the number of days with a granulocyte count of <1000 or even 2000 are probably not demonstrating a clinically significant benefit that will translate into a decreased incidence of fever and infection. One may even question the value of endpoints of 500 absolute neutrophils, although this is the most common number reported. As we shall see, however, perhaps the best study demonstrating the benefit of G-CSF in chemotherapy-induced neutropenia actually induced a median nadir of approximately 100 absolute neutrophils.

The correlate of this discussion is that Phase I trials with both GM-CSF and G-CSF clearly demonstrated that in prolonged bone marrow aplasia, i.e. more than a week, neither cytokine affected the duration of very severe granulocytopenia defined as an ANC less than 100. However, both growth factors can accelerate the recovery phase of neutrophil counts significantly. Thus, the higher the neutrophil level selected to report the effect of CSF's on number of days with neutropenia, the more impressive the difference will appear relative to the control arm. However, if the most significant neutrophil count for infection risk is 200, for example, rather than 500, the difference in the number of days to reach a ANC of 200 will be much less than that for days to reach 500 or even 1000. Clearly, the most useful method would be to report the days of neutropenia at not only <1000 or <500 ANC, but also <200 ANC and <100 ANC. Unfortunately, very few studies provide this data.

XII. Congenital Chronic Neutropenia

Patients with chronic congenital neutropenias are frequently at risk for recurrent infections, particularly those with chronic counts of < 500 ANC/mm^3. These patients are predisposed to skin infections such as furuncles and cellulitis, infections of the oral and gingival mucosa, pneumonia, liver abscess, otitis media, and septicemia. Table 7 lists some of the conditions in the differential diagnosis of severe chronic neutropenia, both congenital and acquired (105). Another group of patients with no history of infection and somewhat higher neutrophil counts have been termed chronic benign neutropenia and clearly do not require treatment with CSF's.

TABLE
Differential Diagnosis of Severe Chronic Neutropenia

Acquired	Congenital
Idiopathic	Myelokathexis
Large-granular lymphocyte-mediated neutropenia	Shwachman-Diamond syndrome
Vitamin B_{12} or folate deficiencies	X-linked agammaglobulinemia
Drug-induced hypersplenism	Dysgammaglobulinemia
	Glycogenosis—type IB
	Cartilage-hair hypoplasia syndrome
	Dyskeratosis congenita
	"Lazy leukocyte" syndrome
	Chediak-Higashi syndrome

Table 7

A separate entity from those listed in the

Table is Kostmann's syndrome or severe congenital agranulocytosis (106). These children have severe neutropenia at birth, usually less than 200 ANC, frequent infections, increased risk of early death, and autosomal recessive inheritance. The bone marrow is unique in demonstrating an arrest of myeloid maturation at the promyelocyte-myelocyte stage.

The results of treatment with CSF's for severe congenital neutropenias, particularly with G-CSF, have been very impressive. Ganser et al. (107) treated four patients with GM-CSF and noted increased white blood cell counts in all cases and resolution of infections. In another single case, Vadhan-Raj et al. (108) treated one patient with chronic neutropenia with IV GM-CSF and observed an increase of eosinophils and monocytes, but not neutrophils. The patient experienced a marked decrease in the incidence of infection, however, despite the persistence of neutropenia. Finally, Welte et al. (109) have described an additional 5 patients treated with GM-CSF. Although all 5 had rises in their white blood cell count, in four of the five the increase was again attributable to eosinophils. Two patients nevertheless resolved chronic gingivostomatitis. However, one patient's severe lung disease from anaerobic Peptostreptococcus did not improve and one patient developed a Staph aureus paronychia while on treatment.

Interestingly, these five patients were crossed over to subsequent treatment with G-CSF. As in the examples shown in Fig 11, the four patients who were not responsive to GM-CSF had impressive rises in the neutrophil count with G-CSF. No severe bacterial infections occurred during therapy and the patient with the severe pneumonia had a dramatic clearing of lung infiltrates. Long term treatment was well tolerated and the white counts remained elevated as long as treatment was continued. One patient on G-CSF did develop a leukocytoclastic vasculitis that occurred whenever the ANC was >1000, but has not had a recurrence of the skin infiltration with counts maintained in the 200-800 range.

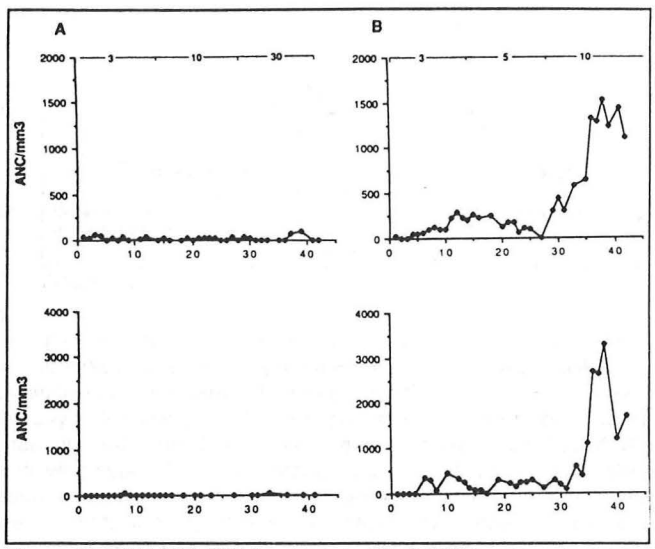

Figure 11. (A) GM-CSF treatment; (B) G-CSF treatment.

Studies by other investigators have confirmed the marked benefit seen with G-CSF. Bonilla described five patients with Kostmann's syndrome who all achieved ANC's greater than 1000 within 10 days of starting treatment, with resolution of chronic infections and a marked reduction in the incidence of new infections and requirement for antibiotics (110). Figure 13 illustrated the response of the ANC in these patients to G-CSF. Since these original reports, larger cooperative trials in both the United States (105, 111-112) and Japan (113, 114) have confirmed the beneficial effects of G-CSF in this group of patients. In 41 patients in the United States, only one failed to respond (105),

while in Japan 15 of 17 cases responded well to therapy (114). Not surprisingly, recent studies have shown that these patients do not have a defect of the G-CSF receptor (115).

Finally, a few case reports have documented benefit from GM-CSF and G-CSF for neutropenia resulting from glycogen storage disease type IB (116, 117). Although one patient had to discontinue GM-CSF therapy due to severe local side effects, this patient was successfully treated with G-CSF. One additional case of neutropenia due to myelokathexis has been reported to benefit from GM-CSF, although the development of bone marrow fibrosis after 4 months of continuous therapy necessitated temporary discontinuation of treatment (118).

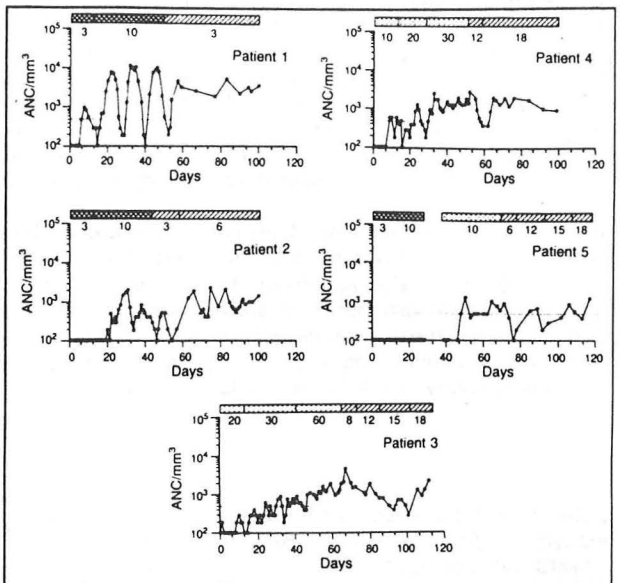

Figure 2. Absolute Neutrophil Counts (ANC) per Microliter of Peripheral Blood in the Study Patients during rhG-CSF Administration.

Doses (in micrograms per kilogram per day) are shown below the bars. Crosshatching denotes intravenous bolus infusion, stippling intravenous continuous infusion, and hatching subcutaneous administration.

Figure 12

XIII. Acquired Neutropenia and Aplastic Anemia

In contrast to the experience with congenital neutropenias, the responses to GM-CSF and G-CSF in acquired aplastic anemia have been more variable. Most patients treated with GM-CSF experienced an increase in both leucocyte count and bone marrow cellularity (77, 119-122). The responses were generally not sustained, however, and the white count returned to baseline after discontinuation of therapy. Moreover, significant improvements in neutrophil counts appears generally to be limited to patients with initial white cell counts greater than 100 ANC; patients with very severe neutropenia or very hypocellular bone marrows did not respond (120). Very few patients have experienced a response in other hematopoietic lineages, as judged by a decrease in transfusion requirements or platelet counts, although one elderly patient with apparent aplastic anemia appears to have had a trilineage response to GM-CSF that was sustained for more than a year (123). Randomized trials have not been reported to definitively assess whether treatment with GM-CSF can decrease the long-term morbidity and mortality of this disease.

Somewhat more encouraging results have been reported in 8 children with aplastic anemia who were refractory to standard treatments, including antithymocyte globulin (124). With progressive escalation of drug doses to as high as 32 μg/kg/day, six of the children had a rise in ANC of more than 500 within 4 weeks. No infections occurred during the month of treatment, one patient maintained the increased ANC for 2 months and another for more than a year after treatment was discontinued. At the highest dose, one child developed hypoxemia, bilateral pleural effusions, and pericardial effusion, which resolved after diuresis and reduction of drug dose.

More recently limited experience with G-CSF has been described. A Japanese groups has demonstrated that 12 of 20 children treated with G-CSF with variable increases in neutrophil counts (125, 126). As with GM-CSF, responses were limited to increases in neutrophils and no lasting benefit was seen. Similar results were reported in the Japanese literature by another group (127). A small number of cases treated with G-CSF and erythropoietin, or very long courses of G-CSF have had sustained responses in more than one cell lineage (128, 129), although it is difficult to distinguish these cases from spontaneous recovery of the bone marrow. Finally, the combination of cyclosporine A and G-CSF has been reported efficacious in a few patients with severe aplasia (130, 131).

Other causes of isolated, acquired neutropenia in the adult have also been treated with recombinant growth factors. Two cases of idiopathic neutropenia associated with recurrent infections have responded well to G-CSF (132, 133). One case of agranulocytosis associated with a proliferation of large granular lymphocytes markedly improved the ANC from less than 200 to 2000 during the course of treatment (134). Two out of three cases in children were also reported to correct with G-CSF (105) A previous case of neutropenia associated with large granular lymphocytes did not respond to GM-CSF (135), and one case of neutropenia associated with Felty's syndrome improved with GM-CSF (96).

XIV. AIDS

Experience in AIDS has been primarily with GM-CSF. This cytokine has been given to AIDS patients not only to ameliorate the neutropenia that occurs with virus infection and AZT therapy, but also in an attempt to enhance the immune function by stimulation of macrophage activity. Clearly, this latter effect will not be expected for G-CSF. Although initial reports raised concerns that GM-CSF might in fact increase the replication of the HIV virus (136, 137), several clinical studies have not shown any significant change in the levels of the p24 antigen or recovery of virus from cultures of peripheral blood monocytes (76, 138, 139). Long-term administration of GM-CSF for over 8 months was reported to be well tolerated (138).

Administration of GM-CSF in AIDS patients with leukopenia results in marked increases in numbers of circulating neutrophils, eosinophils, and monocytes, as originally reported by Groopman and colleagues in the New England Journal of Medicine (76). In fact, this was the first reported trial of GM-CSF in human clinical trials. More recently, the same investigators have reported in Phase I/II trials that GM-CSF can ameliorate the toxicity of chronic AZT administration (139), or the combination of AZT and interferon alpha in the treatment of Kaposi's sarcoma (140, 141). Similar results have been reported by other investigators with GM-CSF, AZT, and interferon (141), and GM-CSF for ganciclovir-induced neutropenia (142).

Although experience with G-CSF is more limited, several recent reports demonstrate that G-CSF is also effective in ameliorating neutropenia in AIDS (143) and toxicity from AZT (144, 145).

XV. Myelodysplasia

The treatment of cytopenias resulting from a variety of preleukemic states collectively termed the myelodysplastic syndromes with GM-CSF and G-CSF have been the subject of intense investigation and are well reviewed in several recent articles (146-148). Table 8 lists the subtypes of MDS agreed upon by a French-American-British group. Notice that the percentage of blasts increases in the RAEB (refractory anemia with excess blasts) and the RAEB-T (refractory anemia with

TABLE 1.

The FAB subtypes of MDS

Type	*Peripheral blood*	*Bone marrow*
RA	< 1% blasts; reticulocytopenia, macrocytosis or normochromic/ normocytic	Usually erythroid hyperplasia with dyserythropoiesis; <5% blasts
RARS	< 1% blasts; dimorphic red cell morphology	As in RA, but Type III sideroblasts ≥15% of erythroid precursors
RAEB	<5% blasts; cytopenias in 2 or 3 cell lines	5-20% blasts; 2 or 3 cell lines showing dyspoiesis
RAEB-T	5-29% blasts, or any Auer rods	20-30% blasts; any Auer rods; otherwise as RAEB
CMMoL	<5% blasts; >1x10⁹/1 monocytes	1-20% blasts; monocytosis

Table 8

excess blasts in transition). One obvious concern in treating these patients is that the CSF's could accelerate the growth of the leukemia cells, many of which have receptors for these factors, as previously discussed.

Thus far, five major trials of GM-CSF in myelodysplasia have been reported (119, 149-152). The results of these studies are summarized in Table 9. Generally treatment was relatively short-term, i.e. 1 to 2 weeks. Overall, most patients had predictable rises in granulocyte counts which were not sustained after discontinuation of the treatment, although in one study two patients had an elevation persisting for more than 6 months (152). A small number of patients had improvements in either reticulocytes, transfusion requirements, or platelet count. About 25% of the patients had a increase in marrow blasts which generally reversed after discontinuation of therapy, although some patients underwent progression to AML, particularly those with higher blast percentages prior to growth factor therapy. Thus, most studies now limit CSF's in myelodysplasia to patients with blasts less than 15% of the marrow. A sixth recent study from M.D.Anderson of 29 patients with myelodysplasia has emphasized the use of "low-dose" GM-CSF, i.e. 5-10 $\mu g/M^2$, as opposed to the usual doses of > 120 $\mu g/M^2$. In this study, 14/29 patients responded to this very low dose therapy without any side-effects (153, 154). A randomized multi-institutional trial of GM-CSF treatment

Table 1. Effects of Recombinant Human GM-CSF and G-CSF in Phase I/II Clinical Trials of Patients with Myelodysplastic Syndromes

	GM-CSF[23,27]	G-CSF[31,32]
Short-term treatment duration	7 to 14 days ×1-5 courses daily, IV or subcutaneously 30-750 μg/m²	42 to 56 days daily subcutaneously
Daily dose	30-750 μg/m²	0.1-3 μg/kg
Number of patients	45	18
FAB subtypes RA/RAEB-RAEB-t/CMML	14/26/5	2/16/0
Responses		
Neutrophils	38 (84%)	16 (89%)
Reticulocytes	14	5
Platelets	8	1
Marrow maturation	9	16
Increased blasts	12	4
Progression to AML	7	5
Long-Term Treatment Duration	2-9 weeks[27]	6-28 months[32]
Persistent neutrophil responses/patients	1/5[27]	10/11[27]

Table 9

vs. observation in MDS has been reported in abstract form (155). Twenty-one patients with MDS, all with less that 15% blasts, have received GM-CSF. Nearly all had at least a two fold increase in granulocyte counts, and in eight cases the responses lasted more than 6 months. No improvements in platelet or hemoglobin levels have been seen. The impact of therapy on infections and progression to AML has not yet been reported.

One disappointing aspect of these GM-CSF trials is that nearly all of the increases in granulocyte numbers appear to have come from the neoplastic clone, as judged by cytogenetic and molecular analysis. Thus, although the growth factor can induce differentiation of the abnormal cells, it does not favor proliferation of the normal clones and therefore does not lead to a remission of the patient's disease. While this finding appears to be a generally valid rule, two cases who achieved what appears to be a remission of their myelodysplasia, one non-clonal and one still clonal, have been reported (156, 157). On the other hand, administration of GM-CSF has been reported to enhance neutrophil function (158) and increase expression of the complement receptor (159) in these patients who frequently have defective granulocyte function.

Side-effects of GM-CSF in these studies have generally been the same as those reported for other patients. However, in one study a small number of patients appeared to have significant increases in marrow fibrosis (160). Spleen enlargement was also apparently more prominent in these patients (161), and one case has been reported who perforated an unsuspected cecal granulocytic sarcoma while receiving GM-CSF. Finally, a few pilot studies have been reported in MDS using a combination of low-dose cytosine arabinoside and GM-CSF (163, 164). Use of the CSF's in AML and in an attempt to cycle leukemic cells to achieve greater cell killing will be discussed more extensively in the section on leukemias.

Finally, G-CSF has also been used to treat patients with myelodysplasia, particularly at Stanford (165-167) and in Japan (168-170). Table 9 compares the American results with G-CSF with those reported for GM-CSF. Generally, the results are comparable with both factors. Cytogenetic abnormalities, if present, did not improve despite the increase in neutrophil counts. No significant changes in eosinophil, platelet, monocyte, or lymphocyte counts were seen. Toxicity was minimal. One case with a rise in platelets and red cell values during treatment with G-CSF has recently been described (171).

XVI. Cyclic Neutropenia - A model for chemotherapy-induced neutropenia?

Cyclic neutropenia is a rare hematologic disorder characterized by recurrent episodes of severe neutropenia at 18-21 day intervals (172). The disorder is benign, although recurrent skin infections, chronic gingivitis, cervical adenopathy, and subcutaneous abscesses are a frequent complication of the neutropenic episodes. Approximately 300-400 patients in the United States are estimated to have this usually congenital disorder. The etiology in uncertain, although an autosomal dominant pattern of inheritance is observed in the majority of families. Although steroids, androgens, and plasmapheresis have been tried in the past, none of these therapies alters the cycling of cell counts or the nadirs of granulocytes which are usually < 200 ANC.

A similar disease is also known to occur in grey collie dogs, although the length of the cycle is different. Indeed, the original use of G-CSF for these disorders was reported in these dogs (173). When G-CSF (5 μg/ kg s.c. bid) was given to animals with cyclic neutropenia, the leukocyte counts continued to cycle with the same period. However, the cycling occurred at a new, higher level of

granulocytes, sufficiently high to eliminate the periods of significant neutropenia.

Shortly after these results in the dog model were described, the same investigators published their results with G-CSF in six human cases of cyclic neutropenia in children (174). As shown in Fig. 13, G-CSF raised the granulocyte level in these patients without eliminating the cycling. However, the elevation was sufficient in these children to greatly reduce or eliminate the number of days with ANC of <200. Most importantly, the frequency of mucositis and infection was markedly reduced. Indeed, in the first 40 months of treatment, no typical mouth ulcers or bacterial infections occurred, and the chronic gingivitis improved. Similar results with G-CSF have been reported by a group at the University of Michigan (105), and in numerous case reports and small series of patients from Japan, Europe, and Scandinavia (113, 114, 175-181).

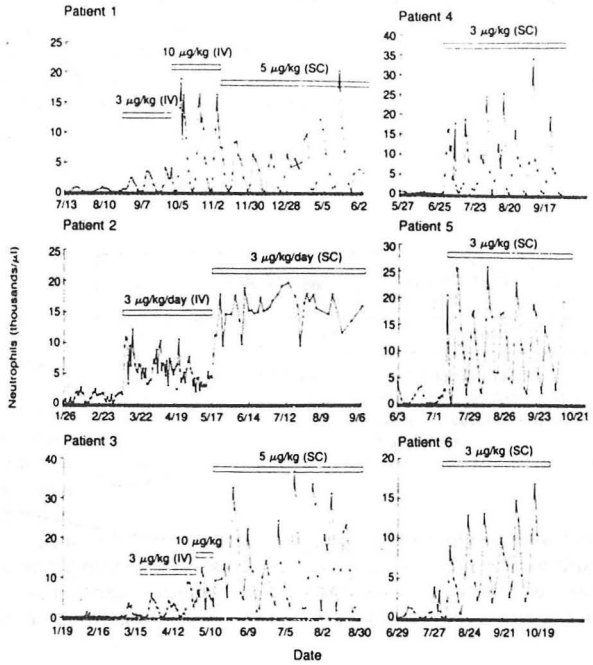

Figure 1. Neutrophil Counts Performed Daily or Every Other Day.
The counts for each patient are shown as thousands of neutrophils per microliter. The dosage of G-CSF is indicated above each rectangle (representing a treatment period). At least six weeks of observation preceded each patient's treatment. For Patient 1, the first four months are shown, as well as months 8 and 9 of treatment. IV denotes intravenous, and SC subcutaneous.

Figure 13

GM-CSF has also been administered to children with cyclic neutropenia, albeit with less success. In the dog model it was originally observed that GM-CSF did not significantly alter the cycling of the granulocytes (173), and that it increased the peaks of neutrophil counts but without affecting the duration or severity of the nadir (182). Two patients treated with GM-CSF have been reported to develop only eosinophilia, without significant effect on the neutrophil count (183, 184). However, one recent case treated with very low doses of GM-CSF has been reported to respond with a complete elimination of cycling and no significant eosinophilia (185).

Although cyclic neutropenia is a rare disorder, the periodicity of the neutrophil counts and the accompanying remarkable changes in marrow cellularity and proliferation appear to be a possible model of the changes that occur with cycles of chemotherapy. In this regard, one observation in cyclic neutropenia that may be particularly relevant is that the most clinical benefit is achieved when the G-CSF is started early in the cycle or given continuously. What is much less effective is to give G-CSF at the beginning of the neutrophil nadir. This model of administration is similar to what has been used for chemotherapy treatment, i.e. start the growth factor within 24-48 hrs after completing

drug therapy, rather than waiting until 10-14 days after treatment when the nadir will generally occur. Although it seems reasonable to start growth factors early, the real question is whether there is any benefit identifiable when G-CSF or GM-CSF is started at the time of severe granulocytopenia and fever. As we shall see later, very little data is available to address this point, although clinically we are frequently faced with a septic patient admitted with very low granulocytes following chemotherapy and asked to decide whether to give CSF's. Of course, with relatively few side-effects, the underlying issue here is cost, which will also be discussed subsequently.

XVII. Chemotherapy-Induced Myelosuppression

A. Introduction

Chemotherapy-induced myelosuppression, with its resultant fever, infection, sepsis, hospitalization, antibiotic therapy, and possible death, is one of the major limitations faced by the oncologist using cytotoxic therapy. In many cases, an inadequate white blood cell count is the only abnormality that prevents the administrations of full doses of chemotherapy, resulting in either delays in drug delivery or reductions in drug dose. Although one cannot always prove that less intensive therapy necessarily results in a lower remission and/or cure rate in every clinical situation, most oncologists strongly believe that a reduction or delay in drug therapy does compromise patient outcome. The evidence that higher drug doses result in higher cure rates in lymphoma, Hodgkin's disease, and breast cancer, for example, was the subject of my previous Grand Rounds on autologous bone marrow transplantation, and will not be reviewed here.

SUPPRESSION OF NEUTROPHILS BY DIFFERENT CYTOTOXIC AGENTS

Action	Time Lapse	Agents
Immediate	0–48 hours	Hydroxyurea, radiation
Early	1–3 weeks	Alkylators, anthracyclines
Delayed	6–8 weeks	Nitrosoureas, mitomycin C
Latent	—	Busulphan

Table 10

As most of you know, the time of neutrophil nadir with most chemotherapeutic agents in 10-14 days, although a few drugs have relatively immediate effects, a few delayed effects, and one drug, Busulfan, appears to produce a latent marrow injury to primitive stem cells (Table 10). In general, most studies with the CSF's have examined drug regimens that produce the classic kinetics of marrow injury, starting the cytokine within 24-48 hours after completion of chemotherapy and continuing daily treatment until after the resolution of the neutropenia, usually discontinuing drug at a total white count of 10,000. In addition, different drugs used in current chemotherapy regimens differ in their toxicity to the bone marrow. Table 11 list those drugs that produce little, moderate, and severe marrow injury. Not surprisingly, some of our most effective agents, such as Adriamycin and cyclophosphamide are highly marrow toxic.

PROPENSITY OF CYTOTOXIC AGENTS TO CAUSE NEUTROPENIA

Absent to mild	Moderate	Severe
Vincristine	Vinblastine/vindesine	Alkylating agents
Bleomycin	Actinomycin	Anthracyclines D
L-asparaginase	Epipodophyllotoxins	Nitrosoureas
Hormones	Cisplatin	Mitomycin C
Tamoxifen	Dacarbazine	Pyrimidine analogues
	Methotrexate	Hydroxyurea
	Purine analogues	
	Hexamethylmelamine	
	Procarbazine	

Table 11

Because neutropenia is such a major factor in cancer chemotherapy, the use of CSF's to reduce this toxicity has been the subject of intense investigation in the last 5 years. Both GM-CSF (186-211) and G-CSF (82, 83, 85, 212-223) have been administered to patients receiving both standard and increased doses of chemotherapy for a variety of solid tumors and lymphomas. Initial studies, of course, were usually uncontrolled or compared with historical controls. Later studies used the patients as their own control in some cases, giving the CSF in either the first or second cycle of the same regiment and comparing the severity of neutropenia, number of febrile days with neutropenia, number of days receiving antibiotics, increase in total hospital days, and tumor response and overall survival. Finally, more recently randomized double-blind placebo controlled trials have been reported.

B. GM-CSF

The first major study of GM-CSF described 16 non-randomized patients with sarcoma who served as their own controls, receiving GM-CSF in the first cycle at doses from 4-32 μg/kg but not in the second (186). The GM-CSF cycles had shorter periods of neutropenia, with 3.5 days of <500 ANC for cycle 1 and 7.4 days for cycle 2. There was statistically significant difference in the nadir ANC, and no difference in the number of hospital admissions for fever and neutropenia, however. An additional difficulty with this study is the tendency of patients to have progressive worsening of their cytopenias with each subsequent course of chemotherapy. Thus, cycle 2 might have had lower counts even if no GM-CSF had been given in cycle 1, although the authors argued that historical controls receiving the same treatment demonstrated no differences between the first and second cycle.

Two other studies using a similar design in patients with Hodgkin's disease, one giving the GM-CSF (2-16 μg/kg) in the first cycle of MOPP therapy and alternating thereafter (199) and the other giving GM-CSF (2-8 μg/kg) in the second cycle and alternating (203), have been reported. Interestingly both papers found that GM-CSF was only effective in shortening neutropenia at the higher doses, i.e. 8-16 μg/kg. There was no difference in the incidence of infection in either study. One reason for these results may have been that both chemotherapy regimens give drugs on both day one and day 8 of each 18 day cycle, thus the GM-CSF was not started until relatively late in the treatment course.

Timing of GM-CSF treatment may also have been sub-optimal in two other trials. In a double-blind randomized trial, Biesma reported 30 patients that were randomized to treatment with GM-CSF plus antibiotics or antibiotics alone (188). However, the GM-CSF (2.8 μg/kg) was not started until the actual hospitalization for fever! With this dose schedule, the recovery from mean nadir at admission of <100 ANC was not statistically different, although once the ANC was greater than 1000 the values in the GM-CSF group were consistently higher. In addition, GM-CSF did not shorten the days of fever or antibiotic administration. More recently, the same group reported 15 patients with ovarian cancer receiving carboplatin and cyclophosphamide with the GM-CSF being increased with each cycle from 1.5 to 3 to 6 μg/kg, and the patients randomized to GM-CSF or placebo (194). Again, the GM-CSF was not started until day 6 and given until day 12. Although there was a significant decrease in the incidence of ANC <500 at the highest dose of GM-CSF (20 of 22 placebo cycles vs. 5 of 17 high GM-CSF cycles), there was no difference in the need to reduce chemo doses or delay therapy. The effect on days of fever was not reported.

More positive results were described in another study that reported 22 patients with various malignancies who were given GM-CSF for the second cycle of treatment if the first resulted in an

ANC <1000 (190). In this study, the mean nadir was 100 ANC in the first cycle and 840 in the second ($p<0.01$). In this study the number of days with fever and antibiotics was also significantly reduced. In another study of 23 patients with non-Hodgkin's lymphoma, 23 received GM-CSF after several cycles of chemo with Mitoxantrone and cytosine arabinoside, while 14 patients were treated with chemo alone (197). The duration of neutropenia, defined as days with an ANC <500 was significantly reduced, but the difference in infection rate was not statistically significant. Of interest, the incidence of mucositis was significantly reduced in the GM-CSF group, an observation made in other studies as well.

In 25 children with solid tumors receiving 5 day courses of cisplatin and etoposide, GM-CSF was studied by progressive escalation of the dose starting at day 6 of each cycle and comparing the results with previous cycles at lower doses (195). The results showed a 50% reduction in median days with fever and neutropenia at the higher GM-CSF doses ($\geq 750\ \mu g/m^2$). Finally, 30 patients with AIDS-associated non-Hodgkin's lymphoma receiving CHOP chemotherapy were randomized to receive either GM-CSF from days 4 to 13 or placebo (202). There were statistically significant reductions in nadir, duration of neutropenia, and days hospitalized for fever and neutropenia (4.9 vs. 1.8). There was no difference in response rates or survival.

Overall, it seems likely that GM-CSF does reduce the incidence and duration of neutropenia, as defined by ANC <500. However, the extent of the benefit on fever, antibiotic use, hospitalization, and survival benefit is less certain. Phase III randomized trials are in progress, but pending their outcome, the FDA has not yet approved GM-CSF for the amelioration of chemotherapy-induced myelosuppression. It is also unclear whether GM-CSF will eventually allow escalations in the doses of chemotherapy, but promising trials have been reported (189, 191), although one group was unable to escalate the dose of Melphalan with GM-CSF support.

C. G-CSF

The initial studies were single arm studies with patients serving as their own controls (212, 213). In the study reported in the New England Journal of Medicine, G-CSF was given in the first cycle of M-VAC therapy for bladder cancer, with no cytokine in the second cycle. Although again one could argue that counts would have inevitably been lower in the 2nd cycle, the G-CSF cycle had 90% reductions in the number of days with an ANC <1000 and <500, but only the difference in the ANC <1000 was statistically significant. The days of antibiotic used for fever and neutropenia was significantly reduced, however, and the patients able to receive the drug dose planned for day 14 of the treatment cycle was higher. As seen with GM-CSF, the incidence of mucositis was also significantly less. The other study looked at patients with small cell lung cancer receiving CAE therapy (Cytoxan, Adriamycin, and Etoposide) and achieved similar results. There was no difference in survival, however. Other studies have also reported shorter neutropenic periods with G-CSF (214) and the ability to give chemotherapy at 14 day instead of the usual 21 day intervals (216).

The double-blind randomized placebo controlled study which gained FDA approval for G-CSF is that reported by Crawford et al. in the New England Journal of Medicine last year (223). In this study, approximately 200 patients with small cell lung cancer were randomized to receive up to 6 cycles of chemotherapy (cyclophosphamide, doxorubicin, etoposide) with or without G-CSF. However, if a patient developed a febrile neutropenic episode, a cross-over to open label G-CSF was

allowed. The results of this study for the first cycle are shown graphically in Fig. 14 and on the title page of this protocol. The duration of ANC < 500 was reduced from 6 days to 3 days by treatment with G-CSF from day 4 to day 17 of the 21 day cycle. During cycles of blinded treatment, the days of antibiotics, the days of hospitalization, and the confirmed infection incidence was reduced approximately 50% in the G-CSF group. The only side-effect was bone pain in about 20% of the patients. Table 12 shows the results for the first cycle of therapy only.

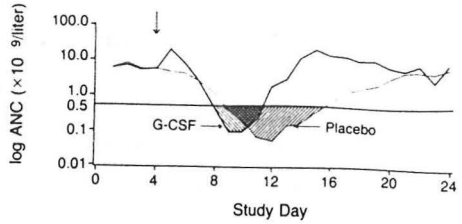

Figure 3. Median Absolute Neutrophil Count (ANC) in the Study Groups during Cycle 1.
The counts are shown on a linear scale (top) and log scale (bottom). The arrow denotes the start of placebo or G-CSF administration. The hatching highlights the degree and duration of neutropenia (counts $<0.5\times10^9$ per liter).

Figure 14

Table 2. Indicators of Neutropenia during Cycle 1 (Double-Blinded Administration).

Indicator	Placebo*	G-CSF*	Adjusted P Value†
Incidence of neutropenia with fever — %	57 (102)	28 (92)	<0.001
Incidence of absolute neutrophil count $<0.5\times10^9$/liter — %	98 (102)	84 (93)	0.001
Median neutrophil nadir — 10^{-9}/liter	0.036 (98)	0.068 (86)	0.004
Median duration — days			
Neutrophil count $<0.5\times10^9$/liter	6.0 (94)	3.0 (86)	<0.001
Fever with neutropenia‡	5.0 (57)	4.0 (25)	NS

*Values in parentheses are numbers of patients.
†Adjusted for disease status and center by the Cochran–Mantel–Haenszel adjusted chi-square or the Wilcoxon rank-sum test. NS denotes not significant.
‡Among patients who had fever with neutropenia.

Table 12

Although this study is impressive, and clearly convinced the FDA to approve G-CSF, a few concerns do remain. The statistical significance of rate of culture-confirmed infections is not given and the event itself was relatively rare, i.e. 13.3 % in the placebo group vs. 6.5% in the G-CSF group. Secondly, the "majority" of these confirmed infections were bacteremias or respiratory tract infections, but apparently an uncertain proportion were less serious infections. Given this low incidence of culture positive fevers, it is not surprising that G-CSF had no effect on overall mortality or survival. The high rate of fever without infection is not surprising, and G-CSF clearly reduced these significantly, but the frequency of these may have been high in this patient population who were instructed to take their temperature daily, regardless of symptoms. In routine clinical practice, many of these fevers may have gone undetected.

XVIII. Bone Marrow Transplantation

A. GM-CSF in Autologous Bone Marrow Transplantation

In contrast to G-CSF, GM-CSF has received approval from the FDA for use in autologous bone marrow transplantation. Since myelosuppression is the dose-limiting toxicity for many chemotherapy regimens, in order to treat certain tumors that are resistant to conventional doses of chemotherapy it has proven necessary to "rescue" the bone marrow function by the infusion of the patient's own bone marrow cells which were collected and stored prior to the chemotherapy. Even with this infusion of bone marrow cells, the recovery of adequate granulocytes requires anywhere from 15 to 25 days, depending on the particular chemotherapy regimen used.

In this clinical setting, a number of trials using historical controls established that recovery of

>500 ANC was clearly accelerated in patients receiving GM-CSF (224-229). One negative report involved "purged" bone marrow that had been treated with a drug to remove residual cancer cells, and thus may not have been very good bone marrow for reconstitution (230). In most of these studies, the number of febrile days was reduced and the length of hospitalization shortened compared to historical controls. No difference in survival has been reported, however. With the exception of one study (224), recovery of platelets and red cells was also not affected by GM-CSF. Mucositis and liver and kidney complications of transplantation appear to be reduced in frequency.

Recently, several randomized trials of GM-CSF in ABMT have been reported (231-235). Nemunaitis et al. described 128 patients with lymphoid malignancies with 65 receiving GM-CSF for 21 days and 63 receiving placebo. Consistent with the earlier trials, GM-CSF patients reached a ANC of 500 7 days earlier than placebo patients. Figure 15 shows the actual mean ANC in the two groups. The number of days with fever did not differ between the two groups, although the GM-CSF had fewer documented infections (11 vs. 19, $p=0.1$). The number of days of antibiotic administration was reduced significantly from 27 to 24, and GM-CSF patients required 6 fewer days in the hospital. There was no difference in patient survival at 100 days. A smaller study with 69 lymphoma patients reported similar results, with patients achieving an ANC Of 500 at 12 days as opposed to 16 days for placebo, and a statistically significant difference in bacterial infections (234). Time to platelet independence, duration of hospital stay, and survival were not affected. Finally, Gulati and Bennett (235) reported on 24 patients, 12 randomized to each arm, and found shorted duration of neutropenia and platelet transfusion, and an 8 day shorter hospitalization. However, infection, response rate, and survival were the same in the two groups. One problem with this last study is that 58% of the placebo group received concomitant radiation therapy while only 17% of the GM-CSF group got radiation therapy.

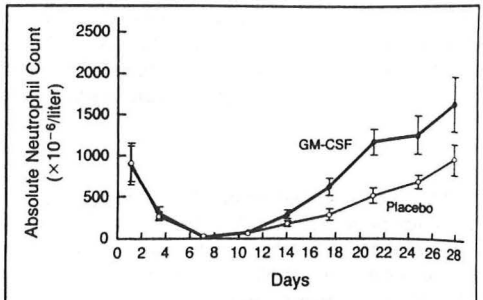

Figure 2. Mean Daily Absolute Neutrophil Counts in 65 Patients Who Received rhGM-CSF and 63 Patients Who Received Placebo.

Figure 15

The long-term follow-up of patients receiving GM-CSF has been described (236). There were no late graft failures, and no difference in survival, relapse, or long-term marrow function. Three other non-randomized studies have reported promising results with GM-CSF to stimulate bone marrow function in patients with prolonged cytopenias following transplant, presumably due to delayed engraftment or actual graft failure (237-239).

B. GM-CSF in Allogeneic Bone Marrow Transplantation for Leukemia

Fewer studies have been reported with GM-CSF in allogenic transplants because of a concern that GM-CSF might worsen graft-versus-host disease or accelerate leukemic relapse. An initial study in patients with graft failure who had received allogeneic transplants did not observe any exacerbation of GVHD (239). On the other hand, one small study of 10 patients suggested an unusually high severity of GVHD in their patients treated with GM-CSF (240). Two non-randomized studies from Seattle of matched sibling donors (242) and unrelated donors (243) did not observe any increase or worsening of GVHD, as compared to historical controls. These authors also could not conclude that

GM-CSF reduced the duration of neutropenia or incidence of infection relative to the historical controls.

Finally, two randomized trials from European groups have been reported (244, 245). One study randomized 40 patients and observed a ANC recovery to 500 3 days earlier in the GM-CSF group which was not statistically significant, although the median count at day 14 was significantly higher (1900 vs. 460). There was no evidence for higher relapse rate or incidence of GVHD, and the duration of hospitalization was the same in both groups. The second study randomized 57 patients and used T-cell depleted bone marrow grafts. Early neutrophil count, even at > 100 ANC and 300 ANC was significantly faster for patients given GM-CSF, and the incidence of pneumonia was significantly lower in the treated group. The incidence of GVHD and early mortality was not different in the two groups, and relapse rates are not different at 2 years median follow-up.

D. G-CSF for Bone Marrow Transplantation

Results obtained with G-CSF have been more limited but similar to those reported for GM-CSF in autologous transplants (246-251) and allogeneic transplants (252). All of these studies are compared to historical controls. Nearly all of the studies report a reduction in the time to achieve a neutrophil count of 500, and some find significant reductions in antibiotic use (246). However, in this latter study the incidence of fever and bacteremia was similar and there were no effects on platelet or red cell transfusions.

XIX. Peripheral Blood Stem Cells

One additonal novel use for CSF's is in the harvesting of stem cells for autologous transplantation. Particularly where malignant cells may have infiltrated the bone marrow, some investigators believe that peripheral blood stem cells mobilized by treatment with CSF's may be less likely to be contaminated with residual tumor. Both GM-CSF (64, 89, 253) and G-CSF (88) have been shown to increase the levels of hematopoietic progenitor cells in the peripheral blood. Combining CSF's with the recovery phase from chemotherapy provides even a greater augmentation of these circulating cells (254). Small numbers of patients in various phase I trials have been supported through high-dose chemotherapy with cells collected following stimulation with GM-CSF or G-CSF (255-259).

XX. GM-CSF and G-CSF for Leukemia Induction

In addition to their use in accelerating hematopoietic recovery, in leukemia it has been proposed to use CSF's to turn on cell cycling of the leukemic cells and thereby increase anti-leukemic cell killing by subsequent chemotherapy. Although this approach is still clearly in an early investigational phase, a large number of largely in vitro studies appear to demonstrate that most patients leukemia cells can be induced to proliferate after stimulation by CSF's, and that in some cases this cell cycling can increase sensitivity to cytotoxic agents (260-268).

One major randomized clinical study using G-CSF in 108 patients with acute leukemia has been reported in the New England Journal of Medicine (269). G-CSF accelerated granulocyte recovery to an ANC >500 on day 20 in the G-CSF arm and day 28 in the control arm. Platelet recovery was unaffected and the incidence of febrile episodes was the same, although documented infections were significantly less frequent in the G-CSF arm. The rate of relapse was reported to be the same in the

two groups. Several other studies have been reported using GM-CSF as compared to historical controls (270-276). In some of these studies, GM-CSF was also given pre-chemotherapy in order to "recruit" the leukemia cells into cell cycle and increase their sensitivity to cytotoxic drugs. Most of these studies report a reduction in time for neutrophil recovery and no effect on the rate of remission, however, one study from M.D. Anderson observed a lower complete remission rate and lower overall survival for the 56 GM-CSF treated patients as compared to a historical control group of 176 patients (276). Moreover, patients who did not enter a remission with this treatment appears more likely to have persistent leukemia in their marrows rather than prolonged marrow aplasia, suggesting GM-CSF may have accelerated regrowth of residual leukemia. Not surprisingly, the authors suggest caution in the use of GM-CSF in the induction treatment of acute leukemia.

XXI. Conclusions and Recommendations

In conclusion, the value of G-CSF in the congenital neutropenias seems unequivocal. In myelodysplasia, both CSF's can increase neutrophil counts but do not lead to sustained remissions or preferential proliferation of the residual non-neoplastic bone marrow cells. Whether prolonged therapy with CSF's will lead to a reduction in infection and prolonged survival remains to be shown in randomized trials. Given that CSF therapy costs about $200 to $300 per day, the economic benefit of reduced infections may not be sufficient in the absence of evidence for an effect on overall survival.

For cycling marrow states, such as congenital cyclic neutropenia and cancer chemotherapy, G-CSF seems to have been shown to clearly affect the duration of neutropenia and to a lesser extent the depth of the nadir. In one large randomized study, this effect was accompanied by a reduction in infections, antibiotic use, and hospitalization. Again, however, no benefit on survival was seen. In addition, this trial produced a very high incidence of neutropenic fevers, as compared to many of our standard chemotherapy regimens. Thus, it seems warranted at this time to use G-CSF for those regimens which produce a high incidence of hospitalization for fever and neutropenia. Even in this setting, it may not be necessary to use G-CSF on all patients, but limit it to those patients who have one admission for fever and neutropenia, or possibly patients who demonstrate a very low nadir count on a previous cycle, even if fever does not occur. Table 13 lists some of the reported benefits of G-CSF in patients receiving chemotherapy.

Reported Clinical Benefits of Combining G-CSF With Chemotherapy
Reduction in the period of neutropenia
Reduction in the number of febrile days
Fewer infections
Fewer days on antibiotics
Delivery of chemotherapy on schedule
Reduced mucositis
Accelerated monocyte recovery

Table 13

Should G-CSF be given to the patient who is admitted in the midst of fever and neutropenia? Although this question has not been studied rigorously, there is very little data to suggest that growth factors provide significant benefit in this setting. The few studies that have looked at using CSF's at the nadir or starting late in the chemotherapy cycle have usually found that there is not benefit to this late administration. The model of cyclic neutropenia also demonstrated that G-CSF must be started early to achieve a significant reduction in the subsequent nadir count. Thus, although G-CSF is benign treatment, on a purely economic basis, it is currently difficult to justify its routine use in patients admitted with the usual fever without source in the setting of neutropenia that is usually a few days in length.

For now, GM-CSF appears to be limitied to its use in bone marrow transplantation support.

With its greater number of side-effects, and the concern over the inhibition of granulocyte migration, and the recent kinetic studies suggesting much of the increase in granulocytes is accounted for by a longer half-life in the blood, rather than a true increase in total body granulocyte pool, the widespread use of GM-CSF seems to offer no advantage over G-CSF. One area of investigation that may favor GM-CSF, however, is in fungal or parasitic infections where stimulation of macrophage or eosinophil function is needed. In addition, a number of investigators are seeding to augment the anti-tumor immune surveillance functions of macrophages, presumably partly in presenting antigen, by treating patients with GM-CSF.

Finally, the eventual hope of the CSF's is that we will be able to increase drug dose, i.e. achieve dose intensification, or shorten the interval of drug dosing, and thus achieve a greater clinical benefit in terms of survival. Whether such intensification is possible remains remains to be determined in controlled trials. In early studies, thrombocytopenia has become the limiting factor, although 30-50% intensification has been reported in some abstracts from the recent meeting of the Amercian Society of Clinical Oncology. Clearly, the future holds the promise of new cytokines, particularly used in combination with exisiting CSF's to further minimize myelosuppression of whatever cause. For those interested in reading more about the clinical use of G-CSF and GM-CSF, a number of recent review articles may be of interest (277-288).

1. Arai K, Lee F, Miyajima A, et al. Cytokines: Coordinators of immune and inflammatory responses. Ann Rev Biochem 59:783-836, 1990.
2. Williams DE, Park LS, Broxmeyer HE, Lu L. Hybrid cytokines as hematopoietic growth factors. Int J Cell Cloning 9:542-547, 1991.
3. Curtis BM. Williams DE. Broxmeyer HE. Dunn J. Farrah T. Jeffery E. Clevenger W. deRoos P. Martin U. Friend D. et al. Enhanced hematopoietic activity of a human granulocyte/macrophage colony-stimulating factor-interleukin 3 fusion protein. Proceedings of the National Academy of Sciences of the United States of America. 88(13):5809-13, 1991.
4. Ganser A. Janssen JW. Ottmann OG. Seipelt G. Eder M. Becher R. Lindermann A. Herrmann F. Schulz G. Mertelsmann R. et al. In vivo effects of granulocyte-macrophage colony-stimulating factor and interleukin-3 on clonal and non-clonal cell populations in patients with clonal hematopoietic disorders. Leukemia. 5(6):487-92, 1991.
5. Estrov Z. Kurzrock R. Talpaz M. Blake M. Gutterman JU. Granulocyte-macrophage colony-stimulating factor and interleukin-3 in combination: a potent and consistent myelodysplastic syndrome bone marrow stimulant in vitro. Annals of Hematology. 63(6):297-301, 1991.
6. Brugger W. Bross K. Frisch J. Dern P. Weber B. Mertelsmann R. Kanz L. Mobilization of peripheral blood progenitor cells by sequential administration of interleukin-3 and granulocyte-macrophage colony-stimulating factor following polychemotherapy with etoposide, ifosfamide, and cisplatin. Blood. 79(5):1193-200, 1992.
7. Asano S. Human granulocyte colony-stimulating factor: its basic aspects and clinical applications. American Journal of Pediatric Hematology-Oncology. 13(4):400-13, 1991.
8. Demetri GD. Griffin JD. Granulocyte colony-stimulating factor and its receptor. Blood. 78(11):2791-808, 1991
9. Hirano T, Akira S, Taga T, Kishimoto T. Biological and clinical aspects of interleukin 6. Immunology Today 11:443-448, 1990.
10. Oheda M, Hasegawa M, Hattori K, et al. O-linked sugar chain of human granulocyte colony-stimulating factor protects it against polymerization and denaturation allowing it to retain its biological activity. J Biol Chem 265:11432-5, 1990.
11. Koeffler HP, Gasson J, Tobler A. Transcriptional and posttranscriptional modulation of myeloid colony-stimulating factor expression by tumor necrosis factor and other agents. Mol Cell Biol 8:3432, 1988.
12. Ernst TJ, Ritchie AR, Demetri GD, Griffin JD. Regulation of granuloctye- and monocyte-colony stimulating factor mRNA levels in human blood monocytes is mediated primarily at a post-transcriptional level. J Biol Chem 264:5700, 1989.
13. Demetri G, Ernst T, Pratt E II, Zenzie B, Rheinwald J, Griffin J. Expression of ras oncogenes in cultured human cells alters the transcriptional and posttranscriptional regulation of cytokine genes. J Clin Invest 86:1261, 1990.
14. Shaw G, Kamen R. A conserved AU sequence from the 3' untranslated region of GM-CSF mRNA mediates selective mRNA degradation. Cell 46:659, 1986.
15. Koeffler HP, Gasson J, Ranyard J, Souza L, Shepard M, Munker R. Recombinant human TNF alpha stimulates production of granulocyte colony-stimulating factor. Blood 70:55, 1987.
16, Zsebo K, Yuschenkoff V, Schiffer S, Chang D, McCall E, Dinarello C, Brown M, Altrock B, Bacby G Jr. Vascular endothelial cells and granulopoiesis: Interleukin-1 stimulates release of G-CSF and GM-CSF. Blood 71:99, 1988.
17. Oster W, Lindemann A, Mertelsmann R, Herrmann F. Granulocyte-macrophage colony-stimulating factor (CSF) and multi-lineage CSF recruit human monocytes to express granulocyte CSF. Blood 73:64, 1989.
18. Wieser M, Bonifer R, Oster W, Lindemann A, Mertelsmann R, Herrmann F. Interleukin-4 induces secretion of CSF for granulocytes and CSF for macrophages by peripheral blood monocytes. Blood 73:1105, 1989.
19. Hermann F, Cannistra S, Griffin J. T-cell/monocyte interactions in the production of humoral factors regulating human granulopoiesis in vitro. J Immunol 136:2856, 1986.
20. Shirafuji N, Asano S, Matsuda S, et al. A new bioassay for human granulocyte colony-stimulating factor (hG-CSF) using murine myeloblastic NFS-60 cells as targets and estimation of its levels in sera from normal healthy persons and patients with infections and hematological disorders. Exp Hematol 17:116-9, 1988.

21. Motojima H, Kobayashi T, Shimane M, et al. Quantitative enzyme immunoassay for human granulocyte colony-stimulating factor (G-CSF). J Immunol Methods 118:187-92, 1989.
22. Watari K. Asano S. Shirafuji N. Kodo H. Ozawa K. Takaku F. Kamachi S. Serum granulocyte colony-stimulating factor levels in healthy volunteers and patients with various disorders as estimated by enzyme immunoassay. Blood. 73(1):117-22, 1989.
23. Kawakami M, Tsutsumi H, Kumakawa T, et al. Levels of serum granulocyte colony-stimulating factor in patients with infections. Blood 76:1962-4, 1990.
24. Sallerfors B, Olofsson T. Granulocyte-macrophage colony-stimulating factor (GM-CSF) and granulocyte colony-stimulating factor (G-CSF) in serum during induction treatment of acute leukemia. Brit J Haematol 78:, 343-351, 1991.
25. Hammond WP. Csiba E. Canin A. Hockman H. Souza LM. Layton JE. Dale DC. Chronic neutropenia. A new canine model induced by human granulocyte colony-stimulating factor. Journal of Clinical Investigation. 87(2):704-10, 1991.
26. Lord BI, Bronchud MH, Owens S, Chang J, Howell A, Souza L, Dexter TM. The kinetics of human granulopoiesis following treatment with granulocyte colony-stimulating factor in vivo. Proc Natl Acad Sci USA. 86:9499-9503, 1989.
27. Chang JM, Metcalf D, Gonda TJ, Johnson GR. Long-term exposure to retrovirally expressed granulocyte colony-stimulating factor induces a nonneoplastic granulocyte and progenitor cell hyperplasia without tissue damage in mice. J Clin Invest 84:1488, 1989.
28. Lang R, Metcalf D, Cuthbertson R, Lyons I, Stanley E, Kelso A, Kannourakis G, Williamson D, Klintworth G, Gonda T, Dunn A. Transgenic mice expressing a hemopoietic growth factor gene (GM-CSF) develop accumulations of macrophages, blindness, and a fatal syndrome of tissue damage. Cell 51:675, 1987.
29. Larsen A, Davis T, Curtis B, Gimpel S, Sims J, Cosman D, Park L, Sorensen E, March CJ, Smith C. Expression cloning of a granulocyte colony-stimulating factor receptor: A structural mosaic of hemopoietin receptor, immunoglobulin, and fibronectin domains. J Exp Med 172:1559, 1990.
30.. Tweardy DJ. Anderson K. Cannizzaro LA. Steinman RA. Croce CM. Huebner K. Molecular cloning of cDNAs for the human granulocyte colony-stimulating factor receptor from HL-60 and mapping of the gene to chromosome region 1p32-34. Blood. 79(5):1148-54, 1992.
31. Fukunaga R, Ishizaka IE, Nagata S. Purification and characterization of the receptor for murine granulocyte colony-stimulating receptor. J Biol Chem 265:14008, 1990.
32. Gearing DP, Comeau MR, Friend DJ, Gimpel SD, Thut, CJ, McGourty J, Brasher KK, King JA, Gillis S, Mosley B, Ziegler SF, Cosman D. The IL-6 signal transducer, gp130: An Oncostatin M receptor and affinity converter for the LIF receptor. Science 255:1434-1437, 1992.
33. Nair BC, DeVico AL, Nakamura S, Copeland TD, Chen Y, Patel A, O'Neil T, Oroszlan S, Gallo RC, Sarngadharan MG. Identification of a major growth factor for AIDS-Kaposi's sarcoma cells as Oncostatin M. Science 255:1430-1432, 1992.
34. Miles SA, Martinez-Maza O, Rezai A, Magpantay L, Kishimoto T, Nakamura S, Radka SF, Linsley PS. Oncostatin M as a potent mitogen for AIDS-Kaposi's sarcoma-derived cells. Science 255:1432-1434.
35. Fukunaga R, Seto Y, Mizushima S, Nagata S. Three different mRNAs encoding the human granulocyte colony-stimulating factor receptor. Proc Natl Acad Sci USA 87:8702-8706, 1990.
36. Nicola NA, Metcalf D. Binding of the differentiation-inducer, granulocyte colony-stimulating factor, to responsive but not unresponsive leukemic cell lines. Proc Natl Acad Sci USA 81:3765, 1984.
37. Nicola NA, Metcalf D. Binding of ^{125}I-labeled granulocyte colony-stimulating factor to normal murine hematopoietic cells. J. Cell Physiol 124:313, 1985.
38. Souza GLM, Boone TC, Gabrilove J, et al. Recombinant human granulocyte colony-stimulating factor: Effect on normal and leukemic myeloid cells. Science 232:61-65, 1986.
39. Begley CG, Metcalf D, Nicola NA. Primary human myeloid leukemia cells: Comparative responsiveness to proliferative stimulation by GM-CSF or G-CSF and membrane expression of CSF receptors. Leukemia 1:1, 1987.
40. Begley CG, Metcalf D, Nicola NA. Binding characteristics and proliferative action of purified granulocyte colony-stimulating factor (G-CSF) on normal and leukemic human promyelocytes. Exp Hematol 16:71, 1988.
41. Budel LM, Touw IP, Delwel R, Lowenberg B. Granulocyte colony-stimulating factor receptors in human acute

myelocytic leukemia. Blood 74:2668-2673, 1989.
42. Park LS, Waldron PE, Friend D, Sassenfeld HM, Price V, Anderson D, Cosman D, Andrews RG, Bernstein ID, Urdal DL. Interleukin-3, GM-CSF, and G-CSF receptor expression on cell lines and primary leukemia cells: Receptor heterogeneity and relationship to growth factor responsiveness. Blood 74:56-65, 1989.
43. Piao YF, Okabe T. Receptor binding of granulocyte colony-stimulating factor to the blast cells of myeloid leukemia. Cancer Res 50:1671-1674.
44. Kondo S, Okamura S, Asano Y, Harada M, Niho Y. Human granulocyte colony-stimulating factor receptors in acute myelogenous leukemia. Eur J Haematol 46:223-230, 1991.
45. Busolino F, Wang JM, Defilippi P, Turrini F, Sanavio F, Edgell CG, Aglietta M, Arese P, Mantovani A. Granulocyte- and granulocyte-macrophage colony-stimulating factors induce human endothelial cells to migrate and proliferate. Nature 337:471, 1989.
46. Uzumaki H, Okabe T, Sasaki N, Hagiwara K, Takaku F, Tobita M, Yasukawa K, Ito S, Umezawa Y. Identification and characterization of receptors for granulocyte colony-stimulating factor on human placenta and trophoblastic cells. Proc Natl Acad Sci USA 86:9323, 1989.
47. Avalos BR, Gasson JC, Hedvat C, Quan SG, Baldwin GC, Weisbart RH, Williams RE, Golde DW, DiPersio JF. Human granulocyte colony-stimulating factor: Biologic activities and receptor characterization on hematopoietic cells and small cell lung cancer cell lines. Blood 75:851, 1990.
48. Wong GG, Witek JS, Temple PA, et al. Human GM-CSF: molecular cloning of the complementary DNA and purification of the natural and recombinant proteins. Science 228:810-5, 1985.
49. Moonen P, Mermod J, Ernst JF, et al. Increased biological activity of deglycosylated recombinant human granulocyte-macrophage colony-stimulating factor produced by yeast or animal cells. Proc Natl Acad Sci USA 84:4428-31, 1987.
50. Gribben JG, Devereaux S, Thomas NSB, et al. Development of antibodies to unprotected glycosylation sites on recombinant human GM-CSF. Lancet 335:434-7, 1990.
51. 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 74:2634-43, 1989.
52. Gasson JC. Molecular physiology of granulocyte-macrophage colony-stimulating factor. Blood 77:1131-1145, 1991.
53. Hermann F, Oster W, Meuer SC, Klein K, Lindemann A, Mertelsmann R. Interleukin-1 stimulates T lymphocytes to produce GM-CSF. J Clin Invest 81:1415-1418, 1988.
54. Herrmann F, Cannistra SA, Griffin JD. T-cell monocyte interactions in the production of humoral factors regulating human granulopoiesis in vitro. J Immunol 136:2856-2861, 1986.
55. Sieff CA, Niemeyer CM, Mentzer SJ, Faller DV. Interleukin-1, tumor necrosis factor, and the production of colony-stimulating factors by cultured mesenchymal cells. Blood 72:1316-1323, 1987.
56. Sallerfors B, Olofsson T, Lenhoff S. Granulocyte-macrophage colony-stimulating factor (GM-CSF) and granulocyte colony-stimulating factor (G-CSF) in serum in bone marrow transplanted patients. Bone Marrow Transplant 8:191-195, 1991.
57. Addison IE, Johnson B, Devereux S, et al. Granulocyte-macrophage colony-stimulating factor may inhibit neutrophil migration in vivo. Clin Exp Immunol 76:149-53, 1989.
58. Buescher ES, McIlheran SM, Vadhan-Raj S. Effects of in vivo administration of recombinant human granulocyte-macrophage colony-stimulating factor on human neutrophil chemotaxis and oxygen metabolism (letter). J Infect Dis 158:1140-2, 1988.
59. Peters WP, Stuart A, Affronti ML, et al. Neutrophil migration is defective during recombinant human granulocyte-macrophage colony-stimulating factor infusion after autologous bone marrow transplantation in humans. Blood 72:1310-5, 1988.
60. Toner GC, Jakubowski AA, Crown JPA, et al. Preservation of localized leukocyte mobilization (LLM) in pateints receiving recombinant human granulocyte colony-stimulating factor (rhG-CSF). Blood 72:154A, 1988 (suppl 1).
61. Bianco JA. Appelbaum FR. Nemunaitis J. Almgren J. Andrews F. Kettner P. Shields A. Singer JW. Phase I-II trial of pentoxifylline for the prevention of transplant-related toxicities following bone marrow transplantation. Blood 78(5):1205-11, 1991.

62. Montgomery B, Bianco JA, Jacobsen A, Singer JW. Localization of transfused neutrophils to site of infection during treatment with recombinant human granulocyte-macrophage colony-stimulating factor and pentoxifylline [letter]. Blood 78:533-534, 1991.
63. Lord BI, Gurney H, Chang J, Thatcher N, Crowther D, Dexter TM. Haemopoietic cell kinetics in humans treated with rGM-CSF. Int J Cancer 50:26-31, 1992.
64. Aglietta M, Piacibello W, Sanavio F, et al. Kinetics of human hemopoietic cells after in vivo administration of granulocyte-macrophage colony-stimulating factor. J Clin Invest 83:551-7, 1989.
65. Johnson GR, Gonda TJ, Metcalf D, Hariharan IK, Cory S. A lethal myeloproliferative syndrome in mice transplanted with bone marrow cells infected with a retrovirus expressing granulocyte-macrophage colony-stimulating factor. EMBO J 8:441, 1989.
66. Gearing DP, King JA, Gough NM, Nicola NA. Expression cloning of a receptor for human granulocyte-macrophage colony-stimulating factor. EMBO J 8:3667-3676, 1989.
67. Hayashida K, Kitamura T, Gorman DM, Arai I, Yokota T, Miyajima A. Molecular cloning of a second subunit of the receptor for human granulocyte-macrophage colony-stimulating factor (GM-CSF): reconstitution of a high-affinity GM-CSF receptor. Proc Natl Acad Sci USA 87:9655-9659, 1990.
68. Nicola N, Metcalf D. Subunit promiscuity among hemopoietic growth factor receptors. Cell 67:1-4, 1991.
69. Tavernier J. Devos R. Cornelis S. Tuypens T. Van der Heyden J. Fiers W. Plaetinck G. A human high affinity interleukin-5 receptor (IL5R) is composed of an IL5-specific alpha chain and a beta chain shared with the receptor for GM-CSF. Cell. 66(6):1175-84, 1991.
70. Kitamura T. Sato N. Arai K. Miyajima A. Expression cloning of the human IL-3 receptor cDNA reveals a shared beta subunit for the human IL-3 and GM-CSF receptors. Cell. 66(6):1165-74, 1991.
71. Yang YC, Ciarletta AB, Temple PA, Chung MP, Kovacic S, Witek-Geanotti JS, Leary AC, Kriz R, Donahue RE, Wong GG, Clark SC. Human IL-3 (multi-CSF): Identification by expression cloning of a novel hematopoetic growth factor related to murine IL-3. Cell 47:3-10, 1986.
72. Yokota T, Coffman RL, Hagiwara H, Rennick DM, Takebe Y, Yokota K, Gemmel L, Shrader B, Yang G, Meyerson P, Luh J, Hoy P, Pene J, Briere F, Spits H, Bachereau J, deVries J, Lee FD, Arai N, Arai KI. Isolation and characterization of lymphokine cDNA clones encoding mouse and human IgA-enhancing factor and eosinophil colony-stimulating activities; Relationship to interleukin 5. Proc Natl Acad Sci USA 84:7388-7392, 1987.
73. Gasson JC, Kaufman SE, Weisbart RH, Tomonaga M, Golde DW. High-affinity binding of granulocyte-macrophage colony-stimulating factor to normal and leukemic human myeloid cells. Proc Natl Acad Sci USA 83:669, 1986.
74. DiPersio JF. Hedvat C. Ford CF. Golde DW. Gasson JC. Characterization of the soluble human granulocyte-macrophage colony-stimulating factor receptor complex. Journal of Biological Chemistry. 266(1):279-86, 1991.
75. Baldwin GC, Gasson JC, Kaufman SE, et al. Nonhematopoietic tumor cells express functional GM-CSF receptors. Blood 73:1033-7.
76. 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. New England Journal of Medicine 317:593-8, 1987.
77. Vadhan-Raj S, Buescher S, LeMaistre A, et al. Stimulation of hematopoiesis in patients with bone marrow failure and in patients with malignancy by recombinant human granulocyte-macrophage colony-stimulating factor. Blood 72:134-41, 1988.
78. 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 Int Med 110:357-64.
79. Devereux S, Linch DC, Campos Costa D, et al. Transient leucopenia induced by granulocyte-macrophage colony-stimulating factor [letter]. Lancet 2:1523-4, 1987.
80. Morstyn G, Lieschke GJ, Cebon J, et al. Early clinical trials with colony-stimulating factors. Cancer Invest 7:443-56, 1989.
81. Morstyn G, Campbell L, Souza LM, et al. Effect of granulocyte colony-stimulating factor on neutropenia induced by cytotoxic chemotherapy. Lancet 1:667-672, 1988.

82. Gabrilove JL, Jakubowski A, Scher H, et al. Effect of granulocyte colony-stimulating factor on neutropenia and associated mobidity due to chemotherapy for transitional cell carcinoma of the urothelium. New England Journal of Medicine 318:1414-1422, 1988.
83. Bronchud MA, Gillio AP, Ruggeiro M, et al. Phase I/II study of recombinant granulocyte colony-stimulating factor in patients receiving intensive chemotherapy for small cell lung cancer. Br J Cancer 56:809-813, 1987.
84. Asano S, Shirafuji N, Watari K, et al. Phase I clinical study for granulocyte colony-stimulating factor. Behring Inst Mitt 83:222-228, 1988.
85. Morstyn G, Campbell L, Lieschke GJ, et al. Abrogation of chemotherapy induced neutropenia by subcutaneously administered granulocyte colony-stimulating factor (G-CSF) with optimization of dose and duration of therapy. J Clin Oncol 7:1554-1562, 1989.
86. Campbell LJ, Maher DW, Tay DLM, Boyd AW, Rockman S, McGrath K, Fox RM, Morstyn G. Marrow proliferation and the appearance of giant neutrophils in response to recombinant human granulocyte colony-stimulating factor (rhG-CSF). Br J Haematol 80:298-304, 1992.
87. Teshima T, Shibuya T, Harada M, Taniguchi S, Nozaki M, Mori T, Mori Y, Tamai H, Niho Y. Effects of G-CSF, GM-CSF, and IL-5 on nuclear segmentation of neutrophils and eosinophils in congenital or acquired Pelger-Huet anomaly. Exp Hematol 19:322, 1991.
88. Duhrsen U, Villeval JL, Boyd J, Kannourakis G, Morstyn G, Metcalf D. Effects of recombinant granulocyte colony-stimulating factor on hematopoeitic progenitor cells in cancer patients. Blood 72:2074, 1988.
89. Socinski M, Cannistra S, Elias A, Antman K, Schnipper L, Griffin J. Granulocyte-macrophage colony-stimulating factor expands the circulating haemopoietic progenitor cell compartment in man. Lancet 1:1194, 1988.
90. Glaspy JA, Baldwin GC, Robertson PA, el al. Therapy for neutropenia in hairy cell leukemia with recombinant human granulocyte colony-stimulating factor. Ann Intern Med 109:789-795, 1988.
91. Ross HJ. Moy LA. Kaplan R. Figlin RA. Bullous pyoderma gangrenosum after granulocyte colony-stimulating factor treatment. Cancer. 68(2):441-3, 1991.
92. Lindemann A, Herrmann F, Oster W, et al. Hematologic effects of recombinant human granulocyte colony-stimulating factor in patients with malignancy. Blood 74:2644-2651, 1989.
93. Jaiyesimi I. Giralt SS. Wood J. Subcutaneous granulocyte colony-stimulating factor and acute anaphylaxis [letter] New England Journal of Medicine. 325(8):587, 1991.
94. Ward JC, Gitlin JB, Garry DJ, Jatoi A, Luikart SF, Zelickson BD, Dahl MV, Skubitz KM. Epidermolysis bullosa acquisita induced by GM-CSF: a role for eosinophils in treatment-related toxicity. Br J Haematol 81:27-32, 1992.
95. Horn TD. Burke PJ. Karp JE. Hood AF. Intravenous administration of recombinant human granulocyte-macrophage colony-stimulating factor causes a cutaneous eruption. Archives of Dermatology. 127(1):49-52, 1991.
96. Hazenberg BPC, Van Leeuwen MA, Van Rijswijk MH, Stern AC, Vellenga E. Correction of granulocytopenia in Felty's syndrome by granulocyte-macrophage colony-stimulating factor, simultaneous induction of interleukin-6 release and flare-up of the arthritis. Blood 74:2769-70, 1989.
97. de Vries EG. Willemse PH. Biesma B. Stern AC. Limburg PC. Vellenga E. Flare-up of rheumatoid arthritis during GM-CSF treatment after chemotherapy [letter] Lancet. 338(8765):517-8, 1991.
98. Hoekman K, von Blomberg-van der Flier BM, Wagstaff J, Drexhage HA, Pinedo HM, Reversible thyroid dysfunction during treatment with GM-CSF. Lancet 338(8766):541-2, 1991.
99. Nathan FE, Besa EC. GM-CSF and accelerated hemolysis [letter] New England Journal of Medicine. 326:417, 1992.
100. Shibuya A, Taguchi K, Kojima H, Abe T. Inhibitory effect of granulocyte-macrophage colony-stimulating factor therapy on the generation of natural killer cells. Blood. 78(12):3241-7, 1991.
101. Dorshkind K. In vivo administration of recombinant granulocyte-macrophage colony-stimulating factor results in a reversible inhibition of primary B lymphopoiesis. J Immunol 146:4204-4208, 1991.
102. Bodey GP, Buckley M, Sathe YS, Freireich EJ. Quantitative relationships between circulating leukocytes and infection in patients with acute leukemia. Ann Intern Med 64:328, 1966.
103. Esparza L, Yap HY, Smith T, Blumenschein G, Bodey GP. Quantitative relationship between degree of

myelosuppression and infection in patients with metastatic breast cancer. Proc Am Soc Clin Onc 2:89, 1983.
104. Louria DB. Infectious complications of neoplastic disease. Introduction and epidemiology. Am J Med 76:414-420, 1984.
105. Boxer LA, Hutchinson R, Emerson S. Recombinant human granulocyte colony-stimulating factor in the treatment of patients with neutropenia. Clinical Immunology and Immunopathology 62:S39-S46, 1992.
106. Kostmann R. Infantile genetic agranulocytosis (agranulocytosis infantilis hereditaria). Acta Paediatr 105:(Suppl)1-78, 1956.
107. Ganser A, Ottman OG, Erdmann H, Schulz G, Hoelzer D. The effect of recombinant human granulocyte-macrophage colony-stimulating factor on neutropenia and related morbility in chronic severe neutropenia. Ann Intern Med 111:887-892, 1989.
108. Vadhan-Raj S, Jeha S, Buescher S, LeMaistre A, Yee G, Lu L, Lloreta J, Hoots WK, Hittelman WN, Gutterman JU, Broxmeyer HE. Stimulation of myelopoiesis in a patient with congenital neutropenia: Biology and nature of response to granulocyte-macrophage colony-stimulating factor. Blood 75:858-864, 1990.
109. Welte K, Zeidler C, Reiter A, Müller W, Odenwald E, Souza L, Riehm H. Differential effects of granulocyte-macrophage colony-stimulating factor and granulocyte colony-stimulating factor in children with severe congenital neutropenia. Blood 75:1056-1063.
110. Bonilla MA, Gillio AP, Ruggeiro M, Kernan NA, Brochstein JA, Abboud M, Fumagalli L, Vincent M, Gabrilove JL, Welte K, Souza LM, O'Reilly RJ. Effects of recombinant human granulocyte colony-stimulating factor on neutropenia in patients with congenital agranulocytosis. New England Journal of Medicine 320:1574-1580, 1989.
111. Dale D, Hammond W, Gabrilove J, Jakubowski A, Bonilla M, O'Reilly R, Andersen C, Vincent M, Welte K. Long term treatment of severe chronic neutropenia with recombinant granulocyte colony-stimulating factor. 76:139a, 1990.
112. Weston B. Todd RF 3d. Axtell R. Balazovich K. Stewart J. Locey BJ. Mayo-Bond L. Loos P. Hutchinson R. Boxer LA. Severe congenital neutropenia: clinical effects and neutrophil function during treatment with granulocyte colony-stimulating factor. Journal of Laboratory & Clinical Medicine. 117(4):282-90, 1991.
113 Hirashima K, Yoshida Y, Asano S, Takaku F, Omine M, Furusawa S, Abe T, Abe T, Dohy H, Tajiri M, et al. Clinical effect of recombinant human granulocyte colony-stimulating factor (rhG-CSF) on various types of neutropenia including cyclic neutropenia. Biotherapy. 3(4):297-307, 1991.
114. Ueda K, Hanawa Y, Takaku F, Asano S, Tsukimoto I, Tsuchida M, Sato T, Ohira M, Hoshi Y, Nishihira K, et al. The effect of recombinant human granulocyte colony-stimulating factor (rG-CSF) on childhood neutropenias [Jpn] Rinsho Ketsueki - Japanese Journal of Clinical Hematology. 32(3):212-20, 1991.
115. Kyas U, Pietsch T, Welte K. Expression of receptors for granulocyte colony-stimulating factor on neutrophils from patients with severe congenital neutropenia and cyclic neutropenia. Blood. 79(5):1144-7, 1992.
116. Hurst D, Lipani J, Becker J, Kilpatrick L, Douglas S. Recombinant human granulocyte colony-stimulating factor treatment of neutropenia in glycogen storage disease IB. Blood 76:148a, 1990.
117. Schroten H, Roesler J, Breidenbach T, Wendel U, Elsner J, Schweitzer S, Zeidler C, Burdach S, Lohmann-Matthes M-L, Wahn V, Welte K. Granulocyte and granulocyte-macrophage colony-stimulating factors for treatment of neutropenia in glycogen storage disease type Ib.
118. Hess U, Ganser A, Schnurch H-G, Seipelt G, Ottmann OG, Falk S, Schulz G, Hoelzer D. Myelokathexis treated with recombinant human granulocyte-macrophage colony-stimulating factor (rhGM-CSF). British J Haematol :254-256, 1990.
119. Antin JH, Smith BR, Homes W, Rosenthal DS. Phase I/II study of recombinant human granulocyte-macrophage colony-stimulating factor in aplastic anemia and myelodysplastic syndrome. Blood 72:705-713, 1988.
120. Nissen C, Tichelli A, Gratwohl A, Speck B, Milne A, Gordon C, Smith E, Schaedelin J. Failure of recombinant human granulocyte-macrophage colony-stimulating factor therpay in aplastic anemia patients with very severe neutropenia. Blood 72:2045-2047, 1988.
121. Vadhan-Raj S, Buescher S, Broxmeyer HE, LeMaistre A, Lepe Zuniga JL, Ventura G, Jeha S, Horwitz LJ, Trujillo JM, Gillis S, et al. Stimulation of myelopoiesis in patients with aplastic anemia by recombinant human granulocyte-macrophage colony-stimulating factor. New England Journal of Medicine 319:1628-1634, 1988.

122. Champlin RE, Nimer SD, Ireland P, Oette DH, Golde DW. Treatment of refractory aplastic anemia with recombinant human granulocyte-macrophage colony-stimulating factor. Blood 73:694-699, 1989.
123. Kurzrock R, Talpaz M, Gomez JA, Estey EH, O'Brien S, Hirsch-Ginsberg C, Koller C, Freireich EJ, Gutterman JU. Differential dose-related haematological effects of GM-CSF in pancytopenia: evidence supporting the advantage of low- over high-dose administration in selected patients. British Journal of Haematology. 78(3):352-8, 1991.
124. Guinan EC, Sieff CA, Oette DH, et al. A phase I/II trial of recombinant granulocyte-macrophage colony-stimulating factor for children with aplastic anemia. Blood 76:1077-82, 1990.
125. Kojima S, Fukuda M, Miyajima Y, Matsuyama T, Horibe K. Treatment of aplastic anemia in children with recombinant human granulocyte colony-stimulating factor. Blood. 77(5):937-41, 1991.
126. Kojima S, Matsuyama T, Miyazaki T, Sakurada K, Shike S, Okino E, Akabane T, Nakahata T, Okuni M, Mugishima H, et al. Treatment of aplastic anemia with KRN8601 (rhG-CSF) [Jpn] Rinsho Ketsueki - Japanese Journal of Clinical Hematology. 31(7):929-36, 1990.
127. Asano S, Hirashima K, Yoshida Y, Takaku F, Miyazaki T, Omine M, Furusawa S, Abe T, Mori M, Arimori S, et al. Clinical effect of recombinant human granulocyte colony-stimulating factor on aplastic anemia [Jpn] Rinsho Ketsueki - Japanese Journal of Clinical Hematology. 31(9):1456-62, 1990.
128. Bessho M, Toyoda A, Itoh Y, Sakata T, Kawai N, Jinnai I, Saito M, Hirashima K. Trilineage recovery by combination therapy with recombinant human granulocyte colony-stimulating factor (rhG-CSF) and erythropoietin (rhEpo) in severe aplastic anemia. Br J Haematol 80:409-411, 1992.
129. Sonoda Y, Yashige H, Fujii H, Tsuda S, Maekawa T, Misawa S, Abe T. Bilineage response in refractory aplastic anemia patients following long-term administration of recombinant human granulocyte colony-stimulating factor. European Journal of Haematology. 48(1):41-8, 1992 .
130. Kojima S, Fukuda M, Miyajima Y, Matsuyama T. Cyclosporine and recombinant granulocyte colony-stimulating factor in severe aplastic anemia. [letter] New England Journal of Medicine 323:920-921, 1990.
131. Bertrand Y, Amri F, Capdeville R, Ffrench M, Philippe N. The successful treatment of two cases of severe aplastic anaemia with granulocyte-colony stimulating factor and cyclosporine A. British Journal of Haematology. 79(4):648-9, 1991.
132. Jakubowski AA, Souza L, Kelly F, Fain K, Budman D, Clarkson B, Bonilla MA, Moore MAS, Gabrilove J. Effects of human granulocyte colony-stimulating factor in a patient with idiopathic neutropenia.
133. Furukawa T, Takahashi M, Moriyama Y, Koike T, Kurokawa I, Shibata A. Successful treatment of chronic idiopathic neutropenia using recombinant granulocyte colony-stimulating factor. Annals of Hematology 62:22-4, 1991.
134. Kaneko T, Ogawa Y, Hirata Y, Hoshino S, Takahashi M, Oshimi K, Mizogichi H. Agranulocytosis associated with granular lymphocyte leukaemia: Improvement of peripheral blood granulocyte count with human recombinant granulocyte colony-stimulating factor (G-CSF). Br J Haematol 74:121-122, 1990.
135. Thomssen C, Nissen C, Gratwohl A, Tichelli A, Stern A. Agranulocytosis associated with T-gamma-lymphocytosis: no improvement of peripheral blood granulcyte count with human recombinant granulocyte-macrophage colony-stimulating factor (GM-CSF). Br J Haematol 71:157-158, 1989.
136. Folks TM, Justement J, Kinter A, Dinarello CA, Fauci AS. Cytokine-induced expression of HIV-1 in a chronically infected promonocyte cell line. Science 238:800-802, 1987.
137. Koyanagi Y, O'Brien WA, Zhao JQ, Golde DW, Gasson JC, Chen ISY. Cytokines alter production of HIV from primary mononuclear phagocytes. Science 241:1673-1675, 1988.
138. Mitsuyasu R, Levine J, Miles SA, DeLeo M, Oette D, Golde D, Groopman S. Effects of long term subcutaneous (SC) adminstration of recombinant granulocyte-macrophage colony-stimulating factor (GM-CSF) in patients with HIV-related leukopenia. Blood 72:356a, 1988.
139. Levine JD, Allan JD, Tessitore JH, Falcone N, Galasso F, Israel RJ, Groopman JE. Recombinant human granulocyte-macrophage colony-stimulating factor ameliorates zidovudine-induced neutropenia in patients with acquired immunodeficiency syndrome (AIDS)/AIDS-related complex. Blood 78(12):3148-54, 1991.
140. Scadden DT, Bering HA, Levine JD, Bresnahan J, Evans L, Epstein C, Groopman JE. GM-CSF as an alternative to dose modification of the combination zidovudine and interferon-alpha in the treatment of AIDS-associated Kaposi's sarcoma. American Journal of Clinical Oncology 14 Suppl 1:S40-4, 1991.

141. Scadden DT, Bering HA, Levine JD, Bresnahan J, Evans L, Epstein C, Groopman JE. Granulocyte--macrophage colony-stimulating factor mitigates the neutropenia of combined interferon alfa and zidovudine treatment of acquired immune deficiency syndrome-associated Kaposi's sarcoma. Journal of Clinical Oncology 9(5):802-8, 1991.
141. Davey RT Jr, Davey VJ, Metcalf JA, Zurlo JJ, Kovacs JA, Falloon J, Polis MA, Zunich KM, Masur H, Lane HC. A phase I/II trial of zidovudine, interferon-alpha, and granulocyte-macrophage colony-stimulating factor in the treatment of human immunodeficiency virus type 1 infection. Journal of Infectious Diseases 164(1):43-52, 1991.
142. Hardy WD. Combined ganciclovir and recombinant human granulocyte-macrophage colony-stimulating factor in the treatment of cytomegalovirus retinitis in AIDS patients. Journal of Acquired Immune Deficiency Syndromes 4 Suppl 1:S22-8, 1991.
143. Kimura S, Matsuda J, Ikematsu S, Miyazono K, Ito A, Nakahata T, Minamitani M, Shimada K, Shiokawa Y, Takaku F. Efficacy of recombinant human granulocyte colony-stimulating factor on neutropenia in patients with AIDS. Aids 4(12):1251-5, 1990.
144. Miles SA, Mitsuyasu RT, Moreno J, Baldwin G, Alton NK, Souza L, Glaspy JA. Combined therapy with recombinant granulocyte colony-stimulating factor and erythropoietin decreases hematologic toxicity from zidovudine. Blood 77(10):2109-17, 1991.
145. van der Wouw PA, van Leeuwen R, van Oers RH, Lange JM, Danner SA. Effects of recombinant human granulocyte colony-stimulating factor on leucopenia in zidovudine-treated patients with AIDS and AIDS related complex, a phase I/II study. British Journal of Haematology 78(3):319-24, 1991.
146. Greenberg PL. Treatment of myelodysplastic syndromes with hemopoietic growth factors. Seminars In Oncology. 19(1):106-14, 1992 Feb.
147. Ganser A, Hoelzer D. Treatment of myelodysplastic syndromes with hematopoietic growth factors. Hematology/Oncology Clinics of North America 6:633-653, 1992.
148. Yoshida Y. Japanese experience in the treatment of myelodysplastic syndromes. Hematology/Oncology Clinics of North America 6:673-685, 1992.
149. Vadhan-Raj S, Keating M, LeMaistre A, Hittelman W, McCredie K, Trujillo JM, Broxmeyer HE, Henney C, Gutterman JU. Effects of recombinant human granulocyte-macrophage colony-stimulating factor in patients with myelodysplastic syndromes. New England Journal of Medicine 317:1545-1552.
150. Ganser A, Völkers B, Greher J, Ottmann OG, Walther F, Becher R, Bergmann L, Schulz G, Hoelzer D. Recombinant human granulocyte-macrophage colony-stimulating factor in patients with myelodysplastic syndromes--a phase I/II trial. Blood. 73(1):31-7, 1989 Jan.
151. Herrmann F, Lindemann A, Klein H, Lübbert M, Schulz G, Mertelsmann R. Effect of recombinant human granulocyte-macrophage colony-stimulating factor in patients with myelodysplastic syndrome with excess blasts. Leukemia. 3(5):335-8, 1989 May.
152. Thompson JA, Lee DJ, Kidd P, Rubin E, Kaufmann J, Bonnem EM, Fefer A. Subcutaneous granulocyte-macrophage colony-stimulating factor in patients with myelodysplastic syndrome: toxicity, pharmacokinetics, and hematological effects. Journal of Clinical Oncology. 7:629-37, 1989.
153. Kurzrock R, Talpaz M, Gomez JA, Estey EH, O'Brien S, Hirsch-Ginsberg C, Koller C, Freireich EJ, Gutterman JU. Differential dose-related haematological effects of GM-CSF in pancytopenia: evidence supporting the advantage of low- over high-dose administration in selected patients. British Journal of Haematology. 78(3):352-8, 1991 Jul.
154. Estey EH, Kurzrock R, Talpaz M, McCredie KB, O'Brien S, Kantarjian HM, Keating MJ, Deisseroth AB, Gutterman JU. Effects of low doses of recombinant human granulocyte-macrophage colony stimulating factor (GM-CSF) in patients with myelodysplastic syndromes. British Journal of Haematology. 77(3):291-5, 1991 Mar.
155. Schuster M, Thompson J, Larson R, Allen S, O'Laughlin R, Israel R, Fefer A. A randomized trial of subcutaneous granulocyte-macrophage colony-stimulating factor versus observation in patients with myelodysplastic syndrome or aplastic anemia. Proc Am Soc Clin Oncol 9:205, 1990.
156. Vadhan-Raj S, Broxmeyer HE, Spitzer G, et al. Stimulation of nonclonal hematopoiesis and suppression of the neoplastic clone after treatment with recombinant human granulocyte-macrophage colony-stimulating factor in a patient with a therapy-related myelodysplastic disorder. Blood 74:1491-1498, 1989.

157. Bessho M, Itoh Y, Kataumi S, Kawai N, Matsuda A, Jinnai I, Saitoh M, Hirashima K, Minamihisamatsu M. A hematological remission by clonal hematopoiesis after treatment with recombinant human granulocyte-macrophage colony-stimulating factor and erythropoietin in a patient with therapy-related myelodysplastic syndrome. Leukemia Research. 16(2):123-31, 1992.
158. Verhoef G, Boogaerts M. In vivo administration of granulocyte-macrophage colony-stimulating factor enhances neutrophil function in patients with myelodysplastic syndromes. British Journal of Haematology. 79(2):177-84, 1991
159. Moore FD, Jack RM, Antin JH. Peripheral blood neutrophils in chronically neutropenic patients respond to granulocyte-macrophage colony-stimulating factor with a specific increase in CR1 expression and CR1 transcription. Blood 79:1667-1671, 1992.
160. Antin JH, Weinberg DS, Rosenthal DS. Variable effect of recombinant human granulocyte-macrophage colony-stimulating factor on bone marrow fibrosis in patients with myelodysplasia. Experimental Hematology. 18(4):266-70, 1990 May.
161. Delmer A, Karmochkine M, Cadiou M, Gerhartz H, Zittoun R. Recurrent spleen enlargement during cyclic granulocyte-macrophage colony-stimulating factor therapy for myelodysplastic syndrome. American Journal of Hematology. 34(1):73-4, 1990 May.
162. Evans C, Rosenfeld CS, Winkelstein A, Shadduck RK, Pataki KI, Oldham FB. Perforation of an unsuspected cecal granulocytic sarcoma during therapy with granulocyte-macrophage colony-stimulating factor [letter] New England Journal of Medicine. 322(5):337-8, 1990.
163. Gerhartz HH, Visani G, Delmer A, Zwierzina H, Ribeiro M, Jacobs A, Marcus R, Baumelou M, Fiére D, Labar B, et al. Low-dose Ara-C plus granulocyte-macrophage colony-stimulating factor for the treatment of myelodysplastic syndromes. EORTC Leukemia Group. Bone Marrow Transplantation. 4 Suppl 3:36-7, 1989 Dec.
164. Brito-Babapulle F, Lord JA, Whitmore DN. Treatment of RAEB-t with intensive chemotherapy and GM-CSF. Leukemia Research. 13(7):605-7, 1989.
165. Negrin RS, Haeuber DH, Nagler A, Olds LC, Donlon T, Souza LM, Greenberg PL. Treatment of myelodysplastic syndromes with recombinant human granulocyte colony-stimulating factor. A phase I-II trial. Annals of Internal Medicine. 110(12):976-84, 1989 Jun 15.
166. Negrin RS, Haeuber DH, Nagler A, Kobayashi Y, Sklar J, Donlon T, Vincent M, Greenberg PL. Maintenance treatment of patients with myelodysplastic syndromes using recombinant human granulocyte colony-stimulating factor. Blood. 76(1):36-43, 1990.
167. Greenberg P, Negrin R, Nagler A, Vincent M, Donlon T. Effects of prolonged treatment of myelodysplastic syndromes with recombinant human granulocyte colony-stimulating factor. International Journal of Cell Cloning. 8 Suppl 1:293-300; discussion 300-2, 1990 Jan.
168. Kobayashi Y, Okabe T, Ozawa K, Chiba S, Hino M, Miyazono K, Urabe A, Takaku F. Treatment of myelodysplastic syndromes with recombinant human granulocyte colony-stimulating factor: a preliminary report. American Journal of Medicine. 86(2):178-82, 1989 Feb.
169. Ohyashiki K, Ohyashiki JH, Toyama K, Takaku F. Hematologic and cytogenetic findings in myelodysplastic syndromes treated with recombinant human granulocyte colony-stimulating factor. Japanese Journal of Cancer Research. 80(9):848-54, 1989 Sep.
170. Yoshida Y, Hirashima K, Asano S, Takaku F. A phase II trial of recombinant human granulocyte colony-stimulating factor in the myelodysplastic syndromes. British Journal of Haematology. 78(3):378-84, 1991 Jul.
171. Washizuka T, Koike T, Toba K, Nagai K, Takahashi M, Shibata A. A rise of erythrocytes and platelets in a patient with myelodysplastic syndrome during the administration of G-CSF [letter] American Journal of Hematology. 39(2):153-4, 1992 Feb.
172. Dale DC, Hammond WP 4th. Cyclic neutropenia: a clinical review. Blood Reviews 2(3):178-85, 1988.
173. Lothrop CD Jr, Warren DJ, Souza LM, Jones JB, Moore MA. Correction of canine cyclic hematopoiesis with recombinant human granulocyte colony-stimulating factor. Blood 72(4):1324-8, 1988.
174. Hammond WP 4th, Price TH, Souza LM, Dale DC. Treatment of cyclic neutropenia with granulocyte colony-stimulating factor. New England Journal of Medicine 320(20):1306-11, 1989.
175. Hanada T. Ono I, Nagasawa T. Childhood cyclic neutropenia treated with recombinant human granulocyte colony stimulating factor. British Journal of Haematology 75(1):135-7, 1990.

176. Hanada T , Ono I. Disappearance of neutrophil oscillations in a child with cyclic neutropenia after treatment with recombinant human granulocyte colony-stimulating factor [letter] European Journal of Haematology 45(3):181-2, 1990.
177. Sugimoto K, Togawa A, Miyazono K, Itoh K, Amano M, Chiba S, Hino M, Mizoguchi H, Takaku F. Treatment of childhood-onset cyclic neutropenia with recombinant human granulocyte colony-stimulating factor [letter]. European Journal of Haematology 45(2):110-1, 1990.
178. Hirashima K, Yoshida Y, Asano S, Takaku F, Omine M, Furusawa S, Abe T, Abe T, Dohy H, Tajiri M, et al. Clinical effect of recombinant human granulocyte colony-stimulating factor (rhG-CSF) on various types of neutropenia including cyclic neutropenia. Biotherapy 3(4):297-307, 1991.
179. Locatelli F, Pedrazzoli P, Zecca M, Maccario R, Giorgiani G, Prete L, Nespoli L, Severi F. Recombinant human granulocyte-macrophage colony stimulating factor (rHuGM-CSF) in cyclic neutropenia. Haematologica 76(3):238-9, 1991.
180. Tsunogake S, Nagashima S, Maekawa R, Takano N, Kajitani H, Saito K, Enokihara H, Furusawa S, Shishido H. Myeloid progenitor cell growth characteristics and effect of G-CSF in a patient with congenital cyclic neutropenia. Int J Hematol 54(3):251-6, 1991.
181. Danielsson L.Harmenberg J. Intermittent rG-CSF treatment in cyclic neutropenia [letter] Eur J of Haematol 48(2):123-4, 1992.
182. Hammond W, Donahue R, Dale D. Purified recombinant human granulocyte-macrophage colony-stimulating factor stimulates granulocytopoiesis in canine cyclic hematopoiesis. Blood 68:165, 1986. (abstr, suppl).
183. Wright DG, Oette DH, Maleck HL. Treatment of cyclic neutropenia with recombinant human granulocyte-macrophage colony-stimulating factor (rh-GM-CSF). Blood 74 (Suppl):863a, 1989.
184. Freund MR, Luft S, Sch:ober C, Heussner P, Schrezenmaier H, Porzsolt F, Welte K. Differential effect of GM-CSF and G-CSF in cyclic neutropenia [letter] Lancet 336(8710):313, 1990.
185. Kurzrock R, Talpaz M, Gutterman JU. Treatment of cyclic neutropenia with very low doses of GM-CSF [letter] American Journal of Medicine 91(3):317-8, 1991.
186. Antman KH, Griffen JD, Elias A, Socinski MA, Ryan L, Cannistra SA, Oette D, Whitley M, Frei E III, Schnipper LE. Effect of recombinant human granulocyte-macrophage colony-stimulating factor on chemotherapy-induced myelosuppression. New England Journal of Medicine 319:593-598.
187. Ardizzoni A. Sertoli MR. Corcione A. Pennucci MC. Baldini E. Intra E. Ferrarini M. Rosso R. Mazzanti P. Pistoia V. Accelerated chemotherapy with or without GM-CSF for small cell lung cancer: a non-randomised pilot study. European Journal of Cancer. 26(9):937-41, 1990.
188. Biesma B. de Vries EG. Willemse PH. Sluiter WJ. Postmus PE. Limburg PC. Stern AC. Vellenga E. Efficacy and tolerability of recombinant human granulocyte-macrophage colony-stimulating factor in patients with chemotherapy-related leukopenia and fever. European Journal of Cancer. 26(9):932-6, 1990.
189. Gianni AM, Bregni M, Siena S, Orazi A, Stern AC, Gandola L, Bonadonna G. Recombinant human granulocyte-macrophage colony-stimulating factor reduces hematologic toxicity and widens clinical applicability of high-dose cyclophosphamide treatment in breast cancer and non-Hodgkin's lymphoma. J Clin Oncol 8:768-778, 1990.
190. Hermann F, Schulz G, Wieser M, Kolbe K, Nicolay U, Noack M, Lindemann A, Mertelsmann R. Effect of GM-CSF on neutropenia and related morbidity induced by myelotoxic chemotherapy. Am J Med 88:619-624, 1990.
191. Logothetis CJ, Dexeus FH, Sella A, et al. Escalated therapy for refractory urothelial tumors: Methotrexate-vinblastine-doxorubicin-cisplatin plus unglycosylated recombinant human granulocyte-macrophage colony-stimulating factor. J Natl Cancer Inst 82:667-672, 1990.
192. Steis RG, BanderMolen LA, Longo DL, Clark JW, Smith II JW, Kopp WC, Ruscetti FW, Creekmore SP, Elwood LJ, Jursey J, Urba WJ. Recombinant human granulocyte-macrophage colony-stimulating factor in patients with advanced malignancy: A phase Ib trial. J Natl Cancer Inst 82:697-703, 1990.
193. Steward WP, Scarffe JH, Dirix LY, Change J, Radford JA, Bonnem E, Crowther D. Granulocyte-macrophage colony-stimulating factor (GM-CSF) after high-dose melphalan in patients with advanced colon cancer. Br J Cancer 61:749-754, 1990.
194. de Vries EG. Biesma B. Willemse PH. Mulder NH. Stern AC. Aalders JG. Vellenga E. A double-blind

placebo-controlled study with granulocyte-macrophage colony-stimulating factor during chemotherapy for ovarian carcinoma. Cancer Research. 51(1):116-22, 1991.
195. Furman WL. Fairclough DL. Huhn RD. Pratt CB. Stute N. Petros WP. Evans WE. Bowman LC. Douglass EC. Santana VM. et al. Therapeutic effects and pharmacokinetics of recombinant human granulocyte-macrophage colony-stimulating factor in childhood cancer patients receiving myelosuppressive chemotherapy. Journal of Clinical Oncology. 9(6):1022-8, 1991 Jun.
196. Harstrick A. Schmoll HJ. Bokemeyer C. Metzner B. Illiger HJ. Berdel W. Ostermann H. Manegold C. R:ath U. Siegert W. et al. Cisplatin/etoposide/ifosfamide stepwise dose escalation with concomitant granulocyte/macrophage-colony-stimulating factor for patients with far-advanced testicular carcinoma. Journal of Cancer Research & Clinical Oncology. 117 Suppl 4:S198-202, 1991.
197. Ho AD. Del Valle F. Haas R. Engelhard M. Hiddemann W. R:uckle H. Schlimok G. Thiel E. Andreesen R. Fiedler W. et al. Sequential studies on the role of mitoxantrone, high-dose cytarabine, and recombinant human granulocyte-macrophage colony-stimulating factor in the treatment of refractory non-Hodgkin's lymphoma. Seminars In Oncology. 17(6 Suppl 10):14-8; discussion 18-9, 1990 Dec.
198. Hoekman K. Wagstaff J. van Groeningen CJ. Vermorken JB. Boven E. Pinedo HM. Effects of recombinant human granulocyte-macrophage colony-stimulating factor on myelosuppression induced by multiple cycles of high-dose chemotherapy in patients with advanced breast cancer. Journal of the National Cancer Institute. 83(21):1546-53, 1991.
199. Hovgaard DJ. Nissen NI. Effect of recombinant human granulocyte-macrophage colony-stimulating factor in patients with Hodgkin's disease: a phase I/II study. Journal of Clinical Oncology. 10(3):390-7, 1992.
200. Havemann K. Klausmann M. Wolf M. Fischer JR. Drings P. Oster W. Effect of rhGM-CSF on haematopoietic reconstitution after chemotherapy in small-cell lung cancer. Journal of Cancer Research & Clinical Oncology. 117 Suppl 4:S203-7, 1991.
201. Jost LM. Pichert G. Stahel RA. Placebo controlled phase I/II study of subcutaneous GM-CSF in patients with germ cell tumors undergoing chemotherapy. Annals of Oncology. 1(6):439-42, 1990.
202. Kaplan LD. Kahn JO. Crowe S. Northfelt D. Neville P. Grossberg H. Abrams DI. Tracey J. Mills J. Volberding PA. Clinical and virologic effects of recombinant human granulocyte-macrophage colony-stimulating factor in patients receiving chemotherapy for human immunodeficiency virus-associated non-Hodgkin's lymphoma: results of a randomized trial. Journal of Clinical Oncology. 9(6):929-40, 1991.
203. Oza AM. Leahy M. Dorey E. Davis CL. Amess J. Horton M. Rohatiner AZ. Wrigley PF. Lister TA. Recombinant human granulocyte macrophage colony stimulating factor following alternating non cross resistant chemotherapy in Hodgkin's disease. Hematological Oncology. 9(6):337-47, 1991.
204. Pujol JL. Tabah I. Michel FB. The use of GM-CSF as adjunctive therapy in small cell lung cancer. Presentation of the interim analysis of a large phase II trial. Pathologie Biologie. 39(9):960, 1992.
205. Rusthoven J. Levin L. Eisenhauer E. Mazurka J. Carmichael J. O'Connell G. Bryson P. Hirte H. Koski B. Two phase I studies of carboplatin dose escalation in chemotherapy-naive ovarian cancer patients supported with granulocyte-macrophage colony-stimulating factor. Journal of the National Cancer Institute. 83(23):1748-53, 1991.
206. Schmid L. Th:urlimann B. M:uller M. Senn HJ. Recombinant human granulocyte-macrophage colony-stimulating factor for the intensification of cytostatic treatment in advanced cancer. Recent Results In Cancer Research. 121:173-81, 1991.
207. Schmoll HJ. Bokemeyer C. Harstrick A. Illiger HJ. Metzner B. Ruther U. Osternamm A. Preiss J. Wilke H. Hohnloser J. et al. Dose escalation of cisplatin (P), etoposide (E), and ifosfamide (I) with GM-CSF for advanced germ cell tumors. Pathologie Biologie 39(9):961, 1992.
208. Steward WP. Verweij J. Somers R. Blackledge G. Clavel M. Van Oosterom AT. Greifenberg B. Soedirman J. Thomas D. Van Glabbeke M. et al. Doxorubicin plus ifosfamide with rhGM-CSF in the treatment of advanced adult soft-tissue sarcomas: preliminary results of a phase II study from the EORTC Soft-Tissue and Bone Sarcoma Group. Journal of Cancer Research & Clinical Oncology. 117 Suppl 4:S193-7, 1991.
209. ten Bokkel Huinink WW. Clavel M. Rodenhuis S. Guastalla JP. Franklin HR. Koier JJ. Vlasveld T. Dalesio O. van Tinteren H. Pinedo HM. Mitoxantrone and GM-CSF: a phase I study with an escalated dose of mitoxantrone in breast cancer. Pathologie Biologie. 39(9):962, 1992.

210. Walsh C. Blum RH. Oratz R. Goldenberg A. Downey A. Speyer JL. Phase I study of doxorubicin, ICRF-187 and granulocyte/macrophage-colony-stimulating factor. Journal of Cancer Research & Clinical Oncology. 118(1):61-6, 1992.
211. Poplin E, Smith H, Behrens B, Redman B, Flaherty L, Neidhart J, Alberts D. SWOG 8825: Melphalan GM-CSF: A Phase I study. Gyn Oncol 44:66-70, 1992).
212. Bronchud MH, Potter MR, Morgenstern G, Blasco MJ, Scarffe JH, Thatcher N, Crowther D, Souza LM, Alton NK, Testa NG, et al. In vitro and in vivo analysis of the effects of recombinant human granulocyte colony-stimulating factor in patients. Br J Cancer 58:64-69, 1988.
213. Gabrilove JL, Jakubowski A, Fain K, Grous J, Scher H, Sternberg C, Yagoda A, Clarkson B, Bonilla MA, Oettgen HF, et al. Phase I study of granulocyte-colony-stimulating factor in patients with transitional cell carcinoma of the urothelium. J Clin Invest 82:1454-1461, 1988.
214. Morstyn G, Campbell L, Souza LM, Alton NK, Keech J, Green M, Sheridan W, Metcalf D, Fox R. Effect of granulocyte colony-stimulating factor on neutropenia induced by cytotoxic chemotherapy. Lancet i:667-672, 1988.
215. Ema H, Suda T, Sakamoto S, Tomonaga T, Tsunoda J, Muroi K, Komatsu N, Miwa A, Ohsaka A, Yoshida M, et al. Effects of the in vivo administration of recombinant human granulocyte colony-stimulating factor following cytotoxic chemotherapy on granulocytic precursors in patients with malignant lymphoma. Jpn J Cancer Res 80:577-582, 1989.
216. Bronchud MH, Howell A, Crowther D, Hopwood P, Souza LM, Dexter TM. The use of granulocyte colony-stimulating factor to increase the intensity of treatment with doxorubicin in patients with advanced breast and ovarian cancer. Br J Cancer 60:121-125, 1989.
217. Neidhart J, Mangalik A, Kohler W, Stidley C, Saiki J, Duncan P, Souza L, Downing M. G-CSF decreases duration of cytopenia in patients receiving dose intensive therapy with cyclophosphamide (C), etoposide (E), and cisplatin (P). J Clin Oncol 7:1685-1692, 1989.
218. Eguchi K, Sasaki S, Tamura T, Sasaki Y, Shinkai T, Yamada K, Soejima Y, Fukuda M, Fujihara Y, Kunitou H, et al. 1. Dose escalation study of recombinant human granulocyte-colony-stimulating factor (KRN8601) in patients with advanced malignancy. Cancer Res 49:5221-5224, 1989.
219. Yoshida T. Nakamura S. Ohtake S. Okafuji K. Kobayashi K. Kondo K. Kanno M. Matano S. Matsuda T. Kanai M. et al. Effect of granulocyte colony-stimulating factor on neutropenia due to chemotherapy for non-Hodgkin's lymphoma. Cancer. 66(9):1904-9, 1990.
220. Kudoh S. Fukuoka M. Negoro S. Tanaka H. Kusunoki Y. Matsui K. Masuda N. Takifugi N. Itoh K. Nishioka M. et al. Weekly dose-intensive chemotherapy in patients with small-cell lung cancer: a pilot study. American Journal of Clinical Oncology. 15(1):29-34, 1992.
221. Kotake T. Miki T. Akaza H. Kubota Y. Nishio Y. Matsumura Y. Ota K. Ogawa N. Effect of recombinant granulocyte colony-stimulating factor (rG-CSF) on chemotherapy-induced neutropenia in patients with urogenital cancer. Cancer Chemotherapy & Pharmacology. 27(4):253-7, 1991.
222. Oyami A, Ota K, Asano S, Takaku F, Yoshida Y, Uzuka Y, Omine M, Furusawa S, Takatani O, Sawada U, et al. A double-blind, cross-over clinical trial of recombinant human G-CSF on neutropenia induced by chemotherapy for non-Hodgkin's lymphoma [Jpn] Nippon Gan Chiryo Gakkai Shi - Journal of the Japan Society for Cancer Therapy 25:2533-48, 1990.
223. Crawford J. Ozer H. Stoller R. Johnson D. Lyman G. Tabbara I. Kris M. Grous J. Picozzi V. Rausch G. et al. Reduction by granulocyte colony-stimulating factor of fever and neutropenia induced by chemotherapy in patients with small-cell lung cancer [see comments] New England Journal of Medicine. 325(3):164-70, 1991.
224. Nemunaitis J, Singer JW, Buckner CD, Hill R, Storb R, Thomas ED, Appelbaum F. Use of recombinant human granulocyte-macrophage colony-stimulating factor in autologous bone marrow transplantation for lymphoid malignancies. Blood 72:834-6, 1988.
225. Brandt SJ, Peters WP, Atwater SK, Kurtzberg J, Borowitz M, Hones R, et al. Effect of recombinant human granulocyte-macrophage colony-stimulating factor on hematopoietic reconstitution after high dose chemotherapy and autologous bone marrow transplantation. New England Journal of Medicine 318:869-76, 1988.
226. Link H, Freund M, Kirchner H, et al. Recombinant human granulocyte-macrophage colony-stimulating factor (rhGM-CSF) after bone marrow transplantation. Behring Inst Mitt 83:313-9, 1988.

227. Devereaux 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 4:49-54, 1989.
228. Visani G. Tosi P. Gamberi B. Cenacchi A. Mazzanti P. Stabilini C. Bandini G. Mazza P. Gherlinzoni F. Cavo M. et al. Accelerated hemopoietic recovery after chemotherapy and autologous bone marrow transplantation in hematological malignancies using recombinant GM-CSF: preliminary results obtained in 14 cases. Haematologica. 75(6):551-4, 1990 Nov-Dec.
229. Lazarus HM, Anderson J, Chen MG, Variakojis D, Mansour EG, Oette D, Arce CA, Oken MM, Gerson SL. Recombinant human granulocyte-macrophage colony-stimulating factor after autologous bone marrow transplantation for relapsed non-Hodgkin's lymphoma: Blood and bone marrow progenitor growth studies. A Phase II Eastern Cooperative Oncology Group trial. Blood 78:830-837, 1991.
230. 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 73:849-857.
231. Visani G. Gamberi B. Greenberg P. Advani R. Gulati S. Champlin R. Hoglund M. Karanes C. Williams S. Keating A. et al. The use of GM-CSF as an adjunct to autologous/syngeneic bone marrow transplantation: a prospective randomized controlled trial. Bone Marrow Transplantation. 7 Suppl 2:81, 1991.
232. Gorin NC. Coiffier B. Hayat M. Philip T. Vernant JP. Herv:e P. Colombat P. Boivin P. Slavin S. Eug`ene-Jolchine I. RHU GM-CSF shortens aplasia duration after ABMT in non-Hodgkin's lymphoma: a randomized placebo-controlled double-blind study. Bone Marrow Transplantation. 7 Suppl 2:82, 1991.
233. Nemunaitis J. Rabinowe SN. Singer JW. Bierman PJ. Vose JM. Freedman AS. Onetto N. Gillis S. Oette D. Gold M. et al. Recombinant granulocyte-macrophage colony-stimulating factor after autologous bone marrow transplantation for lymphoid cancer. New England Journal of Medicine. 324(25):1773-8, 1991 Jun 20.
234. Advani R. Chao NJ. Horning SJ. Blume KG. Ahn DK. Lamborn KR. Fleming NC. Bonnem EM. Greenberg PL. Granulocyte-macrophage colony-stimulating factor (GM-CSF) as an adjunct to autologous hemopoietic stem cell transplantation for lymphoma. Annals of Internal Medicine. 116(3):183-9, 1992 Feb 1.
235. Gulati SC. Bennett CL. Granulocyte-macrophage colony-stimulating factor (GM-CSF) as adjunct therapy in relapsed Hodgkin disease Annals of Internal Medicine. 116(3):177-82, 1992 Feb 1.
236. Nemunaitis J. Singer JW. Buckner CD. Mori T. Laponi J. Hill R. Storb R. Sullivan KM. Hansen JA. Appelbaum FR. Long-term follow-up of patients who received recombinant human granulocyte-macrophage colony stimulating factor after autologous bone marrow transplantation for lymphoid malignancy. Bone Marrow Transplantation. 7(1):49-52, 1991.
237. Brandwein JM. Nayar R. Baker MA. Sutton DM. Scott JG. Sutcliffe SB. Keating A. GM-CSF therapy for delayed engraftment after autologous bone marrow transplantation. Experimental Hematology. 19(3):191-5, 1991 Mar.
238. Vose JM. Bierman PJ. Kessinger A. Coccia PF. Anderson J. Oldham FB. Epstein C. Armitage JO. The use of recombinant human granulocyte-macrophage colony stimulating factor for the treatment of delayed engraftment following high dose therapy and autologous hematopoietic stem cell transplantation for lymphoid malignancies. Bone Marrow Transplantation. 7(2):139-43, 1991 Feb.
239. Nemunaitis J, Singer JW, Buckner CD, et al. The use of recombinant human granulocyte-macrophage colony-stimulating factor in graft failure folowing bone marrow transplantation. Blood 76:245-53, 1990.
240. Atkinson K. Biggs JC. Downs K. Juttner C. Bradstock K. Lowenthal RM. Dale B. Szer J. GM-CSF after allogeneic bone marrow transplantation: accelerated recovery of neutrophils, monocytes and lymphocytes. Australian & New Zealand Journal of Medicine. 21(5):686-92, 1991 Oct.
241. Powles R. Smith C. Milan S. Treleaven J. Millar J. McElwain T. Gordon-Smith E. Milliken S. Tiley C. Human recombinant GM-CSF in allogeneic bone-marrow transplantation for leukaemia: double-blind, placebo-controlled trial. Lancet. 336(8728):1417-20, 1990 Dec 8.
242. Nemunaitis J, Buckner CD, Appelbaum FR, Higano CS, Mori M, Bianco J, Epstein C, Lipani J, Hansen J, Storb R, Thomas ED, Singer JW. Phase I/II trial of recombinant human granulocyte-macrophage colony-stimulating factor following allogeneic bone marrow transplantation. Blood 77(9):2065-71, 1991.
243. Nemunaitis J, Anasetti C, Storb R, Bianco JA, Buckner CD, Onetto N, Martin P, Sanders J, Sullivan K,

Mori M, Shannon-Crocy K, Bowden R, Appelbaum FR, Hansen J, Singer JW. Phase II trial of recombinant human granulocyte-macrophage colony-stimulating factor in patients undergoing allogeneic bone marrow transplantation from unrelated donors. Blood 79:2572-2577, 1992.
244. De Witte T, Gratwohl A, Van Der Lely N, Bacigalupo A, Stern AC, Speck B, Schattenberg A, Nissen C, Gluckman E, Fibbe WE. Recombinant human granulocyte-macrophage colony-stimulating factor accelerates neutrophil and monocyte recovery after allogeneic T-cell-depleted bone marrow transplantation. Blood. 79(5):1359-65, 1992.
245. Kodo H, Tajika K, Takahashi S, et al. Acceleration of neutrophilic granulocyte recovery after bone marrow transplantation by administration of recombinant human granulocyte colony-stimulating factor. Lancet 2:38-39, 1988.
246. Sheridan WP, Mortsyn G, Wolf M, et al. Granulocyte colony-stimulating factor and neutrophil recovery after high-dose chemotherapy and autologous bone marrow transplantation. Lancet 2:891-895, 1989.
247. Taylor KMcD, Jagannath S, Spitzer G, et al. Recombinant human granulocyte colony-stimulating factor hastens granuloctye recovery after high dose chemotherapy and autologous bone marrow transplantation in Hodgkin's disease. J Clin Oncol 7:1791-1799, 1989.
248. Peters W. The effect of recombinant human colony-stimulating factors on hematopoietic reconstitution following autologous bone marrow transplantation. Semin Haematol 26(suppl 2):18-23, 1989.
249. Asano S, Masaoka T, Takaku F. Beneficial effect of recombinant human glycosylated granulocyte colony-stimulating factor in marrow-transplanted patients: results of multicenter phase II-III studies. Transplantation Proceedings 23:1701-3, 1991.
250. Takahashi S. Asano S. Masaoka T. Takaku F. Niitsu Y. Shibuya A. Saito H. Sekine I. Sanpi K. Hanada R. et al. Clinical evaluation of recombinant human granulocyte colony-stimulating factor (rhG-CSF) in autologous bone marrow transplantation [Jpn] Rinsho Ketsueki - Japanese Journal of Clinical Hematology. 32(3):221-6, 1991.
251. Gianni AM. Siena S. Bregni M. Lombardi F. Gandola L. Valagussa P. Bonadonna G. Prolonged disease-free survival after high-dose sequential chemo-radiotherapy and haemopoietic autologous transplantation in poor prognosis Hodgkin's disease. Annals of Oncology. 2:645-53, 1991.
252. Masaoka T, Takaku F, Kasto S, et al. Recombinant human granulocyte colony-stimulating factor in allogeneic bone marrow transplantation. Exp Hematol 17:1047-1050, 1989.
253. Villeval JL, Duhrsen U, Morstyn G, Metcalf D. Effect of recombinant human GM-CSF on progenitor cells in patients with advanced malignancies. Br J Haematol 74:36, 1990.
254. Haas R, Ho AD, Bredthauer U, Cayeux S, Egerer G, Knauf W, Hunstein W. Successful autologous transplantation of blood stem cells mobilized with recombinant human granulocyte-macrophage colony-stimulating factor. Exp Hematol 18, 94-98, 1990.
255. Korbling M, Holle R, Haas R, Knauf W, Dorken B, Ho AD, Kuse R, Pralle H, Fliedner TM, Hunstein W. Autologous blood stem-cell transplantation in patients with advanced Hodgkin's disease and prior radiation to the pelvic site. J Clin Oncol 8:978-985, 1990.
256. Gianni AM, Tarella C, Siena S, Bregni M, Boccadoro M, Lombardi F, Bengala C, Bonadonna G, Pileri A. Durable and complete hematopoietic reconstitution after autografting rhGM-CSF exposed peripheral blood progenitor cells. Bone Marrow Transplantation 6:143-145, 1990.
257. Ho AD, Haas R, Korbling M, Dietz M, Hunstein W. Utilization of recombinant human GM-CSF to enhance peripheral progenitor cell yield for autologous transplantation. Bone Marrow Transplantation 7(Suppl 1):13-7, 1991.
258. Fukuda M, Kojima S, Matsumoto K, Matsuyama T. Autotransplantation of peripheral blood stem cells mobilized by chemotherapy and recombinant human granulocyte colony-stimulating factor in childhood neuroblastoma and non-Hodgkin's lymphoma. Br J Haematol 80:327-331, 1992.
259. Brugger W, Bross K, Frisch J, Dern P, Weber B, Mertelsmann R, Kanz L. Mobilization of peripheral blood progenitor cells by sequential administration of interleukin-3 and granulocyte-macrophage colony-stimulating factor following polychemotherapy with etoposide, ifosfamide, and cisplatin. Blood 79:1193-1200.
260. Butturini A. Santucci MA. Gale RP. Perocco P. Tura S. GM-CSF incubation prior to treatment with cytarabine or doxorubicin enhances drug activity against AML cells in vitro: a model for leukemia chemotherapy.

Leukemia Research. 14(9):743-9, 1990.
261. Suzuki T. Morio T. Tohda S. Nagata K. Yamashita Y. Imai Y. Aoki N. Hirashima K. Nara N. Effects of interleukin-6 and granulocyte colony-stimulating factor on the proliferation of leukemic blast progenitors from acute myeloblastic leukemia patients.Japanese Journal of Cancer Research. 81(10):979-86, 1990.
262. Motoji T. Watanabe M. Uzumaki H. Kusaka M. Fukamachi H. Shimosaka A. Oshimi K. Mizoguchi H. Granulocyte colony-stimulating factor (G-CSF) receptors on acute myeloblastic leukaemia cells and their relationship with the proliferative response to G-CSF in clonogenic assay. British Journal of Haematology. 77(1):54-9, 1991.
263. Kondo S. Okamura S. Asano Y. Harada M. Niho Y. Human granulocyte colony-stimulating factor receptors in acute myelogenous leukemia. European Journal of Haematology. 46(4):223-30, 1991.
264. Ishikawa J. Yoshimura M. Matsunashi T. Tominaga N. Teshima H. Hiraoka A. Nakamura H. Shibata H. Masaoka T. Takaku F. Clinical effect of granulocyte colony-stimulating factor on neutrophils and leukemic cells in myelogenous leukemia: analysis. Japanese Journal of Clinical Oncology. 21(3):169-75, 1991.
265. Koistinen P. Wang C. Curtis JE. McCulloch EA. Granulocyte-macrophage colony-stimulating factor and interleukin-3 protect leukemic blast cells from ara-C toxicity. Leukemia. 5(9):789-95, 1991.
266. Aglietta M. De Felice L. Stacchini A. Petti MC. Bianchi AC. Aloe Spiriti MA. Sanavio F. Apra F. Piacibello W, Stern AC. et al. In vivo effect of granulocyte-macrophage colony-stimulating factor on the kinetics of human acute myeloid leukemia cells. Leukemia 5:979-84, 1991.
267. Lemoli RM. Gulati SC. Strife A. Lambek C. Perez A. Clarkson BD. Proliferative response of human acute myeloid leukemia cells and normal marrow enriched progenitor cells to human recombinant growth factors IL-3, GM-CSF and G-CSF alone and in combination. Leukemia. 5(5):386-91, 1991.
268. Estrov Z, Estey EH, Andreeff M, Talpaz M, Kurzrock R, Reading C, Deissroth AB, Gutterman JU. Comparison of in vivo and in vitro effects of granulocyte-macrophage colony-stimulating factor (GM-CSF) in patients with acute myeloid leukemia. Exp Hematol 20:558-564, 1992.
269. Ohno R, Tomonaga M, Kobayashi T, Kanamaru A, Shirakawa S, Masaoka T, Omine M, Oh H, Takeo N, Sakai Y, Hirano M, Yokomaku S, Nakayama S, Yoshida Y, Miura AB, Morishima Y, Dohy H, Niho Y, Hamajima N, Takaku F. Effect of granulocyte colony-stimulating factor after intensive induction therapy in relapsed or refractory acute leukemia. New England Journal of Medicine 323:871-877, 1990.
270. Teshima H, Ishikawa J, Kitayama H, et al. Clinical effects of recombinant human granulocyte colony-stimulating factor in leukemia patients: a phase I/II study. Exp Hematol 17:853-858, 1989.
271. Bettelheim P. Valent P. Andreeff M. Tafuri A. Haimi J. Gorischek C. Muhm M. Sillaber C. Haas O. Vieder L. et al. Recombinant human granulocyte-macrophage colony-stimulating factor in combination with standard induction chemotherapy in de novo acute myeloid leukemia. Blood. 77(4):700-11, 1991.
272. Cannistra SA. DiCarlo J. Groshek P. Kanakura Y. Berg D. Mayer RJ. Griffin JD. Simultaneous administration of granulocyte-macrophage colony-stimulating factor and cytosine arabinoside for the treatment of relapsed acute myeloid leukemia. Leukemia. 5(3):230-8, 1991.
273. B:uchner T. Hiddemann W. Koenigsmann M. Z:uhlsdorf M. W:ormann B. Boeckmann A. Freire EA. Innig G. Maschmeyer G. Ludwig WD. et al. Recombinant human granulocyte-macrophage colony-stimulating factor after chemotherapy in patients with acute myeloid leukemia at higher age or after relapse. Blood. 78(5):1190-7, 1991.
274. Kantarjian HM. Estey EH. O'Brien S. Anaissie E. Beran M. Rios MB. Keating MJ. Gutterman J. Intensive chemotherapy with mitoxantrone and high-dose cytosine arabinoside followed by granulocyte-macrophage colony-stimulating factor in the treatment of patients with acute lymphocytic leukemia. Blood. 79:876-81, 1992.
275. Kantarjian HM. Talpaz M. Kontoyiannis D. Gutterman J. Keating MJ. Estey EH. O'Brien S. Rios MB. Beran M. Deisseroth A. Treatment of chronic myelogenous leukemia in accelerated and blastic phases with daunorubicin, high-dose cytarabine, and granulocyte-macrophage colony-stimulating factor. Journal of Clinical Oncology. 10(3):398-405, 1992.
276. Estey E, Thall PF, Kantarjian H, O'Brien S, Koller CA, Beran M, Gutterman J, Deisseroth A, Keating M. Treatment of newly diagnosed acute myelogenous leukemia with granulocyte-macrophage colony-stimulating factor (GM-CSF) before and during continuous infusion high-dose ara-C + Daunorubicin: Comparison with patients treated without GM-CSF. Blood 79:2246-2255, 1992.

277. Antman KH. G-CSF and GM-CSF in clinical trials. Yale Journal of Biology & Medicine. 63(5):387-410, 1990.
278. Cebon JS. Morstyn G. The potential role of granulocyte-macrophage colony stimulating factor (GM-CSF) in cancer chemotherapy. Cancer Surveys. 9(1):131-55, 1990.
279. Scarffe JH. Kamthan A. Clinical studies of granulocyte colony stimulating factor (G-CSF). Cancer Surveys. 9(1):115-30, 1990.
280. Davis I. Morstyn G. The role of granulocyte colony-stimulating factor in cancer chemotherapy. Seminars In Hematology. 28(2 Suppl 2):25-33, 1991.
281. Rabinowe SN. Nemunaitis J. Armitage J. Nadler LM. The impact of myeloid growth factors on engraftment following autologous bone marrow transplantation for malignant lymphoma. Seminars In Hematology. 28(2 Suppl 2):6-16, 1991.
282. Sakamoto KM. Golde DW. Gasson JC. The biology and clinical applications of granulocyte-macrophage colony-stimulating factor. Journal of Pediatrics. 118(3):S17-20, 1991.
283. Sakamoto KM. Gasson JC. Clinical applications of human granulocyte-macrophage colony-stimulating factor. International Journal of Cell Cloning. 9(6):531-41, 1991.
284. Furman WL. Crist WM. Potential uses of recombinant human granulocyte-macrophage colony-stimulating factor in children. American Journal of Pediatric Hematology-Oncology. 13(4):388-99, 1991.
285. Scarffe JH. Emerging clinical uses for GM-CSF. European Journal of Cancer. 27(11):1493-504, 1991.
286. Sheridan WP. The role of colony-stimulating factors in bone marrow transplantation. Cancer Investigation. 9(2):221-8, 1991.
287. Nemunaitis JJ. Singer JW. Sanders JE. The use of recombinant human granulocyte macrophage colony-stimulating factor in autologous bone marrow transplantation. Bone Marrow Transplantation. 7 Suppl 3:24-7, 1991.
288. Miles SA. The use of hematopoietic growth factors in HIV infection and AIDS-related malignancies. Cancer Investigation. 9(2):229-38, 1991.