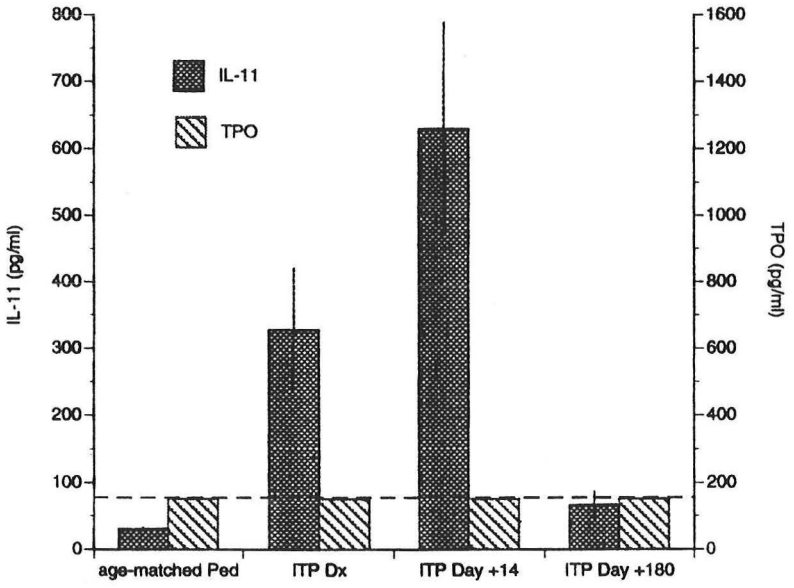
G-CSF and Thrombopoietin:

Hematopoietic Growth Factors to the Rescue



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Areas of Interest: Post-translational lipid modifications of proteins/enyzmes of lipid metabolism/clinical hematology

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Introduction

The hematopoietic growth factors were last reviewed in the Internal Medicine Grand Rounds five years ago. At that time, Roger Fleischman reviewed the biology and clinical use of G-CSF and GM-CSF. I thought that this would be a good time to revisit this topic, because I think some important questions have arisen concerning the indications for the colony-stimulating factors, and these questions commonly come up on ward rounds. In addition, the American Society of Clinical Oncology has released guidelines for the use of recombinant G-CSF, and I thought that it might be helpful to review these guidelines and the rationale for them with you today. The first growth factor to affect the megakaryocytic line, IL-11, was approved by the FDA just a few weeks ago, and the long-awaited growth factor, thrombopoietin, should be released shortly, and I thought I would also provide an introduction to these new growth factors.

My talk will be divided into two parts: one part on G-CSF and one part on thrombopoietin and IL-11. Part I will focus on the clinical use of G-CSF. In part II, I will review the discovery and biology of the thrombopoietic growth factors, and explore their early clinical use.

The Hematopoietic System: A Dynamic, Multifunctional Organ

Fig. 1 is a schematic that reflects our current understanding of hematopoiesis and the factors that regulate it. The main point to be drawn from this diagram is that it is a vastly complex system, comprised of many distinct cell types, and acted upon by a very large array of soluble growth factors. These factors may work alone or in concert, on one target cell or several, and may have effects on cell proliferation, cell survival, or both. The growth factors may each have unique sites of production, different modes of regulation, and may be important in maintaining a basal level of hematopoiesis, or in expanding cell populations in response to physiologic stress.

Although the system is complex, a clearer picture is beginning to emerge through the painstaking dissection of each of these components of the pathway. The work has been facilitated by methods for culturing bone marrow-derived cells developed in the 1960's and 1970's, and by new technologies, such as flow cytometry, monoclonal antibodies (as markers for cell types), and recombinant DNA techniques that allow for the production of useful quantities of the various growth factors. Also, our understanding of the mechanisms involved (and even the identification of some of these growth factors) has been aided by new knowledge about how proliferative signals are transduced in cells, and also by the realization that cell numbers may also be regulated by cell death, through the process of apoptosis. As we shall examine, these advances are very rapidly coming to find applications in the clinical arena.

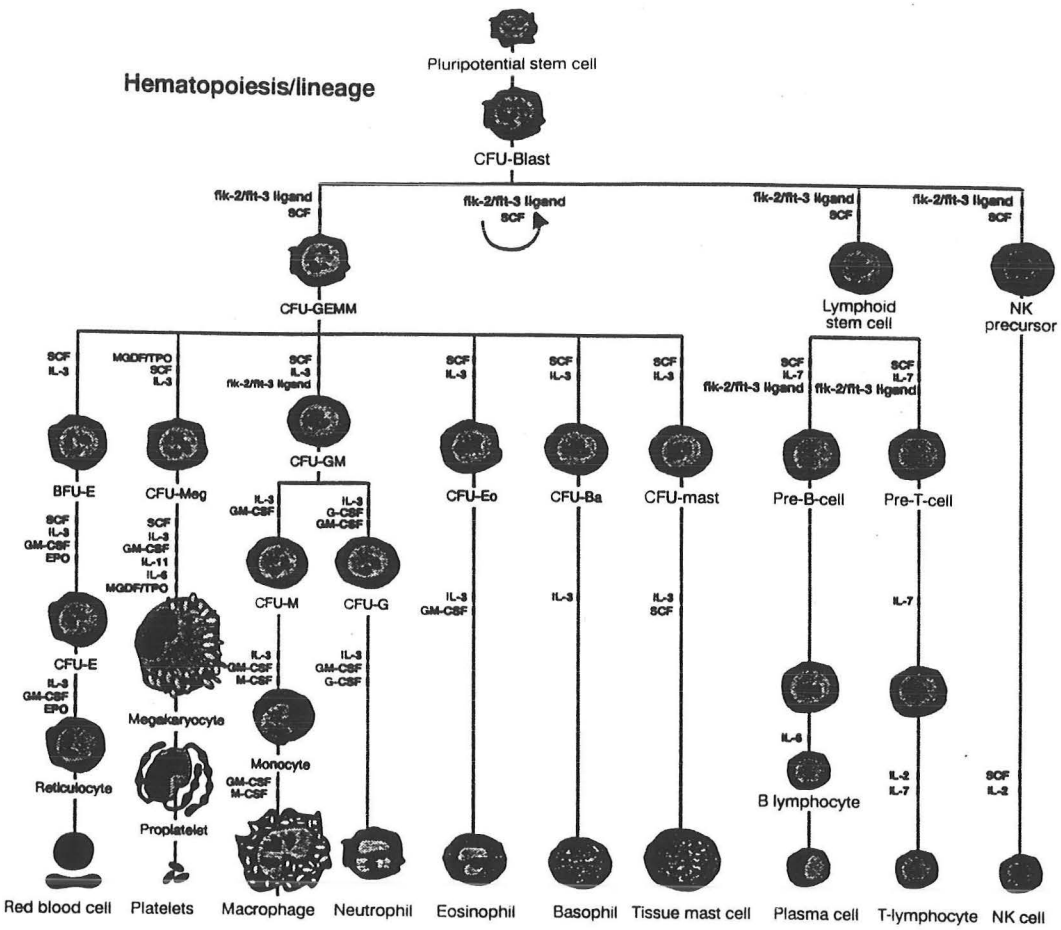


Fig. 1. The control of hematopoiesis. From (1).

Part I: Granulocyte Colony-Stimulating Factor (G-CSF)

G-CSF: A Major Regulator of Granulopoiesis

G-CSF is a glycosylated polypeptide with very specific effects on granulocyte maturation and function (reviewed in (2)). It is only one of four different cytokines that were identified as potentially useful factors for stimulating the growth and differentiation of myeloid cells in the 1970's and 1980's (3). In cell culture systems, granulocyte-monocyte colony-stimulating factor (GM-CSF) and interleukin-3 (IL-3, or multi-CSF) were the most potent stimulators of granulopoiesis, whereas G-CSF and M-CSF (monocyte colony-stimulating factor) were relatively weak, but still considered to be interesting based on their relatively specific effects on the granulocytic and monocytic lineages, respectively.

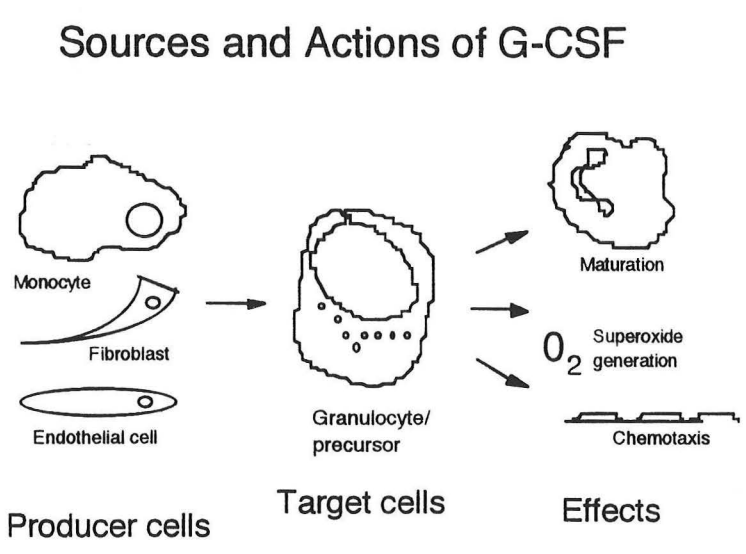
Where does G-CSF fit in this grand scheme of hematopoiesis? The most compelling clues come from studies of genetically engineered ("knock-out") mice, which have targeted deletions in

their G-CSF genes. The results of similar experiments in which the other putative mediators of myelopoiesis have been disrupted are also listed in Table 1 for comparison.

Disrupted Gene	Phenotype
G-CSF	neutropenia
GM-CSF	alveolar proteinosis
M-CSF	osteopetrosis
IL-3	none

Table 1. Phenotypes in mice with targeted disruption of cytokine genes. All four cytokines have potent effects on myelopoiesis in bone marrow cultures. The effects of targeted deletions in mice were surprising. Data adapted from (3).

One major conclusion from these studies is that G-CSF is a major regulator of neutrophil counts, but that it is not absolutely required for granulocyte differentiation or maturation, because otherwise normal neutrophils are produced, but only in decreased numbers. Another conclusion is that GM-CSF has no irreplaceable role in regulating the basal numbers of granulocytes or monocytes, but that it does have a unique role in regulating the function of certain populations of macrophages. Likewise, M-CSF is required for the formation of only certain monocyte populations (osteoclasts). The role of IL-3, despite its potent effects on early hematopoiesis, is unclear. It may be important, but other factors can substitute for its function.



The major cell types that express and secrete G-CSF are monocytes/macrophages, fibroblasts, and endothelial cells, but the relative contributions of these cell types is unclear. G-CSF levels rise during infection (4), and the synthesis and secretion of G-CSF increases in response to TNFα and IL-1 treatment of many cell lines. The increase is mediated by both transcriptional and post-transcriptional mechanisms (5). The response to inflammatory/infectious mediators is consistent with the role of G-CSF as a regulator of neutrophil numbers and with its effects in enhancing neutrophil function.

G-CSF acts specifically on cell surface receptors that are present only on granulocytes and their precursors (5). The G-CSF receptor is a member of the cytokine receptor superfamily, and it has a cytoplasmic domain that interacts with tyrosine kinases. Distinct portions of the cytoplasmic domain are responsible for transducing maturation-promoting and proliferative signals (reviewed in (6). These signals eventually result in the activation of nuclear transcription factors that control the

maturation and proliferation of the neutrophil progenitors. Both the classical Ras/MAP kinase signalling pathway and the JAK-STAT signalling pathway are activated upon stimulation of the G-CSF receptor. The mechanism of the activation is an active area of investigation (6). Point mutations in the G-CSF receptor have been described in subgroups of patients with severe, congenital neutropenia and rare patients with acute myelogenous leukemia. Constitutive activation of the G-CSF receptor through the JAK2 kinase provides an explanation of this phenomenon (7).

When G-CSF binds to neutrophilic progenitors, the progenitors are stimulated to divide. In addition, their maturation is shortened from five days to one day. G-CSF also has positive effects on nearly every neutrophil function that can be measured--chemotaxis, adherence, phagocytosis, antibody-dependent cytotoxicity, superoxide generation, and killing of microorganisms (reviewed in (8)).

The specificity of G-CSF for its receptor, and the restriction of the expression of the G-CSF receptor to neutrophils and their precursors, are the major reasons that G-CSF has edged out the other cytokines listed in Table 1 in the race for a clinically useful drug. For example, GM-CSF has actions on other cell types. For reasons that are unclear, it causes fevers in a proportion of patients, and an increased incidence of a capillary leak syndrome that appears to be mediated by other cytokines (9). GM-CSF may still prove to be useful in some situations (for example, there is some evidence that GM-CSF may be helpful in preventing fungal infections in severely immunocompromised patients, as in the setting of allogeneic BMT), but the utility of the agent will have to be weighed against the somewhat greater toxicity. IL-3, which has effects on many different hematopoietic precursors, has shown quite severe toxicity (capillary-leak syndome) in Phase I-II trials . M-CSF is still in the early stages of clinical development.

Potential clinical applications for G-CSF are shown below. Each of these will be discussed in turn, but the most important of these to the internist is its use in ameliorating chemotherapy-induced neutropenia.

Potential Clinical Applications for G-CSF

- Stimulation of Granulopoiesis
  - Chemotherapy-induced neutropenia
  - Aplastic Anemia
  - Myelodysplastic Syndromes
  - Congenital Neutropenia
- Expansion and Recruitment of Circulating Stem Cells
  - Peripheral Blood Stem Cell Transplantation
- Activation of Neutrophil Function
  - Infection
  - AIDS
  - Cancer
  - Leukocyte dysfunction

Clinical Use of G-CSF: Dose and Administration, Toxicity, and Cost

G-CSF was approved by the FDA in 1991 for the prevention of febrile neutropenia due to cytotoxic chemotherapy. The dose recommended by the manufacturer is 5 μg/kg/day, although lower doses may be effective (10-13). No upper limit of dose has been established, because counts of 200,000 have been achieved in normal individuals receiving G-CSF without ill effect. However, higher levels do not achieve superior results. Dose reductions for renal or hepatic function are unnecessary. The subcutaneous route is most effective in normal individuals but the i.v. route is also acceptable. The optimal time for initiation is 24 to 72 h following chemotherapy, and a "window" of 24 h before or after chemotherapy should be allowed to prevent possible increased toxicity. It should be avoided in patients receiving concomitant chemotherapy and radiation therapy. The manufacturer recommends continuing G-CSF until an ANC of 10,000/μl is reached, but it has been common practice to stop at a level of 4,000 μl or even lower with clear evidence of neutrophil recovery, as one can expect the neutrophil count to double daily. For this reason, patients on G-CSF should be closely monitored (in order to minimize cost). The average wholesale cost of G-CSF is $141.00 per 300 μg vial.

The toxicity of G-CSF is reported to be mild. From 15 to 40% of patients complain of medullary bone pain, and this is usually easily relieved with acetaminophen. The pain occurs just shortly after administration, and just before the onset of neutrophil recovery (14). Other rare reactions include acute febrile neutrophic dermatosis (Sweet syndrome) (15), rashes, and rare allergic reactions. Splenomegaly and splenic infarction have been described (16), but are exceedingly rare. Common laboratory abnormalities include elevations in lactate dehydrogenase, uric acid, and serum and leukocyte alkaline phosphatase, probably due to increased myeloid cell turnover. False positive tests for hepatitis surface antigen, due to a cross-reaction between E. coli proteins (contaminants in the production of G-CSF) and reagents used to detect HBSAg have recently been reported (17). Anti-G-CSF antibodies do not develop with any frequency, and doses as high as 100 μg/kg/day have been given without toxicity.

Indications for G-CSF: The American Society of Clinical Oncology Guidelines

The American Society of Clinical Oncology (ASCO) recently reviewed all of the clinical trials on the use of G-CSF in common clinical situations in order to assist physicians in making decisions concerning its use. A panel of 25 experts in clinical medicine, clinical research, CSF use, bone marrow transplantation, infectious diseases, basic research, statistics, and medical economics reviewed all of the available studies, and rated the evidence according to established criteria. The outcomes measured were absolute neutrophil counts, rates of febrile neutropenia, antibiotic therapy requirements, need for hospitalization, economic impact, and maintenance of chemotherapy dose and schedule as intermediate endpoints. Survival, quality of life, and costs were primary endpoints. Background data that was considered included the myelotoxicity of chemotherapy regimens, toxicity of G-CSF, and cost and alternatives to therapy. Guidelines for dosing, timing of administration, route and duration were also covered, but these closely reflected the manufacturer's guidelines, as described above. One the next page is a summary of the guidelines. The basis for these guidelines will be discussed in detail.

ASCO Recommendations for the Use of Hematopoietic Colony-Stimulating Factors (CSFs): Evidence-Based, Clinical Practice Guidelines

1. For previously untreated patients receiving most chemotherapy regimens, primary [prophylactic] administration of CSFs should not be used routinely. Primary administration of CSFs should be reserved for patients expected to experience febrile neutropenia with an expected incidence of ≥ 40%.

2. Primary [prophylactic] CSF administration may be warranted in patients at higher risk for chemotherapy-induced infectious complications, even though the data supporting such use is not conclusive.

Such risk factors might include preexisting neutropenia due to disease, prior chemotherapy or irradiation, a history of previous febrile neutropenia, or [other] conditions, such as decreased immune function, open wounds, or already active tissue infections.

3. CSFs can decrease the probability of febrile neutropenia in subsequent cycles of chemotherapy after a documented occurrence in an earlier cycle, and its use may be considered if prolonged neutropenia is causing dose reductions or delays in chemotherapy. However, in the absence of clinical data supporting maintenance of chemotherapy dose-intensity, physicians should consider chemotherapy dose reduction as an alternative to the use of CSFs.

4. CSFs in afebrile neutropenic patients is not recommended.

5. For the majority of patients with febrile neutropenia, the available data do not clearly support the routine initiation of CSFs as adjuncts to antibiotic therapy.

However, the use of CSFs may be reasonable in certain high-risk patients (pneumonia, hypotension, multi-organ dysfunction, or fungal infection) although the benefits have not been definitively proved.

6. CSFs can achieve modest decreases in the duration of neutropenia when begun shortly after the completion of AML induction therapy, but beneficial CSF effects on duration of hospitalization, incidence of severe infection, response rates, and long-term survival have yet to be completely determined.

7. CSFs are recommended for patients undergoing autologous bone marrow cell transplantation, and may be useful in the setting of allogeneic BMT and peripheral-blood progenitor cell transplantation. CSF are effective in mobilizing peripheral blood stem cells for transplantation.

1. Primary [prophylactic] administration of CSFs should be reserved for patients expected to experience febrile neutropenia with an expected incidence of ≥ 40%.

There is a clear quantitative relationship between the number of circulating leukocytes and the risk of infection (18), as illustrated in this classic study published in 1966. Therefore, preventing neutropenia in chemotherapy-treated patients would seem to be a reasonable goal.

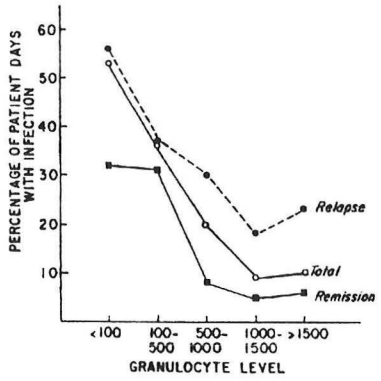


Fig. 2. Relationship between neutrophil counts and infection in patients treated with cytotoxic chemotherapy. From (18).

Three major studies have provided strong evidence that G-CSF may be useful in abrogating the incidence of neutropenic fever in chemotherapy patients (19-21). The three studies involved patients with small cell lung cancer (19, 20) and non-Hodgkin's lymphoma (21), in whom chemotherapy was highly likely to produce neutropenia. The studies showed nearly identical results.

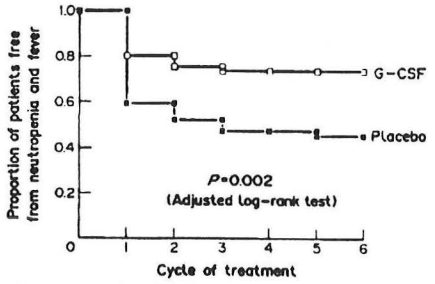


Fig. 2. Proportion of patients in each treatment group who did not experience at least one episode of fever concurrent with neutropenia (ANC less than 1.0 × 10⁹/l and fever ≥ 38.2°C). Data shown by cycle.

Fig. 3. Proportion of small cell lung cancer patients free of neutropenic fever.

Fig. 3 shows a Kaplan-Meier plot of the proportion of patients free of fever with neutropenia over the course of six treatment cycles for small cell lung cancer. There were no meaningful differences in tumor response rates or survival between the groups receiving G-CSF or placebo. G-CSF reduced the occurrence of febrile neutropenia by 50%. In two of these studies, daily neutrophil counts were determined throughout each cycle of chemotherapy. G-CSF in this study reduced the depth of the nadir and decreased the length of neutropenia. In the other study, the depth of the nadir was the same, but occurred one day earlier, and the duration of neutropenia was reduced (Fig. 4).

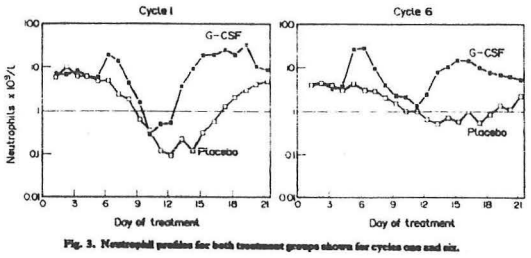


Fig. 4. Neutrophil profiles for subjects treated with G-CSF or placebo. G-CSF reduces the depth and duration of the nadir. From (20).

In one of the studies, a randomized trial of G-CSF in patients receiving chemotherapy for non-Hodgkin's lymphoma, careful attention was paid to treatment delays and reductions in dose intensity (21). G-CSF reduced the number of days that treatment was delayed by about half, and reduced the number of patients requiring dose reductions from 33% to 10%. None of these studies reported a difference in infectious mortality, response rates or survival.

Few outpatient regimens induce greater than the 40% rate of grade IV myelosuppression that was seen in these initial studies. Shown below is a summary of the myelotoxicity of several commonly-used chemotherapy regimens. Only therapies for AML and small cell lung cancer produce a 40% incidence of neutropenia.

Table 2. Incidence of Hematologic Toxicities Associated with Selected Chemotherapy Regimens Adapted from (22).

Cancer	Regimen	No. of pts.	%Grade IV leukopenia	%infection	%infectious death
AML	AraC/DNR	163	93	64	12
Lung (SCLC)	CAE	102	98	13	3
	CAV	156	52	16	4
	cis-p/etop	159	38	8	6
Ovary	CTX/carbo	144	28	7	1.4
Bladder	M-VAC	126	24	6	3
Lymphoma	MOPP	123	22	2	1
	ABVD	115	3	2	0
	CHOP	216	22	5	1
Testicular	PEB	77	16	3	0
Breast	CAF	32	9	3	0
Colorectal	5-FU/lev	181	8	6	-
Head/neck	5FU/carb	86	2	1	1.2

The American Society of Clinical Oncology recommendation to treat prior to chemotherapy was based upon two cost-benefit analyses (23, 24), which were in turn based on cost considerations derived from the above three studies (>40% incidence of neutropenia). The cost-benefit analyses concluded that G-CSF would not be cost-effective prior to chemotherapy regimens that produce less than a 20% incidence of neutropenia, and that the situation between 20 and 40% was unclear. They considered it unlikely that the drug would be less effective at lower rates of

neutropenia, only that more patients would have to be treated to achieve the benefit. (If G-CSF were as cheap as vitamins, every patient would be treated).

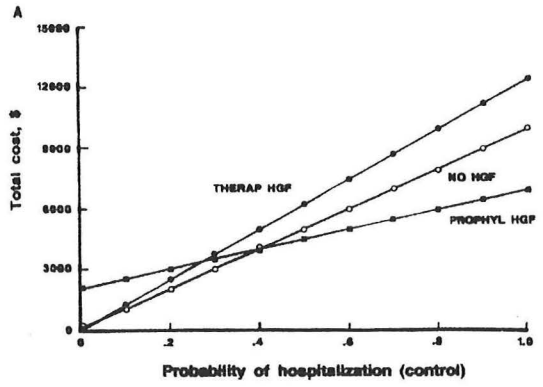


Fig. 5. Analysis of the cost vs. benefit for prophylactic G-CSF, therapeutic G-CSF, and no G-CSF as a function of the probability of hospitalization.

There are several difficulties with this analysis. First, opportunity costs (for example, the costs associated with the patient being away from work or home) are not included in the analysis. These are real costs and it seems that some analysis of the impact of these costs should have been considered. Also, costs for diagnostic and monitoring tests, cultures, drug levels, and consultations were not included. Finally, the assumption was made that the reduction in episodes of febrile neutropenia would be proportional at all levels of risk--for example, in the three large studies, the risk of neutropenia fell from 40% to 20%, a 50% reduction. The assumption is that at 20% risk, the rate will fall to 10%, but for less myelosuppression regimens, it is conceivable that the risk could fall incrementally, instead of proportionally--from 20% to 0%. This occurrence would favor G-CSF.

The conclusion of one of these studies (24) was that for risks of 20-40%, G-CSF would be favored only if hospital costs exceed $1500 per day, or if the cost of G-CSF were lowered to $150 per day and treatment could be limited to 5 days. Some regimens that would produce this degree of neutropenia would include M-VAC, a standard treatment for bladder cancer, and treatments for intermediate and high-grade lymphoma, and standard treatments for ovarian cancer (22).

I would like to see more controlled trials of G-CSF in less myelosuppressive regimens, and a re-evaluation of the minimum effective dose. Early clinical studies suggested that doses one-tenth that currently recommended may be effective. In that case, the use of G-CSF would be increasingly favored. It is clear that when the cost of G-CSF falls, its use in preventing chemotherapy-induced neutropenia will become routine.

2. Primary [prophylactic] CSF administration may be warranted in patients at higher risk for chemotherapy-induced infectious complications, even though the data supporting such use is not conclusive.

Such risk factors might include preexisting neutropenia due to disease, prior chemotherapy or irradiation, a history of previous febrile neutropenia, or in [other] conditions, such as decreased immune function, open wounds, or already active tissue infections.

In addition to the "40%" rule, the above circumstances were felt to be appropriate for G-CSF therapy, although data is inconclusive or does not exist. The special case of febrile neutropenia in a previous course is actually covered in statement #3. The utility in AIDS patients has been well-documented with respect to GM-CSF, with a demonstrated reduction in the rate of febrile neutropenia from 67% to 27% (25).

3. CSF can decrease the probability of febrile neutropenia in subsequent cycles of chemotherapy after a documented occurrence in an earlier cycle, and its use may be considered if prolonged neutropenia is causing dose reductions or delays in chemotherapy. However, in the absence of clinical data supporting maintenance of chemotherapy dose-intensity, physicians should consider chemotherapy dose reduction as an alternative to the use of CSFs.

There is evidence that G-CSF can decrease the probablility of febrile neutropenia in subsequent cycles of chemotherapy if one episode has already occurred (19), based on an evaluation of patients that crossed-over from placebo to G-CSF in that study. While there is not a randomized trial that has addressed this, it would seem reasonable.

The second part of this recommendation could be regarded as controversial. For curable malignancies, dose-intensity would be considered by many to be a very important consideration. More data are needed to settle this question.

4. CSF in afebrile neutropenic patients is not recommended.

No data exists one way or the other. This is not much of an issue because the patient will either quickly recover, or quickly become febrile, and so #5 will apply.

5. For the majority of patients with febrile neutropenia, the available data do not clearly support the routine initiation of CSFs as adjuncts to antibiotic therapy.

However, the use of CSFs may be reasonable in certain high-risk patients (pneumonia, hypotension, multi-organ dysfunction, or fungal infection) though the benefits have not be definitively proved.

This is good evidence that G-CSF is of only marginal benefit in this setting. Six prospective, randomized trial of G-CSF (or GM-CSF) have shown only slight improvements in the number of days of neutropenia and days of hospitalization, averaging only one day (26-30). Rates of mortality due to infection were very low, but more importantly, not clearly affected by treatment. However, in some trials, G-CSF significantly decreased the likelihood of prolonged hospitalization and reduced the need for empiric antifungal therapy (26). This suggests that a small proportion of patients with prolonged neutrophil recovery may benefit, but how does one identify this small proportion of patients?

Data from M.D. Anderson indicates that of the 3% of patients who die from febrile neutropenia, 83% have pneumonia (22). Patients with invasive fungal infections also have a poor prognosis. G-CSF may be a wise choice in these patients, until there is data to the contrary.

6. CSF can achieve modest decreases in the duration of neutropenia when begun shortly after the completion of AML induction therapy, but beneficial CSF effects on duration of hospitalization, incidence of severe infection, response rates, and long-term survival have yet to be completely determined.

Growth factors after chemotherapy for AML are well-tolerated, do not stimulate leukemic growth, and shorten the duration of neutropenia by 5-7 days (9, 31). Some studies have shown reduction in infection rates, antibiotic usage and duration of hospitalization, but no effect on early mortality, complete remission rate or overall survival. No cost-benefit studies to address this issue have been performed, but I think most oncologists consider a 5-7 day reduction in the duration of neutropenia worth the additional cost. Again, as costs fall, their use will be increasingly favored.

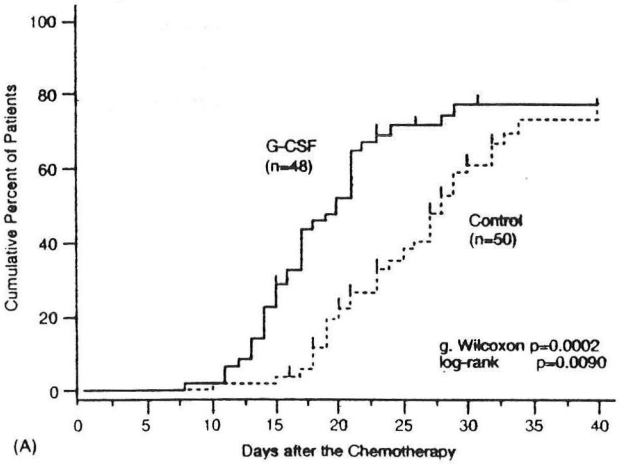


Fig. 6. The recovery of neutrophils to greater than 500/μl after induction therapy for AML. From (32).

7. CSFs are recommended for patients undergoing autologous bone marrow cell transplantation, and may be useful in the setting of allogeneic BMT and peripheral-blood progenitor cell transplantation. CSF are effective in mobilizing peripheral blood stem cells for transplantation.

The issues in terms of recovery of neutrophils in patients undergoing ABMT are similar to those in the treatment for AML. Over a dozen randomized, controlled trials have compared the used of CSFs vs. no CSFs, and there has been evidence in several of these trials for a shorter duration of hospitalization, antibiotc use, and infection (reviewed in (22)). However, the most recent exciting development in transplant research, which was completely unexpected, is that G-CSF mobilizes peripheral stem cells in normal, healthy people, so that the yield of stem cells from a blood donor is about fifteen to thirty-five-fold higher than the yield from an operative bone marrow harvest (reviewed in (33)). This finding has already revolutionized the practice of bone marrow transplantation. In recent data from the ASH meetings, the combination of pretreatment of donors and pretreatment of the patient has reduced the period of neutropenia from an original 21 days to about 9 days. With the addition of a donor granulocyte transfusion at day three after transplant, the period of neutropenia can be limited to less than 24 hours!

The result is that the autologous bone marrow transplant will become entirely an outpatient procedure, for both donor and recipient. Allogeneic transplants, with the attendant problems of graft vs. host disease and immune suppression will continue to be challenging.

Other Indications for G-CSF Therapy

Rare Hematologic Diseases (MDS and AA). G-CSF has been shown to be useful in some MDS patients who have recurrent infections due to neutropenia. A multicenter, randomized trial (34) has addressed the interesting question as to whether G-CSF might impact the natural history of advanced MDS (refractory anemia with excess blasts or in transformation). Unfortunately, there was no difference in time to progression or survival.

In general, patients with very severe marrow hypoplasia, such as those with aplastic anemia, do not respond well to G-CSF. In addition, these patients are a somewhat heterogeneous group. A few reports of responses have appeared, but results thus far have been disappointing.

AIDS. G-CSF has been used to to improve tolerance to ganciclovir and other antiviral drugs in AIDS patients (35). Once again, cost, and not effectiveness, is the issue. Interestingly, doses of 0.3 to 1.0 μg/kg/day or even 5 μg/kg per week (in two divided doses) were effective in this setting. GM-CSF prior to chemotherapy in AIDS patients has proven to be effective with a demonstrated reduction in the rate of febrile neutropenia from 67% to 27% (25). It is likely that G-CSF would be as equally effective in this setting.

Enhancing neutrophil function. This is a completely open question. A few preclinical trials have shown survival benefits in animals treated with G-CSF and pneumonia or sepsis (reviewed in (8)).

G-CSF: Summary

G-CSF is a potent cytokine with specific effects on neutrophil generation, maturation and function. It reduces the incidence of chemotherapy-induced neutropenia by at least 50% when given shortly after chemotherapy, and shortens the duration of neutropenia in patients undergoing myeloablative therapy by about 5-7 days. The unexpected effect of G-CSF in expanding and mobilizing bone marrow stem cells to the periphery is having an enormous impact on the practice of bone marrow transplantation. Many other potential uses of G-CSF remain to be explored.

Part II. Thrombopoietic Growth Factors (Thrombopoietin and IL-11)

Overview of Thrombopoiesis

Our understanding of megakaryocyte and platelet production has lagged behind that of erythrocyte and leukocyte production, mainly due to difficulties in studying the process in cell culture. A megakaryocyte development and promoting factor (MGDF) had been identified in serum from animals made thrombocytopenic by toxins a long time ago, but its unequivocal identification and characterization did not come until very recently (see (36) for a historical review). The availability of the thrombopoietic growth factors (the discovery of which will be covered in more detail below) has now made this problem much more tractable, and we have recently learned much about megakaryocyte development and platelet production, including some new and unexpected findings.

The "classic" model of hematopoiesis has held that the production of mature cells may be divided into two stages--an early proliferative stage, and a late maturation stage. "Late-acting" factors, such as erthropoietin, G-CSF, and the putative thrombopoietin, were not expected to have effects on early progenitor cells, but only on the terminal stages of maturation. This model has required reexaminination, because the "late-acting factors" have been found to have profound effects on even the earliest progenitor cells. This has certainly proven to be true in the case of thrombopoietin (37).

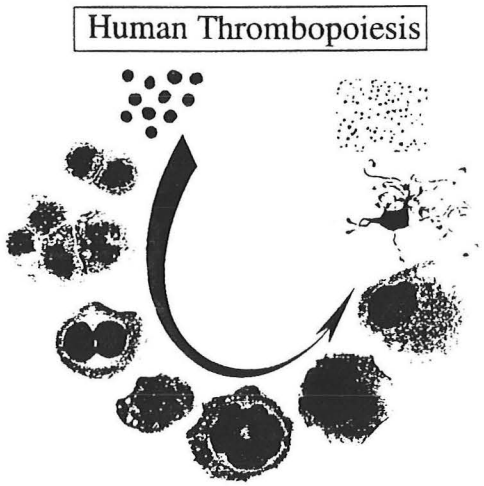


Fig. 7. Megakaryocyte growth and platelet production under the influence of thrombopoietin. From (38).

Fig. 7 is an illustration of megakaryoctye growth, development and platelet production as influenced by thrombopoietin. These are actual photographs taken from cultures of bone marrow stem cells (CD34+) stimulated with 10 ng/ml of thrombopoietin throughout 12 days of culture. The megakaryocyte is a very unusual cell, in that it is polyploid, and undergoes five rounds of mitoses without cell division, in a process called endomitosis. The ploidy of the cell may be as high as 64N before it undergoes a further process of fragmentation and platelet formation. This

process was recently shown to occur by the extension of beautiful, swirling tendrils of cytoplasm that form small blebs that are shed into the circulation as mature platelets.

The discovery of c-mpl, the thrombopoietin receptor

In 1986, Wendling and coworkers in France described a retrovirus that induces an acute myeloproliferative disease in mice (39). The transforming viral oncogene, named v-mpl, was described in 1990 (40). These investigators sequenced the envelope gene of a biologically active c-mpl clone, and found within this region a novel oncogene (v-mpl, for myeloproliferative) that was fused in frame with two parts of the Friend murine leukemia virus envelope gene. The MPLV envelope region encoded an env-mpl fusion polypeptide with the characteristics of a transmembrane protein.

v-mpl shared strong sequence homology with other members of the hemotopoietic superfamily, and it was postulated that the virus had transduced a novel hemotopoietic growth factor receptor (and indeed it had). The characteristics of this superfamily include a conserved arrangment of pairs of cysteines in the amino terminal binding region, a W-S-X-W-S motif in the extracellular domain near the transmembrane region, and the lack of a protein kinase consensus motif in the cytoplasmic domain.

Expression was confined to the spleen, bone marrow, and fetal liver. Remarkably, this growth factor receptor oncogene generated immortalized cell lines of different lineages. Analysis of bone marrow cells infected with the helper-free retrovirus indicated that v-mpl promoted the proliferation and terminal differentiation of both committed and multipotential stem cells. The stage was set for a discovery.

The human and mouse cellular homologs of v-mpl were cloned and their gene structures elucidated (41-45). Using the cloned cDNA, it was determined that c-mpl is expressed only in primitive stem cells (CD34+), megakaryocytes and platelets. This was the key finding, because it suggested that c-mpl could potentially be the long-awaited thrombopoietin, as indeed, it turned out to be. Furthermore, anti-sense RNA to c-mpl inhibited the formation of CFU-MK (megakaryocyte colony forming units) (46). This suggested that c-mpl was indeed a growth factor receptor, possibly for the long-awaited thrombopoietin. This observation set the stage for the race to find the ligand for c-mpl, the putative thrombopoietin.

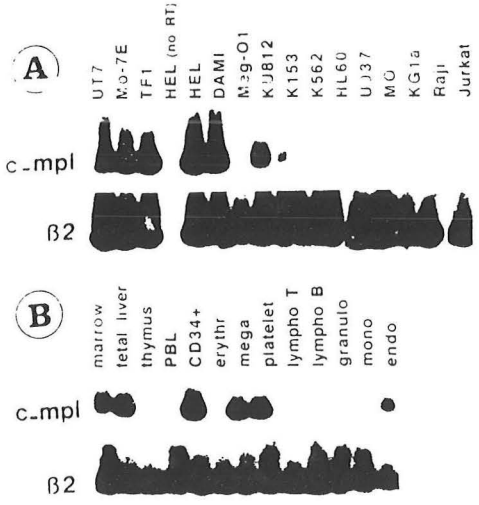


Fig. 8. Expression of c-mpl by human leukemic cell lines and normal purified hematopoietic cells. From (46).

Using three different stategies, five different groups nearly simultaneously obtained cDNA clones for the mpl ligand. Two groups relied on conventional chromatography; their cloning was independent of the c-mpl observation. Three groups used information about c-mpl to purify the c-mpl ligand.

Purification and Cloning of Thrombopoietin

Genentech: immobilized recombinant MPL receptor + irradiated pigs (47). (3 μg from 5 liters, 4 million fold purification)

Amgen: immobilized recombinant MPL receptor + irradiated dogs (48, 49)

Zymogenetics + Kaushansky, University of WA: functional expression cloning from a mutagenized cell line containing the mpl receptor and showing autocrine growth (50, 51).

Kuter, Beeler, and Rosenberg (MGH/Harvard): standard chromatography from thrombocytopenic sheep (52).

Kato, T. (Kirin): standard chromatography from thrombocytopenic rats (53).

Fig. 9. Strategies used to identify and clone the c-mpl ligand (thrombopoietin).

Experiments in mice quickly confirmed that the c-mpl ligand was indeed a major regulator of platelet numbers.

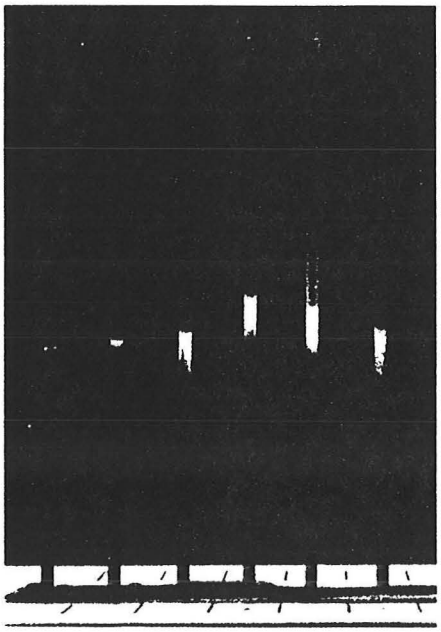


Fig. 10. Hematocrits of peripheral blood from control mice and mice treated with seven daily intraperitoneal injections of recombinant c-Mpl ligand. From (54).

Thrombopoietin: The cDNA and protein

The mpl-ligand (thrombopoietin) is encoded in a 1.7 kb cDNA that predicts a primary translation product of 353 amino acids. Cleavage of a signal peptide generates a mature polypeptide of 332 amino acids, to produce a mature precursor protein of 38 kDa. The first half of the molecule is 23% identical and 50% similar to erythropoietin. Six potential glycosylation sites are found in the carboxyl terminal half, and at least some of these are utilized. Dibasic cleavage sites in the C-terminal half indicate that these may be sites for further proteolytic processing to produce a mature form of thrombopoietin.

Thrombopoietin purified by affinity chromatography from thrombocytopenic dogs is a 23 kDa protein (49) and by conventional chromatography from the sheep is 31 kDa. The processing events that lead to the mature molecule that circulates in humans are not fully understood.

Biological activities of thrombopoietin

Thrombopoietin acts by binding to c-mpl as a homodimer, where it activates the receptor through non-receptor tyrosine kinases . It can activate JAK2 and TYK2 in certain cell lines, and JAK2 alone in other cell lines. The transcription factors STAT1 and STAT5A and 5B become activated by phosphorylation and transmit a proliferative signal to the nucleus. The PI-kinase signalling system may also be activated during thrombopoietin signalling (55). Studies of truncation mutants suggest that different cytoplasmic domains of c-mpl transmit separate proliferative and differentation-promoting signals (56).

Thrombopoietin increases megakaryocyte size, shifts them to higher ploidy classes, and causes them to express lineage specific markers, such as glycoprotein IIb/IIIa and glycoprotein I (reviewed in (38)). Under the microscope, thrombopoietin-treated megakaryocytes display a demarcation membrane system, form proplatelet processes, and fragment into normally-functioning platelets (57).

However, some expected properties of thrombopoietin suggest that it acts much earlier in hematopoiesis than originally expected. It cooperates with erythropoietin to expand the erythroid compartment, and with either IL-3 or stem cell factor to expand the granulocyte-monocyte line. In addition, it acts alone on the very primitive stem cells (CD38+, 34-) to suppress apoptosis of these cells, and thereby expands the stem cell pool (58). This role of thrombopoietin is further supported by experiments in genetically engineered mice, that lack either thrombopoietin (59) or its receptor (60). These animals are severely thrombocytopenic (6% of normal) and have decreases in the number of all progenitor cells (in colony assays) and a decrease in absolute neutrophil counts by 50% (60).

When thrombopoietin is administered to mice, monkeys, or humans, platelet levels begin to rise within 3-5 days and can reach up to 5-10 times normal values. Fig. 11 shows a dose-response curve of a recombinant PEGylated thrombopoietin in normal humans. In this study, the agent was given daily for ten days. The peak occurred six days after the drug was stopped, and counts of 1.5 million were reached at a dose of 1.0 μg/kg/day. The stage has been set for the use of thrombopoietin in the clinical arena.

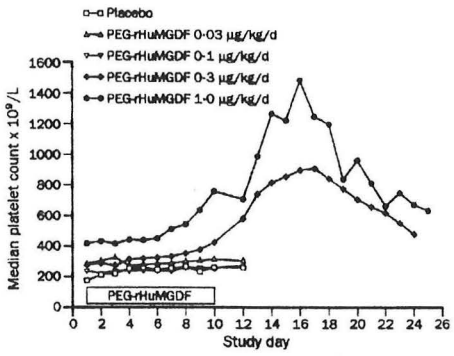


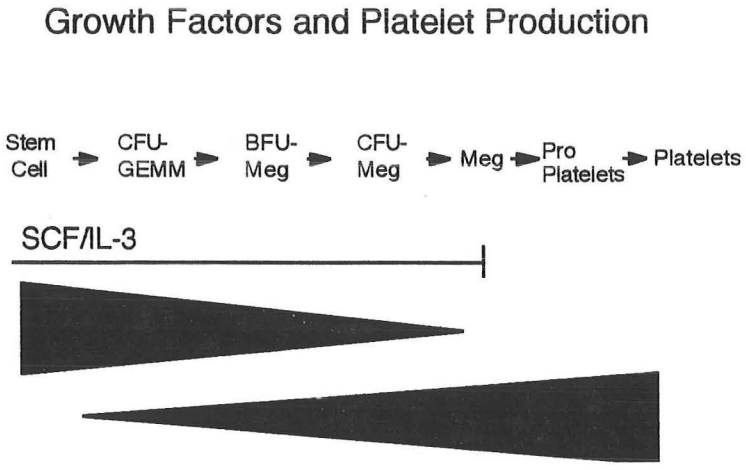
Fig. 11. Responses to thrombopoietin. From (71).

IL-11, An Early Regulator of Megakaryopoiesis

The second newly-described thrombopoietic factor to be covered in this Grand Rounds is IL-11. IL-11 (which has just been approved by the FDA for treatment of chemotherapy-related thrombocytopenia) was first isolated in 1990 as a hematopoietic cytokine with thrombopoietic activity. It is a 199-amino acid polypeptide (19 kDa) that is expressed in many cells types and is induced by IL-1. It has many complex and cell-type specific hematopoietic effects (reviewed in (61)). Specifically, it induces the proliferation of stem cells, multipotent and committed cells in synergy with other factors, and acts synergistically with IL-3, thrombopoietin, and stem cell factor to stimulate megakaryopoiesis. It also acts synergistically with erythropoietin to stimulate erythropoiesis, myelopoiesis and lymphopoiesis. These observations are consistent with a role for IL-11 as a regulator of early hematopoiesis . IL-11 has effects on non-hematopoietic tissues, such as bronchial and gastrointestinal epithelium. In animal studies, IL-11 not only improved the recovery of megakaryopoiesis and myelopoiesis in mouse models of bone marrow transplantation, but also seemed to have beneficial effects in protecting from mucositis and GI toxicity. It also has had beneficial effects in mouse models of acute colitis, toxic shock, and streptococcal sepsis (reviewed in (61)).

Synergy between thrombopoietin and IL-11

Both thrombopoietin and IL-11 promote megakaryocytic proliferation, and they both act on early progenitor cells. Thrombopoietin has additional actions that promote megakaryocytic maturation and platelet production. This has lead to a simple model in which both factors act simultaneously, but in which they play more important roles at different times during megakaryocyte maturation.



Thrombopoietin, IL-ll, and the Regulation of Platelet Counts

Sources of thrombpoietin and IL-11. To understand how these two factors may regulate platelet numbers, it may be important to know where they are produced. Thrombopoietin is made primarily in the liver (hepatocytes) and kidney (epithelial cells of the proximal convoluted tubule) (62). The kidney is unlikely to be a physiologically important source, because platelet numbers do decrease in nephrectomized individuals. However, since its receptor is highly expressed on platelets, it would seem to be of interest as to whether thromboietin deficiency could play some role in the functional platelet defect seen in uremia. Recent results in liver transplant patients indicate that thrombopoietin levels are subnormal (undetectable) in cirrhotics, and rise to normal levels after transplant. The recovery of platelet counts parallels the production of thrombopoietin (63). Thus, it seems that thrombopoietin deficiency may contribute to the thrombocytopenia of liver disease. Thrombopoietin was recently shown to be produced by bone marrow stromal cells (64), and there is a suggestion that mRNA expression for thrombopoietin may be increased in bone marrow stromal cells (but not in liver or kidney cells) in thrombocytopenic patients, both with aplasia and immune mechanisms. This observation in intriguiging but awaits confirmation (only one patient of each type was examined).

IL-11 is made in a large variety of tissues, including neurons, fibroblasts, epithelial cells, muscle cells, osteoblasts, endothelial cells, and spermatids. Most cell lines that respond to inflammatory mediators, such as IL-1 and TNFα, will increase the production of IL-11 in response to these mediators .

Thrombopoietin and IL-11: Evidence for different modes of regulation. Several clinical observations have helped define the roles of these two growth factors in thrombopoiesis. First of all, both factors rise dramatically in patients or animals that are rendered thrombocytopenic by cytotoxic agents (thus the use of aplastic dogs or sheep for the purification of thrombopoietin). However, human thrombopoietin levels are high when thrombocytopenia is due

to megakaryocyte deficiency, and low when due to platelet destruction (65). How can this phenomenon be explained? Platelets bind thrombopoietin with high affinity (100-400 pM) and internalize and degrade it (66). It has been suggested that thrombopoietin may be constitutively expressed and secreted, and that serum levels are regulated by the sequestration and degradation of thrombopoietin bound to platelets (67). However, since the platelet receptor is of the same high affinity as the target progenitor cell, it is difficult to understand how this could operate as an efficient regulatory phenomenon. An alternative explanation is that thrombopoeitin secretion is regulated by a paracrine mechanism operating between megakaryocyte progenitor cells and bone marrow stromal cells. Under circumstances where the progenitors cells are depleted (aplastic states), thrombopoietin levels would rise. However, under conditions where progenitors are increased (immune thrombocytopenia), levels would not rise. Clearly, more work will be needed to sort this out.

Fig. 12 may hold the key to understanding what happens in immune thrombocytopenia, and how platelet counts recover in this situation. The figure shows that IL-11 levels rise acutely and dramatically in the setting of ITP, while thrombopoietin levels remain remarkably constant. These results suggest that thrombopoietin plays a role in the maintenance of a basal level of platelet production at the level of the megakaryocyte precursor, and that IL-11 is responsible for the increased number of platelets in response to increased platelet destruction. Whether the increase in IL-11 levels is due to an autocrine feedback loop involving platelet mass, or involves an inflammatory cytokine generated during the immune-mediated process, is unknown. It also could be argued that thrombopoietin production goes up in the bone marrow stroma in response to immune thrombocytopenia, but that this is not reflected in serum levels because the increased numbers of progenitor cells "soak up" the available hormone or that it is otherwise unavailable. More work will be needed before the mechanisms that regulate platelet numbers are fully understood.

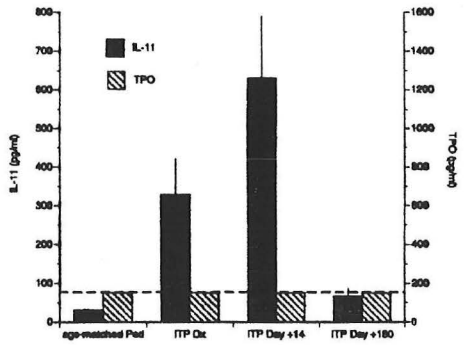


Fig. 12. Endogenous IL-11 levels from pediatric acute ITP patients at various times after diagnosis. From (79).

The Therapeutic Uses of Thrombopoietin and IL-11.

Thrombopoietin. To date, there have been three placebo-controlled trials that have begun to look at the clinical uses of thrombopoietin. All of these studies were Phase I-II trials, in which safety, and not efficacy, were the endpoints. Two studies were done in collaboration with Amgen and examined the dose response of a PEG-modified truncated human recombinant thrombopoietin (which they call megakaryocyte development and growth factor, or MGDF) in

patients with a variety of advanced cancers (68, 69). In the first study, which was originally published as a brief report (70), a dose-dependent and lineage-restricted increase in platelet counts was seen. The treatment was generally well tolerated, with the exception that two thrombotic events occurred in the treated group (one pulmonary embolus and one minor superficial thrombophlebitis). These occurred when the platelet counts were normal and the relationship to treatment is unclear. Recovery of platelet counts to baseline following chemotherapy was shortened from 18 to 22 days (68). No effects on platelet function in these patients were seen, as reported separately (71).

The second study using the Amgen preparation (69) was a dose-escalation study in 53 patients with lung cancer who were treated with carboplatin and paclitaxel. Again, platelet counts recovered much more quickly with MGDF, but the degree of thrombocytopenia was not clinically significant, so efficacy was not assessed. Again, two thrombotic events were seen in the treated group, and not the placebo group, but the groups were not of equal size, and the significance of the observation is unclear. Acute phase reactants were measured and found not to be elevated.

In the third study (72), a single dose of human thrombopoietin (Genentech) was administered to 12 patients with sarcoma (no chemotherapy given). At a dose of 2.4 μg/kg, platelet counts rose to a peak of up to 3.6 fold at 12 days. Platelet function was assessed to be normal and no adverse reactions were reported.

These studies demonstrate that thrombopoietin is probably safe for use in humans, although there was quite a bit of variation in individual response to dose. Efficacy in the setting of chemotherapy-induced thrombocytopenia was not addressed, as none of the patients developed thrombocytopenia that would require treatment.

A report has appeared in abstract form concerning the use of thrombopoietin in ovarian cancer patients receiving carboplatin, that reports a reduced incidence of platelet transfusions, but the details are not yet available (73).

Thrombopoietin (in the form of PEG-MGDF) has recently been studied in unpublished trials for the improvement of platelet pheresis yields in normal donors (74). At well tolerated doses that increased the platelet count to an average of 600,000, the average yield from a platelet pheresis donor could be increased about 2.5-3 fold. This translated to an increase in the increment in the recipient from 11 to 44,000. This is a big improvement in platelet yields, but continued success along these lines will require a demonstration of a high margin of safety, since this involves treating normal donors. The use of thrombopoietin in pretreatment and post-treatment of transplant recipients is under active investigation, but results are not yet available.

IL-11. Two randomized controlled trials of IL-11 (Neumega™, Genetics Institute) for the prevention of chemotherapy-induced thrombocytopenia have been published. The first involved 93 patients with a variety of cancers who had demonstrated a need for platelet transfusions in a previous cycle of chemotherapy (75). Patients were randomized to receive placebo or one of two levels of IL-ll once daily for 14 to 21 days beginning 1 day after chemotherapy. At the higher dose, 30% of patients avoided transfusion vs. 4% in the placebo group. Five patients in the treated group vs. one patient in the placebo group had transient atrial arrhythmias, and five patients in the treated group vs. one in the placebo group had syncope or near syncope (but it was not clear whether these were the same five patients!). These side effects

were attributed to volume expansion, as IL-11 has been shown to cause hemodilution by stimulating renal sodium reabsorption (76).

In the second study (77), performed by the same research group, 77 women with advanced breast cancer receiving dose-intensive chemotherapy were randomized to placebo or 50 μg/kg/day of IL-11. Fifty-nine percent of the control group vs. 32% of the treated group required a platelet transfusion, as shown below.

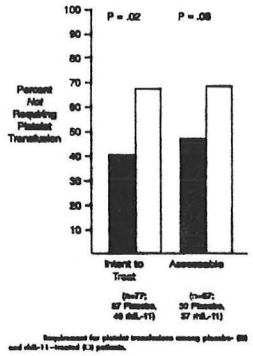


Fig. 13. Requirement for platelet transfusion among placebo (solid bars) and IL-11 treated groups (open bars). From (77).

There was fairly high incidence of edema (63% vs. 14%), pleural effusion (18% vs 0%) and dyspnea (48% vs. 19%) in the treated group.

These two studies have an important limitation. The level of platelet count at which to transfuse was chosen as 20,000/μl, which is a higher threshold than may be indicated. A landmark study in 1991 has demonstrated the safety of withholding platelet transfusion to a level below 10,000, as long as there is no fever or bleeding manifestations (78).

Therefore, it is unclear whether the IL-11 would be of benefit at this lower threshold. In addition, in contrast to the case with G-CSF (and perhaps similar to GM-CSF), there were significant side effects to therapy--the average patient dropped the hematocrit 20% due to volume retention, and there was a disturbingly high incidence of both dyspnea and pleural effusion. These side effects, coupled with the cost ($235 average wholesale price per day vs. $700 per platelet transfusion) may dampen enthusiasm for this drug in this setting.

Summary and conclusions

IL-11 and thrombopoietin cooperate in megakaryocyte development, maturation, and platelet production. Both act on early progenitor cells, especially in synergy with other hematopoietic growth factors. IL-11 acts primarily on early megakaryotic development, and thrombopoietin has strong effects on later megakaryocyte development, maturation and platelet production. The levels of both factors increase dramatically in response to marrow aplasia. IL-11 may play a special role in the elevation of the platelet count in response to immune platelet destruction. IL-11 has been approved for use in decreasing platelet transfusion requirements after chemotherapy, but chemotherapy-induced thrombocytopenia is a relatively rare occurrence (outside

the setting of myeloablative therapy), and results concerning its use in other settings are not yet available. Thrombopoietin and PEG-MDGF, a modified form of thrombopoietin, are still under active clinical development.

This is an exciting time in hematopoietic growth factor research. Clinicians and basic scientists will be kept very busy for the conceivable future, discovering new factors and new uses for growth factors as their biological activities are explored.

References

1. Glaspy, J., M. W. Davis, W. R. L. Parker, M. Foote, and I. McNiece. 1996. Biology and clinical potential of stem-cell factor. *Cancer Chemother. Pharmacol.* 38 (Suppl.):S53-S57.

2. Welte, K., J. Gabrilove, M. H. Bronchud, E. Platzer, and G. Morstyn. 1996. Filgrastim (r-metHuG-CSF): The first 10 years. *Blood.* 88:1907-1929.

3. Metcalf, D. 1997. Suppression or overexpression of genes encoding myeloid growth factors or their receptors. *Hematol Cell Ther.* 39 (2):98-101.

4. Cebon, J., J. E. Layton, D. Maher, and G. Morstyn. 1994. Endogenous haemopoietic growth factors in neutropenia and infection. *Brit. J. Haematol.* 86:265-274.

5. Demetri, G. D. and J. D. Griffin. 1991. Granulocyte colony-stimulating factor and its receptor. *Blood.* 78:2791-2808.

6. Avalos, B. R. 1996. Molecular analysis of the granulocyte colony-stimulating factor receptor. *Blood.* 88:761-777.

7. Rauprich, P., B. Kasper, N. Tidow, and K. Welte. 1995. The protein tyrosine kinase Jak2 is activated in neutrophils from patients with severe congenital neutropenia. *Blood.* 86:4500-4505.

8. Spiekermann, K., J. Roesler, A. Emmendoerffer, J. Elsner, and K. Welte. 1997. Functional features of neutrophils induced by G-CSF and GM-CSF treatment: differential effects and clinical implications. *Leukemia.* 11:466-478.

9. Geller, R. B. 1996. Use of cytokines in the treatment of acute myelocytic leukemia: A critical review. *J. Clin. Oncol.* 14:1371-1382.

10. Bronchud, M. H., J. H. Scarffe, and N. Thatcher, et al. 1987. Phase I/II study of recombinant human granulocyte colony-stimulating factor in patients receiving intensive chemotherapy for small cell lung cancer. *Brit. J. Cancer.* 56:809-813.

11. Maugard-Louboutin, C., C. Chastang, and B. Chevallier, et al. 1993. Dose-effect relationship of granulocyte colony stimulating factor (G-CSF): PE 2601 in patients with advanced breast carcinoma treated by intensive chemotherapy. *Proc. Am. Soc. Clin. Oncol.* 12:90 (abstr).

12. Eguchi, K., S. Sasaki, and T. Tamura. 1989. Dose escalation study of recombinant human granuloctye colony-stimulating factor (KRN8601) in patients with advanced malignancy. *Cancer Res.* 49:5221-5224.

13. Eguchi, K., T. Shinkai, and Y. Sasaki, et al. 1990. Subcutaneous administration of recombinant human granulocyte colony-stimulating factor (KRN8601) in intensive chemotherapy for patients with advanced lung cancer. *Jpn. J. Cancer Res.* 81:1168-1174.

14. Miller, L. L. 1993. Current status of G-CSF in support of chemotherapy and radiotherapy. *Oncology.* 10:67-88.

15. Jain, K. W. 1996. Sweet's syndrome associated with granulocyte colony-stimulating factor. *Cutis.* 57:107-110.

16. Litam, P. P., H. D. Friedman, and T. P. Loughran. 1993. Splenic extramedullary hematopoiesis in a patient receiving intermittently administered granulocyte colony-stimulating factor. *Ann. Int. Med.* 118:954-955.

17. Mair, D. C., M. E. Brecher, E. Hom, H. G. Owen, and T. C. Shea. 1996. False-positive hepatitis B surface antigen screening test results in patients receiving granulocyte-colony-stimulating factor. *Transfusion.* 36:948-951.

18. Bodey, G. P., M. Buckley, and Y. S. Sathe, et al. 1966. Quantitative relationships between circulating leukocytes and infection in patients with acute leukemia. *Ann. Int. Med.* 64:328-340.

19. Crawford, J., H. Ozer, R. Stoller, D. Johnson, G. Lyman, I. Tabbara, J. Grous, V. Picozzi, G. Rausch, R. Smith, W. Gandishar, A. Yahanda, M. Vincent, M. Stewart, and J. Glaspy. 1991. Reduction by granulocyte colony-stimulating factor of fever and neutropenia induced by chemotherapy in patients with small cell lung cancer. *N. Engl. J. Med.* 325:164-170.

20. Trillet-Lenoir, V., J. Green, C. Manegold, J. Von Pawel, U. Gatzemeier, B. Lebeau, A. Depierre, P. Hohnson, G. Decoster, D. Tomita, and C. Ewen. 1993. Recombinant granulocyte colony-stimulating factor reduces the infectious complications of cytotoxic chemotherapy. *Eur. J. Cancer.* 29A:319-324.

21. Pettengell, R., H. Gurney, and J. A. Radford, et al. 1992. Granulocyte colony-stimulating factor to prevent dose-limiting neutropenia in non-Hodgkin's lymphoma: a randomized controlled trial. *Blood.* 80:1430-1436.

22. Anonymous. 1994. American Society of Clinical Oncology recommendations for the use of hematopoietic colony-stimulating factors: Evidence-based, clinical practice guidelines. *J. Clin. Oncol.* 12:2471-2508.

23. Glaspy, J., G. Bleecker, J. Crawford, R. Stoller, and M. Strauss. 1993. The impact of therapy with filgrastim (recombinant granulocyte colony-stimulating factor) on the health care costs associated with cancer chemotherapy. *Eur. J. Cancer.* 29A Suppl. 7:S23-S30.

24. Lyman, G. H., C. G. Lyman, R. A. Sanderson, and L. Balducci. 1993. Decision analysis of hematopoietic growth factor use in patients receiving cancer chemotherapy. *J. Natl. Cancer Inst.* 85:488-492.

25. Kaplan, L. D., J. O. Kahn, and F. Crowe, et al. 1991. 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. *J. Clin. Oncol.* 9:929-940.

26. Maher, D. W., G. J. Lieschke, and M. Green, et al. 1994. Filgrastim in patients with chemotherapy-induced febrile neutropenia: a double-blind, placebo-controlled trial. *Ann. Int. Med.* 121:492-501.

27. Mayordomo, J. I., F. Rivera, M. T. Diaz-Puente, and e. al. 1995. Improving treatment of chemotherapy-induced neutropenic fever by administration of colony-stimulating factors. *J. Natl. Cancer Inst.* 87:803-808.

28. Riikonen, P., U. M. Saarinen, and A. e. a. Makipernaa. 1993. rhGM-CSF in the treatment of fever and neutropenia: A double-blind, placebo-controlled study in children with malignancy. *Proc. Am. Soc. Clin. Oncol.* 12:442.

29. Anaissie, E., S. Vartivarian, and G. P. e. a. Bodey. 1996. Randomized comparison between antibiotics alone and antibiotics plus granulocyte-macrophage colony stimulation factor (E. coli-derived) in cancer pateints with fever and neutropenia. *Amer. J. Med.* 100:17-23.

30. Biesma, B., E. G. de Vries, and P. H. e. a. Willemse. 1990. Efficacy and tolerability of recombinant human granulocyte-macrophage colony-stimulating factor in patients with chemotherapy-related leukopenia and fever. *Eur. J. Cancer.* 26:932-936.

31. Johnson, P. R. E. and J. A. L. Yin. 1997. The role of granulocyte- and granulocyte-macrophage colony-stimulating factors in the treatment of acute myeloid leukemia. *Brit. J. Haematol.* 97:1-8.

32. Ohno, R., M. Tomonaga, and T. Kobayashi, et al. 1990. Effect of granulocyte colony-stimulating factor after intensive induction therapy in relapsed or refractory acute leukemia. *N. Engl. J. Med.* 323:871-877.

33. Brown, R. A., D. Adkins, L. T. Goodnough, J. S. Haug, G. Todd, M. Wehde, D. Hendricks, C. Ehlenbeck, L. Laub, and J. DiPersio. 1997. Factors that influence the collection and engraftment of allogeneic peripheral blood stem cells in patients with hematologic malignancies. *J. Clin. Oncol.* 15:3067-3074.

34. Greenberg, P., K. Taylor, and R. e. a. Larson. 1994. Comparison of four categorized risk scoring systems for analyzing outcome in G-CSF-treated and observed patients with advanced myelodysplastic syndrome (MDS). *Blood.* 84:25a.

35. Hermans, P. 1995. Haematopoietic growth factors as supportive therapy in HIV-infected patients. *AIDS.* 9 (suppl 2):S9-S14.

36. Levin, J. 1995. An overview of thrombopoietin: with a historical perspective. *C R Acad Sci III.* 318 (5):609-18.

37. Kaushansky, K. 1997. Thrombopoietin: understanding and manipulating platelet production. *Annu. Rev. Med.* 48:1-11.

38. Hunt, P. 1995. The physiologic role and therapeutic potential of the Mpl-ligand in thrombopoiesis. *Stem Cells.* 13 (6):579-87.

39. Wendling, F., P. Varlet, M. Charon, and P. Tambourin. 1986. A retrovirus complex inducing an acute myeloproliferative leukemic disorder in adult mice. *Virology.* 149:242-246.

40. Souyri, M., I. Vigon, J.-F. Penciolelli, J. M. Heard, and P. Tambourin. 1990. A putative truncated cytokine receptor gene transduced by the myeloproliferative leukemia virus immortalizes hematopoietic progenitors. *Cell.* 63:1137-1147.

41. Alexander, W. S. and A. R. Dunn. 1995. Structure and transcription of the genomic locus encoding murine c-Mpl, a receptor for thrombopoietin. *Oncogene.* 10 (4):795-803.

42. Mignotte, V., I. Vigon, E. Boucher de Crevecoeur, P. H. Romeo, V. Lemarchandel, and S. Chretien. 1994. Structure and transcription of the human c-mpl gene (MPL). *Genomics.* 20 (1):5-12.

43. Skoda, R. C., D. C. Seldin, M. K. Chiang, C. L. Peichel, T. F. Vogt, and P. Leder. 1993. Murine c-mpl: a member of the hematopoietic growth factor receptor superfamily that transduces a proliferative signal. *Embo J.* 12 (7):2645-53.

44. Vigon, I., J. P. Mornon, L. Cocault, M. T. Mitjavila, P. Tambourin, S. Gisselbrecht, and M. Souyri. 1992. Molecular cloning and characterization of MPL, the human homolog of the v-mpl oncogene: identification of a member of the hematopoietic growth factor receptor superfamily. *Proc Natl Acad Sci U S A.* 89 (12):5640-4.

45. Vigon, I., C. Florindo, S. Fichelson, J. L. Guenet, M. G. Mattei, M. Souyri, D. Cosman, and S. Gisselbrecht. 1993. Characterization of the murine Mpl proto-oncogene, a member of the hematopoietic cytokine receptor family: molecular cloning, chromosomal location and evidence for a function in cell growth. *Oncogene.* 8 (10):2607-15.

46. Methia, N., F. Louache, W. Vainchenker, and F. Wendling. 1993. Oligodeoxynucleotides antisense to the proto-oncogene c-mpl specifically inhibit in vitro megakaryocytopoiesis. *Blood.* 82 (5):1395-401.

47. de Sauvage, F. J., P. E. Hass, S. D. Spencer, B. E. Malloy, A. L. Gurney, S. A. Spencer, W. C. Darbonne, W. J. Henzel, S. C. Wong, and W. J. Kuang, et al. 1994. Stimulation of megakaryocytopoiesis and thrombopoiesis by the c-Mpl ligand [see comments]. *Nature.* 369 (6481):533-8.

48. Bartley, T. D., J. Bogenberger, P. Hunt, Y. S. Li, H. S. Lu, F. Martin, M. S. Chang, B. Samal, J. L. Nichol, and S. Swift, et al. 1994. Identification and cloning of a megakaryocyte growth and development factor that is a ligand for the cytokine receptor Mpl. *Cell.* 77 (7):1117-24.

49. Hunt, P., Y. S. Li, J. L. Nichol, M. M. Hokom, J. M. Bogenberger, S. E. Swift, J. D. Skrine, A. C. Hornkohl, H. Lu, and C. Clogston, et al. 1995. Purification and biologic characterization of plasma-derived megakaryocyte growth and development factor. *Blood.* 86 (2):540-7.

50. Lok, S. and D. C. Foster. 1994. The structure, biology and potential therapeutic applications of recombinant thrombopoietin. *Stem Cells.* 12 (6):586-98.

51. Foster, D. C., C. A. Sprecher, F. J. Grant, J. M. Kramer, J. L. Kuijper, R. D. Holly, T. E. Whitmore, M. D. Heipel, L. A. Bell, and A. F. Ching, et al. 1994. Human thrombopoietin: gene structure, cDNA sequence, expression, and chromosomal localization. *Proc Natl Acad Sci U S A.* 91 (26):13023-7.

52. Kuter, D. J., D. L. Beeler, and R. D. Rosenberg. 1994. The purification of megapoietin: a physiological regulator of megakaryocyte growth and platelet production. *Proc. Natl. Acad. Sci. U.S.A.* 91:11104-11108.

53. Kato, T., K. Ogami, Y. Shimada, A. Iwamatsu, Y. Sohma, H. Akahori, K. Horie, A. Kokubo, Y. Kudo, and E. Maeda. 1995. Purification and characterization of thrombopoietin [published erratum appears in J Biochem (Tokyo) 1996 Jan;119(1):208]. *J Biochem (Tokyo).* 118 (1):229-36.

54. Lok, S., K. Kaushansky, R. D. Holly, J. L. Kuijper, C. E. Lofton-Day, P. J. Oort, F. J. Grant, M. D. Heipel, S. K. Burkhead, J. M. Kramer, and et al. 1994. Cloning and expression of murine thrombopoietin cDNA and stimulation of platelet production in vivo [see comments]. *Nature.* 369 (6481):565-8.

55. Sattler, M., R. Salgia, M. A. Durstin, K. V. Prasad, and J. D. Griffin. 1997. Thrombopoietin induces activation of the phosphatidylinositol-3' kinase pathway and formation of a complex containing p85PI3K and the proto-oncoprotein p120CBL. *J Cell Physiol.* 171 (1):28-33.

56. Drachman, J. G. and K. Kaushansky. 1997. Dissecting the thrombopoietin receptor: functional elements of the Mpl cytoplasmic domain. *Proc Natl Acad Sci U S A.* 94 (6):2350-5.

57. Cramer, E. M., F. Norol, J. Guichard, J. Breton-Gorius, W. Vainchenker, J. M. Masse, and N. Debili. 1997. Ultrastructure of platelet formation by human megakaryocytes cultured with the Mpl ligand. *Blood.* 89 (7):2336-46.

58. Borge, O. J., V. Ramsfjell, L. Cui, and S. E. Jacobsen. 1997. Ability of early acting cytokines to directly promote survival and suppress apoptosis of human primitive CD34+CD38- bone marrow cells with multilineage potential at the single-cell level: key role of thrombopoietin. *Blood.* 90 (6):2282-92.

59. de Sauvage, F. J., S.-M. Luoh, and K. Carver-Moore, et al. 1995. Deficiencies in early and late stages of megakaryocytopoiesis in TPO-KO mice. *Blood.* 86 (255a).

60. Alexander, W. S., A. W. Roberts, N. A. Nicola, R. Li, and D. Metcalf. 1996. Deficiencies in progenitor cells of multiple hematopoietic lineages and defective megakaryocytopoiesis in mice lacking the thrombopoietic receptor c-Mpl. *Blood.* 87 (6):2162-70.

61. Du, X. and D. A. Williams. 1997. Interleukin-11: Review of molecular, cell biology, and clinical use. *Blood.* 89:3897-3908.

62. Sungaran, R., B. Markovic, and B. H. Chong. 1997. Localization and regulation of thrombopoietin mRNa expression in human kidney, liver, bone marrow, and spleen using in situ hybridization. *Blood.* 89 (1):101-7.

63. Peck-Radosavljevic, M., J. Zacherl, Y. G. Meng, J. Pidlich, E. Lipinski, F. Langle, R. Steininger, F. Muhlbacher, and A. Gangl. 1997. Is inadequate thrombopoietin production a major cause of thrombocytopenia in cirrhosis of the liver? *J Hepatol.* 27 (1):127-31.

64. Guerriero, A., L. Worford, H. K. Holland, G.-R. Guo, K. Sheehan, and E. K. Waller. 1997. Thrombopoietin is synthesized by bone marrow stromal cells. *Blood.* 90:3444-3455.

65. Emmons, R. V., D. M. Reid, R. L. Cohen, G. Meng, N. S. Young, C. E. Dunbar, and N. R. Shulman. 1996. Human thrombopoietin levels are high when thrombocytopenia is due to megakaryocyte deficiency and low when due to increased platelet destruction. *Blood.* 87 (10):4068-71.

66. Fielder, P. J., A. L. Gurney, E. Stefanich, M. Marian, M. W. Moore, K. Carver-Moore, and F. J. de Sauvage. 1996. Regulation of thrombopoietin levels by c-mpl-mediated binding to platelets. *Blood.* 87 (6):2154-61.

67. Eaton, D. L. and F. J. de Sauvage. 1997. Thrombopoietin: the primary regulator of megakaryocytopoiesis and thrombopoiesis. *Exp Hematol.* 25 (1):1-7.

68. Basser, R. L., J. E. J. Rasko, K. Clarke, J. Cebon, M. D. Green, A. P. Grigg, J. Zalcberg, B. Cohen, J. O-Byrne, D. M. Menchaca, R. M. Fox, and C. G. Begley. 1997. Randomized, blinded, placebo-controlled phase I trial of pegylated recombinant human

megakaryocyte growth and development factor with filgrastim after dose-intensive chemotherapy inpateints with advanced cancer. *Blood.* 89:3118-3128.

69. Fanucchi, M., J. Glaspy, J. Crawford, J. Garst, R. Figlin, W. Sheridan, D. Menchaca, D. Tomita, H. Ozer, and L. Harker. 1997. Effects of polyethylene glycol-conjugated recombinant human megakaryocyte growth and development factor on platelet counts after chemotherapy for lung cancer [see comments]. *N Engl J Med.* 336 (6):404-9.

70. Basser, R. L., J. E. J. Rasko, and K. Clarke, et al. 1996. Thrombopoietic effects of pegylated recombinant human megakaryocyte growth and deveopment factor (PEG-rHuMGDF) in patients with advanced cancer. *Lancet.* 348:1279-81.

71. O'Malley, C. J., J. E. J. Rasko, and R. L. Basser, et al. 1996. Administration of pegylated recombinant human megakaryocyte growth and development factor to humans stimulates the production of functional platelets that show no evidence of *in vivo* activation. *Blood.* 88:3288-3298.

72. Vadhan-Raj, S., L. J. Murray, C. Bueso-Ramos, S. Patel, S. P. Reddy, W. K. Hoots, T. Johnston, N. E. Papadopolous, W. N. Hittelman, D. A. Johnston, T. A. Yang, V. E. Paton, R. L. Cohen, S. D. Hellmann, R. S. Benjamin, and H. E. Broxmeyer. 1997. Stimulation of megakaryocyte and platelet production by a single dose of recombinant human thrombopoietin in patients with cancer [see comments]. *Ann Intern Med.* 126 (9):673-81.

73. Fyfe, G., D. Jones, and M. Ashby et al. 1998. Experience with rhTPO in Genentech-supported trials. *ASH abstracts, no. 22.*

74. Kuter, D., J. McCullough, and J. Romo, et al. 1998. Treatment of platelet donors with pegylated recombinant human megakaryocyte growth and development factor (PEG-rHuMDGF) increases circulating platelet counts and platelet pheresis yields and increases platelet increments in recipients of platelet transfusions. *ASH abstract #2579.*

75. Tepler, I., L. Elias, and J. W. I. Smith, et al. 1996. A randomized placebo-controlled trial of recombinant human interleukin-11 in cancer patients with severe thrombocytopenia due to chemotherapy. *Blood.* 87:3607-3614.

76. Ault, K. A., J. Mitchell, and C. Knowles, et al. 1994. Recombinant human interleukin eleven (NEUMEGA, rhIL-ll growth factor) increases plasma volume and decreases urine sodium excretion in normal human subjects. *Blood.* 84:276a.

77. Isaacs, C., N. J. Robert, and A. Bailey et al. 1997. Randomized placebo-controlled study of recombinant human interleukin-11 to prevent chemotherapy-induced thrombocytopenia in patients with breast cancer recieving dose-intensive cyclophosphamide and doxorubicin. *J. Clin. Oncol.* 15:3368-3377.

78. Gmur, J., J. Burger, U. Schanz, J. Fehr, and A. Schaffner. 1991. Safety of stringent prophylactic platelet transfusion policy for patients with acute leukaemia. *Lancet.* 338:1223-1226.

79. Chang, M., Y. Suen, G. Meng, J. S. Buzby, J. Bussel, V. Shen, C. Van de Ven, and M. S. Cairo. 1996. Differential mechanism in the regulation of endogenous levels of thrombopoietin and interleukin-11 during thrombocytopenia: Insight into the regulation of platelet production. *Blood* 88:3354-3362.