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Review paper

Can hematopoietic growth factors be used to improve the success of cytotoxic chemotherapy?

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Hematopoietic growth factors (HGFs) have provided oncologists with powerful tools to investigate questions of chemotherapy dose and treatment outcome in cancer patients. Agents such as recombinant granulocyte colony-stimulating factor (e.g. G-CSF; filgrastim) significantly accelerate neutrophil recovery after chemotherapy and therefore allow the delivery of a planned dose on time. Moreover, it is possible to investigate the effects of escalated dose chemotherapy with HGF support. This can be done using the HGF alone or in conjunction with stem cell rescue. HGFs significantly reduce morbidity following bone marrow transplantation (BMT) and may also be used to mobilize peripheral blood progenitor cells (PBPC) to support high-dose chemotherapy. Growth factor-mobilized PBPC have practical and clinical advantages over BMT and may be a more effective method of allowing the delivery of high-dose therapy, but for some patients (who for reasons not yet clear, display a poor mobilization response) a combination of autologous bone marrow and PBPC might be more effective at reconstituting hematopoiesis. Whether more intensive treatment approaches will significantly improve survival remains to be determined.

Key words: G-CSF, GM-CSF, hematopoietic growth factors, HGF, high-dose chemotherapy.

Introduction

Significant advances in the efficacy of combination chemotherapy regimens have been limited during the last two decades by two inter-related problems, namely multi-drug resistance and drug toxicity. Drug resistance may be present at the time of diagnosis, or it may develop following exposure to chemotherapy.[1] Circumvention of drug resistance with inhibitors of the recently identified multi-drug resistance protein, p-glycoprotein, is theoretically attractive and undergoing early clinical testing.[2] However, a more common school of thought is to escalate the dose of chemotherapy in an attempt to erradicate all chemosensitive cells and, perhaps, overcome the resistance of malignant cells to conventional doses. Since many conventional chemotherapy regimens are given at, or close to, the maximum tolerated dose, the scope for dose escalation is severely limited by increased toxicity. The most common obstacle to more effective chemotherapy delivery is severe myelosuppression, which increases the patient's susceptibility to severe infection and compromises the delivery of planned chemotherapy.[3] Attempts to escalate dose intensity, therefore, require effective hematopoietic support to ensure prompt restoration of normal hematopoiesis. Previously, this has only been possible with bone marrow transplantation (BMT), a risky and morbid procedure, restricted to specialist units. The introduction of recombinant HGFs has provided oncologists with powerful tools to manipulate hematopoiesis and, thus, the ability to enhance the delivery of chemotherapy at standard or escalated doses.

The recombinant human HGFs, in particular granulocyte colony stimulating factor (G-CSF) and granulocyte-macrophage colony stimulating factor (GM-CSF), significantly accelerate neutrophil count recovery following chemotherapy and, although neither agent abolishes the neutrophil nadir completely, both significantly reduce the period of neutropenia following myelosuppressive chemotherapy.[4,5] A number of other HGFs, including macrophage colony-stimulating factor (M-CSF), interleukin-3 (IL-3), interleukin-1 (IL-1), and stem cell factor (SCF), are also in the early stages of clinical development, and may prove to be useful additions to the armament of agents that can be used to stimulate myelopoiesis.[6–9]

The clinical efficacy of filgrastim (recombinant methionyl human-G-CSF) has been shown in the

setting of standard-dose chemotherapy[4,10] and following high-dose chemotherapy with BMT.[11] Two randomized, double-blind, placebo-controlled trials in small-cell lung cancer patients have shown that filgrastim significantly reduces neutropenia-associated complications by about 50%.[4,10] In the one study where it was evaluated, the use of filgrastim resulted in better on-time delivery of full-dose chemotherapy (cyclophosphamide, doxorubicin, etoposide) compared with placebo.[10] Recombinant GM-CSF has also been shown to reduce neutropenia following standard chemotherapy[5] and high-dose therapy with BMT.[12]

HGFs have also allowed the development of novel therapeutic strategies such as dose escalation without marrow support and high-dose therapy with peripheral blood progenitor cell (PBPC) rescue. The use of filgrastim, or another HGF, to mobilize PBPC in order to support the delivery of high-dose therapy is perhaps the most promising strategy currently under investigation.[13] Autologous PBPC rescue may have important practical and clinical advantages over traditional hematopoietic rescue with autologous BMT and may be considered for use in conjunction with, or as an alternative to, conventional marrow transplantation.

This article reviews the evidence supporting the current enthusiasm for using HGFs to investigate questions about the dose–response dogma, and examines the differential pharmacology of the available HGFs in order to show that for optimum therapeutic use, they should not be regarded as similar agents; instead, their use should be tailored according to characteristic pharmacological effects.

Rationale for investigating dose–response

The evidence for a dose–response relationship was first demonstrated by Skipper *et al.* in animal experiments using tumor models such as L1210 leukemia.[14]

The dose–response relationship, clearly shown in animal studies, has been more difficult to confirm in the clinical setting. Nevertheless, numerous retrospective analyses and a limited number of prospective studies have indicated the importance of dose in several chemotherapy-sensitive malignancies, including non-Hodgkin's lymphoma,[15] Hodgkin's disease,[16,17] small-cell lung cancer,[18] breast cancer,[19] ovarian cancer[20] and testicular cancer.[21] While retrospective studies such as those conducted by Hryniuk[22] may lead to erroneous conclusions,[19] there is evidence from prospective studies confirming the importance of dose. Budman *et al.* recently showed the importance of dose of standard adjuvant chemotherapy in 1572 patients with stage II, node-positive breast cancer.[23] In the study, conducted by the Cancer and Leukemia Group B, patients receiving a low-dose regimen of cyclophosphamide, doxorubicin and 5-fluorouracil (300/30/300 mg/m^2 for 4 months) had a statistically significant reduction in disease-free and overall survival compared with those receiving either a moderate dose (400/40/400 mg/m^2 for 6 months) or a high dose (600/60/600 mg/m^2 for 4 months). Although still only preliminary, these results imply sub-optimal dosing may adversely affect the outcome of chemotherapy.

Sub-optimal dose intensity may result from dose reduction or delay due to chemotherapy-related toxicity. In chemotherapy-responsive malignancies, for example lymphomas, it is important to preserve dose intensity and HGFs may play a role here by limiting dose reductions or cycle delays as a result of myelosuppression.[24] On the other hand, they may have a more limited role in patients receiving chemotherapy palliatively, unless there is clear evidence that there is dose-related palliation.[25] The ability of HGFs to preserve chemotherapy delivery is comparatively straightforward to demonstrate in clinical trials, but it will be more difficult to establish whether the modest improvements in dose intensity achieved will translate into clinically significant increases in response rate or overall survival.

It has been postulated that current chemotherapy doses fall on the linear phase of the dose–response curve, and therefore, it would be reasonable to expect that further increases in dose intensity may produce meaningful gains in response.[26] It seems logical therefore, to conduct controlled trials to investigate whether high dose-intensive regimens will produce improvements in overall survival for appropriately selected chemo-sensitive malignancies.

However, it is difficult to be confident that increasing chemotherapy dose intensity may produce better results. The difficulties in drawing conclusions from available data are compounded by a lack of uniformity in quantifying chemotherapy dose intensity.[25] The way dose is expressed has important implications for assessing the quality and quantity of the response to anticancer treatment. There are several measurements used to express the amount of drug delivered:

(i) Dose intensity: amount of drug delivered per unit time, expressed as, for example, mg/m^2/week, regardless of the schedule or route of administration.
(ii) Relative dose intensity: amount of drug delivered per unit time relative to an arbitrarily chosen standard single drug, or for a combination regimen, the decimal fraction of the ratio of the test regimen to the standard regimen.
(iii) Total dose: the amount of drug given per dose (mg/m^2) multiplied by the total number of doses.
(iv) Cumulative dose: this method graphically represents both dose intensity and total drug delivery and is useful when analyzing trials designed to isolate treatment variables and improve the therapeutic index of available drugs.[27]

It is clearly desirable for future studies investigating dose–response to state clearly how 'dose intensity' is calculated and for standards to be adopted.[28] Since it has become possible to reduce the severe hematological toxicities associated with high-dose chemotherapy by the combined use of recombinant HGFs and bone marrow (with or without circulating progenitor cells), several groups are investigating high-dose chemotherapy as *consolidation* of a complete response in several chemo-sensitive malignancies (including Hodgkin's disease and non-Hodgkin's lymphomas). So far, the best evidence in favor of this approach (which can be defended on theoretical grounds if there is true minimal residual disease which is still chemosensitive) comes from the experience in the acute myeloid leukemia (AML) model. Cycles of standard intensive combination chemotherapy regimens for one to two years generally produce between 20 and 30% survival at three years.[29] However, allogeneic BMT in adult patients with AML in first remission has yielded approximately 40% disease-free long-term survival, or even 60–70% in patients less than 20 years of age.[30] This important finding recently earned ED Thomas the Nobel Prize for Medicine.

It has been argued that to achieve substantial therapeutic gains, large increases in dose intensity may be needed.[31] A mathematical model has been described for rationally selecting cytotoxic drugs and dosages for combination regimens, based on the antitumor activities of the drugs, given as single agents, and their organ-specific maximum tolerated doses. The model does not assume that the underlying dose–response curve is steep nor that maximally dose-intense regimens are clinically appropriate in all situations. On the basis that many of the successes of combination chemotherapy can be attributed to the achievement of a higher equivalent cytotoxic dose by the combination of drugs with primarily non-overlapping toxic effects, the model allows the selection of optimal combinations of drugs based on knowledge of their relative potencies and maximally tolerated doses. The information required can be deduced from phase II and III studies of the drugs in question. It is then possible to construct a graph like the one shown in Figure 1a, which shows the organ-system toxicities for drugs given at doses producing a given response rate. If a particular toxicity is unique to a drug the line will be at 90° to the axis for that drug, whereas if the drugs share the toxicity the line will slope between the two axes. The shaded area on the graph represents tolerable combinations and the upper boundary of the shaded area shows possible maximally tolerated combinations. The optimal combination occurs at the intercept of the boundary lines. This technique is also applicable to combinations of more than two drugs using linear programming.[31] In the example shown in Figure 1a, the optimal combination of cisplatin and cyclophosphamide results in a total equivalent dose intensity of 2.0, representing an unpromising increase of only 16% over full-dose cisplatin alone. However, if a HGF is used to eliminate the bone marrow constraint for cyclophosphamide (Figure 1b), both drugs can be given in full dose, producing a total equivalent dose of 2.9, representing a 65% improvement. Such a model may be worthy of clinical evaluation. However, use of a HGF would have no effect on drug combinations limited by non-hematological toxicities.

In general, there is increasing opinion that the optimal use of two or three cytotoxic drugs can lead to better results than the sub-optimal use of more drugs. One of the recent encouraging results comes from the European Osteosarcoma Intergroup (EOI)[32] which showed that a brief intensive chemotherapy regimen of only two drugs (doxorubicin and cisplatin) used as adjuvant chemotherapy in operable osteosarcoma of the limbs can lead to very good long-term results (57% disease-free survival at 5 years), certainly comparable to those achieved in cooperative group studies of longer, more complex and more toxic multi-agent chemotherapies, although a formal prospective comparison has not yet been reported.

Clearly there is sufficient reason to continue to explore the use of HGFs to preserve chemotherapy

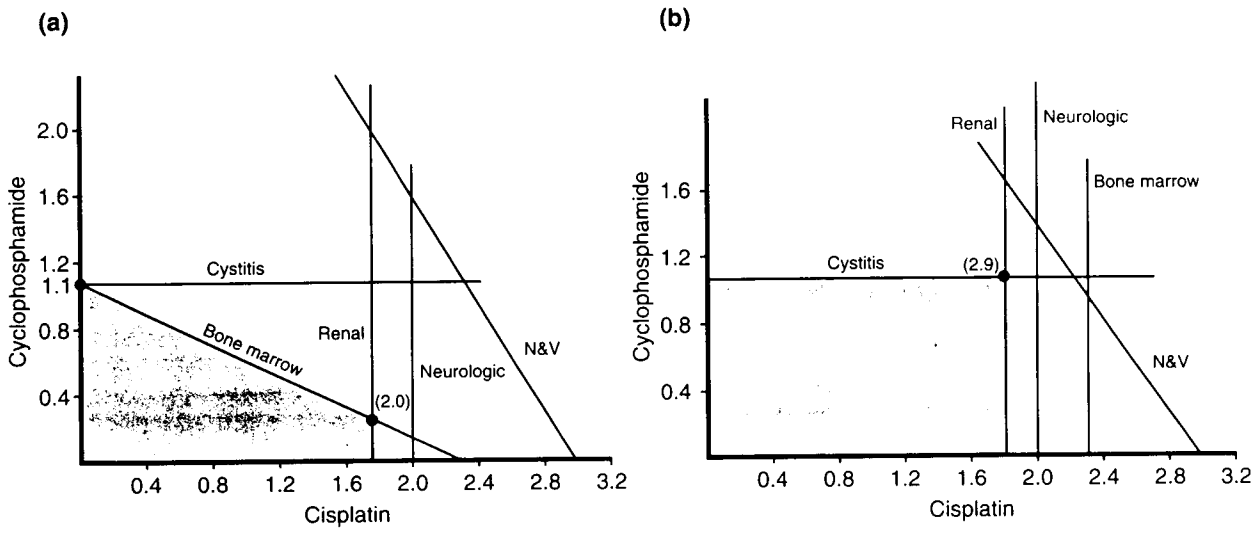

Figure 1. a Calculation of optimum combination of cisplatin/cyclophosphamide based on organ-system toxicities; **b** Optimum combination as in **a**, redrawn to show effect of eliminating the bone marrow constraint for cyclophosphamide. Modified from Simon & Korn (1990).[31] (N&V = nausea and vomiting).

dose intensity or to allow significant dose escalation. Clinical studies should be prospective and randomized to allow definitive conclusions to be drawn. Furthermore, the appropriate hematopoietic rescue strategy needs to be determined. This involves not only the choice of HGF, but also whether to use it alone or in combination with stem cell rescue. Moreover, the source of stem cells needs to be addressed, i.e. whether to use BMT alone, BMT plus PBPC or PBPC alone. To help answer some of these questions it is necessary to understand the differential pharmacology of HGFs.

Differences in pharmacology

HGFs are differentiated by their characteristic effects on the cells of the hematopoietic system. Some factors are lineage restricted, acting exclusively on a particular cell lineage. For example, the actions of G-CSF are restricted almost exclusively to the neutrophil lineage, while M-CSF acts upon macrophage precursor cells and erythropoietin upon erythroid precursors.[33] GM-CSF has broader activity, stimulating progenitors that give rise to neutrophils, monocytes and eosinophils.[33] There are also HGFs such as IL-3 that act on the early multipotent progenitor cells, giving rise to cells of both erythroid and myeloid lineages.[33] SCF stimulates pluripotential cells, making them more responsive to the effects of more lineage-restricted growth factors.[34] For example, SCF alone has no colony-stimulating activity when incubated with marrow cells, but if G-CSF is added, there is an increase in neutrophil colony formation that is greater than if G-CSF is used alone.[34]

Another pluripotent factor under clinical investigation is IL-1.[8] The administration of IL-1α to patients is associated with fever, chills, headache, nausea, vomiting and myalgias. At doses of only 0.3 μg/kg or higher, dose-limiting toxicities were frequent, including severe hypotension, myocardial infarction, confusion, severe abdominal pain, and renal insufficiency. On the other hand, an *i.v.* bolus infusion of recombinant human M-CSF at 30 μg/m^2/day or higher was associated with a peculiar syndrome of ocular or periorbital inflammation, with iridocyclitis as the most severe manifestation.[35]

Since each HGF has a specific biological property, it is important to determine the clinical goal before selecting the appropriate factor to provide the desired response. In this respect, 'early-acting' should not be confused with 'fast acting'. HGFs acting upon early progenitors produce a delayed rise in mature peripheral blood cells when compared with more lineage-restricted, or 'late-acting' factors (Figure 2). Following damage to the hematopoietic system, recovery takes place initially from the more lineage-restricted progenitor cells which are primed to undergo rapid proliferation and development. Thus, if a fast action on neutrophils is required, a recombinant G-CSF such as filgrastim should be used. Whereas if a broad spectrum of cells is required an earlier-acting

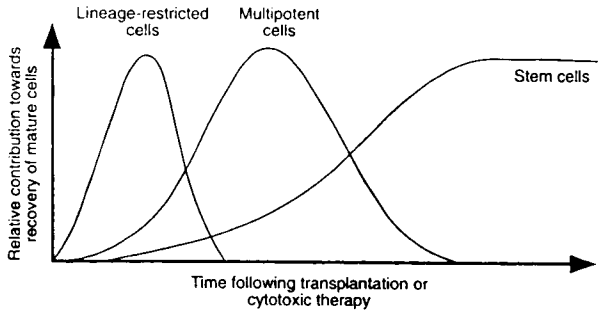

Figure 2. Relative contribution of progenitor cells towards the recovery of mature blood cells following myelosuppression.

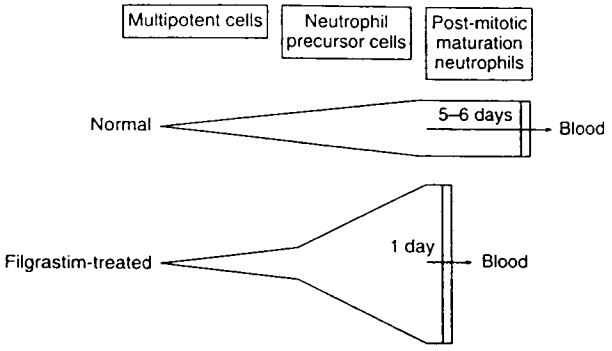

Figure 3. Schematic representation of the effects of filgrastim on hematopoiesis.

factor, or combination of factors may be more appropriate.

Recombinant G-CSF (e.g. filgrastim) and GM-CSF have been studied most extensively to date, but no direct comparative studies have been conducted to determine which HGF provides optimal hematopoietic recovery under different circumstances. However, experimental and clinical evidence shows clear difference between the two agents.[36,37] Kinetic studies by Lord *et al.* have shown that G-CSF (filgrastim) produces a greater and more rapid increase in neutrophil count than GM-CSF (Table 1).[38] As shown in Figure 3, filgrastim produces an increase in peripheral blood neutrophil count by increasing the number of neutrophils being produced and by reducing the maturation time from 5 days to 1 day. These kinetic observations suggest that if dose intensity is to be increased by decreasing the interval between chemotherapy cycles it would appear to be more logical to use filgrastim, rather than recombinant GM-CSF, because of its more rapid effect.[37]

Most clinical experience has been obtained with filgrastim (a non-glycosylated recombinant G-CSF), but a glycosylated form is also in development.[39] Available data indicate that glycosylation of G-CSF is not important for biological activity and consequently it is unlikely that there will be clinically important differences between non-glycosylated and glycosylated forms.[40] Similarly, glycosylation does not appear to be important for the biological efficacy of GM-CSF.[41]

Table 1. Comparison of G-CSF- and GM-CSF-stimulated neutrophils[38]

Neutrophil production	Normal volunteers	GM-CSF	G-CSF
Maximum count ($\times 10^{-6}$/ml)	5.2	17.0	35.0
Appearance in peripheral blood (days)	4–7	4.5–6.5	1–2
Peripheral blood half-life, $t_{1/2}$ (h)	83	48	7.6
Amplification enhancement factor	1	1.5	9.4
Extra amplification divisions	0	0.6	3.2

Neither G-CSF nor GM-CSF stimulate platelet recovery following damage to the hematopoietic system, so alternative strategies are required to support the delivery of chemotherapy regimens where thrombocytopenia becomes dose limiting. Recombinant IL-3 has been studied for its activity on megakaryocytes, but in early clinical studies it has demonstrated only weak effects on platelet count.[42] Investigators are also looking at the combination of two or more growth factors, which may produce a synergistic response, and hence promote platelet as well as neutrophil recovery. For example, the effect of GM-CSF + IL-3 on neutrophil and platelet recovery has been investigated.[43] Results showed that while neutrophil recovery rate was accelerated in the patients receiving GM-CSF + IL-3, compared with patients not receiving cytokines, the rate of recovery was not significantly faster than that observed in patients receiving only GM-CSF. Furthermore, platelet recovery was not different between the three groups.

It is also important to be aware that combinations of HGFs may produce unexpected effects and their clinical use must therefore be regarded as strictly experimental. A recent report showed that a combination of GM-CSF and IL-3 delays platelet recovery after an autologous BMT (ABMT).[44] Such observations may be relevant to clinical trials of the IL-3 GM-CSF fusion molecule known as PIXY-321. Moreover, the sequential use of GM-CSF followed by IL-3 could be counterproductive on theoretical grounds because the increased differ-

entiation of the precursor pool induced by GM-CSF might reduce the pool of IL-3 sensitive cells.

Thus, even if the administration of combinations of HGFs is a logical approach on theoretical grounds, at present no combination has been described which is clinically superior to G-CSF (or GM-CSF) alone in the treatment of prophylaxis of chemotherapy-associated myelosuppression. A combination of a myeloid growth factor and a true megakaryocyte colony-stimulating factor would undoubtedly have clinical relevance, but has not yet been unequivocally proven. The most promising and consistent technique available to accelerate platelet recovery is the use of PBPC mobilized by a HGF. G-CSF, GM-CSF and IL-3 have all been used to generate autologous PBPC for use in transplantation. Sheridan *et al.* gave 17 patients with non-myeloid malignancies G-CSF (filgrastim) at 12 μg/kg/day for 6 days and collected progenitor cells on days 5, 6 and 7 by leukapheresis.[45] Granulocyte-macrophage progenitors increased 58-fold and erythroid progenitors increased 24-fold.

Another strategy to generate PBPC is to give the HGF after treatment with cytotoxic agents, such as cyclophosphamide, to enhance the chemotherapy-induced increase in peripheral blood progenitors.[46] This approach has been investigated in pilot studies using G-CSF, GM-CSF and IL-3.[47,48] However, at present there are no direct comparative data to indicate which is the most effective HGF in terms of numbers of PBPC collected or rate of hematological recovery following transplantation. One study demonstrated that both G-CSF (filgrastim) and GM-CSF increase the production of granulocyte-macrophage colony-forming units (GM-CFU), but no direct comparison was made in this investigation because different CD34+ assessment techniques were used.[48]

The choice of HGF for different clinical settings is also influenced by the tolerability profile of the agents. It appears that the broader acting factors such as GM-CSF are associated with a wider range of side effects than lineage-restricted factors such as G-CSF.[49] This may be due to the induction of secondary cytokine effects following stimulation of monocytes.[50] In general however, the HGFs are well tolerated, particularly when compared with toxic effects of escalated doses of chemotherapy.

Dose intensification without marrow support

The ability of the HGFs to reduce hematological toxicity offers the opportunity to improve the delivery of cancer chemotherapy, both on time and at full dose. In turn, attention has focused on the possibility of improving outcome by further escalating the dose of chemotherapy using HGF support. There have been numerous pilot studies conducted with HGFs and dose-intensive therapy (Table 2),[51-67] and although investigators have expressed optimism about the preliminary results achieved, no definitive conclusions have yet been reached. The majority of patients included in these studies had chemo-responsive advanced malignancies, including lymphoma, breast cancer, ovarian cancer, soft tissue sarcomas and testicular cancer. Although it was possible to show high response rates, no significant impact on overall survival has yet been reported.[52] This approach may be used as a convenient form of cytoreduction and to demonstrate chemosensitivity prior to consolidation with high-dose chemotherapy and bone marrow or PBPC rescue.

Another very important therapeutic variable (which largely determines therapeutic efficacy) besides dose intensity, is total cumulative dose for any particular active drug. For example, 490 patients with advanced ovarian cancer were

Table 2. Studies of hematopoietic growth factors to support delivery of dose-intensive chemotherapy

Reference	Malignancy (no. of patients)	Daily dose of G-CSF or GM-CSF (μg/kg/day)*
G-CSF		
[51]	Various advanced (10)	20, 40, or 60
[52]	Breast and ovarian (21)	10
[53]	Breast cancer (24)	10
[54]	Breast cancer (16)	4
[55]	Urothelial cancer (19)	5
[56]	Urothelial cancer (35)	(375 μg)
[57]	SCLC (64)	(50 μg/m^2)
[58]	Soft tissue sacroma (8)	5 or 10
[59]	Breast cancer (45)	2
GM-CSF		
[60]	Various advanced (23)	(500–1000 μg/m^2)
[61]	Urothelial cancer (32)	(250 μg/m^2)
[62]	SCLC (10)	5
[63]	Testicular cancer (37)	10
[64]	Ovarian cancer (22)	10
[65]	Soft tissue sarcoma (52)	(250 μg/m^2)
[66]	Breast cancer (18)	(250 μg/m^2)
[67]	Acute lymphocytic leukemia (34)	(125 μg/m^2)

* Unless shown in parentheses.
SCLC = small-cell lung cancer.

randomized to receive either a low-dose intensity cyclophosphamide and cisplatin combination or a moderately high-dose intensity combination of these two drugs.[68] No significant difference in overall treatment outcome was noted, but the total dose of cisplatin (400 mg/m^2) was the same in both arms of the study. However, Kaye *et al.*[69] have recently shown in a similar population of patients that a high-dose intensity and a higher total dose of cisplatin (500 mg *vs* 300 mg received total dose) were associated with a highly significant survival advantage. Thus here the total dose of cisplatin seems to be more important than the dose intensity alone. Further increases in total dose of cisplatin are not feasible unless new methods are devised to limit neurotoxicity, but carboplatin total doses are not under a similar non-hematological toxic constraint.

The general conclusions that can be drawn from these preliminary studies are that HGFs improve the delivery of high-dose chemotherapy regimens and will allow an increase in dose intensity for single agents of up to fourfold, before other toxicities intervene. However, it is still too early to determine if a significant improvement in overall survival can be obtained from the increase in dose intensity achieved using HGFs. Although it is clear that HGFs facilitate the delivery of high-dose therapy at levels previously only feasible in specialized transplant centers, further prospective, randomized trials need to be conducted to determine if these new strategies may be integrated into standard clinical practice.

High-dose therapy with BMT

Evidence from the BMT setting suggests it is sometimes possible to overcome apparent drug resistance by increasing dose intensity. BMT offers the possibility of delivering a three- to eightfold higher dose intensity per treatment course. In patients with very advanced lymphoma, increases in dose, particularly in alkylating agents, have produced a significant number of patients who are free of disease and may in fact be cured.[70] The 3-year freedom from progression (FFP) probability of survival is usually less than 10% in patients with advanced high-grade NHL (non-Hodgkin's lymphoma) who fail to respond completely to initial therapy or who relapse following CR (complete remission). However, high-dose therapy and BMT can lead to 35–50% 3-year FFP survival in non-controlled small studies with high-dose chemotherapy and ABMT in sensitive relapse,[71] with a significantly superior survival also reported from large non-randomized studies.[72] However, few randomized studies have been conducted to confirm the apparent benefits of BMT, and the real benefits of high-dose chemotherapy are almost exclusively restricted to chemosensitive relapse.

Clinical studies have shown that filgrastim and recombinant GM-CSF successfully accelerate neutrophil recovery following myeloablative therapy and BMT.[11,12,73] A decrease in the period of neutropenia is associated with a reduction in the use of parenteral antibiotics and the days of hospitalization. GM-CSF is also reportedly associated with improved survival in patients with poor graft function after transplantation.[74] No comparative studies have been conducted to determine whether G-CSF or GM-CSF is the more effective agent for use following high-dose therapy and BMT. Based on present data there is no evidence for a 'monocyte advantage' with GM-CSF, or a 'speed advantage' with G-CSF.

The use of HGFs has clearly reduced overall morbidity in the BMT setting, but whether these agents will also reduce overall mortality remains to be seen. However, despite HGF use, BMT will remain a difficult procedure restricted to specialized centers.

High-dose therapy with PBPC rescue

A PBPC infusion represents an alternative source of hematopoietic progenitor cells and may be used in conjunction with, or instead of, BMT to support the delivery of intensive chemotherapy.[75] The advantages of PBPC over BMT can be summarized as: lower procedure-related morbidity; faster recovery of neutrophils and platelets; a postulated lower burden of malignancy and an increase in the proportion of immunocompetent cells in the graft. The comparative disadvantages of PBPC include: variability in response between patients, a laborious procedure and that the long-term permanency of engraftment has not been fully evaluated. It is not yet clear why some patients exhibit a poor response to mobilization procedures (or even fail to respond) although previous extensive chemotherapy or radiotherapy are probably contributing factors. Finally, it has not yet been unequivocally shown that previous high-dose chemotherapy with PBPC rescue does not compromise future bone marrow recovery following further chemotherapy (for relapse or disease progression) more than usually anticipated.

Before the use of HGFs, stimulation of the progenitor cell pool was only feasible by administration of a chemotherapy agent.[75] Stimulation of PBPC by a marrow-toxic drug has a number of disadvantages. Firstly, although such stimulation may result in a 100-fold increase in the number of GM-CFUs, the kinetics of this expansion are extremely variable from one procedure to the next. Secondly, the doses of chemotherapy required for an acceptable overshoot of GM-CFU cells may result in toxicity such as neutropenic fever and sepsis. Finally, patients who have received a large amount of prior chemotherapy may not respond to this technique. HGFs produce a more consistent and prolonged increase in PBPC and do not produce the severe side effects that may be associated with chemotherapy.[75] Some investigators have nevertheless used cytotoxic agents plus HGFs to maximize the yield of PBPC whilst delivering standard-dose chemotherapy early in the treatment course prior to the initiation of dose-intensive treatment.[48]

There has been a plethora of pilot studies using HGF-mobilized PBPC to support the delivery of high-dose therapy.[45,46,76–80] Although these studies have not yet identified the optimal dose and schedules for the different growth factors, they have clearly shown the clinical efficacy of PBPC in restoring hematopoiesis. Sheridan *et al.* treated patients with high-dose chemotherapy followed by BMT and daily administration of G-CSF (filgrastim). One series of patients also received filgrastim-mobilized PBPC collected prior to high-dose chemotherapy.[11,45] The addition of PBPC was associated with a significant acceleration in platelet recovery compared with BMT plus filgrastim (Figure 4). As a result, the requirement for platelet transfusions was reduced to 24 units compared with 85 units with BMT alone. Neutrophil recovery was similar in patients with and without PBPC, but in both groups recovery was faster than in historical controls who only received BMT.

A study by Peters *et al.* suggested that intensive chemotherapy at doses requiring hematopoietic support may be used to increase disease-free survival in patients with high-risk primary breast cancer.[81] The treatment regimen involved induction therapy with four cycles of CAF (cyclophosphamide, doxorubicin, 5-fluorouracil) followed by high-dose cyclophosphamide, cisplatin and carmustine plus autologous bone marrow rescue, with or without PBPC generated by G-CSF (filgrastim) or GM-CSF. The disease-free survival of 72% at 5 years compares very favorably with rates of 24–31% observed in historical control series.

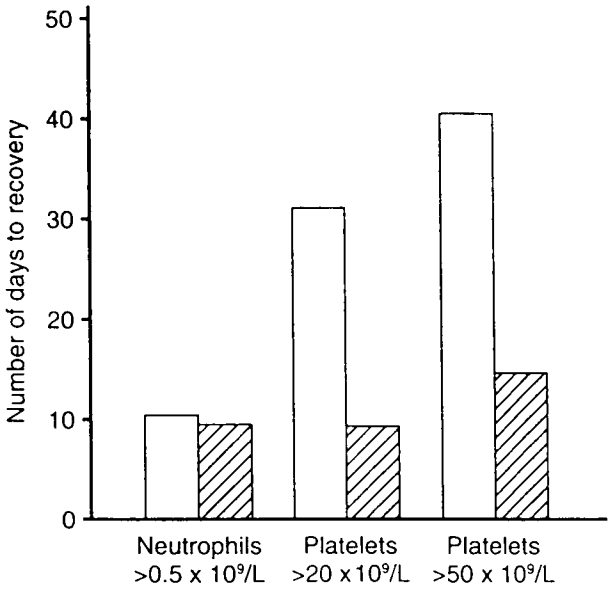

Figure 4. Neutrophil and platelet recovery in patients treated with autologous bone marrow transplant and filgrastim with (▨) and without (□) filgrastim-mobilized peripheral blood progenitor cells. Data from Sheridan *et al.* (1992).[45]

A novel high-dose sequential (HDS) regimen involving the use of GM-CSF and PBPC with BMT has been developed in patients with breast cancer and lymphoma by Gianni *et al.*[47] PBPC harvested after treatment with GM-CSF and cyclophosphamide were reinfused with bone marrow following high-dose chemotherapy (vincristine, methotrexate, cisplatin and melphalan). A second course of GM-CSF was also given. Hematological toxicity was manageable and rapid platelet recovery was reported. The response rate appears promising and results presented recently in patients with breast cancer are encouraging.[82] In the study, 48 patients with breast cancer involving more than 10 nodes were treated with HDS chemotherapy. This regimen is completed after 8 weeks and the patient only needs to be hospitalized for 33 days. A further 37 patients received conventional adjuvant chemotherapy and served as concurrent controls. The two groups were comparable, except the HDS group contained a higher number of patients with a poor prognosis (>20 nodes). After 2 years, relapse-free survival was significantly higher in patients who received HDS chemotherapy (93%) compared with concurrent controls (43%).

PBPC mobilized by G-CSF or GM-CSF may be used without bone marrow to restore hematopoiesis.[78,79,83] Although formal comparative studies

have not been published, it appears that mobilized PBPC alone are as effective as mobilized PBPC plus BMT in accelerating neutrophil and platelet count recovery.[83]

G-CSF-mobilized PBPC have been used to allow the delivery of high-dose therapy in children, in whom preserving dose intensity is of paramount importance owing to the potential curability of many pediatric tumors.[84] The study included seven children with advanced neuroblastoma or non-Hodgkin's lymphoma. PBPC collected after stimulation with chemotherapy and G-CSF (filgrastim) were reinfused after myeloablative chemo-radiotherapy. Hematopoietic reconstitution appeared to be stable over the observation period of up to 22 months.

Preliminary data also suggest that PBPC autotransplantation may be associated with a better treatment outcome than BMT.[85] An analysis of event-free survival results from patients with intermediate-grade lymphoma treated with high-dose chemotherapy with hematopoietic rescue (ABMT, PBPC or allogeneic BMT) has shown that use of PBPC may be superior to BMT in selected patients. A total of 158 patients with intermediate-grade NHL were classified as good or poor prognosis groups based on recognized prognostic factors. Event-free survival was significantly different between these risk groups. In patients with a poor prognosis there was no significant difference in event-free survival between BMT patients and PBPC patients. However, in good prognosis patients, a significant advantage in 3 year event-free survival was observed for PBPC (70%) compared with BMT (32%). The explanation for this effect is unclear, but as this was not a randomized study, interpretations may be open to question.

Clearly the use of PBPC is a promising approach for delivering high-dose chemotherapy more safely and, hopefully, more effectively. However, the technique remains experimental and further studies are required. The long-term effects of using PBPC without BMT must be determined before the clinical use of PBPC is confirmed. Furthermore, the need for additional HGF therapy after PBPC transplantation should be evaluated in prospective studies. If the initial encouraging results are confirmed, the use of PBPC may eventually supersede BMT and thus make high-dose chemotherapy a realistic option for a larger number of patients. Another promising area for clinical research is in improving the determination of PBPC yield and thus minimizing the number of leukapheresis procedures required. It should be possible to achieve hematological recovery using PBPC collected from a single leukapheresis procedure instead of the three or more procedures currently used. Such a step would make the procedure more amenable to integration into standard practice so that high-dose chemotherapy would be feasible on an out-patient basis. The use of the CD34 antigen as a marker for stem cells may prove to be more useful than the traditional and slow GM-CFU assay.[86]

Selection of CD34 cells

Effective collection of CD34+ cells may be improved using positive selection techniques based upon monoclonal antibody technology.[87] Hematopoietic cells from the marrow or peripheral blood are mixed with a monoclonal antibody that selectively binds to the CD34+ cells. The cells then pass through a column containing beads which bind to the monoclonal antibody/CD34+ complex, while the remaining cells pass through the column unhindered. The CD34+ cells are then released by agitation. The main advantages of positive selection are a postulated lower burden of malignant cells and a reduction in the volume of the graft from 200 to 5 ml. Preliminary results from a clinical study comparing the use of CD34+ enriched marrow cells in conjunction with post-transplant G-CSF (filgrastim) or recombinant GM-CSF show that neutrophil and platelet recovery is faster in filgrastim-treated patients.[87] The feasibility of amplifying *ex vivo* progenitor and pluripotent stem cells collected by leukapheresis and subsequently exposed to a variety of growth factors *in vitro* is being explored by several groups, and an interesting activity has been reported for the combination: SCF, IL-1β, IL-6, IL-3 and erythropoietin.[88]

Discussion

Although it is now well established that HGFs significantly reduce the morbidity of anticancer therapy, it is not yet clear whether their use will improve overall survival. In theory, it is conceivable that by reducing toxicity-related deaths, HGFs may make a small but positive impact on survival. However, this hypothesis needs to be evaluated in prospective studies. A more real impact is promised by using HGFs to improve the delivery of anticancer therapy. Preserving the dose intensity of standard-dose regimens has been demonstrated with G-CSF (filgrastim),[10,24] but it seems unlikely

that modest improvements in dose intensity achieved with this approach will have a major impact on overall survival. It may be necessary to escalate chemotherapy dose substantially to achieve real gains. While G-CSF or GM-CSF alone can support the delivery of escalated-dose therapy, the levels achieved with this type of hematopoietic support are still below those achieved with stem cell support.

The morbidity of BMT has been significantly reduced following the introduction of G-CSF and GM-CSF.[11,12] However, perhaps more importantly, oncologists can now use HGFs to mobilize PBPC to support the delivery of high-dose therapy. With these new tools it should be possible to design and conduct prospective, randomized trials to answer important questions concerning dose and response. This will not be a straightforward exercise since there are many complex variables that need to be controlled: the choice of regimen for the control arm; standardized definitions of dose; dose intensity and total dose; and the choice of end-points (response rate or overall survival). It may be difficult to recruit patients into the control arm of such studies if they are aware of anecdotal evidence from pilot studies supporting the superiority of the new 'more intensive' therapy. Furthermore, it is extremely difficult to recruit patients with similar characteristics, e.g. age, gender and stage of malignancy. This suggests a meta-analysis may be a useful approach.

Although these new techniques for allowing effective chemotherapy delivery are promising, it is important to bear in mind that increasing dose intensity will also mean greater toxicity. Furthermore, although HGFs support hematopoietic recovery, they do not prevent non-hematological complications. Future studies should therefore establish doses, schedules and chemotherapy combinations to account for the change in the maximum tolerated dose of agents.

High-dose therapy is an expensive procedure and the use of HGFs will add direct costs. However, by reducing overall morbidity, it may be possible to show an overall cost benefit.[89,90] Investigators should be aware of economic implications and cost-effective analysis should become an integral component of future research.

It is also important to realize that dose intensification is not appropriate in all situations. Identification of patients who will benefit from dose intensity is therefore important. The identification of sub-groups based on known prognostic factors is a priority in this regard. For example, high-grade NHL patients can be divided into two categories.[91] The first category, characterized by stage IV disease, high serum markers and bulky tissue mass may be candidates for high-dose therapy. However, the second category, namely patients with non-bulky disease, normal markers and less than or equal to stage III disease, may respond well to conventional CHOP-like chemotherapy, without being exposed to the risks of intensive treatment. Similar considerations, based on prognostic factors and clinical situations, undoubtedly apply to other malignant diseases. We should be careful not to group all patients with metastatic breast cancer irrespective of their disease-free interval following primary treatment, biological characteristics of their tumors and metastatic sites. It has been known for some time that metastatic bone disease, visceral metastasis, a localized soft-tissue recurrence or disseminated high tumor volume disease are not identical clinical entities.

Conclusion

HGFs have so far posed more questions than they have provided answers. "What would you do if this were your . . . wife, sister, mother, self?",[92] will remain a difficult question to answer. Moreover, it has been stated that nearly 80% of American physicians suggest that women with locally advanced breast cancer should be treated within a non-randomized trial of high-dose chemotherapy and ABMT despite the present lack of uncontroversial evidence that this approach is more beneficial than conventional therapy.[93] Nevertheless, following positive pilot studies, it should now be possible with formal large comparative studies and/or meta-analysis of smaller studies, to harness these tools to determine whether they can give a new lease of life to conventional chemotherapy regimens and improve upon current results.

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