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Special Paper

A European Perspective on Haematopoietic Growth Factors in Haemato-Oncology: Report of an Expert Meeting of the EORTC

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INTRODUCTION

THE FAST development from the laboratory to clinical application of the haematopoietic growth factors (HGFs) is a rare event. The HGFs represent a group of cytokines with well-defined effects on the haematopoietic system. They enable clinicians to modulate physiological and pathological processes and thus manage previously unresolved therapeutic problems. These advances were made possible by the progress of recombinant DNA technology.

Until now, three factors have been registered for clinical application: the myeloid growth factors G-CSF and granulocyte/macrophage colony stimulating factor (GM-CSF) and erythropoietin (EPO), the main regulator of erythroid growth. Thrombopoietin (or megakaryocyte growth and differentiation factor) has been cloned recently and early clinical trials have already been launched. Only limited data are available regarding the combination of HGFs with one another or with earlier acting factors like stem cell factors (SCFs) that are able to prime haematopoietic progenitor cells for response to later acting factors like EPO. The recently cloned growth factor FLT 3 ligand stimulates the growth of primitive haematopoietic progenitor cells through synergistic interactions with multiple other cytokines.

Myeloid growth factors shorten significantly the duration of neutropenia following chemotherapy, while the nadir usually remains unaffected [1, 2]. It is also well known that prophylactic use of HGFs after myelosuppressive chemotherapy leads to a reduction in the incidence of febrile episodes, the use of antibiotics and the number of days of hospitalisation for fever and/or infection [2–4]. EPO exerts its maximal activity at the level of the more differentiated erythroid precursor cells. It is registered in most European countries for the treatment of renal anaemia. In more recent years, it has also been convincingly demonstrated that the majority of patients suffering from anaemia due to malignant disease may benefit from treatment with EPO.

HGFs were developed with the idea of revolutionising cancer therapy by reducing side-effects of cytotoxic agents and thus allowing an increment of cytotoxic dose per time unit. Furthermore, even more ambitious approaches were investigated, e.g. the induction of haematopoiesis in primary bone marrow disorders, or using growth factors to trigger resting myeloid cells into cell cycle, thus making them more amenable to cytoreductive therapy. After almost one decade of clinical research, there is no doubt that several of the initial hopes have been fulfilled but that many questions remain unanswered.

When given in the recommended dosage, HGFs are relatively safe drugs with limited toxicities. HGFs can be self-injected subcutaneously by the patient. However, HGFs are expensive and their non-critical application may contribute to the economical burden of our health care systems.

In recent years, numerous publications have described the biology, clinical use and cost-effectiveness of HGFs. This review aims to update current knowledge of clinical HGF research from a European perspective. In order to address these questions and to provide haemato-oncologists with an update for clinical application of the myeloid growth factors and EPO, the EORTC Biological Therapeutics Development Group (BTDG) convened a meeting of experts. This meeting was supported by a BIOMED grant of the European Commission (Organization of preclinical and clinical research on anticancer therapy with biological response modifiers, No. BMH1-CT94-1587).

The goal of our report was not to define strict guidelines but to give an overview of the indications, for which HGFs are authorised or have been used in clinical trials in the various European countries and describes recommendations for clinical indications based on the review of data from controlled clinical trials.

It must be emphasised that clinical application of a haematopoietic growth factor will always depend on the

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physician's judgement with respect to the individual patient and the clinical situation. However, our update on clinical HGF research may contribute to decision-making by the responsible physician, providing sound information on the currently available data surrounding still unresolved questions.

CLINICAL APPLICATION OF ERYTHROPOIETIN IN CHRONIC ANAEMIA OF CANCER

Recombinant human erythropoietin (EPO) is the main stimulant of erythropoiesis. It exerts its maximal activity at the level of the more differentiated erythroid progenitor and precursor cells [5] by enhancing proliferation, inhibiting apoptosis, and stimulating haemoglobin synthesis, as well as differentiation of red blood cells. It is produced in response to tissue hypoxia, mainly (85–90%) by renal tubular interstitial cells [6] and to a minor degree also by liver and bone marrow (BM) macrophages [7].

Red cell transfusion carries the risk of transmission of infections as well as transfusion reactions. Administration of EPO has convincingly been shown to increase haematocrit and decrease transfusion requirement in 40–70% of patients with chronic anaemia of cancer [8, 9]. It also prevents or ameliorates anaemia in patients undergoing chemotherapy with or without cisplatin [8, 10] and improves haemoglobin levels in HIV infected patients experiencing anaemia while receiving zidovudine treatment [11]. EPO reduces red cell transfusion requirements in patients undergoing allogeneic bone marrow transplantation [12] but not in those subjected to transplantation of autologous bone marrow or peripheral blood stem cells. EPO administration also appears to be a safe and effective method of increasing red cell mass during radiation therapy [13, 14].

The starting dose applied in most studies in patients with anaemia of cancer or chemotherapy-induced anaemia or for zidovudine-associated anaemia in AIDS patients is 150 U/kg, 3×/week. In case of insufficient response, this dose may be extended up to 300 U/kg or even higher.

The benefits to patients who are responsive to EPO are not only normalisation of anaemia and reduction or prevention of transfusion dependency but also a substantial improvement of physical activity, performance status and subjective sense of well-being, i.e. a general improvement in quality of life [15]. In one study, a significantly longer survival time was found in responders to EPO treatment as compared to non-responders [16]. In spite of this observation, it is still unclear whether the improvement status observed in many responding patients has any impact on response to cancer treatment, thus improving prognosis and survival of the underlying malignancy.

Response to EPO treatment is most likely in patients with inadequately low levels of endogenous EPO. Usually, an absolute endogenous EPO level < 100 mU/ml is considered inadequate in anaemic cancer patients [7], but calculation of the individual patient's degree of anaemia and EPO level may yield more accurate predictions. If the EPO response usually seen in patients with an equivalent degree of anaemia caused by iron deficiency or blood loss is taken as the 'expected' level (E) and compared to the observed EPO level (O) of that patient, the O/E ratio is often decreased in anaemia of chronic disease [17]. Approximately 70% of patients with chronic anaemia of cancer and an O/E ratio < 0.9 have been reported to respond to EPO treatment in an ongoing trial. A higher predictive accuracy has been provided by a recently published algorithm which employs the endogenous EPO levels obtained after 2 weeks of treatment and the observed change from the baseline haemoglobin level after 2 weeks of treatment (Figure 1) [18]. With the help of this algorithm, response can be predicted with more than 90% accuracy after 2 weeks of EPO treatment, which seems to be clinically useful.

Apart from local skin reactions at the injection site observed in a small proportion of patients, EPO treatment is very well tolerated by cancer patients. In particular, episodes of hypertension, which are a common threat under rhuEPO therapy for anaemia of chronic renal failure, occur only rarely during EPO treatment of the indications listed above.

For the time being, generally accepted recommendations for rhuEPO treatment have not been firmly established in the field of haematology/oncology. Randomised trials have shown that erythropoietin treatment leads to an increase of haematocrit and decrease of transfusion requirement in anaemic cancer patients with and without chemotherapy. Prospective trials, however, which include a cost-effectiveness evaluation of EPO administration versus red cell transfusion, are unfortunately lacking. Tentatively, the use of rhuEPO may be considered in (a) patients with chronic, symptomatic anaemia of cancer and low endogenous EPO levels, (b) in facilitating autologous blood donation in patients scheduled for elective surgery if their requirement of blood transfusions is highly probable and (c) in particular for Jehovah's witnesses and

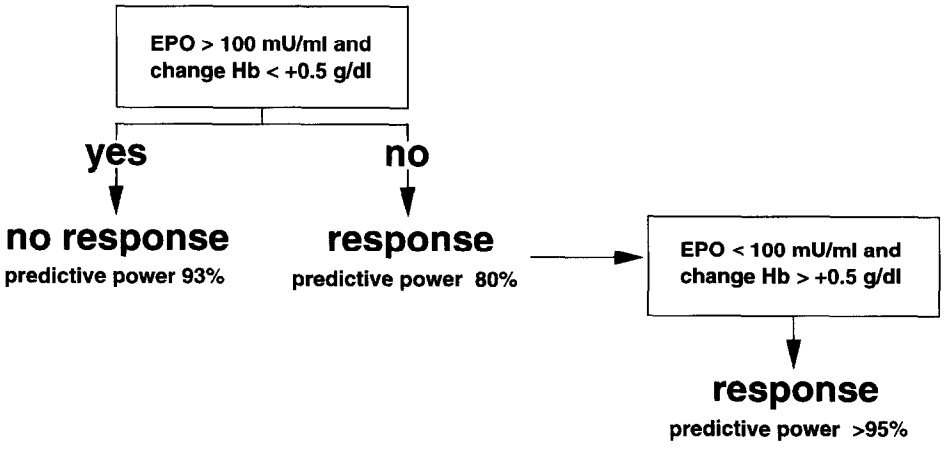

Figure 1. Prediction of response to rhuEPO treatment in patients with chronic anaemia of cancer. Hb, haemoglobin.

Table 1. Generally accepted recommendations for use of recombinant human erythropoietin in the field of haematology/oncology

Indications
Patients with chronic symptomatic anaemia of cancer and low endogenous EPO levels with and without chemotherapy
Facilitating autologous blood donation in patients scheduled for elective surgery if their requirement of blood transfusions is highly probable
Jehovahs witnesses rejecting allogeneic blood, if the necessity of blood transfusions is foreseeable

other persons who reject allogeneic blood, if the necessity of blood transfusions is foreseeable (Table 1). The prevention of chemotherapy induced anaemia in patients who are likely to suffer from symptoms of anaemia such as those with pre-existing cardiovascular disease, coronary heart disease, etc. is still debatable as the respective randomised trials have not been finalised.

SEVERE APLASTIC ANAEMIA

Bone marrow transplantation is the treatment of choice for young patients with severe aplastic anaemia (SAA) and an HLA-identical sibling donor. For those patients without a suitable donor or beyond the age of 50 years, antilymphocyte globulin (ALG) or antithymocyte globulin (ATG) in combination with or without cyclosporin, androgens or prednisone remains the current therapy. Several recombinant human HGFs have been used in aplastic anaemia in an attempt to increase peripheral blood values, to reduce the risks of complications caused by pancytopenia and eventually to induce a sustained multilineage response. Despite many reports, results are not yet conclusive.

Erythropoietin

In severe aplastic anaemia (SAA), single cases of response to EPO have been published, but no well-designed prospective studies have been performed. A response was observed in 3 out of 7 patients treated with doses of EPO up to 24 000 U twice weekly, but only during treatment with EPO [19]. In patients pretreated with ALG or with additional treatment of cyclosporin, sustained multilineage responses following therapy with EPO have been published. EPO may reduce the need for red cell transfusions in a small subgroup of patients with late clonal complications of SAA and low EPO levels [20].

Myeloid growth factors

Several phase III studies with G- and GM-CSF have been published [21–25]. In a prospective randomised placebo-controlled multicentre trial [21], patients with newly diagnosed SAA were treated with ALG, followed by GM-CSF or placebo. In the GM-CSF group, significantly higher counts of neutrophils and monocytes were observed. Only patients with some residual marrow function showed an increase in neutrophil counts. After withdrawal, leukocyte counts declined rapidly and were no longer different in the two study groups. The number of days with fever was significantly reduced in the GM-CSF group and a trend was observed for fewer days on antibiotics in the GM-CSF group. Platelet and red cell transfusion requirements were not different for the two groups and the overall treatment response and survival were also identical. Children with SAA treated solely with GM-CSF did not show an improvement in the disease nor clearance of infections [22]. Thus, GM-CSF without immunosuppression should not be given to SAA patients.

Single cases of successful treatment with G-CSF of patients with SAA have been reported. Some described a trilineage response following long-term application of G-CSF alone or in combination with EPO. Interpretation of these data is difficult, as most patients were pretreated with ALG and/or cyclosporin. In a larger study, 6 out of 10 children with SAA showed an increase in neutrophils and clearance of their infections after treatment with G-CSF [23]. In a pilot study of the EBMT Working Party for SAA, the use of long-term G-CSF in combination with ALG, cyclosporin and methylprednisolone in 40 patients with newly diagnosed SAA led to a trilineage reconstitution in 82% of the patients and they became transfusion independent [24]. Another study of 45 children treated with G-CSF reported an increase in neutrophils in 43 of the 45 patients and a trilineage response in 7, 2 of whom without any other therapy [25].

Conclusion

SAA is not caused by a deficiency of any known HGF. Nevertheless, HGFs have been administered in an attempt to increase peripheral blood values, to reduce the complications of pancytopenia and eventually to alter the course of the disease. So far, in most patients with SAA treated with G-CSF and GM-CSF, a transient increase in neutrophil counts has been observed, the extent of the rise being proportional to the amount of residual myelopoiesis. Early acting growth factors (e.g. IL-3, IL-6 and SCF) have not yet been shown to be effective in the treatment of SAA [26–29]. There is no clear evidence yet, despite the claims of several case reports, that treatment with HGFs have changed the course of the disease. In addition, substantial side-effects can be observed with the administration of HGF. The currently available recombinant human HGFs are interesting tools to investigate regulation of haematopoiesis and promising agents to improve outcome of patients with SAA. Despite almost a decade of investigation, clear answers are lacking. Patients with newly diagnosed SAA should not be treated with growth factors outside a clinical protocol. Inappropriate use of HGFs could reduce the excellent chance of long-term cure after allogeneic BMT or delay administration of effective immunosuppressive therapy. In contrast, well-designed clinical trials will allow us to establish the role of HGFs and their combination and further improve the current results.

MYELODYSPLASTIC SYNDROMES

At present, many studies have been performed with EPO, GM-CSF [30–33], G-CSF [34, 35] and IL-3 [36] in patients with a myelodysplastic syndrome and a low risk of developing leukaemia (MDS-LR), either alone [30–35] or in combination with low-dose Ara-C in MDS at a high risk of developing leukaemia (MDS-HR) [37, 38].

Erythropoietin

In patients with a low risk of developing leukaemia (MDS-LR), the main clinical problem is the dyserythropoietic anaemia requiring regular red cell transfusions and finally leading to iron overload. The effect of EPO on this anaemia is not very favourable. It has been reported that approximately 15–20% of the patients respond to dosages of EPO that are

commonly used in anaemia due to renal disease [39]. Administration of very high dosages of EPO in combination with one of the myeloid growth factors may improve response rates up to almost 50% [40]. Response means transfusion independence, not necessarily normalisation of the haemoglobin content. Although very high dosages of EPO in combination with another HGF may be more promising, financial limitations prohibit further exploration in most centres.

Myeloid growth factors

GM-CSF and G-CSF have been used in Europe and the U.S. Neutrophilia, eosinophilia (related to GM-CSF) and increases in reticulocyte, lymphocyte and monocyte counts have been reported in several phase II and III studies [32, 38]. These effects are generated at relatively low dosages of these HGFs independently of the route of administration. A response rate of 70% has been reported in large series of patients treated with GM-CSF [32]. The effects of GM-CSF on neutrophils were seen within 1 week from the start of the administration and disappeared within 4 weeks after discontinuation. Observed side-effects at these low dosages were mild. No increased risk of transformation to leukaemia has been reported in the MDS-LR series. The effects of G-CSF are similar to those described for GM-CSF. Long-term administration of low-dose GM-CSF or G-CSF appears to be promising as far as haematopoietic activity and side-effects are concerned. The effects of GM-CSF or G-CSF on the incidence of infection have been evaluated in several phase II trials as well as in randomised trials. Results of these trials have shown an improved phagocytosis and bacterial killing by HGF administration with a positive impact on the incidence of infectious episodes [34, 37]. Effects of GM-CSF and G-CSF on platelet counts and haemoglobin levels are rare. The role of IL-3 and IL-6 is unclear, but not very promising. The role of HGFs in myelodysplastic syndromes at high risk of developing leukaemia is even more unclear. In this setting, HGFs have been given in phase II and III studies in combination with low-dose Ara-C or in combination with intensive induction chemotherapy courses. All these policies lead to haematopoietic improvement in approximately 50% of the patients. It is still unclear whether this is the result of the cytostatic treatment or due to the combination with one of the growth factors G-CSF, GM-CSF or IL-3.

Interleukins

Interleukin-3 has been administered to few patients with MDS-LR [38]. Whereas side-effects appeared to be more severe (headache, fever, chills, skin reactions), neutrophil responses were less prominent than after G/GM-CSF administration. Platelet counts or reticulocyte numbers increased in only a small number of patients.

The effect of IL-6 on platelet counts is disputable. One study in the United States [41] of recombinant human interleukin-6 in patients with myelodysplastic syndromes and thrombocytopenia showed an increase in platelet numbers. Side-effects including fever, chills and flu-like symptoms were already present at very low dosages.

Conclusion

The role of myeloid HGFs in myelodysplastic syndromes at low risk of developing leukaemia is rather disappointing. GM-CSF and G-CSF cause an increase of neutrophils and their administration may only be indicated in patients with repetitive infectious episodes and neutropenia. Since most results in patients with acute leukaemia treated with chemotherapy and HGFs did not show an additional effect of the HGF on treatment response, it is not very likely that a positive effect of G-CSF, GM-CSF or IL-3 would be expected in patients with high-risk MDS treated in a similar way.

ACUTE LEUKAEMIA

In acute myeloid leukaemia (AML), leukaemic blast cells express receptors for HGFs, with interindividual variability in number, type and affinity of receptors [42]. Treatment of AML with HGFs has theoretically two kinds of potential therapeutic effects. First, they can recruit leukaemic cells into cycle, thus enhancing their sensibility to chemotherapy [43–45]. Therefore, their administration before and during chemotherapy may lead to increased leukaemic cell kill. Second, when administered after the cytotoxic courses, they may stimulate the normal haematopoietic progenitors, thus accelerating haematopoietic recovery and reducing the morbidity and eventually mortality from infection. The presence of receptors for HGFs in both normal and leukaemic cells could challenge these therapeutic effects by two possible adverse consequences: (1) when given before and during chemotherapy, recruitment of normal pluripotent stem cells in cycle may increase their fraction exposed to cycle-dependent cytotoxic drugs and results in more prolonged marrow aplasia; (2) when administered after the chemotherapy courses, stimulation of residual leukaemic clones may occur, with a risk of resistance to induction treatment or early relapse. Whether given during or after the induction courses, the HGFs may prevent chemotherapy-induced apoptosis of leukaemic cells [46, 47].

HGFs in acute myeloblastic leukaemia

Several pilot studies combining GM-CSF or G-CSF with chemotherapy during induction treatment of AML indicated that HGFs may accelerate the recovery of neutrophils, and/or result in a higher complete remission (CR) rate, when compared to historical controls [48–51]. Results of randomised multicentre trials using GM-CSF or G-CSF have recently been published [52–59] (Table 2). Unfortunately, the designs were quite different from one study to another, thus making comparisons difficult. In addition, few attempts have been made to assess separately the two biological effects: the priming effects by administration before and during the chemotherapy courses, and the acceleration of normal haematopoietic recovery by administration after chemotherapy. Recruitment of leukaemic cells into cycle has been observed by cell kinetics methods in some studies [44]. The optimum dose schedules of HGFs for priming of the leukaemic cells remains largely unknown. The administration of GM-CSF for several days before the start of chemotherapy may also induce a hyperleukocytosis with eventually pulmonary infiltrates [60]. An early start of GM-CSF, 4–7 days before induction chemotherapy, might explain the relatively low CR rate in patients reported by Estey and associates when compared to a group of matched historical controls [61].

A significantly shorter duration of neutropenia was observed in some studies with GM-CSF [55] or G-CSF [52, 57], administered after the induction chemotherapy courses. This slightly accelerated recovery did not result in a significant reduction of the rate of documented infections or mortality during hypoplasia. Only the ECOG Cooperative

Table 2. Phase III trials using G-CSF or GM-CSF in relation to remission induction chemotherapy for AML

References	HGF used	Schedule of HGF in relation to chemotherapy (day = 0)	Number of patients	Complete remission in HGF/placebo (%)	Rate of ANC recovery
Ohno [52]	G-CSF	day 2 until recovery	108*	50/36	++
Witz [54]	GM-CSF	day 0 until recovery	163	63/67	NS
Stone [55]	GM-CSF	day 8 until recovery	347	49/53	NS
Drombret [56]	G-CSF	day 8 until day 28	173†	70/47	++‡
Rowe [57]	GM-CSF	day 11 until recovery	118	61/46	++
Heil [58]	GM-CSF	day 2 until recovery	80	81/79	NS
Zittoun [59]	GM-CSF	day 1 until day 7/day 28 (factorial 2×2)	102	47/74	NS

ANC, absolute neutrophil count; AML, acute myeloid leukaemia. *AML and acute lymphoblastic leukaemia, relapse or refractory; HGF, haematopoietic growth factor; NS, difference not significant. †Elderly AML. ‡No difference for frequency and severity of infections.

study showed a significant decrease of grade 4–5 infections, with a trend to a lower therapy-related mortality and a higher CR rate [55]. The reasons for this discrepancy with the other studies on GM-CSF [54, 56–58] remain to be explored. The results of a randomised study of the EORTC Leukemia Cooperative Group [59] are a matter of concern. In this study, using a 2×2 factorial design, the CR rate was significantly lower in AML patients who received GM-CSF after the chemotherapy courses when compared to the control group. By contrast, there was no difference in the CR rate whether or not patients received GM-CSF before and during the chemotherapy courses. However, positive results were published in favour of a combination with G-CSF by Dombret and colleagues who used this HGF in elderly AML patients from day 9 until day 28 or earlier in case of haematopoietic recovery [56]. These authors observed a significantly higher CR rate in patients receiving G-CSF, without reduction of the mortality rate from infection. The higher CR rate was related to a lower resistance of leukaemia, especially in patients with adverse prognostic factors. These results raised the hypothesis of an antileukaemic effect of G-CSF by stimulation of terminal differentiation of the residual leukaemic cells. If confirmed, they indicate positive biological effects of G-CSF in patients with AML.

HGFs in acute lymphoblastic leukaemia

In acute lymphoblastic leukaemia (ALL), the use of G-CSF, and, eventually, GM-CSF, might be beneficial during induction and consolidation of ALL. Although functional G-CSF and GM-CSF receptors have been observed sometimes in ALL, especially in the biphenotypic acute leukaemias and Phl-positive ALL [62], there is generally no evidence of growth stimulation of leukaemic lymphoblasts by G-CSF, either *in vitro* [63] or in clinical trials [64]. In a recent randomised study of the German group, G-CSF, administered during the second part of the induction course, significantly reduced the duration of neutropenia, with less severe bacterial infections and earlier completion of the induction programme [65]. Opportunistic infections—especially aspergillosis—are a major concern during the treatment of ALL, probably due to intensification of chemotherapy and concomitant treatment with immunosuppressive agents. Systematic use of HGFs along with other prophylactic measures may reduce the rate of opportunistic infections.

Conclusion

In AML, further randomised studies comparing the various HGFs at different doses schedules are warranted. Outside such controlled prospective studies, the use of HGFs should be avoided in AML, at least during the induction period, thus supporting the recent recommendations of an expert panel of the American Society of Clinical Oncology updated in 1996 [66, 67]. In ALL, these restrictions are more relative and flexible.

PERIPHERAL STEM CELL TRANSPLANTATION IN HAEMATOLOGY AND ONCOLOGY

A somewhat unanticipated benefit from the introduction of HGFs was the discovery that they mobilised haematopoietic progenitors from the marrow into the peripheral blood stream. These cells can be harvested by one of several apheresis procedure(s) and cryopreserved to be reinfused after high-dose therapy. In the last 5 years, peripheral blood is rapidly replacing bone marrow as the preferred source of stem cells for autologous transplantation after myeloablative therapy. The most striking finding of PBSC transplantation is the faster trilineage haematological reconstitution of hematopoiesis after high-dose chemotherapy as compared to ABMT [68]. In a randomised study [68] comparing autologous BM and PBSC transplantation in lymphoma patients, both time to platelet recovery and time to neutrophil recovery above was significantly reduced in the PBSC transplantation group. In addition, the relatively low burden of the harvest procedure may explain the increased popularity of PBSC. Furthermore, total costs of PBPC autografting are lower than those of ABMT [69]. Peripheral blood stem cells can be mobilised by chemotherapy [70–72], chemotherapy followed by HGFs [73–75] and HGFs alone [76–79]. Despite the increasing knowledge of progenitor–stroma interaction, the mechanisms of stem cell mobilisation remain undefined. Other mechanisms beside proliferation seem to be involved. In primates, antibodies to the adhesion molecule VLA-4 cause a release of stem cells into the peripheral blood [80]. The release of stem cells into the blood may be the result of a perturbation of the adhesive interactions between these cells and the marrow stroma that, in steady-state conditions, serve to restrict haematopoietic stem cells to the bone marrow and keep them in a resting state [81]. To quantitate PBSC, the number of granulocyte-macrophage colony-forming units (CFU-GM) and the number of CD34 positive cells may be used. The CD34 assay is a more reproducible assay and probably also more reliable. The threshold dose of CD34 positive cells necessary for prompt engraftment is approximately $2–5 \times 10^6$/kg, depending on the amount and duration of previous chemotherapy before transplantation [82–85].

The exact function of CD34 is not known. Three possible functions of this sialomucin may involve adhesion [86], signalling [87] or growth-factor presentation. Recent

experiments [88] showed that in mice lacking CD34, haematopoiesis was delayed in developing embryoid bodies. CD34-deficient haematopoietic progenitors were unable to expand in liquid cultures in response to HGFs. These data indicate that CD34 is involved in the proliferation and/or maintenance of the haematopoietic progenitor cells in embryos and adults.

PBSC mobilisation by HGFs

Several HGFs including G-CSF [76, 79, 89, 90], GM-CSF [77, 91, 92] and other HGFs such as IL-3, IL-6, EPO, stem cell factor, PIXY 321 have been used to mobilise stem cells into the blood. The best studied single mobilising agents are G-CSF and GM-CSF. Administration of G-CSF [89] and GM-CSF [91] induce an up to 100-fold increase of circulating stem cells in patients with various malignancies. Chao [93] reported that patients treated with G-CSF mobilised PBSC had a more rapid engraftment than patients receiving non-G-CSF mobilised PBSC. For G-CSF, a clear dose-response effect could be shown for dosages of 5, 10 and 24 μg/kg/day [76, 94]. The same effect was reported for the use of GM-CSF at dosages of 125 and 250 $\mu g/m^2$/day [77]. In most circumstances, a dosage of 5 μg/kg is adequate for both G- and GM-CSF. Combinations of HGFs are also effective in mobilising PBSC. Theoretically, cytokines that stimulate early progenitors, such as IL-3, SCF and the FLT3 ligand, may expand the pool of progenitors responsive to the late acting haematopoietic growth factors. When given as a sole agent, these early acting cytokines have only limited stem cell mobilising potential, but when given in combination, e.g. IL-3+GM-CSF [95], IL-3+G-CSF [96], SCF+G-CSF [97] synergistic effects on PBSC mobilisation have been observed. Whether these combinations of HGFs offer clinically relevant advantages over the use of G-CSF or GM-CSF alone has not yet been demonstrated.

The approach of using a combination of chemotherapy and growth factors was first reported by Gianni and colleagues, who treated patients with cyclophosphamide and GM-CSF [98]. Other agents such as etoposide and cytarabine combined with HGFs are similarly effective [99]. The combination of chemotherapy and HGFs is more effective in mobilisation of PBSC than chemotherapy alone [76, 98, 100, 101]. Brugger and associates [95] showed that GM-CSF plus IL-3 in addition to chemotherapy further increased PBSC mobilisation as compared to chemotherapy alone or chemotherapy with GM-CSF.

Stem cell mobilisation in allogeneic healthy donors is also possible by administration of G-CSF or GM-CSF alone. Preliminary data suggest that rapid and sustained haematopoietic engraftment without a dramatically increased incidence of acute graft versus host disease occurs despite a higher number of donor T-cells infused [79, 102–104].

Use of HGFs after PBSC infusion

Spitzer [105] has shown that administration of a combination of G-CSF and GM-CSF after PBSC infusion shortens the duration of granulocytopenia by several days, but it has no influence on the duration of thrombocytopenia. Others [106, 107] have shown that G-CSF failed to improve haematopoietic reconstitution following myeloablative chemotherapy and PBSC transplantation. Therefore, the clinical benefit of HGF administration has not yet been demonstrated and may depend on individual clinical circumstances. Currently, phase III randomised placebo-controlled trials are underway to determine the clinical benefit, optimal dosage and schedule of HGF administration after PBSC transplantation.

Conclusions

G-CSF and GM-CSF should be considered equally effective in mobilising short- and long-term repopulating stem cells to the peripheral blood to be used for autologous transplantation. G-CSF and GM-CSF in combination with chemotherapy are more effective than chemotherapy alone in mobilising PBSC. The clinical benefit of HGF administration after PBSC infusion has not yet been demonstrated. Until sound data are available, after PBSC transplantation HGFs cannot be recommended outside clinical protocols.

PROPHYLACTIC AND THERAPEUTIC USE

Neutropenia and infection are the most important dose-limiting toxicities for the majority of chemotherapy regimens. The risk of infection is directly related to the duration and to the degree of neutropenia [108]. The magnitude of myelosuppression depends on the type and intensity of chemotherapy. The administration of HGFs produces a rapid and substantial increase in peripheral blood neutrophil count by amplifying neutrophil production and decreasing the time to release of mature neutrophils [104]. In this way, the use of HGFs may reduce the duration of chemotherapy-related neutropenia and the risk of infection. However, few combination chemotherapy regimens induce more than a 40% rate of grade 4 myelosuppression, which was the criterium for use of HGFs by the expert panel of the American Society of Clinical Oncology [66, 67, 110]. Furthermore, the infection-related mortality due to febrile neutropenia is low, ranging from 0 to 7%. The duration of hospitalisation for febrile neutropenia can exceed 1 week with consequent reduction in terms of quality of life for the patients, and in delay or dose reduction for subsequent chemotherapy. It might be justified to use HGFs with the intent to improve patients quality of life, to reduce the hospital cost and to improve the chemotherapy delivery.

Primary prophylaxis

Three prospective, randomised placebo-controlled trials analysed the impact of HGFs on febrile neutropenia incidence during standard-dose chemotherapy. Two of these trials used chemotherapy consisting of cyclophosphamide, doxorubicin and etoposide (CDE) in patients with small cell lung cancer (SCLC) and one used cyclophosphamide, doxorubicin, vincristine, bleomycine, etoposide and prednisolone (VAPEC-B) in patients with NHL [111–113]. In all these trials, neutrophil recovery occurred faster with HGF use and the relative incidence of febrile neutropenia was reduced by approximately 50%. In the SCLC trials, HGF administration was associated with a decrease of antibiotic use and hospitalisation. Pettengell and associates randomised 80 patients with NHL to receive VAPEC-B chemotherapy with or without G-CSF. Patients randomised to receive G-CSF achieved a greater dose intensity than control patients, but this did not result in significant differences in drug toxicity (other than neutropenia), intravenous antibiotic usage or hospitalisation between the two groups. These three randomised studies did not report a difference in infection-related mortality, tumour response rate and patient survival between HGF and placebo-treated subjects.

Bajorin and associates [114] recently published the results of a multicentre randomised study on patients with advanced or relapsed germ cell tumour receiving conventional dose ifosfamide plus cisplatin in combination with etoposide or vinblastine. One hundred and eight patients were enroled and randomised to receive GM-CSF after chemotherapy in order to assess the impact of GM-CSF on the severity of neutropenia and the incidence of infectious complications due to the treatment planned. The use of GM-CSF was associated with a clinically significant reduction in the incidence of infections only during the first cycle of chemotherapy, while this benefit was not observed during subsequent cycles. The cost benefit of HGF prophylactic use seemed related to the risk of hospitalisation, to the rate of risk reduction and finally to the costs of the single institution [115, 116]. With these assumptions, the use of HGFs was felt to be justified only if the baseline risk of febrile neutropenia exceeded more than 40% and the cost of hospitalisation in single institutions was considered high. In summary, the use of HGFs as primary prophylaxis is not recommended for all patients receiving the first course of standard chemotherapy (Figure 2). In those patients with known poor bone marrow tolerance, HGF prophylaxis may be considered upfront.

Secondary prophylaxis

For secondary prophylaxis, it is appropriate to prescribe HGFs for those patients who have had infections or neutropenia (ANC < 1000 cells/μL) exceeding 7 days during the first course of chemotherapy (Figure 3). In those patients, the subsequent full-dose treatment could expose them to life-threatening complications or alternatively if the dose was to be reduced it would lead to a decrease in expected long-term success rates. This approach is recommended when the aim of treatment is curative such as in germ cell cancers or lymphomas [110, 68].

Febrile neutropenia

Applying HGFs only in the case of infection during neutropenia would reduce the number of patients exposed to HGFs and might restrict the costs of treatment. Six randomised studies using HGFs in patients with febrile neutropenia have been conducted (Table 3) [117–122]. The studies differed with respect to patient categories and treatment protocols. In three studies a significant advantage in terms of neutropenic fever was observed for GM-CSF or G-CSF [118–120]. In none of the studies a significant difference was found in infection-related mortality between HGF and placebo-treated patients. Therefore, for the majority of patients with febrile neutropenia, the available data do not clearly support the routine use of HGFs as adjuncts to antibiotic therapy. The results of the studies do not exclude that a subgroup of patients, e.g. with pneumonia, sepsis syndrome or fungal infection, may benefit from the use of HGFs.

Afebrile neutropenia

The use of HGFs in afebrile neutropenic patients has been investigated in at least one placebo-controlled randomised trial [123] in several tumour types and lymphomas. Although it shortened the duration of severe chemotherapy-induced neutropenia, it had no impact on clinical outcome.

COMPARATIVE DIFFERENCES IN THE CLINICAL SPECTRA OF G-CSF AND GM-CSF

While both factors have been shown to ameliorate the complications of neutropenia, there are potentially important clinical differences in their haematopoietic spectrum, in their toxicity and in the potential they possess to induce other biological effects.

Preclinical and early clinical studies indicated that the activity of G-CSF was essentially confined to neutrophils and their precursors [124, 125]. It was therefore anticipated that

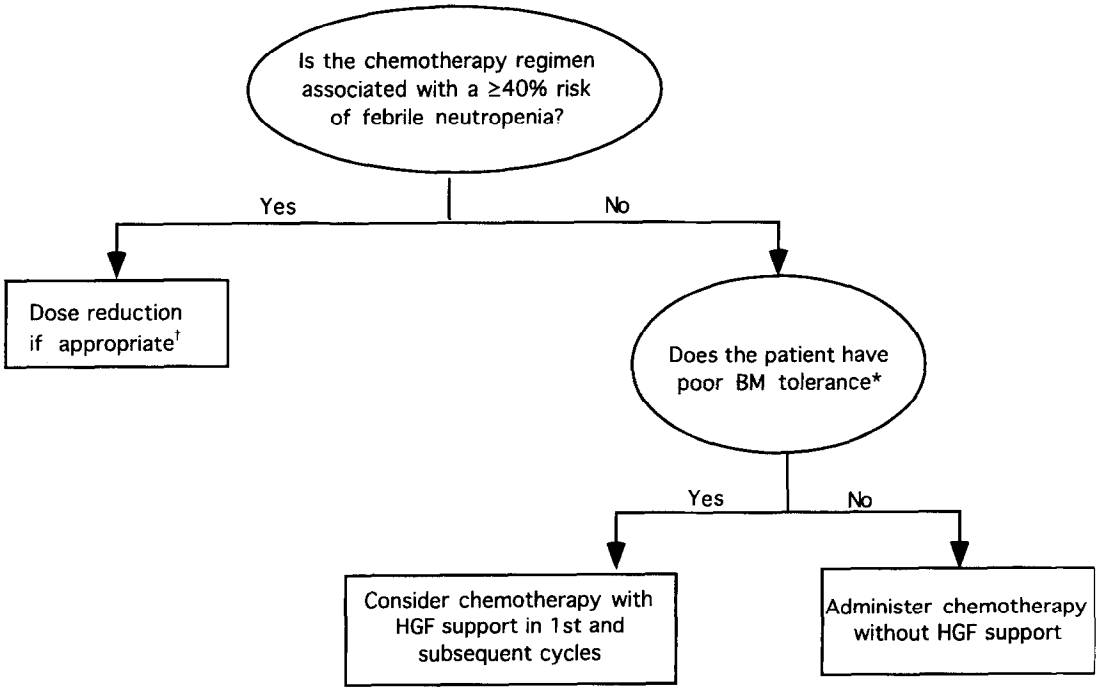

Figure 2. Administration of myeloid HGFs in the primary prevention of febrile neutropenia. *BM heavily infiltrated by malignancy, myelodysplasia or previously treated by chemotherapy or radiography. †Dose reduction may be appropriate if it does not compromise treatment goal.

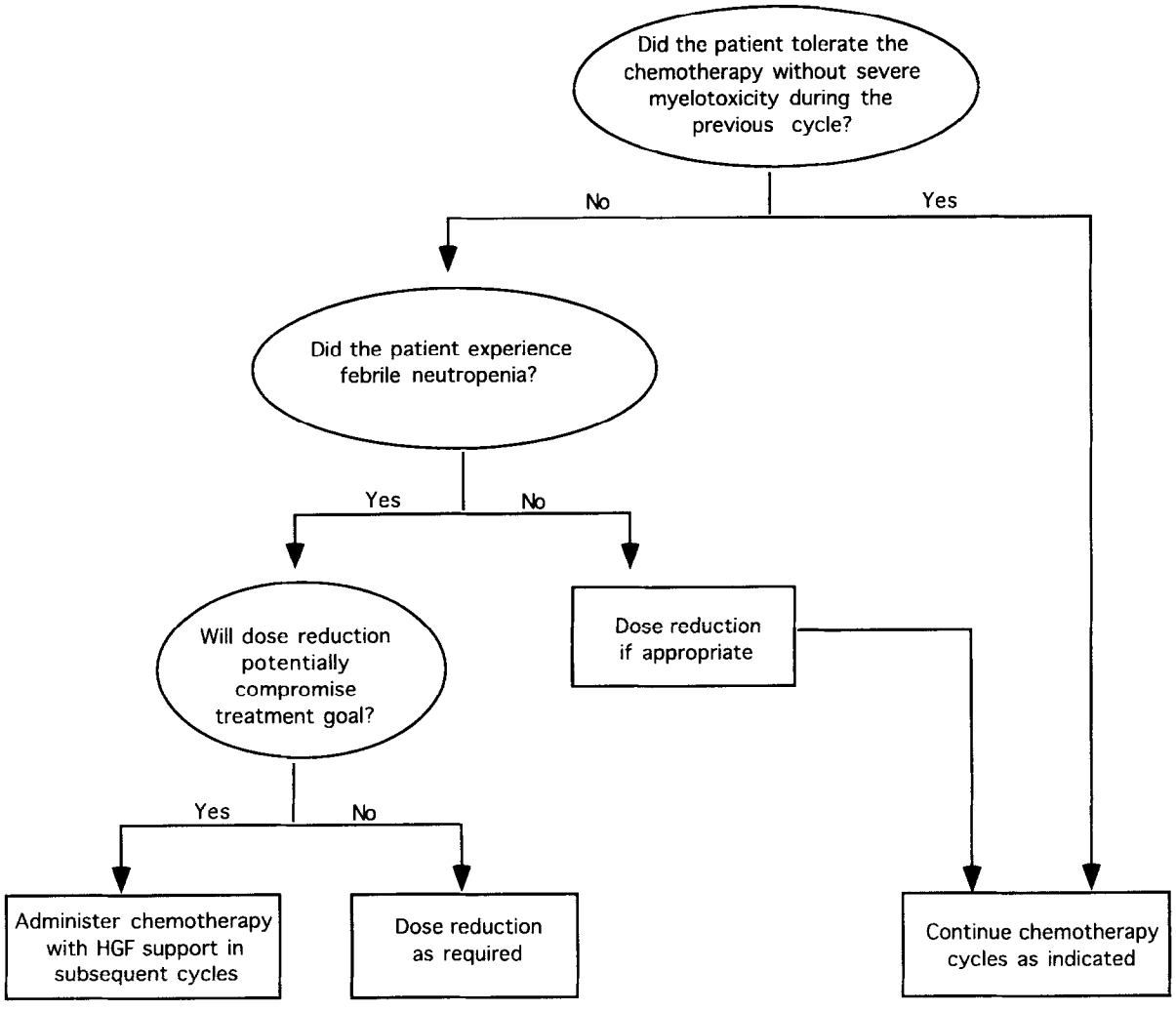

Figure 3. Administration of myeloid HGFs in the secondary prevention of febrile neutropenia.

Table 3. Number of observed episodes of febrile neutropenia and infectious mortality in studies using HGF in febrile neutropenia

References	HGF used	Number of observed patients with febrile neutropenia		Infectious mortality		
		HGF	placebo	HGF (%)	placebo (%)	*P*
Biesma [117]	GM-CSF	15	15	3	0	ns
Riikonen [118]	GM-CSF	28	30	0	0	ns
Maher [119]	G-CSF	109	107	7	3	ns
Mayordomo* [120]	G-CSF and GM-CSF	39 and 39	43	7 and 3	3	ns
Anaissie† [121]	GM-CSF	50	50	2	6	ns
Vellenga [122]	GM-CSF	65	69	1	2	ns
Collective analysis		345	314	4	2	ns

*Study with three arms (respectively G-CSF, GM-CSF and placebo). †Study without placebo control. ns, not significant.

the less lineage-restricted activity of GM-CSF might offer clinical advantages over G-CSF, especially in terms of antimicrobial activity. A review of the many trials in which these agents have been used in patients receiving cytotoxic chemotherapy does not seem to confirm such an advantage [126, 127]. Also in prospective trials in which patients with febrile [128] or non-febrile neutropenia [129] were randomly assigned to receive either G-CSF or GM-CSF, there has been no difference of efficacy. There have also been suggestions that GM-CSF might have a clinically meaningful thrombopoietic effect, but confirmation is lacking [130].

Another potentially important difference in the activity of these agents concerns their effects on the kinetics of haematopoietic progenitors. Both factors induce progenitors into cycle, but following cessation of GM-CSF, a phase of profound kinetic quiescence occurs during which the cells may be less vulnerable to chemotherapy [131]. The wider spectrum of biological activity of GM-CSF compared to G-CSF

raised the possibility that GM-CSF might have other meaningful clinical activities. There has been particular interest in the possibility that this agent might exert an independent antitumour effect. No such anticancer activity has been confirmed in the trials which have been performed [66]. The local administration of GM-CSF, but not G-CSF, was also shown to promote wound healing in a rat model [132].

G-CSF and GM-CSF exist in a glycosylated and a non-glycosylated form. Although the glycosylated and non-glycosylated forms of G-CSF have been shown to differ biologically *in vitro* [133], so far no clinical study has shown a relevant difference between the use of glycosylated versus non-glycosylated products.

Toxicity

When compared to other drugs in use in oncology, both GM-CSF and G-CSF have quite favourable toxicity profiles [126, 134]. G-CSF is a particularly well-tolerated agent. The only side-effect which has been reported frequently is bone pain with an incidence of 14% in patients treated with chemotherapy to 80% in donors for allogeneic transplantation [135–137]. Children who receive G-CSF for primary neutropenic disorders occasionally develop splenomegaly which may result in thrombocytopenia. Rarely, acute flare-ups of pre-existing inflammatory diseases can occur, as can Sweet's syndrome or acute neutrophilic dermatosis [138]. The pattern of toxicity of GM-CSF is more typical of that which has been reported for the administration of other cytokines, with fever and chills occurring relatively often and with occasional patients (especially at higher doses and with intravenous administration) developing pleural and pericardial effusions [139]. However, in one prospective randomised trial in which the administration of G-CSF was compared to that of GM-CSF, no differences in toxicity were reported [129].

THE IMPORTANCE OF DOSE IN CANCER CHEMOTHERAPY

Since many conventional chemotherapy regimens are given at, or close to, the maximum tolerated dose, the scope for dose escalation is limited by increased toxicity. The most common obstacle to more effective chemotherapy delivery is severe myelosuppression. Attempts to escalate the dose intensity of chemotherapy require effective haematopoietic support to ensure prompt restoration of normal haematopoiesis. Previously this has only been possible with bone marrow transplantation (BMT) restricted to specialised hospital units. The introduction of recombinant HGFs and in particular recombinant G-CSF and GM-CSF has provided haematologists and oncologists with powerful tools to manipulate haematopoiesis and thus be able to enhance the delivery of chemotherapy at standard or escalated doses [140]. It is difficult to predict whether increased dose intensity will produce better results [136].

Prospective randomised trials of dose intensity were obviously limited by myelotoxicity until the advent of recombinant HGFs. Nevertheless, at least two different questions can be asked in this context: (a) How does a reduction in standard-dose chemotherapy affect response rate and survival? (b) How does an increase in the dose of chemotherapy affect response rate and survival?

The first question is concerned with the concept of a 'threshold', or minimum effective dose. It is common practice in oncology to delay treatment or reduce dose after chemotherapy-induced toxicity, but no clinical trials have convincingly demonstrated that this option is without risk. Probably the first solid evidence for the existence of such a 'threshold' in the adjuvant treatment of breast cancer has come from a recent Cancer and Leukemia Group B (CALBG) study that enroled 1572 patients with stage II, node-positive breast cancer [141]. All patients received adjuvant CAF (cyclophosphamide, doxorubicin and 5-FU) chemotherapy every 4 weeks at one of three dose rates (mg/m^2) high (600/60/600), moderate (400/40/400) and low (300/30/300). Patients on the high-dose arm and the low-dose arm were treated with 4 cycles (4 months) of chemotherapy, and the dose of each drug given in the low-dose arm was exactly half that given to those in the high-dose arm. As a result, the cumulative dose of cyclophosphamide, doxorubicin and 5-fluorouracil given to patients in the low-dose arm was half that given to those in the high-dose arm. In contrast, patients randomised to the moderate-dose arm of the study received six cycles of chemotherapy, but the dose of cyclophosphamide, doxorubicin and 5-FU on each cycle was two-thirds that given to patients on the high-dose arm. As a result, the cumulative doses of each drug given to patients on the moderate- and high-dose arms were identical, but the rate at which the drugs were administered was faster for patients in the high-dose arm. At a median follow-up of 3.4 years, disease-free survival and overall survival were significantly better in the high-dose group compared with the low-dose group, although toxicity was also greater.

The second question that should be addressed by prospective studies is the one currently attracting more attention: does an increase in the dose of chemotherapy affect response rate and, above all, survival? Most oncologists feel that only if a clear benefit in terms of disease-free survival or overall survival can be proven, then these toxic and expensive therapies can be justified.

Haematological malignancies

High-dose chemotherapy seems to be justified in acute myeloid leukaemias (in first or second remission). For example, in a large multicentric randomised protocol of the EORTC and GIMEMA Cooperative Groups [142], designed to compare prospectively conventional intensive chemotherapy versus consolidation with autologous BMT (ABMT) or allogeneic BMT (allo BMT), at a median follow-up of 3 years, the results appeared satisfactory, with a disease-free survival (DFS) of the intensive chemotherapy arm (29% at 4 years) at least equivalent to the previous studies with less intensive regimens, and a DFS in the allo-BMT and ABMT of 54% and 49%, respectively. The two BMT arms gave significantly better results than the intensive chemotherapy arm ($P = 0.04$). The main reason for failure was relapse in both the ABMT and chemotherapy arm, while treatment-related mortality was higher in the allo-BMT arm.

Mounting evidence (including preliminary data from randomised studies) indicate that ABMT should be performed in relapsed chemosensitive Hodgkin's disease and NHL, or even up-front as consolidation of first remission in poor-prognosis NHL, and in some acute lymphoblastic leukaemias with poor prognosis features. For example, with conventional CHOP-like chemotherapy regimens or more intensive weekly regimens, the five-year survival rate for intermediate- and high-grade NHLs, a classical model of a chemosensitive and chemocurable disease, is only approximately 50%. Moreover,

the 10% of patients who fail to respond to initial therapy, or the 30% who relapse after complete remission, have an extremely poor prognosis with a DFS usually less than 10% at 3 years. Philip and associates [143] have recently presented the final analysis of a randomised study with 216 patients with relapsed intermediate or high-grade NHL. All patients in this multicentric study had previously a complete response (CR) to a doxorubicin-containing regimen, no neurological and bone marrow involvement and no major organ dysfunction. All patients received two courses of a semi-intensive regimen (DHAP chemotherapy) and 109 patients (with CR or a very good partial response) were randomised to receive either four further courses of DHAP plus radiotherapy to involved sites or radiotherapy plus high-dose chemotherapy (BEAC) with ABMT rescue. No difference in terms of prognostic factors at inclusion were observed between the two groups (LDH level, tumour size, histological groups). Four toxic deaths were observed in the BMT arm (out of 50 patients actually transplanted) and none in the chemotherapy arm. All patients were included in the final analysis and DFS was 46% in the BMT arm versus only 12% in the control arm at 5 years follow-up. Similarly, differences were also significant in terms of overall survival (OS): 53% versus 32% at 5 years. The authors concluded that high-dose chemotherapy and BMT should now be regarded as standard therapy in sensitive relapse of NHLs, but BMT is not able to salvage chemotherapy failures at subsequent relapse.

In multiple myeloma patients, the question of whether early intensive therapy with autologous stem cell support offers advantage over conventional treatment has been recently addressed by Attal and collegues [144]. In a randomised trial, melphalan 140 mg/m^2 plus TBI 800 cGy was compared with standard vincristine/melphalan/cyclophosphamide/prednisone–vincristine/carmustine/doxorubicin/prednisone (VMCP-VBAP) in 200 patients. The high-dose arm produced a higher complete response rate (22% versus 5%), longer EFS (27 months versus 18 months) and OS (probability of 5 year survival of 52% versus 12%), respectively.

Solid tumours

In numerical terms, the impact of high-dose chemotherapy is likely to be more important for some chemosensitive solid tumours and, in particular, for cancer of the breast. Following some pilot studies, several randomised studies are ongoing for 10 or more positive axillary lymph nodes and in some studies also patients with more than 4 to 10 positive lymph-nodes are included.

Five year follow-up is now available from at least two non-randomised studies: (1) Peters and associates [145], at Duke University, North Carolina, U.S.A. treated 85 patients with 10 or more axillary lymph nodes with adjuvant high-dose cyclophosphamide, cisplatin and carmustine (CPB) and autologous BMT after CAF adjuvant chemotherapy. The median age was 38 years, the median number of involved axillary lymph nodes was 14 and therapy-related mortality was 12%. Although the risks of comparison to historical controls have been discussed, the five-year DFS in this series was 71% (95% confidence interval: 53–84%), compared to only 28–34% in historical published studies. Differences were also statistically significant in terms of overall survival.

In a similar study, but using a novel high-dose sequential combination chemotherapy (HDS), Gianni and associates [146] reported only 1 procedure-related death (out of 67 patients) and a DFS at 5 years of 56% (overall survival 78%) versus 33–41% in the best historical series (overall survival 60%). If only patients with 10–15 axillary nodes were analysed and patients with more advanced disease were excluded, then the figures became 64% for DFS and 83% for OS in the HDS group.

Other potential but still speculative indications for dose-intensive chemotherapies in solid tumours and haematological malignancies are currently being tested in the clinic and include chronic myeloid leukaemia (CML) [147] and chronic lymphocytic leukaemia (CLL) in young patients [148], relapsed or poor-prognosis germ-cell cancers [149], several paediatric tumours (including stage IV neuroblastomas) [150], advanced epithelial ovarian cancer in young women [151], advanced melanoma and brain tumours. For some common epithelial cancers, like colorectal cancer, response rates but not survival have been increased by high-dose chemotherapy [152]. Similarly, more than 200 patients worldwide with limited stage small cell lung cancer (an example of a chemosensitive but not usually chemocurable tumour) have received high-dose chemotherapy with autologous BMT, but to date, median disease-free survival and overall survival have been comparable to those associated with standard chemotherapy [153]. Low-grade NHL is another example of an initially chemosensitive disease, where high-dose chemotherapy has not yet been shown to definitely improve survival [154].

For the time being, it seems reasonable to conclude on these grounds that HGFs may support the delivery of full-dose chemotherapy in chemosensitive malignancies and may prevent suboptimal treatment due to underdosing. This ought to be particularly important if the main objective is cure or long-term survival. Currently, use of these growth factors (with or without cellular support) in the context of high-dose chemotherapy should be limited to experimental protocols. This is also the opinion of the Accreditation Subcommittee of the European Group for Blood and Marrow Transplantation (EBMT) [155].

CONCLUSION

This review deals with the results of clinical studies with HGFs that have been registered for clinical application: erythropoietin (EPO) and the myeloid growth factors G- and GM-CSF. In responding patients, the use of EPO is able to increase quality of life in patients with chronic symptomatic anaemia of cancer by treatment or prevention of chemotherapy-induced anaemia. Furthermore, application of EPO is able to facilitate autologous blood donation in patients scheduled for elective surgery if their requirement of blood transfusions is highly probable, and in particular for Jehovah's witnesses and other persons who reject donations of allogeneic blood.

Currently, the major indications of G-CSF and GM-CSF are in the prophylaxis of neutropenic fever in patients who are treated with regimens which are likely to produce this complication, and in preventing the recurrence of neutropenic fever in patients who developed an infection in a previous cycle, and in whom dose reduction would be inappropriate. It has been shown in randomised studies that these drugs will increase the ability to administer chemotherapy without dose reductions and delays, which is probably important in the curative setting, but not crucial in the palliative setting. The phase III studies done in this context were not designed to

look for differences in survival but to detect differences in the frequency and severity of febrile neutropenia. Restriction of HGF administration to secondary prevention (e.g. following at least one episode of severe marrow aplasia) is usually recommended. Primary prophylaxis should only be considered in some cases, e.g. in patients with poor bone marrow tolerance. The routine use of HGFs in the treatment of established febrile neutropenia is not indicated. However, patients with life-threatening syndromes of neutropenic infections, e.g. septicaemia, bacterial pneumonia or with fungal infections, might benefit from their application. The use of HGFs in afebrile neutropenic patients has been investigated in at least one placebo-controlled randomised trial in several tumour types and lymphomas and, although it shortened the duration of severe chemotherapy-induced neutropenia, it did not impact on clinical outcome.

The use of HGF mobilised stem cells for stem cell rescue after high-dose chemotherapy has dramatically reduced the transplant-related morbidity. This may lead to an increased application in the treatment of chemosensitive solid tumours as well as haematological neoplasms. Results of randomised studies should be awaited before conclusions can be drawn on the efficacy of HGF support after PBSC reinfusion.

More research is warranted to improve the pharmacoeconomic evaluation in clinical practice. These ought to include not only cost-minimisation analysis (e.g. selection of the least costly intervention among those shown to be of equal benefit) or cost-effectiveness (e.g. comparison of interventions where benefit can be expressed in one common natural unit like life-years saved), but also cost-utility analysis (e.g. quality adjusted life-years) and cost-benefit analysis (comparison of interventions, where costs and benefits are measured in financial terms and net gains/losses can be calculated) [149].

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