Summary, discussion and future perspectives

The scope of this thesis is the use of hematopoietic growth factors (HGF’s) after ‘standard’ chemotherapy with the aim to ameliorate bone marrow suppression. The thesis is divided in two sections regarding interleukin-3 (IL-3) and interleukin-6 (IL-6) respectively.

**Interleukin-3.** In vitro, IL-3 stimulates the proliferation and differentiation of uncommitted and committed bone marrow precursors, which was found to result in the stimulation of myelopoiesis, erythropoiesis and megakaryopoiesis in vivo. It was the stimulation of thrombopoiesis which raised interest for the clinical use of recombinant human IL-3 (rhIL-3), with the aim to especially prevent chemotherapy induced thrombocytopenia. In chapter 1 an overview is given of the use of rhIL-3 in clinical oncology and the supposed role of endogenous IL-3 in allergic processes.

The occurrence of allergy-like side effects after rhIL-3 administration in various studies was the reason for a collaborative investigation of the Divisions of Medical Oncology and Allergy, of the University Hospital Groningen, into the cause of these phenomena, as reported in chapter 2. The role of IL-3 as a mediator in inflammation and allergic diseases is supported by numerous data from in vitro, pre-clinical and clinical work. Side effects frequently reported after rhIL-3 administration to humans are flu-like symptoms, fever, headache and myalgia. Less frequently, allergy-like side
effects such as facial erythema, urticaria, conjunctivitis, dyspnea and edema of the lips and eyelids were observed mostly after several courses of treatment. Furthermore, rhIL-3 induced an eosinophilia even when the myelosuppression of the preceding chemotherapy was still apparent. The objective of our controlled study was to evaluate the in vivo effects of rhIL-3 administration on eosinophil activation. This study was a part of a large randomized, multicenter, placebo-controlled trial, in which rhIL-3 or placebo was administered after carboplatin-cyclophosphamide based chemotherapy for ovarian cancer. Activation of eosinophils was determined by the fraction of hypodense cells, the CD11b expression and their capacity to express CD11b after priming with N-formyl-methionyl-leucyl-phenylalanine (fMLP) and platelet-activating factor (PAF). It was demonstrated that eosinophils were activated and primed in patients receiving rhIL-3. Whether this activation could result in the above mentioned allergy-like side effects remained unclear, as these allergic symptoms were not observed in the first course of treatment, but mostly after several courses of treatment.

In chapter 3 the results of a phase I study are reported in which rhIL-3 was administered subcutaneously to patients treated with cyclophosphamide and carboplatin for their ovarian cancer. In this study two dose levels of rhIL-3, 5 and 10 µg/kg/day, were compared to establish the optimal dose. Traditionally this chemotherapy regimen was administered every 4 weeks, but because of the supposed bone marrow stimulating effects of rhIL-3 the interval was reduced to 3 weeks. No significant differences were observed in hematopoietic effects between the two rhIL-3 dose levels used. However, it appeared that more patients could be treated on a three-weekly basis compared to an earlier treated group of patients treated with chemotherapy alone. It was evident that the 10 µg/kg/day rhIL-3 dose was associated with a higher frequency of side-effects, mainly due to flu-like symptoms. Therefore a dose of 5 µg/kg/day was recommended for a large randomized, multicenter, phase III trial.

The results of a combination of rhIL-3 and rhG-CSF vs just rhG-CSF, after a paclitaxel based chemotherapeutic regimen for ovarian cancer are reported in chapter 4. As the response rates in relapsed ovarian cancer tend to be low, there is a continuing search for more effective therapies in this group of patients. Paclitaxel is a
new and effective chemotherapeutic agent, which has recently become available for clinical use. It will inhibit the depolymerization of microtubuli formed during cell mitosis, which constitutes a unique cytotoxic mechanism and it appeared to be active in cisplatin resistant ovarian cancer. A phase III study in patients with primary ovarian cancer showed a substantial increase in median survival compared to a standard therapy [1]. However, a considerable fraction of patients will ultimately die of their disease. In an effort to improve response rates paclitaxel was combined with ifosfamide and cisplatin, supported by HGF. The purposes of this study were to evaluate toxicity and efficacy of the cytotoxic regimen and to evaluate whether a combination of HGF’s would allow synergistic hematopoietic effects after chemotherapy. The rationale for the combination of rhIL-3 and rhG-CSF is based on the premise that rhIL-3 stimulates the uncommitted precursors, thereby providing a substrate for the proliferative effect of rhG-CSF. A 67% tumor response was achieved in the evaluable patients, with substantial toxicity as a trade off. However, no additive or synergistic effects were observed for the combination of rhIL-3 and rhG-CSF compared to rhG-CSF alone, although there was a tendency for a faster platelet recovery.

The papers presented in this section permit the following conclusions. RhIL-3 administration resulted in eosinophil stimulation, supposedly related to the occurrence of allergy-like side effects found after prolonged rhIL-3 administration. The use of rhIL-3 after standard chemotherapy resulted in a shorter treatment interval. This allowed an increase in dose-intensity compared to historical controls, but confirmatory phase III trials are lacking at this moment. Finally, the combination of rhIL-3 and rhG-CSF did not result in additive or synergistic hematopoietic effects.

**Interleukin-6.** The reasons for the clinical interest in rhIL-6 are comparable to those for rhIL-3. In chapter 5 an overview is given of the use of rhIL-6 in clinical oncology. This pleiotropic cytokine can stimulate hematopoiesis. Its effects on stimulation of thrombopoiesis initiated research aiming at reducing chemotherapy induced thrombocytopenia. Anemia is a consistent finding when rhIL-6 is administered to animals and man and is probably partly due to hemodilution. Another aspect which raised cli-
nical interest is the tumor-cell growth inhibiting effects which were observed in some in vitro and pre-clinical experiments.

In chapter 6 the results of a rhIL-6 dose-finding study after chemotherapy are reported. This study was a sequel to the work published by van Gameren et al.[2] which concerned the administration of the same dose levels of rhIL-6 to the same group of patients before chemotherapy. Nineteen breast- or non small cell lung cancer patients received rhIL-6 in doses ranging from 0.5-20.0 µg/kg/day (in 6 dose levels) after mitoxantrone and thiopeta. Dose-limiting toxicity was observed at 20 µg/kg/day and consisted of headache, fever and nausea. At rhIL-6 doses of 10 and 20 µg/kg/day a faster platelet recovery was observed compared to the lower dose levels, without affecting platelet nadir.

The results of the administration of a combination of recombinant human granulocyte-macrophage colony-stimulating factor (rhGM-CSF) and rhIL-6 before and after chemotherapy are given in chapter 7. RhGM-CSF has stimulating activity on early as well as on late bone marrow progenitors. Based on in vitro and preclinical work it was hypothesized that combination with a late acting cytokine like rhIL-6 might yield hematopoietic synergism. In this study, seven patients were treated with a combination of rhIL-6 and rhGM-CSF before and after mitoxantrone/thiopeta chemotherapy. A historical group of patients treated identically with rhIL-6 only, served as controls. Before chemotherapy the activity of rhIL-6 and rhGM-CSF was evident, but following chemotherapy the combination did not result in enhanced hematopoietic recovery. Furthermore, the combination of rhIL-6 and rhGM-CSF was associated with a higher frequency of side-effects than rhIL-6 alone.

The last part of the second section is reserved for a case report (chapter 8). This case report demonstrates the similarity in symptoms in patients with Castleman’s disease and patients receiving rhIL-6. Castleman’s disease is a lymphoproliferative disorder of uncertain etiology. There is mounting evidence of the pathogenetic role of IL-6 in this disorder. Malaise, fatigue, low-grade fever, acute phase responses, hypergammaglobulinemia and anemia were observed in this patient and were thought to be associated with IL-6 overproduction of the involved lymph nodes. As to the cause of the anemia in this patient, which normalized after treatment, it was
considered the result of a hypoproliferative bone marrow, based on ferrokinetic studies, in the absence of a serum inhibitor and an increased plasma volume.

In conclusion, rhIL-6 administration induced a faster platelet recovery after chemotherapy, without affecting platelet nadir. The combination with rhGM-CSF proved to be ineffective and resulted in substantial toxicity. Prominent side-effects of rhIL-6 administration or overproduction of IL-6 are general malaise and anemia based on hemodilution with depressed erythropoiesis.

**Discussion and future perspectives**

The cytokines, rhIL-3 and rhIL-6, the subject of this thesis were only partially successful in ameliorating chemotherapy-induced thrombocytopenia and controlled phase III trials, have not been published until now. In the case of rhIL-6, it is remarkable that there is such a discrepancy between the in vitro effects and the in vivo data. An explanation could be a diminished biological availability. Clearance studies have demonstrated a rapid disappearance of injected IL-6 [3], which could be retarded by using polyethylene glycol conjugated IL-6 (pegylated IL-6), resulting in enhanced thrombopoiesis [4,5]. The marked thrombopoietic activity of pegylated IL-6 compared to native IL-6 merits further studies in man.

Among IL-3 and IL-6, other cytokines such as stem cell factor (SCF) or c-kit ligand, IL-11 and leukemia inhibiting factor (LIF) affect thrombopoiesis. However, in vitro, none of these cytokines or combinations were able to fully develop platelets from megakaryocyte progenitors. In this respect, the recently discovered c-Mpl ligand [6-9], thrombopoietin (TPO), may be a major breakthrough. Several studies suggest that TPO is the primary regulator of thrombopoiesis, and that it supports the proliferation, differentiation, and maturation of megakaryocytes (MK) and their precursors, resulting in their terminal fragmentation into platelets, as reviewed by Kaushansky [10]. Due to complex interactions, in vivo, between bone marrow cells and constitutive and inducible cytokines, it is difficult to analyze the individual contribution of various cytokines to thrombopoiesis. Therefore, sophisticated in vitro
systems, to minimize external factors, have been used to test the functional capacity of individual or combinations of cytokines. In one of these studies, blocking TPO-activity virtually eliminated MK despite the presence of IL-6, IL-11 and c-kit ligand. IL-3 supported the proliferation and the initial stages of MK-differentiation in the absence of TPO, but full maturation was dependent on TPO [11]. In another in vitro study with highly purified CD 34+MK progenitors, TPO was the principal factor controlling the proliferation and maturation of MK, however, the availability of IL-3, IL-6 and SCF appeared to be critical. Interestingly, IL-3 inhibited both the maturation and polyploidization of MK induced by TPO, thus conserving the immature MK-compartment [12]. Although there are strong arguments which favor the idea that thrombopoiesis is fully dependent on TPO, this is still unclear. TPO-receptor (Mpl-R) deficient mice have their megakaryocyte and platelet levels reduced to 15% compared to controls. Suggesting that other pathways to produce platelets may exist [13], IL-3 in combination with IL-6 or more likely IL-11 are the possible candidates for an alternative pathway [10].

These and other in vitro results paved the way for pre-clinical studies, demonstrating evident thrombopoietic activity of TPO in a mouse model and in non-human primates respectively before and after chemotherapy [14,15]. To overcome problems with the stability of TPO, biochemically modified recombinant human TPO, or megakaryocyte growth and development factor (MGDF Amgen), is currently used in man. The first studies in man have confirmed the thrombopoietic activity of MGDF [16] even after chemotherapy [17]. The results of these studies are rewarding, but controlled trials using more thrombotoxic regimens are needed to establish the true value of MGDF. In addition, unwanted proliferative effects of TPO/MGDF in hematological as well as in solid tumors should be looked for carefully, as acute myeloblastic leukemia cells appeared to proliferate in response to TPO [18].

In the near future, pending phase I and II trials with MGDF, combinations with other thrombopoietic cytokines like rhIL-3 or a myeloid stimulating cytokine may be worthwhile to consider. Combining rhIL-3 and MGDF would be interesting due to the fact that rhIL-3 generates immature MK-progenitors, which could serve as a substrate for MGDF. Combinations with rhGM-CSF or rhG-CSF may be interesting in avoiding both chemotherapy induced thrombocytopenia and neutropenia. In
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myelosuppressed mice the combination of TPO and G-CSF has demonstrated synergism with respect to neutrophil recovery [19], and GM-CSF has augmented the stimulation of TPO on both thrombocytes and reticulocytes [20].

Despite the pivotal role of TPO in thrombopoiesis, it remains to be awaited whether significant chemotherapy dose intensification will be achieved with the use of this cytokine alone or in combination after chemotherapy. Based on the results with other hematopoietic growth factors, it might be expected that the impact of such an increase in dose intensity will probably be too small to have significant effects on survival. Consequently, other methods to increase chemotherapy dose intensity will remain necessary. In this light, the role of HGF’s in dose-intensive chemotherapy will be briefly discussed next, as this may have important implications for the near future.

The clinical value of dose-intensive chemotherapy in solid tumors is still largely unknown. Various publications have demonstrated increased response rates and better overall survival for certain tumor types [21-23], whereas others found no benefit [24,25]. Escalation or intensification of chemotherapy dose beyond the ‘standard’ dosing range requires effective hematologic support. This was initially based on autologous bone marrow transplantation and is currently rapidly replaced by peripheral stem cell autotransplantation. The mobilisation of peripheral stem cells commonly exploits the combination of postchemotherapy rebound and HGF support. The use of HGF’s in this respect is different compared to the use described in this thesis, which aimed at preventing myelosuppression after chemotherapy. The most extensively studied mobilizing HGF’s are rhGM- and especially rhG-CSF, which may induce up to a 100 fold increase in peripheral progenitor cells [26,27]. However, there are indications that combination with a more early acting cytokine such as rhIL-3 yields a higher number of peripheral progenitor cells, detected as CD34+ cells [28,29]. The effects of rhIL-6 in this respect proved to be disappointing, as only minor increases in peripheral progenitors were observed compared to rhGM- and rhG-CSF [30]. Preliminary results indicate that MGDF may also enhance the mobilisation of pluripotent peripheral blood progenitor cells by chemotherapy and rhG-CSF [31].

Mobilisation of peripheral blood progenitors requires the passage of a large volume of blood for leukopheresis, inherently carrying the risk of tumor cell contamination.
A method to minimize this risk is to stimulate the proliferation of these blood progenitor cells ex-vivo with the use of HGF’s, coming from a relatively small volume of blood. A pilot study has demonstrated its feasibility after high-dose chemotherapy and controlled studies are now necessary to assess its value [32,33].

The role of HGF’s in the hemopoietic recovery after high dose chemotherapy is still unclear, as various studies have provided contradictory results [34-36]. However, the combined use of rhG-CSF and rhIL-3 resulted in an increased proliferation and concentration of bone marrow progenitors [37] and subsequently demonstrated an accelerated hematopoietic recovery compared to rhG-CSF alone [38].

Based on phase I-II trials the thrombopoietic effects of rhIL-3 and rhIL-6 after standard chemotherapy are limited to a faster platelet recovery, without affecting the platelet nadir. Although as yet no phase III data are available, a closer adherence to standard chemotherapy schedules will probably be the best achievable result of the prophylactic use of rhIL-3 and rhIL-6. However, the modification of cytokines, like pegylation, may be a new approach to improve the early results. Regarding the use of HGF’s in harvesting peripheral blood progenitors, the combination of rhIL-3 with a myeloid HGF appeared to be superior to harvesting following a myeloid HGF alone. Finally, ex-vivo generation of peripheral blood progenitors is a new way of providing hematologic support after myeloablative chemotherapy, in which existing and new HGF’s will play a crucial role.

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