Hematopoietic effects of recombinant human interleukin-3 and interleukin-6

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Chapter 1

Interleukin-3: its role in the physiopathology of allergy and clinical use in oncology

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Abstract

Interleukin-3 (IL-3) is a hematopoietic growth factor with multilineage stimulatory activity in vitro. In addition IL-3 displays a broad range of effects on mature cells especially during allergic processes. With regard to its supposed stimulatory effect on platelets, recombinant human (rh) IL-3 is now evaluated in the clinic without and after standard chemotherapy. Also in vivo a multilineage effect is observed, with in some studies an increase in leucocytes, neutrophils, eosinophils, monocytes, reticulocytes and platelets. RhIL-3 can reduce the chance of chemotherapy postponement due to insufficient bone marrow recovery. The elimination half life after subcutaneous treatment is 2.15-4.8 hours with excellent bioavailability. The data of rhIL-3 after bone marrow transplantation are currently too preliminary to draw firm conclusions. Phase-III-studies to clarify this and other questions are ongoing.

Introduction

Interleukin-3 (IL-3) is a glycoprotein that stimulates the proliferation and differentiation of multipotent as well as committed progenitors of the various hematopoietic lineages [1,2]. In the body IL-3 is produced by T-lymphocytes, natural killer cells, mast cells and eosinophils [3-8]. The gene for IL-3, which is located on the long arm of chromosome 5, was cloned in 1988 [9,10]. The human IL-3 receptor (IL-3R) consists of the alpha and common beta (beta c) subunits [11]. Beta c, which does not bind IL-3 itself, forms a high affinity receptor with IL-3R alpha [12,13]. The respective receptors for IL-5 and granulocyte-macrophage colony-stimulating factor (GM-CSF) also contain the high affinity beta c receptor [13]. No IL-3 is detectable in circulation in humans under normal conditions. After chemotherapy IL-3 was observed in circulation, which correlated with platelet counts [8]. In vitro IL-3 promotes the survival, proliferation and differentiation of multipotent hematopoietic stem cells and of the committed progenitor cells of the megakaryocyte, granulocyte/macrophage, erythroid, eosinophil, basophil and mast cell lineages [1,2,9,10]. Furthermore it has been shown to be a potent stimulator of megakaryopoiesis in vitro [14-16].

This article mainly highlights the role of rhIL-3 in clinical oncology. However, preclinical data concerning the role of IL-3 in allergies are also being reviewed for general purposes, i.e. reporting on its role in human disease and especially with regard to observed/supposed side effects of rhIL-3 when used therapeutically.

Interleukin-3 and allergies

A great deal of research has been performed on the role of IL-3 in allergic diseases. Allergic reactions can be divided into two phases, both of which are under control of cytokines produced by T helper lymphocytes of the Th_2 phenotype. The first phase is the immediate type of reaction, in which histamine and other mediators are released from mast cells via cross-linking of IgE-receptors [17,18]. The second phase, the so-called late reaction, occurs 5 to 8 hours later and is characterized by the appearance of

eosinophilic granulocytes [19-21]. Over the past few years, evidence for the role of IL-3, IL-4, IL-5 and GM-CSF in both phases of allergic reactions has been increasing. T cell clones of atopic subjects, for instance, have shown to produce predominantly these Th_2-type cytokines [22]. The role of IL-3 in this area will now be considered in more detail.

The production of IL-3 in vivo during allergic inflammation has been suggested in some recent studies. In a study by Kay and coworkers, skin biopsies from human allergic subjects were hybridized with ^{35}S-labelled RNA probes for a number of cytokines [23]. After an allergen-induced late reaction, mRNA for IL-3 was detected indicating that after a late allergic reaction IL-3 is produced. In another study from the same group, it was shown that in subjects with mild allergic asthma, the number of cells positive for mRNA for IL-3 in bronchoalveolar-lavage was increased when compared to control subjects [24]. In addition, one of the eosinophil lifespan-extending factors, present in T cell supernatants and sera of allergic asthmatics was identified as IL-3 [25]. In vitro, it has been shown that IL-3 can augment the spontaneous IgE synthesis by isolated B cells from allergic subjects [26]. The role of IL-3 in allergic diseases is further supported by its in vitro effects on eosinophils and basophils.

Basophils are postulated to be the circulating effector cells of allergy. Comparable to mast cells, they can be triggered by cross-linking of surface IgE-receptors [18]. Analysis of mediator releasing during allergic reactions in rhinitis patients, for instance, has revealed that basophils and not mast cells are activated during the late phase response [27]. IL-3 has been shown to directly cause the release of histamine from basophils of allergic asthmatic subjects [28]. In a subset of subjects where this did not occur, it was possible to enhance the histamine-releasing effect of anti-IgE with low concentrations of IL-3 (0.3 and 3 ng/ml). These direct and indirect releasing activities of IL-3 did not coexist in basophils of a single donor. The direct releasing activity of IL-3 may be dependent on the presence of a subset of IgE that supports release by histamine-releasing factors [29]. This was demonstrated by passively sensitizing basophils from IL-3-nonresponders with this particular type of IgE which could help to explain the rather erratic in vivo results with IL-3. After sensitization, these basophils did respond to IL-3. The enhancing effect of IL-3 has also been

observed in combination with the complement component C3a, C5a, f-methionyl-leucyl-phenylalanine (fMLP), calcium-ionophore A23187 and IL-8, which do not induce histamine-release by themselves [30-33]. Also, basophils have shown to produce leukotriene C4 upon stimulation with C5a and IL-8, only after pre-incubation with IL-3 [30,33]. These and other studies indicate that IL-3 renders the basophil susceptible to IgE-independent stimuli for the release of histamine and leukotrienes. Histamine release by IL-3 alone may be possible only after sensitization with a subset of IgE which would help to explain the rather erratic in vivo results with IL-3. Furthermore, recruitment of basophils to sites of allergic inflammation may also be increased by IL-3, mediated via the up-regulation of the adhesion molecule CD11b, the α-chain of the complement receptor type 3 (mac-1) [34].

Eosinophils migrate to the site of allergic inflammation during the late reaction. The appearance of eosinophils after a late phase allergic response was shown in a number of studies [20,21,35,36]. During migration these eosinophils become activated and may release superoxide anions, leukotrienes, platelet-activating factor (PAF), and enzymes such as eosinophil derived neurotoxin (EDN), eosinophil peroxidase (EPO), and major basic protein (MBP) [35,37-39]. These substances may subsequently damage tissues and thus cause clinical symptoms [39,40]. IL-3 may affect eosinophils during infiltration and activation. Production of IL-3 at the site of allergic inflammation may help attract eosinophils from the blood stream. Although IL-3 itself is not a strong chemoattractant for eosinophils, picomolar concentrations are enough to enhance the chemotactic effect of PAF, fMLP and IL-8 [41]. Furthermore, the eosinophils of allergic asthmatic individuals exhibit an increased chemotactic response towards PAF, which resembles the response of in vitro primed eosinophils of non-allergic control subjects [42-44]. In a study by Moser et al., the migration of human eosinophils across cytokine-activated endothelial cell monolayers was found to be increased after incubation with IL-3 [45]. In the same study, it was found that the migration of eosinophils from subjects with allergic asthma was increased when compared to eosinophils from control subjects. Infiltration of tissue involves adhesion of eosinophils to endothelial cells [46]. From in vitro experiments it has become clear that IL-3 is able to increase the expression of adhesion-molecules such as CD11b and Intercellular Adhesion Molecule 1 (ICAM-1)

on the surface of eosinophils [47-49]. In addition, it has been found that, in contrast to eosinophils from control subjects, eosinophils from allergic patients show an increased expression of CD11b after stimulation in vitro or after allergen exposure [50,51].

Furthermore, IL-3 is able to enhance the production of tissue-damaging substances. In vitro, IL-3 primes the respiratory burst, degranulation and leukotriene C4 production of eosinophils, probably via induction of tyrosine kinase activity [52-55]. Enhanced functional responsiveness of eosinophils after exposure to IL-3 has also been found with respect to the killing of Candida albicans and antibody-coated Schistosoma mansoni larvae [52,54]. In a recent study, IL-3 was shown to induce the expression of CD69. CD69 triggers aggregation and mediator release of platelets, and is therefore a possible receptor for eosinophil activation [56]. Furthermore, eosinophils incubated with IL-3 sediment at low density when centrifuged on a discontinuous gradient of Percoll [54-57]. This so-called hypodense phenotype is characteristic for activated eosinophils [58].

A number of parameters of eosinophils from non-allergic subjects, which are stimulated in vitro by IL-3, have found to be increased in peripheral eosinophils of allergic patients without prior incubation. This indicates an in vivo exposure of these eosinophils to cytokines such as IL-3. Functional parameters such as respiratory burst, degranulation and leukotriene production by eosinophils of allergic patients have found to be enhanced [59-61]. Furthermore, a number of studies have indicated that eosinophils of allergic individuals are hypodense, although the studies on this subject are controversial [62-66]. This controversy is possibly caused by patient selection and differences in isolation procedures [66]. After infiltration and activation of eosinophils in allergic tissue, IL-3 may prolong the survival of eosinophils, which may enable them to persist in the tissue for a longer period [54,57]. These survival-enhancing properties have been detected in sera and T cell supernatants form allergic asthmatic subjects [25]. The described effects of IL-3 may be amplified by the fact that eosinophils and mast cells are capable of producing IL-3, although this has to be further evaluated [5,7,67].

Interleukin-3 and hematopoiesis

Potential interest for the clinical use of recombinant human IL-3 (rhIL-3) was especially raised by the fact that IL-3 is a potent stimulator of megakaryopoiesis in vitro and therefore might be interesting to prevent trombocytopenia [14-16]. IL-3 has been expressed in various systems such as mammalian cells, yeast, B. licheniformis and E. coli.

Studies with rhIL-3 in animals. Studies in murine and primate models with rhIL-3 showed an effect on myelopoiesis, megakaryopoiesis and erythropoiesis [68-71]. There was also a clear effect on basophils and eosinophils [72,73]. When IL-3 was administered for 7 days followed with GM-CSF for 4 days, a pronounced effect was observed on leucocytes with an increase in neutrophils, banded neutrophils, eosinophils, lymphocytes, monocytes, and basophils [72]. In primates the sequential administration of IL-3 and IL-6 resulted in an increased effect on trombopoiesis [74]. The effect of rhIL-3 after chemotherapy consisting of cyclophosphamide and 5-fluorouracil was evaluated in primates [75]. A higher neutrophil nadir count and a reduced period of neutropenia was observed. During white cell recovery there was a pronounced eosinophilia and basophilia. Due to the large variation in platelet recovery in the control animals the effect on platelets was difficult to interpret. Side effects were facial and extremity swelling as well as a pruritic rash. In mice IL-3 was combined with IL-6 after treatment with 5-fluorouracil [76]. This combination showed a synergistic effect on platelet recovery.

Studies with rhIL-3 in humans. The initial studies with IL-3 alone in humans were performed especially in patients with impaired bone marrow function due to e.g. aplastic anemia and myelodysplastic syndrome [77-81]. They therefore do not necessarily all show the full potential of this cytokine. In studies with subcutaneously (sc) administered IL-3, from the second week an increase in leucocyte and platelet counts was observed. The neutrophil increase was dose dependent and in this setting

for platelet effects 250 μg/m^2/day and 500 μg/m^2/day were equally effective. In a phase I study in which rhIL-3 was administered as a daily 4-hour intravenous (iv) infusion, no clear dose response effect was observed [81]. Aglietta et al. administered rhIL-3 to chemotherapy naive patients with neoplastic disease and normal hematopoiesis for seven days sc at doses of 0.25-10 μg/kg/day [82]. In this study no effect was observed on peripheral blood counts on day 7 for platelets, erythrocytes, neutrophils, or lymphocytes. However, a mild monocytosis and basophilia (day 7 at 10 μg/kg/day maximum value 157 % compared to baseline value) occurred. After an initial decrease of eosinophils during the first hours after start of treatment there was a 460% increase of eosinophils compared to baseline values at 10 μg/kg/day. The fact that only effects were observed for certain cell types may be due to the relatively short treatment period and the fact that no data are supplied for the period after day 7. In the same setting however, D'Hondt et al. recently reported that they observed in patients with small cell lung carcinoma, treated with rhIL-3 continuously iv for 7 days during the prechemotherapy period, an effect on day 8 on leucocytes, neutrophils, monocytes and eosinophils [83]. They observed a significant dose dependent effect on platelet counts on day 15.

RhIL-3 was sc as well as iv in general well tolerated up to a dose of 250 μg/m^2/day or 10 μg/kg/day. Side effects were fever, headache and neck rigidity. Less frequently were observed, chills, flushing, bone pain, rash, nausea vomiting and peripheral edema. Dose limiting toxicity was reported at 500 μg rhIL-3 /m^2/day due to severe headache, neck rigidity, bone pain, decrease in platelet counts, vomiting, nausea and recurrent urticaria. RhIL-3 administered as iv bolus injection is reported in the literature with a dose of 125 μg/m^2/day. This caused transient acracyanosis and chills in all three patients. A phase-I-study has been performed in which rhIL-3 was administered for five days followed by rhGM-CSF for 10 days [84]. This combination showed no superior effect on myelo- or trombopoiesis compared to rhGM-CSF or rhIL-3 alone. The fact that rhIL-3 was administered for a relatively short period may be the reason that the effect on platelets was missed.

Since 1992 a number of studies have been published on rhIL-3 administered after chemotherapy. Three full papers have been published on ovarian carcinoma patients. The fact that especially the bone marrow toxic chemotherapeutic drugs carboplatin

and cyclophosphamide are relevant drugs for this tumor type makes this setting relevant for a potential application of rhIL-3. Twenty chemotherapy-naive patients with advanced ovarian cancer eligible for treatment with six cycles carboplatin-cyclophosphamide every four weeks (day 1) were studied [85]. Five patients received 1, 5, 10 or 15 µg/kg/day rhIL-3 during seven days (days 5-11) in cycles 1, 3 and 5 by continuous iv infusion or once daily sc administration. In control cycles 2, 4 and 6 no rhIL-3 was administered. RhIL-3 increased the recovery of leucocyte, neutrophil and platelet counts, especially at 5, 10 and 15 µg/kg rhIL-3 and also increased basophil, eosinophil, monocyte and lymphocyte counts at these dose steps. Effects on reticulocytes were limited. No difference in efficacy between sc and iv rhIL-3 treatment was found. Less chemotherapy postponement for insufficient bone marrow recovery was necessary after cycles during which IL-3 was administered then after control cycles. Platelet transfusions were required in 7/45 control cycles versus 3/50 rhIL-3 cycles. RhIL-3 up to doses of 10 µg/kg/day could be administered without severe side effects. At 15 µg/kg/day rhIL-3 headache was dose-limiting. In these patients a pharmacokinetic analysis was performed. Mean steady-state concentrations (C_{ss}) during continuous iv infusion ranged from 183 pg/ml (1 µg/kg/day) to 2214 pg/ml (15 µg/kg/day) and were linearly related to dose. The total body clearance was 5 ml/min/kg. Elimination $T_{1/2iv}$ was in the range of 0.43-0.88 hours. Following sc injection, the maximum rhIL-3 plasma concentration ranged from 206 pg/ml (1 µg/kg/day) to 6930 pg/ml (15 µg/kg/day). Elimination $T_{1/2sc}$ ranged from 2.15-4.8 hours. Based on trough levels of the seven days sc course, no accumulation occurred. Bioavailability of sc administered rhIL-3 was nearly 100%. It was concluded that the iv route of administration appears to have no advantages over the sc route. Furthermore, in future clinical trials twice daily sc administration of rhIL-3 should be considered [86]. Based on the above data a study, in which 17 patients were entered, was designed to determine if rhIL-3 would allow chemotherapy administration every three weeks [87]. Cyclophosphamide was administered in a dose of 750 mg/m^2 and carboplatin was dose adjusted to creatinine clearance: 60-80 ml/min: 257 mg/m^2, 80-120 : 300 mg/m^2, 120-140: 340 mg/m^2, >140: 385 mg/m^2 in 6 cycles. RhIL-3 (5 or 10 µg/kg/d) was given sc day 2-11 in each cycle. Urticaria occurred at 5 µg rhIL-3 in two patients and at 10 µg in five patients. In four episodes dyspnea and/or oedema was

observed. These reactions occurred during cycle 3-6 and was controlled with antihistamine and prednisolone. No platelet transfusions were required. In 61% of cycles it was possible to give a chemotherapy dose intensification of 33%. Currently a large phase-III-study is ongoing in which first line carboplatin and cyclophosphamide are administered every three weeks with or without rhIL-3 support. Rusthoven et al. administered rhIL-3 days 2-9 in cycle one or two consisting of carboplatin (350 mg/m^2) every 28 days [88]. The maximum tolerated dose was 250 µg/rhIL-3/m^2/day. They observed no beneficial effect on blood counts. The fact that monotherapy was used and that the dose of carboplatin used in this study was relatively low might be the reason why no protective effect was observed. Dercksen et al. treated 15 patients with high dose monotherapy of carboplatin (800 mg/m^2) every 28 days [89]. Starting with the second cycle, 5 µg/kg/day rhIL-3 was added to this treatment, with different timing and duration of rhIL-3. A total of 27 cycles were completed in 12 patients. RhIL-3 in the second cycle significantly reduced the neutrophil nadir and the duration of neutropenia. Less platelet transfusions were required after the second cycle with rhIL-3 than in the first cycle without rhIL-3.

There are three full papers published on the addition of rhIL-3 to the chemotherapy of patients with small cell lung cancer (SCLC). We have treated 15 relapsed SCLC patients with (B. licheniformis-derived) rhIL-3 (1-16 µg/kg/day sc) following chemotherapy [90]. The compiled data from 8 and 16 µg/kg demonstrated that the recovery of leucocytes, neutrophils and platelets was accelerated compared to a previous control cycle. D'Hondt et al. performed a study with rhIL-3 in patients with SCLC treated with carboplatin, VP16-213 and epirubicin [83]. After the first chemotherapy course no rhIL-3 was administered and after the second course seven days rhIL-3 (dose range 0.25- to 10 µg/kg/day) or placebo was administered iv to a total of 28 patients. Following the second cycle of chemotherapy, the platelet recovery was faster than after the first course and there was less chemotherapy postponement due to myelotoxicity. In another study 12 patients with small cell carcinomas of different origin were treated with rhIL-3 at 0.125, 5 and 7.5 µg/kg/day sc after the second but not after the first cycle of carboplatin, ifosfamide and VP16-213 [89]. In the eleven patients in which the hematopoietic effects of the first cycle could be compared with the second cycle no benificial effect of rhIL-3 was observed.

Gianni et al. [91] treated 22 previously untreated breast carcinoma patients with high-dose cyclophosphamide (7 g/m^2/day). The rhIL-3 doses used were 1, 2.5, 5 and 10 µg/kg/day administered iv. The doses up to 5 µg/kg/day were acceptable, at 10 µg/day nausea, vomiting and headache were dose limiting. At 5 µg/kg/day rhIL-3 accelerated granulocyte, platelet and reticulocyte recovery compared to matched historical controls treated without cytokine infusion. Compared to the historical control patients, fewer red blood cell transfusions were required. Substernal pain clearly related to rhIL-3 infusion was reported as the only worrisome toxicity in two patients at 2.5 and 5 µg/kg/day.

These early phase-I/II-studies demonstrated that a dose of 5-10 µg/kg/day rhIL-3 can affect the duration of chemotherapy-induced neutropenia and thrombocytopenia. However, the effect on the neutrophil and the platelet nadir counts seems limited as could be expected from the early phase I/II trials without chemotherapy demonstrating a relatively late effect on the leucocyte and platelet counts. Therefore, depending on the chemotherapy schedule, rhIL-3 may be of greater value in reducing the duration of myelosuppression, rather than in elevating the nadir counts. If the interval between chemotherapy courses is too small and rhIL-3 exposure too short observe an IL-3 effect.

Side effects of rhIL-3 after chemotherapy in the effective dose range were frequent. Both the toxicity profile and the incidence of side effects were very similar to those in the phase-I/II-studies with or without chemotherapy. This is underscored by the fact that in the only study in which patients were treated with rhIL-3 before and after chemotherapy no difference in toxicity was observed in the two settings. Variations in the different studies may also be influenced by the disease of the patient and the route of administration. Theoretically it can be speculated that during treatment of chemotherapy-induced myelosuppression, rhIL-3 may enhance the functions of eosinophils and basophils. The increased mediator release of these cells may cause damage of normal tissue, as is seen in allergic diseases. Observed side effects in phase-I/II-studies such as rash and facial erythema may be caused by local mediator release induced or enhanced by rhIL-3. However, there was no increase of urinary histamine metabolites found during treatment [78,86]. In contrast, release of leukotrienes in tissues is supported by the fact that during rhIL-3 treatment an increase in urinary

leukotrienes has been found to occur, before peripheral leucocytes increased [92]. Understanding of the mechanisms that cause the release of mediators can help to avoid unnessary toxicity of rhIL-3. Due to its pleitropic effects the range of observed side effects of rhIL-3 is larger than reported for rhG- and rhGM-CSF [93].

The effect of rhIL-3 as well as rhGM-CSF after high dose cyclophosphamide (7 g/m^2) on hematopoiesis and microenviroment in human bone marrow was studied by Orazi et al [94]. Patients received 1-5 µg/kg/day IL-3 iv for 14 to 18 days or GM-CSF 5.5 µg/kg/day for 14 days. Effects were evaluated versus treatment with cyclophosphamide alone. Bone marrow (BM) cellularity and myeloid/erythroid ratio were lower in the IL-3 treated group than in the rhGM-CSF treated group, but it was higher in both groups than in the control group. Frequency of $CD34^+$ BM cells was unchanged after rhIL-3 and decreased after rhGM-CSF. The combination of rhIL-3 followed by rhGM-CSF was also studied in patients on chemotherapy treatment [95,96]. Brugger et al. [95] treated cancer patients with chemotherapy followed by rhIL-3 (250 $\mu g/m^2$/day on days 1-5 sc) and rhGM-CSF (250 $\mu g/m^2$/day on days 6-15 sc). Results were compared with patients given rhGM-CSF after chemotherapy and patients treated with chemotherapy only. The patients treated with a hematopoietic growth factor demonstrated a faster neutrophil recovery compared to patients not treated with a growth factor. There was no difference in neutrophil recovery between the rhIL-3 plus rhGM-CSF group and the rhGM-CSF group. In general, the platelet recovery was not hastened by the administration of either the combination of rhIL-3 plus rhGM-CSF or rhGM-CSF alone, but accelerated platelet recovery occurred only in a few intensively pretreated patients.

Effect of rhIL-3 on bone marrow composition and peripheral stem cell harvesting. Treatment of patients with rhIL-3 without chemotherapy resulted in bone marrow cell proliferation by increasing the percentage of bone marrow progenitors in S-phase. The most sensitive progenitors were the megakaryocyte progenitors [82]. The maximum value of peripheral blood colony forming units occurred after seven days of rhIL-3 on day 12 since start of rhIL-3 treatment for CFU-GEMM, and BFU-E and day 15 for CFU-GM [83]. Probably rhIL-3 may on its own not

be able to give sufficient peripheral stem cell increase for a stem cell transplantation in every setting. After chemotherapy rhIL-3 combined with e.g. GM-CSF resulted in more progenitor cells than with GM-CSF alone [97].

RhIL-3 and bone marrow transplantation. Only preliminary data from studies with rhIL-3 alone [98] or combined with G-CSF or GM-CSF after bone marrow transplantation are available [99,100]. It is currently difficult to draw conclusions considering the potential role of IL-3 in this setting. Data from randomized phase-III-studies are eagerly awaited.

The duration of IL-3 administration in some studies was relatively short. In view of its delayed hematological effects, combinations with other growth factors may be more effective. The fact that fever was a side effect in studies with chemotherapy without bone marrow reinfusion might be a complicating factor in the transplant situation. Crump et al. [101] used in patients with delayed engraftment after autologous bone marrow transplantation IL-3 and in some patients together with GM-CSF. They observed only limited benefit, namely a transient increase in neutrophils and eosinophils.

RhIL-3 effects on tumor cells. In vitro IL-3 can stimulate proliferation of malignant cells. One out of 11 small cell lung carcinoma cell lines was stimulated by rhIL-3. This cell line also had receptors for IL-3 [102]. Also in other solid tumor cell lines effects are observed [103,104]. RhIL-3 is known a stimulator for myeloid leukemic cells. This aspect is used as stimulatory factor to induce S-phase in these cells and thus make them more sensitive for chemotherapy [105-107].

Conclusion

IL-3 is a hematopoietic growth factor with multilineage activity. These effects are also observed after treatment with rhIL-3 with or without chemotherapy. Its exact role for the clinic is currently further elucidated with the help of phase-III-studies.

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