Upsala J Med Sci 101: 121–148, 1996

# Hematopoietic Effects and Clinical Application of Granulopoietic Growth Factors

Martin Höglund

*Department of Internal Medicine, University Hospital, Uppsala, Sweden*

## INTRODUCTION

Granulocyte colony-stimulating factor (G-CSF)[1] regulates the production of neutrophil granulocytes, whereas granulocyte-macrophage colony-stimulating factor (GM-CSF)[2] stimulates multiple lineages of hematopoiesis including neutropoiesis and eosinopoiesis. Both these hematopoietic growth factors (HGF) have complex and yet not fully characterised effects on granulocyte function. G-CSF and GM-CSF are used to reduce chemotherapy related neutropenia and to mobilise blood progenitor cells (BPC) for autologous transplantation[3]. Recently, G-CSF has also been administered to healthy donors for collection of BPC and granulocytes, respectively[4,5].

The present thesis focuses on G-CSF - its effect on neutrophil function, use in remission induction chemotherapy for ALL and for mobilisation of blood progenitor cells in normal donors. In addition, the effects of GM-CSF on the regenerating granulopoiesis after ABMT is studied and discussed.

ABBREVIATIONS: ABMT=autologous bone marrow transplantation; ABPCT= autologous blood progenitor cell transplantation; ALL=acute lymphoblastic leukaemia; AML=acute myeloblastic leukaemia; ANC=absolute neutrophil count; BFU-E=burst-forming unit-erythrocyte; BPC=blood progenitor cell; BPI=bactericidal permeability-increasing protein; CD=cluster of differentiation; CFU-GM=colony-forming unit-granulocyte/monocyte; ECP=eosinophil cationic protein; ELISA=enzyme-linked immunosorbent assay; EPO= erythropoietin; f-MLP=formyl-methionyl-leucyl-phenylalanin; G-CSF= granulocyte colony-stimulating factor; GM-CSF=granulocyte-macrophage colony-stimulating factor; GVHD=graft versus host disease; HGF=hematopoietic growth factor; HNL=human neutrophil lipocalin; ICAM=intercellular adhesion molecule; IL=interleukin; LF=lactoferrin; LTC-IC=long-term culture initiating cell; M-CSF= macrophage colony stimulating factor; mab=monoclonal antibody; MPO=myeloperoxidase; P=plasma; PMA=Phorbol myristate acetate, S=serum; SCF=stem cell factor; TNF=tumour necrosis factor; TPO=trombopoietin.

## HEMATOPOIESIS

Adult hematopoiesis (Fig 1) derives from a small pool of pluripotent hematopoietic stem cells (PHSC) residing mainly in the bone marrow [6,7]. PHSC are cells with extensive proliferative potential and with capacity to self-renewal and to differentiate into all hematopoietic lineages [8]. There is no established assay for the quantification of these cells.

At a given time, the vast majority of PHSC are non-dividing, whereas a small proportion is proliferating to give rise to differentiated progenitor cells [9]. Lineage-committed progenitor cells, defined by the ability to form colonies in semisolid culture assays (CFU-GM, BFU-E etc), matures into morphologically recognisable precursor cells (myeloblasts, erytroblasts, megakaryoblasts etc) [10]. Following terminal differentiation, mature blood cells are released into the circulation.

The hematopoiesis is regulated by its interaction with stromal cells, molecular components of the extracellular matrix and with HGF [11,12]. The role of inhibitors of hematopoiesis is less well defined than that of stimulators[13]. HGFs are soluble glycoproteins which stimulate the production, survival and activity of hematopoietic cells[10]. HGF act either locally in the bone marrow microenvironment or systemically as circulating "hormones"[11,13]. Other common features are pleiotropy, overlapping activities and the ability to act both directly on hematopoioetic target cells and indirectly by inducing the production of other cytokines[9].

More than 20 HGF have been biochemically characterised and genetically cloned. HGF presently used in clinical practice or trials include EPO, TPO, G-CSF, GM-CSF, M-CSF, SCF, IL-2, IL-3, IL-6 and IL-11.

## BLOOD PROGENITOR CELLS

Hematopoietic progenitor cells, including primitive progenitors, are present in the circulation at a low frequency [14,15]. The proportion of progenitor cells (CD34+) among mononuclear cells has been estimated to 0.1% in blood and 1-2% in the marrow [16]. Recent *in vitro* studies indicate that the proliferative capacity of blood CD34+ cells is similar to that of bone marrow CD34+ cells [17]. Steady state blood mononuclear cells are capable of restoring myeloablated hematopoiesis in experimental animals [18,19] and in humans [20].

## NEUTROPOIESIS

Neutrophil production

An adult of average size has to produce approximately $1x10^{11}$ granulocytes per day simply to replace normal losses [21]. This production may be increased at least 10-fold under conditions of stress such as acute infections. Of the total pool of neutrophil granulocytes, only 5-10% are found in the circulation[22].

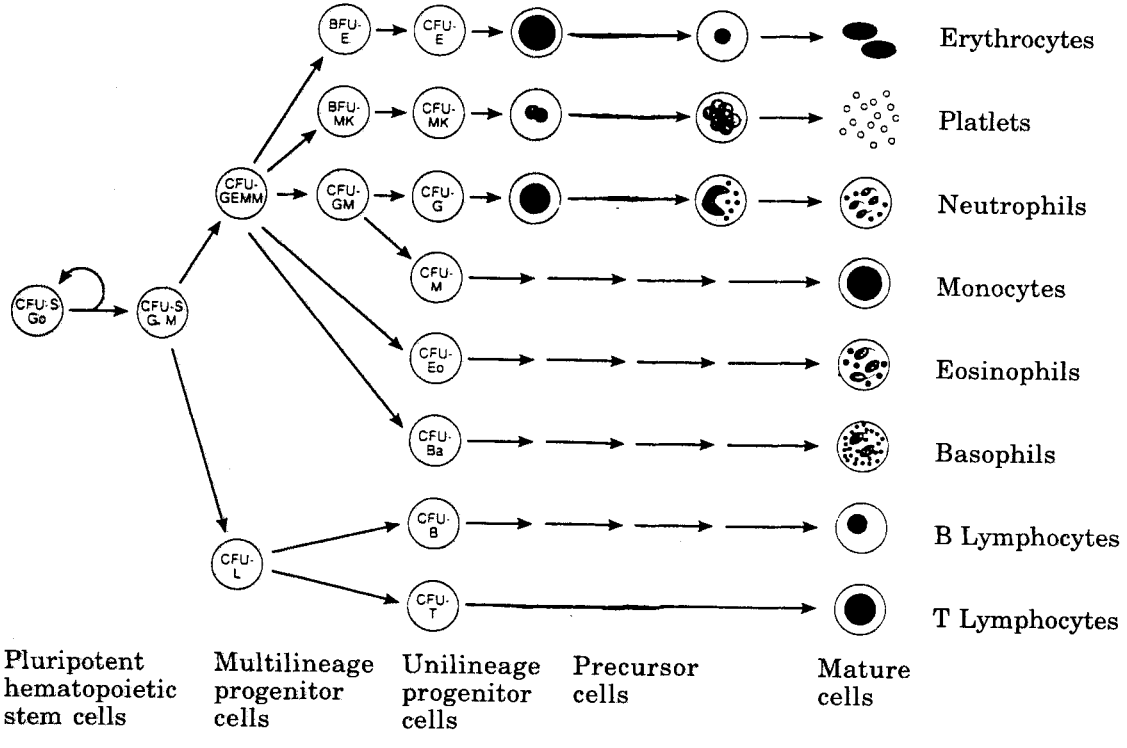


**Figure 1.** Model of hematopoiesis. Modified after Hoffman R, Benz E-JJ, Shattil SJ, Furie B, Cohen H, ed. Hematology: Basic principles and practice. 1:st ed. New York: Churchill Livingstone Inc.1991.

The earliest *morphologically* recognisable neutropoietic cell is the myeloblast [23]. Differentiation from myeloblast to mature granulocyte includes four to eight cell divisions and occur normally over 7-10(14) days [9]. Half of this time is spent in the post-mitotic compartment (neutrophil storage pool) as metamyleocytes, band and segmented neutrophils, respectively [21]. Mature neutrophil granulocytes egress from the bone marrow into the circulation through the endothelial lining of marrow sinusoids into the blood stream [24]. In the circulation, neutrophils normally spend 6-8 hours ($t_{1/2}$) before being eliminated in the liver and spleen.

Neutrophil function.

Neutrophil granulocytes play a key role in the first line defense against bacterial and fungal infections [25]. Neutrophils, including immature forms, may be released from the marrow in response to cytokines or other mediators of inflammation[26]. Further steps in the neutrophil inflammatory response are adhesion, migration, phagocytosis, degranulation and microbial killing.

Adhesion of granulocytes to endothelial cells is mediated by the induction and activation of adhesion molecules on endothelial cells and on granulocytes [27-29]. Neutrophil granulocytes are able to adhere to the endothelial cell adhesion molecules P-selectin, E-selectin and ICAM-1. Adhesion to ICAM-1 is mediated through the adhesion molecules LFA-1 (CD11A/CD11B) and Mac-1 (CD11b/CD18) on granulocytes, adhesion to P-selectin by PSGL-1 (P-selectin glycoprotein ligand 1) and adhesion to E-selectin by sialyl-Lewis$^x$ [30,31]. Granulocyte adhesion to ICAM-1 is necessary for the subsequent transendothelial migration of granulocytes.

Once in the tissues, neutrophils are attracted to the site of inflammation by chemotactic factors, which are generated by the microrganisms or by the inflammatory reaction itself [32]. Activated neutrophils adhere to opsonised microorganisms by surface receptors for complement (CR1, CR3) and the constant region of IgG (FcγRI-III), respectively [33,34]. Following phagocytosis, microorganisms are finally killed by the cytotoxic reactive oxygen metabolites of respiratory burst [35], and by the action of certain granule proteins such as MPO, lysozyme, LF, BPI and defensins (oxygen-independent microbiocidal killing) [36].

Neutrophil granule proteins.

Neutrophil granulocytes contain primary (azurophilic) and secondary (specific) granules [23]. Primary granules are formed at the myeloblast-promyelocyte stage of differentiation and contain MPO, lysozyme, cathepsin G, elastase, defensins as well as other antimicrobiocidal agents [37]. Secondary granules are formed at the myelocyte-metamyelocyte stage and contain a number of different proteins including LF, specific collagenase and lysozyme [23,38]. Gelatinase-containing granules are considered as a subpopulation of secondary granules [39]. Secondary granules contain in their membrane several

adhesion- and chemotactic receptors, which are mobilised to the cell surface upon neutrophil activation [40].
MPO catalyses the reaction of peroxide with chloride to form hypochlorous acid, which is highly toxic for a variety of microorganisms [41]. LF inhibits the growth of ingested microorganisms by sequestering free iron [42] and has been implied a role as inhibitor of granulopoiesis, although the latter remains controversial [43].
HNL is a recently characterised N-glycosylated protein with a molecular weight of 45 kDa (unreduced). It was isolated from neutrophil secondary granules [44]. HNL is identical to the previously described neutrophil gelatinase-asscociated lipocalin, and is in part covalently associated with gelatinase [44,45]. HNL and LF have a different subcellular distribution, and may be released differently upon neutrophil activation [46].

Serum/plasma levels of neutrophil granule proteins.
Neutrophil granule proteins are secreted upon activation and phagocytosis, or may be passively released due to cell damage or death [47]. Activated neutrophils secrete their granules into the phagosome as well as into the extracellular environment. Sensitive immunoassays have been developed for measuring MPO, LF and HNL, respectively, in cells as well as in various body fluids [48,49]. Circulating levels of MPO and LF reflect the neutropoietic activity of bone marrow [48]. Blood measurements of these granule proteins may be used to monitor bone marrow regeneration after chemotherapy including (A)BMT [50,51].
Blood levels of neutrophil granule proteins are not only influenced by the number and turnover of neutrophils, but also by their *in vivo* secretory activity [52]. Thus, highly elevated serum/plasma levels of LF and HNL are observed in conditions with neutrophil activation such as acute infections [53,54]. S-HNL has been shown useful in disciminating between bacterial and viral infections [54].
Serum levels of granule proteins are the result of plasma levels plus what has been secreted from the neutrophils *ex vivo* in the test tube, partly as a consequence of factors produced during coagulation [55]. This *ex vivo* secretion is more pronounced when the neutrophils have been activated *in vivo*.

## GRANULOPOIETIC GROWTH FACTORS

G-CSF and GM-CSF are the two major granulopoietic growth factors. GM-CSF stimulates proliferation and differentiation of hematopoietic cells mainly of the granulo-monocytopoietic and eosinophilic lineages [56]. Apart from its effects on progenitor and precursor cells, GM-CSF is a potent activator of mature neutrophils, eosinophils and monocytes [2,57,58]. Its physiological role is unclear, although studies of the GM-CSF knock-ut mouse suggest that it is important for macrophage function [59]. Therapeutically, recombinant GM-CSF

is mainly used to stimulate neutropoiesis in myelodysplastic syndrome, following chemotherapy and after bone marrow transplantation [3,60,61]. Its clinical application has been hampered by dose-dependent side effects such as fever. The immunomodulating effects of GM-CSF are presently being explored in clinical trials [62].
This thesis focuses on G-CSF, which is therefore discussed below in more detail.

## G-CSF - PRECLINICAL CHARACTERISTICS

### Identification

In the mid-1960s cell culture assays for hematopoietic progenitor cells were developed [63,64]. From studies using these assays it became clear that survival, proliferation and differentiation of hematopoietic cells are dependent on the presence of humoral factors - named colony-stimulating-factors (CSFs). Further purification of these factors turned out to be extremely difficult [65]. Thus, the different CSFs were long defined as biological activities depending on the assay system used.
In 1980, G-CSF was identified by the capacity of postendotoxin treated mice sera to induce differentiation of the murine myelo-monocytoid cell line WEHI-3B(D+) [66]. Further studies showed that this factor selectively stimulated the formation of granulocytic colonies from normal murine hematopoetic progenitor cells, and it was after further purification named "G-CSF"[67]. In 1986, human G-CSF was ultimately purified to homogeneity from CSF-producing tumour cell lines [68,69]. Subsequently, a cDNA encoding human G-CSF was isolated from the bladder carcinoma cell line 5637 and expressed in E coli by Souza et al [70]. Independently, a cDNA for G-CSF was isolated from the squamous carcinoma cell line CHU-2 and expressed in chinese hamster ovary cells by Nagata et al [71].
During the period 1984-1987, the genes encoding GM-CSF, M-CSF, EPO and IL-3, respectively, were also cloned [72-74]. These breakthroughs enabled the large scale production of HGF for *in vivo* studies. Already in 1987 the first clinical trial with recombinant G-CSF was reported [75].

### Structure

Native human G-CSF is an *o*-glykosylated 20 kD polypetide consisting of 174 amino acids and a carbohydrate chain which accounts for ≈4% of the molecular weight [70,76]. It is encoded by a single gene located on chromosome 17q11-22. Before secretion, the G-CSF molecule is glykosylated. Highly purified human G-CSF and recombinant G-CSF have identical biological activity [77,78].
The chinese hamster ovary derived G-CSF (rHuG-CSF; lenograstim) is structurally identical with the naturally ocurring G-CSF [79], whereas the E-coli derived recombinant form (r-metHuG-CSF; filgrastim) has an extra amino

acid at the N-terminal end and is non-glykosylated [70]. The function of the glycosylated part of the molecule is unknown, although it has been hypothesised that the sugar chain confers a greater resistence to inactivation and enzymatic degradation [80,81].

Cellular production.
G-CSF is produced by monocytes-macrophages, fibroblasts and endothelial cells [82]. Small amounts of G-CSF may also be produced by the neutrophils themselves [83]. *In vitro*, bacterial endotoxins [84], IL-1 [85] and TNFα [86] may induce the synthesis of G-CSF by fibroblasts and endothelial cells. G-CSF is also produced by monocytes-macrophages after stimulation by T-cell derived cytokines, such as IL-3 and GM-CSF [87]. Malignant cells producing G-CSF are responsible for the paraneoplastic leukemoid reaction sometimes seen in assocation with certain tumours such as malignant melanoma, hepatoma and sarcoma [1].

Receptor
Like other cytokines, G-CSF excerts its actions by binding to high-affinate receptors [88]. The G-CSF receptor (G-CSF-R) is a single 813 amino acid polypeptide and consists of a cytoplasmatic, a transmembrane and an extracellular domain[89,90]. Recent findings indicate that the cytoplasmic region proximal to the membrane transduces proliferative and survival signals, whereas the distal C-terminal region transduces maturation signals and supressess proliferative signals [91,92].
G-CSF-receptors are present in the neutrophil lineage on cells from the myeloblast to the segmented neutrophil granulocytes. Mature neutrophils are reported to have the greatest number of receptors (50-500/cell) [93]. Monocytes and their precursors possess a small number of these receptors.
AML clonogenic cells commonly express a low levels of G-CSF receptors. However, the presence of these receptors *per se* does not predict that the AML cells proliferate in response to G-CSF [94].

Circulating levels
In healthy individuals, serum levels of endogenous G-CSF are usually low, i.e. below the detection limit of most currently used methods (<30-50 pg/ml ) [95]. High blood levels of G-CSF have been shown in the early phase of bacterial infections [53,96,97] as well as in various conditions associated with severe neutropenia [95,98-100]. Decreasing S-G-CSF preceedes or parallells neutrophil recovery after stem cell transplantation [101,102]. High levels of S-G-CSF during neutropenia may be due to increased production, decreased clearence or both [97].
Notably, serum levels during severe infections are comparable with what is observed after treatment with standard doses of recombinant G-CSF.

Physiological role.
Recent experiments with the G-CSF gene knock-out mice (G-CSF-/-) suggest that G-CSF is of key importance both in maintaining steady-state hematopoiesis and in the hematopoietic response to environmental stress[103]. Thus, G-CSF-/- mice have a severe chronic neutropenia, a reduction in the mobilisable neutrophil reserve and a reduced marrow granulopoiesis. In addition, G-CSF -/- mice show a reduced ability to control infections, suggesting that G-CSF is necessary for the normal emergency granulopoietic response.

*In vitro* effects
G-CSF stimulates proliferation, differentiation and survival of neutrophil progenitor and precursor cells[10,104]. G-CSF also enhcances mature neutrophil survival [105], adherence [106] and primes the respiratory burst [107-109].
G-CSF stimulates the growth of committed myeloid progenitors (CD34+CD33-), whereas cultures enriched for primitive progenitors (CD34+CD33-) show no or minimal response to stimulation with G-CSF alone [110,111]. However, G-CSF primes primitive cells to increased response to "early acting cytokines" such as IL-1, IL-3, IL-6 and SCF [112].

Pharmacological effects
Administration of G-CSF induces an acute margination of neutrophils, as manifested by a transient drop in ANC between (15)30-90 minutes post s.c. injection [75,83,113]. The intial drop is followed by a sharp increase in ANC, starting 2-3 hours and reaching peak levels at about 12 hours post injection [114]. This early increase may be due to mobilisation of neutrophils from the bone marrow storage pool [115]. Following the continued administration of G-CSF, there is a dose-dependent progressive neutrophilia plateauing after 7-8 days [83,116,117]. After discontinuation of G-CSF, ANC return to preteatment values within 3-4 days.
More detailed information about the effects of G-CSF on the neutropoiesis comes from cell kinetic studies in humans [75,118,119] and mice [120], using tritiated thymidine ({$^3$H}TdR) and autoradiography. Thus, G-CSF was shown to increase the proportion and proliferation rate of neutrophilic precursors, and to reduce the myelocyte-to-blood transit time to 1-2 days (normal 4-5 days) [118,119]. These results further suggest that G-CSF excerts its proliferative effect mainly on the stage of myeloblast-promyleocyte, whereas effects on more primitive progenitors are limited [75,118]. G-CSF has no appearent impact on the circulation half-life of neutrophils [123].
G-CSF causes a dose-dependent increase in the monocyte count which is modest relative to the neutrophil resoonse. High doses (≥10 μg/kg/d) of G-CSF may be associated with a transient decrease in platlet count [83,121].
The effect of G-CSF on progenitor cell mobilisation is discussed below.

Effects on neutrophil function

Neutrophils collected from patients or healthy volunteers receiving single or repeated doses of G-CSF show increased adherence [106], enhanced phagocytosis [122] and increased superoxide anion ($O_2^-$) production in response to f-MLP [123]. G-CSF increases the surface expression of adhesion molecules [124-126] and Fc receptors for IgG [114,127]. Results with respect to effects on neutrophil chemotaxis are inconsistent [128-130].

Some of the observed effects on mature neutrophils might be indirect or reflect the incresing immaturity of the neutrophil population following G-CSF [114,130].

## G-CSF - CLINICAL APPLICATIONS

G-CSF is presently used in a number of different clinical settings, ranging from generally accepted/formally approved (i.e. congenital neutropenia, post chemotherapy, post ABMT, mobilisation of PBPC for autologous transplantation) to clearly experimental indications (infections in non-neutropenic patients) (Table I).

**Table I. Indications for clinical use of G-CSF**

| | |
|---|---|
| CHEMOTHERAPY INDUCED NEUTROPENIA | |
| standard chemotherapy | 131-133 |
| ABMT | 134 |
| ABPCT | 135 |
| alloBMT | 136 |
| MOBILISATION OF BPC | |
| patients | 137,138 |
| healthy donors | 4,139 |
| MOBLISATION OF GRANULOCYTES* | 5,121 |
| CHRONIC NEUTROPENIA | |
| congenital | 140,141 |
| cyclic | 142 |
| idiopathic | 140 |
| OTHER | |
| myelodysplastic syndrome | 143 |
| aplastic anaemia | 144 |
| AIDS | 145 |
| non-neutropenic infections** | 146 |

*for granulocyte transfusion ** no definite clinical results available

In line with the present thesis, the following discussion will be focused on the use of G-CSF to reduce infectious morbidity after chemotherapy induced neutropenia, and for mobilisation of blood progenitor cells.

## CHEMOTHERAPY INDUCED NEUTROPENIA

Infections during neutropenia

Chemotherapy induced neutropenia is frequently complicated by infections or fever suspected of infection [147]. The risk of infection correlates with the depth and duration of neutropenia [25]. Apart from neutropenia (ANC<0.5x$10^9$/l), other factors related to the underlying disease or its treatment may increase the incidence of infections. These include mucositis, immunosuppression, defect granulocyte function and the use of indwelling intravenous catheters. Prophlyactic antibiotic treatment may decrease the risk of gram negative infections in neutropenic patients, although its role is still controversial [148].

Patients presenting with "febrile neutropenia" (fever and ANC<0.5x$10^9$/l) are usually readmitted for treatment with broad-spectrum intravenous antibiotics. This therapy is generally continued until resolution of clinical symtoms *and* neutropenia [149]. The cause of fever cannot be identified in 60-70% patients presenting with febrile neutropenia [150].

Both infections and prolonged neutropenia *per se* may also lead to delays and dose-reduction of chemotherapy, thereby potentially compromising treatment efficacy.

G-CSF after standard dose chemotherapy in solid tumours

Treatment with G-CSF after myelosuppresive chemotherapy shortens the period of neutropenia and time to neutrophil recovery[75]. G-CSF may thus reduce delays in chemotherapy and prevent dose reductions [151]. No advantage of G-CSF treatment in terms of tumour respons or survival has yet been shown [133]. Several ongoing studies explore the use of G-CSF in the context of dose escalation.

Three randomised trials in patients with solid tumours have demonstrated that the administration of G-CSF after chemotherapy may reduce the incidence of febrile neutropenia by approximately 50 %. Patients receiving G-CSF requried fewer days in hospital due to infections and had less days with antibiotics. It is not clear whether the number of days with fever (i.e. irrespective of ANC) was reduced. Importantly, in all these trials the incidence of febrile neutropenia in the control group was as high as >40% [131,152,153].

G-CSF seems to have no impact on infectious morbidity after less myelosuppressive chemotherapy [133]

G-CSF in acute leukemia.

In AML, the use of growth factors has been restricted due to concern about the possible stimulation of leukaemia cells by such treatment [154]. However, recent

studies show that patients receving G-CSF after induction chemotherapy for AML have a similar or even better complete remission rate compared with controls [155-158]. Still, no impact on treatment-related mortality or long-term survival has been demonstrated. G-CSF reduces chemotherapy related neutropenia, but an impact on infectious morbidity (i.e. incidence of infections, fever) has not been consistently shown [156,158].
There are yet few data regarding the possible value of granulopoietic growth factors during induction treatment of ALL. In a recently published study, the administration of G-CSF during the second phase of remission induction chemotherapy had no clear impact on infectious morbidity [159].

G-CSF after stem cell transplantation
Treatment with G-CSF after ABMT shortens time to neutrophil engraftment (ANC>0.1x10$^9$/l) by 3-4 days, whereas time to neutrophil recovery (ANC>0.5x10$^9$/l) is reduced by with approximately one week [134,160]. Doses of G-CSF above 5 μg/kg/d does not seem to confer any advantage in terms of neutrophil recovery [161]. Uncontrolled trials suggest that the start of G-CSF treatment may be delayed until six days post ABMT [162].
Results from two phase III trials indicate that treatment with G-CSF after ABMT reduces neither the incidence of infections nor the number of days with fever [134,163]. The lack of impact of G-CSF on infections might reflect the fact that patients still are profoundly neutropenic during the first week post ABMT [137]. Nevertheless, patients receiving G-CSF after ABMT have fewer days on antibiotics and total parenteral nutrition and require shorter time in hospital post transplant. This may reflect an impact of G-CSF on the duration or severity of infections and/or the fact that clinical decisions are influenced by knowledge of the neutrophil count.
G-CSF has a modest effect on neutrophil recovery (ANC>0.5x10$^9$/l) after autologous blood progenitor cell transplantation[135]. An impact on infectious morbidity has not been shown.
Treatment with G-CSF after allogeneic BMT hastens neutrophil recovery without adversely affecting the incidence or severity of acute GVHD [134,136]. Reduction in infectious morbidity has not been clearly shown.

## BLOOD PROGENITOR CELLS - MOBILISATION AND TRANSPLANTATION

Mobilisation of BPC
Mobilisation of hematopoietic progenitor cells may be induced by a number of different agents, including endotoxin, dextran, chemotherapy and various HGFs (reviewed) [164,165]. Depending on the stimuli used, mobilisation occurs within 30 min (IL-8) [166] or may take up to three weeks (chemotherapy) [167]. The mechanisms responsible for progenitor cell peripheralisation are not well

known and may be different for different stimuli [168]. Recent experiments indicate that cytoadhesion molecules may play an important role [169].
Hematopoietic recovery from myelosuppressive chemotherapy is associated with a transient increase in circulating progenitors [167]. Single high-dose cyclophosphamide or other cytotoxic regiments thus enhances the collection of blood progenitor cells for autologous transplantation [170]. Mobilisation outcome after chemotherapy relates to the degree of myelosuppression [171]. Combining chemotherapy and HGFs (G-CSF, GM-CSF, IL-3+GM-CSF) have synergistic effects, and this strategy is today widely used for mobilisation of progenitor cells in cancer patients [138,172,173].
A number of different HGFs (i.e. G-CSF, GM-CSF, EPO, IL-1, IL-3, IL-8, IL-11, SCF) are capable of mobilising blood progenitor cells [166,168,173-178]. Some of these agents are obviously not sufficiently effective (EPO, IL-3) or too toxic (IL-1) to become clinically useful as single agents. Combinations of different cytokines, such as G-CSF and SCF, may act synergistically [179].
Clinical experience of HGF alone for mobilisation of PBPC is mainly confined to G-CSF and GM-CSF [180,181]. Recent findings indicate that G-CSF mobilises CD34+ cells more efficiently than GM-CSF, although GM-CSF appears to mobilise a higher proportion primitive CD34+ cell subsets (CD34+CD38-)[182]. Due to its lower toxicity, G-CSF is preferred in healthy donors.

Mobilisation with G-CSF
Daily administration of G-CSF significantly increases the number of circulating progenitors, including cells of the granulocyte-macrophage, erythroid and megakaryocyte lineages [174,183,184]. Thus, Dührsen et al observed an up to 100-fold increase in colony-forming cells in cancer patient receiving G-CSF at the dose of 3 µg/kg/d or higher. Typically, progenitor cells start to increase 2-3 days after initiating G-CSF peaking another two days later [185,186]. Extending G-CSF treatment beyond 6-7 days does not further improve the mobilisation [184]. A correlation between the dose of G-CSF and increase in progenitor cells has been shown in animals [187,188], although human data are conflicting [4,189].
A minority of patients mobilises poorly in response to G-CSF (or other regimens). Previous chemotherapy and pelvic irridiation, respectively, adversely effect the outcome of stem cell mobilisation [190,191]. In healthy donors, factors predicting for low yield of CD34+ cells are not defined, although some studies suggest a negative correlation with age [4,117].
Previous reports suggest that G-CSF mobilises exclusively highly committed progenitors of the granulocyte and monocyte lineages [192]. However, several recent studies also demonstrate mobilisation of phenotypically primitive progenitor cells (CD34+CD38-) [17,182,193]. The capacity of G-CSF mobilised progenitors to generate and sustain hematopoiesis *in vitro* has been reported comparable with BM cells on a cell to cell basis [17,194]. G-CSF mobilised progenitor cells have the capacity to sustain syngeneic engraftment in myeloablated mice [195].

Collection of BPC

Hematopoietic stem cells coseparates with monocytes and lymphocytes on centrifugal gradients. The technology of large-scale mononuclear cell collection by continous flow blood cell separators was developed during the 1970s and early 1980s [196]. A typical leukapharesis session implies the processing of 2-3 times the normal blood volume with a flow rate of 50-80 ml/min and a collection efficiency of usually 50-70% [197,198]. Peripheral veins or various forms of dual lumen venous catheters are used as vascular access. Major side-effects of the leukapheresis procedure are uncommon, although non-trivial catheter related complications occasionally occurs [139].

Enumeration of BPC

The CD34 antigen is expressed by hematopoietic progenitor cells, endothelial cells and myeloid tumor cells. Hematopoietic cells expressing CD34 includes both primitive and committed progenitor cells [199,200]. Progenitors capable of forming colonies in semisolid culture assays reside in the CD34+ population [201]. $CD34^+$ seleceted bone marrow and blood cell grafts, respectively, are capable of reconstituting hematopoiesis in patients receiving myeloablative chemotherapy [202,203].

Flow cytometric enumeration of $CD34^+$ cells, using fluorochrome-conjugated anti-CD34 mabs, has become a standard method for evaluating the quality of blood cell grafts and for monitoring of progenitor cell mobilisation [204]. Several reports confirm a good correlation between numbers of CD34+ cells and CFU-GM [204,205]. Although CD34-measurements have less interlaboratory variability than the CFU-GM assay, lack of standardisation still makes it difficult to compare results from different centers [206].

In the setting of autologous blood progenitor cell transplantation, the number of CD34+ cells infused correlates with the rate of hematopoietic (neutrophil and platelet) reconstitution. [191,207,208]. However, increasing the dose of progenitor cells above a certain "upper threshold" may not further improve the rate of hematopoietic recovery [209]. Blood cell grafts containing less than 1-$2.0 \times 10^6$ $CD34^+$ cells/kg tend to be associated with delayed platlet recovery [191,210].

The majority of CD34+ cells are lineage committed [16]. Primitive progenitors may be enumerated using the LTC-IC assay or other *in vitro* culture methods [211]. However, these methods are too complicated for routine clinical use. Alternatively, phenotypically primitive cells may be quantified by multiparameter flow cytometry [212]. Thus, primitive CD34+ cell subsets are defined by the lack of expression of differentiation (CD33 etc) or activation (CD38, HLA-DR) antigens, or by the coexpression of CDw90 (Thy-1) [213-215].

Autologous BPCT

Early experience of autologous BPCT was mainly derived from patients not eligible for bone marrow harvesting due to previous pelvic irradiation or tumor infiltration of the marrow. Collection of steady-state blood stem cells typically required median eight leukaphereses to obtain a yield considered sufficient for transplantation [216]. Hematopoietic reconstitution following transplantation of unmobilised BPC was comparable to ABMT with occasional patients experiencing delayed engraftment [217].

Mobilisation with chemotherapy and/or cytokines makes it possible to collect a larger number of blood stem cells than is contained in a bone marrow harvest, using a limited number of leukaphereses [137,218]. Consequently, high-dose chemotherapy supported with *mobilised* PBPC is asscociated with more rapid lymphocyte, neutrophil and, in particular, platelet recovery compared with ABMT [180,219,220]. A reduced need of platelet transfusions and shorter time in hospital post transplant has been shown in unrandomised studies [221]. Thus, blood cell grafts have gradually replaced bone marrow in most categories of patients undergoing high-dose chemotherapy. Paradoxically, the original hypothesis that blood cell transplants are less likely to be contaminated by tumour cells is yet unproven [222].

Allogeneic BPCT.

Allogeneic BPCT may have advantages over allogeneic BMT in terms of more rapid hematopoietic and/or immunological reconstitution. The possibility to obtain a larger number of progenitors may be particularly important in the settings of graft failure and donor-recepient mismatch, respectively [223,224]. In some donors, the avoidance of bone marrow harvest/general anaestesia might be preferable. Despite these potential advantages, allogeneic PBCT did not appear on the clinical arena until very recently. Reasons for this include reluctance to mobilise healthy donors with G-CSF, uncertainty about long-term engraftment capacity of blood stem cells and the supposed increased risk of GVHD [225].

G-CSF mobilised blood cell grafts contain about 1 log more T-cells than an average bone marrow graft [4]. Theoretically, the high number of T-cells (and NK-cells) may be advantagous from the point view of GVL [4]. Whilst murine studies demonstrate a correlation between the number of infused T-cells and development of GVHD [226], this has not been uniformly shown in humans. [227]. Thus, it has been hypothesised that the risk of GVHD might not increase above a certain threshold level of T-cells [228].

The possible effects of G-CSF on T-cells are of interest in this context. Thus, in a recent study in mice, it was shown that treating donors with G-CSF modulates donor T-cells toward production of type-2 cytokines, which is associated with a reduced severity of acute GVHD. Survival was improved in recepients of G-CSF treated donors [229].

The number of hematopoietic progenitor cells required for rapid and sustained allogeneic engraftment has not yet been established, present recommendations

of ≥4-5 CD34+ cells/kg being based on extrapolations from the autotransplant setting [4,230]. In healthy donors, such a number of progenitors may be collected by 1-3 leukapheresis after mobilisation with G-CSF 5-16 μg/kg/d for 4-6 days [4,139,231]. G-CSF has been generally well tolerated, although "bone pain" seems to be more frequent in normal donors than in cancer patients receiving G-CSF[139]. The optimal dosage of G-CSF for mobilisation of BPC in healthy donors has not yet been defined [225].
Clinical experience of alloBPCT is rapidly growing. Recently published small studies suggest that sibling donor transplantation of blood progenitor cells restores hematopoiesis at least as rapid as bone marrow allografts without increasing the risk of acute GVHD. Sustained allogeneic engraftment has been documented up to two years post transplant.[230-232]. The impact of BPCT on the incidence/severity of chronic GVHD and disease-free survival, respectively, is yet not known. This, as well as the value of T-cell depletion by CD34-enrichment, is being evaluated in ongoing Phase III trials.

---

**The effect of rGM-CSF on neutrophil and eosinophil regeneration after ABMT as monitored by circulating levels of granule proteins.**
(paper I)

Rational
Blood levels of neutrophil granule proteins reflect the neutropoietic acitivity of bone marrow. We have previously shown that blood measurements of MPO and LF may be used to monitor the activity of regenerating neutropoiesis after ABMT.
The major aim of this study was to investigate the effects of rGM-CSF on the granulopoiesis after ABMT.

Materials and methods
Twenty-two consecutive adult patients with non-myeloid malignancies were randomised to receive either rGM-CSF 10 μg/kg or placebo from day +1 after ABMT until ANC>$1x10^9$/l for four consecutive days. P-MPO, S-MPO, P-LF, S-LF and S-ECP were determined daily during a period of 3-4 weeks by means of sensitive radioimmunoassays.

Results and discussion.
Following neutrophil engraftment (ANC>$0.1x10^9$/l) at median day 11 (GM) and 12 (placebo), respectively, ANC increased more rapidly in the GM-CSF group. P-MPO and S-MPO did not differ between the two groups during the early post engraftment period (d+11-19), suggesting that GM-CSF had no effect on neutropoietic proliferation during this period. These findings suggest that GM-CSF after ABMT increases the blood number of neutrophils by other mechanisms such as prolonging the half-life in circulation and early release from bone marrow [233].

In the GM-CSF group, S-ECP increased during the second and third week post ABMT, which corresponded to an increase in eosinophil counts. This indicates that GM-CSF stimulates eosinophil recovery without causing an activation of the eosinophils.

Conclusions.
GM-CSF increases ANC after ABMT through other mechanisms than stimulation of neutropoietic proliferation. GM-CSF stimulates eosinophil recovery after ABMT.

**The effect of granulocyte colony-stimulating factor (G-CSF) on the degranulation of secondary granule proteins from human neutrophils *in vivo* may be indirect.**

(paper II)

Rational
Single injections of G-CSF result in neutrophil activation and degranulation. The effects of repeated administration of G-CSF on neutrophil degranulation are less well known. LF and HNL are proteins contained in different subpopulations of the secondary granules of neutrophil granulocytes
The aim of the present study was to investigate the effects of G-CSF on neutrophil secondary degranulation *in vivo* and *in vitro*. We were also interested in the possible involvement of other cytokines in the activation of neutrophils during administration of G-CSF.

Material and methods.
Heatlhy male volunteers (aged 21-35) received G-CSF (lenograstim) 7.5-10 μg/kg s.c. once daily for six days (day 1-6). Blood samples were obtained at day 1-8, 12 and at follow up 3±1 weeks after the last injection. HNL and LF were determined in serum and plasma by RIA, and TNF-α (free TNF-α+TNF-α bound to soluble receptors in samples) in plasma using a commercial ELISA. Granulocytes were separated from heparinised blood by density gradient centrifugation over 67% Percoll, and assayed for intracellullar content of HNL and LF, respectively. Separated granulocytes were stimulated with FMLP and PMA, and the supernatants assayed for granule proteins (HNL, LF, MPO).

Results and discussion.
Following G-CSF 7.5 μg/kg sc (n=8), blood HNL, LF and neutrophils increased with a similar kinetics, i.e. an initial rapid rise was followed by a plateau day 2-7 and a subsequent return to baseline.
At day 5, separated neutrophils showed an enhanced release of HNL and LF, respectively, in response to f-MLP compared to neutrophils obtained at baseline (day 1). By contrast, f-MLP induced release of HNL and LF, respectively, was only marginally enhanced by preincubation of *normal* neutrophils with G-CSF *in vitro*. Taken together, these findings indicate the effect of G-CSF on secondary degranulation is mainly indirect.

Following G-CSF 10 μg/kg (n=2), P-TNF-α increased about 25-fold. TNF-α started to increase at day 2 and peaked at day 6. Intriguingly, the elevated P-TNF-α levels were temporarily correlated to other signs of neutrophil activation. We therefore speculate that TNF-α may be partly responsible for the activation of neutrophils during administration of G-CSF.

Conclusions

G-CSF enhances release of secondary granule proteins from neutrophil granulocytes *in vivo*. The effect of G-CSF on secondary degranulation is mainly indirect. G-CSF induces an increase in P-TNF-α.

## Effects of *in vivo* administration of G-CSF on neutrophil functions in healthy volunteers

(paper III)

Rational

G-CSF has multiple effects on mature neutrophil function *in vitro* and *in vivo*. Previous studies of *in vivo* effects of G-CSF on neutrophil function have given conflicting results, particularly with respect to chemotaxis. G-CSF in increasingly used in healthy donors to enhance the collection of granulocytes for transfusion.

The major aim of this study was to further characterise the effects of G-CSF, administered as repeated s.c. injections to healthy volunteers, on neutrophil functions.

Materials and methods

G-CSF was administered s.c. once daily for six days (day 1-6) to healthy male volunteers. Neutrophil migration (modified Boyden chamber), chemiluminescence (CL), adherence to nylon fibers and phagocytosis of IgG- and IgG-C3-coated particles were investigated before, day 2, day 5 and three weeks after G-CSF 7.5-10 μg/kg/d (n=12). Neutrophil surface expression of adhesion molecules and Fcγ-receptors, respectively, was measured by flow cytometry before, day 5, day 8 and three weeks after G-CSF 3-5 μg/kg/d (n=12). Results obtained after G-CSF were compared to baseline using Wilcoxon's signed rank test.

Results and discussion

G-CSF decreased neutrophil chemotaxis towards zymosan activated serum, f-MLP and IL-8, respectively. Chemotaixs was reduced both at day 2 and 5. The negative effective on chemotaxis could only partly be explained by a reduced chemokinesis

G-CSF increased neutrophil adherence and phagocytosis of both IgG-coated and IgG-C3-coated particles.

G-CSF (3-5 μg/kg) enhanced the neutrophil surface expression of adhesion molecules (CD11b/CD18, CD35) and of Fcγ-receptors (CD32, CD64), respectively.

G-CSF had no effect on lucigenin enhanced CL, irrespective of whether the neutrophils had been activated by PMA or serum-opsonised zymosan. Contrary to previous reports, this suggests that G-CSF *in vivo* has no effect on the $O_2^-$ producing enzyme complexes of the respiratory burst.

Conclusions,
G-CSF *in vivo* impairs neutrophil chemotaxis but enhances neutrophil adherence and phagocytosis. G-CSF *in vivo* does not stimulate the respiratory burst

**G-CSF reduces neutropenia but not infectious morbidity during induction therapy for acute lymphoblastic leukemia**
(paper IV)

Rational
Remission induction chemotherapy for adult ALL is associated with a high incidence of infections during the neutropenic phase.
The aim of this study was to assess the value of G-CSF (lenograstim) in reducing neutropenia (duration, severity) and infectious morbidity (incidence of severe infection, duration of fever) in adult patients with ALL receiving remission induction chemotherapy.

Materials and methods.
Consecutive patients with newly diagnosed ALL were randomised to receive (n=41) or not receive (n=39) G-CSF (lenograstim) during 28 days of remission induction chemotherapy. G-CSF 5 μg/kg was administered as sc injections once daily at days 3-14 and 17-28. All patients were hospitalised and received prophylactic antibiotics (norfloxacin) during this period. Blood counts, fever and clinical symtoms were assessed daily.

Results and discussion.
G-CSF was well tolerated and did not appear to adversely affect disease outcome. Compared to the untreated control group, G-CSF reduced the duration of severe neutropenia (ANC<0,5x$10^9$/l) from 20 to 14 days (p=0.0135). However, G-CSF had no impact on the incidence of severe infections (septicaemia, pneumonia, abscess), days with fever nor days with i.v. antibiotics.
The incidence of severe infections in this study was (in both arms) only about 20% compared to 45% in a previous trial with the same chemotherapy. Improved supportive care including use of prophylactic antibiotics might explain this difference.
The "discrepancy" between the effect of G-CSF on neutropenia and the lack of impact on infectious morbidity is consistent with the findings of other studies of G-CSF after chemotherapy. We cannot exclude that subgroups of ALL patients might benefit from the addition of G-CSF. However, based on the

results of this study we cannot recommend the routinely use of G-CSF during remission induction chemotherapy for ALL

Conclusions
G-CSF shortens the duration of neutropenia but does not reduce infectious morbidity in patients with ALL undergoing remission induction chemotherapy.

**Dose Dependent Mobilisation of Haematopoietic Progenitor Cells in Healthy Volunteers Receiving Glycosylated rHuG-CSF**
(paperV)

Rational
The optimal dosage of G-CSF for blood progenitor cell mobilisation in healthy donors has not been defined. Previous studies of a possible dose-response relationship have given conflicting results. It is not clear to which extent G-CSF mobilises primitive progenitor cells.
The primary objective of this trial was to study whether increasing the dose of lenograstim (glycosylated G-CSF) improves the mobilisation of progenitor cells in healthy volunteers. Major secondary objectives were to investigate the G-CSF induced mobilisation of primitive CD34+ cell subsets, and the safety and tolerability of lenograstim when administered to healthy subjects.

Materials and methods
Twenty-four healthy male volunteers, aged 20-34 years, were included. Four groups of six volunteers received lenograstim once daily for six days (days 1-6) at a dose of 3, 5, 7.5 and 10 μg/kg/d s.c., respectively. All subjects underwent a 10-L leukapheresis on day 5, 6 or 7. CD34+ cells were enumerated by flow cytometry using directly conjugated mab (HPCA-2). CD34+ cells in bone marrow (BM) aspirates obtained prior to G-CSF and in leukapheresis products (LP), respectively, were further immunophenotyped.

Results and discussion
Lenograstim was overall well tolerated. The majority of volunteers reported bone pain, which tended to be more intense in the group receiving 10 μg/kg. The intensity of bone pain and the extreme leukocytosis (WBC> $60x10^9/l$) in some volunteers receiving 10 μg/kg prevented further escalation of lenograstim.
Maximal mobilisation of progenitor cells occurred on day 5 or 6. Lenograstim 10 μg/kg produced significantly higher peak levels of CD34+ cells, BFU-E and CFU-GM, respectively, compared with each of the other doses ($p<0.05$, ANOVA). A good correlation was observed between the number of CD34+ cells in blood and apheresis product, respectively, ($r=0.95$, $p=0.0001$).
A comparison of steady state BM CD34+ cells and LP CD34+ cells in paired samples for each individual, showed a higher proportion of primitive CD34+ cell subsets (CDw90+, HLA-DR-, CD45RA-, CD33-) among LP CD34+ cells.

Taken together these results suggest that lenograstim 10 μg/kg/d is an optimal dose for mobilisation of progenitor cells in healthy donors. Corroborating our findings, a recent study showed that filgrastim 10 μg/kg in healthy volunteers was tolerable and mobilised progenitor cells more efficiently than 3-5 μg/kg [234]. None of these two studies have explored dose levels above 10 μg/kg.

Conclusions

Lenograstim 10 μg/kg/d to healthy volunteers is well tolerated and mobilises progenitor cells more efficiently than 3, 5 and 7.5 μg/kg/d, respectively. G-CSF favors mobilisation of primitive CD34+ cell subsets. Lenograstim 10 μg/kg/d for 5-6 days should provide an efficient mobilisation of progenitor cells in most healthy donors.

**Glycosylated G-CSF is more potent than non-glycosylated G-CSF in mobilisation of peripheral blood progenitor cells in healthy volunteers**

(paper VI)

Rational

*In vitro* bone marrow colony assay studies indicate that lenograstim (glycosylated G-CSF) is more potent than filgrastim (non-glycosylated G-CSF). However, such a difference has not yet been shown *in vivo*.

The primary objective of this trial was to compare the efficacy of equivalent doses (μg) of lenograstim and filgrastim G-CSF in mobilising CD34+ cells. Major secondary objectives were to compare the kinetics of the increase in $CD34^+$ cells in blood, and to assess the safety and tolerability of the two drugs given to healthy subjects.

Materials and methods

Thirty-two healthy male volunteers, median age 27 years (19-44), were randomised to receive either lenograstim 10 μg/kg followed by filgrastim 10 μg/kg or vice versa with a wash-out period of minimum four weeks. Both drugs were administered as s.c. injections once daily for five days (day 1-5). CD34+ cells were enumerated at days 1 and 4-8. All flow cytometric analyses were performed blindly by one and the same investigator.

Results and discussion

There was no evidence of a carry-over effect for any of the predefined efficacy variables. $CD34^+$ cells were mobilised with a similar kinetics, peaking at median day 6 (5-6) for both drugs. A significant difference in favor of lenograstim was shown for peak number of $CD34^+$ cells/μl blood (104±38 vs 82±35, mean±1SD, $p<0.0001$, paired t-test, n=30) and number of CFU-GM/μl blood at day 6 (14.6±8.4 vs 10.2±4.6, $p<0.0001$), respectively. There was no difference in day 6 number of $CD3^+$ cells. Both drugs were generally well tolerated and did not differ with respect to number of adverse events.

The results show that there is a difference in *in vivo* potency between lenograstim and filgrastim. Its seems reasonable to assume that the greater potency of lenograstim is related to its being glycosylated, although the physiological significance of the carbohydrate chain of G-CSF molecule is unknown. As for clinical implications, the finding may be relevant for the dosing of G-CSF for mobilisation of progenitor cells in healthy donors.

Conclusion.
Lenograstim 10 μg/kg/day mobilises blood progenitor cells more efficiently than filgrastim 10 μg/kg/day in healthy volunteers, indicating a difference in *in vivo* potency between the two G-CSFs.

## GENERAL CONCLUSIONS

**GM-CSF after ABMT**
- increases neutrophil counts through other mechanisms than stimulation of neutropoietic proliferation
- stimulates eosinophil regeneration

**G-CSF *in vivo***
- enhances the release of secondary granule proteins from neutrophil granulocytes
- increases the plasma level of TNF-α
- impairs neutrophil chemotaxis
- enhances neutrophil adherence and phagocytic capacity
- has no effect on the respiratory burst

**G-CSF during standard induction chemotherapy for ALL**
- reduces the period of neutropenia
- no difference in infectious morbidity could be shown

**G-CSF 3-10 μg/kg/d to healthy volunteers**
- favors mobilisation of primitive $CD34^+$ cell subsets

**Glycosylated G-CSF (lenograstim) 10 μg/kg/d to healthy volunteers**
- is well tolerated
- mobilises progenitor cells more efficiently than 3, 5 and 7.5 μg/kg/d
- mobilises progenitor cells more efficiently compared to an identical (μg) dose of non-glykosylated G-CSF (filgrastim)

## REFERENCES


1. Demetri G, Griffin J: Granulocyte Colony-Stimulating Factor and Its Receptor. Blood 78:2791, 1991

2. Gasson J: Molecular Physiology of Granulocyte-Macrophage Colony-Stimulating Factor. BLOOD 77:1131, 1991

3. Lieschke G, Burgess A: Granulocyte colony-stimulating factor and granulocyte-macrophage colony-stimulating factor. New Engl J Med 327:28, 1992

4. Dreger P, Haferlach T, Eckstein V, Jakobs S, Suttorp M, Löffler H, Müller-Ruchholtz W, Schmitz N: G-CSF-mobilized peripheral blood progenitor cells for allogeneic transplantation: safety, kinetics of mobilization, and composition of the graft. Br J Haematol 87:609, 1994

9. Emerson SG. The stem cell model of hematopoiesis. In: Hoffman R, Benz E-JJ, Shattil SJ, Furie B, Cohen H, ed. Hematology: Basic principles and practice. 1:st ed. New York: Churchill Livingstone Inc.1991,: 72.

10. Metcalf D: The molecular control of cell division, differentiation commitment and maturation in haematopoietic cells. Nature 339:27, 1989

14. McCredie K, Hersh EM, Freireich EJ: Cells Capable of Colony Formation in the Peripheral Blood of Man. Science 171:293, 1971

16. Bender J, Unverzagt K, Walker D, Lee W, Van Epps DE, Smith D, Stewart CC, To LB: Identification and comparison of CD34-positive cells and their subpopulations from normal peripheral blood and bone marrow using multicolor flow cytometry. Blood 77:2591, 1991

17. To LB, Haylock DN, Dowse T, Simmons PJ, Trimboli S, Ashman LK, Juttner CA: A comparative study of the phenotype and proliferative capacity of peripheral (PB) $CD34^+$ cells mobilized by four different protocols and those of steady-state PB and bone marrow $CD34^+$ cells. Blood 84:2930, 1994

20. Kessinger A, Armitage JO, Landmark JD, Weisenburger DD: Reconstitution av Human Hematopoietic Function with Autologous Cryperserved Circulating Stem Cells. Exp Hematol 14:192, 1986

21. Robinson W, Mangalik A: The kinetics and regulation of granulopoiesis. Seminars in Hematology 12:7, 1975

23. Bainton D, Ullyot J, Farquhar M: The development of neutrophilic polymorphonuclear leukocytes in human bone marrow. Origin and content of azurophil and specific granules. J Exp Med 134:907, 1971

25. Bodey G, Buckley M, Sathe Y, Freireich E: Quantitative relationshsips between circulating leukocytes and infection in patients with acute leukemia. Ann Intern Med 64:328, 1966

27. Butcher E: Leukocyte-endothelial cell adhesion as an active, multi-step process: A combinatorial mechanism for specificity and diversity in leukocyte targeting. Avd. Exp. Med. Biol. 323:181, 1992

33. Springer T: Adhesion receptors of the immune system. Nature 346:425, 1990

44. Xu SY, Carlson M, Engström Å, Garcia R, Peterson C, Venge P: Purification and chracterization of a human neutrophil lipocalin (HNL) from the secondary granules of human neutrophils. The Scandinavian Journal of Clinical&Laboratory Investigation 54:365, 1994

48. Olofsson T, Olsson I, Venge P, Elgefors B: Serum myeloperoxidase and lactoferrin in neutropenia. Scandinavian Journal of Haematology 18:73, 1977

49. Xu SY, Peterson C, Carlson M, Venge M: The development of an assay for neutrophil lipocalin - to be used as a specific marker of neutrophil activity in vitro and in vivo. Journal of Immunological Methdos 171:245, 1994

50. Öberg G, Venge P: Bone-marrow regeneration after therapy-induced hypoplasia monitored by serum measurements of lactoferrin, lysozyme and myeloperoxidase. Scand J of Haematology 37:130, 1986

51. Öberg G, Simonsson B, Smedmyr B, Tötterman TH, Venge P: Myeloid regeneration after bone-marrow transplantation monitored by serum measurements of myeloperoxidase, lysozyme and lactoferrin. Eur J of Haematology 38:356, 1987

53. Pauksen K, Elfman L, Ulfgren A-K, Venge P: Serum levels of granulocyte-colony stimulating factor (G-CSF) in bacterial and viral infections, and in atypical pneumonia. Bristish Journal of Haematology 88:256, 1994

54. Xu SY, Pauksen K, Venge P: Serum measurements of human neutrophil lipocalin (HNL) discriminate between bacterial and viral infections. The Scandinaivan Journal of Clinical&Laboratory Investigation 55:125, 1995

57. Lopez A, J Williamson JG CG Begley, JM Harlan, SJ Klebanoff, A Waltersdorph, G Wong SC Clark, MA Vadas: Recombinant human granulocyte-macrophage colony stimulating factor stimulates in vitro mature neutrophil and eosinophil functions, surface receptor expression and survival. J Clin Invest 78:1220, 1986

59. Stanley E, Lieschke G, Grail D, Metcalf D, Hodgson G, Gall J, Maher D, Cebon J, Sinickas V, Dunn A: Granulocyte-macrophage colony-stimulating factor-deficient mice show no major perturbation of hematopoiesis but develop a characteristic pulmonary pathology. Proc natl Acad Sci USA 91:5592, 1994

61. Gerhartz H, Engelhard M, Meusers P, Brittinger G, Wilmanns W, Schlimok G, Mueller P, Huhn D, Musch R, Siegert W, Gerhartz D, Hartlapp J, Thiel E, Huber C, Peschl C, Spann W, Emmerich B, Schadek C, Westerhausen M, Pees H-W, Radtke H, Engert A, Terhardt E, Schick H, Binder T, Fuchs R, Hasford J, Brandmeier R, Stern A, Jones T, Ehrlich H, Stein H, Parwaresch M, Tiemann M, Lennert K: Randomised, double-blind, placebo-controlled, phase III study of recombinant human granulocyte-macrophage colony stimulating factor as adjunct to induction treatment of high-grade malignant non-Hodgkin´s lymphoma. Blood 82:2329, 1993

63. Bradley T, Metcalf D: The growth of mouse bone marrow cells in vitro. Aust J Exp Biol Med Sci 44:287, 1966

66. Burgess A, Metcalf D: Characterisation of a serum factor stimulating the differentiation of myelomonocytic leukemia cells. International Journal of Cancer 26:647, 1980

67. Nicola N, Metacalf D, Matsumoto M, Johnson G: Purification of a factor inducing differentiation in murine myelomonocytic leukemia cells. Identification as granulocyte colony-stimulating factor. Journal of Biological Chemistry 258:9017, 1983

70. Souza L, Boone T, Gabrilove J, Lai P, Zsebo K, Murdock D, Chazin V, Bruszewski J, Lu H, Chen K, Barendt J, Platzer E, Moore M, Mertelsmann R, Welte K: Recombinant human

granulocyte colony-stimulating factor: Effects on normal and leukemic myeloid cells. Science 232:61, 1986

71. Nagata S, Tsuchiya M, Asano S, Kaziro Y, Xamazaki T, Yamamoto O, Hirato Y, Kubota N, Ohedd M, Nomura H, Ono M: Molecular cloning and expression of cDNA for human granulocyte colony-stimulating factor. Nature 319:415, 1986

75. Bronchud M, Scarffe J, Thatcher N: Phase I/II study of recombinant human granulocyte colony-stimulating factor in patients receiving intensive chemotherapy for small cell lung cancer. Br. J Cancer 56:809, 1987

76. Oheda M, Hase S, Ono M: Structure of the sugar chains of recombinant human granulocyte colony-stimulating factor produced by Chinese hamster ovary cells. J Biochem 103:544, 1988

80. Oh-Eda M, Hasegawa M, Hattori K, Kuboniwa H, Kojima T, Orita T, Tumonou K, Yamazakai T, Ochi N: O-Linked Sugar Chain of Human Granulocyte Colony-stimulating Factor Protects against Polymerizatioin and Denaturation Allowing It to Retain Its Biological Activity. The Journal of Biological Chemistry 265:11432, 1990

81. Kishita M, al e: Stability of G-CSF in serum. The Clinical Report 26:1, 1992

88. Olsson I, Gullberg U, lantz M, Richter J: The receptors for regulatory molecules of hematopoiesis. European Journal of Haematology 48:1, 1992

89. Fukunaga R, Ishizaka-Ikeda E, Nagata S: Purification and characterization of the receptor for murine granulocyte colony-stimulating factor. J Biol Chem 265:14008, 1990

91. Dong F, van Buitenen C, Pouwels K, Hoefsloot L, Löwenberg B, Touw I: Distinct cytoplasmic regions of the human granulocyte colony-stimulating factor receptor involved in induction of proliferation and maturation. Mol Cell Biol 13:7774, 1993

94. Löwenberg B, Touw I: Hematopoietic growth factors and their receptors in acute leukemia. Blood 81:281, 1993

95. Watari K, Asano S, Shirafuji N, Kodo H, Ozawa K, Takaku F, Kamachi S: Serum granulocyte colony-stimulating factor levels in helathy volunteers and patients with various disorders as estimated by enzyme immunoassay. Blood 73:117, 1989

103. Lieschke GJ, Grail D, Hodgson G, Metcalf D, Stanley E, Cheers C, Fowler KJ, Basu S, Zhan YF, Dunn AR: Mice Lacking Granulocyte Colony-Stimulating Factor Have Chronic Neutropenia, Granulocyte and Macrophage Progenitor Cell Deficiency, and Impaired Neutrophil Mobilization. Blood 84:1737, 1994

105. Begley C, Lopez A, Nicola N, Warren D, Vdas M, Sanderson C, Metcalf D: Purified colony-stimulating factors enhance the survival of human neutrophils and eosinophils in vitro: a rapid and sensitive microassay for colony-stimulating factors. Blood 68:162, 1986

114. Kerst M, de Haas M, van der Schoot E, Slaper-Cortenbach I, Kleijer M, von dem Borne A, van oers R: Recombinant Granulocyte Colony-Stimulating Factor Administration to Healthy Volunteers: Induction of Immunophenotypically and Functionally Altered Neutrophils via an Effect on Myeloid Progenitor Cells. Blood 82:3265, 1993

115. Katoh M, Shirai T, Shikoshi K, Ishii M, Saito M, Kitigawa S: Neutrophil kinetics shortly after initial administration of recombinant granulocyte colony-stimulating factor: neutrophil alkaline phosphatase activity as an endogenous marker. Eur J Haematol 49:19, 1992

118. Lord B, Bronchud M, Owens S, Chang J, Howell A, Souza L, Dexter T: The kinetics om human granulopoiesis following treatment with granulocyte colony-stimulating factor in vivo. Proc Natl Acad Sci USA 86:9499, 1989

120. Lord BI, Molineux G, Pojda Z, Souza LM, Mermod JJ, Dexter TM: Myeloid Cell Kinetics in Mice Treated With Recombinant Interleukin-3, Granulocyte Colony-Stimulating Factor (CSF), Granulocyte-Macrophage CSF In Vivo. Blood 77:2154, 1991

121. Bensinger WI, Price TH, Dale DC, Appelbaum FR, Clift R, Lilleby K, Williams B, Storb R, Thomas ED, Buckner D: The Effects of Daily Recombinant Human Granulocyte Colony-Stimulating Factor Administration on Normal Granulocyte Donors Undergoing Leukapheresis. Blood 81:1883, 1993

127. Repp R, Valerius T, Sendler A, Gramatzki M, Iro H, Kalden J, Platzer E: Neutrophils Express the High Affinity Receptor for IgG (FcgammaR1, CD64) After In Vivo Application of Recombinant Human Granulocyte Colony-Stimulating Factor. Blood 78:885, 1991

131. Trillet-Lenoir V, Green J, Manegold C, Von Pawel J, Gatzemeier U, Lebeua B, Depierre A, Johnson P, Decoster G, Tomita D, Ewen C: Recombinant Granulocyte colony stimulating factor reduces the infectious complications of cytotoxic chemotherapy. Eur J Cancer 294:319, 1993

134. Gisselbrecht C, Prentice HG, Bacigalupo A, Biron P, Milpied N, Rubie H, Cunningham D, Legros M, Pico JL, Linch DC, Burnett AK, Scarffe JH, Siegert W, Yver A: Placebo-controlled phase III trial of lenograstim in bone-marrow transplantation. The Lancet 343:696, 1994

137. Sheridan W, Wolf M, Lusk J, layton JE, Souza L, Morstyn G, Dodds A, Maher D, Green M, Fox R: Granulocyte colony-stimulating factor and neutrophil recovery after high-dose chemotherapy and autologous bone marrow transplantation. The Lancet 891, 1989

140. Dale D, Bonilla M, Davis M: A randomised controlled phase III trial of recombinant human granulocyte colony-stimulating factor (filgrastim) for treatment of severe chronic neutropenia. Blood 81:2496, 1993

146. Nelson S: Role of granulocyte colony-stimulating factor in the immune response to acute bacterial infection in the non-neutropenic host. Clin Infect Dis 18:S197, 1994

147. Pizzo P: Management of fever in patients with cancer and treatment-induced neutropenia. N Engl J Med 328:1323, 1996

152. Crawford J, Ozer h, Stoller R, Johnson D, Lyman G, Tabbara I, Kris M, Grous J, Picozzi V, Ruasch G, Smith R, Gradishar W, Yahanda A, Vincent M, Stewart M, J G: Reduction by granulocyte colony-stimulating factor of fever and neutropenia induced by chemotherapy in patients with small-cell lung cancer. The New Engl of Medicine 325:164, 1991

158. Dombret H, Chasttang C, Fenaux P, Reiffers J, Bordessoule D, Boubdallah R, Mandelli F, Ferrant A, Auzanneau G, Tilly H, Yver A, Degos L: A controlled study of recombinant human granulocyte colony-stimulating factor in elderly patients after treatment for acute myelogenous leukemia. N Engl J Med 332:1678, 1995

159. Ottman O, Hoelzer D, Gracien E: Concomitant colony-stimulating factor and induction chemoradiotherapy in adult acute lymphoblastic leukemia. Blood 86:444, 1995

165. To L. Mobilizing and collecting blood stem cells. In: Gale RP, Juttner C, Henon P, ed. Blood stem transplants. New York, US: Cambridge University Press, 1994: 56.

166. Laterveer L, Lindley I, Heemskerk D, Camps J, Pauwels E, Willemze R, Fibber W: Rapid mobilization of hematopoietic prgoenitor cells in rhesus monkeys by a single intravenous injection of interleukin-8. Blood 87:781, 1996

167. Richman CM, Weiner RS, Yankee RA: Increase in Circulating Stem Cells Following Chemotherapy in Man. Blood 47:1031, 1976

169. Papayannopoulou T, Nakamato B: Peripheralization of hemopoietic progenitors in primates treated with anti-$VLA_4$ integrin. Proc. Natl. Acad. Sci. USA 90:9374, 1993

170. To LB, Sheppherd KM, Haylock DN, Dyson PG, Charles P, Thorp DL, Dale BM, Dart GW, Roberts MM, Sage RE, Juttner CA: Single High Doses of Cyclophophamide Enable the Collection of High Numbers of Hemopoietic Stem Cells from the Peripheral Blood. Exp. Hematology 18:1990

172. Pettengell R, Morgenstern GR, Woll PJ, Chang J, Rowlands M, Young R, Radford JA, Scarffe JH, Testa NG, Crowther D: Peripheral Blood Progenitor Cell Transplantation in Lymphoma and Leukemia Using a Single Apheresis. Blood 82:3770, 1993

174. Dührsen U, Villeval J-L, Boyd J, Kannourakis G, Morstyn G, Metcalf D: Effects of recombinant human granulocyte colony-stimulating factor on hematopoietic progenitor cells in cancer patients. Blood 72:2074, 1988

175. Socinski MA, Cannistra SA, Elias A, Antman KH, Lowell S, Griffin JD: Granulocyte-Macrophage Colony Stimulating Factor Expands the Circulating Haemopoietic Progenitor Cell Compartment In Man. The Lancet May 28:1194, 1988

180. Sheridan WP, Begley CG, Juttner CA, Scer J, To LB, Maher D, McGrath KM, Morstyn G, Fox RM: Effect of peripheral-blood progenitor cells mobilised by filgrastim (G-CSF) on platelet recovery after high-dose chemotherapy. The Lancet 339:640, 1992

182. Lane T, Law P, Maruyama M, Young D, Burgess J, Mullen M, Mealiffe M, Terstappen L, Hardwick A, Moubayed M, Oldham F, Corringham R, Ho A: Harvesting and enrichment of hematopoietic progenitor cells mobilized into the peripheral blood of normal donors by granulocyte-macrophage colony-stimulating factor (GM-CSF) or G-CSF: potential role in allogeneic marrow transplantation. Blood 85:275, 1995

184. Matsunaga T, Sakamaki S, Kohgo Y, Ohi S, Hirayama Y, Niitsu Y: Recombinant human granulocyte colony-stimulating factor can mobilize sufficient amounts of peripheral blood stem cells in healthy volunteers for allogeneic transplantation. Bone Marrow Transplantation 11:103, 1993

188. Roberts AW, Metcalf D: Granulocyte colony-stimulating factor induces selective elevations of progenitor cells in the peripheral blood of mice. Exp Hematol 22:1156, 1994

189. Stoppa AM, Blaise D, Viens P, Baume D, Mannoni P, Novakovitch G, David FA, Yver A, Maraninchi D: Phase I study of in vivo lenograstim (rHuG-CSF) for stem cell collection demonstrates improved neutrophil recovery after autologous bone marrow transplantation. Bone Marrow Transplantation 13:541, 1994

191. Bensinger WI, Longin K, Appelbaum F, Rowley S, Weaver C, Lilleby K, Gooley T, Lynch M: Peripheral blood stem cells (PBSCs) collected after recombinant granulocyte colony stimulating factor (rhG-CSF): an analysis of factors correlating with the tempo of engraftment after transplantation. Br J Haematol 87:825, 1994

193. Tjønnfjord G, Steen R, Evensen S, Thorsby E, Egeland T: Characterization of $CD34^+$ peripheral blood cells from healthy adults mobilized by recombinant human granulocyte colony-stimulating factor. Blood 84:2795, 1994

194. Demuynck H, Pettengell R, de Campos E, Dexter TM, Testa NG: The Capacity of Peripheral Blood Stem Cells Mobilised with Chemotherapy plus G-CSF to Repopulate Irradiated Marrow Stroma *in vitro* is Similar to that of Bone Marrow. Eur J. Cancer 28:381, 1992

195. Molineux G, Pojda Z, Hampson IN, Lord BI, Dexter TM: Transplantation potential of peripheral blood stem cells induced by granulcyte colony-stimulating factor. Blood 76:2153, 1990

199. Civin C, Strauss L, Brovall C, Fackler M, Schwartz J, Shaper J: Antigenic analysis of hematopoiesis. III. A hematopoietic progenitor cell surface antigen defined by a monoclonal antibody raised against KG-1a cells. The Journal of Immunology 133:157, 1984

200. Krause D, Fackler M, Civin C, May W: Review: CD34: Structure, biology, and clinical utility. Blood 87:1, 1996

202. Berenson RJ, Bensinger WI, Hill RS, Andrews RG, Garcia-Lopez J, Kalamasz DF, Still BJ, Spitzer G, Buckner CD, Bernstein ID, Thomas ED: Engraftments After Infusion of CD34+ Marrow Cells in Patients with Breast Cancer or Neuroblastoma. Blood 77:1717, 1991

204. Siena S, Bregni M, Brando B, Belli N, Ravagnani F, Gandola L, Stern A, Lansdorp P, Bonadonna G, Gianni A: Flow cytometry for clinical estimation of circulating hematopoietic progenitors for autologous transplantation in cancer patients. Blood 77:400, 1991

205. Bender JG, Lum L, Unverzagt KL, Lee W, Van Epps D, George S, Coon J, Ghalie R, McLeod B, Kaizer H, Williams SF: Correlation of colony-forming cells, long-term culture initiating cells and $CD34^+$ cells in apheresis products from patients mobilized for peripheral blood progenitors wih different regimens. Bone Marrow Transplantation 13:479, 1994

206. Johnsen HE (for the laboratories associated to the Nordic Myeloma Study Group): Report from a Nordic workshop on $CD34^+$ cell analysis: technical recommendations for progenitor estimation in leukapheresis from multiple myeloma patients. Journal of Hematotherapy 4:21, 1995

211. Sutherland HJ, Eaves CJ, Eaves AC, Dragowska W, Lansdorp PM: Characterization and partial purification of human marrow cells capable of initiating long-term hematopoiesis in vitro. Blood 74:1563, 1989

215. Baum CM, Weissman I, Tsukamoto A, Buckle A-M, Peault B: Isolation of a candidate human hematopoietic stem-cell population. Proceedings of the National Academy of Science, USA 89:2804, 1992

216. Kessinger A, Armitage J, Smith D, Landmark J, Bierman P, Weisenburger D: High-dose therapy and autologous peripheral blood stem cell transplantation for patients with lymphoma. Blood 74:1260, 1989

220. Roberts MM, To LB, Gillis D, Mundy J, Rawling C, Ng K, Juttner CA: Immune reconstitution following peripheral blood stem cell transplantation, autologous bone marrow transplantation and allogeneic bone marrow transplantation. Bone Marrow Transplantation 12:469, 1993

221. Peters WP, Rosner G, Ross M, Vredenburgh J, Meisenberg B, Gilberg C, Kurtzberg J: Comparative Effects of Granulocyte- Macrophage Colony-Stimulating Factor (GM-CSF) and

Granulocyte Colony-Stimulating Factor (G-CSF) on Priming Peripheral Blood Progenitor Cells for Use with Autologous Bone Marrow After High-Dose Chemotherapy. Blood 81:1709, 1993

222. Brugger W, Bross K, Glatt M, Weber F, Mertelsmann R, Kanz L: Mobilization of Tumor Cells and Hematopoietic Progenitor Cells Into Peripheral Blood of Patients with Solid Tumours. Blood 83:636, 1994

228. Kernan N, Collins N, Juliano L, Cartagena T, Dupont B, O´Reilly R: Clonable T lymphocytes in T cell-depleted bone marrow transplants correlate with development of graft-v-host disease. Blood 68:770, 1986

229. Pan L, Delmonte Jr J, Jalonen CK, Ferrara JLM: Pretreatment of Donor Mice With Granulocyte Colony-Stimulating Factor Polarizes Donor T Lymphocytes Toward Type-2 Cytokine Production and Reduces Severity of Experimental Graft-Versus-Host Disease. Blood 86:4422, 1995

230. Körbling M, Przepiorka D, Huh YO, Engel H, van Besien K, Giralt S, Andersson B, Kleine HD, Seong D, Deisseroth AB, Andreeff M, Champlin R: Allogeneic blood stem cell transplantation for refractory leukemia and lymphoma: potential advantage of blood over marrow allografts. Blood 85:1659, 1995

231. Bensinger WI, Weaver CH, Appelbaum FR, Rowley S, Demirer T, Sanders J, Storb R, Buckner CD: Transplantation of allogeneic peripheral blood stem cells mobilized by recombinant human granulocyte colony-stimulating factor. Blood 85:1655, 1995

232. Schmitz N, Dreger P, Suttorp M, Rohwedder E, Haferlach T, Löffler H, Hunter A, Russell NH: Primary transplantation of allogeneic peripheral blood progenitor cells mobilized by filgrastim (granulocyte colony-stimulating factor). Blood 85:1666, 1995

*A complete list of references may be obtained from the author.*