Migration of human hematopoietic progenitor cells
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Citation for published version (APA):
Chapter 1

General Introduction
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1. INTRODUCTION

Hematopoietic stem cell transplantation is performed to rescue a patient’s hematopoietic system after myeloablative chemo- or radiotherapy. A special feature of hematopoietic stem cell transplantation is the migration of intravenously infused hematopoietic stem- and progenitor cells (HPC) from peripheral blood to bone marrow, a process referred to as homing. Despite the clinical and biological significance, the (adhesive) mechanism(s) by which the process of homing of HPC to the bone-marrow microenvironment are mediated, are still not completely understood. Improvement of the efficacy of stem cell transplantation might be achieved by modulating the ability of stem cells to home to the bone marrow. For this purpose, a better understanding of the mechanisms involved in homing is necessary.

This thesis focuses on the migration of various sources of normal and malignant HPC. Special emphasis is placed on Stromal Cell Derived Factor-1 (SDF-1) and its receptor CXCR-4, because this chemokine has been described as an important chemoattractant for normal and malignant HPC. The following introduction provides an overview of normal and malignant hematopoiesis and of mechanisms involved in migration of hematopoietic stem cells.

2. HEMATOPOIESIS

All mature blood cells are derived from hematopoietic stem cells (HSC). In healthy adults, hematopoiesis takes place in the bone marrow (BM), where the majority of hematopoietic stem cells are located. These stem cells are pluripotent, i.e. they have the capability of self-renewal as well as the capacity to differentiate into committed progenitor cells. Further maturation and proliferation of the progenitor cells leads to the production of mature cells of various lineages, which are released into the peripheral blood. Stem cells and progenitor cells express the CD34 antigen, which is one of the most common markers used to characterize and to isolate these cells. During differentiation of the HPC the expression of CD34 antigen gradually diminishes, i.e. mature cells do not express the CD34 antigen anymore.

However, a recent study showed reversible expression of CD34 by murine hematopoietic stem cells, suggesting that CD34 may be a marker of activated stem
cells but not necessarily of all stem cells [1]. Recent work indicates that human CD34+ stem cells may also exist, and are able to induce hematopoietic activity after transplantation. [2-5]. Further investigation is necessary to determine whether human CD34+ and CD34- stem cells are representing the same cell population and the CD34 antigen is just a marker for activated stem cells.

3. STEM CELL TRANSPLANTATION

In patients undergoing chemo- and/or radiotherapy, hematopoiesis is seriously disturbed. Bone-marrow function in these patients can be reconstituted by bone-marrow transplantation (BMT) and by the more recently developed method of cord blood or peripheral blood stem-cell transplantation (PBSCT). BMT or PBSCT may be allogeneic (from an HLA-matching donor) or autologous (from the patient him-or herself). For allogeneic PBSCT, Granulocyte-Colony-Stimulating Factor (G-CSF) and for autologous PBSCT, G-CSF in combination with chemotherapy is usually applied to mobilize CD34+ cells into peripheral blood (PB) [6,7]. After reinfusion, CD34+ cells derived from peripheral blood, bone marrow or cord blood (CB) home to the bone marrow, and stem cells from all three sources are able to reconstitute BM function [8,9].

Several factors determine the outcome as measured by hematopoietic recovery of stem cell transplantations. The number of transplanted CD34+ cells seems to be the major factor [10]. The threshold quantity of PB CD34+ cells needed for transplantation is generally thought to lie between 2.5 and 5.0 x 10^6 cells/kg bodyweight [10-12]. There is a relationship between the number of CD34+ cells transplanted and the time required for hematological reconstitution. Patients who received a greater number of CD34+ cells/kg had shorter recovery times than patients grafted with smaller number of CD34+ progenitor cells [13-15].

3.1 Sources of stem cells

Three different sources of stem cells are used for stem cell transplantation, i.e bone marrow, mobilized peripheral blood (PBSC), and umbilical cord blood (UCB). Bone marrow transplantation has been used since the late 1950s [16,17]. The ability of PBSC to reconstitute hematopoiesis has been shown by several groups in 1986 [18-21]. PBSC were collected during steady-state hematopoiesis or following cytotoxic chemotherapy, since cytokines were not available at that time. In 1989 the first transplantation of cytokine-mobilized PBSC was reported [22]. The number of circulating HPC can be increased by a number of stimuli, including
chemotherapy and cytokines such as G-CSF, granulocyte-macrophage colony stimulating factor (GM-CSF), stem cell factor (SCF), thrombopoietin (TPO), and IL-8 [23-31]. Several studies have shown that patients receiving mobilized HPC experienced a significantly faster neutrophil and platelet recovery than historical controls who received autologous bone marrow transplantation [26,32,33]. Mobilized PBSC are also widely used for allogeneic grafting, although an increased risk of chronic graft-versus-host disease (GVHD) has been reported [34,35]. Mobilized HPC are the most common source of stem cells nowadays, since large numbers of CD34+ cells can easily be harvested.

In the early 1980s it has been discovered that UCB contains high numbers of HPC [36,37]. An advantage of the use of CB cells could be that they are immunologically immature and therefore less likely to cause GVHD [38]. Subsequently, in 1988 the first successful CB transplant was performed in a Fanconi's anemia patient [39]. Until now, approximately 1,000 CB transplants have been reported in literature [40-43]. Initially, it was assumed that only children and small-size adults could be transplanted with UCB, because a single UCB collection might not contain enough repopulating cells to transplant an adult patient, but clinical data have shown that cord blood stem cell grafts are capable of inducing stable engraftment, despite the very low numbers of CD34+ cells present [42].

Moreover, various in vitro and in vivo assays have been developed to study differences between stem cells of various sources to repopulate the hematopoietic system. Over the last decade, several groups have developed functional in vivo assays to quantitate the number of primitive human severe combined immune deficiency (SCID) repopulating cells (SRCs), based on their ability to repopulate bone marrow of intravenously transplanted SCID or nonobese diabetic (NOD/SCID) mice [44,45]. It was observed that UCB stem cells, unlike PB or BM derived stem cells, are capable of engrafting NOD/SCID mice without the administration of exogenous cytokines [46]. Using the NOD/SCID model several groups have shown that SRC are enriched in UCB compared with adult BM or mobilized peripheral blood [47,48]. Noort et al. have shown that the repopulating ability and homing potential in NOD/SCID mice of CD34+ cells isolated from UCB was higher than that of BM-derived cells [49]; similar results were shown by Holyoake et al [50]. In contrast to the SRC frequency, which is normally determined 35 days after transplantation, the seeding efficiency of human stem cells is usually analyzed 24 hours after transplantation [51]. Using this assay, van
Hennik et al did not observe any significant differences in the seeding efficiencies of UCB, BM and PBSC [52].

4. Homing

Homing of HPC can be considered as a multistep process, in which various adhesion molecules present on both HPC and endothelial cells are involved, similar to what has been found for transendothelial migration of leukocytes at places of inflammation (Figure 1) [53,54]. The first step in migration consists of tethering and rolling of HPC along the endothelium [55-57]. The adhesion molecules known to be involved in these processes are described below. Chemokines (chemotactic cytokines) or growth factors located on the endothelial surface or bound to proteoglycans in the endothelial extracellular matrix, activate $\beta_1$- and $\beta_2$-integrins on the rolling HPCs, leading to firm adhesion to Ig-like ligands on the endothelial cells [58-60]. Chemokines or other growth factors can activate integrins by binding to their specific receptors, which are either seven-transmembrane spanning G-protein coupled or cell surface growth factor receptors [61]. Subsequently, the firm

![Figure 1. Sequential steps in transendothelial migration. Abbreviations: HPC= hematopoietic progenitor cell; BMEC= bone marrow endothelial cell.](image-url)
adhesion is followed by transendothelial migration of HPC and finally leads to anchoring of the HPC in the bone-marrow microenvironment [62-64]. Adhesion molecules involved in transendothelial migration of HPC were unidentified at the start of this project. The anchoring of HPC depends mainly on adhesion via integrins to stromal cells and to extracellular matrix proteins [65-67]. This multistep process, involving the different adhesion molecules that are activated by a range of modifiers, may explain selective migration at specific places. However, Rood et al. did not obtain any indication that BM endothelial cells express a specific adhesion molecule with respect to homing of HPC [68].

4.1 Adhesion Molecules
Since adhesion molecules play an important role in the trafficking of HPC cells during the mobilization and homing processes, we here summarize the adhesion molecules present on HPC or endothelial cells. The adhesion molecules can be divided in the following superfamilies: Selectins, Integrins, Immunoglobulin superfamily, Sialomucins, CD44 antigens and Cadherins.

4.1.1 Selectins
Selectins are transmembrane cell surface proteins of which three family members have been identified so far; E(CD62E)-, L (CD62L)- and P(CD62P)-selectin. Selectins are important for the initial rolling of leukocytes along the vessel wall [69]. E-Selectin is expressed on activated endothelial cells, P-Selectin on endothelial cells (activated with thrombin or histamine) and platelets and L-selectin on leukocytes, including HPC [70]. All selectins bind to sialylated carbohydrates; i.e. sialyl Lewis\(^x\) (sLe\(^x\)) or its isoform sLe\(^a\) [71]. L-selectin binds to the mucins CD34, GlyCAM, MadCAM-1 [72-74]. CD34 is expressed on endothelium and may therefore serve as an endothelial ligand for L-selectin. A novel ligand for L-selectin has just been discovered [75]. This hematopoietic cell L-selectin ligand (HCLL) is expressed by normal and leukemic hematopoietic progenitors and mediates L-selectin-dependent cell-cell adhesive interactions (Sackstein). Moreover, L- P-and E- selectin can bind to P-selectin glycoprotein ligand-1 (PSGL-1), expressed by leukocytes, including HPC [76-81]. Another ligand for E-selectin is E-selectin ligand (ESL-1) expressed on myeloid cells [82,83].

Since bone marrow endothelial cells have been described to constitutively express high levels of E-selectin, this molecule is thought to play a role in adhesion and transendothelial migration of HPC [84-86]. A recent \textit{in-vitro} study indeed indicated that SDF-1-induced migration of human CD34\(^-\) cells is mediated by E-
selectin [87]. Moreover, studies in murine models have indicated that E-selectin [88] and P-selectin [88,89] are involved in the initial adhesion and rolling of HPC on the bone-marrow endothelial cells (BMEC).

In addition, Dercksen et al. showed for patients undergoing PBSCT, that the number of L-selectin positive CD34^+ cells is a better predictor of rapid recovery than the total number of CD34^+ cells. This suggests that L-selectin is involved in the homing of the CD34^+ cells after PBSC transplantation [90].

### 4.1.2 Integrins

Integrins are a family of heterodimeric cell surface molecules consisting of a noncovalently bound α- and β-chain. Thus far, 17 α-subunits and 8 β-subunits have been defined, which in combination can result in at least 23 different integrin heterodimers [91-93]. The integrins mediate adhesion of HPC to extracellular matrix proteins (ECM) such as fibronectin, collagen, laminin or thrombospondin, or cell-surface-expressed cell adhesion molecules (CAM) i.e. VCAM and ICAMs [94,95]. The integrins can be divided into 8 subfamilies on the basis of the different β-subunits. Of the β₁-integrins or Very Late Antigens (VLA’s), mainly α₄β₁ and α₅β₁ are expressed on HPC [66]. Furthermore, HPC express two β₂-integrins LFA-1 (α₅β₂) and Mac-1 (α₅β₂) [66,95]. β₂-integrins mediate cell-cell adhesive interactions by binding to immunoglobulin superfamily molecules on other cells, such as endothelial cells [96].

Moreover, blocking antibodies against β₂-integrins inhibit spontaneous transmigration of HPC through monolayers of bone marrow endothelial cells in an in-vitro system [63,64], while during SDF-1-induced transendothelial migration of murine HPC, VLA-4 and VCAM-1 are involved [97]. In vivo experiments suggest a relatively predominant role of β₁-integrins in the retention of HPC in the marrow and homing of HPC to the marrow.[54,98,99]. This idea stems from the observation that circulating CD34^+ cells express VLA-4 at a lower level than CD34^+ cells residing in the BM. This suggests that the release of CD34^+ cells and the ability to circulate is related to the presence and expression level of VLA-4 [90, 100-104]. In addition, treatment of human CD34^+ cells with antibodies against β₁-integrins prevented engraftment in NOD/SCID mice and sheep [105,106].

### 4.1.3 Immunoglobulin superfamily

Immunoglobulin superfamily antigens are characterized by repeats of immunoglobulin-like domains. Adhesion mediated by these molecules is either
homophilic (between two identical molecules) or heterophilic (between two different molecules). PECAM-1 (CD31) is an example of the first group. VCAM, ICAM-1,-2 and -3, are examples of the second group; they bind to integrins. PECAM-1 is expressed on platelets, endothelial cells and leukocytes, including HPC [107]. ICAM-1 is constitutively expressed on leukocytes and endothelial cells, and activation of the endothelial cells by LPS, IL-1 or TNF-α induce expression of ICAM-1 [108]. ICAM-2 is expressed on endothelial cells and leukocytes [109], while ICAM-3 is mainly expressed on leukocytes [110]. VCAM-1 is expressed at low levels on endothelial cells (upregulated by LPS, IL-1 or TNF-α), but is constitutively expressed on bone-marrow endothelial cells [84]. A relatively new family member is CD166 (ALCAM), a transmembrane protein expressed on 40-70% of CD34+ cells from BM or mobilized PB [111-112]. Almost all primitive (CD38+, thy-1+) CD34+ cells express CD166. It is probably involved in homophilic interactions between early hematopoietic progenitors.

4.1.4 Sialomucins

The mucin-like molecules represent an emerging family of transmembrane glycoproteins expressed by tissues of the hematopoietic system [113]. Mucin-like molecules all share the common characteristic of being highly glycosylated polypeptides, containing predominantly O-linked carbohydrate side-chains [113].

Several sialomucins have been found on HPC, including the stem cell antigen CD34 [114], CD43 [115,116], CD45RA [117] and PSGL-1 [56,80] and CD164 [118]. Other important members of this family that are not expressed on HPC include GlyCAM-1 and MadCAM-1, ligands for L-selectin (see paragraph 4.1.1). Moreover, CD34 is also expressed on endothelial cells [119]. Although the precise function of CD34 remains unclear, several studies show that it can act as an adhesion molecule [72,120,121].

4.1.5 CD44

CD44 is a highly glycosylated surface molecule with various isoforms arising from differences in glycosylation and alternative splicing [122]. Normal and malignant HPC express CD44, which supports adhesion to hyaluronan (HA) and fibronectin [123-126]. Furthermore, CD44 is involved in the recirculation of lymphocytes through the binding to vascular addressins on high endothelial venules [127]. In CD44 knock-out mice, progenitor cell egress from the BM appears to be defective [128]. CD44 functions as a cell-cell adhesion receptor as well as an extracellular matrix receptor.
4.1.6 Cadherins
Cadherins constitute a major class of adhesion molecules that support calcium-dependent, homophilic cell-cell adhesion in all solid tissues of the body [129]. The cadherin family can be divided into two subfamilies, the classic cadherins and the protocadherins; each of the subfamilies can be further subdivided [93]. The only cadherins strongly expressed on endothelial cells are Neural (N)- and Vascular Endothelial (VE) cadherin. Recently, Cadherin-13 was found in bovine aortic endothelium [130]. N-cadherin is expressed on adult neural tissue, muscle cells and endothelial cells [93], whereas (VE)-cadherin is expressed on all types of endothelium. VE-cadherin is localized in the intercellular junctions, and its expression is required for the control of vascular permeability and vascular integrity [131]. Moreover, epithelial (E) cadherin has been described to be expressed on erythroid cells, mainly on normoblasts and erythroblasts [132].

4.2 Migration and motility
Prior to migration in response to a chemotactic stimulus, leukocytes display a complex repertoire of motility-associated processes, including shape changes, polarization of signaling complexes, actin polymerization and polarized adhesion. Subsequent acto-myosin based contractility moves the cell forward, in the direction of the source of the chemotactic gradient. Chemokines bind to their receptors, which thereby trigger signaling cascades that lead to directed motility. We here summarize the role of chemokines involved in migration of HPC and the cytoskeletal changes necessary for this process.

4.2.1 Chemoattractants and chemokines in historical perspective
There are two classes of chemoattractants, the classical leukocyte chemoattractants such as fMLP, LTB₄, C5a and PAF, and the chemotactic cytokines (chemokines). Classical leukocyte chemoattractants act broadly on neutrophils, eosinophils, basophils and monocytes [133]. Chemokines are a group of small (8-14kDa), structurally related molecules that regulate cell trafficking of various types of leukocytes through interactions with a subset of seven-transmembrane, G-protein-coupled receptors [134,135]. About 40 different chemokines have now been identified in humans, but new chemokines are still discovered in a rapid pace.

Chemokines have been divided into two major subfamilies on the basis of the arrangement of the two N-terminal cysteine residues, depending on whether the first two cysteine residues have an amino acid between them (CXC) or are adjacent (CC). [61,134]. Two other sub-families of chemokines have been described: C and
CX3C, but they both consist of only one member each [134]. Chemokine receptors are also divided into subfamilies, i.e. the CXCR, CCR, CR and CX3CR family [134,136]. Recently, new nomenclature has been proposed in which the R (of receptor) is replaced by a L (of ligand) to designate the chemokines [134]

Although several factors have been identified as chemoattractants for leukocytes, until 1997 only limited information was available on chemoattractants for HPC. Till that time, a broad range of chemotattractants and growth factors had been tested for their chemotactic effect on HPC. No chemotactic effects were found with fMLP, Rantes, Gro-α, MIP-1α, MIP-1β, MCP-1, GM-CSF, IP-10, IL-1α, IL-1β, IL-4, IL-6, IL-8, eotaxin, TNF-α, IFN-γ [62,137]. In contrast, stem cell factor (SCF) and IL-3 were reported as a chemoattractant for murine HPC [138], but these factors only slightly induced migration of human HPC [139-140]. Cherry et al. showed that murine bone marrow stromal cell lines produce a chemotactic factor for mouse hematopoietic stem cells [141]. Furthermore, Schweitzer et al. observed that supernatant of bone marrow cultures enhanced the migration of HPC through bone-marrow endothelium, providing evidence for the production of (a) chemotactic factor(s) for human HPC as well [137]. At that time it was postulated that one of these chemotactic factors might be hepatocyte growth factor/scatter factor (HGF/SF), which is produced by murine stromal cells as well as by human bone marrow stromal cells [142,143]. At the start of this project, HGF/SF was a promising candidate to induce migration of normal and malignant hematopoietic progenitor cells. HGF/SF is described below.

4.2.1.1 HGF/SF
Hepatocyte growth factor /scatter factor (HGF/SF) is a multifunctional cytokine that regulates cell growth, motility, migration and angiogenesis [144-146]. HGF/SF is produced by a variety of mesenchymal cells as a single chain pro-HGF/SF, which is enzymatically cleaved into biologically active HGF/SF consisting of an α- and a β-chain [147-151]. The receptor for HGF/SF is encoded by the MET proto-oncogene, c-MET, which is a cell surface tyrosine-kinase receptor also consisting of an extracellular α- and a transmembrane β-chain [152].

The ligand and its receptor play a role in dissemination of multiple solid tumors. HGF/SF is produced by tumor cells or stromal cells within the tumor and in addition a role has been proposed to HGF/SF and c-MET in hematopoiesis [143,153-155]. CD34+ HPC express the c-MET receptor, and it was observed that HGF/SF promoted survival of human HPC and could be detected in the supernatant
Chapter 1

of bone-marrow stromal cells [142,156]. Moreover, HGF/SF and c-MET seem to be involved also in malignant hematopoiesis, because it has been reported that leukemic and lymphoma cells produce HGF/SF [157]. Furthermore, significant amounts of HGF/SF have been detected in blood and bone-marrow plasma of leukemic patients [158,159]. Overexpression of c-MET, leading to activation of the proto-oncogene, has also been detected in some cases of human leukemia and lymphoma [159,160].

4.2.1.2 Chemokines for HPC

In 1997, the first powerful chemoattractant for CD34⁺ cells was described, i.e. stromal cell-derived factor-1 (SDF-1, also known as PBSF), produced by stromal cells, including those from the bone marrow [62,140]. It has subsequently been shown that SDF-1 activates integrins on HPC and induces transendothelial migration of HPC in vitro [62,161]. SDF-1 is classified as a CXC-chemokine and is also a chemoattractant for monocytes and lymphocytes [162]. The receptor for SDF-1 is a G-protein-coupled receptor, called Fusin, LESTR or CXCR-4 [163-166]. In SDF-1 or CXCR-4 knock-out mice, hematopoietic precursors do not shift to the bone-marrow during fetal development, suggesting that SDF-1 plays an important role in the migration of HPC to the BM [167-169]. Recently, Peled et al. found SDF-1 and CXCR-4 to be critical for murine bone marrow engraftment by SCID-repopulating stem cells [170]. They also demonstrated that migration of CD34⁺CD38⁻loa cells to SDF-1 in vitro correlated with in-vivo engraftment and stem cell function in NOD/SCID mice.

Furthermore, Aiuti et al. have described that chemotaxis of CD34⁺ cells to SDF-1 was enhanced by pretreatment of the cells with IL-3 or SCF [62]. Recently, two studies appeared both reporting an enhancing effect of chemotaxis of CD34⁺ cells by a combination of SDF-1 and SCF compared to SDF-1 alone [140,171]. The enhanced chemotaxis observed with the combination of SDF-1 and SCF can possibly be explained by cooperativity in downstream signaling pathways [171].

The CC chemokine Macrophage inflammatory protein-3β (MIP-3β, also known as CKβ-11 or ELC) and its receptor CCR-7 were originally described to be important in migration of B and T cells [172,173]. A few studies have shown that MIP-3β can also act as chemoattractant for a small subset of human CD34⁺ cells [174,175]. In contrast to SDF-1, which attracts multiple types of HPC, MIP-3β attracted mainly HPC restricted to macrophage differentiation [175]. Recently, another CC chemokine Secondary lymphoid-tissue chemokine (SLC, also known
as exodus2/6ckine/TCA4), has been shown to be a specific agonist for CCR-7
[176,177], and similar to MIP-3β it was found to be mainly attractive for
macrophage progenitors [178].

4.2.2 Cytoskeleton
Chemokines that bind to G-protein-coupled receptors trigger signaling cascades
that lead to directed motility. This signaling is still poorly understood but probably
involves Rho-like GTPases that regulate temporally and spatially coordinated actin
polymerization in concert with integrin-mediated adhesion [179-181]. Shape
to changes and migration requires dynamic rearrangements of the cytoskeleton and
modulation of cell adhesion [182,183]. These temporal and spatial reorganizations
of cell structure and cell contacts are controlled by extracellular signals including
growth factors, chemokines and adhesion molecules.

The cytoskeleton consists mainly of three components: actin filaments,
microtubules, and intermediate filaments [182-184]. Reorganization of actin
filaments and cell-substratum contacts is believed to be involved in cell motility
[185]. Actin at the area of the leading edge is thought to be continuously
depolymerizing and repolymerizing during cell movement [184].

5 MALIGNANT HEMATOPOIEISIS

5.1 Acute myeloid leukemia (AML)
Acute myeloid leukemia (AML) is caused by malignant counterparts of
hematopoietic progenitor and precursor cells. AML is characterized by an increase
in the number of immature myeloid cells in the marrow and an arrest in their
maturation, finally resulting in hematopoietic insufficiency, with or without
leukocytosis. [186]. AML is a heterogeneous disease, caused by a variety of
pathogenic mechanisms [186]. In contrast to normal hematopoiesis, immature cells
in AML leave the bone marrow, and these cells may anchor in extramedullar
locations, such as in liver and spleen. This may reflect differences in the control of
migration of leukemic cells in comparison with non-malignant cells.

The most commonly used method of classification has been developed by the
French-American-British (FAB) group, and divides AML into distinct subtypes
(M0-M7) that differ with respect to the particular myeloid lineage involved and the
degree of leukemic cell differentiation [187-191]. In addition, cytogenetic analysis
of leukemic blasts has resulted in the identification of specific chromosomal
aberrations in about half of patients with AML [192-194]. For example, in 98% of the patients suffering from acute promyelocytic leukemia (AML-M3), a t(15;17;q22;q21) translocation has been observed.

AML treatment usually includes intensive chemotherapy, alone or in combination with autologous or allogeneic stem cell transplantation. Despite treatment, the survival rate among patients who are less than 65 years of age is only 40 percent [195]. Patients older than 60 years generally have an even poorer prognosis, with a probability of survival at five years of less than 10 percent [195].

Similar to what has been found for normal hematopoietic progenitor cells, Möhle et al. recently described that leukemic blasts from patients with acute myeloblastic leukemia (AML) express variable amounts of CXCR-4 [161]. This expression might be an indication that the SDF-1/CXCR-4 pathway plays a role in the trafficking of leukemic cells. Further research is necessary to establish this hypothesis (this thesis).

5.2 Myelodysplastic syndrome (MDS)
MDS are a heterogeneous group of clonal hematological disorders characterized by cytopenias and dysplastic changes of hematopoietic cells, due to a defect of the stem cells [196]. The MDS are classified into five subgroups i.e.: refractory anemia (RA), RA with ring sideroblasts (RARS), RA with excess blasts (RAEB), RAEB in transformation (RAEBt) and chronic myelomonocytic leukemia (CMML).

About half of the patients are over 70 years old, and males are more commonly affected. MDS is considered pre-leukemic since a significant percentage of patients develop AML [197]. Conversion to AML is diagnosed when the percentage myeloblasts in the marrow exceed 30 % [186]. Similar to what has been found in AML patients, approximately half of the MDS patients bear specific cytogenetic lesions such as deletions of (parts of) chromosome 5 and/or 7 [198]. A variety of treatments has been attempted for MDS patients, depending on the severity of the disease. This varies from transfusion of red cells and platelets to intensive chemotherapy or bone-marrow transplantation.

6. SCOPE OF THIS THESIS
The aim of this study was to unravel different mechanisms involved in migration of hematopoietic progenitor cells during homing to the bone marrow. A better understanding of the mechanisms involved in homing is necessary to improve the efficiency of stem cell transplantation.
Since different sources of CD34<sup>+</sup> cells are available for stem cell transplantation, we first established the *in-vitro* migratory capacity of CB-, PB- and BM-derived CD34<sup>+</sup> cells over FN-coated filters. For this purpose, an improved Transwell assay was developed for which low numbers of cells are needed. Furthermore, we investigated the role of CXCR-4 expression on these cells and the integrins involved in SDF-1-induced migration (chapter 2). Subsequently, we investigated to what extent the *in-vitro* migratory capacity of PB-derived CD34<sup>+</sup> cells is related to hematopoietic recovery after autologous stem cell transplantation (chapter 3).

Large differences in migration between CD34<sup>+</sup> cells of various sources and within one source (CB, PB, BM) were observed. Therefore, we investigated in chapter 4 whether differences in migratory ability of various sources of CD34<sup>+</sup> cells could be explained by differences in actin polymerization. For movement of cells in the direction of a chemotactic gradient, cells have to be able to change their cytoskeleton. Rearrangement of the actin cytoskeleton has been described as an early cellular response during chemotactic activation. In the present study we analyzed the extent and kinetics of SDF-1-induced actin polymerization in CB and PB-derived CD34+ cells and studied whether this correlated with the migratory capacity of these cells.

In the process of homing, CD34<sup>+</sup> hematopoietic progenitor cells migrate across the bone-marrow endothelium in response to SDF-1. To study the process of homing in a situation that better resembles the *in vivo* situation, we have developed an *in-vitro* transendothelial migration assay. In this assay, we investigated the role of adhesion molecules on the CD34<sup>+</sup> cells as well as on the endothelial cells (chapters 5 and 6).

Acute myeloid leukemia (AML) cells represent malignant counterparts of hematopoietic progenitor and precursor cells. Because leukemic and lymphoma cells produce HGF/SF, we investigated whether HGF/SF affects the function of leukemic cells. Migration of leukemic cells induced by HGF/SF was investigated in an *in-vitro* assay similar to the assay for normal HPC (chapter 7).

Möhle et al. previously showed that leukemic blasts from patients with acute myeloblastic leukemia (AML) express variable amounts of CXCR-4 [161]. In the present study, we compared spontaneous and SDF-1-induced migration of leukemic cells from the peripheral blood or the bone marrow derived of 26 patients. Besides migration and CXCR-4 expression, these cells were also analyzed for cell cycle and phenotypical analysis, to determine which other factors could
play an important role in the trafficking of malignant hematopoietic cells (Chapter 8). In Chapter 9 the results of the preceding chapters are discussed and summarized.

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Chapter 1


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General Introduction


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General Introduction


General Introduction
