Autologous stem cell transplantation (auto-SCT) has an important role in the treatment of multiple myeloma (MM) and in many patients with non-Hodgkin lymphoma (NHL). Various mobilization methods are used to harvest the stem cells from peripheral blood. The effects of the mobilization regimens on the graft cellular composition and the effects of the graft composition on post-transplant recovery and outcome have been unclear. These were the issues addressed in this series of studies performed as a part of the prospective GOA (Graft and Outcome in Autologous transplantation) study.
Graft and Outcome in Autologous Stem Cell Transplantation
JAAKKO VALTOLA

Graft and Outcome in Autologous Stem Cell Transplantation

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ABSTRACT

High-dose therapy (HDT) supported by autologous stem cell transplantation (auto-SCT) has an established role in the treatment of multiple myeloma (MM) and non-Hodgkin lymphoma (NHL). The landscape for blood stem cell mobilization has changed during the last decade, mainly due to the introduction of the novel mobilizing agent plerixafor to clinical use. This series of studies was performed within a prospective multicenter GOA (Graft and Outcome in Autologous transplantation) study, where the aim was to evaluate the effect of various mobilization methods, including plerixafor in poor mobilizers, on blood graft cellular composition, post-transplant recovery and outcome after auto-SCT.

The use of plerixafor in chemomobilized NHL patients was associated with higher numbers of T and B lymphocytes and NK cells and an increased proportion of primitive CD34+CD133+CD38- cells from the total CD34+ cells in the grafts. In plerixafor-mobilized patients, hematological recovery after auto-SCT was slightly slower for platelet engraftment, but the NK cell recovery was faster. There was a correlation between higher amounts of lymphocytes in the grafts and a more rapid lymphocyte recovery after auto-SCT. Outcome was not affected by the use of plerixafor.

In NHL patients a more rapid early immune recovery (absolute lymphocyte count on day +15 post-transplant (ALC-15) \( \geq 0.5 \times 10^9/L \)) was favorable in regard to progression-free survival (PFS), and in patients with aggressive lymphomas also with respect to overall survival (OS). In multivariate analysis a higher number of CD34+ and CD34+CD133+CD38- cells in the grafts and the use of plerixafor for mobilization were prognostic for ALC-15 \( \geq 0.5 \times 10^9/L \).

Analysed as part of the randomized MM-02 study, the blood grafts of MM patients mobilized with low-dose cyclophosphamide (CY) plus granulocyte-colony-stimulating factor (G-CSF) contained significantly more CD34+ cells than the grafts of MM patients mobilized with G-CSF alone. In grafts mobilized with G-CSF alone the proportion of CD34+CD133+CD38- cells from all CD34+ cells and the absolute numbers of T and B lymphocytes as well as NK cells were significantly higher. G-CSF alone mobilization was associated with a higher ALC-15 count and more rapid NK cell recovery at three and six months post-transplant. Outcome was not affected by the method of mobilization.

Blood grafts of chemomobilized MM patients and MM patients mobilized with G-CSF alone contained higher proportions of CD34+CD133+CD38- cells and more T and B lymphocytes as well as NK cells if also plerixafor was administered. In patients mobilized with G-CSF alone the graft composition was comparable except for the higher number of CD3+CD4+ lymphocytes in patients receiving also plerixafor. Hematological and immune recovery as well as outcome were comparable, but CD3+CD4+ lymphocyte recovery was more rapid in the plerixafor-mobilized patients. No difference in PFS or OS was observed according to the use of plerixafor in MM patients.

The method of blood stem cell mobilization substantially affects autologous graft composition, which may influence the post-transplant recovery. The optimal mobilization method for various disease entities is still unknown and warrants further studies together with functionality analyses of the mobilized and recovering lymphocytes.
National Library of Medicine Classification: QU 325, WH 140, WH 525, WH 540, WO 660
Medical Subject Headings: Transplantation, Autologous; Stem Cell Transplantation; Autografts; Multiple Myeloma; Lymphoma, Non-Hodgkin; Heterocyclic Compounds; Cyclophosphamide; Granulocyte Colony-Stimulating Factor; B-Lymphocytes; T-Lymphocytes; Killer Cells, Natural; Antigens, CD34; Lymphocyte Count; Disease-Free Survival; Prognosis; Survival Rate; Graft Survival; Prospective Studies; Humans
Korkea-annoksisella solunsalpaajahoidolla (intensiivihoido) ja tämän jälkeen potilaalta itseltään kerättyjen kantasolujen palauttamisella (autologinen kantasolusirto) on keskeinen merkitys erityisesti multippelin myelooman (MM) ja non-Hodgkin-lymfooman (NHL) hoidossa. Kantasolusirteiden keräämistä edeltävän ja keräyksen mahdollistavan kantasolujen periferiseen veneen mobilisoinnin käytäntöä ovat muuttuneet viime vuosina. Erityisesti uusimmasta käyttöön tulleesta lääkkeestä, pleriksaforista, on muodostunut tärkeä apu huonosti kantasolujen mobilisoiville potilaalle. Tässä tutkimussarjassa selvitettiin prospektiivisesti, osana Graft and Outcome in Autologous transplantation (GOA) -tutkimusta, eri mobilisaatiomenetelmien – mukaan lukien pleriksafori huonosti mobilisoivilla potilailla – vaikutusta kantasolusirteiden solukoostumukseen sekä siirteen vaikutusta intensiivihoidon jälkeiseen toipumiseen ja ennusteeseen.


NHL-potilailla nopean varhaisen immunologisen palautumisen (arvioituna veren lymphosyyttimäärän ≥ 0.5x10⁹/L pv+15 siirrosta, ALC-15) todettiin ennustavan pidempää siironjälkeistä tautivapaata aikaa (IPS) ja aggressiivisissa tauhmuoduissa myös pidempää kokonaiselossaaloaikaa (OS). Monimutuuttoja- analyysissä ALC-15 ≥ 0.5x10⁹/L suhteen ennustelleislisiä tekijöitä osoittautuivat siirteen suurempi CD34⁺- ja CD34⁺/CD133⁺/CD38⁻-solumäärä sekä pleriksaforin käyttö.


immunologinen toipuminen erosivain CD3⁺CD4⁺ -lymfosyyttien osalta pleriksaforia saaneiden eduksi. Pleriksaforin käyttämisen ei todettu vaikuttavan siirronjälkeiseen ennusteeeseen.

Mobilisaatiomenetelmät vaikuttavat siirteen solukoostumukseen ja tällä taas on merkitystä siirronjälkeisen toipumisen kannalta. Kunkin tautientiteen suhteen optimaalisen mobilisaatiostrategian selvittäminen vaatii lisätutkimuksia erityisesti kerättyjen lymfosyyttipopulaatioiden funktionaalisilla tutkimuksilla, mutta myös kantasaosisiirron jälkeisen immunijärjestelmän toipumisen osalta.

Luokitus: QU 325, WH 140, WH 525, WH 540, WO 660
Yleinen Suomalainen asiasanasto: kantasolujen siirto; kantasolut; myeloma; non-Hodgkin-lymfoomat; pleriksafori; sytostaattihoito; lääkehoito; kasvutekijät; lymfosyytit; valkosolut; toipuminen; henkiinjääminen
Acknowledgements

The Graft and Outcome in Autologous transplantation (GOA) study, which served as the basis of this thesis was initiated at Department of Medicine, Kuopio University Hospital in 2012. The most work of the thesis was also carried out at the Department of Medicine, Kuopio University Hospital. Also the Department of Clinical Microbiology, University of Eastern Finland was essentially involved during the course of the study from 2014 to 2018. Also, the Central hospitals of Jyväskylä, Joensuu, Savonlinna and Mikkeli as well as the University Hospitals of Oulu, Tampere and Turku were considerably involved in the study.

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Kuopio, July 2018

Jaakko Valtola
List of the original publications

This dissertation is based on the following original publications:


The publications were adapted with the permission of the copyright owners. The original publications are later referred by their Roman numerals.
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<th>Abbreviation</th>
<th>Definition</th>
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<tbody>
<tr>
<td>7-AAD</td>
<td>7-aminoactinomycin D</td>
</tr>
<tr>
<td>ALC-15/30</td>
<td>absolute lymphocyte count on day +15/30 post-transplant</td>
</tr>
<tr>
<td>ALDH</td>
<td>aldehyde dehydrogenase</td>
</tr>
<tr>
<td>AraC</td>
<td>high-dose cytarabine</td>
</tr>
<tr>
<td>ASBMT</td>
<td>American Society of Blood and Marrow Transplantation</td>
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<tr>
<td>Auto-SCT</td>
<td>autologous stem cell transplantation</td>
</tr>
<tr>
<td>B</td>
<td>blood</td>
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<tr>
<td>BEAC</td>
<td>carmustine, etoposide, cytarabine, cyclophosphamide</td>
</tr>
<tr>
<td>BEAM</td>
<td>carmustine, etoposide, cytarabine, melphalan</td>
</tr>
<tr>
<td>BM</td>
<td>bone marrow</td>
</tr>
<tr>
<td>CD</td>
<td>cluster of differentiation (surface antigen of cell)</td>
</tr>
<tr>
<td>CFU-GM</td>
<td>Colony Forming Unit-Granulocyte Macrophage</td>
</tr>
<tr>
<td>CHOP</td>
<td>cyclophosphamide, doxorubicin, vincristine, prednisolone</td>
</tr>
<tr>
<td>CHOEP</td>
<td>cyclophosphamide, doxorubicin, etoposide, vincristine, prednisolone</td>
</tr>
<tr>
<td>CI</td>
<td>confidence interval</td>
</tr>
<tr>
<td>CNS</td>
<td>central nervous system</td>
</tr>
<tr>
<td>CI</td>
<td>confidence interval</td>
</tr>
<tr>
<td>CNS</td>
<td>central nervous system</td>
</tr>
<tr>
<td>CXCR</td>
<td>chemokine receptor</td>
</tr>
<tr>
<td>CY</td>
<td>cyclophosphamide</td>
</tr>
<tr>
<td>DC</td>
<td>dendritic cell</td>
</tr>
<tr>
<td>DHAP</td>
<td>dexamethasone, high-dose cytarabine, cisplatin</td>
</tr>
<tr>
<td>DLBCL</td>
<td>diffuse large B-cell lymphoma</td>
</tr>
<tr>
<td>DMSO</td>
<td>dimethyl sulfoxide</td>
</tr>
<tr>
<td>EBMT</td>
<td>European Society for Blood and Marrow Transplantation</td>
</tr>
<tr>
<td>EFS</td>
<td>event-free survival</td>
</tr>
<tr>
<td>FL</td>
<td>follicular lymphoma</td>
</tr>
<tr>
<td>FLT3</td>
<td>fms-like tyrosine kinase 3</td>
</tr>
<tr>
<td>FMG</td>
<td>Finnish Myeloma Group</td>
</tr>
<tr>
<td>FSC</td>
<td>forward scatter</td>
</tr>
<tr>
<td>G-CSF</td>
<td>granulocyte colony-stimulating factor</td>
</tr>
<tr>
<td>GOA</td>
<td>Graft and Outcome in Autologous transplantation study</td>
</tr>
<tr>
<td>HD-MEL</td>
<td>high-dose melphalan</td>
</tr>
<tr>
<td>HDT</td>
<td>high-dose therapy</td>
</tr>
<tr>
<td>HL</td>
<td>Hodgkin lymphoma</td>
</tr>
<tr>
<td>HR</td>
<td>hazard ratio</td>
</tr>
<tr>
<td>HSC</td>
<td>hematopoietic stem cell</td>
</tr>
<tr>
<td>ICE</td>
<td>ifosfamide, carboplatin, etoposide</td>
</tr>
<tr>
<td>IMF</td>
<td>International Myeloma Foundation</td>
</tr>
<tr>
<td>IMWG</td>
<td>International Myeloma Working Group</td>
</tr>
</tbody>
</table>
ISHAGE  International Society of Hematotherapy and Graft Engineering
KUH  Kuopio University Hospital
La-CD34+  number of CD34+ cells in the leukapheresis product
LVL  large-volume leukapheresis
MCL  mantle cell lymphoma
MM  multiple myeloma
MM-02  Multiple Myeloma 02 study (by the FMG)
NHL  non-Hodgkin lymphoma
NK  natural killer
OR  odds ratio
OS  overall survival
OUH  Oulu University Hospital
PFS  progression-free survival
PTCL  peripheral T-cell lymphoma
R  rituximab
RVD  lenalidomide, bortezomib, dexamethasone
SDF-1  stroma-derived factor 1
SSC  side scatter
TBV  total blood volume
TUH  Tampere University Hospital
TYKS  Turku University Hospital
VCD  bortezomib, cyclophosphamide, dexamethasone
VD  bortezomib, dexamethasone

VCAM-1  vascular cell adhesion molecule 1
VLA  very late antigen
## 1 Introduction

The main purpose of any cancer treatment is to eliminate malignant cells without being too toxic or harmful for the patients. In hematological malignancies, the most common methods to achieve this goal include chemotherapy, radiotherapy, antibody-mediated treatments, novel targeted drugs and high-dose therapy (HDT) supported by stem cell transplantation.

The first attempts to introduce autologous stem cell transplantation (auto-SCT) into clinical practice were performed in the 1970s [Appelbaum et al. 1978]. Over the following decades more clinical experience was attained, and the methods for the mobilization, collection, and analysis of the stem cells evolved. Consequently, the number of auto-SCTs has been on a steady rise since the 1990s [Passweg et al. 2012]. In 2016 altogether 25,995 autologous transplantations were reported to the European Society of Blood and Marrow Transplantation (EBMT) [Passweg et al. 2018]. According to the EBMT registry, the most common indications for auto-SCTs were multiple myeloma (MM) (11,551 transplantations, 44%) and non-Hodgkin lymphoma (NHL) (6498 transplantations, 25%). Only a small proportion of patients received grafts harvested from bone marrow (BM), and almost 99% of autologous grafts were collected from peripheral blood.

To enable a successful engraftment after auto-SCT, an adequate number of CD34+ cells is needed in the graft. This, on the other hand, requires a successful outcome in the apheresis procedures which, in turn, is dependent on a proper mobilization of the CD34+ cells from the marrow to the circulation. The mobilization is usually performed by using a granulocyte colony-stimulating factor (G-CSF) alone or in a combination with chemotherapy (chemomobilization). However, poor mobilization is a significant clinical problem in 5-30% of patients when mobilized using the traditional methods [Pusic et al. 2008, Jantunen and Kvalheim 2010, Wuchter et al. 2010]. The most novel clinically available method to boost the mobilization process is use of plerixafor, a selective and reversible chemokine receptor 4 (CXCR4) antagonist.

The number of CD34+ cells in the grafts has traditionally been the most important parameter of the graft quality as a higher number of CD34+ cells has been correlated with more rapid engraftment and hematological recovery after high-dose therapy [Tricot et al. 1995, Weaver et al. 1995, Ketterer et al. 1998, Johnsen et al. 1998, Allan et al. 2002, Nieboer et al. 2004, Zubair et al. 2006, Klaus et al. 2007, Stiff et al. 2011, Russell et al. 2015], and in some studies also with better progression-free survival (PFS) or even improved overall survival (OS) after auto-SCT [Gordan et al. 2003b, Toor et al. 2004, Pavone et al. 2006, O’Shea et al. 2006, Yoon et al. 2009]. The generally accepted minimum number of CD34+ cells for a single transplant is $2 \times 10^6$/kg CD34+ cells, even though the optimal number may be higher [Siena et al. 2000, Giralt et al. 2009]. However, there is also a significant number of other cell types in the blood grafts, and the grafts have been, for example, reported to possess up to 20 times more lymphocytes than CD34+ cells [Varmavuo et al. 2012a, Varmavuo et al. 2012b]. These other cell types seem to have an important role in the post-transplant phase. For example, the higher number of infused lymphocytes has been associated with improved outcome [Porrata et al. 2004a, Porrata et al. 2004b, Katipamula et al. 2006, Porrata et al. 2008, Hiwase et al. 2008b, Porrata et al. 2016]. However, there has been a lack of prospectively collected data on the blood graft cellular composition regarding the various lymphocyte subsets and their effects on hematological and immune recovery after auto-SCT.

The mobilization methods traditionally used in NHL and MM patients have been reported to bear a unique effect on the cellular composition of the blood grafts, e.g. in regard to the number of more primitive CD34+ cells [Möhle et al. 1994, Varmavuo et al. 2012b, Varmavuo et al. 2013],
the total lymphocyte count and the number of various lymphocyte subsets [Holtan et al. 2007, Hiwase et al. 2008a, Hiwase et al. 2008b, Varmavuo et al. 2012a, Varmavuo et al. 2012b, Gaugler et al. 2013]. There are also a few reports on the effects of plerixafor on the blood graft cellular composition. These preliminary studies revealed plerixafor not only to be an efficient CD34+ mobilizer, but that it may also affect the blood graft composition in a unique manner [Holtan et al. 2007, Varmavuo et al. 2012a, Varmavuo et al. 2012b, Varmavuo et al. 2013]. Nevertheless, there have been no prospective studies comparing the effects of the various mobilization methods on the blood graft cellular composition.

The GOA study, which serves as the backbone for this dissertation (later referred to study), was conducted to prospectively investigate the blood graft cellular composition in patients with MM and NHL after various mobilization methods and to evaluate the correlations of blood grafts on the hematological and immune recovery and outcome after auto-SCT. In the first study, the aforementioned topics were studied in regard to the use of plerixafor in patients with NHL who mobilized poorly and in the fourth study the same scheme was performed in MM patients. In addition, the factors affecting early post-transplant immune recovery and subsequently its clinical significance in patients with NHL were analysed. Finally, patients with MM were randomly assigned to granulocyte-colony stimulating factor ± cyclophosphamide (CY) mobilization after induction therapy and the blood graft cellular composition and post-transplant recovery were analysed according to the randomized mobilization arms.
2 Review of the literature

2.1 AUTOLOGOUS STEM CELL TRANSPLANTATION (AUTO-SCT)

2.1.1 Current indications
Since the first clinical studies introducing the possible benefits of auto-SCT in patients with malignant lymphoma [Appelbaum et al. 1978], the evidence on behalf of its advantages has grown dramatically. Nowadays auto-SCT is considered a standard of care in various hematological malignancies [Ljungman et al. 2010, Majhail et al. 2015]. In recent years, also new indications for auto-SCT have emerged, for example AL amyloidosis [Jaccard et al. 2007, Rosengren et al. 2016], systemic sclerosis [van Laar et al. 2014, del Papa et al. 2017, Sullivan et al. 2018], multiple sclerosis [Muraro et al. 2017] and systemic lupus erythematosus [Leone et al. 2017].

Alongside with the increase in the number of conditions potentially benefiting from auto-SCT, the absolute number of transplant procedures performed has been steadily rising in Europe [Passweg et al. 2012, Passweg et al. 2018] as well as globally [Niederwieser et al. 2016]. According to the most recent survey by the EBMT, the main indications for auto-SCT are MM, NHL and Hodgkin lymphoma (HL) [Passweg et al. 2018]. According to the same survey, in 2016 over 11,000 transplantations were performed for patients with MM, more than 6000 transplantations for NHL patients and around 2000 transplantations for patients with HL [Passweg et al. 2018]. However, the registry covers data mainly from the European transplant centers reporting their activity to the registry and it also includes data from few non-European centers. In the US the transplantation statistics are very much alike as a similar increase in the total number of auto-SCTs performed has been observed and the proportion amongst the various transplantation indications is comparable [D’Souza et al. 2017].

2.1.2 Non – Hodgkin lymphoma
NHLs constitute a heterogenous group of lymphoid malignancies [Morton et al. 2007, Swerdlow et al. 2016], diffuse large B-cell lymphoma (DLBCL) being the most common histological subtype and accounting for up to 30-35% of all cases [Morton et al. 2007]. The incidence of DLBCL increases with age and during the last decades the general incidence of DLBCL has raised dramatically, probably in part due to the larger cohorts of the elderly in general population and the enhanced diagnostic techniques [Fisher and Fisher 2004, Teras et al. 2016].

Despite its aggressive behavior, in the majority of patients DLBCL is considered curable with the anthracycline-based, monoclonal CD20 antibody-containing immunochemotherapy regimens. The combination of cyclophosphamide, doxorubicin, vincristine and prednisone (CHOP) has been the most commonly used chemotherapy for decades and the addition of CD20 antibody rituximab (R) to chemotherapy in the first-line treatment has improved the outcomes [Coiffier et al. 2002]. Besides the R-CHOP regimen, other regimens, such as the combination of dose-adjusted etoposide, prednisone, vincristine, cyclophosphamide, doxorubicin and rituximab (DA-EPOCH-R) have been studied particularly in high-risk patients, with comparable or even better results in comparison to R-CHOP [Wilson et al. 2012]. Furthermore, the combination of dose-intensive rituximab, doxorubicin, cyclophosphamide, vindesine, bleomycin and prednisone (R-ACVBP) has previously been reported to improve PFS compared with R-CHOP in certain patient populations [Rêcher et al. 2011]. In high-risk patients the regimen of rituximab plus hyperfractionated cyclophosphamide, vincristine, doxorubicin and dexamethasone (R-CVAD) alternating with high-dose methotrexate plus cytarabine has been
reported superior to R-CHOP-21 [Oki et al. 2013] and the addition of etoposide to R-CHOP (R-CHOEPEP) has also been promising in young patients with high-risk DLBCL [Gang et al. 2012, Vitolo et al. 2013]. Collectively, according to various studies, more intensive first-line treatment may be warranted especially in high risk and double or triple hit lymphomas [Zahid et al. 2017] and especially in younger patients with high risk DLBCL [Melén et al. 2016].

In relapsed DLBCL the second-line chemotherapy is commonly either a combination of rituximab with ifosfamide, etoposide, and carboplatin (R-ICE) [Kewalramani et al. 2004] or dexamethasone, high-dose cytarabine, and cisplatin (R-DHAP) [Gisselbrecht et al. 2010]. In the CORAL (Collaborative Trial in Relapsed Aggressive Lymphoma) study, these two regimens were compared in a randomized manner, and there was no significant difference in outcome in patients with first relapse or who were refractory after first-line treatment [Gisselbrecht et al. 2010]. However, in a subanalysis R-DHAP was reported to be superior over R-ICE in patients with germinal center B -like DLBCL [Thieblemont et al. 2011]. There is no consensus on the third line treatment of relapsed DLBCL, even though a recent subanalysis of the CORAL trial tried to clarify this issue [van den Neste et al. 2017]. Recently, the Nordic Lymphma Group has launched a phase II study combining pixantrone, bendamustine, etoposide and in CD20 positive tumors also rituximab (PREBEN), in patients with relapsed aggressive lymphomas [clinicaltrials.gov: NCT02678299].

The role of auto-SCT in the first-line treatment of DLBCL has been studied in the pre-rituximab era with contradictory results. Also, the results from more recent studies have been somewhat ambiguous [Kaneko et al. 2015, Cortelazzo et al. 2016, Landsburg et al. 2017, Chiappella et al. 2017], even though significantly improved PFS and OS were reported in young high-IPI-patients receiving intensified immunochemotherapy (R-CHOP) with methotrexate instead of immunochemotherapy (R-CHOP) plus auto-SCT [Strüßmann et al. 2017]. The current opinion is that auto-SCT does not seem to be generally beneficial in first-line treatment [Greber et al. 2008], but in high-risk patients it may be used after first-line treatment [Haioun et al. 2000, Ljungman et al. 2010, Stiff et al. 2013]. However, the benefits of the latter practice have been questioned if the first-line treatment is considered adequately intensive [Landsburg et al. 2017]. Also, according to a recent study, in primary refractory disease auto-SCT seems to be feasible if the salvage chemotherapy yields at least a partial response [Vardhana et al. 2017]. In clinical practice, the present standard of care is to treat DLBCL patients with HDT followed by auto-SCT in relapsed chemosensitive disease [Philip et al. 1995, Caimi et al. 2016], if only partial response is acquired after the first line chemoimmunotherapy [Janitunen and Sureda 2012] and in double or triple hit DLBCLs as part of first-line therapy, according to the discretion of the clinician.

Follicular lymphoma (FL) is a germinal center-derived indolent B-cell lymphoma that by nature tends to have a slow pace of progression. FL is the second most common type of NHL, comprising up to 20-25% of all NHLs [Teras et al. 2016]. FL is considered treatable, but in most scenarios not curable [Lunning et al. 2012], even though currently there might be a paradigm shift as novel treatments have been shown to be very effective [Cabanillas et al. 2013]. In addition, allogeneic transplantation might even provide a cure for some patients with relapsed FL, but is generally used with caution as it is associated with notable non-relapse mortality [Kuruvilla et al. 2016].

Patients with FL have multiple treatment options, including immunochemotherapy [van Oers et al. 2006], radiotherapy and auto-SCT [Kothari et al. 2014]. There are also novel drugs available, such as the phosphatidylinositol 3-kinase delta (PI3Kδ) inhibitor idelalisib [Gopal et al. 2014]. Recently, the combination of obinutuzumab plus bendamustine has been reported to be effective in rituximab-refractory disease [Sehn et al. 2016].

In FL, auto-SCT has been found to improve PFS [Lenz et al. 2004, Deconinck et al. 2005, Ladetto et al. 2008] but not OS [Deconinck et al. 2005, Ladetto et al. 2008, Gyan et al. 2009] when used following the first-line treatment in randomized studies. However, opposing results on the
benefits of auto-SCT in regard to PFS and OS after first-line treatment were reported by Sebban et al. [Sebban et al. 2006]. In their study, chemotherapy + interferon maintenance was reported to be equally effective compared to chemotherapy followed by auto-SCT. In relapsed FL auto-SCT is generally considered as a valid treatment as it has been reported to improve PFS and OS [Schouten et al. 2003, Le Gouill et al. 2011, Kothari et al. 2014], although the optimal timing of auto-SCT is still debated [Montoto et al. 2013, Kuruvilla et al. 2016]. In a recent report with a median follow-up of 12 years, auto-SCT seemed to be a valid treatment option in first, second and third complete remission (CR) [Jiménez-Ubieto et al. 2017]. In the case of FL transforming to a more aggressive lymphoma type, auto-SCT remains a valid treatment option [Hamadani et al. 2008].

*Mantle cell lymphoma* (MCL) accounts for about 7-9% of all NHLs in Europe [Swerdlow et al. 2016] and typically affects elderly males. The modern treatment of MCL is based on a combination of chemotherapy and immunotherapy with rituximab [Eskelund et al. 2016] but according to a recent study, the frontline treatment should be carefully evaluated according to the TP53 status of the patients [Eskelund et al. 2017].

Consolidation with auto-SCT after the first-line treatment has been reported to improve PFS [Dreyling et al. 2005] and to be promising also in regard to OS [Geisler et al. 2008, Geisler et al. 2012]. There is growing evidence that auto-SCT should be considered in transplant-eligible patients in first remission [Dreyling et al. 2005, Geisler et al. 2008], also recommended by a recent consensus project [Robinson et al. 2015]. Also, the use of maintenance therapy with rituximab has been reported beneficial after auto-SCT [Mei et al. 2017, Le Gouill et al. 2017]. However, even though especially the Nordic treatment protocol has been shown to be very effective [Geisler et al. 2012], long-term follow-up has demonstrated the slowly progressing nature of MCL with very late relapses [Eskelund et al. 2016].

*Peripheral T-cell lymphomas* (PTCLs) are a heterogenous group of mature T-cell lymphomas and comprise about 5% of all NHLs [Ellin et al. 2014, Kharfan-Dabaja et al. 2017, Teras et al. 2016]. PTCLs tend to have an aggressive behavior and poor prognosis [Schmitz and de Leval 2017]. Because of the heterogeneity and rarity of the PTCLs in the Western world [Ellin et al. 2014, d’Amore et al. 2012], there has been a lack of prospective studies, and there are still no data from a prospective randomized setting in the first-line treatment. The treatment of PTCLs is chemotherapy-based; in most subtypes CHOP or CHOEP regimens are used [Schmitz and de Leval 2017]. There is also growing evidence on the benefits of auto-SCT in PTCL after first-line treatment [Reimer et al. 2009, d’Amore et al. 2012, Jantunen et al. 2013] but randomized studies are lacking.

### 2.1.3 Multiple myeloma

MM develops from a clonal expansion of specific plasma cells producing excess amounts of monoclonal immunoglobulins and usually causing symptoms related either to extensive bone marrow infiltration, bone lesions or end-organ damage, especially renal failure. The treatment of MM has changed dramatically during the past years as a considerable number of new drugs have become available. The novel drugs used to treat MM mainly fall into the three categories: immunomodulatory agents (thalidomide, lenalidomide, pomalidomide), proteasome inhibitors (bortezomib, carfilzomib, ixazomib) and monoclonal antibodies (elotuzumab, daratumumab). Also, a drug serving as a histone deacetylase inhibitor (panobinostat) and another blocking the anti-apoptotic receptor BCL-2 (venetoclax) have been studied with promising results [Moreau et al. 2016, Kumar et al. 2017]. Still, old drugs like dexamethasone and prednisone and alkylating agents like melphalan and cyclophosphamide have retained their position in the treatment algorithms.

The superiority of auto-SCT over standard chemotherapy was shown before the era the novel myeloma drugs [Attal et al. 1996]. Concurring results have been verified in other prospective randomized studies [Child et al. 2003, Fermand et al. 2005], and auto-SCT is still a standard of
care in transplant-eligible patients [Giralt et al. 2015, Maybury et al. 2016, Attal et al. 2017]. Even though there have been studies questioning the position of auto-SCT over standard chemotherapy [Bladé et al. 2005, Barlogie et al. 2006], its role has also been evaluated in the era of the novel drugs [Palumbo et al. 2014, Gay et al. 2015, Attal et al. 2017] and auto-SCT has been reported to hold its place also in the modern treatment of MM. However, as new drugs are continuously developed and brought into clinical practice, the clinical setting may change rapidly and should be constantly re-evaluated in the future. Of note, these are the issues addressed in two large prospective randomized studies [“Randomized trial of lenalidomide, bortezomib, dexamethasone vs high-dose treatment with SCT in MM patients up to Age 65 (DFCI 10-106)” (clinicaltrials.gov: NCT01208662) and “Study to compare VMP with HDM followed by RVD consolidation and lenalidomide maintenance in patients with newly diagnosed multiple myeloma (HO95)” (clinicaltrials.gov: NCT01208766)].

The role of a second auto-SCT in MM progressing after initial treatment has been recently addressed in a meeting organized by the International Myeloma Foundation (IMF) through its International Myeloma Working Group (IMWG), together with the Blood and Marrow Transplant Clinical Trials Network (BMT CTN), the National Marrow Donor Program (NMDP), the EBMT, and the American Society of Blood and Marrow Transplantation (ASBMT) [Giralt et al. 2015]. The expert committee suggested that auto-SCT should be considered in relapse setting if the patient has not been treated with auto-SCT during first-line treatment or if the remission or response after the first auto-SCT has been 18 months or longer. In the clinical setting, it is a common practice to collect enough CD34+ cells for at least two transplants in patients who are considered fit for another transplant in the upcoming years.

Over the years there has been debate on the use of tandem transplants in MM as the upfront tandem transplantations have been reported to yield good outcomes [Attal et al. 2003, Barlogie et al. 2006, Barlogie et al. 2007]. In a comparison with a patient population treated mainly before the era of the novel myeloma drugs, the tandem transplantation protocol seemed feasible compared to other treatment modalities because PFS and OS were prolonged [Pineda-Roman et al. 2008]. Also, other studies conducted mainly before the introduction of modern drugs have reported tandem transplants to be superior to a single auto-SCT for OS [Attal et al. 2003] and PFS/EFS [Cavo et al. 2007]. Further, in a recent analysis an upfront tandem transplantation improved PFS over those treated with a single auto-SCT [Cavo et al. 2016]. However, in another recent analysis with a median follow-up over 11 years, a single transplantation was reported non-inferior to a protocol implementing two transplantations in regard to event-free survival and OS [Mai et al. 2016]. Still, performing tandem transplantations may be useful in selected patient populations, e.g. in newly diagnosed high-risk patients with chromosomal translocation within chromosomes 4 and 14 (t(4;14)) or deletion in short arm of chromosome 17 (del(17p)) it has been reported to improve PFS or OS [Moreau et al. 2007, Cavo et al. 2013]. To conclude, there is still no consensus on whether tandem transplantations should be used upfront and in which patient populations, especially because the use of maintenance treatment after auto-SCT seems to be effective at least in standard-risk patients [Cornell et al. 2017, Sengsayadeth et al. 2017].

2.2 MOBILIZATION OF BLOOD GRAFTS IN AUTOLOGOUS SETTING

2.2.1 Mechanisms of mobilization

Hematopoietic stem cells (HSCs) are a unique set of cells possessing an ability to give rise to any line of blood cells and to renew themselves [Lymperi et al. 2010, Mosaad et al. 2014, Yu et al. 2016]. Further, in appropriate circumstances HSCs may also be able to specialize into other non-hematological cell types [Mosaad et al. 2014]. In the steady state, the majority of HSCs
HSCs are anchored to the bone marrow stroma with various ligands and receptors. One of the most important factors retaining HSCs in the bone marrow is considered to be CXC-chemokine ligand 12 (CXCL12)/stroma-derived factor 1 (SDF-1), expressed by osteoblasts, endothelial cells, and reticular cells of the niche stroma [Alvarez et al. 2013]. CXCL12/SDF-1 adheres with the CXCR4 receptor presented on the surface of HSCs [Lapidot et al. 2002, Lymperi et al. 2010, Mosaad et al. 2014]. There are also other factors involved in the retention of HSCs in the niche, such as osteopontin, thrombopoietin, angiopoietin 1, stem cell factor (SCF) and other cell-adhesion molecules like cadherins, selectins, immunoglobulin superfamily, mucin-like family, CD44 family and integrins [Cao et al. 2016, De Grandis et al. 2016].

Homing of the HSCs is a term that refers to the process of CD34+ cells rapidly leaving circulation after the graft infusion [Lapidot et al. 2005]. The HSCs may exit circulation not only to the BM, but also to other organs such as liver and spleen, even though eventually also part of these cells may enter the BM [Lapidot et al. 2005]. The homing of HSCs involves multiple factors, including adhesion molecules such as vascular cell adhesion molecule 1 (VCAM-1), E-selectin/CD62 antigen-like family member E and P-selectins in the stroma [de Grandis et al. 2016]. There are also several important selectin binding ligands on the surface of HSCs affecting the homing process, e.g. P-selectin glycoprotein ligand 1 (PSGL-1) [Katayama et al. 2003] and CD44 [Sackstein et al. 2008]. Ultimately, the HSCs bind to the BM stroma through interaction with SDF-1 and CXCR4. The mobilization of stem cells from the BM into the circulation to facilitate apheresis requires various interventions targeting the complex mechanisms anchoring the HSCs in the BM niches.

2.2.2 Granulocyte colony-stimulating factor (G-CSF)

The first studies to demonstrate the effectiveness of chemokines in the mobilization of blood stem cells were published in the 1980s [Socinski et al. 1988]. In clinical practice G-CSFs have been used for stem cell mobilization since the early 1990s. Nowadays the use of G-CSF alone or after chemotherapy (chemomobilization) is the widely accepted standard of mobilizing stem cells for autologous purposes in patients with MM or NHL [Bensinger et al. 2009, Gertz et al. 2010]. The main advantages of using G-CSF alone mobilization are the ease of use, the predictability of the timing to initiate the apheresis and lower risk for adverse effects than with chemomobilization.

The commonly used G-CSFs have been either non-glycosylated filgrastim or glycosylated lenograstim [Gertz et al. 2010, Duong et al. 2014]. Usually the standard dose for filgrastim is 10μg/kg/day until the apheresis has been completed. Commonly the first apheresis is initiated on day (d) +4-5 from the start of G-CSF.

In recent years biosimilar filgrastim has become available and it has been found to be comparable to the original filgrastim [Bhamidipati et al. 2017]. The use of single-dose pegylated form of filgrastim (pegfilgrastim) has not been officially accepted for mobilization purposes, but according to recent reviews comparing pegfilgrastim to non-pegylated G-CSF, it seems to enable an earlier start of apheresis and require fewer apheresis sessions [Kobbe et al. 2009, Kim et al. 2015]. The comparative usefulness of pegfilgrastim over filgrastim has also been reported by Putkonen et al. in a retrospective single centre study [Putkonen et al. 2009]. Another pegylated G-CSF accepted for treatment of neutropenia after chemotherapy is lipepegfilgrastim and there is at least one ongoing clinical trial on the safety and efficacy of lipepegfilgrastim in the
mobilization of stem cells [“Lonquek for autologous stem cell mobilization” (clinicaltrials.gov: NCT02488382)].

The mechanism of stem cell mobilization by G-CSF is due to its proteolytic action in the bone marrow. Consequently, an increased number of several proteases can be measured in the bone marrow, e.g. matrix metallopeptidase 9 (MMP-9), cathepsin G and neutrophil elastase [Greenbaum and Link 2011, Alvarez et al. 2013]. As the result of the cleavage of e.g. the CXCL12/CXCR4 adhesion mechanism, various cell types egress from the marrow, including CD34+ stem cells [Greenbaum et al. 2011, Bonig and Papayannopoulou 2013, Alvarez et al. 2013].

2.2.3 Chemotherapy plus G-CSF

In patients with lymphoma the mobilization of stem cells has usually been performed by using chemotherapy (together with G-CSF), which serves also as a treatment for the malignancy itself. The collection of stem cells is initiated when the patient is recovering from the chemotherapy-induced cytopenias as at that time there are usually measurable numbers of CD34+ cells in blood [Richman et al. 1976, Abrams et al. 1981]. There is no single chemotherapy regimen recommended for all patients or diseases, but various combinations of cytotoxic drugs such as DHAP (dexamethasone, high-dose cytarabine, cisplatin), ICE (ifosfamide, carboplatin, etoposide) or high-dose cytarabine or etoposide alone may be used, depending on the underlying disease. In MM patients, cyclophosphamide (CY) in conjunction with G-CSF is commonly used and after RVD induction has been reported more effective than mobilization with G-CSF alone [Silvennoinen et al. 2016]. In general, the term chemomobilization is used to refer to a mobilization procedure with any form of chemotherapy plus G-CSF.

The use of chemomobilization compared to G-CSF alone mobilization has been reported to be more effective in regard to the apheresis yields and the number of apheresis sessions needed [Meldgaard Knudsen et al. 2000, Narayanasami et al. 2001, Bensinger et al. 2009, Gertz et al. 2010]. Also, added advantages of using chemomobilization are thought to base on its anti-tumor effects [Gertz et al. 2010] and in lymphoma patients a more intensive chemomobilization regimen has been reported to improve outcome over standard chemomobilization [Damon et al. 2015]. However, in MM patients the use of CY-based mobilization has not been associated with better outcomes over G-CSF alone mobilization [Dingli et al. 2006, Uy et al. 2015]. Furthermore, the use of chemomobilization is associated with common chemotherapy-related adverse effects like febrile neutropenia and infectious complications [Meldgaard Knudsen et al. 2000, Damon et al. 2015], which in turn may lead to prolonged hospitalization [Gertz et al. 2010]. However, the cytotoxic effects of the chemomobilization depend on the regimen used and vary amongst patients by their individual characteristics. Also, another difficulty with chemomobilization may be the difficulty to estimate the optimal timing to initiate apheresis because the length of the cytopenic phase varies individually.

2.2.4 Plerixafor

Use of chemomobilization or G-CSF alone to mobilize CD34+ cells is associated with the clinical problem of a considerable proportion of patients failing to mobilize an adequate graft to support HDT. The proportion of these poor mobilizers is estimated to be 5-30% [Weaver et al. 1995, Pusic et al. 2008, Bensinger et al. 2009, Wuchter et al. 2010, Jantunen et al. 2012]. Historically a common method to overcome this issue has been a re-mobilization either with G-CSF alone or chemomobilization, but these methods have been reported to result in 70-80% failure rates [Pusic et al. 2008]. Bone marrow collections may also be considered in patients who fail to mobilize enough CD34+ cells to peripheral blood [Jantunen & Kvalheim 2010].

The most recent clinically available method to overcome poor mobilization is the novel mobilizing agent plerixafor (formerly known as AMD3100), a small bicyclam molecule originally designed to treat patients with human immunodeficiency virus (HIV) infection [de
Clerq et al. 2009]. However, plerixafor was found to cause marked leukocytosis and in further studies, to mobilize CD34+ cells into the blood stream [Liles et al. 2003, Broxmeyer et al. 2005].

Plerixafor is a reversible antagonist of the CXCR4 receptor, which is widely presented on the surface of cells around the body. It is thought to mainly affect stem cell mobilization by blocking the CXCR4 and SDF-1 interaction [Keating et al. 2011], but the exact pin-point location for this mechanism is still somewhat unclear [Bonig et al. 2013]. In fact, plerixafor has been reported to affect bone marrow homeostasis in multiple ways [Dar A et al. 2011].

The phase III studies of plerixafor were performed in MM [DiPersio et al. 2009b] and NHL patients [DiPersio et al. 2009a]. In these studies, the patients mobilized with G-CSF plus plerixafor yielded significantly more CD34+ cells with fewer apheresis sessions than patients receiving G-CSF plus placebo. Thereafter many studies of plerixafor use in patients who have been proven poor mobilizers have been reported with congruent results [Calandra et al. 2008, Basak et al. 2011, D’Addio et al. 2011, Jantunen et al. 2011b, Hübel et al. 2012, Lanza et al. 2014].

In the majority (approx. 60-80%) of proven or predicted poor mobilizers (defined by low CD34+ cell counts in blood), a successful blood graft collection may be achieved by using plerixafor in conjunction with either chemomobilization or G-CSF [Jantunen and Lemoli 2012, Jantunen et al. 2016]. By the decision of European Medicines Agency (EMA) in 2009, plerixafor is currently indicated in combination with G-CSF to enhance mobilization of hematopoietic stem cell grafts to support subsequent auto-SCT in lymphoma and myeloma patients who mobilize poorly.

The routine use of plerixafor has been restricted especially by its price. Therefore, to maximize the benefits and minimize costs, there has been an interest in generating algorithms for the pre-emptive or just-in-time use of plerixafor. By definition, pre-emptive/just-in-time administration means giving plerixafor if a mobilization failure becomes imminent [Jantunen and Lemoli 2012]. Various algorithms for pre-emptive use have been developed [Jantunen et al. 2012, Sinha et al. 2011, Milone et al. 2014]. Generally, the algorithms are somewhat in line with the guidelines defined by the consensus of the EBMT, which suggest the use of plerixafor if the blood CD34+ count is less than 10 x 10^6/L prior to apheresis. In patients with blood CD34+ cell (B-CD34+) counts of 10-20 x 10^6/L a dynamic approach based on the disease characteristics and prior treatment of the given patient is suggested [Mohty et al. 2014].

2.2.5 Novel mobilization strategies

There are still patients intended for auto-SCT who fail to mobilize enough CD34+ cells even with the combination of G-CSF or chemomobilization plus plerixafor. Thus, there is a constant search for novel methods to mobilize stem cells more effectively. Many of the currently investigated substances intervene with the CXCR4/SDF-1α axis [Domingues et al. 2017], such as POL6326 (balixafortide) [Karpova et al. 2015], TG-0054 (burixafor) [Huang et al. 2009], BKT140 (4F-benzoyl-TN14003) [Peled et al. 2014] and ALT-1188 [Rettig et al. 2013]. These substances have been reported to be effective stem cell mobilizers either as single-agents or together with G-CSF. However, further studies are needed as the current knowledge on these drugs has been mainly derived from murine models and phase I studies in man [Domingues et al. 2017]. Apart from the CXCR4/SDF-1 axis mechanism of action, substances affecting other targets in the niche are also under investigation [Domingues et al. 2017]. Table 1 lists some novel substances intended for stem cell mobilization and with at least preliminary results from studies in man.
Table 1. Novel drugs under investigation for stem cell mobilization.

<table>
<thead>
<tr>
<th>Substance</th>
<th>Mechanism of action</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>POL6326 (balixafortide)</td>
<td>CXCR4 antagonist</td>
<td>Schmitt et al. 2010, Karpova et al. 2015; NCT01841476</td>
</tr>
<tr>
<td>BKT140 (BL8040)</td>
<td>CXCR4 antagonist</td>
<td>Nagler et al. 2010, Peled et al. 2014; NCT01010880</td>
</tr>
<tr>
<td>LY2510924</td>
<td>CXCR4 antagonist</td>
<td>Galsky et al. 2014</td>
</tr>
<tr>
<td>CDX-301 (rhFLT3L)</td>
<td>FLT3 agonist</td>
<td>Anandasabapathy et al. 2015</td>
</tr>
<tr>
<td>Bortezomib</td>
<td>VLA/VCAM-1, proteasome inhibitor</td>
<td>Giglio et al. 2009; NCT02037256</td>
</tr>
<tr>
<td>NOX-A12</td>
<td>anti-SDF-1</td>
<td>Ludwig et al. 2017; NCT01521533</td>
</tr>
<tr>
<td>Meloxicam</td>
<td>NSAID</td>
<td>Hoggat et al. 2013 (NCT02003625); Jeker et al. 2017</td>
</tr>
<tr>
<td>Eltrombopag</td>
<td>TPO receptor agonist</td>
<td>Domingues et al. 2017; NCT01286675</td>
</tr>
</tbody>
</table>

NSAID = non-steroidal anti-inflammatory drug; TPO = thrombopoietin
2.3 BLOOD GRAFTS – APHERESIS, BLOOD CD34+ CELL ENUMERATION AND GRAFT PROCESSING

2.3.1 Blood graft collection

The first evidence of multipotent stem cells circulating in peripheral blood originates from the 1950s [Bond et al. 1958] and the concept of blood stem cells was introduced for the first time in the early 1960s [Goodman and Hodgson 1962]. The development of apheresis systems using the continuous-flow method [Freireich et al. 1965] enabled the processing of large quantities of blood and meaningful collection of blood stem cells with multiple aphereses. The first successful aphereses were reported in 1971 [McCredie et al. 1971] and in the first successful auto-SCTs in the mid-1980s the aphereses were performed in steady state, i.e. without any specific attempts to boost the mobilization of stem cells [Körbling et al. 1981].

As the knowledge of the mobilization methods evolved, aphereses became much more effective [Körbling and Freireich 2011]. Thereafter it was reported that blood-derived grafts were associated with faster hematological recovery and improved immunological recovery after HDT than those harvested from bone marrow [Hénon et al. 1992, Beyer et al. 1995, Schmitz et al. 1996]. Consequently, in the past two decades grafts acquired from peripheral blood have become the standard source for HSCs used for auto-SCT [Passweg et al. 2016]. However, the source of the graft does not seem to affect the outcome after transplantation in the autologous setting [Beyer et al. 1995, Schmitz et al. 1996]. Also, the cellular composition of the mobilized cells and grafts from peripheral apheresis compared to bone marrow grafts have been evaluated [Hassan et al. 1996, Dmytrus et al. 2016]. Peripheral blood grafts have been reported to contain significantly higher numbers of CD3+ T lymphocytes [Hassan et al. 1996], more multipotent progenitors, lymphoid multipotent progenitors as well as erythroid multipotent progenitors [Dmytrus et al. 2016]. In the era when almost all autologous grafts are collected from peripheral blood, bone marrow harvesting of HSCs has been reserved mainly to patients failing blood apheresis, although the usefulness of such practice has been questioned [Goterris et al. 2005]. Also, as plerixafor nowadays gives new hope for the patients mobilizing poorly, the role of bone marrow harvesting in autologous setting is even more questionable [Kanate et al. 2013].

Various commercial enterprises have developed solutions for performing apheresis procedures. These machines implement different software and hardware techniques [Hequet 2015] but share the common principle of separating the blood components into layers by centrifugation and thereafter isolating the HSCs from the buffy coat [Hequet 2015]. Because the apheresis equipment varies by hardware and the softwares can be re-programmed, it is understandable that some differences in the apheresis yields between different apheresis systems have been reported [Ravagnani et al. 1999, Sorensen et al. 2011, Wu et al. 2012]. An important factor affecting the apheresis yield is the volume of blood processed. In many centers 2-3 times the estimated total blood volume (TBV) of the given patient is usually processed during an apheresis session. However, there are reports on the usefulness of larger volumes (large-volume leukapheresis, LVL), using usually 3-6 TBVs [Abrahamsen et al. 2005, Gašová et al. 2010, Bojanic et al. 2011]. LVL may be useful especially in poorly mobilizing patients [Abrahamsen et al. 2005, Gasova et al. 2010, Bojanic et al. 2011] and in patients with MM with the intention to collect enough HSCs for two auto-SCTs [Zubair et al. 2009]. However, a careful patient selection is needed as the LVL may induce clinical problems, e.g., significant electrolyte disturbances and cytopenias [Abrahamsen et al. 2005, Gašová et al. 2010]. Also, the LVL is also more exhaustive procedure for the patients due to the extended length and requires more nursing resources as well as proper anticoagulation.
2.3.2 Enumeration of blood CD34+ cells

Since the early 1990s, the surface antigen CD34+ has been widely used to mark and isolate HSCs for transplantation purposes. CD34+ is a glycoprotein expressed by hematopoietic precursors and stem cells as well as some other tissues, e.g. vascular endothelial cells [Furness and McNagny 2006, Nielsen and McNagny 2008]. In hematopoietic cells CD34+ is considered a marker of primitive cells, because it is lacking from the more mature hematopoietic cells [Furness and McNagny 2006]. The family of CD34+ cell surface proteins have a role in cell trafficking, adhesion and proliferation [Furness and McNagny 2006, Nielsen and McNagny 2008]. In stable circumstances, only approximately 1-9 cells/µl (1-9 x 10^6/L) in blood are CD34 positive and the level remains constant even if the total WBC count varies [Eidenschink et al. 2012]. During the mobilization process, the aim is to expand the number of CD34+ cells in the blood to enable effective apheresis.

To initiate apheresis at an optimal moment, it is necessary to perform prompt analysis of the CD34+ cell counts in the peripheral blood (B-CD34+). B-CD34+ cell counts are used to guide the mobilization, especially the administration of plerixafor in the case of an imminent mobilization failure. In order to ensure an adequate graft for HDT and to decide whether to continue apheresis, it is necessary to measure the number of collected CD34+ cells (La-CD34+) after each apheresis.

The B-CD34+ count correlates with the number of collected CD34+ cells [Armitage et al. 1997, Hollingsworth et al. 1999, Yu et al. 1999, Villa et al. 2012] and is therefore used to guide the initiation of apheresis. In many centers the threshold for initiating apheresis has been set to B-CD34+ ≥ 20 x 10^6/L, but in certain circumstances, especially in poor mobilizers, it may be reasonable to initiate apheresis with lower B-CD34+ values [Jantunen 2008, Jantunen and Kvalheim 2010].

The B-CD34+ and La-CD34+ counts are analysed with flow cytometry [Keeney et al. 2004]. In flow cytometry, single cells in suspension pass a laser beam and thereafter scatter light at variable angles and wavelengths. Scattered light is measured by detectors placed around the stream of analysed fluid. Detectors measure forward scattered light (FSC) and side scattered light (SSC). FSC measures mainly volume and size, and SSC inner structures (ie. granularity) of the cell [Virgo and Gibbs 2012]. To enable more detailed analysis of cells, fluorescent labels or fluorochromes are used. Fluorochromes are substances which emit light at specific wavelengths after they have been excited by a laser beam. They are usually conjugated with antibodies recognizing specific structures on cell membrane or in cell. Each fluorochrome has its unique threshold for excitation and emission thereby enabling analysis of specific structures on cell membrane or in the cell.

The method for the analysis of CD34+ cells by flow cytometry was developed in the 1990s by Siena et al. [Siena et al. 1991]. The protocol generated by ISHAGE has become the golden standard in clinical practice [Sutherland et al. 1996, Gratama et al. 2003]. In the original guidelines two-color immunofluorescence by CD45 FITC/CD34 PE fluorochromes was used because it enabled exact counting of CD34+ cells [Sutherland et al. 1996].

The original ISHAGE guidelines were based on the use of two-platform method, which combines data from a conventional hematology analyser and a flow cytometry [Gratama et al. 1997]. However, this system has been considered somewhat cumbersome and prone to errors [Gratama et al. 1999]. Hence a single-platform method was developed, making it possible to achieve CD34+ cell counts directly from the flow cytometry [Keeney et al. 1998]. The single-platform method is preferred also due to its lower variation [Gratama et al. 1999].

2.3.3 Blood graft processing

After aphereses it is recommended to freeze the grafts without unnecessary delay to avoid any problems in the graft quality [Watts and Linch 2016]. Before freezing, the grafts are processed to achieve an optimal cellular density in the container bags for preservation of the viability of the
cells until the thawing and re-infusion as well as to minimize any adverse events after the graft infusion [Calmels et al. 2007, Bachier et al. 2012]. An important method to protect the cells from the stress caused by the cryopreservation is the addition of a cryopreservative agent, most commonly dimethyl sulphoxide (DMSO) to the apheresis product. DMSO is usually added in a final concentration of 10%. However, in clinical practice there is variation in the concentrations used [Akkök et al. 2009, Morris et al. 2014] and it has been reported that lower concentrations of DMSO (e.g. 5%) may also be adequate for successful cryopreservation [Galmés et al. 1999, Liseth et al. 2009, Akkök et al. 2009]. The infusion of the cryopreserved grafts is associated with a risk of adverse events [Shu et al. 2014]. DMSO has often been considered responsible for many of these [Akkök et al. 2009], even though the reactions may in fact be caused by other factors, e.g. cell debris in the grafts [Calmels et al. 2007, Shu et al. 2014].

The freezing of the grafts before the actual cryopreservation can be done either with an uncontrolled or controlled-rate method. In the former the temperature decreases constantly until the desired level. The main benefits of this system are better affordability and thus accessibility as well as the ease of use, compared to controlled-rate method, which requires more sophisticated equipment and facilities. However, there are apparent benefits of using the controlled-rate method. By lowering the temperature of the apheresis product gradually, the risk of uncontrollable intracellular ice formation is reduced [Watts and Linch 2016]. Still, there is currently no clear consensus of the superiority of one of these methods over the other [Perez-Oteyza et al. 1998, McCullough et al. 2010]. After freezing, the standard is to cryopreserve the grafts in a liquid nitrogen freezer at -170°C or if such is not available, in a mechanical freezer in a temperature of at least -80°C.

The thawing of the grafts is a rapid process usually taking place in the close vicinity of the patient, preferably in the patient room. The common method to thaw the grafts is a sterile water bath at +37°C. Alternatively, a dry-warming method may be used [Röllig et al. 2002]. Regardless of the method used, the thawing must be a quick and strictly sterile procedure and it is recommended to infuse the thawed grafts within 15 minutes.

2.3.4 Cell viability and culture
The quality of the collected grafts may be assessed by analyzing the viability of the cells in the apheresis products. The most common method is to use 7-aminoactinomycin D (7-AAD), which enters non-viable cells and can therefore be used to distinguish these cells from the viable cells [Schmid et al. 1992, Keeney et al. 1998, Akkök et al. 2011]. By flow cytometry with CD34 and CD45 surface antigens and 7-AAD, the viable CD34+ cells can be predictably calculated [Akkök et al. 2011, Watts et al. 2016]. In addition, to ensure the suitability of the grafts for transplantation, the Colony Forming Unit-Granulocyte Macrophage (CFU-GM) assay may be performed [Decot et al. 2012], because it may offer information on the graft quality. However, as the results of CFU-GM assay have been reported to vary among various centers [Ketterer et al. 1998], the standard use of the method has been questioned. Still, it is not clear whether e.g. 7-AAD analysis, which commonly is not a part of clinical practice, would be a more reliable method than CFU-GM assessment to analyse the quality of the grafts [Watts et al. 2016]. In clinical practice, the most relevant indicator of graft quality is a timely engraftment after HDT.

2.4 BLOOD GRAFT CELLULAR COMPOSITION

2.4.1 CD34+ cells and CD34+ subclasses
The number of CD34+ cells in the autologous grafts has been accepted as the most important parameter for graft quality [Jantunen and Fruehauf 2011]. The number of CD34+ cells has been reported to correlate with the time of engraftment after HDT [Tricot et al. 1995, Weaver et al. 1995, Ketterer et al. 1998, Johnsen et al. 1998, Allan et al. 2002, Nieboer et al. 2004, Zubair et al.
In a study including the CD34+ cells, results by Beksac, 45RA has been underlined and a new 6.
The use of plerixafor was associated with a significant

chemomobilized NHL patients [Varmavuo et al. 2010, Jantunen et al. 2011] and the number and proportion of

also lack differentiation markers, thereby representing a population of primitive stem

cells [Terstappen et al. 1991]. These CD34+CD38 cells have been reported to be truly pluripotent and able to differentiate to various cell types [Miller et al. 1999]. The CD34+CD38 cells may have a role at least in the short-term engraftment after auto-SCT [Zubair et al. 2006]. In fact, the minimum desirable number of CD34+CD38 cells to be infused has been proposed to be as low as 0.05 x 10^6 cells/kg [Hénon et al. 1998], which was reported to be the threshold for better short-and long-term hematopoietic recovery.

Also, other markers of cell maturity from patients with NHL have been studied. For example, the surface antigen CD133 has been associated with primitivity and pluripotency [Hess et al. 2006]. According to studies in mice, CD34+CD133+ cells have enhanced engraftment when compared to CD34+CD133- cells [Gordon et al. 2003]. A higher number of transplanted CD34+CD133+ cells has in fact been reported to correlate with better neutrophil and platelet recovery after auto-SCT [Hicks et al. 2007, Camacho et al. 2012]. To enhance the recognition of the primitive HSCs, many other cell surface markers have been studied [Beksac and Preffer 2012] and promising results have been reported in regard to e.g., the ALDH activity of the primitive stem cells [Alison et al. 2010, Beksac and Preffer 2012, Roug et al. 2014]. Quite recently, also the importance of the surface marker CD45RA has been underlined and a new model for human hematopoiesis proposed [Görgens et al. 2013a, Dmytrus et al. 2016].

The mobilization method used has an impact on the CD34+ cell counts in the blood grafts. Currently the most effective way to mobilize the maximum number of CD34+ cells is to use all three of the most common means together, i.e. chemomobilization combined with plerixafor [Dugan et al. 2010, Jantunen and Fruehauf 2011]. The mobilization method seems also to affect the number and proportion of mobilized CD34+ subclasses. For example, the use of G-CSF alone instead of chemotherapy + G-CSF yielded more primitive CD34+ cells [Möhle et al. 1994] and the use of G-CSF + plerixafor vs. G-CSF alone mobilized more primitive CD34+ cells in MM patients [Fruehauf et al. 2009, Taubert et al. 2011]. Further, in MM the proportion of CD34+CD38- cells in the grafts was higher after mobilization with G-CSF +/- CY plus plerixafor (for poor mobilization) in comparison to CY + G-CSF mobilization [Varmavuo et al. 2013]. Interestingly, in the study by Taubert et al. the primitiveness of CD34+ cells in the peripheral blood was further analysed by using ALDH. The use of plerixafor was associated with a significant increase in the number of ALDH^bright and CD34+ cells and CD34+CD38- cells when compared to the cell counts before the plerixafor administration [Taubert et al. 2011], indicating the potential of plerixafor to possibly mobilize a population of very primitive CD34+ cells.

There are similar observations in regard to CD34+ cell maturity from patients with NHL receiving chemomobilization plus plerixafor for poor mobilization compared to chemomobilized NHL patients [Varmavuo et al. 2012b]. However, somewhat opposing results have also been published. In a study including both lymphoma and MM patients, the addition
of plerixafor to G-CSF mobilization yielded smaller proportions (of all graft cells) of CD34+CD38- cells compared to mobilization with G-CSF alone, but nevertheless the proportion of CD34+CD38- cells of all CD34+ cells was higher in the plerixafor-mobilized patients [Roug et al. 2014]. In a recent report, Worel et. al underlined the complexity of hematopoiesis and hence the importance of careful antigen panel selection in the graft analysis [Worel et al. 2017]. In their analysis, CD45RA was included in the antigen panel of the graft analyses and a revised hematopoiesis model [Görgens et al. 2013a, Görgens et al. 2013b] used in the classification of the cell populations. Possibly thereby, the results from that study were contradictory to those reported previously by other investigators: the patients mobilized with G-CSF + plerixafor had more mature CD34+ cells in the apheresis products, and the patients given G-CSF alone had higher numbers of the most primitive progenitors defined as CD133-CD45RA+. The authors speculated that the reason behind these differences was the use of CD45RA in conjunction with CD133 and CD38, because according to the revised hematopoiesis model, only the CD45RA antigen may reveal the true early stage of the hematopoietic stem cell commitment.

2.4.2 Lymphocytes

Lymphocytes are a heterogenous group of blood cells and can be roughly divided into three subgroups: T and B lymphocytes and natural killer (NK) cells. T lymphocytes have a broad spectrum of functions. They are responsible for cell-mediated cytotoxic reactions, regulate immune reactions through cytokine secretion and provide support for optimal B cell function. The main function of B cells is to capture, process and present antigen to e.g., viral infections and against tumor cells. B cells can be further classified to memory T cells, naïve T cells, regulatory T cells and gamma delta T cells as mentioned in the previous chapter for the lymphocytes, also the number of T lymphocytes collected are affected by the mobilization regimen used [Condomines et al. 2006, Jantunen and Fruehauf 2011].
As with the total number of lymphocytes, also higher numbers of CD3+CD4+ T cells in the grafts have been linked to improved EFS and OS after auto-SCT in MM patients [Schmidmaier et al. 2008]. The same investigators also reported an increased CD4/CD8 ratio (but not CD8+ independently) to associate with improved EFS. In the same study, higher numbers of lymphocytes with HLA-DR marker were strongly associated with worse outcome. However, no further classification for this subset was provided.

In another study including patients with various hematological malignancies, the number of infused CD3+CD8+ T lymphocytes was reported to affect the absolute lymphocyte recovery after auto-SCT [Atta et al. 2009], which in turn, at least in NHL patients, might affect the outcome after auto-SCT. The number of CD3+CD8+ T lymphocytes in the apheresis products was higher in those patients who were mobilized with G-CSF alone compared to chemomobilization, in patients presenting higher lymphocyte counts in blood at the time of the apheresis and in patients with more apheresis sessions [Atta et al. 2009]. In another study with MM patients the CD3+CD8+ T lymphocyte count in the blood grafts was positively associated with the lymphocyte recovery after auto-SCT and a lower CD4/CD8 ratio in the graft predisposed to mucositis during auto-SCT [Lee et al. 2012]. Of note, a low CD4/CD8 ratio in blood at the time of engraftment was also associated with increased risk of cytomegalovirus reactivation [Lee et al. 2012], which was a relatively common complication in that study (26% of the patients). Furthermore, in another study, the blood grafts of poor mobilizers were reported to have a higher proportion and higher counts of CD3+CD8+ T lymphocytes [Russell et al. 2015]. In the same study the absolute number and proportion of CD4+ T lymphocytes was higher in the blood grafts of good mobilizers, even though the difference was not statistically significant. It was also reported that a higher graft CD3+CD8+ to CD34+ ratio was associated with faster neutrophil engraftment after auto-SCT especially in poor mobilizers. It was concluded that CD3+CD8+ lymphocytes might have a dual role in mobilization and engraftment. On the one hand, a higher number of CD3+CD8+ lymphocytes might hamper the mobilization of CD34+ cells but on the other hand, a higher proportion to mobilized CD34+ cells may augment the homing process [Russell et al. 2015]. The mechanisms behind these findings are not clear but similar findings of the CD3+CD8+ lymphocytes possibly affecting CD34+ cell homing have been previously reported [Adams et al. 2003].

Another study evaluated the role of CD26+ (dipeptidyl peptidase-4, DPP4) positive T cells in the autologous grafts. The CD26+ receptor has multiple functions in cell trafficking and signaling [Hildebrandt and Schabath 2008] as well as in for example antigen-T-cell and SDF-1/CXCR-4 interaction [Christopherson et al. 2002, Hildebrandt and Schabath 2008]. In the study evaluating the role of CD26+ T cells in the autologous grafts, higher numbers of CD26+ cells were present in the apheresis products of patients encountering any post-transplant event (relapse, disease progression or death). Thus, higher CD26+ T lymphocyte counts in the grafts were reported to associate with worse EFS after auto-SCT [Hildebrandt et al. 2012].

### 2.4.2.2 B lymphocytes

In a study from the pre-rituximab era, the autografts of MM and NHL patients were reported to contain up to 5% of CD19+ cells [Johnsen et al. 1998]. In more recent analyses the blood grafts of NHL patients have been reported to practically lack B cells, probably due to the previous treatment with CD20 antibody rituximab [Varmavuo et al. 2012a, Varmavuo et al. 2012b]. Also in the blood grafts of MM patients the number of CD19+ cells is significantly lower than the number of CD3+CD4+ and CD3+CD8+ T lymphocytes [Varmavuo et al. 2013]. In a heterogenous patient population including poor mobilizers with MM, NHL and Hodgkin lymphoma (HL), the grafts mobilized with G-CSF plus plerixafor were reported to contain significantly more CD19+ lymphocytes than the grafts mobilized with chemotherapy plus G-CSF plus plerixafor [Worel et al. 2016].
The clinical impact of graft B cells to post-transplant outcome has not been extensively studied, but in a study with MM patients a higher CD19+ lymphocyte count in apheresis products was associated with worse outcome [Schmidmaier et al. 2008]. Previously it has been suggested that malignant B cells end up in the grafts of NHL and MM patients [Johnsen et al. 1998] and might therefore provide an explanation for relapses after auto-SCT. On the other hand, the number of CD19+ cells in the grafts has been reported to correlate with the absolute lymphocyte count (ALC) at the time of engraftment [Lee et al. 2012], potentially affecting outcome likewise ALC-15.

2.4.2.3 NK cells

NK cells (CD3-CD16/56+) are a vital part of the innate immune system and function as cytotoxic lymphocytes. They are essential in controlling viral infections and in defence against tumor cells.

The number of NK cells in the blood grafts is affected by the method of mobilization. By combining plerixafor to chemomobilization in NHL patients [Varmavuo et al. 2012a, Varmavuo et al. 2012b], and to G-CSF mobilization in MM patients [Varmavuo et al. 2013], more NK cells may be harvested in poor mobilizers. However, in NHL patients who were not poor mobilizers, the combination of G-CSF plus plerixafor vs. G-CSF alone did not yield more NK cells [Holtan et al. 2007]. In a study including NHL, MM and HL patients deemed as poor mobilizers, the grafts of G-CSF plus plerixafor mobilized patients contained significantly more NK cells than patients mobilized with chemomobilization plus plerixafor [Worel et al. 2016].

There are few studies on the significance of NK cells in the grafts in autologous setting. In a small and heterogenous patient population the correlation of the lymphocyte subsets in the blood grafts and lymphocyte recovery post-transplant was analysed. The number of NK cells in the grafts was found to correlate with the tempo of the lymphocyte recovery after auto-SCT [Porrata et al. 2003]. In addition, NK cells have been reported to recover rapidly after auto-SCT [Porrata et al. 2001b], to be the most important subset of ALC-15 and also predictive of outcome after auto-SCT [Porrata et al. 2008]. Recently in an analysis of NHL patients the higher number of infused NK cells was found to associate with improved outcome after auto-SCT [Porrata et al. 2016].

2.4.3 Dendritic cells

Dendritic cells (DC) function as messengers between the innate and adaptive immune systems by presenting antigens to T and B lymphocytes. The mobilization method used has been reported to affect the proportions of DC subsets in the apheresis products [Bolwell et al. 2003]. In that study, mobilization with G-CSF alone in comparison to chemotherapy plus G-CSF yielded significantly more plasmacytoid (pDC) than myeloid (mDC) dendritic cells in the apheresis products and was thus associated with a greater pDC/mDC ratio. In another study, a higher number of mDCs in the blood grafts as well as the total DC counts in post-transplant blood samples was associated with improved outcome after auto-SCT [Dean et al. 2005]. In lymphoma patients, the combination of G-CSF plus plerixafor has been reported to efficiently mobilize both mDCs and pDCs [Gazitt et al. 2007]. In a recent analysis including various hematological malignancies, the grafts collected after G-CSF plus plerixafor mobilization had a significantly higher proportion of plasmacytoid dendritic cells than grafts mobilized with to G-CSF alone [Gaugler et al. 2013].

2.4.4 Other cell types

Recently there have been studies on the possible effects of monocyte counts and lymphocyte to monocytes ratio in autologous grafts in the post-transplant outcome. A lower number of infused monocytes in conjunction with an adequate number of lymphocytes generating an
optimal ratio (≥1.0) has been reported to associate with improved outcome after auto-SCT in patients with DLBCL [Porrata et al. 2014], HL [Porrata et al. 2015a] and T cell lymphoma [Porrata et al. 2015b]. The mechanisms behind these findings are somewhat unclear, but it has been proposed that a higher number of monocytes might affect the immune responses in unfavorable ways [Porrata et al. 2014]. However, it might be that these positive results in regard to outcome are also due to, or because of, the greater lymphocyte counts in the grafts.

During apheresis, polymorphonuclear leukocytes, especially neutrophils, are also collected in addition to peripheral blood mononuclear cells (PBMCs) [Pierelli et al. 2012]. However, neutrophils have been reported to suffer a major loss during the cryopreservation [Reich-Slotky et al. 2008] and may not have major clinical significance after auto-SCT, even though the granulocytes in the grafts have been claimed to cause some adverse reactions after the graft infusion [Khera et al. 2012].

2.4.5 Tumor cells

Autologous blood grafts may be contaminated with tumor cells [DiPersio et al. 2011]. The range of the tumor infiltration has been estimated to vary from 0.01% up to 10% of the graft cells, and there is no evidence that one mobilization method would be preferable over another in terms of minimizing the mobilization of tumor cells [DiPersio et al. 2011]. Also, the use of plerixafor has been reported safe in MM and NHL patients in regard to the tumor cell contamination of the blood grafts [Fruehauf et al. 2010, Tricot et al. 2010].

There are some studies on the possible effects of tumor cell contamination on the post-transplant outcome. Gertz et al. [Gertz et al. 1997] reported that the circulating monoclonal plasma cells at the time of the apheresis were associated with worse outcome in MM patients. Another study did not find a congruent correlation with the number of tumor cells in the grafts and outcome in patients with either MM or NHL [Ho et al. 2009]. Further, a study including DLBCL and FL patients did not verify a correlation with the number of malignant cells in the grafts and post-transplant outcome [Blystad et al. 2004], corroborated by another study in NHL patients [Demirkazik et al. 2001]. However, there are opposing results in MM [Vogel et al. 2005] and NHL patients [Sharp et al. 1996]. In these studies, there seemed to be a correlation between the number of tumor cells in the grafts and outcome of the patients. Yet another study stressed the importance of the tumor cell burden in the grafts: there was a correlation with the number of tumor cells of the grafts and outcome in MM patients, but only with high numbers of tumor cells [Kopp et al. 2009]. So far, efforts to select tumor-free blood grafts have not been successful, and these measures have not been shown to impact outcome [Stewart et al. 2001, Bourhis et al. 2007]. It has been proposed that the adequate eradication of the underlying disease in the patients before auto-SCT is more important than the tumor cell contamination of the grafts [DiPersio et al. 2011].

2.4.6 Factors affecting mobilization and blood graft composition

In addition to the method of mobilization used, there are also other factors that may alter the course of the mobilization and the cellular composition of the apheresis products. The histology of the underlying malignancy has been proposed as a risk factor for poor mobilization or collection. Especially patients with lymphomas have been reported to possess a higher risk for poor mobilization [Jantunen and Kvalheim 2010, Jantunen et al. 2012], although in some studies the success of mobilization has also been reported to be comparable between NHL and MM patients [Pusic et al. 2008, Wuchter et al. 2010].

Previous chemotherapy may cause damage in the bone marrow, and has been, in fact, reported to impair the mobilization process [Jantunen et al. 2003, Bensinger et al. 2009, Jantunen and Kvalheim 2010, Wuchter et al. 2010, Wu et al. 2016]. Many of the commonly used chemotherapy agents have been reported to hamper stem cell mobilization [Jantunen and Kvalheim 2010], including the novel myeloma drug lenalidomide [Kumar et al. 2007, Popat et
It has been proposed, that a maximum of four lenalidomide-containing treatment cycles should be given before the collection of autografts [Kumar et al. 2009]. In a recent analysis, the previous lenalidomide exposure was associated with a higher number of aphereses needed, but the CD34+ mobilization and yields and the blood graft composition was comparable to lenalidomide-naïve MM patients [Partanen et al. 2017a]. As reported by Porrata et al., preceding chemotherapy also decreases the number of lymphocytes in blood at the time of the apheresis, which in turn may lead to a lower number of lymphocytes in the apheresis product [Porrata et al. 2004b]. Also, the time from the previous chemotherapy has been reported to correlate with the number of lymphocytes in blood at the time of the apheresis [Holtan et al. 2006]. The time from the last chemotherapy cycle may also affect the platelet level in blood at the time of mobilization – a factor predicting the success of apheresis in NHL patients [Kuittinen et al. 2004].

The age of patients may affect the graft composition. An analysis of BM grafts of healthy individuals revealed the proportion of CD34+CD38- and CD34+CD38- cells in the marrow to be significantly higher in elderly (>60 years) than younger (<35 years) patients [Woolthuis et al. 2014], and in the clinical cohort of the same study the long-term hematopoietic engraftment was reported to be slower in the older patients. Also, the collection of CD34+ cells may be less efficient and therefore require more aphereses in elderly patients [Tempescul et al. 2010], which on the other hand might yield more lymphocytes in the grafts. A higher age of the patients at the time of mobilization has been studied as a potential risk factor for poor mobilization. In fact, a higher age has been associated with inferior mobilization in patients with MM [Morris et al. 2003, Musto et al. 2015], lymphomas [Hosing et al. 2009] and in a patient population with mixed hematological diseases [Wu et al. 2016]. However, there are also opposing results indicating that age does not affect mobilization in elderly patients with MM [Jantunen et al. 2006] or NHL [Kuittinen et al. 2004] or in mixed patient populations [Wuchter et al. 2010, Tempescul et al. 2010]. As chemomobilization seems to be a more efficient mobilizer than G-CSF alone also in the elderly [Morris et al. 2003], the inferior collection results in some studies could be due to more frequent use of G-CSF alone mobilization in the elderly.

**2.5 POST-TRANSPLANT HEMATOLOGICAL RECOVERY**

A successful early engraftment after auto-SCT is a sign of the proper function of the infused graft and reduces complications in the post-transplant phase [Ketterer et al. 1998]. The recovery of neutrophils, lymphocytes and platelets is important to reduce post-transplant infectious complications, need for red cell and platelet transfusions and the length of post-transplant hospitalization. The length of the hospitalization, use of antibiotics and blood products is also directly connected to the costs of the transplantation [Varma vuo et al. 2016].

There are many studies concentrating on the factors affecting hematological recovery after auto-SCT and as described in the previous chapters, especially the number of CD34+ cells and CD34+ subclasses seem to be important in that sense. However, the pace of hematological recovery may depend on various factors. For example, the administration of G-CSF after the graft infusion has been reported to improve neutrophil recovery [Klumpp et al. 1995, Hornedo et al. 2002, Trivedi et al. 2009], protect from post-transplant infectious complications and decrease the need of hospitalization [Trivedi et al. 2009]. Even though the use of G-CSF post-transplant seems to improve post-transplant neutrophil recovery, it does not seems to affect the platelet recovery [Klumpp et al. 1995, Hornedo et al. 2002]. Although the extent and type of the previous chemotherapy may delay the hematological recovery [Moskovitz et al. 1998, Gertz et al. 2009], chemotherapy containing mobilization has also been reported comparable to G-CSF mobilization in terms of the pace of engraftment [Menendez et al. 2002, Benyamini et al. 2017].
Also, the effects of plerixafor on the engraftment kinetics have been studied, and generally no changes have been reported [DiPersio et al. 2009a, DiPersio et al. 2009b, Yuan et al. 2017].

2.6 POST-TRANSPLANT IMMUNE RECOVERY

The tempo of immune recovery following auto-SCT seems to be of importance. For example, a more rapid recovery of lymphocytes after auto-SCT has been linked to improved patient outcome by various groups [Porrata et al. 2001a, Kim et al. 2006, Gordan et al. 2003a, Yoong et al. 2005, Hiwase et al. 2008a, Porrata et al. 2008, Kim et al. 2016]. The most widely used timepoint for the analysis of rapid immune recovery is the absolute lymphocyte count +15 days after auto-SCT (ALC-15). Usually a threshold of 0.5 x 10^9/l is used for ALC-15 and values equal or above that level seem to correlate with improved outcome in patients with MM [Porrata et al. 2001a] and NHL [Porrata et al. 2001a, Gordan et al. 2003a, Kim et al. 2004, Porrata et al. 2008]. There are also conflicting results in MM patients. Hiwase et al. [Hiwase et al. 2008a] did not find ALC-15 to be significant in regard to outcome, but they reported a positive correlation with ALC-30 ≥ 1.0 x 10^9/l and outcome. Similar results with ALC-30 at the cut-off level of 1.0 x 10^9/l were reported in another study in patients with T cell lymphoma [Kim et al. 2004]. There are also other studies questioning ALC-15 as a prognostic marker in NHL patients [Torka et al. 2015]. Further, a slightly lower threshold for ALC-15 (0.4 x 10^9/l) has been found predictive of favorable outcome in DLBCL patients [Kim et al. 2016].

There are limited data on the more detailed analysis of the immune recovery following auto-SCT. In a prospective study by Porrata et al., the lymphocyte subsets at d+15 were analysed in NHL patients and the higher NK cell counts were reported to independently predict improved PFS and OS [Porrata et al. 2008]. In NHL patients, a higher proportion of CD3+CD8+ central memory and effector memory T cells at d+100 after auto-SCT were associated with worse PFS and OS [Torka et al. 2015]. In another study with MM patients the lymphocyte recovery after auto-SCT was studied, and the recovery of CD8+ lymphocytes was reported to be significantly faster than the recovery of CD4+ lymphocytes. Therefore, especially the CD4+ regulatory T/CD8+ effector T cell ratio decreased after the transplantation, possibly providing an anti-tumor environment as the number of regulatory T cells is temporarily diminished [Chung et al. 2016]. The patients who eventually relapsed after auto-SCT demonstrated a distinctive immune profile, which could be spotted by analyzing the senescence of T cells after transplantation by certain immune markers. The T cells in the relapsed patients seemed to be senescent/exhausted, possibly due to chronic antigen stimulation [Chung et al. 2016]. These findings might warrant early immune intervention, e.g. PD-1 blockage to restrict the activity of the senescent CD8+ lymphocytes, to improve the control of the underlying malignancy early after auto-SCT.

Factors affecting post-transplant immune recovery have also been studied. The dose of infused CD34+ cells has not been generally associated with the lymphocyte recovery after auto-SCT [Porrata et al. 2004a, Kim et al. 2004, Tiwari et al. 2007, Atta et al. 2009, Hiwase et al. 2008a, Porrata et al. 2015a, Porrata et al. 2016], but there are also opposing results [Blystad et al. 2004, Yoon et al. 2009, Kim et al. 2016]. Previous chemotherapy before stem cell collection has been reported to hinder lymphocyte recovery in patients with MM [Hiwase et al. 2008a] and in HL, fewer previous chemotherapy cycles were associated with ALC-15 ≥ 0.5 x 10^9/l [Porrata et al. 2002]. Correspondingly, in NHL patients a longer time from previous chemotherapy before auto-SCT was found to be beneficial [Holtan et al. 2006]. The dose of infused lymphocytes has been reported to be related to faster immune recovery in patients with MM [Porrata et al. 2004a, Hiwase et al. 2008a] and NHL [Yoong et al. 2005, Porrata et al. 2008a, Porrata et al. 2016]. In patients with NHL a higher number infused CD8+ lymphocytes seem beneficial in regard to faster immune recovery [Atta et al. 2009]. In MM patients the CD8+ and CD19+ lymphocytes in the grafts have been reported to correlate with lymphocyte recovery at the time of engraftment.
[Lee et al. 2012]. On the other hand, a decreased CD4+/CD8+ ratio in the blood grafts has been associated with increased risk of post-transplant infectious complications in MM patients [Lee et al. 2012]. In the same study, multiple correlations with the infused graft and the recovery of the lymphocyte subsets in blood at the time of engraftment were discovered, e.g. the number of NK cells in the graft correlated to the number of NK cells in blood. Also, a higher number of CD3+ lymphocytes, or a lower count of NK cells and a higher CD8+ count or a low CD4+/CD8+ ratio in blood at the time of engraftment cells at engraftment were associated with increased risk of post-transplant infections. An improved recovery of NK cells seems to be beneficial as a higher level at one month after the auto-SCT has also been reported to associate with better PFS in patients with MM [Rueff et al. 2014].

The functional properties of NK cells after auto-SCT have also been studied, and there seem to be differences in the expression profile compared to the steady state NK cells [Jacobs et al. 2015]. In that study NK cells were the main lymphocyte subset at leukocyte level ≥1.0 x10⁹/L post-transplant, and the post-transplant NK cells expressed significantly higher levels of CD57 and killer Ig-like receptors (KIRs) than NK cells studied before auto-SCT and a longer time after the transplantation. Based on the NK cell profile, the authors suggested that the early post-transplant NK cells are actually more immature or new NK cells, instead of activated “old” NK cells. The early NK cell population was also reported to possess effective antitumor activity right after the transplantation [Jacobs et al. 2015].

The reasons behind the evident benefits of early immune recovery to post-transplant outcomes are not clear. The protection from infectious complications right after the auto-SCT may be a reason, but also a more complex immune mechanism targeting the malignant disease has been proposed [Porrata 2010, Chung et al. 2016]. Also, it is not certain if a particular cellular component is more important in ALC-15. For example, a higher number of NK cells has been linked to improved outcome, whereas a higher monocyte count has been found to be disadvantageous [Porrata 2016].

### 2.7 GRAFT COMPOSITION AND OUTCOME

The various graft components have been reported to impact post-transplant outcome. The correlation of the number of the collected or infused CD34+ cells on outcome has been evaluated in multiple studies. In general, a higher amount of CD34+ has been found to be favorable in regard to OS or PFS [Gordan et al. 2003b, Pavone et al. 2006, Yoon et al. 2009, Toor et al. 2004, O’Shea et al. 2006]. However, there are also studies where such correlation has not been verified [Stockerl-Goldstein et al. 2000, Klaus et al. 2007]. The reason for these varying results is unclear, but various reasons have been proposed for the association, e.g., more rapid hematological recovery after a larger CD34+ cell dose and characteristics of the patients mobilizing poorly [Jantunen and Fruehauf 2011]. Also, especially in most of the earlier studies, the only available data on the graft composition has generally been the number of CD34+ cells. Therefore, the possible effect and importance of the other graft constituents explaining the varying results have been left out of the analyses. Also, many studies have not taken into account the heterogeneity of the patient populations, as it seems probable that e.g. the immune recovery after auto-SCT might be of varying importance amongst different diseases like NHL and MM. Further, for example using the CD34+ cell count as a sole parameter of the graft quality may place too much importance on the CD34+ cell count, and many other important factors may remain unnoticed.

The potential importance of the total number of lymphocytes in the grafts for outcome has been discussed in the previous chapters. However, besides the total lymphocyte count, also a higher proportion of helper T cells (CD3+CD4+) in the grafts has been independently linked to improved EFS after auto-SCT in patients with MM [Schmidmaier et al. 2008]. In that study, an
increased CD4/CD8 ratio in the grafts was associated with improved EFS, and a higher CD19+ cell proportion seemed to worsen EFS. Hildebrandt et al. reported that a higher CD26+ T cell number in the grafts was associated with worse EFS in a histologically mixed patient population [Hildebrandt et al. 2012]. Further, in a recent analysis of NHL patients the higher number of infused NK cells was found to be associated with improved outcome after auto-SCT [Porrata et al. 2016]. To conclude, the current knowledge on the possible effects of blood graft composition on outcome is scarce and contradictory.

### 2.8 Plerixafor and Outcome

In the original phase III studies on plerixafor the outcome measures were comparable in both MM and NHL patients within the first 12 months. In a recent update of the original phase III studies [DiPersio et al. 2009a, DiPersio et al. 2009b] the follow-up was extended up to five years [Micallef et al. 2018]. In that report including NHL and MM patients randomly assigned to receive either G-CSF plus plerixafor or G-CSF plus placebo, there were no significant differences in the PFS or OS within the mobilization groups in either disease. Also, recently the preliminary results of the CALM (Collaboration to collect Autologous transplant outcomes in Lymphoma and Myeloma) study [Morris et al. 2011], required by the European Medicines Agency (EMA) to investigate the outcome of patients receiving plerixafor-mobilized grafts vs. otherwise comparable patients mobilized without plerixafor, were reported [Morris et al. 2018, Sureda et al. 2018] and there were no significant differences in PFS or OS in MM or NHL patients receiving plerixafor combined to G-CSF or chemotherapy and G-CSF due to poor mobilization. However, in MM patients the authors speculated there to be a trend for worse outcome in plerixafor mobilized patients.

Besides these reports, the outcome of plerixafor mobilized patients has mainly been reported from rather short perspectives. In a small study with 36 NHL patients, of whom nine received plerixafor, PFS was reported better during the follow-up of 18 months in patients who received G-CSF plus plerixafor instead of G-CSF alone mobilization [Holtan et al. 2007]. Another study with NHL patients reported no difference in outcome during the first 24 months post-transplant in poor mobilizers receiving chemomobilization plus plerixafor vs. chemomobilized patients [Varmavuo et al. 2014]. These results were echoed by our recent analysis of pegfilgrastim plus chemotherapy mobilized NHL patients receiving plerixafor due to poor mobilization [Partanen et al. 2017b]. Otherwise the data including NHL patients is scarce; a small retrospective study including MM and NHL patients provided data on PFS and OS in G-CSF + plus plerixafor (due to poor mobilization) and G-CSF alone mobilized patients [Moreb et al. 2011], but no comparison within the plerixafor +/- groups was performed.

In MM patients, a small and retrospective study compared the PFS of patients receiving either CY plus G-CSF or G-CSF and added plerixafor according to the choice by the clinician [Garfall et al. 2014]. The PFS of plerixafor-receiving patients was reported to be inferior in the total patient cohort but comparable in patients with high-risk cytogenetics.

To conclude, the effects of plerixafor on outcome in NHL and MM patients are gradually being clarified. One of the main findings so far are the neutral results from the studies with the largest set of patients and longest follow-up time [Micallef et al. 2018, Morris et al. 2018, Sureda et al. 2018]. Besides the preliminary results form the CALM study, the outcome results are still somewhat puzzling as the patient populations in most studies do not correspond the real-life usage of plerixafor. Also, the results so far may be distorted by multiple causes, one being the heterogeneity of studies with the selection of plerixafor-patients; some have included standard and some only poor mobilizers, the latter itself being a prognostic factor for poor outcome [Gordan et al. 2003b, Moreb et al. 2017]. Therefore, the final results from the CALM study are awaited with interest.
3 Aims of the study

The aim of this prospective multicenter study was to analyse the effects of various mobilization methods on the blood graft cellular composition, post-transplant hematological and immune recovery as well as outcome in NHL and MM patients.

The more specific aims were:

1. To analyse blood graft cellular composition, post-transplant hematological and immune recovery and outcome in NHL patients receiving either chemomobilization or chemomobilization plus pre-emptive plerixafor for poor mobilization (I)

2. To evaluate the factors affecting early immune recovery in patients with NHL and to assess the importance of early immune recovery for post-transplant outcome (II)

3. To explore in a randomized setting after RVD (lenalidomide, bortezomib, dexamethasone) induction the impact of mobilization with low-dose cyclophosphamide + G-CSF vs. mobilization with G-CSF alone on the blood graft cellular composition, hematological and immune recovery as well as outcome in patients with MM (III)

4. To investigate the effects of mobilization including plerixafor on the blood graft cellular composition, hematological and immune recovery as well as outcome in patients with MM (IV)
4 Patients and methods

4.1 THE GRAFT AND OUTCOME IN AUTOLOGOUS TRANSPLANTATION (GOA) STUDY

4.1.1 Study outline and aims
The Graft and Outcome in Autologous transplantation study was initiated at Kuopio University hospital (KUH) catchment area in May 2012. Later, University Hospitals of Oulu (OUH), Tampere (TUH) and Turku (TYKS) joined the prospective non-interventional study. The latter three centers stopped recruiting new patients by the end of 2015 and in KUH at the end of 2016. In December 2016, altogether 284 patients were included into the study (138 NHL and 146 MM patients).

The main objectives of the GOA study were to analyse the cellular composition of blood grafts after various mobilization methods as well as hematological and immune recovery and outcome in patients with NHL or MM. The inclusion criteria were the diagnosis with NHL or MM and an intention to treat with auto-SCT as well as a collection of an adequate blood graft (≥ 2.0 x10^6/kg CD34+ cells) to support HDT.

In this thesis, the focus was on the effects of plerixafor on the graft composition and post-transplant recovery in NHL (I) and MM patients (IV), factors affecting early immune recovery and its clinical importance in NHL patients (II) and effects of G-CSF vs. G-CSF + CY mobilization on graft composition and post-transplant recovery in MM patients (III).

4.1.2 Patients and methods
The patient population consisted of adult patients with NHL or MM eligible and scheduled for an auto-SCT. The patients were recruited by the physicians in charge of their treatment at the university hospitals performing the aphereses and HDTs. The auto-SCTs were performed in the four university hospitals (Kuopio, Oulu, Tampere and Turku). After the auto-SCT the patients were followed at their local hospitals according to the standard procedures.

The study design was non-interventional and therefore did not implicate specific treatment regimens, method of mobilization – except in MM patients participating the randomized mobilization study (MM-02) by the Finnish Myeloma Group – or type of high-dose therapy. These decisions were done solely by the physician in charge of the treatment of the given patient. There were also no instructions or algorithm for the use of plerixafor except in patients participating the MM-02 study. In practice, it was administered to poorly mobilizing patients. The minimum collection target of CD34+ cells was set to 2.0 x 10^6/kg for a single transplant except in the MM-02 study the target was 3.0 x 10^6/kg for a single transplant and 6.0 x 10^6/kg for tandem transplants.

After auto-SCT the patients were regularly followed at their local hospitals according to the standard procedures. Regarding to the GOA study, during the first year after the transplantation there was a special emphasis on hematological and immune recovery and on the occurrence of infectious complications. The patients will be monitored up to five years from the auto-SCT for progression or relapse.

4.1.3 Study of hematological and immune recovery
Within the first 12 months from the transplantation, hematological recovery was evaluated by analyzing complete blood counts at 15 days (graft infusion on d0) and at 1, 3, 6 and 12 months. The immune recovery was analysed in a proportion of the patients (all MM-02 study patients...
and a proportion of NHL patients recruited at KUH) with flow cytometry of the blood lymphocyte subsets at 1, 3 and 6 months post-transplant (see 4.1.5).

4.1.4 B-CD34⁺ analysis and blood lymphocyte subsets
B-CD34⁺ cell counts were measured daily since the morning of the predetermined, first assumed day of apheresis and the analyses were performed daily until an adequate collection target was achieved or the collections were halted. The B-CD34⁺ enumerations were performed at the laboratories of the university hospitals performing the aphereses. After each collection, the CD34⁺ count of the apheresis product (La-CD34⁺) was analysed in the same manner.

In a subset of NHL and MM patients the lymphocyte subsets in blood were analysed post-transplant. The number of CD3⁺, CD3⁺CD4⁺, CD3⁺CD8⁺, CD19⁺ and NK cells were analysed at the laboratories of the university hospital.

4.1.5 Analysis of cryopreserved blood grafts
After the blood graft collection, additional 0.5ml samples were taken from each apheresis product. DMSO was added in the final concentration of 10% to protect the cells from death or stress caused by the cryopreservation. These samples were stored in liquid nitrogen using a controlled-rate freezing program and maintained in nitrogen freezer at -170°C. No graft manipulation was performed. For further information about the analysis of cryopreserved grafts see 4.5.2.

4.2 PATIENT POPULATIONS

4.2.1 Study I
The patient population consisted of 41 patients with NHL who received auto-SCT at KUH between May 2012 and February 2014 and were followed up to January 31st 2015. All patients received chemotherapy plus G-CSF (pegfilgrastim or filgrastim) to mobilize CD34⁺ cells. Fourteen patients (93%) in the plerixafor group and twenty-four patients (92%) in the control group received pegfilgrastim instead of filgrastim. Fifteen patients (37%) were also given plerixafor because of poor mobilization of CD34⁺ cells and formed the plerixafor group. The control group consisted of 26 patients who were mobilized without plerixafor. The minimum criteria for inclusion into this study were flow cytometric analysis of the graft samples and evaluation of the post-transplant hematological recovery. In 31 patients (76%) also the immune recovery post-transplant was analysed with flow cytometry. The patient characteristics are presented in Table 2.

4.2.2 Study II
The study included 72 patients with NHL who received auto-SCT between May 2012 and October 2014 at Kuopio, Oulu and Tampere University Hospitals and were followed until May 31st 2015. All patients were chemomobilized and 24 patients (33%) received also plerixafor because of poor mobilization. Fifty-one patients (71%) received pegfilgrastim and twenty-one filgrastim. All patients had data available on the graft analysis and hematological recovery and in 44 (61%) patients there was also data on immune recovery – defined as the analysis of the lymphocyte subsets – at least at one month post-transplant. The data on early lymphocyte recovery 15 and 30 days post-transplant was available in 69 patients (96%). The patient characteristics are presented in Table 2.
Table 2. Characteristics of NHL patients in studies I and II.

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Study I Mobilization with plerixafor</th>
<th>Study I Mobilization without p-value plerixafor</th>
<th>Study II All patients</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of patients</td>
<td>15</td>
<td>26</td>
<td>72</td>
</tr>
<tr>
<td>Gender (M/F)</td>
<td>8/7</td>
<td>11/15</td>
<td>NS</td>
</tr>
<tr>
<td>Age (years), range</td>
<td>64 (41 – 73)</td>
<td>62 (19 – 68)</td>
<td>NS</td>
</tr>
<tr>
<td>Histology</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DLBCL</td>
<td>10 (^1)</td>
<td>10 (^1)</td>
<td>32 (^2)</td>
</tr>
<tr>
<td>PTCL</td>
<td>3</td>
<td>7</td>
<td>11</td>
</tr>
<tr>
<td>MCL</td>
<td>2</td>
<td>7</td>
<td>21</td>
</tr>
<tr>
<td>FL</td>
<td>2</td>
<td>2</td>
<td>7</td>
</tr>
<tr>
<td>Other</td>
<td></td>
<td></td>
<td>1 (^3)</td>
</tr>
<tr>
<td>Disease status</td>
<td></td>
<td></td>
<td>NS</td>
</tr>
<tr>
<td>I CR</td>
<td>7</td>
<td>13</td>
<td>34</td>
</tr>
<tr>
<td>I PR</td>
<td>4</td>
<td>9</td>
<td>23</td>
</tr>
<tr>
<td>II CR</td>
<td>3</td>
<td></td>
<td>8</td>
</tr>
<tr>
<td>II PR</td>
<td>3</td>
<td></td>
<td>3</td>
</tr>
<tr>
<td>Relapse/ progression</td>
<td>1</td>
<td>1</td>
<td>4</td>
</tr>
<tr>
<td>Mobilization chemotherapy</td>
<td></td>
<td></td>
<td>NS</td>
</tr>
<tr>
<td>DHAP</td>
<td>6</td>
<td>11</td>
<td>25</td>
</tr>
<tr>
<td>AraC</td>
<td>5</td>
<td>10</td>
<td>31</td>
</tr>
<tr>
<td>ICE</td>
<td>2</td>
<td>2</td>
<td>4</td>
</tr>
<tr>
<td>CHOP/CHOEP</td>
<td>2</td>
<td></td>
<td>4</td>
</tr>
<tr>
<td>Bonn C</td>
<td></td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>BFM A</td>
<td></td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Cyclophosphamide</td>
<td></td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Other</td>
<td></td>
<td></td>
<td>5</td>
</tr>
</tbody>
</table>

p-values 0.05 or more are designated as NS.

\(^1\) Including one patient with primary CNS lymphoma in the plerixafor group and two patients in the control group; \(^2\) Including two CNS lymphomas and three patients with transformation from FL; \(^3\) Burkitt’s lymphoma (transformation from FL).

Bonn C = high-dose cytarabine, vindesine, dexamethasone; BFM A = cytarabine, vincristine, teniposide, methotrexate, ifosfamide.
4.2.3 Study III
This study included 38 patients with newly diagnosed MM. The patients received auto-SCT between August 2013 and May 2015 at the University Hospitals of Kuopio, Oulu, Tampere and Turku and were followed up to October 31st 2015. Alongside the GOA study, the patients participated also the prospective Multiple Myeloma 02 study (MM-02) by the Finnish Myeloma Group (FMG). The aim of the MM-02 study was to analyse the efficacy and safety of lenalidomide, bortezomib and dexamethasone (RVD) induction therapy, to compare a randomly assigned mobilization with either low-dose cyclophosphamide plus G-CSF (arm A) or G-CSF alone (arm B) as well as to analyse the efficacy and safety of lenalidomide maintenance therapy after auto-SCT.

Of the 38 patients, 17 were randomly assigned to arm A and 21 patients to arm B. Two patients in arm B had their grafts collected but eventually received allogeneic transplantation due to disease-related high-risk characteristics and therefore altogether thirty-six patients proceeded to auto-SCT. By the study protocol of MM-02, all patients received three 21-day cycles of RVD induction. Thereafter all patients received high-dose melphalan (200 mg/m^2) on d-2 followed by graft infusion on d0. According to the study protocol of the GOA study, all patients had the data of the collected blood grafts available and the hematological (up to 12 months) and immunological recovery (up to 6 months) was analysed. The patient characteristics of study III are presented in Table 3.

4.2.4 Study IV
This study included 87 adult patients with MM participating the GOA study who received their first auto-SCT between June 2012 and December 2014 and were followed up to October 15th 2017. The participating University hospitals were Kuopio, Oulu, Tampere and Turku. Thirty patients (29%) were also included in the MM-02 study, others received the mobilization by the choice of the physician in charge of their treatment. Plerixafor was given for poorly mobilizing patients without predetermined protocol except in patients participating the MM-02 study. The patient population was divided into two groups according to the use of plerixafor, thus forming the plerixafor group (n = 10) and the control group (n = 77). Graft cellular composition and hematological (up to 12 months) recovery were analysed in all patients and immune recovery (up to 6 months) in 36 patients. The patient characteristics of study IV are presented in Table 3.
Table 3. Characteristics of MM patients in studies III and IV.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Study III</th>
<th>Study IV</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Arm A(^1) n = 17</td>
<td>Arm B(^2) n = 19</td>
<td>Control group (n = 77)</td>
</tr>
<tr>
<td>Gender (M/F)</td>
<td>10/7</td>
<td>8/11</td>
<td>NS</td>
</tr>
<tr>
<td>Age, years (median, range)</td>
<td>58 (49-70)</td>
<td>63 (52-70)</td>
<td>NS</td>
</tr>
<tr>
<td>Paraprotein type, n (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IgG</td>
<td>11 (65)</td>
<td>14 (74)</td>
<td>44 (57)</td>
</tr>
<tr>
<td>IgA</td>
<td>3 (18)</td>
<td>4 (21)</td>
<td>18 (23)</td>
</tr>
<tr>
<td>Light chain</td>
<td>3 (18)</td>
<td>1 (5)</td>
<td>15 (20)</td>
</tr>
<tr>
<td>Light chain type, n (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Kappa</td>
<td>13 (76)</td>
<td>15 (79)</td>
<td>53 (69)</td>
</tr>
<tr>
<td>Lambda</td>
<td>4 (24)</td>
<td>4 (21)</td>
<td>23 (30)</td>
</tr>
<tr>
<td>ISS, n (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>I</td>
<td>4 (24)</td>
<td>10 (53)</td>
<td>21 (27)</td>
</tr>
<tr>
<td>II</td>
<td>11 (65)</td>
<td>8 (42)</td>
<td>34 (44)</td>
</tr>
<tr>
<td>III</td>
<td>2 (12)</td>
<td>1 (5)</td>
<td>9 (12)</td>
</tr>
<tr>
<td>IMWG risk (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Low</td>
<td>3 (18)</td>
<td>1 (5)</td>
<td>3 (4)</td>
</tr>
<tr>
<td>Intermediate</td>
<td>13 (76)</td>
<td>15 (79)</td>
<td>48 (62)</td>
</tr>
<tr>
<td>High</td>
<td>1 (6)</td>
<td>3 (16)</td>
<td>13 (17)</td>
</tr>
<tr>
<td>Mobilization method, n (%)</td>
<td></td>
<td></td>
<td>0.009</td>
</tr>
<tr>
<td>CY + G-CSF</td>
<td>17 (100)</td>
<td>NA</td>
<td>57 (74)</td>
</tr>
<tr>
<td>G-CSF alone</td>
<td>NA</td>
<td>19 (100)</td>
<td>20 (26)</td>
</tr>
<tr>
<td>Plerixafor added</td>
<td>1 (6)</td>
<td>4 (21)</td>
<td></td>
</tr>
</tbody>
</table>

p-values 0.05 or more are designated as NS. \(^1\) = cyclophosphamide (2g/m\(^2\)) + G-CSF; \(^2\) = G-CSF alone; CY = cyclophosphamide (2g/m\(^2\)); G-CSF = granulocyte-colony stimulating factor; ISS = international staging system; NA = not applicable.
4.3 MOBILIZATION AND COLLECTION OF BLOOD GRAFTS

4.3.1 Studies I and II
In patients with NHL the mobilization was performed by using disease-specific treatment protocols. The most commonly used chemotherapy regimens were high-dose cytarabine (AraC) or a combination of cytarabine with dexamethasone and cisplatin (DHAP) (Table 2). All patients received G-CSF, most commonly pegfilgrastim at the dose 6 mg after chemotherapy. For filgrastim the dose was 5 μg/kg/d. In study I, plerixafor was given to 15 out of 41 patients (37%) and in study II to 24 out 72 patients (33%). The most common plerixafor dose was 0.24 mg/kg and it was usually given late in the evening. The patient and mobilization characteristics are presented in more detail in Table 2.

The usual level to start daily B-CD34+ cell measurements was WBC level over 0.5 – 1.0 x 10^9/L, depending on the local practice in the participating hospitals. The common threshold to initiate apheresis was B-CD34+ level 20 x 10^6 /L but 14 patients (34%) in study I and 27 patients (36%) patients in study II had B-CD34+ level below that before the first apheresis (B-CD34+ range 2-19 x 10^6 /L). The minimum collection target was 2.0 x 10^6/kg CD34+ cells. In KUH the collections were initially performed with the COBE Spectra AutoPBSC (COBE Laboratories Inc., Lakewood, CO) and since April 2013 with the Spectra Optia apheresis system (Spectra Optia® Apheresis System, Software 7.2, Terumo BCT Lakewood, CO, USA) using automated software intended for collection of mononuclear cell fraction. At Oulu and Tampere University Hospitals the Spectra Optia Apheresis system was used throughout the study period. The blood volume processed daily was 2-2.5 times the estimated TBV of the given patient.

4.3.2 Studies III and IV
In study III there were 38 patients, of whom 17 (45%) were randomly assigned to mobilization with CY plus G-CSF (arm A). CY 2g/m^2 was administered on day +1 and G-CSF (filgrastim) 5 μg/kg from day +4 onwards. In arm B 21 patients were mobilized with G-CSF alone; filgrastim 10 μg/kg was given from day +1 onwards. The B-CD34+ level ≥ 10 x 10^6 /L at day +10 in arm A and day +5 in arm B was set as a threshold to start apheresis. By the study protocol of MM-02, plerixafor was used in arm A if B-CD34+ was < 10 x 10^6 /L and WBC ≥ 10 x 10^9/L. In arm B plerixafor was given if B-CD34+ was < 10 x 10^6 /L on day +5. Plerixafor was used also if the first apheresis yielded less than 1.0 x 10^6/kg CD34+ cells.

Before the collections, an individual evaluation and decision was made on the assumed number of transplantations in a given patient thereby determining the collection target. For a single transplant, the La-CD34+ goal was ≥ 3.0 x 10^6 /kg and for two transplantations ≥ 6.0 x 10^6 /kg in the MM-02 study. The apheresis machines used are described in paragraph 4.3.1. In addition, in Turku University Hospital the COM.TEC Fresenius (Blood Cell Separator Fresenius Hemo Care GmbH, Bad Homburg, Germany) apheresis machine was used. The daily processed blood volume was 2-3 times the estimated total blood volume of the given patient.

In study IV altogether 60 patients (69%) received CY + G-CSF to mobilize blood grafts and 27 patients G-CSF. Only three patients (5%) mobilized with CY + G-CSF needed plerixafor compared to seven patients (26%) mobilized with G-CSF (p = 0.009).
4.4 HIGH-DOSE THERAPY

4.4.1 Studies I and II
The most common high-dose therapy regimen used was BEAM (carmustine 300 mg/m² on d -6, etoposide 200 mg/m² from d -5 to d -2, cytarabine 300 mg/m² from d -5 to -2 and melphalan 140 mg/m² d -1, graft infusion on d 0), which was given in study I to 39 patients (93%) and in study II to 64 patients (89%). In study II four (6%) patients were treated with BEAC regimen (carmustine 300 mg/m² on d -7, etoposide 200 mg/m² from d -6 to d -3, cytarabine 400 mg/m² from d -6 to -3, and cyclophosphamide 35 mg/kg d -6 to d -3). In study I, three patients (7%) and in study II four patients (6%) received a combination of carmustine (400 mg/m² on d -6) and thiotepa (5 mg/m² on d -5 and -4) due to primary CNS lymphoma. G-CSF was given to all patients after the graft infusion, most commonly pegfilgrastim (93% in study I and 71% in study II).

4.4.2 Studies III and IV
All MM patients received high-dose melphalan (200 mg/m²) on d-2, followed by the graft infusion on d0. In both studies III and IV, for patients participating the MM-02 study, G-CSF (filgrastim) was given according to the study protocol from day +5 onwards if the collected graft had less than 3.0 x 10^6/kg CD34+ cells. Other patients received G-CSF by the decision of the physician in charge. In study III altogether seven patients (19%) and in study IV altogether 66 patients (76%) were given G-CSF after the graft infusion.

4.5 LABORATORY METHODS

4.5.1 Flow cytometric enumeration of the blood CD34+ cells (B-CD34+) and leukapheresis product (La-CD34+)
The B-CD34+ count before the apheresis and the La-CD34+ count after each apheresis session was analysed within few hours by flow cytometry in the laboratory of the hospitals performing the apheresis procedure. The samples were analysed locally to enable the study of fresh samples. The flow cytometers used were FACSCanto (Becton Dickinson, San Jose, CA), FACSCanto2 (Becton Dickinson, San Jose, CA) and Beckman Coulter Navios flow cytometry (Beckman Coulter Inc., Brea, CA). All CD34+ analyses were performed using an ISHAGE protocol with a single-platform method [Gratama et al. 2003].

4.5.2 Graft processing and analysis of graft cellular composition
The collected apheresis products were processed at the stem cell laboratory of the corresponding university hospitals: in Kuopio at the Laboratory Centre of Eastern Finland, in Oulu at the Northern Finland Laboratory Centre Nordlab, in Tampere at Fimlab and in Turku at Tykslab. No graft manipulation was performed. To protect the cells from stress and death caused by the process of cryopreservation, DMSO was added in a final concentration of 10%. To enable the analysis of the cellular composition of the frozen grafts, two 0.5ml tubes were taken from each apheresis product. The apheresis products were frozen using a controlled-rate freezing program and thereafter maintained in a liquid nitrogen freezer at -170°C. Later, the cryopreserved graft samples were thawed at +37°C water bath and analysed at the Department of Microbiology at the University of Eastern Finland with flow cytometry (FACSCanto, Beckton Dickison, San Jose, CA) using the ISHAGE protocol with a single-platform method (Stem-Kit, Immunotech SAS/Beckman Coulter, Marseille, France). All analyses were performed by the same experienced flow cytometrist. Every collected apheresis product from each patient was analysed. The following antibodies against cell surface markers were...
used for the analysis of CD34+ cells and CD34+ subsets (clone designations in parentheses): CD34 (8G12), CD38 (HB7), CD133 (AC133), and CD45 (2D1). Antibodies were acquired from Becton Dickinson (Heidelberg, Germany) except for CD133 (Miltenyi Biotec were GmbH, Bergisch Gladbach, Germany). Viable CD34+ cells were defined by using 7-AAD staining.

To determine the absolute counts of T, B, and natural killer (NK) cells as well as the CD3+CD4+ and CD3+CD8+ subpopulations of T cells in the grafts, CD3/CD8/CD45/CD4 and CD3/CD16+/CD56/CD45/CD19 reagents (BD Multitest, Becton Dickinson) with Trucount tubes (BD Trucount, Becton Dickinson) were used.

4.5.3 Flow cytometric analysis of blood lymphocyte subsets
The immune recovery was analysed by examining blood lymphocyte subsets at 1, 3 and 6 months post-transplant in a group of patients. In NHL patients transplanted at KUH immune recovery was intended to be analysed in about 40 patients included in the study. Otherwise there was no algorithm for the selection of patient for these analyses except for the patients participating the MM-02 study who were included into the analyses. The flow cytometric analyses were performed at the laboratories of the hospitals in charge of the post-transplant follow-up of the given patient. The antigen panel used was the same as described in chapter 4.5.2, except for four patients in study III whose samples were analysed at TUH and the analysis was performed with Beckman Coulter Aquios CL (Beckman Coulter Inc., Brea, CA) using the Aquios Tetra-1 (CD45 FITC/CD3 PC5/CD4 RD1/CD8 ECD) and the Aquios Tetra-2+ (CD45 FITC/CD3 PC5/CD56+/CD16 RD1/CD19 ECD) antibody panels.

4.5.4 Quality control
All transplantation centers participating the GOA study were supervised by the Finnish Medicines Agency (Fimea). In addition, the laboratories of each university hospital participated regularly the CD34+ Stem Cell Enumeration trials and Immune Monitoring Scheme organized by UK NEQAS (www.ukneqasli.co.uk), which include CD34 measurements and lymphocyte subset measurements (both 6 times a year).

4.6 DATA COLLECTION
The clinical information of the patients was gathered from the patient record system of the hospital in charge of the treatment and follow-up of the given patient. All data was collected in a written form to a specifically designed data-collection form and stored in a secure location at Kuopio University Hospital. Any data stored electronically for analysis purposes was unidentifiable and encrypted using AES-256 and RSA algorithms.

4.7 STATISTICAL METHODS
In studies I, II and IV Mann-Whitney U test, Pearson’s chi-square test, Fischer’s exact test for small samples, Spearman’s rank, Cox regression and Kaplan-Meier method were used to analyse the data. In study II, a logistic regression model for binary variables was used. Also, univariate and multivariate analyses were performed. In multivariate analysis, all predictors were included to model and the backward variable selection was carried out. Results from logistic analysis were presented with odds ratios (OR) and 95% confidence intervals (CI) and from Cox regression analysis with hazard ratios (HR) and 95% CIs, respectively. In addition, in study III a logistic regression model was used for binary variables. All p-values were two-tailed and p-values <0.05 were considered statistically significant. All analyses were performed using appropriate software (IBM Statistics v.22 for Macintosh and IBM Statistics version 23 and version 24 for Windows, Armonk, NY).
4.8 APPROVALS

The Research Ethics Committee of Northern Savo Hospital District approved the study protocol for the GOA study (13/2012) as well as the MM-02 study (51/2012). For both studies, a written informed consent was obtained from all patients before the study enrollment. Both studies were conducted according to the Declaration of Helsinki, International Conference of Harmonization and Guidelines for Good Clinical Practice. The MM-02 study was also approved by the Finnish Medicines Agency. The MM-02 study was also registered at www.clinicaltrials.gov (NCT017907379).
5 Results

5.1 BLOOD GRAFT CELLULAR COMPOSITION AND POST-TRANSPLANT RECOVERY IN NON-HODGKIN LYMPHOMA PATIENTS MOBILIZED WITH OR WITHOUT PLERIXAFOR – A PROSPECTIVE COMPARISON (I)

The median B-CD34+ count before the first apheresis (15 vs. 31 x 10⁶/L, p = 0.002), the highest measured median B-CD34+ count during the course of aphereses (20 vs. 49 x 10⁶/L, p = 0.003), the median yield of the first apheresis (1.2 vs. 2.8 x 10⁶/kg, p = <0.001) and the median total yield of CD34+ cells (2.5 vs. 3.9 x 10⁶/kg, p = 0.004) were all lower in the plerixafor group than in the control group. The median number of aphereses was two in the plerixafor group and one in the control group (p = 0.004).

In the analysis of the cryopreserved blood grafts there were significantly less CD34+ cells in the plerixafor group than in the control group (total number: 2.2 x 10⁶/kg (0.8-6.7) vs. 3.5 x 10⁶/kg (1.9-14.9) (p = 0.001) and with 7-AAD: 2.0 x 10⁶/kg (0.6-5.7) vs. 3.1 x 10⁶/kg (1.5-14.3) (p = 0.006). The number of CD34+CD133+CD38− cells was comparable between the groups, but the proportion of these cells from all CD34+ cells was significantly higher in the plerixafor group (3.5 vs. 1.7 %; p = <0.001). The numbers of T lymphocytes and NK cells were all significantly higher in the plerixafor group. For detailed graft analysis, see Table 4.

Table 4. Blood graft cellular composition according to the use of plerixafor in NHL patients.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Mobilization with plerixafor (n = 15)</th>
<th>Mobilization without plerixafor (n = 26)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD34+ w/a 7AAD</td>
<td>2.2 (0.8 – 6.7)</td>
<td>3.5 (1.9 – 14.9)</td>
<td>0.001</td>
</tr>
<tr>
<td>CD34+ w 7AAD</td>
<td>2.0 (0.6 – 5.7)</td>
<td>3.1 (1.5 – 14.3)</td>
<td>0.006</td>
</tr>
<tr>
<td>CD34+CD133+CD38−</td>
<td>0.07 (0.01 – 0.17)</td>
<td>0.05 (0.11 – 0.35)</td>
<td>0.502</td>
</tr>
<tr>
<td>CD34+CD133+CD38− cells from all CD34+ cells (%)</td>
<td>3.5 (0.8 – 10.8)</td>
<td>1.7 (0.44 – 5.3)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>CD3+</td>
<td>160.9 (49.2 – 454.4)</td>
<td>58.6 (10.9 – 415.4)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>CD3+CD4+</td>
<td>81.1 (29.1 – 267.1)</td>
<td>35.2 (7.7 – 114.3)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>CD3+CD8+</td>
<td>75.3 (16.5 – 279.1)</td>
<td>21.0 (3.1 – 301.8)</td>
<td>0.001</td>
</tr>
<tr>
<td>CD19+</td>
<td>0.0 (0.0 – 0.01)</td>
<td>0.0 (0.0 – 3.2)</td>
<td>0.576</td>
</tr>
<tr>
<td>NK</td>
<td>20.4 (0.4 – 39.5)</td>
<td>4.8 (0.6 – 20.7)</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

median (range); w/a = without; w = with

The neutrophil engraftment after HDT was comparable between the groups (nine days), but the platelet engraftment ≥ 20 x10⁹/L was slower in the plerixafor group (15 vs. 12 days, p =
0.044). Also, the neutrophil counts were significantly lower in the plerixafor-mobilized patients at one month post-transplant (2.0 vs. 2.8 x 10^9/L, p = 0.026). The hemoglobin level was higher in the plerixafor group at 12 months (140 vs. 125 g/L, p = 0.048) as well as the NK cell level at one month (0.4 vs. 0.2 x 10^9/L, p = 0.001) post-transplant (study I, Table 3).

The cellular composition of the blood grafts had significant correlations with the hematological and immune recovery after the transplantation, especially within the first three months (study I, Tables 6-7). At the first timepoint (+15 days) there was a correlation with the number of CD34+ cells in the infused grafts and the leukocyte, neutrophil and platelet counts. The aforementioned positive correlation of infused CD34+ cells with the platelet count lasted until six months post-transplant. Also, the number of CD34+CD133+CD38 cells in the grafts had a positive correlation with the leukocyte, neutrophil and platelet counts at +15 days, lymphocyte counts at +3 months and leukocyte and neutrophil counts at 6 months. Further, the number of CD3+, CD3+CD4+ and CD3+CD8+ lymphocytes in the grafts correlated with lymphocyte counts at +15 days and at one month. The correlation was also present at three months with the CD3+ and CD3+CD8+ lymphocyte counts. Interestingly, CD3+CD8+ cells in the graft had a negative correlation with the number of neutrophils at 6 and 12 months. The number of graft NK cells correlated positively with the B lymphocyte count at 3 months and the platelet count at 12 months post-transplant.

The graft cellular content had also several significant correlations with immune reconstitution, especially at one month post-transplant. At that time point the number of CD3+, CD3+CD4+and NK cells in the graft correlated positively with their counterparts in blood samples. Comprehensive correlation analyses of graft composition with hematological and immune reconstitution are presented in Tables 6-7 in the original study I.

The plerixafor group was comparable to the control group in regard to the length of hospitalization, incidence of bacteremia, neutropenic fever or opportunistic infections. There was no difference in the PFS or OS between the groups (study I, Fig 1 and 2).

### 5.2 EARLY IMMUNE RECOVERY AFTER AUTOLOGOUS TRANSPLANTATION IN NON-HODGKIN LYMPHOMA PATIENTS: PREDICTIVE FACTORS AND CLINICAL SIGNIFICANCE (II)

The median number of collected CD34+ cells was 3.5 x 10^6/kg (range 1.6 – 25.7 x 10^6/kg) and the median number of viable CD34+ cells in the grafts after cryopreservation 2.4 x 10^6/kg (range 0.6 – 14.3 x 10^6/kg). The number of various graft components was comparable to study I, considering the patient population mobilized with and without plerixafor (study I, Table 2). The median neutrophil engraftment was ten days and platelet engraftment 14 days. The median ALC-15 in the entire patient cohort was 0.6 x 10^9/L. In the patients receiving plerixafor the median ALC-15 was 0.77 x 10^9/L compared to 0.44 x 10^9/L in patients mobilized without plerixafor (p = 0.091).

In univariate analysis, the prognostic graft components for ALC-15 ≥ 0.5 x 10^9/L were the CD3+CD4+ (p = 0.009) and NK cell counts in the grafts (p = 0.014). Also, the use of plerixafor was associated with higher probability of early immune recovery (p = 0.011). The histology of FL (p = 0.035) or MCL (p = 0.0016) were associated with a decreased probability of ALC-15 ≥ 0.5 x10^9/L. Also in multivariate analysis, the histology of FL (p = 0.005) and MCL (p = 0.003) seemed to decrease the probability of ALC-15 ≥ 0.5 x10^9/L whilst the higher number of CD34+ (p = 0.015) and CD34+CD133+CD38+ cells (p = 0.005) in the grafts and the use of plerixafor (p = 0.004) increased the probability.

The predictive factors for the blood lymphocyte subsets at one month to be above the lower normal range (defined by the Laboratory Centre of Eastern Finland) were also analysed. For B-CD3+ cells the level was ≥ 0.7 x 10^9/L and for B-CD3+CD4+ ≥ 0.3 x 10^9/L, respectively. An
exception for the cut-offs was made for B-NK and B-CD3$^+$CD8$^+$ cells as almost all patients reached the level of normal lower limit (0.09 x10$^9$/L and 0.2 x10$^9$/L, respectively) at one month after auto-SCT. Therefore, the levels were set to the median values (0.2 x10$^9$/L and 1.0 x 10$^9$/L, respectively). In both univariate and multivariate analysis, there was a negative correlation between female gender and B-CD3$^+$ cells ≥ 0.7 x 10$^9$/L (p = 0.024 and p = 0.016, respectively). Age > 60 years (p = 0.033) and higher amount of CD3$^+$ cells in the graft (p = 0.029) were associated with B-CD3$^+$ cell counts ≥ 0.7 x 10$^9$/L in multivariate analysis. In univariate analysis, there was a correlation with age over 60 years and B-CD3$^+$CD4$^+$ cells ≥ 0.3 x 10$^9$/L (p = 0.049). Also the total number of CD3$^+$ (p = 0.050) and CD3$^+$CD4$^+$ (p = 0.023) cells in the grafts was associated with B-CD3$^+$CD4$^+$ cells ≥ 0.3 x 10$^9$/L at one month post-transplant. The number of CD3$^+$CD4$^+$ cells was significant also in multivariate analysis (p = 0.023). NK cells (p = 0.002), CD3$^+$ (p = 0.033) and CD3$^+$CD4$^+$ lymphocytes (p = 0.018) in the grafts were prognostic in regard to B-NK cells ≥ 0.2 x 10$^9$/L at one month post-transplant in univariate analysis. The number of NK cells in the graft retained also a significant correlation in a multivariate analysis (p = 0.048). The use of plerixafor was associated with NK cells ≥ 0.2 x 10$^9$/L both in univariate (p <0.001) and multivariate analysis (p = 0.015). In multivariate analysis, also female gender had a negative association with NK cell counts ≥ 0.2 x 10$^9$/L (p = 0.033). There were no statistically significant correlations for CD3$^+$CD8$^+$ cells ≥ 1.0 x 10$^9$/L.

In the entire patient population ALC-15 ≥ 0.5 x10$^9$/L was associated with improved OS (Log rank, p = 0.021) (Figure 2) but not PFS (Log rank, p = 0.252) (Figure 1). ALC-15 ≥ 0.5 x10$^9$/L also predicted OS in multivariate analysis (HR 0.222, p = 0.022) when age > 60 years, gender, lymphoma histology, disease status at the time of transplantation and use of plerixafor were taken into consideration. When only more aggressive lymphoma histologies (DLBCL, PTCL, Burkitt) were analysed, ALC-15 ≥ 0.5 x10$^9$/L was found to be significant in regard to both PFS (Log rank, p = 0.030) (Figure 3) and OS (Log rank, p = 0.002) (Figure 4).
Figure 1. Progression-free survival of NHL patients according to ALC-15 ≥ 0.5 x10⁹/L.

Figure 2. Overall survival of NHL patients according to ALC-15 ≥ 0.5 x10⁹/L.
Figure 3. Progression-free survival of patients with aggressive lymphoma histologies according to ALC-15 level of $0.5 \times 10^9$/L.

Figure 4. Overall survival of patients with aggressive lymphoma histologies according to ALC-15 level of $0.5 \times 10^9$/L.
5.3 BLOOD GRAFT CELLULAR COMPOSITION AND POST-TRANSPLANT OUTCOMES IN MYELOMA PATIENTS MOBILIZED WITH OR WITHOUT LOW-DOSE CYCLOPHOSPHAMIDE - A RANDOMIZED COMPARISON (III)

The median CD34+ cell yield was $6.7 \times 10^6$/kg in patients mobilized with CY + G-CSF (arm A) and $6.0 \times 10^6$/kg in those mobilized with G-CSF alone (arm B) ($p = 0.064$). There was no significant difference in the use of plerixafor (Arm A 6% vs. Arm B 19%, $p = 0.243$). The blood grafts in arm A had a significantly higher total number of CD34+ cells ($p = 0.001$) as well as viable CD34+ cells (with 7-AAD) ($p = 0.009$). The proportion of CD34+CD133−CD38− cells from all CD34+ cells was significantly higher in arm B (4.1% vs. 2.5%, $p = 0.006$). The amount of CD3+ ($p < 0.001$), CD3+CD4+ ($p = 0.001$), CD3+CD8+ ($p = 0.001$), CD19+ ($p < 0.001$) and NK cells ($p < 0.001$) were also significantly higher in the G-CSF arm. The detailed graft analysis is presented in Table 5.

Table 5. Blood graft cellular composition in MM patients according to the mobilization method after cryopreservation.

<table>
<thead>
<tr>
<th>Variable (x10^6 cells/kg)</th>
<th>CY + G-CSF (arm A), n = 17</th>
<th>G-CSF (arm B), n = 19</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD34+ w/a 7AAD</td>
<td>6.4 (3.1-8.9)</td>
<td>4.1 (1.4-7.3)</td>
<td>0.005</td>
</tr>
<tr>
<td>CD34+ w 7AAD</td>
<td>3.9 (1.4-8.1)</td>
<td>3.0 (0.2-6.2)</td>
<td>0.009</td>
</tr>
<tr>
<td>CD34+CD133−CD38−</td>
<td>0.1 (0.02-0.33)</td>
<td>0.7 (0.01-0.37)</td>
<td>0.561</td>
</tr>
<tr>
<td>CD34+CD133−CD38− cells</td>
<td>2.5 (1.0-6.0)</td>
<td>4.1 (1.0-9.0)</td>
<td>0.006</td>
</tr>
<tr>
<td>from all CD34+ cells (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CD3+</td>
<td>65.1 (28.3-283.0)</td>
<td>215.2 (50.4-683.6)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>CD3+CD4+</td>
<td>45.2 (12.6-156.4)</td>
<td>116.3 (29.4-502.5)</td>
<td>0.001</td>
</tr>
<tr>
<td>CD3+CD8+</td>
<td>23.1 (5.3-133.9)</td>
<td>90.5 (20.8-197.3)</td>
<td>0.001</td>
</tr>
<tr>
<td>CD19+</td>
<td>2.0 (0.46-11.6)</td>
<td>8.7 (0.3-76.7)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>NK</td>
<td>6.8 (0.9-36.1)</td>
<td>31.7 (15.5-144.7)</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

median (range); w/a = without; w = with.
Patients in arm A received a significantly higher number of CD34+ cells (4.4 vs. 3.2 x 10⁶/kg, p = 0.033) after high-dose MEL. There was no difference in the length of hospitalization, incidence of neutropenic fever or tempo of engraftment (neutrophils >0.5 x 10⁶/L (median 12 vs. 13 days in arm A vs. arm B p = 0.379), platelets >20 x10⁹/L (median 12 days in the both groups, p = 0.443)) between the mobilization arms. Lymphocyte recovery at d+15 (0.5 vs. 0.8 x 10⁹/L, p = 0.033) and hemoglobin recovery at three months post-transplant (120 vs. 127 g/L, p = 0.045) were significantly faster in arm B. The total number of T lymphocytes (p = 0.038) and the number of CD3-CD8+ lymphocytes (p = 0.035) was higher in arm A at three months post-transplant. The number of NK cells was higher in arm B at three (p = 0.005) and six (p = 0.014) months post-transplant. For comprehensive analysis of the hematological and immune recovery, see Tables III-IV in study III.

Within the median follow-up time of 474 days in arm A and 433 days in arm B (p = 0.925) there was no difference in PFS (Figure 5) (p = 0.927).

![Figure 5](image_url)

*Figure 5. Progression-free survival of myeloma patients according to the method of mobilization.*
5.4 BLOOD GRAFT CELLULAR COMPOSITION AND POST-TRANSPLANT RECOVERY IN MYELOMA PATIENTS MOBILIZED WITH OR WITHOUT PLERIXAFORE – A PROSPECTIVE COMPARISON (IV)

The number of collected CD34+ cells was comparable between the plerixafor and the control groups (6.0 vs. 6.4 x 10⁶/kg, respectively; p = 0.436). There was also no significant difference in the median number of aphereses performed (two in both groups, p = 0.086). There were no differences between the groups in the number of CD34+ or CD34+CD133−CD38− cells in the apheresis products or in the grafts used after the HDT, but the proportion of CD34+CD133−CD38− cells from all CD34+ cells was significantly higher in plerixafor mobilized grafts (Table 6). Also, the total numbers of other analysed graft components (CD3+, CD3+CD4+, CD3−CD8+, CD19+ and NK cells) were all significantly higher in the plerixafor group (Table 6). The grafts of patients mobilized with G-CSF alone or with G-CSF and plerixafor were comparable in regard to CD34+ and CD34+CD133−CD38− cells as well as other graft components. The only statistically significant difference between the patients mobilized with G-CSF or G-CSF + plerixafor was the higher number of CD3+CD4+ lymphocytes in the grafts used at HDT, favoring G-CSF + plerixafor (Table 7).

The median neutrophil engraftment > 0.5 x 10⁹/L was 14 days in the plerixafor group and 12 days in the control group (p = 0.002). There was no difference in the platelet engraftment (13 vs. 12 days, respectively; p = 0.618). Hematological recovery was comparable between the groups within the first 12 months after auto-SCT (study IV, Table 3). The phase of immune recovery was also comparable between the groups except for the faster recovery of CD3+CD4+ lymphocytes in the plerixafor group at three (medians 0.5 vs. 0.3 x 10⁹/L, respectively; p = 0.035) and six months (medians 0.4 vs. 0.3 x 10⁹/L, respectively; p = 0.033) post-transplant (Table 8).

Patients in the plerixafor group were hospitalized for a significantly longer time (21 vs. 18 days, p = 0.029) during HDT. There were no differences in the incidence of bacteremia (10% vs 26 %, p = 0.410). In the plerixafor group the median follow-up time was 1108 days (342-1654) and in the control group 1203 days (139-1928) (p = 0.406). There was no difference in the incidence of myeloma progression (60 % in both groups, p = 0.633). The median time to progression was 791 days in the plerixafor group and 449 days in the control group (p = 0.291).

In the Kaplan-Meier survival analysis there was no statistically significant difference between the groups in regard to PFS (Figure 6) (p = 0.817) or OS (Figure 7) (p = 0.513).
<table>
<thead>
<tr>
<th>Variable</th>
<th>Apoptosis products used at HDT</th>
<th>Pherexator used</th>
<th>Pherexator not used</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>(n = 10)</td>
<td>(n = 10)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 6. Blood graft cellular composition in MM patients according to pherexator use.
Table 7. Blood graft cellular composition in MM patients mobilized with G-CSF according to the use of plerixafor.

<table>
<thead>
<tr>
<th>Variable</th>
<th>All apheresis products</th>
<th></th>
<th></th>
<th>Apheresis products used at HDT</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>G-CSF plus plerixafor</td>
<td>G-CSF</td>
<td>p-value</td>
<td>G-CSF plus plerixafor</td>
</tr>
<tr>
<td></td>
<td>(n = 7)</td>
<td>(n = 20)</td>
<td></td>
<td>(n = 7)</td>
</tr>
<tr>
<td>CD34+ with 7-AAD</td>
<td>3.1 (1.2-5.1)</td>
<td>2.4 (0.2-6.1)</td>
<td>NS</td>
<td>1.8 (1.2-4.4)</td>
</tr>
<tr>
<td>CD34+CD133+CD38+</td>
<td>0.1 (0.04-0.28)</td>
<td>0.06 (0.005-0.342)</td>
<td>NS</td>
<td>0.07 (0.04-0.14)</td>
</tr>
<tr>
<td>Proportion of CD34+CD133+ CD38- from all CD34+ cells (%)</td>
<td>4.3 (3.1-5.8)</td>
<td>3.7 (1.1-8.8)</td>
<td>NS</td>
<td>2.9 (1.9-5.6)</td>
</tr>
<tr>
<td>CD3+</td>
<td>318.4 (58.3-683.6)</td>
<td>210.4 (50.4-496.5)</td>
<td>NS</td>
<td>247.4 (29.2-388.3)</td>
</tr>
<tr>
<td>CD3+CD4+</td>
<td>198.5 (37.6-502.5)</td>
<td>102.6 (29.4-249.9)</td>
<td>NS</td>
<td>131.6 (18.8-266.2)</td>
</tr>
<tr>
<td>CD3+CD8+</td>
<td>88.1 (21.2-194.1)</td>
<td>91.7 (17.8-242.9)</td>
<td>NS</td>
<td>81.6 (10.6-194.1)</td>
</tr>
<tr>
<td>CD19+</td>
<td>17.3 (1.7-16.7)</td>
<td>9.0 (0.05-61.59)</td>
<td>NS</td>
<td>12.3 (0.8-49.36)</td>
</tr>
<tr>
<td>NK cells</td>
<td>46.6 (2.3-65.3)</td>
<td>28.6 (13.9-144.7)</td>
<td>NS</td>
<td>27.3 (1.15-59.86)</td>
</tr>
</tbody>
</table>

p-values 0.05 or more are designated as NS; HDT = high-dose therapy
Table 8. Immunological recovery in MM patients after auto-SCT according to the use of plerixafor.

<table>
<thead>
<tr>
<th></th>
<th>Plerixafor used, median (range); n</th>
<th>Plerixafor not used, median (range); n</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blood flow cytometry one month after auto-SCT (x 10^9/L)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CD3^+</td>
<td>1.3 (0.8 – 1.7); 4</td>
<td>1.1 (0.3 – 5.9); 19</td>
<td>NS</td>
</tr>
<tr>
<td>CD3^+CD4^+</td>
<td>0.5 (0.3 – 0.8); 4</td>
<td>0.3 (0.1 – 2.0); 19</td>
<td>0.035</td>
</tr>
<tr>
<td>CD3^+CD8^+</td>
<td>0.8 (0.4 – 1.1); 4</td>
<td>0.8 (0.2 – 5.4); 20</td>
<td>NS</td>
</tr>
<tr>
<td>NK</td>
<td>0.5 (0.2 – 0.6); 4</td>
<td>0.4 (0.1 – 0.8); 19</td>
<td>NS</td>
</tr>
<tr>
<td>CD19^+</td>
<td>0.01 (0.0 – 0.3); 4</td>
<td>0.01 (0.0 – 0.1); 19</td>
<td>NS</td>
</tr>
<tr>
<td>CD4/CD8 - ratio</td>
<td>0.5 (0.0 – 1.2); 4</td>
<td>0.4 (0.0-1.2); 19</td>
<td>NS</td>
</tr>
<tr>
<td>Blood flow cytometry three months after auto-SCT (x 10^9/L)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CD3^+</td>
<td>1.1 (0.9 – 1.6); 4</td>
<td>1.2 (0.2 – 2.6); 27</td>
<td>NS</td>
</tr>
<tr>
<td>CD3^+CD4^+</td>
<td>0.4 (0.3 – 0.8); 4</td>
<td>0.3 (0.1 – 0.5); 27</td>
<td>0.033</td>
</tr>
<tr>
<td>CD3^+CD8^+</td>
<td>0.8 (0.5 – 0.9); 4</td>
<td>1.0 (0.2 – 2.1); 27</td>
<td>NS</td>
</tr>
<tr>
<td>NK</td>
<td>0.2 (0.2 – 0.3); 4</td>
<td>0.2 (0.1 – 0.5); 27</td>
<td>NS</td>
</tr>
<tr>
<td>CD19^+</td>
<td>0.1 (0.1 – 0.1); 4</td>
<td>0.1 (0.0-0.4); 27</td>
<td>NS</td>
</tr>
<tr>
<td>CD4/CD8 - ratio</td>
<td>0.6 (0.0 – 0.9); 4</td>
<td>0.3 (0.0 – 1.1); 27</td>
<td>NS</td>
</tr>
<tr>
<td>Blood flow cytometry six months after auto-SCT (x 10^9/L)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CD3^+</td>
<td>1.0 (0.4 – 1.8); 22</td>
<td>1.0 (0.5 – 1.3); 5</td>
<td>NS</td>
</tr>
<tr>
<td>CD3^+CD4^+</td>
<td>0.3 (0.2 – 0.5); 22</td>
<td>0.4 (0.1 – 0.7); 5</td>
<td>NS</td>
</tr>
<tr>
<td>CD3^+CD8^+</td>
<td>0.6 (0.2 – 1.4); 22</td>
<td>0.6 (0.4 – 0.7); 5</td>
<td>NS</td>
</tr>
<tr>
<td>NK</td>
<td>0.2 (0.1 – 0.8); 22</td>
<td>0.3 (0.3 – 0.4); 5</td>
<td>NS</td>
</tr>
<tr>
<td>CD19^+</td>
<td>0.1 (0.0 – 0.5); 22</td>
<td>0.1 (0.0 – 0.1); 5</td>
<td>NS</td>
</tr>
<tr>
<td>CD4/CD8 - ratio</td>
<td>0.4 (0.0 – 1.3); 22</td>
<td>0.5 (0.0 – 1.1); 5</td>
<td>NS</td>
</tr>
</tbody>
</table>

Reference values of blood flow cytometry reported by the Laboratory of Eastern Finland: Blood (B)-T-CD3 0.85–2.28 x10^9/L, B-CD19 0.12–0.43 x10^9 /L, B-T-CD4 0.458–1.406 x10^9/L, B-T-CD8 0.24–0.98 x10^9/L, B-NK 0.08–0.57x10^9/L; p-values 0.05 or more are designated as NS. n = number of observations.
Figure 6. Progression-free survival of the myeloma patients according to the use of plerixafor.

Figure 7. Overall survival of the myeloma patients according to the use of plerixafor.
6 Discussion

The purpose of this study was to prospectively evaluate the effects of various mobilization methods on the blood graft cellular composition, post-transplant recovery and outcome in patients with NHL and MM undergoing auto-SCT. In studies I and IV the effects of plerixafor given due to poor mobilization of CD34+ cells were analysed in NHL and MM patients, respectively. In study II the clinical importance of early post-transplant immune recovery in NHL patients was evaluated and factors affecting the recovery were investigated. The third study was based on the randomized multicenter mobilization substudy of the MM-02 study by the Finnish Myeloma Group and the aim was to investigate the aforementioned matters after RVD induction and mobilization either with CY plus G-CSF or G-CSF alone in MM patients. In all studies data on the cellular composition of the blood grafts after cryopreservation and hematological recovery after auto-SCT were available.

The main findings were that the addition of plerixafor to chemomobilization in NHL patients and chemomobilization or G-CSF mobilization in MM patients has a drastic effect on the graft cellular composition. In MM patients mobilized with G-CSF alone the changes are less pronounced and without statistical significance in comparison to patients mobilized without plerixafor. Further, in MM patients also a mobilization with G-CSF alone alters the graft composition and seems to spare especially the lymphocytes in the grafts compared to mobilization with CY plus G-CSF. Furthermore, according to these results the use of plerixafor may in NHL patients slightly speed up the immune recovery but also slow down the hematological recovery. The immune recovery of MM patients mobilized with G-CSF alone was slightly faster than in patients mobilized with CY plus G-CSF. In MM patients the use of plerixafor also seemed to enhance the recovery of CD3+CD4+ lymphocytes after auto-SCT. In NHL patients ALC-15 ≥ 0.5 x 10^9/L was associated with improved OS. A higher number CD34+ and CD34+CD133+CD38 cells in the grafts and the use of plerixafor for poor mobilization were associated with increased probability of ALC-15 ≥ 0.5 x 10^9/L in NHL patients.

6.1 STUDY DESIGN AND PATIENTS

All NHL and MM patients included in the four studies also participated the prospective GOA study. The majority of the patients were recruited at KUH and may be considered to represent a real-life cohort because during the enrollment period for the GOA study only a few patients treated with auto-SCT at KUH were not included in the study. Also in OUH the recruited patients represented the majority of all NHL and MM patients intended for auto-SCT during the course of the study enrollment. The total number of patients recruited at TUH and TYKS was small and most probably represent more of a random sample of all patients undergoing auto-SCT in these centers. However, the proportion of these patients (TUH, TYKS) relative to the entire patient cohort of the GOA study during the enrollment period is small. An obvious drawback of this kind of multicenter study is the variation in local circumstances outside the main study centers, easily leading to recruitment difficulties and thereby fewer patients enrolled to the study.

The collection of the necessary information of the patients in all centers was performed by distributing the study protocol of the GOA study and the information schedule of the necessary blood samples and by using the structured data collection material in all centers. However, as a
disadvantage of a multicenter study, the data collection seemed to be prone to human errors, commonly appearing as missing data.

Another drawback of the study was the somewhat limited number of patients included in all four studies, especially the number of patients mobilized with plerixafor in study IV. There are two main explanations for this: first, as plerixafor was given only to poor mobilizers, especially the number of MM patients remained low, because MM patients tend to generally mobilize better than NHL patients. Second, for meaningful follow-up times, the last possible transplantation day had to be limited for each study, further reducing the number of patients available. However, considering that this is the first prospective study on the effects of mobilization methods on blood graft cellular composition and post-transplant recovery, the sample sizes can be considered reasonable.

6.2 CLINICAL AND LABORATORY METHODS

As the GOA study was non-interventional and the patients were mainly recruited at KUH and OUH, the premobilization and mobilization phase and HDT represented well the current standard of care of NHL patients in Finland (studies I and II). However, in studies III and IV the interpretation of the results requires more caution as there were also patients participating in the MM-02 study, where patients received three cycles of RVD induction and were thereafter randomly assigned to mobilization with either CY plus G-CSF or G-CSF alone. RVD induction is not generally applicable in clinical practice outside study protocols in Finland, but it may be more effective treatment than e.g. the commonly used VD or VCD inductions [Cavo et al. 2015, Moreau et al. 2016, Chakraborty et al. 2017, Durie et al. 2017]. This might have some effect on the pre- and post-transplant responses and outcome of the patients participating in the MM-02 study, compared to those treated with more conventional regimens. However, in study III all patients received RVD induction and in study IV around 50% of the patients received RVD induction, but the distribution of the various induction regimens was comparable between the groups compared. The possible effects of the various induction therapies on the graft composition in these MM patients remain unclear because the limited sizes of the patient cohorts did not allow meaningful statistical analysis.

The choice of the mobilization regimen in MM patients was based either on the randomization in the MM-02 study or was decided by the physician in charge of the patients’ treatments. In study III the groups were due to randomization well balanced regarding the mobilization regimens. In study IV a significantly higher proportion of patients in the plerixafor group received mobilization with G-CSF alone. This might somewhat distort the results of the graft composition analysis because according to the results from study III, the mobilization with G-CSF alone instead of CY plus G-CSF yielded significantly more T and B lymphocytes and NK cells in the grafts. Similar results on the graft composition after chemomobilization vs. mobilization with G-CSF alone have been recently reported [Worel et al. 2016].

In the GOA study, there was no algorithm for the use of plerixafor in case of poor mobilization. In MM-02 study plerixafor was administered if B-CD34+ was < 10 x 10^6/L and B-WBC was at least 10 x 10^6/L in Arm A or B-CD34+ cell count was < 10 x 10^6/L on day 5 in Arm B. Also, if the yield of the first apheresis was < 1 x 10^6/kg CD34+ cells, plerixafor was given. In practice, the thresholds for plerixafor use in NHL patients in the GOA study were roughly the same as used in MM-02 in arm A. In general, the results with plerixafor-mobilized patients should be considered bearing in mind that the patients receiving plerixafor were de facto poor mobilizers and therefore might possess some patient or disease-related factors, possibly altering the pre- and post-transplantation phase as such.

As HDT, all MM patients received MEL 200mg/m² and around 90% of NHL patients received BEAM. All NHL patients received G-CSF after the graft infusion, as did the MM patients if they
participated the MM-02 study and had less than 3.0 x 10⁶/kg CD34⁺ cells in their grafts. For other MM patients, there was no general algorithm of post-transplant G-CSF use, but altogether 73 % of MM patients received G-CSF post-transplant. This may in part explain the slower neutrophil engraftment in MM patients than in NHL patients.

All laboratory methods used were standardized and comparable. The laboratories used in the study also participated in regular quality controls. All analyses of the grafts were performed with the same flow cytometer, with an identical gating protocol and by the same experienced cytometrist.

The graft samples represented all collected apheresis products from all patients. A few patients from OUH were excluded from the study because their samples were lost. The transportation of the graft samples from the apheresis centers to University of Eastern Finland (UEF) was performed as an over-night transportation using dry ice at around -78°C. The transient increase in the storage temperature during the transportation should not compromise the quality of the graft samples as there are reports on long-term use of high temperature freezers (-80°C) instead of liquid nitrogen storage with good results [McCullough et al. 2010]. However, there are no reports on the effects of storage temperature on other graft components besides the CD34⁺ cells. Due to the prospective study setting, the length of the cryopreservation of the actual grafts before the HDT and the graft samples before the analyses was about the same. Also, as graft samples were analysed approximately every 6-12 months, the length of cryopreservation was comparable among the patients in the various study groups.

6.3 BLOOD GRAFT CELLULAR COMPOSITION, HEMATOLOGICAL AND IMMUNE RECOVERY

6.3.1 NHL patients mobilized with or without plerixafor (study I)
As discussed in the previous chapters, plerixafor has been shown to be an effective mobilizer of CD34⁺ cells in combination with G-CSF or chemotherapy plus G-CSF in patients with NHL. However, data on the long-term hematological and immune recovery has been limited. Also, there have only been some small retrospective analyses on the blood graft cellular composition of plerixafor-mobilized NHL patients.

In study I, the blood grafts of the plerixafor-mobilized patients had significantly less CD34⁺ cells but the proportion of the more primitive CD34⁺CD133⁺CD38⁻ cells from all CD34⁺ cells was significantly higher than in the control group. Similar findings have been previously reported [Varmavuo et al. 2012b]. Except for the slower platelet engraftment in the plerixafor group, there were no differences in the course of hematological recovery according to the plerixafor use. Congruent results for the effect of plerixafor on engraftment and hematological recovery have been previously presented [Varmavuo et al. 2014, Girbl et al. 2014] even though in another study a delay in the platelet and neutrophil recovery was observed [Alexander et al. 2011]. The roughly comparable post-transplant recovery of the plerixafor-treated patients may be considered promising because the patients in the plerixafor group were deemed poor mobilizers, a characteristic which itself might negatively influence the hematological recovery due to lower CD34⁺ cell counts in the grafts.

Besides the CD34⁺ cells, there were other differences in the graft composition. The numbers of T lymphocytes and NK cells was significantly higher in the plerixafor-mobilized grafts. These results echoed the ones reported from a retrospective setting in chemomobilized NHL patients [Varmavuo et al. 2012a, Varmavuo et al. 2014].

The total number of lymphocytes, the number of CD3⁺CD4⁺ and CD3⁺CD8⁺ lymphocytes as well as the number of NK cells in the grafts correlated positively with the pace of the recovery of T cell subsets, NK cells and ALC-30 counts post-transplant and also, except for the NK cells, with ALC-15 counts. These results are of significant interest for several reasons. For example,
the number of infused lymphocytes has been denoted as a prognostic factor after auto-SCT in lymphoma patients [Porrata et al. 2004b, Katipamula et al. 2006]. Moreover, higher number of NK cells in the grafts has been reported to associate with faster ALC recovery after auto-SCT [Holtan et al. 2007, Porrata et al. 2003] and the importance of higher ALC-15 in NHL patients has also been reported to associate with improved outcome [Porrata et al. 2001a, Joao et al. 2006, Porrata et al. 2008].

The NK cell recovery was significantly faster in the plerixafor group at one month after auto-SCT. Otherwise there were no differences between the groups in the pace of immunological recovery. As NK cells have been denoted to be the most important lymphocyte subset of the immune recovery in regard to survival after auto-SCT [Porrata et al. 2008, Porrata et al. 2010], the difference observed in the NK cell recovery may be of special interest and should be analysed in a larger set of patients and also at an earlier timepoint – for example at day+15 post-transplant – than we did in this study.

An enhanced immune recovery after auto-SCT has been associated with improved outcome in some studies [Porrata et al. 2001a, Gordan et al. 2003b, Katipamula et al. 2006] and usually the threshold of ALC-15 ≥ 0.5x10^9/l has been used. In study I that lymphocyte threshold for day+15 was exceeded (median) only in the plerixafor group, even though there was no statistically significant difference in the ALC-15 counts between the groups.

As the GOA study was non-interventional and several hospitals with different clinical practices were included in study I, the patients received a variety of mobilization regimens. Therefore, even though there was no statistically significant difference between the groups in the use of the various chemotherapies, that might be an aspect potentially distorting the results. Of note, in the present study plerixafor was used only to augment poor mobilization, but it may be anticipated that in standard mobilizers the addition of plerixafor to chemomobilization increases not only the number of collected CD34+ cells, but also the number of collected lymphocytes and various lymphocyte subsets and NK cells, as was the case with NHL patients receiving G-CSF with or without plerixafor [Holtan et al. 2007]. In fact, the only currently available data on the use of plerixafor with chemomobilization in normal mobilizers suggests an increase in the total number of CD34+ cells in comparison to chemomobilization alone [Dugan et al. 2010]. There are no data available on various lymphocyte subset in grafts collected from patients who mobilize adequately with chemotherapy plus G-CSF, but still receive plerixafor.

6.3.2 MM patients mobilized with G-CSF with or without CY (study III)

In study III the blood graft composition and hematological and immune recovery of MM patients receiving mobilization with either CY plus G-CSF or G-CSF alone after three cycles of RVD induction were compared. The patients participated in the MM-02 study by the Finnish Myeloma Group and were randomly assigned to either of the mobilization arms.

Following chemomobilization more CD34+ cells were collected, and also after cryopreservation there were significantly more CD34+ cells in the chemomobilized grafts than in grafts mobilized with G-CSF alone. There was no difference in the absolute number of CD34+CD133+CD38+ cells in the grafts, but the proportion of these cells from all CD34+ cells was significantly higher in the grafts mobilized with G-CSF alone. This difference might explain the comparable engraftment kinetics between the groups, even though the median amount of infused CD34+ cells was lower in patients mobilized with G-CSF alone: the more primitive CD34+CD38+ cells in the autologous grafts have been proposed to positively affect the post-transplant engraftment [Hénon et al. 1998]. Probably due to the use of CY, the chemomobilized grafts had significantly lower NK cell and lymphocyte counts.

NK cell recovery was notably slower in the chemomobilized patients at three and six months post-transplant, and also at one month post-transplant there was a borderline (although not statistically significant) difference. These finding may be caused by the previously mentioned,
cytotoxic effect of CY on the lymphocyte subsets in the grafts. Whether the number of NK cells or other graft components truly affect the pace of immune recovery in MM patients is unclear and warrants further studies. Means to enhance the NK cell recovery might be beneficial as NK cells have been proposed to possess antymyeloma effects [Davies et al. 2001, El-Sherbiny et al. 2007] and could thereby aid the post-transplant disease control. In fact, the post-transplant level of NK cells above 0.1 x 10^9/L in blood at one month has been associated with improved PFS in MM patients [Rueff et al. 2014].

There may also be other important aspects in the immune recovery. In a recent analysis on the immune profile of MM patients in long-term remission [Arteche-Lopez et al. 2017], there seemed to be distinct differences in the profile of these MM patients in comparison to healthy controls: a lower percentage of CD4+ lymphocytes and an increase in the percentage of CD4- and CD8+ T effector memory cells. It would be intriguing to find out whether such differences exist also in non-relapsing vs. relapsing MM patients in the long run, and also if there are other differences in the immune profile in regard to the absolute numbers of the cells or in their expression profile.

In study III, the pace of CD3+CD8+ recovery was faster in CY plus G-CSF mobilized patients at three months after the auto-SCT. The role of CD8+ T cells in long-term control of MM might be of importance because as mentioned in the previous paragraph, an increased number of CD8+ cells was found in MM patients with long remission compared to healthy controls [Arteche-Lopez et al. 2017]. Nonetheless, these observations should be repeated in sole MM patient populations and probably more information of the immune profile should be gathered as the immune landscape in MM seems to be rather complex [Dosani et al. 2015].

As reported in a study comparing chemomobilization with G-CSF mobilization [Desikan et al. 1998], the hematological recovery was roughly comparable between the groups. ALC-15 was significantly higher in the G-CSF group, probably due to the higher number of infused lymphocytes [Hiwase et al. 2008a], CD3+CD8+ lymphocytes [Atta et al. 2009] and NK cells [Porrata et al. 2003], which all have been associated with improved lymphocyte recovery after auto-SCT. The importance of ALC-15 after auto-SCT has been reported in many malignancies, especially in NHL, where higher ALC-15 has been associated with improved PFS and even OS [Porrata et al. 2001a, Joao et al. 2006, Porrata et al. 2008]. Also in study II ALC-15 ≥ 0.5 x 10^9/L was a prognostic marker in NHL patients. In patients with MM there are fewer data on the effects of ALC-15 but some results supporting its importance have been presented [Porrata et al. 2001a, Kim et al. 2006]. More prospective studies are needed to evaluate the importance of the immune recovery in patients with MM. In study III the median follow-up was too short for meaningful analysis of PFS after auto-SCT, especially because lenalidomide maintenance therapy was used in all patients.

### 6.3.3 Patients with MM mobilized with or without plerixafor (study IV)

The use of plerixafor in patients with MM has been reported to be safe and effective in both first-line mobilization and re-mobilization [Calandra et al. 2008, DiPersio et al. 2009b, Sahin and Demirer 2017]. The engraftment and post-transplant hematological recovery has also been regarded comparable to patients mobilized without plerixafor [DiPersio et al. 2009b, Alexander et al. 2011, Worel et al. 2011, Micalef et al. 2013, Shaughnessy et al. 2013, Russell et al. 2013]. In study IV, the effects of plerixafor on the graft composition and immune recovery were analysed for the first time in a prospective setting in myeloma patients.

The addition of plerixafor to mobilization with CY plus G-CSF vs. G-CSF alone resulted in significantly higher numbers of T and B lymphocytes and NK cells in the collected grafts. Congruent results have been reported in a smaller retrospective study, where the number CD19+ and NK cells was higher in the plerixafor-mobilized grafts [Varmavuo et al. 2013]. However, in study IV the mobilization regimens were unequally distributed as more patients in the plerixafor group received mobilization with G-CSF alone. This may have affected the
results, because as in study III the grafts of MM patients mobilized with G-CSF alone contained significantly more T and B lymphocytes and NK cells than grafts mobilized with CY plus G-CSF. Furthermore, in another study the apheresis products obtained from mobilization with G-CSF plus plerixafor contained significantly more B lymphocytes and NK cells than apheresis products following chemomobilization plus plerixafor [Worel et al. 2016]. Therefore, a further analysis of patients mobilized with G-CSF alone was performed according to the use of plerixafor. The G-CSF plus plerixafor-mobilized grafts contained more CD34+ cells, even though the difference was not statistically significant. The absolute T lymphocyte (except for CD3'CD8' cells) and NK cell counts were also higher, but the differences were not statistically significant. The only statistically significant difference was the higher number of CD3'CD4' cells in the grafts of the G-CSF plus plerixafor group, but only in the grafts used following HDT.

Based on the highest median number of CD34+ cells in the apheresis products as the benchmark of mobilization efficacy, the combination of CY plus G-CSF and G-CSF plus plerixafor seemed to be the most effective of the various mobilization methods studied. However, considering the other graft constituents, the cytotoxic effect of CY was notable and the total number of CD3' T lymphocytes was lowest in the group constituting mainly of patients receiving CY plus G-CSF. In fact, the highest median number of CD3' T lymphocytes and NK cells was observed in the patients receiving G-CSF plus plerixafor. In MM patients there are no previous graft comparisons of G-CSF vs. G-CSF plus plerixafor available, but in NHL patients the T and NK cell counts have also been reported to be higher in a similar comparison with standard mobilizers [Holtan et al. 2007]. Hence, it seems that in MM patients the effect of plerixafor on the graft cellular composition might be present regardless of the mobilization regimen used and is especially notable in regard to other graft components besides the CD34+ cells.

The neutrophil engraftment was slightly slower, and at d+15 platelet counts were somewhat lower in the plerixafor group. A similar delay in platelet and neutrophil recovery has been reported earlier in plerixafor-mobilized MM patients [Alexander et al. 2011], but in most studies the engraftment and hematological recovery have been comparable in patients receiving plerixafor as part of their mobilization regimen [DiPersio et al. 2009b, Tricot et al. 2010, Worel et al. 2011, Micallef et al. 2013, Shaughnessy et al. 2013, Russell et al. 2013]. Interestingly, also in study I with NHL patients the platelet and neutrophil recovery was in a similar manner slower in the plerixafor group. Therefore, the differences observed in the pace of hematological recovery might also be a result of some patient-related factors rather than, for example, due to differences in the graft composition. As support for that hypothesis, in study IV the number of infused CD34+ (unlike in study I) and CD34'CD133'CD38' cells was comparable between the groups and, in fact, the proportion of the CD34'CD133'CD38' cells was higher in the plerixafor group. According to previous studies, these graft components should actually augment the hematological recovery [Zubair et al. 2006]. Therefore, the observed slower hematological recovery was probably due to the characteristics of the plerixafor-mobilized patients who were – unlike in some earlier plerixafor studies – poor mobilizers. It may be speculated whether the pace of hematological recovery was actually slightly improved by the use of plerixafor or by some of the alterations in the graft composition.

The pace of the immune recovery was comparable between the groups except for the CD3'CD4' cells, which recovered significantly faster during the first three months post-transplant in the plerixafor group. Whether this is due to the higher total number of T lymphocytes or some of their subsets in the plerixafor-mobilized grafts is unclear. Previously, the higher lymphocyte counts in the grafts have been associated with more rapid lymphocyte recovery after auto-SCT [Porrata et al. 2004a, Hiwase et al. 2008a]. In study IV, however, the total lymphocyte recovery was comparable at all timepoints even though there were more lymphocytes in the infused, plerixafor-mobilized grafts. The observed improvements in CD3'CD4' lymphocyte recovery might therefore be for example due to a more profound
mobilization of this specific lymphocyte subset. On the other hand, the bone marrow of patients with various plasma cell dyscrasias has previously been reported to possess higher numbers and functionally altered sets of various T lymphocytes [Pérez-Andres et al. 2006], a factor potentially affecting also the mobilization, collection and recovery phase. The role of CD3+CD4+ lymphocytes in myeloma is currently unclear. For example, in an analysis of the immune profile of MM patients in long-term complete remission lower B-CD4+ lymphocyte counts were observed compared to healthy donors [Arteche-Lopez et al. 2017] and recently a report underlined the cunning interaction, or recruiting, of the T-helper cells and myeloma cells [Wang et al. 2017].

In a previous study [Lee et al. 2012] there was also a correlation with the CD19+ and CD8+ lymphocytes in the autologous blood grafts and blood lymphocyte counts at the time of engraftment. However, in study IV there was no significant difference in the median ALC-15 level between the mobilization groups even though the patients in the plerixafor group received several times more lymphocytes of all studied subsets. As the immune profile of T and B lymphocytes and NK cells after auto-SCT might be important for long-term outcome [Arteche-Lopez et al. 2017], further studies on the characteristics of the immune cells mobilized and collected after plerixafor injection are warranted.

6.4 EARLY IMMUNE RECOVERY AND ITS CLINICAL SIGNIFICANCE IN PATIENTS WITH NHL (II)

Previous reports have underlined the importance of early lymphocyte recovery after auto-SCT in patients with various hematological malignancies, especially NHL [Porrata et al. 2008, Porrata et al. 2001a, Gordan et al. 2003a]. Therefore, the purpose of study II was to investigate the importance of early immune recovery and especially the factors enhancing it in NHL patients.

As in some earlier studies [Porrata et al. 2008, Porrata et al. 2001a, Joao et al. 2006], in study II ALC-15 ≥ 0.5 x 10^9/L independently predicted better OS after auto-SCT in patients with NHL. However, unlike in several previous reports [Porrata et al. 2008, Porrata et al. 2001a, Gordan et al. 2003a, Joao et al. 2006], there was no significant association with the PFS. Because of the limited median follow-up time, the prognostic potential of ALC-15 ≥ 0.5 x 10^9/L was also analysed separately in patients with aggressive lymphoma subtypes. In these patients also the PFS was significantly improved if ALC-15 was ≥ 0.5 x 10^9/L. The exact mechanism for this improvement remains unclear, but the enhanced lymphocyte recovery might promote a better disease control by a putative graft-versus-tumor effect [Porrata 2010]. Immune skewing after HDT might also have some effect, as has been speculated in autoimmune diseases [Muraro et al. 2014]. It is also possible that the better or more vital early immune recovery reflects the intensity of the earlier treatments – a factor itself possibly indicating a less aggressive disease and therefore an indicator of better outcome.

The use of plerixafor has not been previously found to affect post-transplant engraftment or lymphocyte recovery [DiPersio et al. 2009a, Varmavuo et al. 2014]. Interestingly, in study II the median ALC-15 ≥ 0.5 x 10^9/L was observed only in the plerixafor group, and in the multivariate analysis the use of plerixafor was associated with increased probability of ALC-15 ≥ 0.5 x 10^9/L. The use of plerixafor yielded more NK cells in the blood grafts and improved the NK cell recovery, as has been reported previously [Varmavuo et al. 2012b, Varmavuo et al. 2012a] and also in study I. Consequently, because a small study earlier pinpointed the NK cells as the most important lymphocyte subset in ALC-15 [Porrata et al. 2003], an extended follow-up might reveal effects on the prognosis of these patients. Aside the use of plerixafor and some patient related factors, in multivariate analysis the amount of CD34+ and CD34+CD133+CD38- cells in the
grafts were associated with ALC-15 ≥ 0.5 x 10^6/L. Basically this may be considered to be in line with the generally agreed importance of the adequate CD34⁺ cell counts in the grafts for e.g. post-transplant recovery (see 2.4.1.). However, the optimal number of CD34⁺ cells in the grafts still remains unclear and should probably be evaluated in conjunction with the more primitive subsets that have been, as discussed previously, reported to affect the hematological recovery [Hénon et al. 1998, Zubair et al. 2006].

6.5 Outcome in Patients Mobilized With or Without Plerixafor (I, IV)

Most of the studies on plerixafor have concentrated on the mobilization efficacy and short-term hematological recovery in patients with NHL and MM. The effects of plerixafor on outcome after auto-SCT has been less clear. However, recently published results from the long-term follow-up of the patients included in the original plerixafor phase III studies with MM and NHL patients [DiPersio et al. 2009a, DiPersio et al. 2009b] showed no difference in the PFS or OS according to the use of plerixafor with G-CSF compared to mobilization with G-CSF alone [Micallef et al. 2018]. The obvious limitation of that study was the patient selection (not deemed poor mobilizers), which does not depict the real-life usage of plerixafor. Earlier, another study with the same limitation of patient selection and a rather modest patient number, the PFS of G-CSF plus plerixafor mobilized NHL patients was reported better than in patients mobilized with G-CSF alone [Holtan et al. 2007].

Recently the preliminary results of the CALM study organized by the EBMT and required by the EMA to evaluate the outcome of MM and NHL patients receiving plerixafor-mobilized grafts vs. those receiving grafts mobilized with other methods were published [Morris et al. 2018, Sureda et al. 2018]. In both NHL and MM patients the PFS and OS of patients receiving plerixafor to augment poor mobilization were reported comparable to patients mobilizing adequately with other methods. However, even though without statistical significance, the authors speculated on the trend of inferior survival in MM patients receiving plerixafor [Morris et al. 2018].

In our retrospective analysis with poor mobilizers the use of plerixafor in mobilization did not affect outcome compared to mobilization with other methods in NHL patients [Varmavuo et al. 2014]. Recently, similar results were obtained from a study in NHL patients mobilized with chemotherapy plus pegfilgrastim who received plerixafor for poor mobilization [Partanen et al. 2017b]. Furthermore, in contrast to the other studies, in a small study with MM patients, the PFS of patients receiving G-CSF plus plerixafor was inferior to those mobilized with CY plus G-CSF [Garfall et al. 2014].

In the present study with NHL patients (study I), there was no significant difference in the PFS or OS, but there seemed to be a positive trend in the plerixafor group for the PFS. The possible reasons for this notion are not clear, but it may be speculated to be due to the alterations in the graft composition and immune recovery (for example the tempo of early immune recovery). In study IV there were also no significant differences amongst the groups, but especially the OS in plerixafor-mobilized patients needs to be re-evaluated in a larger set of patients and with a longer follow-up.

Collectively, the results from studies I and IV encourage the use of plerixafor in poorly mobilizing NHL and MM patients as it does not seem to be associated with a negative impact on outcome. In fact, the use of plerixafor might even have some positive effects on the outcomes, because the patients’ survival was not inferior compared to normal mobilizers, even though the poor mobilization itself is associated with an inferior prognosis [Gordan et al. 2003b, Moreb et al. 2017]. In addition, the use of plerixafor gives hope for the significant proportion of poorly mobilizing NHL and MM patients who otherwise would have inadequate mobilization
of CD34+ cells to proceed to auto-SCT with its potential benefits. The possible effects of plerixafor on outcome in poor mobilizers will hopefully be clarified by the upcoming final results of the CALM study.
7 Conclusions

The main findings and conclusions of this series of studies were:

1. Use of plerixafor in chemomobilized NHL patients to augment poor mobilization led to a higher proportion of CD34^+133^+CD38^- cells relative to all CD34^+ cells and to a higher total number of T lymphocytes, CD3^+CD4^+, CD3^+CD8^+ and NK cells in the blood grafts. Platelet engraftment was slightly slower and NK cell recovery faster in the plerixafor-mobilized patients. The use of plerixafor did not seem to influence PFS or OS.

2. In NHL patients, ALC-15 ≥ 0.5 x 10^9/L was associated with improved OS and in patients with aggressive NHL subtypes also with improved PFS. In multivariate analysis the number of CD34^+ and CD34^+133^+CD38^- cells in the grafts after cryopreservation and the use of plerixafor for poor mobilization were associated with an increased probability of ALC-15 ≥ 0.5 x 10^9/L.

3. The mobilization of MM patients with CY plus G-CSF yielded significantly more CD34^+ cells in the apheresis products, but the proportions of CD34^+133^+CD38^- cells relative to all CD34^+ cells in the grafts and the absolute number of T and B lymphocytes as well as NK cells in the grafts were lower compared to mobilization with G-CSF alone. The engraftment and hematological recovery were comparable, but the NK cell recovery was faster in patients mobilized with G-CSF alone.

4. The use of plerixafor in MM patients to augment poor mobilization led to a higher proportion of CD34^+133^+CD38^- cells relative to all CD34^+ cells and to a higher total number of T lymphocytes as well as CD3^+CD4^+, CD3^+CD8^+, CD19^+ and NK cells in the grafts. The use of plerixafor did not affect engraftment or hematological recovery, but the recovery of CD3^+CD4^- T cells was faster in plerixafor-mobilized patients. There seemed to be no significant difference in PFS or OS according to the use of plerixafor.
8 Future perspectives

In less than ten years from its introduction to clinical use, plerixafor has become a crucial part of the mobilization strategy for hard-to-mobilize NHL and MM patients. So far, it has been found to be safe in these patient populations in regard to the post-transplant recovery and outcome. In fact, it seems that the use of plerixafor might even reduce the previously reported negative effects of poor mobilization on outcome. So far, the use of plerixafor has been reported to be safe also with a substantial follow-up time [Micallef et al. 2018] and the final results form the CALM study by the EBMT [Morris et al. 2018, Sureda et al. 2018] are just around the corner and will bring even more information on the post-transplant outcome of plerixafor-mobilized patients in real-life usage. So far, the main limitation for the use of plerixafor has been its price, narrowing the use mainly to poor mobilizers. However, the costs might decrease in the future due to the use of improved algorithms [Jantunen et al. 2012, Micallef et al. 2013, Milone et al. 2014], and accessibility may become more widespread.

As the graft composition has been shown to be considerably affected by plerixafor use, the next phase should be to further subclassify the lymphocytes collected to find out if also the number and ratio of their subsets is altered. Moreover, functional and expression profile testing could be carried out as it has already been reported that plerixafor may also modify the functional and expression profiles. The objective could be to gain an even more thorough understanding of the graft constituents that are altered by the method of mobilization and whether these alterations affect the post-transplant recovery and outcome. Furthermore, whether the alterations in the amounts and functionality of various graft composition are of importance in standard mobilizers should also be evaluated.

The importance of immune recovery in NHL patients has been previously reported, but there are currently limited data identifying whether the pace of immune recovery is of importance in MM patients. Also, the factors altering the course of immune recovery in MM patients are not known. Furthermore, in earlier studies the NK cells have been reported to be of importance in MM [Davies et al. 2001, El-Sherbiny et al. 2007] and the NK cells may be of significant interest in the era of immunomodulatory drugs commonly used in both pre- and post-transplant phase [Dosani et al. 2015, Balasa et al. 2015]. Hence, whether the NK cell recovery could be altered (as seems to be the case in NHL patients) and thereby the effects of immunotherapy possibly enhanced, should be evaluated. Also, other aspects of the immunological profile of MM patients should be more thoroughly studied, because, for example, the previously reported MM cell interactions with T-cells in the bone marrow [Wang et al. 2017] may be of the utmost importance in the post-transplant disease control.

To date, the analyses of the blood grafts have been performed by the best available knowledge of the blood cell maturation process. However, in recent years a novel method to analyse the primitive subsets of CD34+ cells has been proposed. In fact, a new model of human hematopoiesis underlining the importance of cell surface marker CD45RA has been proposed [Görgens et al. 2013a, Dmytrus et al. 2016]. Especially if this method of classifying the primitive cells is validated by other study groups, the type and number of primitive CD34+ cells in the grafts after various mobilization methods could be re-evaluated by the novel methods.

Finally, in the future the ultimate objective should be the understanding of the optimal strategy for the induction treatments and mobilization methods to harvest the ideal amounts and ratios of different cell types which, in turn, possess the best functional capacities for proper post-transplant recovery, a fertile ground for consolidation therapies and optimal circumstances for long-term outcome. The next phase in the autograft engineering should be to evaluate if
there are optimal thresholds for the various graft constituents (CD34+ and NK cells, T lymphocytes) in regard to post-transplant events in NHL and MM patients. Ideally, after obtaining such information on the optimal graft composition in various malignancies, a prospective study with pre-defined collection targets for the most important graft components should be carried out. The mobilization should be accomplished by using the most suitable strategy to tailor an optimal graft. However, the future of autograft engineering may not lie only in the absolute numbers of various cell types, but also in the fine tuning of the cell-to-cell interactions in the bone marrow stroma, lymph organs and other affected tissues as well on the correct triggering and modulation of the immune system.
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Autologous stem cell transplantation (auto-SCT) has an important role in the treatment of multiple myeloma (MM) and in many patients with non-Hodgkin lymphoma (NHL). Various mobilization methods are used to harvest the stem cells from peripheral blood. The effects of the mobilization regimens on the graft cellular composition and the effects of the graft composition on post-transplant recovery and outcome have been unclear. These were the issues addressed in this series of studies performed as a part of the prospective GOA (Graft and Outcome in Autologous transplantation) study.