High-Dose Therapy and Autologous Peripheral Blood Stem Cell Transplantation in Patients with Multiple Myeloma

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Abstract Since its introduction in 1983, high-dose therapy followed by autologous peripheral blood stem cell transplantation is a pillar of the treatment of patients with multiple myeloma. In the last decades, a multitude of clinical trials helped to improve strategies based on high-dose therapy and autologous stem cell transplantation resulting in a continuously prolongation of overall survival of patients. In this chapter we will review the progress, which has been made in order to enhance the mobilisation of autologous stem cells and increase the effectiveness of this treatment.

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T. Moehler and H. Goldschmidt (eds.), *Multiple Myeloma*, Recent Results in Cancer Research 183, DOI: 10.1007/978-3-540-85772-3_10,

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10.1 Introduction

High-dose chemotherapy (HDT) and autologous peripheral blood stem cell transplantation (SCT) has improved response rates and duration of survival in patients with multiple myeloma (MM) (Bensinger 2008). Recent research concentrating on the pathogenesis and the molecular basis of the disease has led to the development of novel agents, which are not only targeting tumor cells but also stromal cells supporting tumor cell growth. Three of these novel agents, thalidomide (Glasmacher et al. 2006), lenalidomide (Dimopoulos et al. 2007; Weber et al. 2007), and bortezomib (Richardson et al. 2005) are remarkably effective in reducing the malignant cell clone. This had led to the incorporation of these novel agents not only in salvage, but also into first-line protocols resulting in an improvement of treatment outcome. In the following, we will review the clinical trials that are based on high-dose therapy and autologous peripheral blood SCT in patients with MM.

10.2 Peripheral Blood Stem Cell Mobilization

10.2.1 The Role of Adhesion Molecules

Whereas in the beginning of HDT and autologous SCT bone marrow was the main source for blood stem cell transplantation, blood-derived stem cell grafts have replaced bone marrow. Before we present the various methods used for the mobilization of peripheral blood stems cells, a few remarks are necessary concerning our current understanding of the bone marrow's stem cell niche as first proposed by Schofield and colleagues (Schofield 1983). This niche is a very special place within the bone marrow microenvironment as it guarantees the lifelong maintenance of the most primitive hematopoietic stem cell (HSC) population. The various components of the niche regulate the finely tuned balance between self-renewal and differentiation along the respective hematopoietic lineages. Apart from osteoblast and endothelial cells, the bone marrow contains a broad variety of different stromal cells, including fibroblasts and adipocytes, which form a highly specialized micro architecture as it is needed for the differentiation of the various hematopoietic progenitor and precursor cells. Apart from the production of hematopoietic growth factors and cytokines by the stromal cells of the bone marrow microenvironment, they mediate adhesive interactions essential for migration, circulation, and proliferation of HSC (Verfaillie et al. 1994; Prosper et al. 1998; Whetton and Graham 1999). The receptor and ligands involved include members of the ß1 and ß2 integrin family, selectins and super immunoglobulin families (Fig. 10.1) (Soligo et al. 1990; Verfaillie et al. 1991; Simmons et al. 1992; Teixido et al. 1992; Liesveld et al. 1993; Kinashi and Springer 1994). Some of their natural ligands are expressed on endothelial cells or other stromal cells. The binding partners may also represent compounds of the extracellular matrix of the bone marrow microenvironment.

In the following, we will present some of the receptors and ligands of the bone marrow microenvironment, which are involved in migration and mobilization of HSC. There is an L-selectin (CD62L) recognizing carbohydrate residue on endothelial cells. This interaction mediates the initial attachment of leukocytes to endothelial cells, a process termed as tethering. L-selectin is highly expressed on circulating $CD34^+$ stem and progenitor cells implying an essential role for homing of stem cells following transplantation (Mohle et al. 1995). The ß1 integrins very late antigen 4 ([VLA-4] CD29/CD49d) and VLA-5 (CD29/CD49e) are heterodimers permitting adhesion of hematopoietic progenitor cells to different

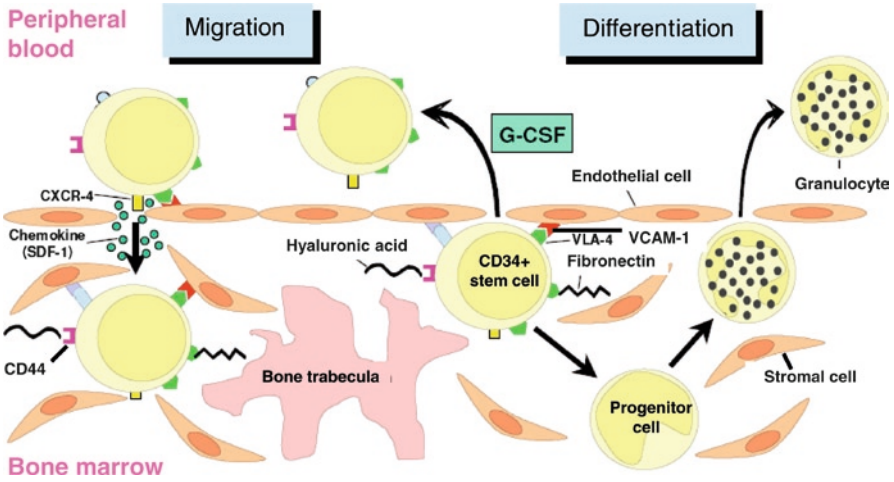

Fig. 10.1 Role of adhesion molecules and their ligands for mobilization of CD34⁺ hematopoietic stem cells

compounds of the bone marrow stroma. In particular, the VLA-4-mediated interaction between hematopoietic stem cells and bone marrow stroma is of functional relevance for hematopoiesis as well as for mobilization and homing of CD34⁺ cells (Prosper et al. 1998; Miyake et al. 1991; Yanai et al. 1994; Hamamura et al. 1996). We observed that circulating CD34⁺ cells express VLA-4 at a lower level in comparison to CD34⁺ cells in the bone marrow. The release of CD34⁺ cells and their migratory capacity is apparently related to the expression level of VLA-4 (Prosper et al. 1998; Mohle et al. 1995; Leavesley et al. 1994; Yamaguchi et al. 1998; Bellucci et al. 1999; Lichterfeld et al. 2000). Looking at circulating CD34⁺ cells from peripheral blood during G-CSF-enhanced marrow recovery in comparison to CD34⁺ cells from steady-state bone marrow we found a significantly reduced functional state of the VLA-4 receptor (Lichterfeld et al. 2000). Moreover, the number of circulating CD34⁺ cells during marrow recovery was inversely related to the activation state and not to the expression level of VLA-4. This observation clearly suggests that the functional state of VLA-4 in circulating CD34⁺ cells is different from that in bone marrow CD34⁺ cells.

Besides VLA-4, the ß2 integrin leukocyte function-associated molecule-1 ([LFA-1], CD18/CD11a) mediates interactions between CD34⁺ hematopoietic progenitor cells and bone marrow stroma. On circulating CD34⁺ cells, LFA-1 had a lower expression level than on bone marrow-derived CD34⁺ cells (Mohle et al. 1995). Functionally, the adhesion to and migration through an endothelial cell layer could be inhibited using LFA-1-directed blocking monoclonal antibodies (Mohle et al. 1995, 1997). There is also a relationship between cell adhesion and signal transduction pathways, which are activated by cytokines (Hughes and Pfaff 1998). Other adhesion molecules relevant in the context of mobilization and homing are the platelet endothelial cell adhesion molecule-1 (PECAM, CD31) and CD44. It is also strongly expressed on CD34⁺ hematopoietic stem and progenitor cells. The ligands of CD44, hyaluronic acid, and osteopontin are components of the stromal microenvironment. Monoclonal antibodies directed against CD44 lower the adhesion of CD34⁺ cells to bone marrow stroma, induce the mobilization of progenitor cells in mice, and prevent hematopoiesis in long-term bone marrow cultures (Miyake et al. 1990; Khaldoyanidi et al. 1996, 1997; Oostendorp et al.

1998; Rosel et al. 1999). Beside growth factors and adhesion molecules, the alpha chemokine CXCL12 also known as stromal-derived factor 1 (SDF-1) plays a relevant role in blood stem cell migration (Aiuti et al. 1997). The cellular receptor of SDF-1 is CXCR-4, which functions as coreceptor for T cell-tropic HIV-1 strains (Bleul et al. 1996). CXCR-4 is expressed in $CD34^+$ cells dependent on the degree of differentiation. The subset of $CD34^+/CD38^{low}$ and $CD34^+/HLA\text{-}DR^{low}$ cells representing a population of more immature progenitor cells stain brightly positive for CXCR-4, whereas a lower level of CXCR-4 expression was observed on the population of $CD34^+/CD38^{bright}$ and $CD34^+/HLA\text{-}DR^{bright}$ cells (Deichmann et al. 1997; Viardot et al. 1998). SDF-1 acts as chemoattractant for hematopoietic stem cells (Aiuti et al. 1997; Mohle et al. 1998; Voermans et al. 1999).

10.2.2 The Role of Hematopoietic Growth Factors

In the vast majority of patients and normal donors hematopoietic growth factors are used for the mobilization of peripheral blood stem cell and progenitor cells. Our first studies addressing the mobilizing ability of hematopoietic growth factors were carried using granulocyte macrophage colony-stimulating factor (GM-CSF). In a group of 11 patients with different types of hematological malignancies and a history of extensive previous cytotoxic chemotherapy, an approximately 18-fold increase of circulating colony-forming units granulocyte macrophage (CFU-GM) in comparison to baseline values was observed (Socinski et al. 1988). An even fivefold greater enhancement was observed when GM-CSF was administered following cytotoxic chemotherapy to increase the natural rebound of circulating $CD34^+$ cell during hematopoietic recovery (Socinski et al. 1988). Looking at the mobilizing capacity of G-CSF in comparison to GM-CSF, no significant difference became apparent between these two growth factors (Winter et al. 1996; Hohaus et al. 1998). In the following studies, peripheral blood stem cell mobilization was performed in the context of a cytotoxic chemotherapy. This kind of peripheral blood stem cell mobilization is generally associated with a lower likelihood of harvesting malignant cells, particularly if the malignancy proved to be chemosensitive (Hohaus et al. 1993; Haas et al. 1992, 1994a, 1997). The advantage of a chemotherapy-based peripheral blood stem cell mobilization is best reflected by the data of a study with an intraindividual comparison (Mohle et al. 1994). In that setting, we observed a sevenfold greater yield of $CD34^+$ cells per leukapheresis after G-CSF-supported chemotherapy compared with steady-state administration of G-CSF at a dose of 5 μg/kg/day.

In order to get a better understanding of the processes underlying peripheral blood mobilization, we and other groups looked for differences between $CD34^+$ cells from bone marrow and peripheral blood during G-CSF-enhanced marrow recovery and found a 3.7-fold greater peak concentration of $CD34^+$ cells in the peripheral blood during G-CSF-supported recovery in comparison with bone marrow samples from steady-state hematopoiesis (Haas et al. 1995). Independent of the method used for peripheral blood stem cell mobilization the vast majority of circulating $CD34^+$ cells were found to be in the G_0/G_1 phase, including a greater proportion of more primitive $CD34^+$ cells. This could be concluded from the results of functional assays enumerating the long-term culture-initiating cells and pre-CFU-GM (Tarella et al. 1995). The functional data were in line with findings made by immunophenotyping demonstrating that a greater proportion of mobilized peripheral blood stem cells expressed the early stem cell-associated antigen Thy-1 in comparison with bone marrow (Fig. 10.2) (Haas et al. 1995).

We addressed this aspect on a molecular level and assessed the gene expression of 1,185 genes in highly enriched bone marrow

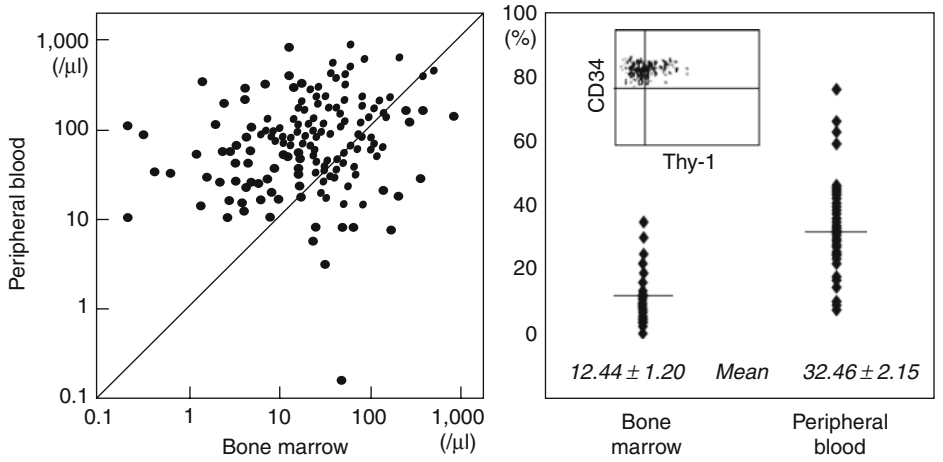

Fig. 10.2 Intraindividual comparison between CD34$^+$ cells from peripheral blood and bone marrow. *Left*: Concentration of CD34$^+$ cells in bone marrow samples before the start of cytotoxic chemotherapy and in peripheral blood (peak level) obtained during cytokine-enhanced marrow recovery. The mean concentration of CD34$^+$ cells was 2.3-fold greater in peripheral blood compared to bone marrow samples. *Right*: Proportion of CD34$^+$/Thy-1$^+$ cells in bone marrow samples from 20 patients before mobilization and 48 leukapheresis products collected during G-CSF-enhanced marrow recovery post-chemothreapy

CD34$^+$ or G-CSF-mobilized peripheral blood CD34$^+$. Using cDNA array technology we found that 65 genes were significantly differentially expressed. These data molecularly confirmed and explained the finding that CD34$^+$ cells residing in the bone marrow cycle more rapidly, whereas circulating CD34$^+$ cells consist of a higher number of quiescent stem and progenitor cells. All together, these results are a strong basis for the preferential use of blood-derived progenitor cells rather than bone marrow for autologous or allogeneic transplantation since the more primitive hematopoietic progenitor cells or even stem cells are particularly relevant for sustained long-term hematopoiesis following myeloablative conditioning therapy (Haas et al. 1995; Dercksen et al. 1995).

The composition of the various CD34$^+$ cell subsets also depends on whether G-CSF is administered during steady-state hematopoiesis or following cytotoxic chemotherapy. In a study including patients with acute leukemia, Hodgkin's disease, non-Hodgkin's lymphoma, or MM, the amount of CD34$^+$ cells collected post-chemotherapy was 5.7-fold greater in comparison with a peripheral blood stem cell harvest obtained during steady state (Haas et al. 1995). In particular, the mean proportion of more primitive CD34$^+$ progenitors lacking HLA-DR or CD38 expression was smaller in patients with peripheral blood stem cell collection following G-CSF-supported chemotherapy than during steady-state mobilization. Considering the greater number of CD34$^+$ cells mobilized in total, the absolute amount of CD34$^+$/HLA-DR$^-$ cells was still 2.3-fold greater post-chemotherapy. On the other hand, the proportion of lineage-committed CD34$^+$/CD33$^+$ cells was significantly enhanced post-chemotherapy in comparison with steady-state mobilization. These data are in line with findings of another group showing that CD34$^+$ cells, mobilized following G-CSF during steady state, contained a

greater proportion of $CD38^-$ cells than $CD34^+$ cells mobilized by other regimens (To et al. 1994).

10.2.3 The Role of Cytotoxic Stem Cell Mobilization

Irrespective of the growth factor used and the particular mode of application, there is always a wide variation in the mobilization efficacy between normal volunteers as well as among patients (Roberts et al. 1995). Individual factors or characteristics associated with peripheral blood stem cell mobilization in patients are essentially the dose of cytotoxic chemotherapy administered for mobilization, the underlying disease and the cumulative amount of previous cytotoxic treatment, as well as previous radiotherapy. For instance, administration of 7 g/m^2 cyclophosphamide in comparison with 4 g/m^2 resulted in significantly greater peak levels of $CD34^+$ cells in the peripheral blood of patients with MM (Goldschmidt et al. 1996). Goldschmidt et al. treated 103 myeloma patients with 7 g/m^2 cyclophosphamide followed by daily 300 μg G-CSF to harvest peripheral blood progenitor cells (Goldschmidt et al. 1997). Peripheral blood stem cell autografts containing >2.0 × 10^6 $CD34^+$ cells per kg body weight were obtained at the first attempt from 90 of 100 evaluable patients. The most significant factor predicting impairment of peripheral blood stem cell collection was the duration of previous melphalan treatment. In multivariate discriminate analysis, treatment with melphalan during the most recent chemotherapy cycles prior to mobilization and previous radiotherapy had a marginally significant negative influence on the efficacy of peripheral blood stem cell collection. The functional capacity of $CD34^+$ cells to restore hematopoiesis after myeloablative treatment was not reduced related to the duration of melphalan exposure. At the time of best response to conventional treatment, a median paraprotein reduction of 21% was achieved following high-dose cyclophosphamide. Two heavily pre-treated patients died, and one patient developed pulmonary toxicity WHO grade IV following high-dose cyclophosphamide. Potential transplant candidates should undergo mobilization and harvesting of PBPC before melphalan-containing treatment. Combinations of hematopoietic growth factors and their dose-modifications should be investigated to improve PBPC collection and to allow a dose reduction of the mobilization chemotherapy. Another study reported on the G-CSF-related mobilization efficiency in 120 patients with MM who received cytotoxic chemotherapy (Martin-Murea et al. 1998). Three schedules of G-CSF administration starting 24 h after the end of chemotherapy were used: (a) a standard dose of 300 μg/day until the completion of peripheral blood stem cell collection, (b) dose escalation from 300 to 600–1,200 μg/day during marrow recovery, (c) 600 or 1,200 μg/day starting 24 h after cytotoxic chemotherapy. As a result, the individual dose per kg bodyweight varied between 2.83 and 23.08 μg. No relationship was found between the dose of G-CSF administered and the peak level of circulating $CD34^+$ cells or the $CD34^+$ cell counts recorded over the entire collection period. In another retrospective study, including 61 patients with lymphoma, we looked for patient-associated factors that may influence the yield of $CD34^+$ cells following G-CSF-supported cytotoxic chemotherapy (Haas et al. 1994a). We found that previous cytotoxic chemotherapy and irradiation adversely affected the yield of $CD34^+$ cells. As consequence, we proposed to harvest peripheral blood stem cells as early as possible during the course of the disease to ensure a yield sufficient to support HDT.

The minimum quantity of $CD34^+$ cells needed for transplantation is generally accepted to lie between 2.5 and 5.0 × 10^6/kg body weight (Hohaus et al. 1993; Haas et al. 1994b; Reiffers et al. 1994). In the context of these analyses, a relationship was found between the number of $CD34^+$ cells transplanted and the time required

for hematological reconstitution. Not surprisingly, patients who received a greater number of $CD34^+$ cells/kg needed shorter recovery times than patients grafted with a smaller number of $CD34^+$ progenitor cells (Hohaus et al. 1993; Bensinger et al. 1994; Weaver et al. 1995; Ketterer et al. 1998). Following the successful experience made with peripheral blood stem cells in the context of autografting, this source of hematopoietic stem and progenitor cells also serves for allogeneic transplantation (Dreger et al. 1994; Bensinger et al. 1995; Korbling et al. 1995; Schmitz et al. 1995).

In the following, we will address the aspect of contamination of the autograft with malignant cells. Gene-marking studies in patients with acute myeloid leukemia and neuroblastoma have shown that malignant cells reinfused along with leukapheresis products may contribute to relapse. Thus, a reduction in the number of malignant cells in autografts is desirable. Cremer et al. analyzed the percentage of malignant cells and the number of $CD34^+$ peripheral blood stem cells in leukapheresis products mobilized by G-CSF alone compared with high-dose cyclophosphamide plus G-CSF in patients with MM (Cremer et al. 1998). A quantitative polymerase chain reaction assay involving CDR3-specific primers based on the method of limiting dilutions was used to determine the tumor loads of leukapheresis products. Sixteen autografts from eight patients with MM were analyzed intraindividually in matched pairs. The percentage of malignant cells was lower in leukapheresis products obtained after cyclophosphamide administration ($p=0.017$; median 0.0067 vs 0.009%), whereas the number of $CD34^+$ cells was higher ($p=0.012$; median 0.3 vs 0.095%). The calculated number of malignant cells per $CD34^+$ cell was significantly lower in leukapheresis products after cytotoxic mobilization as well ($p=0.017$). We conclude that mobilization by cyclophosphamide plus G-CSF leads to a lower number of malignant cells per $CD34^+$ cell in LPs compared with G-CSF alone.

10.2.4 The Role of Pegfilgrastim for Stem Cell Mobilization

Progress in the field of peripheral blood stem cell mobilization has been made by a chemical modification of G-CSF, i.e., the pegylation of filgrastim. Different from the original compound PEG-filgrastim is characterized by a significantly longer half-life because of a substantially reduced renal elimination (Zamboni 2003). In the first study including patients with different types of hematological malignancies, we could demonstrate safety and efficacy of this new compound in mobilizing a sufficient number of $CD34^+$ cells required for at least one autologous transplantation (Steidl et al. 2005). In a subsequent study, pegfilgrastim was given at two different dose levels for PBPC mobilization in patients with stage II or III MM (Bruns et al. 2006). Four days after cytotoxic therapy with cyclophosphamide (4 g/m^2), a single dose of either 6 mg pegfilgrastim or 12 mg pegfilgrastim or daily doses of 8 µg/kg unconjugated G-CSF were administered. Pegfilgrastim was equally potent at 6 and 12 mg with regard to mobilization and yield of $CD34^+$ cells. Pegfilgrastim in either dose was associated with a more rapid white blood count recovery ($p=0.03$) and an earlier performance of the first apheresis procedure ($p<0.05$) in comparison to unconjugated G-CSF. There was no difference regarding $CD34^+$ cell maximum and yield. We therefore concluded that a single dose of 6 mg pegfilgrastim is equally potent as 12 mg for mobilization and harvest of peripheral blood stem cells in patients with MM. In the context of pegfilgrastim, it was interesting to note that the pegfilgrastim-exposed $CD34^+$ cells had a subset composition different from that of filgrastim-mobilized $CD34^+$ cells, i.e., a greater proportion of more primitive $CD34^+$ cells as characterized by the lack of CD38 expression (Fig. 10.3) (Bruns et al. 2008). The different subset composition was accompanied by a significantly different gene expression profile reflecting the

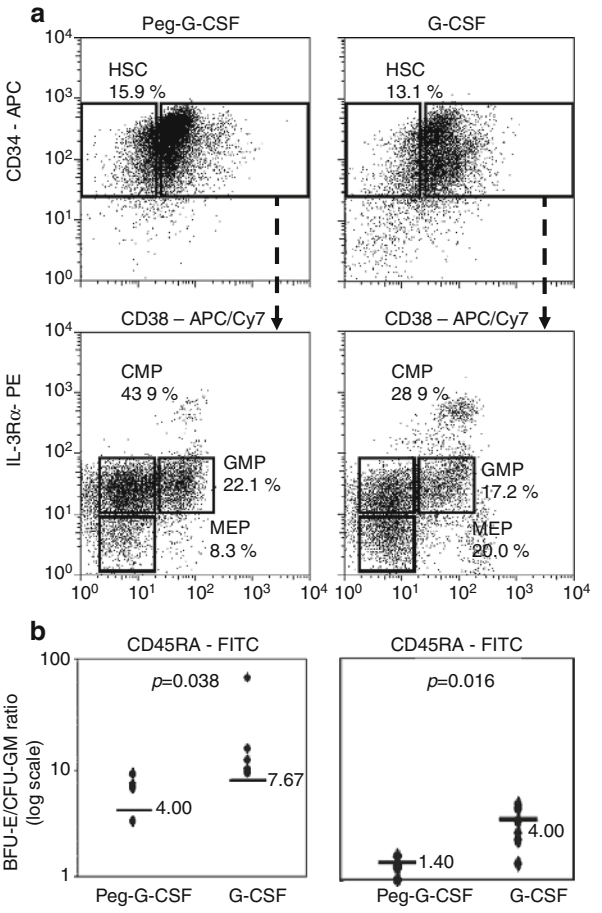

Fig. 10.3 (**a**) Different patterns of hematopoietic stem and progenitor cells in the peripheral blood of patients stimulated with either Peg-G-CSF (*left*) or G-CSF (*right*). Immunomagnetic selection of $CD34^+$ cells followed by multicolor flow cytometry was utilized to analyze hematopoietic stem and progenitor cell subsets. After gating on viable cells and lineage-depletion subfractions of hematopoietic stem cells (Lin^-, $CD34^+$, $CD38^-$), common myeloid progenitors (Lin^-, $CD34^+$, $CD38^+$, IL-3Ra$^+$, $CD45RA^-$), granulocyte monocyte progenitors (Lin^-, $CD34^+$, $CD38^+$, IL-3Ra$^+$, $CD45RA^+$), and megakaryocyte erythrocyte progenitors (Lin^-, $CD34^+$, $CD38^+$, IL-3Ra$^-$, $CD45RA^-$) were determined. (**b**) Clonogenic assays of mononuclear cells (*left*) and purified $CD34^+$ cells (*right*) of patients mobilized by either Peg-G-CSF or G-CSF. Mononuclear cells from apheresis products of patients mobilized with either Peg-G-CSF or G-CSF were seeded in semisolid growth medium containing stem cell factor, GM-CSF, colony-stimulating factor, interleukin-3, interleukin-6, and erythropoietin.

preponderance of a more immature $CD34^+$ cell subset on the level of the transcriptome. For instance, the $CD34^+$ cells mobilized by pegylated G-CSF had higher expression levels of genes indicative of early hematopoiesis, including HOXA9, MEIS1, and GATA3. We found lower expression of genes characteristic of erythroid and later stages of myeloid differentiation and a lower functional burst-forming unit erythroid/colony-forming unit-granulocyte-macrophage ratio. Consistently, greater numbers of hematopoietic stem cells and common myeloid progenitors and fewer megakaryocyte-erthrocyte progenitors were found in the pegylated-G-CSF-mobilized $CD34^+$ cells. Additionally, sorted pegylated-G-CSF-mobilized hematopoietic stem cells displayed higher expression of HOXA9 in comparison to G-CSF-mobilized hematopoietic stem cells. In line with the gene expression data, $CD34^+$ cells mobilized by pegylated G-CSF, as well as sorted hematopoietic stem cells, showed a significantly greater cell cycle activity. Thus, stimulation with pegylated-G-CSF or G-CSF results in different expression of key regulatory genes and different functional properties of mobilized hematopoietic stem cells as well as their progeny, a finding that might be relevant for the application of these cells in blood stem cell transplantation.

This can be concluded from the results of a recent clinical trial in which the authors found significantly greater leukocyte, reticulocyte,

and platelet counts on day 100 after initial engraftment following transplantation of pegfilgrastim-mobilized autografts compared to grafts mobilized by unconjugated G-CSF (Vanstraelen et al. 2006). Of interest, the number of pegfilgrastim mobilized CD34$^+$ cells transplanted was even smaller than the number of G-CSF-mobilized cells ($p = 0.0575$). Hence, it was assumed that different biological functions of pegfilgrastim-mobilized cells may have accounted for these observations (Vanstraelen et al. 2006). Searching for the underlying mechanism that may explain the different transcriptional and functional phenotypes of pegfilgrastim-mobilized cells, it has been previously shown in a murine G-CSF receptor knock-out model that pegfilgrastim and G-CSF exert their pharmacological effects via the same G-CSF receptor (Kotto-Kome et al. 2004). Thus, the different effects of G-CSF and pegfilgrastim are apparently not related to activation of different receptors. Interestingly, in a recent randomized clinical trial, the effect of continuous intravenous administration versus daily single subcutaneous doses of G-CSF on CD34$^+$ cell mobilization was examined (Lee et al. 2005). The authors found that CD34$^+$ cell peak concentrations were reached 2 days earlier following continuous intravenous G-CSF administration compared to daily subcutaneous injections. These findings and the mobilization kinetics observed following the administration of pegfilgrastim suggest that the time-course of stimulation (pulsatile vs. continuous), rather than a dose-related mechanism, might account for the distinct effects of pegfilgrastim and G-CSF on hematopoietic stem and progenitor cells.

10.3 High-Dose Therapy and Autologous Stem Cell Transplantation

10.3.1 The Beginning of High-Dose Therapy in the 1980

Dr. Solly published the first well-documented case of a patient with MM in 1844, and treatment consisted of rhubarb and orange peel (Solly 1844). Dr. Thomas Watson used an alternative treatment 1 year later, who prescribed steel and quinine after application of phlebotomy in a similar patient (Macintyre 1850). It was nearly 100 years later that Blokhin et al. (1958) reported the effective application of melphalan in a small series of patients, and this was the beginning of modern chemotherapy as treatment in patients with MM (Fig. 10.4). Another

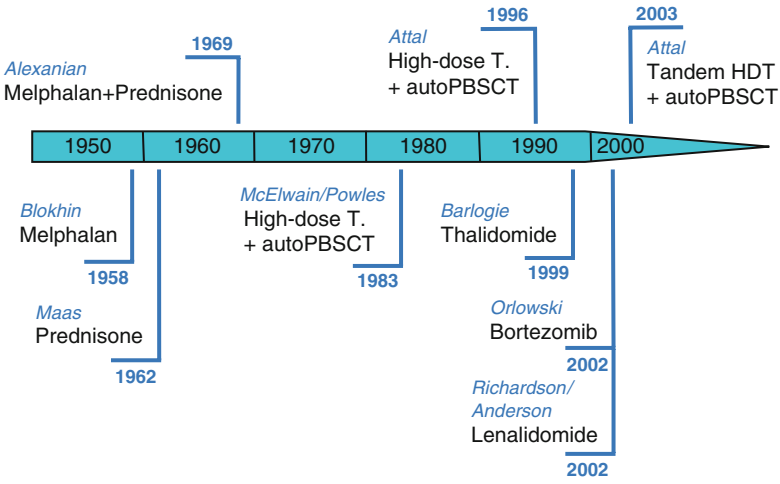

Fig. 10.4 Timeline of the treatment of patients with multiple myeloma

important step was the introduction of steroids (Maas 2008). Taken together, the combination of melphalan and prednisone was established by Alexanian et al. in 1969 (Alexanian et al. 1969). In a randomized trial of 183 patients with MM, a survival benefit of 6 months could be observed with melphalan and prednisone in comparison to melphalan alone. Based on this study, the classic Alexanian protocol became the standard of care for patients with MM for nearly 30 years. Several trials compared different combination chemotherapies to melphalan and prednisone in a randomized fashion, and various combinations were associated with higher response rates or a more rapid induction of remission (Myeloma Trialists' Collaborative Group 1998). Still, no combination could show a survival advantage, and thus melphalan and prednisone remained the gold standard of myeloma therapy for decades. Median overall survival in this time was approximately 3 years.

The change in treatment standards began when Mc Elwain and Powles (1983) reported on a patient with plasma cell leukemia who achieved a complete remission after treatment with 140 mg/m^2 melphalan. In the following, the group from the Royal Marsden Hospital showed a dose-effect of melphalan in patients with MM. While a complete remission could only be observed in 5% of patients following conventional chemotherapy, administration of 140 mg/m^2 melphalan induced complete remissions in 35% of patients (Cunningham et al. 1994). This treatment was associated with prolonged cytopenias, resulting in a high treatment-related mortality. Despite this experience, several other investigators further increased the dose of melphalan and transplanted autologous blood stem cells in order to reduce the toxicity of the procedure. In that respect, Barlogie and coworkers were pioneers, who developed "Total Therapy," an intensive treatment regimen using HDT and autologous PBSCT (Barlogie et al. 1999).

10.3.2 The Role of Purging of the Autograft

MM is characterized by a various degree of peripheral blood and bone marrow involvement with malignant plasma cells. Therefore, hematopoietic stem cell grafts often contain tumor cells. Using qualitative IgH PCR, it has been shown that almost all unselected leukapheresis products contained cells belonging to the myeloma clone (Corradini et al. 1995, 1999; Martinelli et al. 2000). As a consequence, several groups tried to reduce the number of tumor cells in autografts (Vescio et al. 1999; Lemoli et al. 1999; Stewart et al. 2001). The most widely used in vitro purging method is the positive selection of CD34$^+$ progenitor cells using immunomagnetic beads. Still, clone-specific IgH rearrangements were detectable in most CD34$^+$-enriched leukapheresis products (Bird et al. 1994; Abonour et al. 1998; Lemoli et al. 1996; Johnson et al. 1996) as well as in autografts obtained after negative selection of lineage-positive cells, even despite the combined use of positive selection of CD34$^+$ cells and depletion (Lemoli et al. 1999; Barbui et al. 2002; Tricot et al. 1998). The use of quantitative IgH PCR on samples from blood stem cell harvests has provided the means to accurately quantify the success of in vitro purging procedures. A reduction of three log of contaminating myeloma cells has been demonstrated in most studies (Barbui et al. 2002; Schiller et al. 1995; Thunberg et al. 1999), which could be further increased using experimental small-scale CD34$^+$ separation systems (Voena et al. 2002; Cremer et al. 1997). Different in vivo purging strategies also did not result in a complete elimination of clonotypic cells in stem cells harvests. It could be shown that the number of malignant cells was significantly lower in leukapheresis products obtained after cytotoxic mobilization than after steady-state mobilization (Cremer et al. 1998). Repeated courses of mobilization chemotherapy led to a median

reduction of myeloma cells of 0.2 log per cycle (Ladetto et al. 2002). There were no differences in the number of myeloma cells in leukapheresis products obtained at different days during the harvesting period (Ladetto et al. 2002; Zhou et al. 2003; Kiel et al. 1998; Lincz et al. 2001). A median 15-fold higher proportion of tumor cells was found in bone marrow harvests than in peripheral blood leukapheresis products (Ladetto et al. 2002; Vescio et al. 1996), which resulted in equal total clonotypic cell numbers in BM or PB autografts, because of the increased total number of required cells for peripheral blood SCT.

The prognostic value of IgH PCR of stem cell harvests is questionable. In one study, patients, who received leukapheresis products with no evidence of residual myeloma cells as assessed by IgH PCR, were more likely to obtain a CR following transplantation and had a borderline significant longer progression-free and overall survival (Lopez-Perez et al. 2000, 2001). Others could not consequently reproduce this finding (Mitterer et al. 2001; Galimberti et al. 2003). It is conceivable, that the possibility to purge an autograft to PCR negativity is a reflection of a lower tumor burden in vivo and therefore associated with a better prognosis, while the tumor cells infused in patients with PCR positive autografts per se are not of relevance. This is in line with clinical findings showing, that the use of selected autografts did not have a significant benefit regarding the event-free or overall-survival of patients in three randomized multicenter trials (Vescio et al. 1999; Stewart et al. 2001; Lemoli et al. 2000; Bourhis et al. 2007). Moreover, the incidence of infections and delayed engraftment is greater in patients receiving $CD34^+$ selected PB stem cell grafts in comparison to those who autografted using unselected leukapheresis products. Because of these results and the high costs of the selection procedure, today $CD34^+$ cell selection of autografts has been abandoned in case of MM.

10.3.3 The Role of the Conditioning Regimen

The reason, why a reduction of graft contamination by myeloma cells does not improve disease control, most probably is the failure of HDT to eradicate the malignant cells in patients to a level below the number of reinfused tumor cells. Thus, great effort has been undertaken to further improve the conditioning regimen of HDT and autologous SCT.

Melphalan has been given as monotherapy, in combination with total body irradiation (TBI) and in combination with other chemotherapeutic agents. TBI was used in analogue to regimen in patients with leukemia because MM is a radiosensitive malignancy. In a retrospective study, we evaluated the efficacy and toxicity of a high-dose melphalan-based therapy with or without TBI followed by peripheral blood SCT in patients with MM (Goldschmidt et al. 1998). Between June 1992 and June 1996, 104 patients with a median age of 51 years underwent transplantation at the University of Heidelberg. Fifty patients were treated with TBI plus melphalan 140 mg/m^2 while 54 patients received melphalan 200 mg/m^2. Following peripheral blood stem cell autografting, the median time to attainment of platelets $\geq 20 \times 10^9$/L and neutrophils $\geq 0.5 \times 10^9$/L was 11 and 14 days, with no difference between the treatment groups. In the TBI group significantly longer periods of total parenteral nutrition were required due to the occurrence of severe mucositis. Two patients from the TBI group died of transplantation-related complications. Following high-dose treatment, remission state improved in 43 out of 102 patients. No statistically significant advantage in reaching complete or partial remission was observed with TBI and high-dose melphalan compared to the treatment with high-dose melphalan alone. The optimal high-dose treatment, with particular reference to the inclusion or omission of TBI, should be prospectively investigated. These findings were confirmed by a

prospective randomized study (Moreau et al. 2002). Patients randomly assigned to melphalan 200 mg/m^2 in this study had significantly faster hematologic recovery, less transfusion requirements, a lower incidence of severe mucositis, and had to stay a shorter period of time in hospital. While the median duration of event-free survival was similar in both arms, the 4 years estimate for overall survival was significantly better in patients receiving melphalan 200 mg/m^2 with 66% versus 46%. In accordance to this result, the EBMT presented registry data on 2,404 patients with an autologous transplantation for MM showing that patients, who had received preparative regimen without TBI, had a significantly longer overall survival (Bjorkstrand 2001). As a consequence, preparative regimens including TBI are not recommended.

Hypothesizing a positive dose-response relationship several groups tried to further increase the dose of HDT regimen. Several groups obtained dose intensification by a more intensive chemotherapeutic regimen (Fenk et al. 2005a; Anagnostopoulos et al. 2004; Abraham et al. 1999; Martinelli et al. 2003). So, patients with advanced myeloma were included in a pilot study and received idarubicin 60 mg/m^2, melphalan 200 mg/m^2, and cyclophosphamide 120 mg/kg (Heyll et al. 1997). Seven of eight patients in the pilot study achieved a near complete remission, and the toxicity observed appeared to be acceptable. There was no toxic death, but severe mucositis and fever of unknown origin were observed in all patients. However, when this regimen was compared to melphalan 200 mg/m^2 in a randomized trial for previously untreated patients, the rate of near complete remissions was higher with the dose intense regimen with 30% versus 10%, but did not translate in a better event-free or overall survival (Fenk et al. 2005a). Moreover, the intensive regimen was associated with a significantly increased toxicity in terms of severe mucositis followed by infectious complications associated with a treatment-related mortality of 20%. In line with this finding, other studies using different dose-escalated conditioning regimen have also shown an increased mortality without improvement of event- free or overall survival (Anagnostopoulos et al. 2004; Abraham et al. 1999; Martinelli et al. 2003). In the light of these data, the generally accepted high-dose therapy for patients with MM in our days is melphalan 200 mg/m^2.

Currently studies are under way, which combine bortezomib with melphalan as part of the high-dose therapy. Promising results with high CR rates in relapsing and refractory patients have been reported so far (Roussel et al. 2008), but further randomized studies have to confirm this preliminary data in the future.

10.3.4 Supportive Care During High-Dose Chemotherapy

With melphalan 200 mg/m^2 treatment-related mortality of HDT is relatively low and mainly related to hematological toxicity associated with febrile neutropenia and mucositis. The majority of severe infectious complications result from grade IV neutropenia. Without administering hematopoietic growth factors, increased levels of G-CSF have been observed in patients during the early phase of marrow aplasia following HDT and autologous SCT (Haas et al. 1993). During later periods of marrow reconstitution after HDT, the use of recombinant human G-CSF has been shown to accelerate neutrophil engraftment and to decrease the duration of febrile neutropenia, which resulted in a reduced risk of treatment-related infections (Valteau-Couanet et al. 2005; Olivieri et al. 2004).

Pegfilgrastim, a pegylated derivate of filgrastim is characterized by a prolonged plasma half-life in vivo due to decreased renal clearance. It has a similar effect on neutrophil recovery as the usual filgrastim in patients receiving conventional chemotherapy (Holmes et al. 2002; Johnston et al. 2000). Pegfilgrastim is given only once following the end of cytotoxic chemotherapy, which is obviously advantageous and

more convenient for the patient. Pegfilgrastim administration following HDT with autologous transplantation leads to elevated plasma levels of pegfilgrastim, which are inversely related to the number of neutrophils (Fig. 10.5) (Fenk et al. 2006). Pegfilgrastim levels are more than 100-fold higher than physiological G-CSF levels observed after HDT with autologous SCT (Haas et al. 1993) and approximately tenfold higher than G-CSF levels after daily G-CSF application (Piccirillo et al. 1999). The duration of severe neutropenia is 5 days shorter in patients receiving pegfilgrastim compared to those without growth factor. The susceptibility to pegfilgrastim stimulation is reflected by an approximately fivefold rise of the absolute neutrophil count on the day following the administration of pegfilgrastim. Patients showing this kind of response to pegfilgrastim have a particularly short period of neutropenia. Our findings are in line with other groups (Vanstraelen et al. 2006; Staber et al. 2005; Jagasia et al. 2005). In addition, they showed that there is no difference between the daily administrations of G-CSF versus the single injection of pegfilgrastim with regard to the time needed for neutrophil recovery. Despite the shortened duration of neutropenia, the duration of the stay in hospital is not shortened since there is no difference with respect to platelet recovery, infectious complications, mucositis, and the need for parenteral nutrition between the patients receiving pegfilgrastim and those without. The rate and severity of mucositis can be reduced by the addition of human recombinant keratinocyte growth factor, which is a stimulator of the mucosal stem cells (Kobbe et al. 2006).

10.3.5 High-Dose Chemotherapy Is Superior to Conventional Chemotherapy

Since the introduction of HDT up to now, at least 20,000 patients with MM were treated with HDT and autologous SCT according to a European Blood and Marrow Transplant (EBMT) registry study (Bjorkstrand and Gahrton 2007). The first randomized study to demonstrate the superiority of HDT in comparison to conventional chemotherapy was from the French "intergroupe francophone de myelome" (IFM) and included 200 untreated patients, who were younger than 65 years without severe renal impairment (Attal et al. 1996). In this trial, the rate of complete remissions was significantly enhanced from 5% with conventional chemotherapy to 22% with HDT and autologous SCT. The improved response rate translated into a significantly longer median event-free survival of 44 months following HDT versus 18 months in the control arm. There was also a significantly longer median overall survival of 57 versus 44 months. The British Medical Research Council (MRC) (Child et al. 2003) published similar results 7 years later. Therefore, HDT supported by autologous PBSCT became the standard therapy for young patients with multiple myeloma and normal renal function. Later, there were five other studies comparing single HDT and autologous PBSCT with conventional chemotherapy (Fermand et al. 1998, 2005; Palumbo et al. 2004; Blade et al. 2005; Barlogie et al. 2006a). These studies were confirmatory with regard to the two studies from France and the UK. Still there were some concerns because of the lack of a significant survival benefit. Looking at the results of all randomized trials a greater rate of complete remissions in the patients receiving HDT could be observed in six of seven studies (Attal et al. 1996; Child et al. 2003; Fermand et al. 1998, 2005; Palumbo et al. 2004; Blade et al. 2005). In contrast, a longer event-free survival was found in five of seven studies (Attal et al. 1996; Child et al. 2003; Fermand et al. 1998, 2005; Palumbo et al. 2004), while a longer overall survival was noted in three of seven studies (Attal et al. 1996; Child et al. 2003; Palumbo et al. 2004). In a meta-analysis of

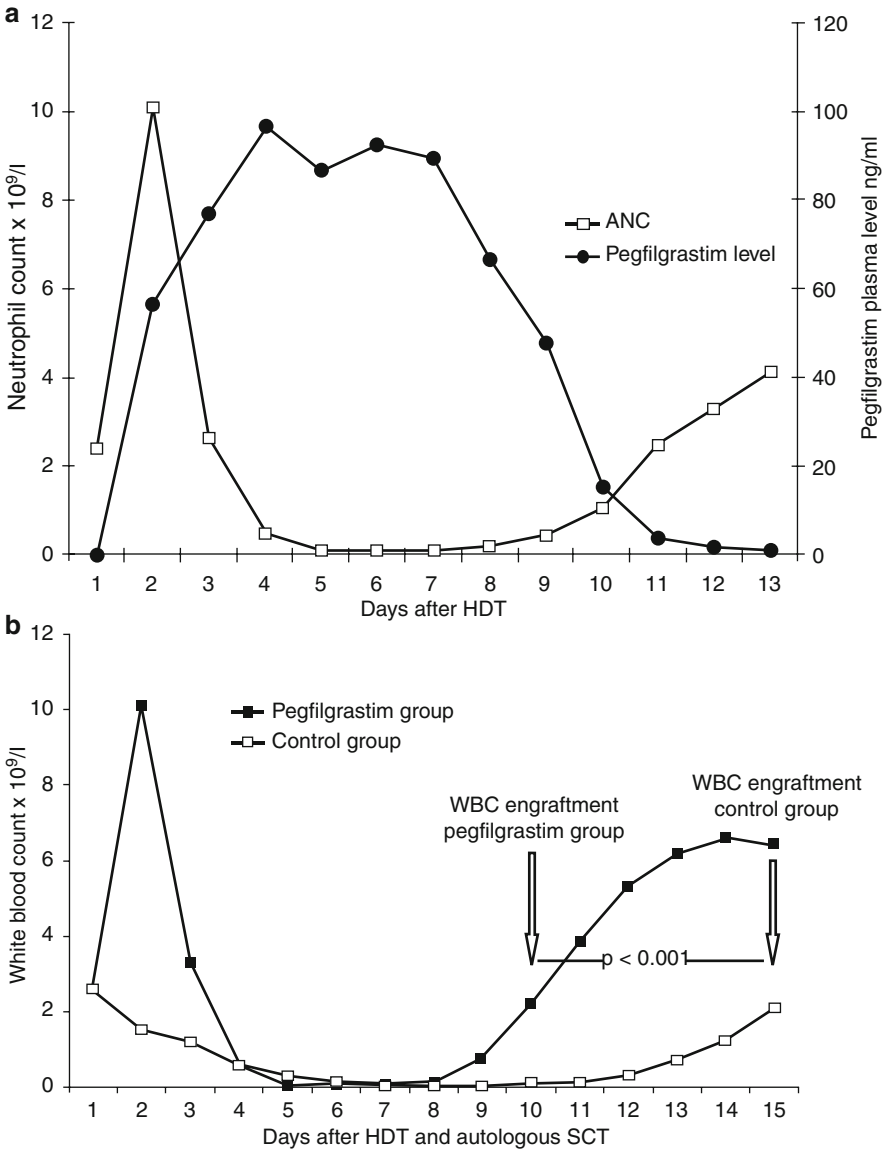

Fig. 10.5 (**a**) Neutrophil counts and pegfilgrastim levels of patients receiving 6 mg pegfilgrastim on day+1 after HDT and autoloogus SCT are shown. (**b**) Comparison of time to leucocyte recovery after HDT and autologous SCT of patients who received pegfilgrastim and a historical control group withour hematopoietic growth factor support

nine HDT trials (Koreth et al. 2007), which also included studies with older patients who had received double intermediate-dosed conditioning regimen, HDT was superior to conventional therapy as far as event-free was concerned but not with regard to survival. The duration of survival is influenced by the use of more or less effective salvage therapies, in particular since the introduction of novel agents (Kumar et al. 2008). Therefore, it is not surprising that HDT followed by autologous SCT remains the therapy of first choice for patients eligible for this treatment procedure.

10.3.6 Timing of High-Dose Chemotherapy

In principle, HDT with autologous PBSCT can be performed frontline as consolidation therapy following induction therapy or as salvage treatment at the time of relapse after a conventional first-line therapy. Two studies have addressed this question in a randomized fashion (Fermand et al. 2005; Barlogie et al. 2006a). Both studies did not observe a difference with regard to overall survival. In the study of Barlogie et al. (2006a), there was also no difference with regard to event-free survival. In contrast, Fermand et al. (2005) observed a significantly longer time of event-free survival in the "early" treatment group of patients with 39 versus 13 months in those receiving HDT following a previous relapse. More important, the time spent without therapy was longer in the "early" treatment group with 28 versus 22 months. In none of the studies, a comparison was made with respect to quality of life. It should be also considered that patients not receiving up-front HDT are usually on therapy for a longer period of time which is associated with a greater risk that organ complications acquired along the conventional therapy hamper a "late" HDT. In addition, there is a higher risk for developing a secondary myelodysplastic syndrome because of the long exposition to low-dose alkylating agents. Therefore, HDT as first-line therapy is recommended, while despite this general recommendation HDT is of therapeutic efficacy at any stage of the disease. In the light of this statement, peripheral blood stem cell collection should be performed following induction therapy, irrespective whether an "early" or "late" HDT is envisaged.

10.3.7 Tandem Autologous Transplantation

The experience with HDT and autologous SCT showed that patients achieving a complete or at least very good partial response had a longer overall survival than patients, who achieved only a partial remission (Lahuerta et al. 2008). In order to accomplish a CR in as many patients as possible dose intensification by means of sequential cycles of HDT and autologous PBSCT was proposed by several investigators. In particular, Barlogie and coworkers at the University of Arkansas (Barlogie et al. 1997) introduced double or tandem HDT as part of the "Total Therapy" program. The French IFM (Attal et al. 2003) was the first group to demonstrate feasibility and superiority of a tandem HDT in comparison to a single HDT in a randomized trial for patients up to the age of 60 years. Of all patients, 75% underwent a second transplantation and the treatment-related mortality was less than 5%. The 7-year event-free and overall survivals were 20% versus 10% and 42% versus 21% in favor for the tandem transplantation. However, the median difference in event-free and overall survival was – despite the statistical significance – only 2 and 10 months, respectively. Moreover, in a subgroup analysis the benefit of a second HDT was restricted to patients who did not achieve at least a very good partial response.

Five other randomized studies also investigated the efficacy of tandem versus single HDT

and autologous PBSCT. In a recently published meta-analysis of all six trials (Kumar et al. 2009), taking into account 1,803 patients a significantly better response rate was obtained following tandem HDT that was associated with a significantly higher treatment-related mortality. As far as event-free and overall survivals were concerned there was no statistically significant difference whether one or two HDT were performed. Excluding one study from the meta-analysis in which single HDT in combination with thalidomide maintenance treatment was compared to tandem HDT without thalidomide resulted in a significant change in the hazard ratio favoring tandem transplantation with respect to EFS. Further, data in this meta-analysis did not permit a subgroup analysis according to the response following the first HDT. In conclusion, the therapeutic benefit of tandem HDT is not entirely clear. As with single HDT the role of tandem HDT has to be readdressed in the light of the availability of new therapeutic compounds.

10.3.8 The Role of Induction Treatment

The novel agents such as thalidomide (Glasmacher et al. 2006), bortezomib (Richardson et al. 2005) and lenalidomide (Dimopoulos et al. 2007; Weber et al. 2007) have shown high efficacy in patients with relapsed or refractory MM. Therefore, several investigators have moved these agents from the relapsed setting into front-line therapy. In the context of HDT and autologous PBSCT all three drugs have been used for induction therapy either in combination with dexamethasone or with conventional chemotherapy or with each other.

Studies using thalidomide in combination with dexamethasone and/or chemotherapy (Rajkumar et al. 2006; Cavo et al. 2005; Macro et al. 2006; Lokhorst et al. 2008) as induction therapy demonstrated higher response rates before HDT. Nevertheless, this early difference was neutralized by HDT, as response rates after HDT were not different anymore. Only a longer follow-up will show whether this minor benefit will translate into a longer time of event-free and overall survival. The incidence of deep-vein thrombosis is higher with thalidomide combinations necessitating the use of prophylactic anticoagulation.

Induction treatment with bortezomib results not only in a significant improvement of remission rates before transplant, difference that persists following HDT. The rate of at least very good partial responses with bortezomib and dexamethasone in the IFM trial in comparison to VAD is also significantly greater with 68% versus 47% (Harousseau et al. 2006). Preliminary data also show a benefit in terms of event-free survival. In addition, the high efficacy in patients with extramedullary disease favors this combination. The problem with an induction treatment including bortezomib is the high incidence of peripheral neuropathy, which develops in 46% of all patients including 7% with WHO grade 3 and 4.

Induction treatment with lenalidomide in combination with dexamethasone induces remissions in the majority of patients. A randomized comparison (Rajkumar et al. 2006) of lenalidomide with low-dose dexamethasone versus lenalidomide with high-dose dexamethasone resulted in a comparable therapeutic efficacy, but the toxicity was markedly reduced in patients receiving low-dose dexamethasone.

A longer follow-up is needed to estimate the therapeutic potential of the new drugs with regard to overall survival of patients. Until then, combinations with lower dosages of novel agents should be examined in order to reduce the toxicity of induction therapy without compromising the efficacy. Taken together, for patients outside of clinical trials, it is a reasonable approach to begin with conventional induction therapy and only change treatment to novel agents in case of

unresponsiveness. This way, undue toxicity can be avoided. For high-risk patients with extramedullary disease or abnormal karyotype novel agents may also be considered as up-front therapy (Laura et al. 2006; Jagannath et al. 2007; Bahlis et al. 2006). In all studies with novel agents, peripheral blood stem cells could be collected for the majority of patients, while their number was reduced in comparison to patients receiving the conventional type of induction therapy. Therefore, a discontinuation of the novel agents is recommended before PBSC mobilization is initiated.

10.3.9 The Role of Consolidation or Maintenance Treatment

Besides induction therapy novel agents were also used after transplantation in order to further reduce residual tumor cells and prolong the duration of disease control. Maintenance therapy with interferon (INF) alpha or corticosteroids is associated with severe constitutional symptoms leading to a reduced quality of life. In addition, INF alpha has only a little effect on the course of the disease, if any effect at all (Myeloma Trialists' Collaborative Group 2001). Thus, with the availability of novel agents INF, alpha and corticosteroids are not used for maintenance therapy anymore.

Introduced by the Arkansas group (Barlogie et al. 2006b), thalidomide was continuously administered at different doses from the start of induction therapy, throughout tandem HDT and following HDT until disease progression. In a randomized comparison with patients not receiving thalidomide, treatment with thalidomide resulted in higher rates of complete remission with 62% versus 44%, while the 5-years survival rate was also superior with 56% versus 44%. Still, overall survival was not different in both treatment arms. After a follow-up time of 72 months, the group of patients with cytogenetic abnormalities showed had a better overall survival when they had received thalidomide (Barlogie et al. 2008).

The use of thalidomide only after HDT seems to be more effective. In a randomized study from the French IFM, 597 patients were randomized between three kind of maintenance therapies after tandem HDT and PBSCT (Attal et al. 2006). Patients received pamidronate, pamidronate plus thalidomide, or nothing. the Patients receiving thalidomide had the longest event-free survival at 3 years with a proportion of 52% versus 36% and an overall survival rate at 4 years of 87% versus 75%. Only patients with chromosome 13 deletion or with achievement of a complete or very good partial remission did not benefit from thalidomide treatment. Other studies (Abdelkefi et al. 2008; Spencer et al. 2006; Fenk et al. 2005b) have confirmed these results. Abdelkefi et al. (2008) showed that a maintenance therapy with thalidomide over a period of 6 months after a single HDT and autologous PBSCT is even superior to a tandem HDT without maintenance therapy.

The major side effect of thalidomide is a severe polyneuropathy forcing approximately 60% of the patients to discontinue the therapy (Barlogie et al. 2006b). This toxicity is very disadvantageous as patients, who are able to tolerate thalidomide for more than 10 months have a statistically significantly longer survival time than patients who had to abandon thalidomide due to adverse events (Lilienfeld-Toal et al. 2007). In addition, there is no consensus about the optimal treatment schedule and dose. Lenalidomide provides a useful alternative, as it is more potent in vitro and less toxic than thalidomide. It is also effective in high-risk patients with chromosome 13 deletions (Bahlis et al. 2006). Therefore, lenalidomide has the potential to improve remission rates, event-free, and overall survival following HDT without relevant toxicity. In principle, bortezomib

may also be considered for maintenance, although a greater risk of developing polyneuropathies has to be envisaged in comparison to lenalidomide.

Another experimental alternative for maintenance therapy after HDT could be an isotype vaccination with dendritic cells (Abdalla et al. 2007; Curti et al. 2007; van Rhee 2007; Bogen et al. 2006). DC based vaccines for patients with malignant diseases generated under different culture conditions have been investigated for more than a decade. Despite these efforts, clinical results of DC vaccination studies showed therapeutic efficacy only in a limited number of patients so far (Nestle et al. 2005). In search of an alternative way for DC generation, we examined the molecular and functional characteristics of dendritic cells generated with interferon and GM-CSF (IFN-DC) and compared the results with dendritic cells generated with the classical protocol using IL-4 and TNF-alpha (IL-4/TNF-DC) (Korthals et al. 2007). We could show that both, IFN-DC and IL-4/TNF-DC, display typical DC characteristics, but also have distinct molecular and functional phenotypes. Our results from gene expression analysis show that IFN-DC have signs of a pronounced maturation state and an increased migratory capacity to the lymph nodes in comparison to IL-4/TNF-DC. Strikingly, IFN-DC showed a more plasmacytoid phenotype associated with NK cell characteristics on a molecular and protein level as well as a functional cytotoxic activity against tumor cells. We found a significant upregulation of 32 genes strongly related to NK cell functions in IFN-DC compared to IL-4/TNF-DC. These include NK cell receptors NKp80, NKp44, NKp46, and NKG2D that are synergizing the cytotoxic activity of NK cells (Bryceson et al. 2006; Moretta et al. 2001), as well as CD56 and cytotoxic effector molecules such as granzymes and TRAIL. Indeed, on protein level, we could detect intracellular pools of TRAIL and granzyme B in IFN-DC. Finally, as a further corroboration of the suggested cytotoxic capacity, IFN-DC, but not IL-4/TNF-DC, was able to kill K562 cells in vitro. These findings are of particular interest, as a new murine DC cell population has been recently described, termed interferon-producing killer dendritic cells (IKDC), that express molecular markers of plasmacytoid DC and NK cells (Chan et al. 2006; Taieb et al. 2006). IKDC exhibit specific cytolytic activity upon contact with tumor cells or activation with CpG oligonucleotides and subsequently upregulate costimulatory molecules, migrate to the lymph nodes and present antigen to T cells. Indeed, nine of the genes specifically expressed by IKDC, including granzymes, NKG2D, NKp46, and CD49b as determined by microarray analysis (Chan et al. 2006), were also differentially expressed by IFN-DC in comparison to IL-4/TNF-DC. Together with the pronounced migratory potential and the cytotoxic capacity of IFN-DC, the similarities between IFN-DC and mouse IKDC suggest that also in humans a molecular and functional relationship exists between DC and NK cells. In conclusion, IFN-DC, which can not only stimulate T cells but also can kill tumor cells by themselves, should be evaluated in clinical vaccination trials (Fig. 10.6).

10.3.10 Prognostic Factors

There are a number of prognostic factors, which can be used at the time of first diagnosis to estimate the risk of relapse. Among those are an advanced age, renal dysfunction, high ß2-microglobulin, low albumin, high CRP or LDH levels, thrombocytopenia, high plasma cell labeling index and most importantly chromosomal abnormalities with t(4;14) as the worst prognostic marker. The usefulness of gene expression studies defining high-risk profiles has to be evaluated prospectively. All these prognostic markers may

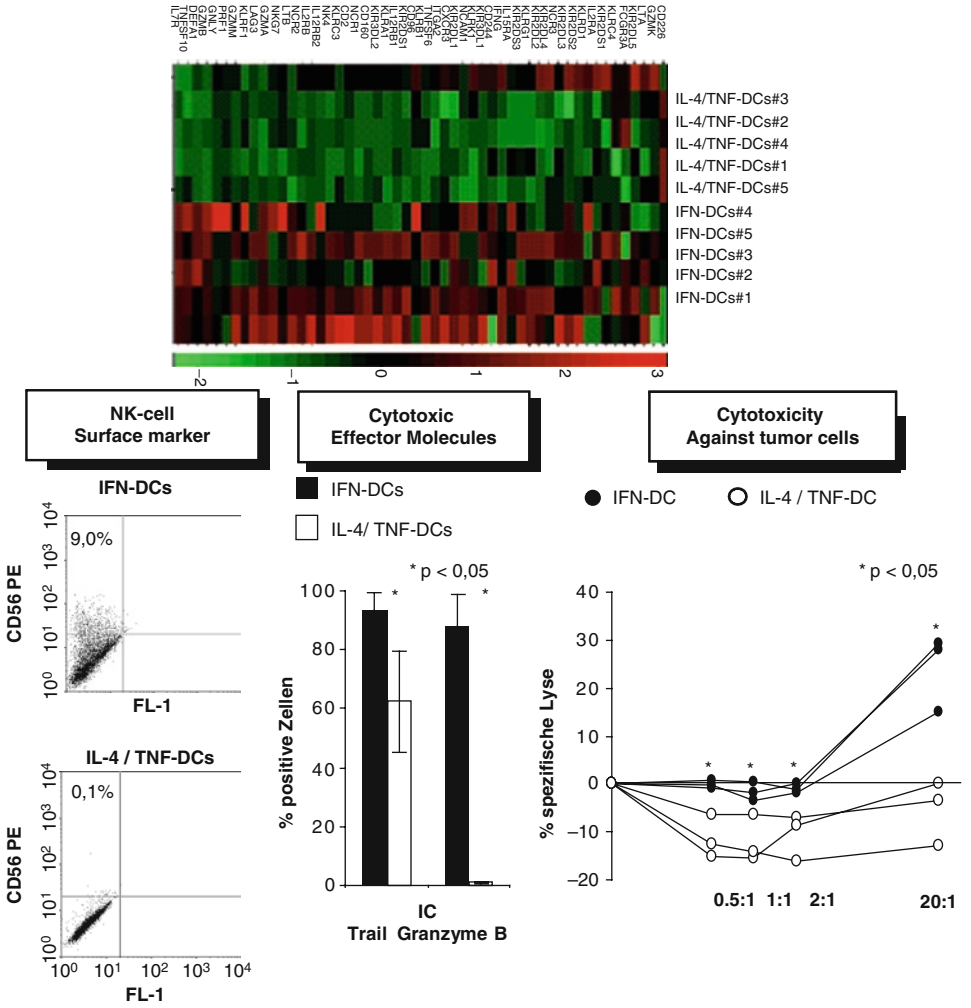

Fig. 10.6 IFN-DC have novel molecular, phenotypical and functional characteristics in comparison to IL-4/TNF-DC. (**a**) Hierarchical cluster analysis of 52 genes related to NK cell function for IFN-DC and IL-4/TNF-DC preparations with expression levels obtained by Affymetrix microarray analysis. (**b**) Expression of NK cell surface markers and (**c**) cytolytic effector molecules by DC as analysed by flow cytometray. CD56 and intracellular expression of TRAIL and granzyme B by IFN-DC and IL-4/TNF-DC. (**d**) Cytolytic activity of DC. Specific lysis of tumor cells by DC was measured by flow cytometric detection of propidium iodide uptake after coculture with K562 cells

be considered for clinical decision making in order to allocate patients to more or less intensive treatment regimen. Assessment of response by conventional diagnostic procedures is a dynamic parameter (Lahuerta et al. 2008). As mentioned above patients achieving at least a very good partial response after the first HDT have no further benefit from a second one (Attal et al. 2003). However, the prognostic implication of complete response is limited. Patients

with a prior history of monoclonal gammopathy of undetermined significance (MGUS) or smoldering MM have the same treatment outcome after HDT as patients achieving a complete response even when they show a clear M-protein spike in the electrophoresis (Pineda-Roman et al. 2007). These patients had "returned"to their prior MGUS stage following eradication of the transformed MM tumor cell population as result of HDT. In this particular group of patients the achievement of CR or failure to reach this aim is not of prognostic relevance. Another group of patients with a disease type resembling a high-grade non-Hodgkins lymphoma may present with high levels of free light chains in the serum. Even if these patients achieve a complete remission, they have a very poor prognosis (van Rhee et al. 2007).

Another possibility to assess response during the course of therapy is the measurement of minimal residual disease (MRD) on a molecular level. This method is more sensitive and specific to detect tumor cells of clonal origin (Fenk et al. 2004a). It provides a quantitative estimate of the risk of relapse and may permit therapeutic decisions, as shown for patients with acute lymphoblastic or chronic myeloid leukemia (Szczepanski et al. 2001). The detection of MRD is of particular relevance, as novel agents are very effective in reducing the number of malignant cells and thus are leading to a higher rate of patients with very good partial and complete remissions. For patients with MM, a molecular remission as shown by qualitative immunoglobulin heavy chain (IgH)-PCR is associated with a better event-free survival after myeloablative allogeneic PBSCT (Corradini et al. 2003). Following HDT and autologous SCT bone marrow, samples of 87% of patients remain PCR-positive (Corradini et al. 1999). Therefore, a quantitative method is necessary. Using a limiting dilution assay for IgH-PCR, Bakkus et al. (2004) identified a threshold level of 0.015% clonotypic cells in bone marrow samples obtained 3 months after HDT and autologous SCT as prognostically relevant for the EFS. Another group used multiparameter flow cytometry with a detection threshold of 10^{-4} (0.01%) which is in the same range and reported similar results. Using real-time quantitative RCR as a third method, we defined a cut-off value of 0.03% clonotypic cells in the bone marrow determined before HDT and autologous PBSCT (Fenk et al. 2004b) as a prognostic marker for the probability of EFS. Patients falling below this threshold after induction and mobilization chemotherapy not only had longer EFS, but also a better OS than patients with values did above this cut-off level. The MRD level was found to be prognostically relevant independent of ISS stage, cytogenetics, and the kind of maintenance therapy. These results imply that induction therapy before HDT with SCT has to be improved at least for those patients with high MRD levels. Therefore, MRD monitoring provides a rationale for a patient-tailored therapy dependent on the individual response to a given treatment.

Another alternative to MRD monitoring may be gene expression profiling after a given drug is administered. Gene expression studies were performed 48 h after a test dose of bortezomib was applied to 142 untreated patients in order to determine whether any MM- or microenvironment-associated changes with prognostic implication could be observed (Shaughnessy et al. 2008). A high-risk score defined by the upregulation of proteasome genes after bortezomib application was associated with an extremely poor survival of less than 24 months and was an independent prognostic parameter in multivariate analysis. This kind of analysis will help us to get a better understanding of the pathophysiology of MM and mechanisms of drug resistance to novel drugs.

10.3.11 Targeted Versus High-Dose Chemotherapy

Novel therapies such as thalidomide, bortezomib, and lenalidomide necessitate redefining the role of HDT and autologous SCT. Further

studies are needed to better understand how to use these agents in conjunction with HDT in patients with multiple myeloma. Some may ask whether in the era of novel agents HDT and autologous blood stem cell transplantation has any role. This opinion is supported by the results of a study comparing tandem HDT with low-dose melphalan and prednisone in combination with thalidomide in elderly patients (Facon et al. 2007). In this study, the combination of conventional chemotherapy with thalidomide was superior with regard to survival. Rather than comparing HDT with novel agents, the therapeutic efficacy of HDT in combination with novel agents should be investigated in order to improve remission rate and ultimately the duration of survival of the patients.

With the availability of a therapy based on a better understanding of the pathophysiology of the disease, we may undergo a transition from the general cytotoxic effect of HDT to an individualized specific effect of a small molecule, antibody, or other biological response modifier aiming at a particular structure within the malignant plasma cell or its precursor. This could be, for instance, the inhibition of pathophysiologically relevant pathways, which govern self-renewal, proliferation, and differentiation of the myeloma cell or protect it from apoptosis. Inhibition of particular pathways, which are known to play a role in MM cell growth, has not been successful so far (Ocio et al. 2008). This may be due to the evolutionary dynamic of human life, which is finding a new way, when one is blocked. More likely, the simultaneous inhibition of different pathways is probably required. One example may be the combined inhibition of the unfolded protein response, which is responsible for the detection and disposal of misfolded proteins. MM cells secrete large amounts of paraprotein, which are correctly folded by the chaperone system. If this process fails, two systems, the proteasome and the aggresome, eliminate the accumulating misfolded proteins. If this process also fails, cell death occurs because of accumulating toxic proteins. Inhibition of the chaperone system is possible with heat-shock protein inhibitors, whereas histone deacetylase inhibitors can inhibit the aggresome. Application of these novel drugs together with the proteasome inhibitor bortezomib has shown very promising results. Whether the inhibition of all three pathways will lead to a sustained clinical effect has to be awaited. As long as there is no effective targeted therapy available, high dose therapy with autologous peripheral blood stem cell transplantation is still the cornerstone of any therapy in combination with thalidomide, lenalidomide, and bortezomib.

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