Intensive Therapy in Multiple Myeloma

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ISBN 90-77017-94-1

Cover and layout by: Christine Segeren

Printed by: Optima Grafische Communicatie, Rotterdam, The Netherlands

Financial support for printing of this thesis by:
Amgen B.V., Breda, The Netherlands
Roche B.V., Mijdrecht, The Netherlands
Novartis Pharma B.V., Arnhem, The Netherlands

Intensive Therapy in Multiple Myeloma

Intensieve behandeling van het multipel myeloom

Proefschrift

ter verkrijging van de graad van doctor aan de
Erasmus Universiteit Rotterdam
op gezag van de
Rector Magnificus

Prof.dr.ir. J.H. van Bemmel

en volgens besluit van het College voor Promoties.

De openbare verdediging zal plaatsvinden op
vrijdag 13 september 2002, om 13.30 uur

door

Christine Maria Segeren

geboren te Geleen

Promotiecommissie

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The work presented in this thesis was financially supported by the Dutch National Health Council (OG 96-020)

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CHAPTER 1

Introduction
To be published as "Chemotherapy for multiple myeloma: from conventional to high-dose treatment"

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European Journal of Cancer, in press

1.1 Multiple myeloma

Multiple myeloma is a malignant plasma cell disorder accounting for 1% of all malignant diseases and about 10% of hematological malignancies. The annual incidence is approximately 0.003% and increases progressively with age. Multiple myeloma is characterized by an abundant clonal proliferation of plasma cells in the bone marrow, which produce a monoclonal heavy and/or light chain immunoglobulin (M-protein), usually IgG or IgA, occasionally IgM, IgD or IgE. This M-protein is present in the serum and/or urine of the majority of the patients. Only 1-2% of patients present with non-secretory multiple myeloma (1).

An M-protein may be present in the serum without evidence of multiple myeloma. This so-called monoclonal gammopathy of unknown significance (MGUS) is present in 3% of persons older than 70 years and in 1% of those older than 50 years. MGUS is characterized by an M-component of less than 30 g/l, less than 10% plasma cells in the bone marrow and the absence of bone lesions. MGUS requires no treatment. Approximately one fourth of these patients ultimately progress into overt multiple myeloma, systemic amyloidosis, macroglobulinemia or a related lymphoproliferative disorder (2,3). Smoldering multiple myeloma is diagnosed when patients have an M-component (> 30 g/l) and more than 10% plasma cells in the bone marrow but few bone lesions and no clinical symptoms. These patients should not be treated, since their condition can remain stable for years without therapy (4).

The most common clinical feature in patients with multiple myeloma is bone pain due to osteolytic lesions or osteoporosis. Other symptoms include anemia due to bone marrow infiltration, renal insufficiency due to hypercalcemia and precipitation of light chains in the kidneys and recurrent bacterial infections caused by suppression of normal immunoglobulin synthesis. Spinal cord compression, hyperviscosity and amyloidosis are relatively rare conditions in multiple myeloma (1).

The most commonly used staging system in multiple myeloma was defined by Salmon and Durie who described three stages (5).

Stage I:	Hb > 6.2 mmol/l and serum Ca < 2.65 mmol/l and normal skeletal X-rays or one solitary lesion and: IgG > 35 g/l but < 50 g/l IgA > 20 g/l but < 30 g/l Urine M-protein > 1 g/24 hours but < 4 g/24 hours
Stage II:	Not stage I or III
Stage III:	Hb < 5.3 mmol/l or serum Ca > 2.65 mmol/l or IgG > 70 g/l or IgA > 50 g/l or urine M-protein > 12 g/24 hours or multiple skeletal lesions
A:	Normal renal function
B:	Abnormal renal function: creatinine ≥ 177 µmol/l

Without treatment the median survival in multiple myeloma is 17 months (6). Local radiotherapy can be used for painful bone lesions, pathological fractures or spinal cord compression but systemic chemotherapy is the treatment of choice. With the introduction of melphalan and prednisolone as remission induction therapy the median survival of patients with multiple myeloma had improved from 17 months to 30 months (7,8). Since then, several other combination chemotherapy regimens have been used in an attempt to

improve the response rate and survival in newly diagnosed patients. However, these regimens were not superior to melphalan and prednisolone (9,10).
In 1983 McElwain and Powles first demonstrated the feasibility of high-dose therapy in multiple myeloma. An escalated dose of melphalan was used in an attempt to overcome primary resistance to conventional doses of alkylating agents. This approach resulted in a complete remission in approximately 35% of the patients (11).
Since then high-dose therapy followed by hemopoietic stem cell rescue has been extensively used in the treatment of multiple myeloma. Many phase I/II trials consisting of heterogeneous groups of patients have been published, which suggest a therapeutic benefit of high-dose treatment. However, selection of patients and the inconsistent criteria of response make it difficult to evaluate these studies. Recently a prospective randomized trial demonstrated that high-dose chemotherapy followed by autologous bone marrow transplantation improved the outcome of newly diagnosed patients with multiple myeloma when compared to conventional combination chemotherapy (12). However, eventually patients continued to relapse after autologous transplantation. Therefore the place of intensified therapy in this disease needs to be defined in more detail.
In this chapter we will review the role of high-dose therapy followed by autologous stem cell transplantation in multiple myeloma. First the biology of the disease will be discussed.

1.2.1 The biology of multiple myeloma

Myeloma cells are the malignant counterpart of normal plasma cells in the bone marrow, which produce IgG and IgA. Normal plasma cells are terminally differentiated and do not proliferate or divide. They develop from plasmablasts in the lymph nodes that have switched from IgM production to IgG or IgA, following antigenic stimulation and somatic mutation of the immunoglobulin genes. Myeloma cells, however differ from plasma cells in that they are more immature. They can be identified by their immunophenotype, which usually is $CD19^-$, $CD28^-$, $CD38^+$, $CD56^+$ and $CD138^+$. From the somatic mutations in the immunoglobulin genes in the myeloma cells one can conclude that the cells have been previously exposed to antigenic stimulation. This would indicate that the myeloma precursor cells have their origin in the lymph node germinal center. Illegitimate switch recombinations are almost invariably observed and these determine the unique clonal character of the disease in each patient. Like normal plasma cells these malignant cells differentiate into plasmablasts, which migrate to the bone marrow (1,13). In many patients this condition may exist for many years without clinical symptoms or progression of the disease, and this is called monoclonal gammopathy of undetermined significance (MGUS) (2,3). The clonal cells produce an unique immunoglobulin, which is called M-component (1).
There is now considerable evidence that the development of multiple myeloma is a multistep process, which requires multiple oncogenic hits (13). The majority of the patients have an aneuploid genotype, which frequently is hyperdiploid (14,15). In individual patients many chromosomal abnormalities may be seen. Commonly a chromosomal break is observed at chromosome 14q32, where the immunoglobulin gene is located. Following translocation to another chromosome and depending on the geographic recombination, the latter gene may fuse to various partner genes, e.g. c-maf (chromosome 16), the Fibroblast Growth Factor Receptor 3 gene (FGFR3) (chromosome

4), bcl-1 (chromosome 11), cyclin D3 (chromosome 6) and c-myc (chromosome 8) (13,16-19). The translocations in the bone marrow myeloma cells are regarded as early oncogenic events that have occurred in the germinal center. An important characteristic of early stage myeloma is that the malignant cells reside exclusively in the bone marrow. For their growth and expansion they depend on the bone marrow microenvironment, which produces interleukin-6, a myeloma plasma cell growth stimulating peptide (20). Myeloma cells express various adhesion molecules such as syndecan-1, LFA-1 and VLA-5 (21,22). Within this close interaction in the microenvironment interleukin-6, the soluble interleukin-6 receptor and interleukin-1β activate osteoclasts, which may resorb bone and may ultimately lead to the lytic bone disease that is typical of multiple myeloma (13).
In 30-50% of the patients deletions of chromosome 13 are observed and these are associated with a stage of aggressive, therapy resistant disease (23-26). Most probable, the loss of (part of) chromosome 13 is a second step towards a stage where myeloma cells become less dependent for their growth on the bone marrow stromal cells. Another indication for a further development of the disease at this stage is the frequent presence of N-ras and K-ras mutations in bone marrow relapses. Finally, in some patients extramedullary relapses are observed that are associated with a complete loss of the adhesion molecules, loss of CD56 and p53 mutations (13,27). The latter condition is designated plasma cell leukemia and is characterized by organ infiltration, presence of malignant cells in the blood, a high proliferation rate and a poor prognosis (1,28).
The recognition that chromosomal translocations and gene activation may play an important role in the development of multiple myeloma has prompted cytogenetic analysis in these patients. The significance of chromosomal abnormalities for the clinical prognosis has now been established.

1.3.1 Prognostic factors in multiple myeloma

In patients with multiple myeloma a wide spectrum in survival is seen ranging from weeks or months towards an indolent course with a survival of more than 10 years. Identification of prognostic factors is important to distinguish patients who would require early aggressive treatment from those submitted to milder forms of therapy. Moreover, the assessment of prognostic factors is important for the comparison of patient groups in different clinical trials. Different prognostic factors may be combined to create clinical staging systems that can be used in prospective trials.
The most widely used staging system is the Durie and Salmon staging system introduced in 1975 (5). According to this system patients are grouped into three stages based on hemoglobin and serum calcium levels, the M-component concentration in serum and/or urine and the extend of lytic bone lesions. Subdivisions according to renal function were made (see earlier). Several other staging systems have been proposed. The Eastern Cooperative Oncology Group (ECOG) classifies patients in good and poor risk categories based on levels of urea, calcium, leukocytes and platelets. The British Medical Research Counsel has proposed to classify patients in stages A, B or C depending on hemoglobin, urea and performance status. Comparison of 7 staging systems showed no important differences in survival curves (29). Since then several other prognostic factors reflecting the biological features of this disease like cytogenetics, β_2-microglobulin, plasma cell labeling index and C-reactive protein have been described. These prognostic factors are

not yet implemented in a staging system but initiatives towards a new international prognostic index for multiple myeloma have been taken (30).
Prognostic factors in multiple myeloma can be divided in categories concerning features, which are characteristic of the malignant clone, features reflecting tumor burden and variables that are not disease related.

1.3.2.1 Cytogenetic abnormalities

Plasma cells from nearly all patients with multiple myeloma have an abnormal DNA content (14,15). According to Lai et al. 58% of the patients exhibit hyperdiploidy, 29% have hypodiploidy and in 10% of patients a normal DNA content is measured (31).
However, with conventional karyotyping an abnormal karyotype is found in only 20-60% of patients (24,31-34). The wide range of abnormal karyotypes found in cytogenetic studies may reflect different stages of disease (31). Moreover, conventional cytogenetic studies are often hampered by the relatively low growth fraction of multiple myeloma metaphase cells (31,35). With fluorescence in situ hybridization techniques (FISH), chromosomal abnormalities can be detected in up to 90% of the myeloma patients (15,36-38). In comparison with conventional cytogenetics, FISH may reveal chromosomal abnormalities not disclosed by conventional cytogenetic analysis (37,39). Novel molecular cytogenetic techniques named multicolor spectral karyotyping (SKY) and multicolor fluorescence in situ hybridization (m-FISH) may enable the identification of complex abnormalities. These methods are based on the co-hybridization of 14 chromosome painting probes to metaphase chromosomes, which allows simultaneous visualization of each chromosome pair by an unique color (40,41).
In multiple myeloma multiple and complex chromosomal abnormalities and gene expression profiles have been described (40,41).
Certain specific abnormalities have been shown to express prognostic significance (see below).

1.3.2.2 Numerical abnormalities

Numerical abnormalities most frequently involve chromosomes 3, 5, 7, 9, 11, 15 and 19 (trisomies) and chromosomes 13 and X (monosomies) (31,33,36,37,42). The combination of trisomies of chromosomes 3, 5, 7, 9, 11, 15, 19 and 21 is often observed, which suggests a consistent pattern of cytogenetic abnormalities during progression of disease (33). In one study a survival advantage was found for hyperdiploid patients when compared to diploid patients (43).

Chromosome 13 and 13q deletions

With conventional karyotyping abnormalities of chromosome 13 are observed in about 20% of newly diagnosed patients and in 36-47% of patients with an abnormal karyotype at diagnosis (24,31,44). Using more sensitive FISH probes this percentage may vary from 30-50% depending on the probes used (26,38,45-47). When a panel of 11 probes extending over the entire q-arm is used, deletions of chromosome 13 may be found in up to 86% of the patients (48).
Which genes deleted and/or altered by deletions of chromosome 13 have a critical role in the oncogenesis of multiple myeloma, is not yet clear. Loss of the tumor suppressor gene Rb-1 may be important but the deletions of chromosome 13 are so large that it is not

certain whether the Rb-1 gene is the key relevant gene (13,23,47,49). In individual patients a marked deletion heterogeneity between myeloma cells with partial deletions, monosomy and sometimes no deletions of chromosome 13 has been described. Therefore it is suggested that a proliferation hierarchy may exist within an individual patient's tumor population depending on the extend of chromosome 13 deletions which may reflect different stages of disease progression (48).
Abnormalities of chromosome 13 are associated with poor event-free and overall survival after conventional dose chemotherapy (26,38). Also after high-dose therapy deletions of chromosome 13 are associated with poor outcome (24,25,46,50). Among 1000 patients receiving melphalan based tandem high-dose therapy, the presence of chromosome 13 abnormalities predicted a 5 year event-free survival of 0% versus 20% in patients without chromosome 13 abnormalities, and an overall survival of 16% as compared to 44% (both $P<0.0001$). Chromosome 13 deletions were not only associated with rapid progression of disease but also drug resistance to chemotherapy (25).

1.3.2.3 Structural abnormalities

Chromosomal rearrangements are frequently seen in multiple myeloma. The most frequent abnormalities involve 1q13, 8q24, 13q14, 14q32 and 17p13.
Due to chromosomal translocations or mutations overexpression, suppression or altered function of oncogenes may arise. In addition, deletions may result in the loss of tumor suppression genes. In B-cell malignancies, such as multiple myeloma proto-oncogene activation by chromosomal translocations frequently involves a rearranged immunoglobulin gene locus, e.g., the IgH locus on one partner and a proto-oncogene on the other. Once the proto-oncogene is juxtaposed to this locus, its expression comes under the control of immunoglobulin promoters and enhancers and consequently the rearranged gene becomes dysregulated. This is for instance the case in c-myc, bcl-1 and FGFR3 deregulation. In contrast, other genes like the ras family may be deregulated because of point mutations (13).

14q32 abnormalities

Abnormalities involving 14q32 are present in 20-40% of the multiple myeloma patients with an abnormal karyotype (13). The translocation breakpoints are located in switch regions of the immunoglobulin heavy chain locus. Among the multiple partner chromosomes 11q13, 4p16, 16q23 and 18q21 have been described but in most cases the partner is not identified (14q32+) (13).
In 25% of multiple myeloma cell lines 14q32 is translocated to 11q13, t(11;14)(q13;q32), the site of the bcl-1/cyclin D gene (51). Translocation t(11;14)(q13;q32) is detected in 5-40% of patients with multiple myeloma using conventional techniques (17). Using fluorescence in situ hybridization this abnormality was found in 7 out of 89 newly diagnosed patients (7.9%) (38). Translocations at 11q13 are associated with lymphoplasmocytic cell morphology, aggressive disease and unfavorable outcome in patients receiving either conventional therapy or high-dose therapy (17,23,24,38).
In 25% of 14q32 abnormalities observed in multiple myeloma cell lines the partner chromosome is 4p16, i.e. t(4;14)(p16;q32), which is associated with increased expression of the fibroblast growth factor receptor 3 (FGFR3) and the Multiple Myeloma SET domain (MMSET) (16). In another 25% of cases the partner chromosome is 16q23, i.e.

t(14;16)(q32;q23), associated with c-Maf expression (16). The incidence of these abnormalities in various series of patients may differ because of the difficulty of detecting certain translocations due to the telomeric position of the IgH gene or the small number of metaphases examined (13,52).
Translocation t(14;18)(q32;q21) is not very common in multiple myeloma but an increased expression of bcl-2 has been described by Durie in 75% of patients (53,54). In studies published so far bcl-2 overexpression in multiple myeloma does not correlate with survival (55).
Recently a novel translocation t(6;14)(p21;q32) was described in 6% of the myeloma patients that is associated with a high expression of cyclin D3 (19). Thus in addition to cyclin D1, cyclin D3 may be oncogenic in multiple myeloma as well.
In 14q32 translocations the fusion may also involve 9p13 (PAX 5), 12p13 and 13p13, Xq28 and 12q24 (56). Using multicolor spectral karyotyping additional new recurring translocations may be identified which may play a role in the oncogenesis of multiple myeloma (57). The exact prognostic implications of these translocations are not yet known.

1.3.2.4 Oncogenes and tumor suppressor genes

Ras oncogenes

The ras family consists of three related oncogenes: N-ras, K-ras and H-ras. Mutations in N- and K-ras are present in up to 47% of the multiple myeloma patients. In the end stage disease this figure increases to 67% (49). K-ras but not N-ras mutations are associated with a shorter median survival in newly diagnosed patients (2.0 vs. 3.7 years, $P<0.02$) as was reported by Eastern Cooperative Oncology Group (58).

P53 tumor suppressor gene

The p53 gene, which is located on chromosome 17, controls the normal cell cycle by regulating transcription and possibly DNA replication (1). In multiple myeloma, p53 mutations are rarely found at diagnosis (59). The frequency increases from 13 to 43% in end stage disease (27). This suggests that p53 mutation is a late event during progressive disease of multiple myeloma. Using interphase fluorescence in situ hybridization deletions of 17p that involved the p53 gene locus, were found in 24.7% of 89 untreated patients. The response rate and survival after conventional chemotherapy were significantly lower in patients with 17p deletions as compared to patients without this deletion (40.0% vs. 73.2%, $P=0.008$ and 16.2 months vs. 51.3 months, $P=0.008$) (38).
Drach et al. found p53 deletions, which were predominantly monoallelic, in 32.8% of newly diagnosed patients and 54.5% of relapsed patients. Patients with a p53 deletion had a significantly shorter survival from time of diagnosis when treated with conventional dose chemotherapy (median 13.9 vs. 38.7 months, $P<0.0001$) (60).

C-myc

C-myc was the first oncogene that was investigated in multiple myeloma because of its oncogenic role in other B-cell malignancies. It plays an important role in controlling proliferation, differentiation and apoptosis. C-myc is activated by chromosome translocation into the switch region of the IgH locus. Although structural chromosomal changes near c-myc are present in only 10% to 20% of tumors, elevated expression of

c-myc may occur frequently (1,13,61). Diverse karyotypic abnormalities of the c-myc locus associated with c-myc dysregulation were seen in 19 of 20 cell lines and approximately 50% of patients with advanced multiple myeloma. These abnormalities include unusual and complex translocations and insertions that often juxtapose myc with an IgH locus. This abnormality occurs as a late event and is associated with tumor progression (62). The involvement and prognostic relevance of c-myc in multiple myeloma has not been settled.

1.3.3 Plasma cell morphology

Increased plasma cell infiltration in the bone marrow can be accompanied by cytological changes. In 1982, Bartl reported that 32% of myeloma patients had plasmablastic morphology with bone marrow examination. These patients had a shorter survival time (19 vs. 41 months) (63).

In 1985 Greipp proposed a simple classification system in which plasma cells were classified as mature, intermediate, immature or plasmablastic according to defined criteria (61,64). The plasmablastic group had a median survival of 10 months as compared to 35 months in the other subgroups ($P<0.05$). The prognostic relevance of plasmablastic morphology was examined in 453 patients enrolled in an Eastern Cooperative Oncology Group Phase III Trial. Patients with plasmablastic morphology had lower hemoglobin and albumin levels, higher calcium and β_2-microglobulin levels and higher plasma cell percentages. They also had a higher plasma cell labeling index, elevated soluble interleukin-6 levels and a greater proportion of ras mutations. The event-free survival and overall survival were shorter in plasmablastic myeloma (1.1 vs. 2.7 and 1.9 vs. 3.7 years, respectively, $P<0.0001$) in an univariate analysis. In a multivariate analysis the plasmablastic morphology remained an independent adverse prognostic factor (61). Plasmablastic morphology was also an independent predictor of poor survival after intensive chemotherapy followed by autologous stem cell transplantation in relapsed or refractory multiple myeloma. In patients with plasmablastic morphology the overall survival was significantly worse as compared to those without (5 vs. 24 months, $P=0.001$) (65). A major limitation of the use of plasmablastic morphology as a prognostic factor is that the interpretation is subjective (61).

1.3.4 Plasma cell labeling index

Several studies have evaluated the prognostic value of the plasma cell labeling index in conventional therapy (66-68). Most groups used a cut-off plasma cell labeling index value of 1% or 2% to identify patients with a poor prognosis.

Boccadoro et al. examined the prognostic significance of the plasma cell labeling index in 107 patients. In rapid responders to conventional chemotherapy the median survival of patients with a plasma cell labeling index $\geq 2\%$ was shorter than of those with a plasma cell labeling index $< 2\%$ (16 months vs. 47 months, $P<0.004$) (66).

Controversy exists about the relevance of the plasma cell labeling index as regards outcome after high-dose therapy. It has been suggested that the adverse prognostic effect of a high plasma cell labeling index is not apparent following high-dose therapy (69). Poor prognosis patients with a high plasma cell labeling index ($> 1.2\%$) treated with high-dose therapy had a significant longer overall survival than with conventional therapy (median 49.5 vs. 32.5 months, $P<0.03$). In patients with a low plasma cell labeling index

the type of treatment did not correlate with the overall survival (61 vs. 54 months, respectively) (69). Gertz et al. reported that a plasma cell labeling index ≥ 0.8% was associated with a poor overall survival after high-dose therapy (70). In both studies β_2-microglobulin remained an adverse prognostic variable. Occasionally patients have a stable plateau phase with minimal numbers of monoclonal plasma cells but a high labeling index. An analysis showed that 57 patients with plateau phase disease and a plasma cell labeling index > 1% had a reduced median time to progression (8 vs. 39 months, P<0.0001) and overall survival (20 vs. 56 months, P<0.0001) when compared to 105 matched controls with a plasma cell labeling index < 1% (71).
Other proliferation assays such as the Ki-67 incorporation assay have a wider variation in results and therefore do not correlate well with the plasma cell labeling index (72). With these assays it may be difficult to identify plasma cells.
Because the measurement of the plasma cell labeling index is relatively complex and time consuming, San Miguel et al. analyzed the cell cycle distribution of bone marrow cells in 120 untreated multiple myeloma patients using a DNA/CD38 double-staining technique in which the proliferative activity of the plasma cells based on their CD38 expression can be discriminated from that of the residual bone marrow cells. A high percentage of plasma cells in the S-phase (> 3%) was an independent prognostic factor for poor overall survival (73).
Both the plasma cell labeling index and the S-phase estimations identify a subgroup of patients with poor prognosis. S-phase assay provides a useful alternative when the plasma cell labeling index is not available and vice versa.

1.3.5 Specific multiple myeloma antigens and adhesion molecules

Myeloma cells resemble their normal counterparts depending on their differentiation stage. Most myeloma plasma cells express mature cell surface antigens like CD38 and PCA-1. Some cells express more immature B-cell antigens like CD10 and CD20. Expression of CD20 appears to correlate with a poor prognosis (74). While plasma cells lack surface immunoglobulin, they invariably express cytoplasmic immunoglobulin (75).
Other unique surface antigens that are expressed in the majority of myeloma cells include CD56, an isoform of the neural cell adhesion molecule NCAM. CD56 may be highly expressed on some myeloma cells but not on normal plasma cells. Extramedullary disease and monoclonal plasma cells in MGUS and myeloma cells at relapse often lack this antigen, which may be consistent with a role as adhesion molecule to bone marrow. The lack of CD56 has been found to be typical for plasma cell leukemia. Forty percent of $CD56^-$ MM patients developed a leukemic phase as compared to 11% of $CD56^+$ multiple myeloma patients (P<0.008) (76). The prognostic relevance of CD56 however is still unclear (49,77,78).
Myeloma cells frequently express CD44 variant isoforms. CD44 mediates the binding of multiple myeloma cells to stroma and it regulates interleukin-6 production. CD44 expression was observed in 36% of newly diagnosed patients (79). Expression of variant isoforms containing the 9v domain is associated with advanced stage of disease, progressive disease and shorter overall survival (79).
CD49e or VLA-5 expression is also correlated with response. A high proportion of $VLA\text{-}5^-$ immature myeloma cells is associated with a poor response to conventional treatment in multiple myeloma (21).

Some myeloma cells express CD40. Activation of CD40 by its ligand CD40L stimulates the secretion of interleukin-6 from myeloma cells suggesting the induction of autocrine interleukin-6 mediated cell growth. The role of CD40 as a prognostic factor is still unclear (13).
CD138 (Syndecan-1) is a heparan sulphate-bearing proteoglycan present on the surface of myeloma cells where it mediates myeloma cell-cell and cell-extracellular matrix contact. Expression of CD138 is lost during progression and this may have prognostic relevance (22).

1.3.6 Glucocorticoid receptor

Glucocorticoids constitute an active component in many chemotherapeutic regimens for multiple myeloma. The therapeutic use of glucocorticoids is based on the induction of apoptosis. This hormone induced cytolytic response is mediated through highly specific glucocorticoid receptors. Activation of the glucocorticoid receptor results in nuclear translocation, binding to a specific DNA sequence called glucocorticoid responsive element and interaction with other transcription factors (80).
The beneficial effects of glucocorticoids are limited due to the occurrence of resistant tumor cell clones, which evolve during glucocorticoid treatment. During treatment the malignant cells may develop resistance to the glucocorticoid-mediated apoptosis (81). Genetic abnormalities of the glucocorticoid receptor are not a common cause of glucocorticoid resistance in hematological malignancies. Small deletions and point mutations have been found in a highly resistant myeloma cell line (82). However, fresh bone marrow samples of patients with glucocorticoid resistant chronic lymphatic leukemia revealed no glucocorticoid receptor point mutation (83). The glucocorticoid induced response is not directly correlated with the number of glucocorticoid receptors in multiple myeloma cell lines. Even in highly refractory patients, who are resistant to high-doses of glucocorticoids, the number of glucocorticoid receptors may be comparable to sensitive patients (84). Therefore it seems likely that the resistance to glucocorticoids is mediated by post-receptor mechanisms or is due to the presence of glucocorticoid receptor isoforms.
In normal glucocorticoid responsive tissues the functional glucocorticoid receptor α and the splice variant glucocorticoid receptor β are expressed. A variant glucocorticoid receptor mRNA, glucocorticoid receptor P or glucocorticoid receptor δ, has been observed in highly resistant myeloma cell lines (85,86). Glucocorticoid receptor P is a truncated glucocorticoid receptor protein of 676 amino acid residues. Glucocorticoid receptor-P differs from glucocorticoid receptor α and glucocorticoid receptor β and is encoded by the exons 2-7 and a part of intron 7 while exons 8 and 9 are missing. Whether the presence of glucocorticoid receptor δ or glucocorticoid receptor P on malignant plasma cells is predictive for resistance to glucocorticoid therapy remains to be elucidated.

1.3.7 β_2-Microglobulin

β_2-microglobulin is the low molecular weight light chain of the HLA complex. β_2-microglobulin is synthesized by all nucleated cells. Serum β_2-microglobulin levels are increased in renal failure due to reduced elimination of β_2-microglobulin (87).

Serum β_2-microglobulin concentrations are often increased in multiple myeloma. Serum β_2-microglobulin levels correlate well with tumor burden (88). Bataille described that a serum β_2-microglobulin of more than 6 µg/l is associated with a poor median survival (18 months vs. 52 months) in patients treated with conventional chemotherapy (88). In a study in 612 patients treated with conventional chemotherapy by the South West Oncology Group, β_2-microglobulin was found to be the most significant adverse prognostic factor (89).
In patients treated with high-dose therapy β_2-microglobulin is also an important predictive factor (12,69,90-92). Low β_2-microglobulin levels ($\leq$ 2.5 mg/l) have been associated with better relapse free and overall survival (90). A low β_2-microglobulin was also found to predict for a greater complete response rate in patients treated with high-dose therapy and stem cell transplantation (93). In a randomized trial of autologous bone marrow transplantation versus conventional chemotherapy by the French Intergroupe Français du Myélome, a low β_2-microglobulin in the serum was the only significant predictor of the probability of attaining a complete or very good partial response (12). In addition, β_2-microglobulin has been demonstrated to be an independent parameter for event-free survival and overall survival. The same observation was made in the double autologous transplantation program performed in Little Rock in Arkansas in which β_2-microglobulin > 4 mg/l and abnormalities of chromosomes 11 and 13 correlated with significantly shorter event-free survival (1.7 vs. 4.2 years) and overall survival (2.1 vs. 7.0 years) (92). Consequently β_2-microglobulin serum levels are useful to distinguish patients according to risk and stratify them to alternative strategies.

1.3.8 Interleukin-6

The pathogenesis of multiple myeloma depends on the presence of a complex network of growth factors, which regulate proliferation, differentiation, and survival of myeloma cells. These cytokines are similar to those mediating proliferation and differentiation in immunoglobulin secreting normal plasma cells.
Evidence that interleukin-6 is involved in the pathogenesis of multiple myeloma comes from several in vitro studies and from clinical observations. Interleukin-6 may induce growth of myeloma cells freshly isolated from patients. Myeloma cells spontaneously produce interleukin-6 and express the interleukin-6 receptor (IL-6R). Anti-interleukin-6 antibodies inhibit growth of myeloma cell lines in vitro (13,20,94-96). Interleukin-6 supports myeloma growth by stimulating proliferation and preventing apoptosis, but it has no effect on the differentiation of myeloma cells.
Stimulation by interleukin-6 requires binding to the interleukin-6 receptor IL-6R. This receptor is composed of two subunits: an interleukin-6 binding protein, the interleukin-6Rα receptor, and interleukin-6β receptor or gp130, the signal-transducing unit of the receptor. Binding of interleukin-6 to interleukin-6Rα induces tyrosine phosphorylation and association with gp-130 and the subsequent formation of gp130 homodimers. The transduction signal is not fully clarified but is mediated through the JAK (Janus kinase)/STAT (signal transducers and activators of transcription) pathway and the RAS dependent MAPK (mitogen-activated protein kinase) pathway. Interleukin-6 protects against anti-FAS induced apoptosis (13).
The soluble form of the interleukin-6 receptor (s-interleukin-6R) has the same affinity for interleukin-6 as the membrane bound interleukin-6R and acts as an agonist (13,97). It

binds to the cytokine with the same affinity as to the membrane form and the resulting complex binds and induces the dimerization of the transducer chains. It is created by alternative splicing or by receptor shedding. Gp-130 also exists in a soluble form, created by alternative splicing or receptor shedding and has an antagonistic function (13).
High serum levels of interleukin-6 and s-interleukin-6 were reported as predictors of poor prognosis (98). Pulkki et al. reported shorter survival in 207 newly diagnosed multiple myeloma patients with raised s-interleukin-6R (99). In a multivariate analysis in 394 patients high serum interleukin-6 and s-interleukin-6R levels were significantly and independently associated with poor prognosis and poor survival (100). However Schaar et al. found no association between high interleukin-6 levels and differences in survival in 60 multiple myeloma patients (101). Interleukin-6 induces the synthesis of C-reactive protein (CRP) by hepatic cells. High serum levels of CRP, measured as a surrogate marker of interleukin-6, may also reflect a poor prognosis (100,102).

1.3.9 Other serum markers

High serum levels of LDH identify patients with an aggressive lymphoma-like like presentation of multiple myeloma (103). Bulky retroperitoneal lymphadenopathy characterizes this variant of multiple myeloma. The level of LDH is of prognostic relevance (104). Because of the small number of patients with elevated serum LDH levels (5-11% of cases) this phenomenon is of limited clinical relevance (105).
High serum levels of type I collagen ICTP reflecting degradation of bone, are associated with poor survival in newly diagnosed patients especially in normocalcemic patients (106,107).
An abnormal low or high serum hyaluronan values at diagnosis are associated with shorter median survival (21.1 vs. 19.7 months, respectively) as compared with intermediate levels (32.6 months) (108).
Recently Seidel et al. found that an elevated serum concentration of hepatocyte growth factor, which has a role in osteolysis, might identify a subgroup of patients with a poor response to melphalan-prednisone treatment and a short survival. However in multivariate analysis elevated hepatocyte growth factor was not of prognostic relevance (109).
The same group described serum syndecan-1, a heparan sulfate proteoglycan, as a new prognostic factor (110). Patients with high syndecan-1 levels had a median survival of 20 months compared to the survival of 44 months in the low syndecan-1 group ($P<0.0001$).
It remains to be determined if these new serum parameters have an independent prognostic relevance in patients with high-dose therapy.

1.3.10 Circulating plasma cells

In multiple myeloma, the peripheral blood is usually not contaminated with large numbers of monoclonal plasma cells, but many studies have shown the presence of small numbers of circulating tumor cells (111-114).
Witzig et al. detected monoclonal plasma cells in the peripheral blood in 80% of 254 newly diagnosed patients. The median percentage of circulating monoclonal cells (cIg positive) was 6%. Among 254 patients (57%) 144 subjects had $\geq$ 4% circulating monoclonal cells. Patients with $\geq$ 4% monoclonal cells had a median survival of 2.4 years as compared to 4.4 years for the patients with less than 4% cells ($P<0.001$). In a

multivariate analysis, the percentage of circulating plasma cells and the bone marrow plasma cell labeling index classified patients into low, intermediate and high-risk groups with an median overall survival of 52, 35 and 26 months, respectively (115). The presence of monoclonal plasma cells in the peripheral blood is also predictive of progression in smoldering multiple myeloma. The median time to progression was 0.75 years in patients with abnormal cells and 2.5 years in patients with normal cells in the peripheral blood ($P<0.01$) (116).

1.3.10 Angiogenesis

Prominent bone marrow vascularization is frequently observed in multiple myeloma. It correlates with a high plasma cell labeling index and may be important for predicting disease progression. Plasma levels of various angiogenetic cytokines such as basic fibroblastic growth factor (bFGF) and vascular endothelial growth factor (VEGF) are elevated in patients with active myeloma (117). High microvessel density is associated with poor prognosis in multiple myeloma. This finding is the rationale for trials with anti-angiogenetic agents such as thalidomide and derivatives.

Until recently the prognosis of patients with multiple myeloma was exclusively defined by clinical variables such as performance status, age and the Durie and Salmon staging system. More recently prognostic factors that reflect the cellular en molecular characteristics of the malignant clone have been introduced. It is expected that cytogenetics combined with markers such as β_2-microglobulin may lead to a new staging system, required to select patients for various treatment options.

1.4 Treatment of multiple myeloma

1.4.1 Response criteria

The first criteria of response were developed by the Committee of the Chronic Leukemia and Myeloma Task Force (CLMTF) of the US. National Cancer Institute. The main parameter for objective response was a 50% reduction of the M-protein concentration in serum and urine (118).

In 1972 the South West Oncology Group (SWOG) defined objective response as at least 75% reduction of the serum M-protein synthesis rate and a decrease of at least 90% of the urinary light chain excretion. Patients with a reduction of the M-protein synthesis between 50% and 74% (SWOG criteria) and a M-protein decrease of less than 50% but with clinical improvement (CLMTF criteria) were considered to have a partial remission (PR) (8).

In subsequent clinical trials the CLMTF and SWOG criteria have been used extensively, often with modifications like addition of response categories such as very good partial response and minimal response.

In 1980 the concept of plateau phase was introduced by Durie et al. (119). A plateau phase is a period of stable disease following chemotherapy of at least 4 to 6 months albeit with persistence of measurable disease with a stable M-protein and a significant number of plasma cells. This definition of plateau phase was later used in the United Kingdom Medical Research Council (MRC) Myelomatosis trials (120).

The CLMTF and SWOG criteria do not include complete response (CR) criteria because CR was rarely observed with the then existing regimens. Following the introduction of

combination chemotherapy complete responses were observed (121). With the use of high-dose therapy followed by stem cell rescue a significant number of patients achieved a complete remission and different criteria of complete response were needed. It is now agreed that a CR should require the absence of a M-protein in serum and/or urine with no detecTable plasma cells in the bone marrow. New criteria of response, relapse and progression after high-dose therapy were recently proposed by 3 transplantation registries in order to bypass methodological inconsistencies (122).
With more and better complete remissions being observed after high-dose therapy the need for the assessment of molecular remissions emerged. With a quantitative PCR based on the amplification of the unique heavy chain sequence of the tumor using patient allele-specific oligonucleotides (IgH ASO PCR) minimal residual disease can be quantified with a sensitivity of 10^{-4} cells (123,124).

1.4.2 Conventional chemotherapy for newly diagnosed patients

Before the introduction of alkylating agents the median survival time for patients with multiple myeloma was at most 17 months (6). With the introduction of melphalan and prednisolone for remission induction the median overall survival improved to 30 months with an overall response rate of 50 to 60% (7,8).
Since then several combination chemotherapy regimens have been used in an attempt to improve survival and response rate. The M-2 protocol which used the combination of melphalan (M), cyclophosphamide (C), prednisone (P), carmustine (BCNU) (B) and vincristine (V) was introduced in 1977 and it resulted in an 87% objective response rate and a median survival of 38 months (125). A response benefit was observed for combination chemotherapy as compared with melphalan and prednisone in a randomized study in 487 patients by Blade et al. (126). The SWOG also reported an improvement in response and survival in patients treated with VCMP/VBAP or VCMP/VCAP as compared to melphalan/prednisone (MP) (127). However, in contrast various other randomized trials showed no difference in survival (10,126,128).
In 1998 the Myeloma Trialists' Collaborative Group published a meta-analysis of 6633 patients treated in 27 randomized trials in which combination chemotherapy and melphalan plus prednisone were compared. There was no difference in mortality between these two therapies. In terms of median survival combination chemotherapy and melphalan/prednison were equivalent (29 months vs. 29 months). However there was a significantly higher response rate with combination chemotherapy as compared to melphalan and prednisone (60% vs. 53.2%, $P< 0.00001$) (10).
The combination of vincristine, adriamycine and high-dose dexamethasone (VAD) was first introduced in patients with refractory multiple myeloma (129). In successive studies VAD has been used as first-line treatment in previously untreated patients. The overall response rate was higher (55-84%) and more rapid than with other combination regimens, but this did not result in a prolongation of survival (36 to 44 months) (121,130,131). Dexamethasone alone may also induce rapid responses with a response rate of 43% and an overall survival similar to VAD (81). Because of the rapid response induction VAD is also often used as remission induction treatment prior to high-dose therapy and autologous transplantation (12,92,132).

1.4.3 Conventional chemotherapy for refractory or relapsing patients

Patients with multiple myeloma who have become refractory after initial response to alkylating agents or who are primary resistant have a poor prognosis.
Their prognosis improved with the introduction of the VAD regimen by Barlogie et al. (129). VAD induced remissions in 32% of primary resistant patients and in 65% of relapsing patients even if they had been treated earlier with a doxorubicine containing regimen (133,134).
Likewise, with alternative combination regimens such as VBAD or VBAP (vincristine, BCNU, doxorubicine and dexamethasone or prednisone) approximately 30% of the patients responded, while higher responses were observed in primary resistant patients as compared with refractory relapse (135,136). VMBCP was shown to be equivalent to VAD for patients who relapsed after cyclophosphamide and prednisone (137).
High-dose dexamethasone alone is also effective in the treatment of resistant myeloma. In patients unresponsive to previous treatment the response was the same as with VAD. In relapsing patients the response to VAD was superior to dexamethasone alone (65% vs. 21%) (133). By combining VAD with cyclophosphamide (HyperCVAD) resistance to VAD or dexamethasone may be overcome in 40% of VAD resistant patients (138).
Failure of response to VAD may be the result of the multidrug resistance phenotype (MDR), which is characterized by the expression of P-glycoprotein. In drug resistant malignant cells P-glycoprotein lowers the intracellular concentration of cytotoxic drugs by pumping these out of the cell. Efforts to overcome MDR include high-dose therapy, regimens of non-cross-resistant cytotoxic drugs and the use of MDR modulators. The use of the non-cross-resistant agents etoposide, dexamethasone, cytarabine and cisplatin (EDAP) resulted in responses of 40% in patients with advanced multiple myeloma, however it was associated with severe myelosuppression and short survival (139). The use of verapamil or quinine for MDR modulation was shown to be disappointing (140). Sonneveld et al. combined VAD with cyclosporin in 21 patients resistant to VAD. The response rate in MDR positive patients was 58% compared with 33% in all patients.
However in a subsequent study in 36 patients no correlation could be found between MDR-1 expression and the response on VAD suggesting other mechanisms than MDR responsible for resistance (141,142).

1.4.4 The role of interferon

In 1979 interferon was introduced in the management of multiple myeloma (143). It was presumed to have synergistic antitumor effects when used in combination with chemotherapy. Following two promising pilot studies in which interferon was added to melphalan or VBMCP resulting in a 75 to 80% response rate, several large prospective randomized trials were performed with conflicting results (Table 1) (144,145). In two studies a higher response rate have been seen with chemotherapy and interferon (146,147). A third study showed a higher complete remission rate for patients treated with BVMCP and interferon, but failed to show an effect on the overall response rate (148). Other studies also failed to demonstrate an effect of interferon on response or survival (144,149,150). Only in one small study a survival benefit was found (146).
Interferon as monotherapy in relapsing and refractory patients results in an overall response rate of only 10 to 20% in these patients (151). Combinations of VAD or dexamethasone with interferon have shown no improvement of response duration or

Table 1. Randomized trials on interferon in induction chemotherapy

Author	No.	Regimen	Interferon	Response	PFS (months)	OS (months)
Abrahamson(233)	35	VAD/IFN	3 MU 3 x week	78%	15	44
	37	VAD		77%	15,ns	44
Cooper(145)	134	MP/IFN	2 MU/m^2	37%	18	36
	134	MP	Day 1,3,5,8,10,12	44%	22,ns	37,ns
Ludwig(150)	125	VMCP/IFN	2 MU	67%	23.2	38.9
	131	VMCP	Day 1-5 each week	61%	15.8,p=0.05	30.2,ns
Montuoro(147)	22	MP/IFN	6 MU/m^2	95%	?	at 80 weeks
	28	MP	Day 1,3,5,8,10,12	68%	?	93% vs. 50%
				p<0.05	p<0.025	p<0.025
Nordic MM Group(151)	286	MP/IFN	5 MU	44%	21	32
	297	MP	3 x weekly until failure	45%	15,p=0.005	29,ns
Österborg(148)	157	MP/IFN	7 MU/m^2	68%	27	
	160	MP	Day 1-5, 22-26	42%	29,ns	
				p<0.0001		
Oken(149)	628	VBMCP/IFN	3 MU/m^2	CR 18%	30	
		VBMCP	3 x weekly	CR 10%	25,ns	

PFS=progression free survival; OS=overall survival;VAD=vincristine,doxorubicin,dexamethasone; IFN=interferon; MU= million units; MP=melphalan, prednisone; ns=not significant; VMCP=vincristine, melphalan, cyclophosphamide,predisone; VBMCP=vincristine,BCNU,melphalan,cyclophosphamide,prednisone

survival when compared with historical controls treated with VAD or dexamethasone alone (152).

Considering the antiproliferative effect of interferon and the reductive effect on the self-renewal of the myeloma cells, there is more rationale for the use of interferon in maintenance therapy than in induction therapy.

Several large randomized studies have been published on the use of interferon as maintenance therapy, but its value remains controversial (Table 2). A significant prolongation of response duration with interferon maintenance was found in 7 randomized trials. This prolongation ranged from 4.2 to 12 months (149,150,153-157). Two smaller studies showed no prolongation of response (158,159). Most studies however fail to show an effect of interferon maintenance on survival (150,153,155,157-159). Only 2 studies showed significant prolongation of survival with the use of interferon maintenance (149,156).

The Myeloma Trialists' Collaborative Group performed a meta-analysis on the role of interferon in multiple myeloma. Data of 4012 patients from 24 trials were gathered, i.e., 2469 patients in 12 induction trials and 1543 patients in 12 maintenance trials. Progression free survival was significantly better with interferon (33% vs. 24% at 3 years, $P<0.00001$) an effect seen in both maintenance trials ($P<0.00001$) and in induction trials ($P=0.0003$). The median time to progression increased with 6 months. It is to be noted that the survival benefit due to the use of interferon is relatively small (53% vs. 49% at 3 years, median survival increase 4 months) in both induction ($P=0.05$) and maintenance trials ($P=0.03$). This benefit was restricted to smaller trials. The effect of interferon was not significantly related to the dose or the duration of interferon or patients' characteristics (160).

Another meta-analysis in 3948 patients tested the differences between interferon and control patients in 17 induction trials (2333 patients) and in 13 maintenance trails (1615 patients). Interferon after induction therapy resulted in a 6.6% higher response rate ($P<0.0002$) and a prolongation of relapse free and overall survival (4.8 and 3.1 months, $P<0.001$ and $P<0.01$, respectively). This analysis also addressed the issue of cost-effectiveness. One-year survival gain was estimated to be $42.482 for induction therapy and $18.968 for maintenance therapy (161). From meta-analyses it was concluded that given the significant, although limited, improvement of clinical outcome and its acceptable cost-effectiveness, interferon treatment of patients with multiple myeloma must be considered. Quality of life and cost utility were also examined in a randomized trial of the Nordic Myeloma Study Group where interferon was added to melphalan and prednisone during induction and maintenance. During the first year of treatment there were more complaints about chills, fever, fatigue, appetite loss and vomiting but the impact on quality of life score was low. After one year there was no difference any more. Costs per quality adjusted life scale were high: $US 110.000,- (162,163). Based on a patients' preference studies, the majority of patients would accept the toxicity and costs of treatment like interferon if a 6 months gain in relapse free or overall survival could be expected (164).

For an overview of interferon trials, see Tables 1 and 2.

Table 2. Randomized trials on interferon as maintenance therapy

Author	No. IFN Controls	Regimen	Interferon 3 x weekly	PFS (months)	OS (months)
Blade(154)	38	VMCP/VBAP	3 MU/m^2	13	38.8
	42		no IFN	7.7,p=0.042	32.7,p=0.12
Browman(155)	85	MP	2 MU/m^2	17	43
	91		no IFN	12,p<0.002	35,p=0.16
Drayson(156)	143	Diverse regimens	3 MU	16.9	41.2
	141		no IFN	12.7,p=0.02	35.4,p<0.57
Ludwig(150)	125	VMCP	2 MU	17.8	50.6
	131		no IFN	8.2,p<0.01	34.4,p<0.05
Mandelli(157)	50	MP of VMCP/	3 MU/m^2	26	52
	51	VBAP	no IFN	14,p=0.0002	39,p=0.05
	286	MP/IFN	5 MU	21	32
Nordic MM Group(151)	297		no IFN	15,p=0.005	29,ns
	52	MP stage II	5 MU	13	45
Peest(160)	65	MP/VBAMDex	no IFN	13,ns	45,ns
Salmon(159)	97	VMCP(S)/VBAP	3 MU	12	32
	96	or VAD	no IFN	11,ns	38,ns
Westin(158)	61	MP	5 MU	13.9	36
	64		no IFN	5.7,p<0.0001	35,ns

IFN=interferon; PFS=progression free survival; OS=overall survival; VMCP=vincristine,melphalan,cyclophosphamide,predisone; VBAP=vincristine,BCNU,doxorubicin,prednisone; MU= million units; MP=melphalan,prednisone; ns=not significant; VBAMDex=Vincristine,BCNU,doxorubicin,melphalan,dexamethasone; VAD=vincristine,doxorubicin,dexamethasone

Table 3. Autologous stem cell transplantation in refractory multiple myeloma

Author	No.	Type of Resistance	Conditioning Regimen	CR	CR + PR	TRM	PFS		OS	
							Median (months)	% PFS (months)	Median (months)	% OS (months)
Alexanian(177)	26	Primary	Various	15%	65%	8%	17	31% (24)	42	72% (24)
	23	Late	Various	0%	61%	17%	5	0% (24)	18	23% (24)
Tricot(181)	31	After Tx	Various	22%	-	-	-	-		78% (18)
Vesole(234)	72	Primary	MF200 + IFN (n=56)	27%	58% iit.	7%	11	25% (36)	19	31% (36)
Fermand(194)	8	Primary	MF140 + hydroxyurea + VP16 + TBI	25%	88%	12%	NR	71% (12)	NR	88% (9-12)
Rajkumar(178)	75	Primary (n=12)	MF140 + TBI	17%	83%	9.3%	26	-	30	-
		Relapse on therapy (n=33)	(n=57)	30%	88%		7		12	
		Relapse off therapy (n=30)		39%	96%		13		21	

CR=complete remission; PR=partial remission; TRM=treatment related mortality; PFS=progression free survival; OS=overall survival; Tx=transplantation; MF= melphalan; IFN=interferon; itt.=intention to treat; TBI=total body irradiation; NR=not reached

1.4.5 New treatment modalities

Thalidomide was recently introduced as an anti-myeloma agent with anti-angiogenic properties. Singhal et al. reported about the use of thalidomide in 84 previously treated patients. The response rate was 32% with 2% complete remissions. In 78% of patients the response was apparent within 2 months (117). At 12 months the event-free survival and overall survival were 22.5% and 58.5%. This promising result was confirmed by studies in refractory and relapsed patients or patients who relapsed after autologous peripheral blood stem cell transplantation (165,166).

1.4.6 Intensive treatment for multiple myeloma

1.4.6.1 High-dose melphalan

As discussed above, conventional chemotherapy rarely leads to complete remissions with no no major gain in survival. In 1983 McElwain and Powles first explored the feasibility of high-dose therapy in an attempt to induce more complete remissions and prolong survival. With high-dose melphalan (HDM 140 mg/m^2 or MF140) 3 of 5 previously untreated patients and 1 of 4 previously treated patients achieved a complete remission. All patients responded to treatment (11).

Next high-dose melphalan was evaluated in 58 patients under 63 years of age. Eleven of 41 previously untreated patients entered a complete remission (27%) and 21 entered a partial remission (51%). The median duration of remission was 19 months. The response rate in 15 previously treated patients was 66% with 2 patients in complete remission (167). However myelosuppresion was severe and 10 patients died from sepsis or hemorrhage.

Cunningham reported an overall response rate after high-dose melphalan of 82% with 32% complete remissions in 63 previously untreated patients. The median duration of the response in this study was 18 months. Nine early deaths occurred due to toxicity (14%) (168).

A high response rate in newly diagnosed patients was also confirmed by Lokhorst et al. (169). The toxicity of high-dose melphalan due to severe myelosuppression can be reduced by the use of growth factors provided that there is enough adequate bone marrow reserve (170,171).

High-dose melphalan without stem cell support proved to be to toxic for upfront treatment. However, it was an early and important step towards the development of high-dose therapy with stem cell rescue. High-dose melphalan is now used as single agent or combined with total body irradiation (TBI) in most conditionings regimens (92,172,173).

1.4.6.2 Intermediate dose melphalan

Lokhorst et al. treated 21 previously untreated and 10 relapsing or refractory patients with 2 or 3 courses of intermediate dose melphalan (IDM or MF 70 mg/m^2) with growth factor support (granulocyte colony-stimulating factor, G-CSF). The objective of this study was to reduce the toxicity of high-dose melphalan, uphold its efficacy and preserve the possibility of stem cell collection. Eighty-five percent of previously untreated patients responded, 18% entered a complete remission. Fifty percent of previously treated patients responded but with high bone marrow toxicity. The overall toxicity was moderate and no serious infections occurred. The majority of patients were treated in the out-patient clinic (174).

Table 4. Autologous bone marrow stem cell transplantation in previously untreated patients

Author	No.	Situation at Tx	Conditioning Regimen	CR	CR + PR	TRM	PFS		OS	
							Median (months)	% (months)	Median (months)	% (months)
Harousseau(187)	35	Responsive	MF140 MF140 ± TBI	34%	94%	6%	28/ -	-	41	-
Attal(235)	31	Responsive to VCMP	MF140 + TBI + IFN	48%	94%	3%	NR/ -	53% (33)	NR	85% (33)
Jagannath(236)	19	Responsive to VAD	MF140 + TBI	37%	58%	5%	21/ -	-	54	-
Cunningham(173)	53	Post-VAMP	MF200	75%	98%	2%	>20/ -		>54	63% (54)
Harousseau(182)	81	Responsive (77%)	MF140± TBI	27%	83%	-	30/ -	26% (48)	39	47% (48)
Alexanian(237)	45	Responsive to VAD	MF140 + TBI (n=24), rest various	45%	89%	11%	NR/ -	58% (36)	50	77% (36)
Bjørkstrand(223)	189	Responsive (n=143) Unresponsive (n=46)	MF ± TBI ± Cy (n=156) Various (n=33)	40%	86%	13%	20/ -	21% (60)	34	38% (60)
Cunningham(221)	84	Post VAMP/ C-VAMP	MF140-200 or Bu + IFN (n=42) - IFN (n=42)	77%	88%	0%	46/39 27/27		78	
Attal(12)	100	Post-VCMP/ VBAP	MF140 + TBI + IFN	22%	81%	2%	- /27	28% (60)	NR	52% (60)

Tx=transplantation; CR=complete remission; PR=partial remission; TRM=treatment related mortality; PFS=progression free survival; OS=overall survival; MF=melphalan; TBI=total body irradiation; VCMP=vincristine,cyclophosphamide,melphalan,prednisone; IFN=interferon; NR=not reached; VAD=vincristine,doxorubicin,dexamethasone; VAMP=vincristine,doxorubicin,melphalan,dexamethasone; Cy=cyclophosphamide; Bu=busulphan; VBAP=vincristine,BCNU,doxorubicin,prednisone

The administration of intermediate dose melphalan was also feasible in older patients with multiple myeloma. Palumbo et al. described 71 patients (median age 64 years) who were treated with 2 or 3 cycles of 100 mg/m^2 melphalan (MF100) followed by stem cell support. Their outcome was compared to 71 matched controls who were treated with oral melphalan/prednisone. Eighty-nine percent of the patients completed the entire program. The complete remission rate was 47% after MF100 and 5% after melphalan/prednison. The median event-free survival was 34 months in the MF100 group as compared to 17.7 months in the melphalan/prednison group (P<0.001). The median overall survival was 56 versus 48 months (P<0.01) (175).
These studies indicate that intermediate dose melphalan or high-dose melphalan are effective salvage regimens in relapsed patients and effective induction regimens in newly diagnosed patients.

1.4.6.3 Autologous stem cell transplantation for refractory multiple myeloma
Autologous stem cell transplantation (auto-SCT) was first performed in patients with refractory multiple myeloma. Responses of 65% to 88% were reported with a median overall survival up to 42 months (Table 3) (176,177). With repeated transplants the median event-free survival and overall survival were 37 months and > 43 months respectively (178). This indicates that tumor resistance can be overcome with high-dose chemotherapy.
The results of transplantation at relapse, either sensitive or refractory, are significantly worse with a median overall survival of 21 months (Table 3) (176-178). However, in a randomized trial comparing high-dose therapy and autologous peripheral stem cell transplantation as up-front or rescue treatment in case of resistance to conventional chemotherapy or relapse, the estimated median overall survival was 64 months in both the early high-dose therapy group and in the late transplantation group (179).
Since auto-SCT is now frequently performed, relapse from transplantation is a new problem. Tricot reported on 94 patients who had relapsed after autologous transplantation. Transplantation performed as primary salvage therapy was associated with a significantly prolonged overall survival as compared to conventional chemotherapy (P=0.009) but selection bias may have been involved. Low β_2-microglobulin (< 2.5 mg/l) and late relapse were significant favorable factors of good outcome (180).

1.4.6.4 Autologous stem cell transplantation as intensification or consolidation treatment
Many phase I and II trials have been performed to study the response rates and response duration after autologous stem cell transplantation for consolidation or intensification after remission induction in newly diagnosed patients. In Table 4 and Table 5 the results of these studies are summarized. In the majority of published studies only patients who had responded to induction chemotherapy were planned to receive high-dose therapy with stem cell transplantation. Harousseau et al. reported a median overall survival of 54 months in 103 patients responding to induction therapy as compared to 30 months in 30 non-responding patients (181). The conditioning regimen before transplantation included high-dose melphalan with or without total body irradiation for the majority of studies. The overall response rate after autologous stem cell transplantation in various studies

have been summarized in Tables 4 and 5 and are on the average about 90%. Complete response rates ranged from 22% to 70%. The high complete response rate was associated with a long progression free survival up to a median of 46 months and a median overall survival of 5 years.

Although the response and overall survival appear to be much better than historical control groups treated with conventional therapy, it is difficult to draw conclusions from these non-randomized trials. The studies used different approaches. Further e.g. due to stricter eligibility criteria, there was an enormous patient selection bias regarding age, performance status and chemosensitive disease. Finally in these studies response criteria also differed.

Some studies compared patient groups with matched controls. Blade et al. reported on 487 patients with symptomatic MM who had entered into a randomized trail to compare VCMP alternating VBAP with MP. A subgroup of 77 patients who could have been candidates for high-dose therapy because of age < 65 years, stages II or III disease, performance status < 3 and response to chemotherapy but who had not received high-dose therapy were compared to selected patients treated with high-dose therapy. The median overall survival from initiation of chemotherapy was 60 months, which was the same after high-dose therapy. The median overall survival of all 487 patients was 29 months. The only independent prognostic factor was renal function at diagnosis (182). This stresses the need for randomized trials.

In a prospective population based study Lenloff et al. compared the impact of high-dose therapy with autologous stem cell support in 274 patients younger than 60 years with 274 matched historic controls derived from earlier Nordic prospective trials on conventional chemotherapy. The overall survival at 4 years was 61% for the high-dose therapy group and 46% for the control group (P<0.001) (173).

Barlogie et al. compared a double autologous stem cell transplantation program with case matched registry data for response rate (86% vs. 52%, P=0.0001), median event-free survival (49 vs. 22 months, P=0.0001) and median overall survival (61% vs. 39%, P=0.01) (91).

The only prospective randomized trial for evaluation of high-dose therapy has been published by the IFM (Intergroupe Français du Myélome). In this IFM90 trial, conventional chemotherapy (4 to 6 cycles of alternating BVAP/VMCP) was used for remission induction in 200 patients. After induction therapy patients were randomized to receive either 4 (additional) courses of conventional chemotherapy or melphalan 140 mg/m^2 (HDM140 or MF140) and TBI (8 Gy) followed by autologous bone marrow transplantation (BMT). The results were published in 1996 and updated later (12,183). High-dose therapy with autologous BMT was superior to conventional chemotherapy. In the high-dose therapy group 38% of the patients had a complete or very good partial remission compared to 14% of the patients treated with conventional chemotherapy (P<0.001). Twenty-six percent of the patients did not undergo the transplantation. The median event-free survival and overall survival were 18 and 42 months respectively in the conventionally treated group and 28 months and 57 months in the high-dose transplant therapy group. The probabilities of event-free survival and overall survival at 6 years were 14% and 28% in the conventional treated group and 25% and 43% in the high-dose therapy group.

Table 5. Autologous peripheral blood stem cell transplantation in previously untreated patients

Author	No.	Mobilization Regimen	Conditioning Regimen	CR	CR + PR	TRM	PFS/EFS		OS	
							Median (months)	% (months)	Median (months)	% (months)
Boccadoro(69)	54	Cy 7 g/m^2 + G-CSF	MF140 + TBI	50%	90%	0	- /34.5	-	70	-
Fermand(238)	63	Mega-CHOP	HDC + TBI	20%	100%	11%	43/ -	42% (60)/ -	59	54% (60)
Majolino(239)	290	Various	Various	40%	90%	3%	-	- /28% (72)		47% (72)
Moreau(207)	142	Various	MF200	-	-	0%	- /20.5			65.5% (45)
Marit(240)	73	Cy 7 g/m^2 +GM-CSF	MF140 + TBI	44%	93%	2%	-	38% (27)/ -	60	
Alegre(219)	259	Various	MF200 or MF + TBI	51%	92%	4%	23	-	35	-
Gianni(241)	13	Cy 7 g/m^2 + G-CSF	MF120 + TBI	77%	92%	0%	38	-	41	-
Lenhoff(174)	348	Cy 4 g/m^2 + G-CSF	MF200	41%	89%	4%	27	39% (36)/ -	NR	71% (36)
Harousseau(182)	133	Various	MF140 or MF + TBI	37%	83%	4%	33	35% (48)/ -	46	43% (60)

CR=complete remission; PR=partial remission; TRM=treatment related mortality; PFS=progression free survival; EFS=event-free survival; OS=overall survival; Cy=cyclophosphamide; MF=melphalan; TBI=total body irradiation; CHOP=cyclophosphamide,doxorubicin,vincristine,prednisone; HDC=carmustine, etoposide; NR=not reached

Thus high-dose therapy significantly improved event-free survival (P=0.01) and overall survival (P=0.03) (184). The major criticism to this study was the relatively small number of patients and the poor response in the conventionally treated patients as compared to the usual results in previous trials of conventional chemotherapy (9,10,185).

1.4.6.5 Double transplantation

The achievement of a complete remission is of critical significance for event-free survival and overall survival after transplantation (12). In an attempt to increase the response rate and to reduce the relapse rate, dose-escalation with repeated transplants was investigated (Table 6).

Harousseau et al. treated 97 patients, 44 with advanced myeloma including 14 primary resistant and 30 relapsing and 53 untreated patients with a first course of high-dose melphalan without stem cell support. In responding patients a subsequent second course with stem cell support was administered. Overall response and complete remission rate were 71% and 25%, respectively after the first course. Only 36% of 69 responders proceeded to a second course due to toxicity. The complete remission rate after second transplant was 34% and the median overall survival was 41 months versus 24 months for all patients (186).

Bjorkstrand et al. performed double transplants in 15 patients. Eleven patients (73%) proceeded to the second transplantation. Eight of these patients were in complete remission after the second transplant. Seven patients continue in complete remission after a median of 23.3 months (187).

The Seattle group reported the results of a phase I-II study of tandem high-dose melphalan 200 mg/m^2 (HDM200 or MF200) in 55 patients. The complete remission rate improved from 15% after the first cycle to 55% after second cycle. The probability of event-free survival and overall survival at 18 months was 76% and 84% (188).

The largest experience with high-dose melphalan and double transplants has been obtained by the group of Barlogie et al. Between 1990 and 1995 231 newly diagnosed patients were treated with remission induction by VAD, high-dose cyclophosphamide and granulocyte-macrophage colony-stimulating factor (GM-CSF) with stem cell collection and a non-cross-resisting regimen of etoposide, dexamethasone, cytarabine and cisplatin (EDAP). The first high-dose regimen consisted of high-dose melphalan 200 mg/m^2 and was repeated if complete or partial remission was obtained. Otherwise the second regimen consisted of total body irradiation and cyclophosphamide. Eighty-eight percent of patients completed induction therapy, while 84% of the patients received a first and 71% a second transplant. Eight percent of patients died due to toxicity. After induction a 65% response rate with a 15% complete response rate (CR) was reached. The response rate increased to 75% (26% CR) after the first transplant and 83% (41% CR) after the second transplant. Median event-free survival and overall survival were 43 and 68 months, respectively. Among the patients reaching a complete response the median complete remission duration was 50 months. The absence of chromosome 11 and 13 abnormalities, low β_2-microglobulin levels at diagnosis and an early onset of complete remission were favorable prognostic factors (92).

These observations suggest that more intensive treatment results in a higher complete remission rate resulting in a prolonged progression free survival and overall survival. However, the role of double transplantation still has to be assessed in randomized trials.

In 1994 the Intergroupe Français du Myélome (IFM) started a randomized trial in 402 previously untreated patients comparing single transplantation (HDM140 and TBI) with double transplantation (HDM140 and HDM140-TBI) after induction with 3 to 4 cycles of VAD. In 2000 an interim analysis was performed with a median follow up of 4 years. Eighty-five percent of patients received the first transplant and 78% of patients in the double transplantation group received a second transplant. Complete remission or a very good partial remission was observed in 39% of patients in the single transplantation group versus 49% in the double transplantation group (P=0.06) The median event-free survival and overall survival were not significantly different between both treatment groups. However, for patients with a β_2-microglobulin of less than 3 mg/l at diagnosis the 3-year overall survival was better after double intensification (69% vs. 84%, P=0.05) (189,190).
At the VIIIth International Myeloma Workshop held in 2001, two other randomized studies were presented which showed no improvement of overall survival with double transplantation. In 1996 a prospective randomized multicenter study was started in Italy. Patients were randomized to receive either HDM200 (n=81) or HDM200 for the first transplantation and melphalan 120 mg/m^2 and busulfan for the second transplantation (n=97). The complete remission rate was higher in the double transplantation group (26 vs. 22%, ns). At a follow-up of 30 months the progression free survival was significantly longer in the double transplantation group (31.5 vs. 20.5, P=0.03). However, event-free and overall survival were not different (29.5 months and 71% probability at 4 years in the double transplantation group vs. 21.5 months and 74%) (191).
Preliminary results of the French 'Myelome autogreffe' group also did not show any benefit from double transplantation over single transplantation (192). Longer follow-up however is required to draw definite conclusions.

1.4.6.6 Time of transplant

Should patients with multiple myeloma receive a transplant early or late in the course of their disease?
Gertz et al. collected stem cells in 118 patients within 6 months of diagnosis followed by transplantation at the time of progression. Of 118 patients, 67 had transplants, 9 died of progression of disease and 42 remained alive in plateau phase. The median overall survival was 58.5 months. They concluded that early cryopreservation of stem cells followed by transplantation at progression is a feasible approach (192).
Fermand et al. reported a multicenter randomized trial about the timing of autologous transplant in 202 patients. Patients received high-dose therapy with stem cell support at diagnosis after induction with VAMP courses or later at disease progression or resistance on VMCP courses or relapse in responders. In all patients stem cells were collected after one cycle of CHOP. At a median follow up of 58 months the estimated median overall survival was 64.6 months in the early transplanted group as compared with 64 months in the late transplanted group. In this study the only advantage of early high-dose treatment was a shorter period of chemotherapy and a better quality of life (179).

Tabel 6. Autologous double transplantation for multiple myeloma

Author	No.	Type of Resistance	Condioning Regimen	CR	CR + PR	TRM	PFS/EFS		OS	
							Median (months)	% (months)	Median (months)	% (months)
Harouseau(187)	53	Newly Diagnosed	MF140 (n=97)	25%	71%	-	20	-	24	-
	44	Primary and relapse	MF140 + TBI (n=38)	65%	92%	8%	28	-	41	-
Björkstrand(188)	15	Newly Diagnosed	MF200 (n=15) MF140 + TBI (n=11)	60%	93%	7%	-	93% (20)/ -	-	92% (20)
Weaver(189)	55	Newly Diagnosed	MF200 (n=55) MF200 (n=38)	15% 55%	-	5%	-	20% (18)/ -	-	84% (18)
Barlogie(92)	231	Newly Diagnosed	MF200 (n=195) MF200 or MF140 + TBI (n=165)	26% 38%	75% 81%	2% 6%	52	-	68	-
Fermand(193)	193	Newly diagnosed randomized	carmustine + etoposide + TBI + MF140 (n=94)	42%	-	9%	-	57% (27)	-	72% (27)
			or MF140 and MF140 + etoposide + TBI (n=99)	37%	-	7%	-	57% (27)	-	72% (27)
Attal(191)	402	Newly diagnosed Randomized	MF140 +TBI (n=200)	39%		1.5%	-/24	31% (36)	48	58% (36)
			or MF140 and MF140 +TBI (n=202)	49%	-	3%	-/30	39% (36)	54	66% (36)
Cavo(192)	192	Newly diagnosed Randomized	MF 200 (n=81) or MF 200 and MF 120 + Bu 12 mg/kg (n=97)	22%	-	-	20.5/21.5	-	-	74% (prob. 48)
				26%	-	-	31.5/29.5	-	-	71% (prob. 48)

CR=complete remission; PR=partial remission; TRM=treatment related mortality; PFS=progression free survival; EFS= event-free survival; OS=overall survival; MF=melphalan; TBI=total body irradiation; Bu=busulphan

1.4.6.7 Source of stem cells and mobilization regimen

The use of peripheral blood stem cells as an alternative source of stem cells for autologous bone marrow transplantation in multiple myeloma was introduced in the late 1980s (193).

Harousseau et al. compared autologous bone marrow and peripheral stem cell transplantation in 132 patients in a retrospective analysis. The median time to neutrophil recovery was significantly shorter in the peripheral stem cell group (13 vs. 20 days, $P<0.001$). There were no differences in response rate, progression free survival and overall survival (194).

Peripheral blood stem cell transplantation has now almost completely replaced autologous bone marrow transplantation. The main advantages of blood stem cells are easier availability, faster hemopoietic recovery and lower contamination of the graft.

Stem cells can be collected following treatment with G-CSF (195,196). G-CSF stimulates the hemopoietic progenitor to migrate to the blood. Stem cell mobilization with a combination of high-dose cyclophosphamide and G-CSF was compared with G-CSF alone in a randomized study by the Arkansas group. G-CSF alone was associated with lower morbidity, shorter duration of mobilization, comparable recovery after transplantation and also lower costs (197).

A common procedure to mobilize stem cells is the combination of cyclophosphamide (3-7 g/m^2) and G-CSF or GM-CSF (Table 5). The optimal dose of cyclophosphamide for mobilization has not been settled. Higher doses of cyclophosphamide (7 g/m^2) are more efficient as compared to lower doses (4 g/m^2) (198). Higher doses could also reduce the contamination of the graft. However higher doses are associated with increased toxicity. Demirer et al. compared cyclophosphamide (4 g/m^2) with G-CSF or GM-CSF, G-CSF alone and cyclophosphamide (4 g/m^2) plus etoposide (200 mg/m^2) with G-CSF. The combination of cyclophosphamide and etoposide with G-CSF was superior to the other regimens based on the mean daily $CD34^+$ cell collection yield (199).

The mobilization effect of stem cell factor was investigated by Facon et al. The addition of stem cell factor (SCF 20 µg/kg/d) to cyclophosphamide (4 g/m^2) and G-CSF (5 $\mu g/m^2$) resulted in a significant increase in $CD34^+$ cell yield and a concomitant reduction in the number of leukapheresis required to collect a sufficient harvest (200). Until now there are no reports about the combination of SCF and G-CSF alone.

Percentage of myeloma infiltration of the marrow, previous radiotherapy and number of prior chemotherapy regimens especially of alkylating agents are important predictive factors for the success of mobilization (199). The most significant predicting factor for inadequate stem cell collection was the duration of previousmelphalan treatment (198). Prior interferon therapy may also have an unfavorable effect on peripheral stem cell collection (201). The speed of engraftment after transplantation was found to be highly correlated with the number of $CD34^+$ cells infused. Tricot et al. analyzed 225 patients with newly diagnosed or refractory multiple myeloma. Prompt engraftment was seen with more than 2 x 10^6/kg $CD34^+$ cells in patients with less than 24 months of chemotherapy whereas in patients with longer exposure to chemotherapy 5 x 10^6/kg $CD34^+$ cells were required for rapid engraftment (50). Marit et al. found that the number of CFU-GM cells, previous use of alkylating agents, response to conventional chemotherapy before priming with cyclophosphamide and interval between diagnosis and priming were important parameters for rapid engraftment (202).

1.4.6.8 Conditioning regimens

The most widely used conditioning regimens for transplantation are HDM200 alone or HDM140 combined with total body irradiation (Tables 4, 5, 6).

Goldschmidt et al. retrospectively compared HDM with or without TBI in 100 patients and found no differences in complete or partial remission between these two groups. The patients in the TBI group required parental alimentation during significantly prolonged periods due to more severe mucositis (203).

The Spanish Registry for Transplantation compared 315 patients treated with HDM200, 127 patients with HDM140 plus TBI and 121 patients with 12 mg/kg busulphan plus HDM140. There were no significant differences of response, event-free survival or overall survival. Treatment related mortality was greater among the group receiving melphalan combined with TBI compared to melphalan alone (9.1% vs. 6.1%) (204).

A registry study of 8362 autotransplants by the European Group for Blood and Marrow Transplantation (EBMT) showed that the use of a non-TBI pretransplant preparative regimen was associated with a significantly better progression free survival and overall survival (205).

The final analysis of a randomized study of the Intergroupe Français du Myélome which compared HDM200 (n=142) with HDM140 and TBI (n=140) reported equal response frequencies in both groups and more toxicity with more treatment related deaths in the TBI group (5% vs. 0%, P=0.07). The overall survival at 45 months was better after HDM200 (65.8% vs. 45.5%, P=0.05) (206). The duration of neutropenia was significantly shorter after HDM200. Duration of thrombocytopenia was also shorter. The incidence of WHO toxicity grade ≥ 3 mucositis was dramatically reduced after HDM200 (42% vs. 71%, P<0.001). HDM200 is thus preferable for myelo-ablative therapy to TBI containing regimens.

Other regimens than those containing melphalan include combinations of thiotepa, busulfan (Bu), cyclophosphomide (Cy), BCNU, etoposide (VP16), dacarbazine (DTIC) (Table 7), but their comparative value has not been critically evaluated.

1.4.6.9 Myeloma cell contamination of the graft

Peripheral blood stem cells are now widely used for autologous transplantation (194). However they carry the risk of disease relapse since stem cell grafts obtained from bone marrow or peripheral blood may be contaminated with myeloma cells.

Several assay techniques have been used for analysis of tumor cell contamination. With immunofluorescence techniques 60% of 47 grafts contained monoclonal plasma cells (207). Using immunoglobulin heavy chain gene fingerprinting, a PCR based technique with a sensitivity of 0.1 to 0.01%, 14 of 32 leukaphereses were shown to be contaminated (208). However, with a quantitative PCR based on the amplification of the unique heavy chain sequence of the tumor using patient allele-specific oligonucleotide primers (IgH ASO PCR) in one series all 15 bone marrow and peripheral blood grafts examined appeared to be contaminated with myeloma cells (209). Peripheral stem cell grafts appeared to be less contaminated with tumor cells using quantitative PCR (1.7-23700 fold fewer myeloma cells) (210).

Table 7. Other conditioning regimens

Author	No.	Type of disease	Conditioning Regimen	CR	CR + PR	TRM	PFS/EFS		OS	
							Median (months)	% (months)	Median (months)	% (months)
Fermand(180)	91	Untreated	Lomustine + VP-16 + Cy + TBI	19%	86%	10%	-/39	-	-	80% (24) 73% (36)
Dimopoulos(242)	40	Various	Thiotepa + Bu + Cy	25%	65%	13%	NR	72% (12)/ -	NR	-
Alegre(243)	24	Responsive	Bu + MF	58%	38%	4%	NR	74% (24)/ -	NR	91% (24)
Bensinger(244)	63	44 refractory 19 responsive	Bu + Cy ± TBI Bu + Cy + Thiotepa	30%	65%	25%	-	30% (36)/ -	-	46% (36)
Schiller(245)	55	Responsive	Bu + Cy + CD34 selection	80%	72%	0%	14/ -	29% (36)/ -	-	47% (36)
Adkins(246)	31	Relapsed or Refractory	Cy + BCNU + VP16 + DTIC	52%	68%	18%	-	42% (12)/ -	-	70% (12)/ -
Long(247)	34	Responsive	Cy + VP16 ± TBI or BCNU	34%	87%	6%	-	26% (48)/ -	-	36% (48)
Lokhorst(133)	50	Responsive	Cy + TBI	24%	76%	6%	34/ -	-	NR	63% (36)
Lahuerta(205)	563	Various	MF200 (n=315) MF140 + TBI (n=127) Bu + MF140 (n=121)	- - -	73% 72% 86%	6.1% 9.1% 6.8%	-	- /16% (60) - /21% (60) - /16% (60)	-	37% (60) 43% (60) 47% (60)
Tribalto(248)	52	Previously Untreated	MF + Bu	31%	75%	-	21/ -	24% (72)/ -	57	48% (60)

CR=complete remission; PR=partial remission; TRM= treatment related mortality; PFS=progression free survival; EFS=event-free survival; OS=overall survival; Cy=cyclophosphamide; TBI=total body irradiation;Bu=Busulphan; NR=not reached; MF=melphalan

Hypothetically, tumor cells in the graft may contribute to relapse after transplantation. Henry et al. suggested that patients transplanted with peripheral blood stem cells containing few tumor cells, had better disease control than those transplanted with higher number of tumor cells in the graft (210). Gertz et al. observed that the presence of monoclonal plasma cells in the graft as detected with immunofluorescence microscopy was associated with a shortened progression free survival after transplantation (70). In an other study the number of reinfused plasma cells had no effect on the outcome of patients treated with intensified chemotherapy and stem cell support (211).
Several attempts have been made to reduce the tumor cell contamination of the grafts either by positive selection of $CD34^+$ cells or by depletion of the malignant cells from the graft using monoclonal antibodies against antigens on the malignant cells. The CD34 antigen is a hallmark of progenitor cells that are capable of engraftment after myeloablative therapy. There is a correlation between the number of $CD34^+$ cells infused and time to recovery after transplant (50). Within the $CD34^+$ fraction $CD34^+CD19^+$ B-cells that express patient specific IgH VDJ gene rearrangements has been found. The involvement of the progenitor cell compartment with clonal progenitors has been considered an impediment to stem cell transplantation in multiple myeloma. However, in most studies $CD34^+$ cells are considered not to be part of the malignant clone (209,212,213). Positive selection of $CD34^+$ cells may reduce contamination of the graft with tumor cells to a median of 3.1 log. Following such procedure the majority of the grafts remained positive after selection as determined by PCR based on the amplification of the unique heavy chain sequence of the tumor using patient allele-specific oligonucleotides (214). Rapid and save recovery after transplantation sustained despite $CD34^+$ selection (214,215).
Tricot et al. purified $CD34^+Thy^+Lin^-$ progenitor cells using a combination of elutriation, chemical lysis and high speed sorting to deplete for monocytes, granulocytes, erythrocytes and platelets (216). The grafts of three patients contained no tumor cells as detected by PCR using CDR III specific primers. However, these transplants were associated with substantially delayed engraftment and the use of this procedure was therefore discontinued.
Whether tumor cells in the graft ultimately contribute to the relapse of multiple myeloma remains an unanswered question. In a multicenter randomized trial Vescio et al. compared $CD34^+$ selected versus unselected autologous stem cell transplantation in 131 patients. There were no differences in engraftment in the 2 groups. Neither were differences noted in clinical response probability, progression free survival and overall survival at one year (214). In the randomized study of Intergroupe Français du Myélome $CD34^+$ selection was optional. The first analysis of this trial showed no significant difference between the two groups regarding response rate, event-free survival and overall survival (184). Lack of benefit of $CD34^+$ selection was also found in 2 other non randomized studies (215,217). This would indicate that relapses after high-dose therapy are not likely to occur from graft contamination.

1.4.6.10 Interferon after high-dose therapy

High-dose therapy appears to result in a higher complete remission rate. In a few uncontrolled studies interferon appeared to induce a prolongation of time to disease progression and better overall survival after autologous transplantation (181,218,219).

So far only one randomized study has been published with interferon maintenance after autologous transplantation (220). Eighty-five patients were randomized to maintenance treatment with 3 x 10^6 Units interferon 3 times a week or no further treatment following HDM200 and autologous stem cell support. The median progression free survival was 46 months in patients with interferon maintenance as compared to 27 months without interferon maintenance (P<0.025). There was also a survival advantage in the interferon group at 52 months after HDM200 (P=0.006). However, at a median time of 77 months the survival advantage had disappeared because of relapse. In a large retrospective registry analysis of the EBMT 473 patients who had received interferon maintenance after autologous transplant were compared with 419 patients who had not been treated with interferon overall survival and progression free survival were significantly better in the interferon group (overall survival, 78 vs. 47 months, P=0.007 and progression free survival, 29 vs. 20 months, P=0.0006). This difference was greater among the patients with a partial or complete remission after transplantation (221). More randomized trails would be needed to establish the role of interferon after high-dose therapy.

1.4.6.11 Allogeneic stem cell transplantation following myelo-ablative conditioning

The role of allogeneic transplantation following myelo-ablative therapy (allo-SCT) has been evaluated in only a few small studies. The major disadvantage of allo-SCT is its limited use because less than 25% of patients have a suitable donor and the procedure is done up to a certain age limit.

Potential advantages of an allo-SCT are that the graft is not contaminated with malignant cells and a graft-versus-myeloma effect may be exploited. However a survival advantage following allogeneic transplantation has not yet been shown.

The EBMT performed a retrospective case matched analysis of 189 myeloma patients treated with allo-SCT compared with an equal number of autologous transplanted patients. The median overall survival was better in the autologous transplanted patients than in the allogeneic transplanted patients (34 vs. 18 months, P=0.001). This survival advantage was only observed in men, not in women. No subgroup was identified which would benefit from allogeneic transplantation. In allogeneic transplanted patients alive at 1 year post-transplant there was a trend for a better long-term overall survival (P=0.09) and a significant prolonged progression free survival (P=0.02) (222). The main reason for the poor overall survival in the allogeneic transplanted patients was a much higher transplant related mortality (41% vs. 13%, P=0.0001), which was not compensated for by a lower relapse or progression rates. The high treatment related mortality (18-57%) following allogeneic transplantation was also reported by several other groups (Table 8) and may be related to a high percentage of heavily pretreated and refractory patients receiving allogeneic transplants, the higher age and poor immune status of these patients. Preliminary results of applying allogeneic transplantation at an earlier phase in the disease also show a significant treatment related mortality (132). The EBMT registry compared the results of allogeneic bone marrow transplantation (n=334) performed in 1983-1993 with allogeneic bone marrow (n=356) and peripheral stem cell transplantation (n=133) in 1994-1998. The median overall survival was 10 months in the earlier period compared to 50 months for patients treated with allogeneic bone marrow transplantation during the later period. This significant improvement over time was related to an improvement of the treatment related mortality (223). The major manifestations of the

treatment related mortality are interstitial pneumonitis, infections and graft-versus-host-disease (GVHD) (222). The reduced treatment related mortality in the recent EBMT analysis was due to fewer deaths of bacterial and fungal infections and interstitial pneumonitis probably as a result of earlier transplantation and less prior chemotherapy.
The complete remission rate that was obtained in most series is 26% to 77% (Table 8). Even molecular remissions as assessed by PCR based on the amplification of the unique heavy chain sequence of the tumor using patient allele-specific oligonucleotide primers were described in 50-75% of patients in complete remission after receiving an allograft and in none of the patients in complete remission following an autograft (124,224). However, eventually the majority of the patients relapsed. The low progression free survival and the high treament related mortality precludes a significant benefit in overall survival in most series (Table 8).
Bensinger showed in a multivariate analysis of 80 allogeneic transplanted patients the following adverse risk factors for outcome: transplantation more than one year after diagnosis, β_2-microglobulin of more than 2.5 mg/l at time of transplant, female patients transplanted from male donors, more than 8 cycles of prior chemotherapy and Salmon and Durie stage III disease at the time of transplant (225). In the EBMT registry favorable prognostic factors were female sex, stage I disease, low β_2-microglobulin, one line of prior treatment before transplant, IgA isotype multiple myeloma and complete remission before transplantation (226).
The EBMT recently performed a case-matched comparison of syngeneic transplantation with autologous and allogeneic transplantation. The overall survival following syngeneic transplantation tended to be better (73 vs. 44 months) and the progression free survival was significantly better (72 vs. 25 months) with autologous transplantation and both were significantly better than compared with allogeneic transplantation. The conclusion of this analysis is that syngeneic transplantation is the treatment of choice if a twin donor is available (227).

1.4.6.12 Allogeneic stem cell transplantation following dose reduced conditioning

More recently new conditioning regimens have been introduced that employ reduced dosages of cytotoxic therapy aiming at intensive immunosuppression rather than on myelo-ablation.
Nonmyelo-ablative therapy may establish stable engraftment after allo-SCT and maintain anti-tumor efficacy with less toxicity. The Little Rock group treated 16 poor risk patients with allo-SCT from an HLA-matched (n=2) or mismatched sibling (n=14) after melphalan 100 mg/m^2. No treatment related mortality was observed at 100 days. At one year 12 patients were still in remission (complete and partial remission). Graft-versus-host-disease was the major problem with this procedure (228).
The use of nonmyelo-ablative therapy and allogeneic stem cell transplantation is currently still in an early stager of development and its value needs to be further explored.

1.4.6.13 Graft-versus-myeloma effect

The presence of a beneficial graft-versus-myeloma effect was recently demonstrated by remissions induced by donor lymphocyte infusions in patients relapsing after allogeneic transplantation. Tricot et al. first described the induction of a complete remission in a patient with a relapse after transplant with donor lymphocyte infusions (229). Lokhorst et

Table 8. Sibling HLA-matched allografts for multiple myeloma

Group	Patients	Regimen	Early deaths	TRM	CR	CR + PR	PFS/EFS % (months) Median (months)	OS% (months) Median (months)
EBMT(223)	189	Cy + TBI ± MF	20%	41%	48%	72%	50% (48)/ -	18 median
SFGM(249)	137	Various	-	57%	51%		33 median for CR patients	28% (60) from diagnosis 41.7% (60) from Tx
Little Rock(250)	97	Various	26%	50%	26%	56%	- /27.5% (36)	12.8% (36)
Seatle(226)	80	Bu + Cy ± TBI	44%	57%	36%	58%	20% (54)/ -	24% (54)
EBMT(227)	162	Various		45%	44%	-	45% (60)/ -	32% (48) 17 median
Vancouver(251)	26	Bu + CF ± MF Cy + TBI	19%	35%	50%	73%	40% (14)/ -	47% (36)
EBMT(224)	334 (1983-1993) 223 (1994-1998) 133 (1994-1998)	Various + ABMT Various + ABMT Various + allo-PBSCT	- - -	38% 21% 25%	53% 54% 50%	- - -	7 median/ - 19 median/ - 15 median/ -	10 median 50 median NR
Toronto(252)	22	Cy + TBI Bu + Cy	27%	54%	45%	-	22% (36)/ -	30% (36)
Nottingham(253)	13	TBI + MF or TBI + Cy	15%	23%	77%	-	69% (36)/ -	69% (36)
HOVON(133)	11	Cy + TBI	-	18%	32%	-	60% (44)/ -	NR

TRM=treatment related mortality; CR=complete remision; PR=partial remission; PFS=progression free survival; EFS= event-free survival; OS=overall survival; Cy=cyclophosphamide; TBI=total body irradiation; MF=melphalan; Tx=transplantation; Bu=busulphan; ABMT=allogeneic bone marrrow transplantation; allo -PBSCT= allogeneic peripheral blood stem cell transplantation; NR=not reached

al. studied 13 patients with a relapse after allogeneic transplantation. Eight of 13 patients responded to donor lymphocyte infusions. Four of these patients achieved a partial remission and 4 other patients attained a complete remission. The highest probability of response was after infusion of 1 x 10^8 T-cells. The major complication of donor lymphocyte infusions was graft-versus-host-disease. Acute and chronic graft-versus-host-disease were seen in 87% and 85% of the responders (230). Recently the same group presented their results of donor lymphocyte infusions in a larger group of patients with a longer follow-up (231). Twenty-seven patients received 52 donor lymphocyte infusions courses at a median of 30 months after allo-SCT. Reinduction therapy was given to 13 patients before donor lymphocyte infusions. A response was observed in 14 (52%) of patients including 6 patients with a complete remission (22%). Five patients remained in remission for more than 30 months after donor lymphocyte infusions. Factors correlated with response were a T-cell dose of 1 x 10^8 cells/kg, response to reinduction therapy and responsive disease before allo-SCT. The median overall survival was 18 months for all patients (11 months for the donor lymphocyte infusions resistant group, not yet reached for the responding patients) (231).

1.5 Aim of this thesis

Multiple myeloma is still an incurable disease. With combination chemotherapy the median survival is about 3 years. High-dose therapy with autologous or allogeneic stem cell support has been used in an attempt to improve response rate and survival. Promising results of this approach have led to even further dose intensification with double high-dose therapy end stem cell support. Randomized studies are needed to address the issue of who might benefit from different treatment modalities. On this basis we conducted a prospective, randomized, multicenter phase III study of high-dose therapy in previously untreated multiple myeloma.

In **chapter 2** the feasibility of vincristine, adriamycin and dexamethasone (VAD) administered as rapid infusion is described as a fast induction regimen in multiple myeloma.

In **chapter 3** a potential explanation for failure of response to dexamethasone is described. The role of a glucocorticoid receptor splice variant in resistance to dexamethasone containing regimen will be discussed.

In **chapter 4** the results are presented of a prospective randomized multicenter study comparing intensified chemotherapy treatment with intensified chemotherapy followed by myelo-ablative therapy in untreated multiple myeloma.

In **chapter 5** the outcome of high-dose therapy with allogeneic stem cell transplantation as compared to intensive treatment alone is described.

In **chapter 6** describes the feasibility of high-dose melphalan divided in 2 cycles to avoid the necessity of reinfusion of tumor contaminated stem cells.

In **chapter 7** the prognostic relevance of cytogenetic analysis in multiple myeloma is discussed.

In **chapter 8** the influence of high-dose therapy on quality of life is evaluated.

In **chapter 9** the cost aspects of high-dose therapy are described.

In **chapter 10** the results and relevance of the presented data will be discussed.

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CHAPTER 2

Vincristine, doxorubicin and dexamethasone (VAD) administered as rapid intravenous infusion for first-line treatment in untreated multiple myeloma

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British Journal of Haematology 1999:105:127-130

Summary

We examined the feasibility to achieve a rapid response in patients with previously untreated multiple myeloma by administering vincristine 0.4 mg and doxorubicin 9 mg/m^2 as a rapid intravenous infusion during 4 days together with intermittent high-dose dexamethasone 40 mg (VAD) for remission induction treatment in patients who were scheduled to receive high-dose therapy.139 patients (86 male, 53 female; median age 53 years, range 32-65 years; Salmon/Durie stage IIA: 42, IIB: one, IIIA: 89, IIIB: seven) were included in a prospective multicenter study in which VAD was administered as remission induction treatment and was followed by intensified treatment. The response was evaluated according to the criteria of the Eastern Cooperative Oncology Group (ECOG). The results of treatment were evaluable in 134 patients. Five patients died before evaluation. Eighty-six patients (62%) achieved a partial response (PR) and 7 patients (5%) achieved a complete response (CR), which equates to a response rate of 67%. The main side effect was mild neurotoxicity, which was observed in 18% of the patients. Fever or infections were reported in 27% of the patients. VAD administered as an outpatient regimen, based on rapid intravenous infusion is an effective induction regimen for untreated myeloma with a 67% response rate and acceptable toxicity.

Introduction

With the introduction of melphalan and prednisolone as remission induction therapy the median survival of patients with multiple myeloma improved from 17 months to a median survival of 30 to 36 months (1). Since then, several combination chemotherapy regimens have been used in an attempt to improve the survival and response rate in newly diagnosed patients with multiple myeloma which did not show superiority over melphalan and prednisolone (1). The combination of vincristine and doxorubicin administered as a continuous infusion together with intermittent high-dose dexamethasone (VAD), a non-alkylating agent-based regimen, induces rapid and marked responses in newly diagnosed patients and in patients with relapsed or refractory myeloma (2,3). Usually vincristine and doxorubicin in this regimen are administered by a continuous infusion via an indwelling catheter, which imposes logistic problems for outpatient administration. We now report the feasibility of administering VAD as an outpatient schedule using 4 days of rapid intravenous infusion in patients with previously untreated multiple myeloma. This regimen was designed in order to investigate the feasibility of achieving a rapid response with outpatient treatment in newly diagnosed patients, while conserving the well-proven efficacy of continuous infusional VAD. In this study only patients < 66 years of age were included, who were scheduled to receive further intensified treatment. No elderly patients were studied.

Methods

Study design

From December 1995 to April 1998 232 patients aged 15-65 years with previously untreated multiple myeloma were registered in a prospective multicenter study for intensified treatment following remission induction treatment. In this study three to four cycles of rapid intravenous VAD were given as standard remission induction treatment to all patients. Thereafter patients were randomized to receive either intensified treatment including peripheral blood stem cell transplantation followed by interferon-α-2a maintenance or intensive chemotherapy without stem cell support followed by interferon-α-2a maintenance. Patients with multiple myeloma stage II or III and younger than 66 years of age were included in the study. Patients with World Health Organization (WHO) performance status of 4 and patients with concomitant severe cardiac, pulmonary, neurologic or metabolic disease were excluded from registration.

Patient Characteristics

139 patients (86 male and 53 female) who had at least 1 year of follow-up after VAD were included in this analysis. All patients received rapid intravenous infusion of VAD as remission induction regimen (Table 1).

All patients gave written informed consent. The study was performed according to the Helsinki agreement. The median age of the patients was 53 years, ranging from 32 to 65 years. According to the Salmon and Durie classification, 42 patients had stage IIA disease and 89 patients had stage IIIA disease, one patient had stage IIB and seven patients had stage IIIB disease (4). Forty patients had IgA, 73 patients IgG and three patients had IgD myeloma. One patient had an IgM M-component and 18 patients had urinary light chain disease. Four patients had non-secretory myeloma.

Before the start of treatment with VAD 46 patients had a WHO performance status of 0, 64 patients a status of 1, 17 patients a status of 2 and eight patients had a WHO performance status of 3. The performance status was not known in four patients.

Treatment regimen

The VAD regimen consisted of vincristine 0.4 mg and doxorubicin 9 mg/m^2, both administered in 100 cc NaCl 0.9% by intravenous rapid infusion (30 minutes each) for 4 consecutive days. Dexamethasone was given orally at a dose of 40 mg on days 1-4, 9-12 and 17-20 during uneven cycles of VAD. The treatment cycles were repeated at 4 weeks intervals. All patients received fluconazole 200 mg/d and trimethroprim sulphamethoxazole 960 mg twice daily as prophylaxis against infections. The use of anti-emetic drugs was allowed. Treatment was given in the outpatient clinic via a peripheral intravenous catheter.

Table 1. Patient characteristics

No. of patients	139
Age (years)	
Median	53
Range	32-65
Sex (male/female)	86/53
M-component	
Immunoglobulin G	73
Immunoglobulin A	40
Immunoglobulin D	3
Immunoglobulin M	1
Light-chain disease	18
Non-secretory myeloma	4
Stage (Salmon & Durie)	
IIA	42
IIB	1
IIIA	89
IIIB	7
Performance status (WHO)	
0	46
1	64
2	17
3	8
Unknown	4

Evaluation of response

The response to VAD was evaluated by follow-up bone marrow samples and follow-up serum and urine M-protein measurements 3-4 weeks after the last VAD cycle. The response to VAD was determined according to the criteria of the Eastern Cooperative Oncology Group (ECOG). Partial response (PR) was defined as a 50% or more decrease of M-protein in serum or urine or > 50% reduction of bone marrow infiltration (in non-secretory myeloma). Complete response (CR) was defined as no M-protein measurable in serum and 10 times concentrated urine by immunofixation analysis and < 5% plasma cells with no abnormal morphology in bone marrow smears. These plasma cells had to be polyclonal by immunofluorescence staining. The median duration of response was not analyzed because induction treatment was directly followed by intensified treatment.

Results

One hundred and thirty-nine patients received a total of 416 cycles of VAD. One hundred and seventeen patients received three cycles of VAD and 13 patients received four cycles. Nine patients received one or two cycles because of early death (four), no response (three) or progressive disease (two). In addition 26 patients received local radiotherapy just before or during the VAD cycles because of local symptoms.

Ninety-three patients (67%) were responsive according to the ECOG criteria (Table 2). 86 patients (62%) achieved a partial remission: 24 patients with stage IIA disease, 55 with stage IIIA and seven with stage IIIB disease. Seven patients (5%) entered a complete remission: four patients with stage IIA and three patients with stage IIIA disease. Thirty-eight patients (27%) showed no response. Three patients were progressive. The response of five patients was not evaluable because of early death due to toxicity or concomitant disease.

Table 2. Response to VAD according to stage of disease

Disease stage	No. patients	CR	PR	NR	ED	PD
IIA	42	4	24	12	1	1
IIB	1	0	0	1	0	0
IIIA	89	3	55	25	4	2
IIIB	7	0	7	0	0	0
Total	139	7	86	38	5	3

CR=complete remission; PR=partial remission; NR=no response; ED=early death; PD=progressive disease

After VAD, 34 patients (25%) had a better WHO performance status than before treatment. In all patients with stage IIB and IIIB disease (median creatinine 269 μmol/l; range 183-814 μmol/l), the renal function normalized. One hundred nineteen of the 139 patients (86%) were able to proceed to high-dose therapy.

In order to determine the toxicity of rapid intravenous infusion of VAD, the WHO toxicity ≥ 2 was recorded during all 416 VAD cycles (Table 3). Significant nausea and vomiting arose in nine cycles (2%). In 10 cycles (2%) patients developed mucositis grade

2 or 3. Liver toxicity maximum grade 3 was seen during eight cycles (2%) and in six cycles (1%) mild to moderate renal function disturbances developed. One patient developed severe renal insufficiency. Overall, neurotoxicity was present in 30/139 patients (22%). Six patients developed neurotoxicity WHO grade 1 (4%), 12 patients neurotoxicity WHO grade 2 (9%) and 12 patients neurotoxicity WHO grade 3 (9%). One patient had cardiac dysrhythmias and one patient had a myocardial infarction. No phlebitis was seen. In two patients cutaneous toxicity WHO grade 2 occurred due to extravasation.

Forty-two infection episodes WHO grade ≥ 2 were reported in 37 patients (27%). Five patients had an infection during two cycles of VAD. Pulmonary infections were most common occurring in 14 episodes. Documented bacteremia was observed in seven episodes. There were 6 cases of bacteremia caused by Gram-positive micro-organisms and one case with a Gram-negative bacteremia. Thirty-one episodes required oral antibiotics and 11 episodes intravenous antibiotics.

Table 3. WHO toxicity ≥ 2 during VAD cycles

	No. of cycles
Nausea and vomiting	9 (2%)
Mucositis	10 (2%)
Liver	8 (2%)
Renal	7 (1%)
Cardiac	2 (0-1%)
	No. of patients
Neurotoxicity	24 (18%)
Infections	37 (27%)

Five patients died during VAD treatment. One patient developed plasma cell leukemia and died of progressive disease. Two patients died of septic shock with acute tubulus necrosis in one patient. One patient died of respiratory insufficiency due to cardiac insufficiency. One patient died of congestive heart failure following myocardial infarction.

Discussion

The VAD regimen was first used in patients with multiple myeloma refractory to alkylating agents by Barlogie et al. in 1984 (2). In successive studies VAD was used as first-line treatment in previously untreated myeloma patients or as remission induction treatment prior to high-dose therapy and autologous bone marrow transplantation (3,5-7). The rationale of continuous VAD regimen was based on the assumption that the addition of corticosteroid pulses in high dosage improves the response rate in patients with refractory myeloma and on in vitro data showing that a better tumor reduction was achieved in myeloma cells by prolonged exposure when compared to short exposure to vincristine (2,3,8-10). In addition, the peak serum concentrations of vincristine and doxorubicin with VAD administered as continuous infusion are low. This fact has been regarded as a major reason why the risk of side effects such as polyneuropathy and

cardiomyopathy is relatively low, while an optimal anti-tumor effect is maintained (2,11). However, it remains to be established if this results in a better anti-tumor efficacy. In contrast to multiple myeloma, in Non-Hodgkin's lymphoma it is attempted to achieve a rapid response by rapid intravenous infusion of the same agents in the CHOP regimen (12).

A disadvantage of the administration of VAD as continuous infusion is the necessity of a central venous catheter, which makes outpatient administration difficult and is associated with catheter-related problems such as sepsis and thrombosis in 24% of the patients treated with VAD as a remission induction therapy (7).

In order to evaluate the feasibility and efficacy of VAD application in a more convenient schedule we administered vincristine and doxorubicin as a rapid intravenous infusion in a large cohort of unselected patients. Potential advantages of this approach were that no central venous catheter was necessary during remission induction that outpatient administration was routinely possible, and that catheter-related infections could be avoided. Thus, the insertion of a central venous catheter could be delayed in patients receiving an autologous stem cell transplantation. VAD was chosen as a remission induction regimen prior to subsequent intensive treatment because it induces rapid responses and is not excessively myelosuppressive.

The response rate on remission induction treatment with only three to four cycles of VAD administered as rapid infusion is 67%. No direct comparison with continuous infusion of VAD is possible because of the absence of randomization for route of administration. Although this study is not fully comparable with earlier studies with respect to number of VAD cycles (six to seven courses vs. three to four courses in our study) and inclusion criteria (median age 53 to 57 years vs. 53 years in our study), it is known that continuous infusion of VAD may result in a 55% to 84% response rate in previously untreated patients (3,5,7). In relapsed and refractory patients the response rate may vary from 50% to 70% (2,13,14). Thus, rapid intravenous VAD (three to four cycles) results in the same response rate as with continuous infusion.

From the present study, no conclusions can be drawn about survival of these patients, since they continued with further treatment. However, the primary aim in these patients was to obtain a rapid response and to clear the clinical symptoms for which three to four cycles seem to be sufficient.

Treatment with VAD is often associated with a high incidence of bacterial infections, which are frequently catheter-related and are facilitated by high-dose steroids. The fact that a central venous catheter is not required for rapid intravenous infusion of VAD and prophylactic antibiotics were administered, may have contributed to the significantly lower incidence of serious infections in our study as compared with earlier studies (27% vs. 54% with antibiotic prophylaxis and 60% without antibiotic prophylaxis) (5,7). Mild to moderate neuropathy also occurred less frequently than the incidence described in patients treated with continuous infusion of VAD (22% vs. 100%) (3,5).

In this study, the upper limit of age was 65 years. However, elderly patients with multiple myeloma are even more vulnerable to (bacterial) infections and neurotoxicity. Therefore the use of this regimen may also benefit patients > 65 years of age.

We conclude that VAD administered as a rapid intravenous infusion is as effective as continuous infusion for remission treatment in previously untreated multiple myeloma. The most notable toxicities associated with VAD, i.e. serious infections and

neurotoxicity, had a significant lower incidence with this regimen. Therefore it can be recommended for convenient outpatient administration in stage II and III multiple myeloma.

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CHAPTER 3

Expression in hematological malignancies of a glucocorticoid splice variant which augments glucocorticoid receptor mediated effects in transfected cells

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Cancer Research 2001:61:3937-3941

Summary
Glucocorticoids play an important role in the treatment of a number of hematological malignancies, such as multiple myeloma. The effects of glucocorticoids are mediated through the glucocorticoid receptor α whose abundance can be modulated by alternative splicing of the glucocorticoid receptor mRNA. Two splice variants of the glucocorticoid receptor mRNA have been described: the glucocorticoid receptor β, which reportedly has a dominant negative effect on the actions of the glucocorticoid receptor α, and the glucocorticoid receptor P, of which the effects are unknown. In this study we have investigated the expression levels of these two splice variants at the mRNA level in multiple myeloma cells and in a number of other hematological tumors. While the glucocorticoid receptor β mRNA was, if at all, expressed at very low levels, considerable amounts (up to 50% of the total glucocorticoid receptor mRNA) glucocorticoid receptor P mRNA was present in most hematological malignancies. In transient transfection studies in several cell types and in multiple myeloma cell lines the glucocorticoid receptor P increased the activity of the glucocorticoid receptor α. These results suggest that the relative levels of the glucocorticoid receptor α and the glucocorticoid receptor P may play a role in the occurrence of glucocorticoid resistance in tumor cells during the treatment of hematological malignancies with glucocorticoids.

Introduction

Glucocorticoids (GCs) constitute an active component of chemotherapeutic regimens for various B-cell lymphoid malignancies, such as Hodgkin's Disease (HD), indolent and aggressive Non-Hodgkin's lymphoma (NHL), multiple myeloma (MM) and acute or chronic lymphocytic leukemia (ALL, CLL) (1-3). In contrast, acute or chronic myeloid leukemia (AML, CML) and preleukemic states such as the myelodysplastic syndromes (MDS) usually do not respond favorably to GC treatment (4). In lymphoid malignancies dexamethasone induces a rapid apoptotic response in vitro, which may be antagonized by interleukin-6 and other cytokines (5). However, the beneficial effects of GC therapy are limited due to the occurrence of resistant tumor cell clones, which evolve during GC treatment. During treatment, the malignant cells may develop resistance to the GC-mediated cytolysis (1).

Genetic abnormalities of the glucocorticoid receptor (GR) are not a common cause of GC resistance in hematological malignancies. Small deletions and point mutations have been found in a highly resistant MM cell line and in other leukemic cell lines (6,7,8). However, analysis of fresh leukemia cell samples obtained from GC resistant patients with CLL has not revealed any GR point mutations (3).

Prolonged exposure of resistant cells to GCs has been reported to up-regulate GR expression (9). However, in these MM cell lines the GC-induced response is not correlated with the number of GRs (9). Even in highly refractory MM patients, resistant to high doses of GCs, the number of GRs per tumor cell is comparable to that in sensitive tumor cells (10). Consequently, it seems likely that resistance to GCs is mediated either by post-receptor mechanisms or that it is associated with the presence of non-functional GR isoforms (9). Hence the question arises which mechanisms are required for GCs to induce apoptosis. It is important to know if, and to what extent abnormal GRs or GR-isoforms are involved in the multi-step process leading to treatment-refractory disease. Several years ago a variant GR mRNA, designated GR-P, was observed in tumor cells obtained from a GC-refractory myeloma patient (11). The GR-P protein is a truncated GR protein of 676 amino acid residues (Figure 1) (11). GR-P differs from GR-α, which is the active form of the GR, and the well-known natural splice variant GR-β as the exons 2-7 and a part of intron 7 encode it and the exons 8 and 9 are missing. GR-α uses the exons 2-8 and part of 9α as coding region whereas GR-β uses the exons 2-8 and part of 9β as coding region, as is indicated in Figure 1 (12). In this study, we demonstrate that GR-P mRNA is present in fresh tumor cells obtained at diagnosis from patients with MM, ALL or NHL who were sensitive to treatment with GCs. In contrast to this, the expression of the GR-β splice variant was very low or undetectable in these samples. Transfection studies in several different cell lines demonstrated that GR-P increases the activity of GR-α in COS cells and in HeLa cells, but not in CHO cells. This suggests that cell-specific factors play a role in this process and that the ratio of GR-P vs. GR-α+β mRNA may reflect different grades of GC responsiveness, a higher ratio corresponding to a higher sensitivity.

Materials and methods

Tumors and tumor cell lines

After having obtained approval from the local Medical Ethics Committee and informed consent from the patients, fresh bone marrow cells were collected at diagnosis from the

posterior iliac crest of 16 previously untreated patients with MM. These patients were included in a clinical study protocol of the Dutch-Belgian Hemato-Oncology Group (HOVON). After marrow collection, these MM patients were treated with 3 cycles of intravenous vincristine, adriamycine, and dexamethasone (VAD). Thereafter, the clinical response was assessed according to the criteria of the Southwestern Oncology Group. For the present analysis patients were classified as responders (those who had achieved a complete response or a partial response), and non-responders. In order to determine the pattern of GR expression in MM patients as compared to other hematological malignancies, we also studied tumor cells obtained from the bone marrow of untreated patients with ALL (n=5) and NHL (n=5), who subsequently showed a clinical response to GC treatment and from patients with AML (n=5), a disease that is unresponsive to GC-treatment. Bone marrow aspirates were collected in Hanks' Hepes medium, and purified using buffy coat or a Ficoll-Hypaque gradient and adherence depletion. Samples with less than 80% tumor cells as determined by microscopy were discarded. Normal lymphocytes were obtained from healthy volunteers and purified by Ficoll-Hypaque gradient centrifugation.

RNA isolation

Total RNA was isolated using the Trizol standard protocol (Life Technologies, inc.,Gaithersburg MD). Dr. Gert-Jan van Steenbrugge (Department of Urology, Erasmus University, Rotterdam) kindly provided total RNA from control tumor cell lines and prostate carcinoma xenografts.

RT-PCR assays

One μg of total RNA was reverse transcribed using 1 pmol oligo-dT primers (Pharmacia Biotech, Roosendaal, The Netherlands), 2.0 units Superscript reverse transcriptase (GIBCO-BRL, Gaithersburg, MD), 0.5 units RNasin (Promega Benelux, Leiden, The Netherlands) and 1mM deoxynucleotide triphosphates in reverse-transcriptase buffer (GIBCO- BRL). The total volume was adjusted with distilled H_2O to 20 μl. One tenth of the RT-reaction mixture was used directly for the PCR reaction in a total volume of 20 μl, with 1.0 unit of SuperTaq polymerase (Sphaero Q, Leiden, The Netherlands), 300 nM of the relevant primers (see below), and SuperTaq PCR buffer (Sphaero Q). The sequences of the oligonucleotide primers #1, 2 and 3 (Pharmacia Biotech, Roosendaal, The Netherlands) to discriminate GR-P from the total GR message, shown in Figure 1, have been described previously (11). The sequences of the oligonucleotide primers # 4, 5 and 6 (Pharmacia Biotech) to discriminate GR-α from GR-β (Figure 1) are as follows: primer #4, 5'-GAATGACTCTACCCTGCATG-3'; primer #5, 5'-TTTCCATTTGAATATTTTGG-3'; and primer #6, 5'-GCTTTCTGGTTTTAACCACA-3'. Upstream primer #4 is common to both GR-α and GR-β and hybridizes to exon 7 sequences, encoding part of the hormone-binding domain of the receptor. The downstream primers for GR-α (#5) and GR-β (#6) are within exon 9α, and exon 9β, respectively. A trace amount (2 μCi) of ^{32}P-labeled dATP was added to the mixture. Samples were heated for 5 minutes at 94°C, then 35 cycles were carried out, consisting of 1 min at 94°C, 1.5 min at 50°C, and 1.5 min at 72°C. This was followed by a final 10 min extension at 72°C. The PCR reaction products were separated on a 6% non-denaturing

poly-acrylamide gel, and the gel was dried and exposed to X-ray film (Fuji, Tokyo, Japan) for signal detection and quantification.

Hormones and substrates

Dexamethasone was purchased from Pharmacin (Zwijndrecht, The Netherlands). D-luciferin was purchased from Sigma (St. Louis, MO).

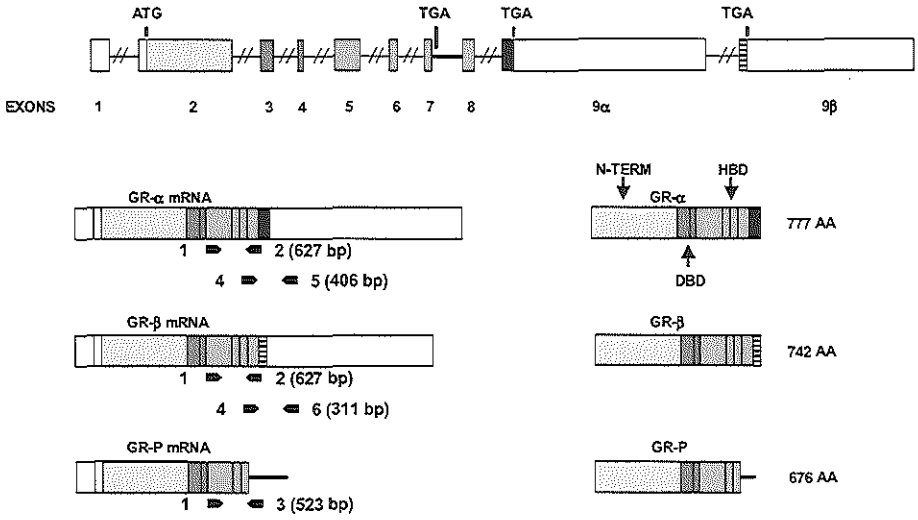

Figure 1. Glucocorticoid mRNA and protein variants and PCR primers

GR mRNAs resulting from alternative splicing of the GR gene and their derived GR-proteins. GR-β differs from GR-α by usage of exon 9β as coding region, and GR-P is an mRNA encompassing part of intron 7, but lacking exons 8 and 9. N-TERM: N-terminal (variable) domain, DBD: DNA binding domain, HBD: hormone binding domain.

The location of the PCR primers used, and the lengths of the amplified fragments are indicated under the mRNA structure. Primer-pairs 1-2 versus 1-3 were used to distinguish GR-α+β from GR-P. Primer-pairs 4-5 versus 4-6 were used to distinguish GR-α from GR-β.

Reporter genes and expression vectors

Dr Ronald Evans (The Salk Institute, La Jolla, CA) kindly provided the pRShGRα expression vector. The hGRdelta expression vector, pcDNA3-hGRdelta was constructed by replacing the ClaI/XhoI fragment of pcDNA3-hGRalpha with hGRdelta cDNA sequence 1525 to 2331 from pRSV-hGRdelta as ClaI/XhoI fragment. pRSV-hGRdelta was in turn derived by replacing the ClaI/XhoI fragment of pRSVhGRalpha (R. Evans) with hGRdelta cDNA sequence 1101 to 2331delta. The 5' sequence of the PCR product was common to all GR splice variants and contained a unique ClaI site. The 3' primer incorporated an XhoI site at the 3' end. The pcDNA3.1 vector, containing the cytomegalovirus (CMV) promoter was purchased from Invitrogen (Groningen, The Netherlands). The MMTV-LUC reporter plasmid was kindly provided by Organon (Oss, The Netherlands).

Cell culture and transfections

Monkey kidney (COS-1), Chinese Hamster Ovary (CHO) and human cervical epithelial carcinoma cells (HeLa) were maintained in DMEM-Ham's F-12 tissue culture medium (Life Technologies, Inc.) supplemented with 5% charcoal-dextran treated FCS (Life Technologies, Inc.). The RPMI 8226 parent cell line was kindly provided by Dr. WS Dalton, Lee Moffitt Cancer Center, Tampa, FL, USA. These cells were grown in suspension culture in DMEM medium with 10% DCC-FCS and 0.1% gentamycin. The UM3 cell line was derived and provided by Dr. H.M. Lokhorst, Department of Hematology, University Hospital Utrecht, The Netherlands. These cells were grown in suspension culture in RPMI 1640 medium with 10% DCC-FCS and 0.1% gentamycin. For transcription regulation studies, cells were plated at 6.0×10^4 cells per well ($3.5cm^2$, COS-1 and HeLa cells) or at 3×10^4 cells per well (CHO cells). They were grown for 24 h and transfected overnight by calcium phosphate precipitation, as described previously (13,14). For luciferase (LUC) assays, cells were transfected with 10 ng/well of pRShGRα and either 0, 1, 5, 10, 50 or 100 ng/well pcDNA3hGRδ plasmid, containing the hGR-P receptor, driven by the CMV promoter. All transfections were supplemented to a total of 100 ng/well of CMV promoter-containing plasmid with the pcDNA3.1 plasmid. In addition to this, 100 ng/well of MMTV-LUC plasmid was added. pTZ carrier DNA was added to bring the total amount of DNA to 2 μg/well. After an incubation period of 24h, dexamethasone was added and after another 24 h the cells were harvested for the LUC assay, as described previously (13,14). RPMI 8226 and UM3 multiple myeloma cells at 10^5 cells per well were transfected in suspension using the FuGENE transfection reagent (Roche Diagnostics Nederland B.V., Almere, The Netherlands) according to the supplier's protocol with a FuGENE:DNA ratio of 6:1. For luciferase (LUC) assays, cells were transfected with 24 ng/well of pRShGRα and either 0, 15, 30, 60, 120 or 240 ng/well pcDNA3hGRδ plasmid, containing the hGR-P receptor, driven by the CMV promoter. All transfections were supplemented to a total of 240 ng/well of CMV promoter-containing plasmid with the pcDNA3.1 plasmid. In addition to this, 100 ng/well of MMTV-LUC plasmid was added. pTZ carrier DNA was added to bring the total amount of DNA to 2 μg/well. After an incubation period of 24h, dexamethasone (100 nmol/L) was added and after another 24 h the cells were harvested for the LUC assay, as described previously (13,14).

Statistical analysis

The data resulting from the transfection studies were analyzed by ANOVA and when significant differences between groups were present, multiple comparisons were carried out using the Student-Newman-Keuls test.

Results

Expression of GR-P in multiple myeloma

We have screened bone marrow samples, obtained before the start of treatment from 16 patients with multiple myeloma for the presence of mRNAs, encoding the GR variants. Of these patients 8 (in the lanes 10, 11, 13, 14, and 16-19 in Figure 2) had shown a rapid response to vincristine/adriamycine/dexamethasone combination therapy, while 8 patients responded only slowly or were refractory (in the lanes 1, 4-9, and 15).

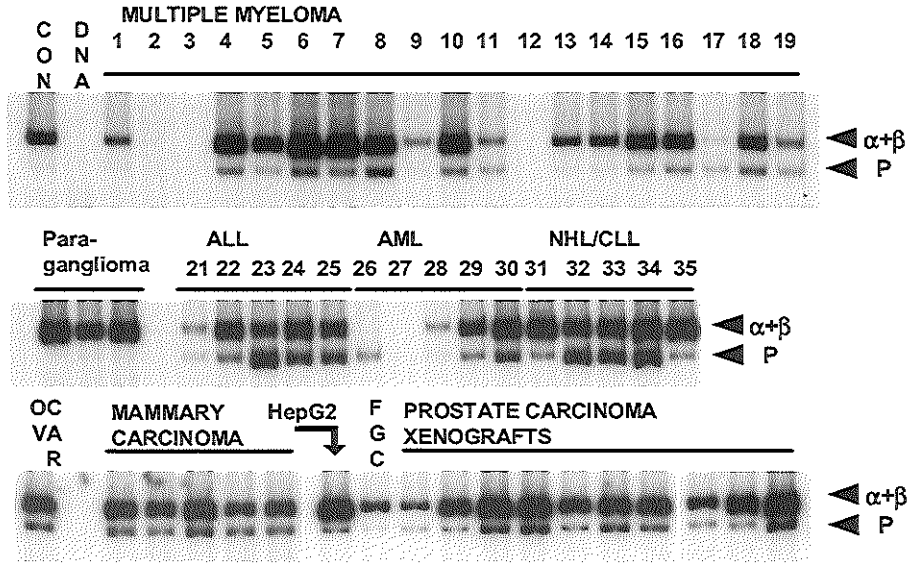

Figure 2. The expression of GR-α+β and GR-P mRNAS in several types of hematological malignancies and in solid tumors

Top panel: multiple myeloma (lanes 1-21). RT-PCR showing GR-α+β (upper signal) and GR-P (lower signal) levels. Abbreviations: CON=Normal lymphocytes, DNA=genomic DNA negative control. Numbers are patient numbers: 1-9 and 15 are GC resistant patients, the others are GC sensitive. Material from patients 2, 3, and 12 (the empty lanes) failed to amplify.

Middle panel: RT-PCR showing GR-α+β and GR-P levels in leukemic cells and in paragangliomas. ALL=acute lymphocytic leukemia, AML=acute myeloid leukemia (material from patient 27 (the empty lane) failed to amplify), NHL=Non-Hodgkin's lymphoma, CLL=chronic lymphocytic leukemia.

Bottom panel: RT-PCR showing GRα+β and GR-P levels in an ovarian tumor cell line (OV-CAR), in several mammary tumor cell lines, in HepG2-cells (HepG2), in LNCaP-FGC (FGC) cells and in prostate carcinoma xenografts, respectively.

The expression of GR-P varied drastically between patients, and in some cases GR-P mRNA levels even exceeded those of GR-α+β: e.g. patient 17, albeit that in this patient the total signal was rather low. Quantification of GR-P versus GR-α+β mRNA-ratios did not show a clear difference between sensitive or less sensitive MM patients.

Separate analysis of the levels of GR-α and GR-β mRNAs showed that GR-β was expressed in all samples at 1 to 5% of the GR-α message (not shown). In 5 samples of normal lymphocytes, GR-P constituted less than 20% of the total GR message (GR-α+ GR-β + GR-P). An example is shown in Figure 2, lane CON.

Expression of GR-P in other hematological malignancies

Expression-levels of the GR-P variant were also determined in purified tumor cells

obtained from the bone marrow samples of patients with ALL, AML, and NHL. Substantial levels of GR-P expression were found in many of the samples (Figure 2). However, as was found in the MM samples, there was a considerable variation in GR-P expression. In ALL patients the relative level of GR-P ranged from 23 % of the total GR message (patient 21) to 54% (patient 23). NHL patients all expressed GR-P at very high levels, as can be observed in patients 32, 33 and 34 (44%, 41% and 43% of the total GR message, respectively).

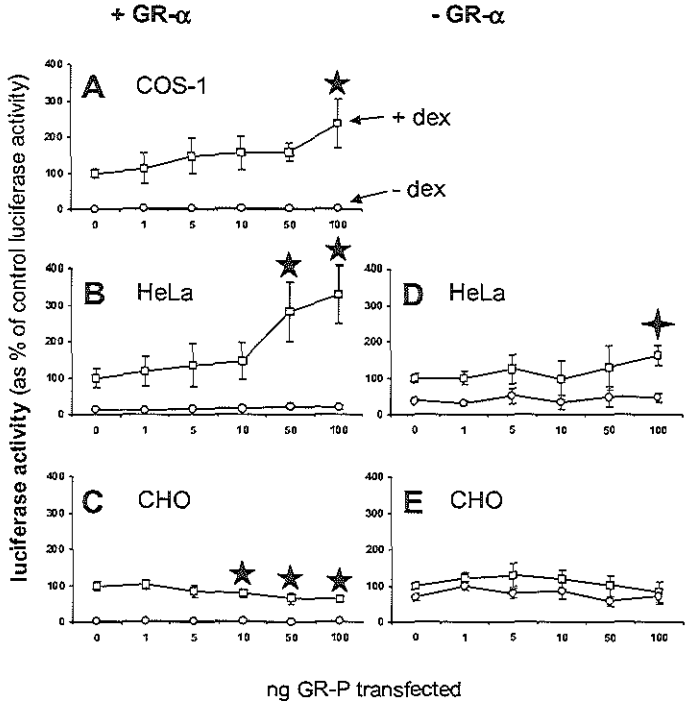

Figure 3. Cell-type specific effects on GR-α activity by GR-P

COS-1 (A), HeLa cells (B,D), and CHO cells (C,E) were transfected with increasing concentrations of the GR-P encoding plasmid pCDNA3hGRδ (0 - 100 ng/well) as indicated, in the presence (A,B,C) or absence (D,E) of 10 ng/well of the GR-α encoding plasmid pRShGRα. Additionally, 100ng/well of the reporter plasmid pMMTV-LUC was transfected (A-E), and the pCDNA3.1 plasmid was co-transfected to obtain a constant total concentration of CMV promoter-containing vector of 100 ng/well in each transfection (A-E). Luciferase activity was measured in supernatants from ligand-untreated cells (O) and in supernatants from cells treated with dexamethasone (100 nmol/L, □). Luciferase activity is shown as a percentage of control values (in the presence of dexamethasone and absence of transfected GR-P), set as 100%. Data are the means of three transfection experiments, each carried out in triplicate; error bars represent standard deviations of the mean. Values that were significantly different (Newman-Keuls test) from the control values (without GR-P) are indicated by ★ ($P<0.05$) and ✦ ($P<0.01$).

In samples from patients with acute myeloid leukemia a hybrid expression pattern of GC variants was observed. Although only 4 out of 5 samples could be analyzed successfully, apparently 2 patients exclusively expressed either only GR-α (patient 28) or only GR-P (patient 26), while in two other samples both GR-α and GR-P were detected (patients 29 and 30). In all these samples GR-β expression was undetectable under these conditions.

Expression of GR-P in other tumors

In samples from paragangliomas, consisting of very slowly dividing cells as opposed to the other tumors, which consist of more rapidly dividing cells, no expression of GR-P was detected (Figure 2). In ovarian carcinoma and mammary carcinoma cell lines, GR-P was expressed at constant levels, at approximately 27% of the total GR mRNA, which is slightly higher than that found in normal lymphocytes (the highest level found was 20%). In the liver cell line HepG2 and in the androgen-sensitive prostate carcinoma cell-line LNCaP-FGC, GR-P was expressed at low levels, comparable to those seen in normal lymphocytes. In prostate carcinoma xenografts, GR-P expression varied from 12% to 30% of the total message (Figure 2). GR-β was not expressed in these tumors.

Effect of GR-P on cell-type specific activity of GR

To study the effect of GR-P on GR-α activity, transient transfection assays were performed using several cell lines of different origins. First, COS-1 cells were co-transfected with 10 ng hGR-α expression plasmid and increasing concentrations (0-100 ng) of GR-P expression plasmid as indicated in Figure 3A. In the absence of GR-P, dexamethasone stimulated GR-α-mediated luciferase expression approximately 100-fold. When GR-P was co-transfected with GR-α, it induced a significant concentration-dependent increase in the dexamethasone-induced luciferase activity, resulting in a maximal stimulation of 2.5-fold over the stimulation by dexamethasone in the absence of GR-P ($P<0.05$). In HeLa cells the stimulatory effect of GR-P was also observed when GR-α was co-transfected ($P<0.01$; Figure 3B). In the absence of exogenous GR-α in HeLa cells, which have functionous endogenous GR-α, a similar trend was observed ($P<0.05$; Figure 3D). Induction levels in HeLa cells in the absence of exogenous GR-α were one third of those observed when GR-α expression-plasmid was present (results not shown). In contrast to the effects of GR-P in COS-1 and HeLa-cells, GR-P had an inhibitory effect in CHO cells (Figure 3C). When CHO cells were co-transfected with GR-P and GR-α, a reduction of the maximal stimulation by 50% was observed, relative to the dexamethasone-induced stimulation in the absence of GR-P ($P<0.01$; Figure 3C). However, in the absence of co-transfected GR-α, CHO-cells showed no response to dexamethasone (Figure 3E), despite the presence of endogenous GR-α in these cells. Most importantly, co-transfection of GR-P also potentiated the GR-α mediated dexamethasone effect significantly ($P<0.01$; Figure 4) in two MM cell lines, RPMI 8226-S (Figure 4, panel A) and UM3 (Figure 4, panel B).

Discussion

The cause of GC resistance in hematological malignancies is unclear. Direct involvement of the glucocorticoid receptor may include genetic alterations in the GR gene or altered expression of the GR or its splice variants. Indeed, genetic alterations in the GR have been reported to occur in a multiple myeloma cell line: a deletion of 8 bp in the 3'-UTR

(exon 9α) of the GR-gene was found in the MM cell line U266 (6). This 8-bp sequence contained an estrogen response element half site (5'-TGACCT-3'), which may serve to interact with regulatory factors, including the estrogen receptor (6). Analysis of a number of GC sensitive and resistant cell lines derived from the clonal leukemic cell line CEM-C7 suggests that mutations in the GR gene frequently cause glucocorticoid resistance in vitro (7,8).

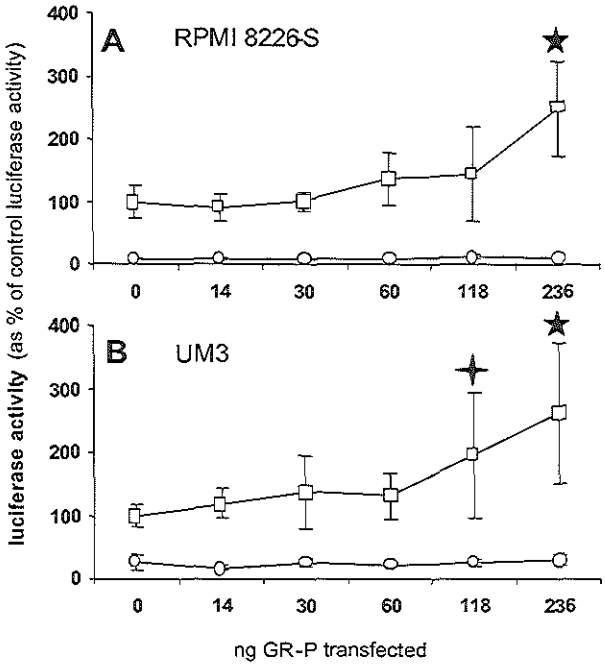

Figure 4. The effects of GR-P in RPMI 8226-S cells and in UM3 multiple myeloma cell lines
RPMI 8226-S cells (panel A) and UM3 cells (panel B) were transfected with increasing concentrations of the GR-P encoding plasmid pCDNA3hGRδ (0-240 ng/well) as indicated, in the presence of 24 ng/well of the GR-α encoding plasmid pRShGRα. Additionally, 100ng/well of the reporter plasmid pMMTV-LUC was transfected and the pCDNA3.1 plasmid was co-transfected to obtain a constant total concentration of CMV promoter-containing vector of 240 ng/well in each transfection. Luciferase activity was measured in supernatants from ligand-untreated cells (O) and in supernatants from cells treated with dexamethasone (100 nmol/mL, □). Luciferase activity is shown as a percentage of control values (in the presence of dexamethasone and absence of transfected GR-P), set as 100%. Data are the means of three (A) or two (B) transfection experiments, each carried out in triplicate; error bars represent standard deviations of the mean. Values that were significantly different (Newman-Keuls test) from the control values (without GR-P) are indicated by ★ (P<0.05) and ✦ (P<0.01).

The lack of apoptotic responses to GC in GC resistant cells is Activator Protein-1 (AP-1) independent but may involve the less effective induction of a labile Nuclear Factor-κ-B (NF-κ-B) inhibitory factor (8). We have recently shown that mutations in the GR gene of

patients with generalized GC resistance may achieve a differential interaction with some of these factors (13).

One report has addressed the question as to whether mutations in the human GR gene can also account for the development of refractoriness to GC-treatment in CLL patients (3). However, none of the resistant patients studied revealed any additional mutations in the GR-gene in their tumor cells. The results suggested that mechanisms other than altered ligand or DNA binding of the receptor should be responsible for the lack of response to dexamethasone therapy (3). Indeed, studies in three MM cell lines, OPM-2, RPMI 8226 and OPM-1, sensitive, partially sensitive and resistant to GCs, respectively, revealed that the number and affinity of GRs in these cell lines did not predict their response to GCs (9). The resistance to glucocorticoid inhibition of cell growth in the OPM-1 and RPMI 8226 cell lines may thus be a post receptor mechanism. Multidrug resistant (MDR) MM cell lines R10, R40 and R60 exhibited a decrease of their absolute GR level upon doxorubicin-treatment (15). In the relatively doxorubicin-sensitive cell line R10 this reduction was not counteracted by GC-treatment. However, in the highly doxorubicin-resistant R40 and R60 cell lines, GR mRNA levels were up-regulated upon stimulation with GCs. GC-treatment may thus be an alternative mechanism for the reversal of MDR (15).

A splice variant of the human glucocorticoid receptor termed GR-P has been identified in multiple myeloma patients and we have investigated expression of its mRNA in untreated hematological malignancies (11). Relative to its expression in normal peripheral blood lymphocytes (10-20% of the total GR mRNA), we observed a consistently high expression of this isoform in myeloma plasma cells. GR-P mRNA was also present in acute lymphoblastic leukemia, Non Hodgkin's lymphoma, and to a lesser extent in acute myeloid leukemia. These levels of expression of the GR-P suggest a potential role for the GR-P, not only in highly refractory MM cells, as has been suggested, but also in relatively sensitive cells. Moreover, GR-P expression was also found, at considerable levels (45-55% of the total GR mRNA) in lymphocytes isolated from normal bone marrow aspirates (not shown) (11).

These data indicate that the GR-P form is uniformly present in malignant hematological cells at diagnosis. So far, its role in these cells is difficult to assess. In our small series of MM patients, refractoriness to GC therapy did not seem to be associated with grossly altered ratios of the GR-P versus GR-α+β mRNA (Figure 2). Possibly, GC treatment may induce some changes in the level of GR-P expression later during the course of the disease. The present data indicate that GR-P expression is not a phenomenon exclusively observed in highly refractory cases of myeloma, as was assumed in earlier studies (11). Although we have focussed on MM in this study, other GC-responsive diseases such as ALL and NHL were also included and essentially the same pattern was observed. In contrast, AML patients showed a more variable pattern, which seems in line with the clinical observation of lower or absent response of these tumors to GC-treatment.

GR-P was also found at a variable degree of expression in a panel of other tumors: in xenografts from prostate carcinomas, and in tumor cell lines from ovarian carcinoma, mammary carcinoma, HepG2-cells and LNCaP-FGC-cells. These data indicate that high levels of GR-P expression are not restricted to hematological malignancies and may be involved in the GC-response of tumors originating from different tissues. Interestingly the GR-P variant was absent in benign neuro-endocrine tumor cells (paragangliomas).

Since it was observed that GR-β was expressed at very low levels in MM, and could not be detected at all in other hematological malignancies or in solid tumors, it can be concluded that the GR-P splice variant is the only one that is predominantly generated in (hematological) malignancies.

We and others (14,16,17) have shown, that GR-β does not inhibit GR effects in COS-1 and in other cells, whereas other groups have shown that the GR-β acts as a dominant inhibitor of GR action (12,18). The observation that the GR-P splice variant investigated in this study, stimulates GR activity in COS-1, HeLa, and MM cells while it inhibits GR-effects in CHO-cells is new. Our experiments in the RPMI 8226-S and UM3 myeloma cell lines (Figure 4) demonstrate that a specific dexamethasone response is mediated through the GR-α and that GR-P may up-regulate activation of the dexamethasone effect. This finding may be of important clinical relevance because of the observed wide variation of GR-P expression in naive myeloma cells obtained from patients.

The effects of GR-P, observed in our transfection experiments are relatively small: 2.5 fold increases only at relatively high concentrations of added GR-P expression plasmid. However, it is possible that in this system the amount of co-transfected GR-α is so high that relatively much GR-P is necessary to make its effects visible. Moreover, the effects observed in CHO cells occur already when GR-α and GR-P are transfected in equal quantities (10 ng each, see Figure 3C). Such equal amounts of both splice variant mRNAs have also been observed in several tumor samples (e.g. Figure 2, lanes 17, 23 and 34). In contrast to the situation in these transfection experiments, where a constant amount of GR-α was used in the presence of increasing concentrations of GR-P, the situation in vivo is different. There the generation of each copy of GR-P mRNA goes at the expense of a copy of GR-α mRNA. Reductions in the number of GR-α molecules per cell have previously been shown to reduce glucocorticoid sensitivity (19).

The mechanisms by which the effects of GR-P occur are unknown. However, the differences observed between the cell-types suggest that the specific cellular environment plays a role in determining the nature of these effects. GR-P may have a conformation that favors dimerization to ligand-bound GR-α, depending upon the relative abundance of the GR-α and GR-P proteins, upon which the dimer translocates to the nucleus to stimulate transcription of target genes in a more efficient manner compared to regular GR-α homodimers. Alternatively, the balance between GR-α and GR-P formation in the in vivo context may modulate the effects of glucocorticoids. In this way decreased expression of GR-P in chemotherapy-resistant hemato-oncological tumor cells could explain enhanced resistance to glucocorticoid therapy. Clinically, such mechanisms are of great importance, since the development of resistance to GCs during treatment contributes to treatment failure. Also, here we have only investigated one mode of glucocorticoid action with respect to the effects of GR-P: stimulating effects of the GR operating via direct interaction of the GR-dimer with discrete sequences in the promoter region of the target gene (the MMTV glucocorticoid responsive elements). It will be interesting to investigate the role of GR-P in systems designed to dissect other modes of GR-action such as in AP-1 and NF-κ-B mediated glucocorticoid signal transduction. We have previously shown that several mutations in the GR-gene, responsible for generalized GC-resistance exhibit different response profiles in such systems (13). Recently it was shown that GR-P does bind to the promoter region of the proopiomelanocortin gene, but apparently does not repress gene expression in that context (20). We are currently also

investigating which external signals influence the relative abundance of the GR splice variants in peripheral mononuclear leukocytes, and if changes in this distribution affect the regulation of endogenous glucocorticoid-regulated genes, such as several cytokine genes.

Acknowledgements
We thank Dr. G.J. van Steenbrugge (Department of Urology, Erasmus University Rotterdam) for making available samples of RNA from control tumor cell lines and from prostate carcinoma xenografts. We also thank T. Buitenweg-de Jong for skillful technical assistance in the isolation of patient RNA samples and in the transfection experiments. C. Berrevoets (Department of Endocrinology and Reproduction, Erasmus University Rotterdam) kindly provided advice and assistance in the transfection experiments.

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CHAPTER 4

Myelo-ablative treatment following intensified chemotherapy in previously untreated multiple myeloma: a prospective randomised phase III study

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Blood, in press

Summary

We compared the efficacy of intensified chemotherapy followed by myelo-ablative therapy and autologous stem cell rescue with intensified chemotherapy alone in newly diagnosed with multiple myeloma. 261 eligible patients with stage II/III multiple myeloma below 66 years were randomized after remission induction therapy with VAD to receive intensified chemotherapy, i.e. melphalan 140 mg/m^2 iv. divided in 2 doses of 70 mg/m^2 iv. (IDM) without stem cell rescue (n=129) or the same regimen followed by myelo-ablative therapy consisting of cyclophosphamide, total body irradiation and autologous stem cell reinfusion (n=132). Interferon-α-2a was given as maintenance. Seventy-nine percent of patients received both cycles of IDM and 79% of allocated patients actually received myelo-ablative treatment. The response rate (CR plus PR) was 88% in the intensified chemotherapy group versus 95% in the myelo-ablative treatment group. CR was significantly higher after myelo-ablative therapy (29% vs. 13%; P=0.002). With a median follow up of 33 months (range: 8-65 months), the event-free survival (EFS) was not different between both treatments (median 21 months vs. 22 months; P=0.28). Time to progression (TTP) was significantly longer after myelo-ablative treatment (25 months vs. 31 months; P=0.04). The overall survival (OS) was not different (50 vs. 47 months; P=0.41). Intensified chemotherapy followed by myelo-ablative therapy when applied as first line treatment for multiple myeloma resulted in a higher complete response rate and a longer TTP. However, it did not result in a better EFS and OS.

Introduction

For more than 30 years melphalan and prednisone has been the standard treatment for multiple myeloma (MM) resulting in a median overall survival of 30 to 36 months (1-4). From 1983 high-dose melphalan (140 mg/m^2, HDM) was used to overcome resistance to conventional doses of alkylating agents (5,6). Since then, high-dose therapy supported by stem cell rescue has been explored in phase I and phase II studies in MM (7-18). Sofar, only one randomized study demonstrated that standard chemotherapy followed by myelo-ablative treatment improved the survival as compared to chemotherapy alone (19). This study has been criticized as a relatively poor response in the conventionally treated patients was observed (20). Based on these results, several randomized studies have addressed the issue of intensive therapy versus standard treatment (21-23).

In November 1995 the Dutch-Belgian HOVON group started a prospective multicenter phase III trial to study the efficacy of myelo-ablative therapy with stem cell rescue added to intensified chemotherapy compared to intensified chemotherapy alone. The study was closed April 1, 2000. We now report the results of the first analysis of this study.

Patients and methods

Criteria for enrollment

Patients up to 65 years of age with previously untreated multiple myeloma and stage II or III A/B disease according to the Salmon and Durie criteria were eligible for the study (24). Criteria for exclusion were WHO performance status 4, severe cardiac, pulmonary, neurologic or metabolic disease, inadequate liver function (i.e. bilirubin ≥ 2.5 times the upper limit of normal value), prior malignant disease except non-melanoma skin tumors or stage 0 cervical carcinoma and prior extensive radiotherapy involving the myelum which could preclude total body irradiation. Hemodialysis or treatment for hypercalcemia with pamidronate was instituted when needed. All patients had given written informed consent before inclusion. The study was performed according to the Helsinki agreement.

Study protocol

The treatment protocol is outlined in Figure 1.

Remission induction treatment

Patients were treated with 3-4 cycles of VAD for remission induction. The VAD regimen consisted of daily doses of vincristine 0.4 mg and doxorubicin 9 mg/m^2 by rapid intravenous infusion for 4 consecutive days as described previously (25). Dexamethasone 40 mg was given orally on days 1-4 on even cycles and on days 1-4, 9-12 and 17-20 on uneven cycles of VAD. The treatment cycles were repeated at 4-weekly intervals. Antibacterial and antifungal prophylaxis was given according to local guidelines.

Randomization

After induction treatment with VAD, patients were randomly assigned to one of the two treatment groups irrespective the response to VAD. Randomization was stratified by center. Exclusion criteria for randomization were WHO performance status 3 or 4, severe cardiac disease (WHO grade ≥ 3), inadequate liver function or persistent serum creatinin ≥ 177 μmol/l. Patients under 56 years of age with an HLA-identical sibling were

candidates for allogeneic stem cell transplantation (allo-SCT), in which case they were not eligible for randomization.

Peripheral stem cell harvest

Four to 6 weeks after the last cycle of VAD peripheral blood stem cells were collected after cyclophosphamide (4 g/m^2 iv., day 1) and granulocyte colony-stimulating factor (G-CSF, Amgen, Thousand Oaks, Ca, 300 µg/day subcutaneously for patients under 75 kg, otherwise 480 µg/day) starting at day 5 until the last day of leukapheresis. Peripheral blood stem cell collection was started as soon as the WBC count reached 1.0 x 10^9/l and ≥ 1% CD34$^+$ cells were present in the peripheral blood. A minimum of 5 x 10^6 CD34$^+$ cells/kg and/or 10 x 10^4 granulocyte-macrophage colony-forming units (CFU-GM)/kg were required in order to proceed to myelo-ablative treatment. Bone marrow harvesting was performed if stem cell collection failed and at least 2 x 10^8/kg nucleated bone marrow cells were harvested.

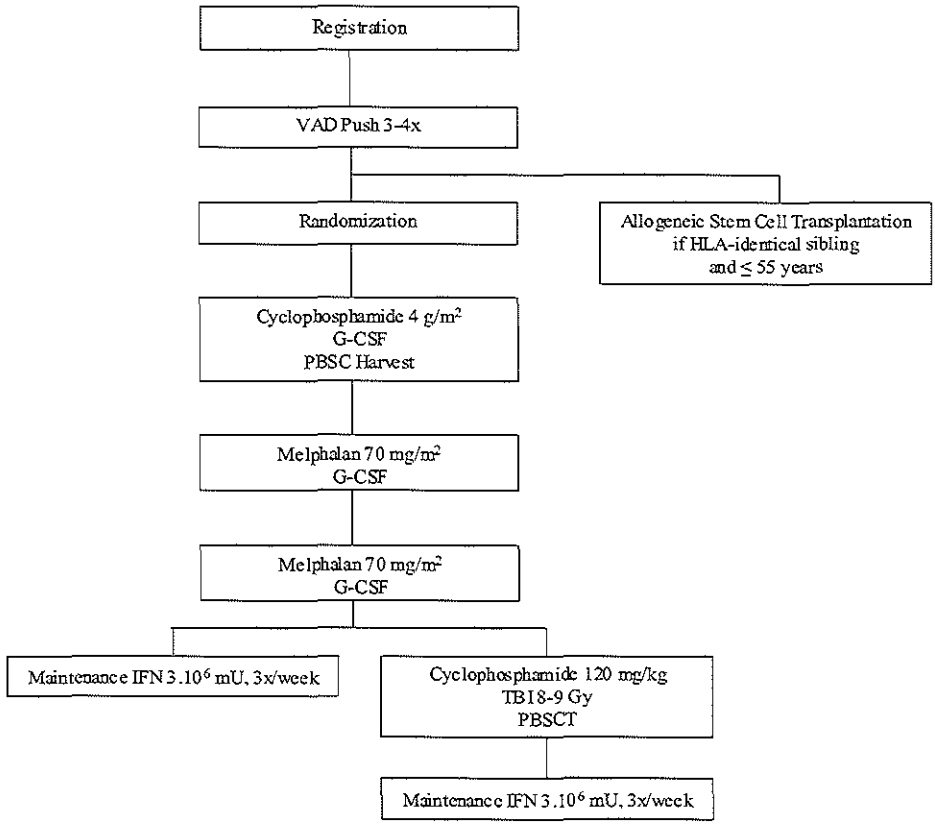

Figure 1. Outline of the study

Intensification with melphalan
Melphalan (140 mg/m^2) was administered, divided in 2 cycles of 70 mg/m^2 iv. (IDM) without stem cell rescue. IDM was given in the outpatient clinic as a slow bolus injection with hyperhydration and forced diuresis as described before (26). Antibacterial and antifungal prophylaxis were given according to local guidelines. Prophylactic G-CSF was started on day +4 after melphalan at a daily dose of 300 to 480 µg subcutaneously and continued until the neutrophil count had recovered to ≥ 1.0 x 10^9/l. Prophylactic platelet transfusions were given once the platelet count dropped to levels ≤ 10 x 10^9/l. A second cycle of IDM with G-CSF was administered maximal 8 weeks after the first IDM except when aplasia persisted (neutrophils ≤ 0.5 x 10^9/l at day 30 and/or platelets ≤ 50 x 10^9/l at day 42). If hematopoietic recovery had not occurred at 8 weeks after IDM I or IDM II autologous stem cell rescue with 2.5 x 10^6/kg CD34$^+$ cells was allowed.

Myelo-ablative treatment and autologous peripheral blood stem cell reinfusion
Patients who were randomized for myelo-ablative treatment proceeded to this regimen if at least a partial remission had been achieved and an adequate stem cell graft was available. Exclusion criteria were WHO performance status ≥ 3, severe organ dysfunction or serum creatinine, bilirubine and transaminases of ≥ 2.5 times the upper limit of normal values. The myelo-ablative regimen consisted of cyclophosphamide 60 mg/kg given twice on 2 consecutive days followed by total body irradiation with lung shielding (9 Gy, lung dose 8 Gy). Fractionated body irradiation of 2 x 5 Gy or 2 x 6 Gy was allowed.

Interferon-α-2a maintenance
Treatment with interferon-α-2a (IFN, 3 x 10^6 Units thrice weekly) was started 60 to 90 days after the second cycle of IDM or 60 to 90 days after transplantation and was continued until relapse or progression in those patients who had reached at least a partial remission and a WHO performance status 0-2 in the absence of severe organ dysfunction with a platelet count > 50 x 10^9/l and a neutrophil count > 1.0 x 10^9/l.

Evaluation of response
Partial response (PR) was defined as a 50% or more reduction of monoclonal immunoglobulins (M-protein) in serum and/or urine or > 50% reduction of bone marrow infiltration in non-secretory myeloma. Complete response (CR) was defined as no M-protein measurable in serum and/or 10 times concentrated urine by immunofixation analysis and < 5% plasma cells which had to be polyclonal by immunofluorescence staining. Relapse from CR was defined as recurrence of monoclonal plasma cells in the bone marrow or recurrence of M-protein in serum and/or urine measured by immunofixation. Progression from PR was defined as a doubling of M-protein on 2 consecutive measurements or an increase of M-protein with deterioration of clinical condition.

Cytogenetic studies
Bone marrow samples were cultured in RPMI with 6-10% serum and in Iscove medium containing interleukin-4 and/or interleukin-6 and harvested after 24 and 96 hours according to standard cytogenetic techniques. The metaphase preparations were stained for RFA-, QFQ-, or GTG-banding and karyotypes were described according to the

international nomenclature (27). Where possible a minimum of 20 cells was analyzed. The presence of a clonal abnormality was defined as two metaphases with the same additional chromosome or the same structural abnormality or as three metaphases missing the same chromosome. When only one abnormal metaphase was found, the clonality of the abnormalities observed had to be confirmed with FISH. Those cases with a normal karyotype where less than 20 cells were analyzed, were considered a failure.

Quality of life study
Quality of life was assessed using the EORTC QLQ-C30 questionnaire (28). This questionnaire includes a global health and quality of life scale, 5 functioning scales (physical, role, cognitive, emotional and social), 3 symptom scales (fatigue, nausea and vomiting) and a number of single items. Questionnaires were presented to patients prior to start of treatment, at evaluation of each treatment phase and every three months thereafter for 2 years. Quality of life measurements were stopped at time of relapse or progression.

Cost analysis
Costs per patient (1998 level) were calculated from start of interferon-α-2a maintenance treatment or myelo-ablative treatment up to 3 years post-randomization or death, if earlier. The medical consumption of a random patient selection from the entire sample served as input for the analysis. Average unit costs for the medical consumption items were based on financial data from the participating hospitals, by following the micro-costing method (29,30).

Statistical analysis
All patients who fulfilled the eligibility criteria after VAD were randomized between the 2 treatment groups. This study was designed to reveal a 15% increase in 2-years event-free survival from randomization from 40% to 55% in the myelo-ablative group. With a two-sided significance level $\alpha = 0.05$ and a power $1 - \beta = 0.80$, 170 patients were required in each treatment group and 178 events had to be observed (31). It was assumed that 90% of the patients should be randomized. Therefore it was intended to enter 400 patients in 4 years time. Ultimately 453 patients were registered, of whom 12 were not eligible. Three hundred and eleven patients (71%) have been randomized, of whom 8 were not eligible for randomization. In order to have complete data of the patients in this analysis, the analysis was restricted to eligible patients who were registered before August 1, 1999. Thus 261 patients were included: 129 in the intensified chemotherapy group and 132 in myelo-ablative treatment group. The present analysis was performed in November 2001. The number of events for event-free survival in the groups was 179, 94 in the intensified chemotherapy group and 85 in the myelo-ablative therapy group.
Patient characteristics between the two treatment arms were compared using Pearson's chi-squared test or Fisher's exact test whichever appropriate, in case of discrete variables, or the Wilcoxon rank-sum test in case of continuous variables. Endpoints in the study included response rate, event-free survival (EFS), time to progression (TTP) and overall survival (OS) from randomization. EFS was determined from the date of randomization until no response after IDM, progression/relapse after previous response or death without progression, whichever came first. Patients who had still no response after IDM were

Table 1. Patient characteristics at time of diagnosis

Characteristics	All registered patients (n=379)	Intensified Chemotherapy alone (n=129)	Myelo-ablative therapy (n=132)
Age in years (median, range)	55 (31-65)	55 (38-65)	56 (32-65)
Male/female	235/144	74/55	81/51
WHO performance:			
0 – 1	297	108	107
2 – 3	78	19	25
Not done	4	2	-
Stage (Salmon & Durie)			
IIA	75	32	27
IIB	5	-	2
IIIA	259	89	93
IIIB	40	8	10
Monoclonal protein			
IgA	98	40	34
IgG	215	70	78
IgD	9	3	3
LcD	42	11	11
Non-secreting myeloma	15	5	6
Hemoglobin ≤ 6.21 mmol/l	136	49	39
Serum calcium >2.65 mmol/l	45	8	12
Serum LDH > ULN	55	23	14
Serum β_2-microglobulin			
0 – 3	155	55	62
> 3 mg/l	170	49	60
Not done	54	25	10
Bone marrow plasma cells			
≤ 50 %	221	75	79
> 50 %	125	38	45
Not done	33	16	8
Number of skeletal lesions			
0	75	31	24
1 – 2	59	18	23
≥ 3	243	80	85
Not done	2	-	-

considered failure at one day after randomization. TTP was calculated from the date of randomization until progression/relapse or death of multiple myeloma. Patients without progression who died from other causes were censored at date of death. OS was measured from date of randomization until death. Patients still alive at the date of last contact were censored. EFS, TTP and OS were estimated by the Kaplan-Meier method, and Kaplan-Meier curves were generated to illustrate differences between the two treatment arms and compared using the log-rank test. The analysis was by intention-to-treat and the patients who were eligible for randomization were analyzed according to the treatment arm they were assigned to.

The quality of life analysis and the cost analysis were performed by applying the Mann-Whitney U-test.

The variables at diagnosis that were included in the analysis of prognostic factors are shown in Table 1. Univariate survival analysis was performed with Cox-regression to determine differences in survival between subgroups. The variables that appeared significant in the univariate analysis were also included in a backward selection multivariate Cox regression. The multivariate Cox regression started with all significant variables, and the variable with the largest P-value was removed, until all remaining variables had a P-value of less than 0.05. All reported P-values are two-sided and a significance level $\alpha = 0.05$ was used.

Results

Patient characteristics

From November 1995 until April 2000, 453 patients from 46 centers were registered. Twelve patients were found not eligible because of double registration (n=2), prior treatment with chlorambucil (n=1), stage I disease or Monoclonal Gammopathy of Undetermined Significance (MGUS, n=3), AL amyloidosis (n=3), prior chemotherapy for lung carcinoma (n=1), extensive radiotherapy precluding total body irradiation (n=1) or poor pulmonary function (n=1). The first analysis included 379 patients registered before August 1, 1999. Eight percent of the 379 patients had progression from prior diagnosed plasma cell disorders: 13 patients from MGUS, 12 from plasmacytoma, 4 from stage I disease and 2 from smoldering myeloma. Two hundred and sixty-eight patients proceeded to randomization. Seven patients were unjustly randomized, i.e. 4 patients had persistent renal failure and in 3 patients allo-SCT was planned. Thus, 261 patients were eligible for randomization. Of these 129 were randomized to intensified chemotherapy alone and 132 patients to myelo-ablative treatment added to intensified chemotherapy. One hundred eleven patients were not randomized. Fifty-four patients had an HLA-identical sibling and proceeded to allo-SCT and 16 patients died before or during VAD. Other reasons were persistent renal failure (n=6), no response to treatment or progressive disease (n=12), excessive toxicity or poor performance status (n=13), refusal (n=7) or other (n=3). Patient characteristics at time of diagnosis are shown in Table 1.

Completion of allocated treatment

In 258 of 261 randomized patients cyclophosphamide and G-CSF were administered. In 10 patients no leukapheresis was performed due to insufficient stem cell mobilization (n=5), toxicity (n=3), early death (n=1) or refusal (n=1). Peripheral blood stem cells were collected in 248 patients and bone marrow was harvested in 2 patients. The median

number of $CD34^+$ cells harvested was 9.9 x 10^6/kg (range: 0.3-76.7 x 10^6/kg). In 242 patients an adequate graft for autografting was obtained.
Of 261, 254 patients received the first cycle of IDM, i.e. 124 patients (96%) in the intensified chemotherapy group and 130 patients (98%) in the myelo-ablative therapy group. Seven patients did not receive melphalan because of progressive or resistant disease (n=2), excessive toxicity (n=3) or refusal (n=2). Of 261 patients, 206 patients (79%) actually received melphalan 140 mg/m^2 divided in 2 cycles, equally divided over the two groups. Forty-eight patients received only one cycle of IDM because of no response or progressive disease (n=10), excessive toxicity or poor performance status (n=27) or other reasons (n=11). One hundred and four randomized patients (79%) proceeded to myelo-ablative treatment with stem cell rescue, 11 patients after 1 cycle of IDM and 93 patients after 2 cycles of IDM. In 13 patients myelo-ablative therapy with stem cell rescue was not performed because of no response or progressive disease (n=3), excessive toxicity or poor performance status (n=5), absence of a graft (n=3) or other (n=2). IFN was started in 91 patients (71%) in the intensified chemotherapy group as compared to 75 patients (57%) in the myelo-ablative therapy group. The median duration of IFN treatment was 8 months, i.e. 12 months in the intensified chemotherapy group and 7 months in the myelo-ablative therapy group (P=0.11; hazards ratio 1.3, 95% confidence interval (CI) = [0.9,1.9]). Reasons for stopping IFN were toxicity (n=72; 29 in the intensified chemotherapy group and 43 in the myelo-ablative therapy group), relapse or progression (n=54; 39 in the intensified chemotherapy group and 15 in the myelo-ablative therapy group), refusal (n=3) or other (n=3).

Table 2. PR or CR reached on protocol

	Intensified Chemotherapy alone (n=129)	Myelo-ablative therapy (n=132)
VAD	71%	69%
IDM I	82%	85%
IDM II	88%	91%
PBSCT		95%
IFN-α-2a	88%	95%

Response
The overall response rate (PR and CR) of all 379 registered patients on VAD was 63% (CR 2%). Twelve patients died before response evaluation (3%), 30% of patients had no response, 2% had progressive disease and of 2% of patients the response was unknown.
The overall response rate after VAD in the 261 randomized patients was 70% (CR 3%), which increased to 90% after 2 cycles of IDM. Five patients being not in PR or CR before myelo-ablative treatment with stem cell rescue achieved a partial response after transplantation. Of 261 randomized patients 239 patients eventually achieved a PR or CR. In patients randomized to intensified chemotherapy the response rate was 88% and in patients randomized to myelo-ablative therapy following intensified chemotherapy the

response rate was 95%. The cumulative response rate after each treatment phase is presented in Table 2.

After 2 cycles of IDM, 10% of patients in the intensified chemotherapy group had achieved a CR as compared to 17% in the myelo-ablative therapy group (P=0.12). The overall CR rate on protocol was 13% in the intensified chemotherapy group and 29% in the myelo-ablative therapy group (P=0.002, Table 3). After myelo-ablative treatment and peripheral stem cell reinfusion the overall response was 95%, while 29% of patients achieved a CR and 66% a PR.

Table 3. CR on protocol

	Intensified Chemotherapy alone (n=129)	Myelo-ablative therapy (n=132)	
VAD	1%	5%	
IDM I	6%	8%	
IDM II	10%	17%	P=0.12
PBSCT		27%	
IFN-α-2a	13%	29%	P=0.002

Event-free survival, time to progression and overall survival

The median follow-up from randomization of the 156 patients still alive was 33 months (range: 8 to 65 months). There was no significant difference in EFS from randomization between the 2 treatment groups. The median EFS was 21 months in the intensified chemotherapy group versus 22 months in the myelo-ablative therapy group (P=0.28; hazard ratio = 0.85, 95% CI = [0.63,1.14], Figure 2).

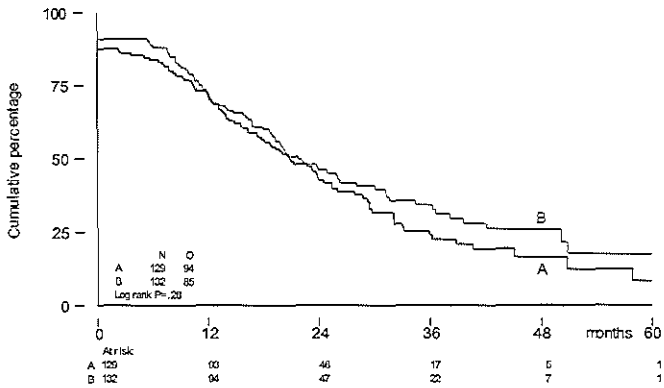

Figure 2. Kaplan-Meier curves of event-free survival after intensified chemotherapy alone (A) and myelo-ablative therapy with autologous stem cell rescue (B).

The TTP was longer in patients with myelo-ablative therapy (25 months vs. 31 months; P=0.04; hazard ratio = 0.70, 95% CI = [0.5,0.98], Figure 3). The median OS from randomization was 50 months in the intensified chemotherapy group versus 47 months in the myelo-ablative therapy group (P=0.41; hazard ratio = 1.17, 95% CI = [0.80, 1.72], Figure 4).

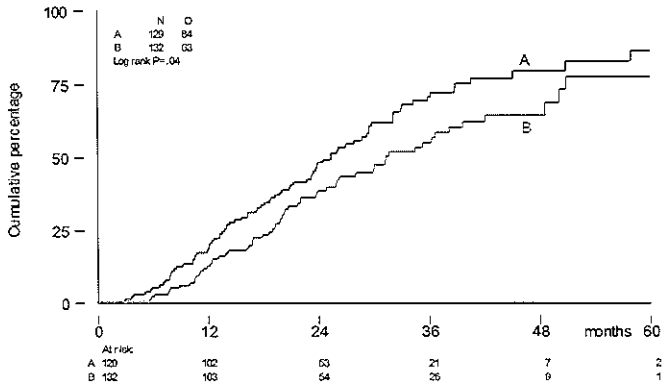

Figure 3. Kaplan-Meier curves of time to progression after intensified chemotherapy alone (A) and myelo-ablative therapy with autologous stem cell rescue (B).

TTP and OS were also calculated for randomized patients who were actually eligible for further treatment after evaluation of the last IDM (91 patients in the intensified chemotherapy group and 106 patients in the myelo-ablative therapy group). TTP from evaluation after IDM was median 22 months in the intensified chemotherapy group and 27 months in the myelo-ablative therapy group (P=0.02; hazard ratio = 0.64, 95% CI = [0.44, 0.94]). The median OS from date of evaluation of the last IDM was not significantly different between the 2 treatment groups (45 months vs. 46 months, P=0.11; hazard ratio = 1.48, 95% CI = [0.92, 2.36]).

Prognostic factors

Univariate Cox regression showed that stage III disease, hemoglobin ≤ 6.21 mmol/l, β_2-microglobulin > 3 mg/l after VAD, elevated LDH, plasma cell labeling index > 1% (all P<0.01), β_2-microglobulin > 3 mg/l at diagnosis and skeletal lesions (P<0.05) were associated with shorter EFS. Treatment arm was not of prognostic relevance. In the multivariate analysis only hemoglobin ≤ 6.21 mmol/l and elevated LDH remained statistically significant (P<0.001, Table 4).

It appeared that the prognostic factors for EFS were also predictive for TTP, in the univariate as well as in the multivariate analysis. This is not completely unexpected, as almost all patients achieved at least a PR before transplantation or start IFN (Table 5). In addition elevated calcium levels and randomization to intensified chemotherapy were adverse prognostic factors for TTP in the univariate analysis.

Univariate Cox regression showed that stage B disease, hemoglobin ≤ 6.21 mmol/l, β_2-microglobulin > 3 mg/l at diagnosis, elevated LDH, multiple skeletal lesions, β_2-microglobulin > 3 mg/l after VAD (all P<0.01), older age and stage III disease (both P<0.05) predicted for inferior OS from randomization. Hemoglobin ≤ 6.21 mmol/l, elevated LDH (both P<0.01), skeletal lesions, stage B disease and β_2-microglobulin > 3 mg/l at diagnosis (P<0.05) remained statistically significant in the multivariate analysis for OS (Table 6).

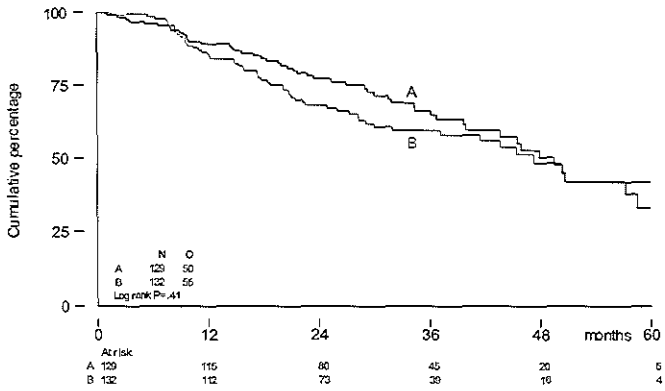

Figure 4. Kaplan-Meier curves of overall survival after intensified chemotherapy alone (A) and myelo-ablative therapy with autologous stem cell rescue (B).

Cytogenetic studies

Cytogenetic analysis was performed in 154/453 registered patients in designated centers. In 128 patients cytogenetic studies were successful. Chromosomal abnormalities were detected in 57/128 patients (45%) of whom 27 patients showed hyperdiploidy (47%), 14 showed hypodiploidy (25%), 6 were pseudodiploid (11%) and 10 were not classifiable (18%).

In 28 of 57 patients chromosome 1p/q abnormalities were found (49%). Monosomy 13 or deletions of 13q were present in 25 patients. There was a strong association between chromosome 1p/q abnormalities and those of chromosome 13 (P<0.001); nineteen patients had both abnormalities. In the multivariate analysis of this group of patients only chromosome 1p/q abnormalities predicted for inferior EFS (P=0.04), TTP (P<0.0001) and OS (P=0.003).

Toxicity and causes of death

Toxicity CTC grade 3 and 4 was observed in 9% of patients during the first cycle of IDM and in 5% during the second cycle of IDM. Mucositis, nausea, vomiting and hemorrhage were the most frequent side effects. The frequency of WHO grade 3 or 4 infections was 13% during both the first and the second cycle of IDM. Myelo-ablative treatment was associated with 45% grade 3 or 4 toxicity. Infection grade 3 or 4 was observed in 44% of the patients.

Treatment related mortality (TRM) was 0.8% after the first cycle and 0.5% after the second cycle of IDM. TRM within 3 months after myelo-ablative therapy was 3.9%.
Fifty patients in the intensified treatment group have died, 34 patients from relapse or progression, 3 from other malignancies, 5 from infections, 1 from interstitial pneumonia, 3 from hemorrhage and 4 patients from other reasons.
In the myelo-ablative therapy group 55 patients have died. Thirty-three patients died from relapse or progression, 4 from interstitial pneumonia, 9 from infections, 3 from respiratory insufficiency, 1 from hemorrhage and 5 from other reasons.

Table 4. Univariate and multivariate cox regression analysis of risk factors for event-free survival

Risk factor	Univariate analysis			Multivariate analysis		
	HR	95% CI	P-value	HR	95% CI	P-value
Stage III disease	1.6	1.1-2.4	0.009			
Hb ≤ 6.21 mmol/l	1.7	1.2-2.3	< 0.001	1.9	1.3-2.6	0.001
β_2-microglobulin > 3 mg/l	1.4	1.0-2.0	0.03			
LDH > ULN	2.2	1.5-3.3	< 0.001	2.5	1.6-3.8	<0.001
Plasma cells LI > 1%	2.6	1.3-5.2	0.005			
β_2-microglobulin > 3 mg/l after VAD	2.0	1.3-3.1	< 0.001			
Skeletal lesions (0 vs. 1-2 vs. ≥ 3)	1.2	1.0-1.5	0.03			
Myelo-ablative treatment	0.9	0.6-1.1	0.28			

Hematological recovery
After the first cycle of IDM, WBC reached ≥ 1.0 x 10^9/l after median 20 days (range: 0-61 days) and platelets ≥ 20 x 10^9/l after median 22 days (range: 0-61 days). After the second cycle of IDM, WBC recovery was identical. Platelet recovery however was slower: median 27 days (range: 0-126 days). Autologous stem cell reinfusion after myelo-ablative treatment resulted in rapid hematopoietic recovery: WBC reached ≥ 1.0 x 10^9/l after median 13 days (range: 0-48 days) and platelets reached ≥ 20 x 10^9/l after median 11 days (range: 0-145 days). Stem cell rescue was given in 8 patients after the first cycle of IDM (7 patients in the intensified chemotherapy group) and in 8 patients after the second cycle of IDM (7 patients in the intensified chemotherapy group) because of persistent bone marrow aplasia.

Quality of life assessment
During follow up quality of life was assessed at 3, 6, 9 and 12 months during the first year of follow-up. During this period functioning was worse in many domains and symptoms were more persistent after myelo-ablative therapy, with the following relevant differences remaining statistically significant even at 12 months: overall quality of life (P<0.05), role functioning (P<0.05) and social functioning (P<0.001). Patients had more financial problems after myelo-ablative treatment (P<0.05). More patients in the myelo-

ablative therapy group had complaints of pain (P<0.05), loss of appetite (P<0.05) and fatigue (P<0.001) when compared to the intensified chemotherapy alone.

Table 5. Univariate and multivariate cox regression analysis of risk factors for time to progression

Risk factor	Univariate analysis			Multivariate analysis		
	HR	95% CI	P-value	HR	95% CI	P-value
Stage III disease	1.9	1.2-2.8	0.004			
Hb ≤ 6.21 mmol/l	1.9	1.4-2.7	< 0.001	2.0	1.4-3.0	<0.001
Calcium > 2.65 mmol/l	1.7	1.1-2.6	0.02			
β_2-microglobulin > 3 mg/l	1.6	1.1-2.3	0.02			
LDH > ULN	2.1	1.3-3.2	0.002	2.2	1.3-3.5	0.002
Plasma cells LI > 1%	2.7	1.3-5.6	0.008			
β_2-microglobulin > 3 mg/l after VAD	1.9	1.2-3.1	0.006			
Skeletal lesions (0 vs. 1-2 vs. ≥ 3)	1.3	1.0-1.6	0.03			
Response on VAD	0.9	0.6-1.3	0.48			
Myelo-ablative treatment	0.7	0.5-1.0	0.04			

Cost analysis

Total costs were calculated from start of IFN maintenance treatment (intensified chemotherapy, n=35) or myelo-ablative treatment (myelo-ablative therapy following intensified chemotherapy, n=37) up to a median follow up of 865 days (range 165-1231) and 844 days (range 37-998; ns), respectively.

Table 6. Univariate and multivariate cox regression analysis of risk factors for overall survival

Risk factor	Univariate analysis			Multivariate analysis		
	HR	95% CI	P-value	HR	95% CI	P-value
Age (continuous)	1.03	1.0-1.07	0.03			
Stage III disease	1.9	1.1-3.2	0.01			
Stage B disease	2.6	1.5-4.7	0.001	2.2	1.0-4.8	0.04
Hb ≤ 6.21 mmol/l	1.8	1.2-2.7	0.004	2.0	1.3-3.2	0.003
β_2-microglobulin > 3 mg/l	2.3	1.5-3.5	< 0.001	1.6	1.0-2.6	0.04
LDH > ULN	2.4	1.5-3.9	< 0.001	2.1	1.2-3.5	0.007
β_2-microglobulin > 3 mg/l after VAD	3.2	1.9-5.3	< 0.001			
Skeletal lesions (0 vs. 1-2 vs. ≥ 3	1.4	1.1-1.9	0.006	1.4	1.0-1.9	0.03
Myelo-ablative treatment	1.2	0.8-1.7	0.41			

Total median costs were significantly higher after myelo-ablative therapy ($27,587; range $16,926-$154,408 vs. $20,180; range $6,220-$55,533; P=0.0001). This was particularly due to a higher number of hospital days (median 38; range 22-187 vs. 0; range 0-95; P=0.0001) of which 29 (range 20-123) were for the recovery hospitalization following the stem cell reinfusion. The most important cost item was a hematology isolation hospital day, at a unit cost of $397. Other important cost items that were significantly higher in after myelo-ablative therapy were costs of blood components ($1,255; range $0-$16,194 vs. $0; range $0-$9,954; P=0.0001), and costs of antibiotics ($1,161; $96-$16,570 vs. $3; $0-$2,976; P=0.0001).

Discussion

The observation that the achievement of a complete remission increases the probability of prolonged EFS and OS in MM has prompted several groups to explore dose-escalation regimens including double transplantation (21-23,32,33).

This randomized study was designed to evaluate whether patients who are treated with high-dose chemotherapy benefit from additional myelo-ablative treatment. Intensified chemotherapy was administered as 2 separate cycles of melphalan 70 mg/m^2 iv. (intermediate dose melphalan, IDM). Myelo-ablative treatment following intensive chemotherapy resulted in a higher CR rate and a longer TTP. EFS and OS however were not improved after a median follow up of 33 months from randomization.

The largest experience with repeated myelo-ablative treatments has been achieved at the University of Arkansas in the Total Therapy Program (32,33). It was observed that the response may increase from 65% after induction to 75% after the first and 83% after the second myelo-ablative treatment including 41% complete responses (33). In a comparative analysis with case matched registered controls receiving conventional chemotherapy, double transplantation was superior with regard to response rate (86% vs. 52%, P=0.0001), median EFS (49 vs. 22 months, P=0.0001) and OS (62 vs. 48 months, P=0.01) (32). However the value of repeated intensive therapy still needs prospective evaluation. In 1994 a randomized study was initiated in France to compare the efficacy of single with double transplantation. After a median follow up of 4 years EFS and OS were comparable (median 24 and 48 months in the single and 30 and 54 months in the double transplantation group) (21). Remarkably, only patients with good prognostic profile, i.e. a β_2-microglobulin < 3 mg/l had a significantly better OS after double transplantation (34). The first interim analysis of the Italian 1996 clinical trial in which single and double intensive therapy were compared in 178 patients did not show a significant difference in complete remissions or OS between both groups after a median follow up of 30 months (22). Preliminary results from the French group "Myélome Autogreffe" also did not show a significant benefit of double intensive therapy over single intensive therapy (23).

Other high-dose therapy studies have shown a longer overall survival than the overall survival observed in patients in our study (11,17,19,21,32). In the present study only patients with stage II and III disease (77% stage III) and patients with stage B disease were included, while patients with stage I disease were excluded. Furthermore the high-risk character of patients that were included in our study is reflected by the high percentage of patients with a β_2-microglobulin > 3 mg/l. In addition, cyclophosphamide and TBI as a myelo-ablative regimen in our study may not have an optimal anti-myeloma effect and may be inferior to high-dose melphalan as is used in most protocols today.

Recently a German study showed that the combination of TBI and cyclophosphamide is inferior to melphalan alone when used as a myelo-ablative regimen before the second transplantation (35). The use of a non-TBI conditioning regimen has also been related with prolonged PFS and OS by the European BMT registry (36). The use of TBI in our study may also have contributed to the reduced quality of life after myelo-ablative treatment when compared to intensified chemotherapy alone and the fact that higher number of patients had to stop IFN due to excessive toxicity.

Recently, a number of studies have shown that chromosome 13 deletions associated with high β_2-microglobulin are strong adverse prognostic factors for EFS and OS after conventional and high-dose therapy including tandem transplantation (33,37-39). In most of these reports, this cytogenetic abnormality has been detected by FISH, which is more sensitive than conventional chromosome techniques. In the present study, initiated in 1995, and solely based on cytogenetic analysis we found that in addition to chromosome 13 deletions, chromosome 1p/q abnormalities were of prognostic relevance with regard to EFS, TTP and OS. This prognostic significance of abnormalities of chromosome 1p/q for outcome in high-dose treatment has not, to our knowledge, been previously reported. In addition, we found a strong correlation between the presence of these two abnormalities, which indicates that the role of chromosome 13/13q deletions for the outcome of treatment in several studies may have to be reevaluated in this respect. However, our results indicate that the outcome of patients with unfavorable prognostic factors including chromosome 13 abnormalities and 1p/q abnormalities will not be improved by intensification alone.

In conclusion, intensified chemotherapy followed by myelo-ablative therapy results in a higher complete response rate and a longer time to progression, but it does not lead to a better event-free and overall survival when applied as first treatment in previously untreated multiple myeloma. In time, a longer follow up of all patients included in the study is awaited. Definition of prognostic factors like β_2-microglobulin and chromosomal aberrations may allow allocating patients in various prognostic subgroups to different therapeutic modalities in order to improve outcome.

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CHAPTER 5

Partially T–cell depleted allogeneic stem cell transplantation as part of first line treatment of multiple myeloma is inferior to intensive treatment alone. Results from a prospective comparison of patients treated in the phase III study HOVON 24 MM

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Submitted

Summary

We determined in a prospective study the efficacy, toxicity and long term outcome of upfront allogeneic stem cell transplantation (allo-SCT) in multiple myeloma (MM) in comparison with intensive treatment alone. In this prospective phase III HOVON 24 MM study, patients responsive to intensive induction treatment, received either interferon-α-2a maintenance (IFN) or a second intensification with myelo-ablative treatment and auto-SCT. Fifty-six patients with an HLA-identical sibling (median age at Tx 48 yrs, range 31-56) were allocated to a partial T-cell depleted allo-SCT after induction therapy. With a median follow-up of 29 months (range 9-48) of patients still alive since allo-SCT, 32 patients have died, 11 (20%) from progressive disease, 19 (34%) from TRM and 2 (4%) from other causes. As compared to an age matched group of 73 patients who were eligible after induction therapy for further treatment (IFN or auto-SCT), median PFS after allo-SCT was 18 months versus 21 months in the auto-SCT/IFN group (P=0.66). Time to progression tended to be somewhat longer after allo-SCT (median 25 vs. 22 months, P=0.08). Median OS after allo-SCT was 25 months versus 47 months in the auto-SCT/IFN group (P<0.001).

In conclusion, this first prospective evaluation of allo-SCT in MM in comparison with intensive treatment does not support the use of T-cell depleted myelo-ablative allo-SCT as part of first-line therapy.

Introduction

High dose chemo-radiotherapy has improved response rate dramatically in multiple mycelia (MM). Especially when applied early in the course of the disease, myelo-ablative treatment followed by autologous stem cell rescue may induce responses in more than 80% of the patients including a considerable number of patients with a complete response (CR) (1-4). So far only one phase III trial has been published indicating that intensive treatment may also improve overall survival (OS) compared to conventional chemotherapy (5). A better survival was also observed by the Nordic Myeloma Study Group which compared patients treated with melphalan 200 mg/m^2 with historical controls (6). However it remains questionable whether chemo-radiotherapy alone can eradicate the clonogenic myeloma cell. There is no plateau in the progression free survival (PFS) and OS curves following autologous stem cell transplantation (auto-SCT) and even patients in so-called CR continue to relapse. This is in accordance with the observation that molecular remissions after myelo-ablative therapy followed by auto-SCT are rare (7).

Recently the existence of a graft-versus-myeloma (GVM) effect was proven by the induction of remissions by donor lymphocyte infusions (DLI) in patients with relapsed MM after allogeneic stem cell transplantation (allo-SCT) (8,9). In a recent update of 27 patients, response to DLI was 52% and 30% of patients attained a CR. In 3 patients a molecular remission after DLI is now sustaining for more than 48 months, suggesting a curative potential of adoptive T-cell therapy in MM (10).

The necessity of performing allo-SCT in MM however is still disputed. Median OS in different reports varies from 18 to 28 months from transplantation (11-16). A survival advantage for patients receiving an allo-SCT compared to patients with the same characteristics treated with auto-SCT and no SCT at all has not been shown. In a retrospective case-matched analysis performed by the European Bone Marrow Transplantation (EBMT) registry the OS of patients receiving auto-SCT was significantly better than of allo-SCT patients. Only for patients alive at 1 year post-transplant OS and event-free survival (EFS) were prolonged after allo-SCT (17). A major reason for the poorer outcome of allo-SCT is the high rate of treatment related mortality (TRM, usually around 40%) which is not compensated for by a higher CR rate and lower relapse rate. An important factor responsible for the excessive toxicity of allo-SCT in MM may be the high percentage of pretreated and refractory disease and the relatively high age of patients included in published studies.

Since 1991 two intensive treatment protocols for MM were performed in The Netherlands and in Belgium under auspices of the Dutch-Belgian Hemato-Oncology Cooperative Study Group HOVON. In the recently closed phase III HOVON MM24 trial with 453 patients, interferon-α-2a (IFN) maintenance was compared to auto-SCT and IFN maintenance following intensive induction therapy with vincristine, adriamycin, dexamethasone (VAD) and intermediate dose melphalan (Melphalan 70 mg/m^2, IDM) (18,19). Patients under 56 years with an HLA-identical sibling could be allocated to allo-SCT after induction therapy. This approach was chosen in order to evaluate the efficacy of early allo-SCT on TRM and its possible favorable effect on long-term outcome in comparison with patients that received intensive treatment with or without auto-SCT.

Patients and methods

Patients and study design of HOVON 24 MM study

Four hundred and fifty-three patients were included in the HOVON 24 MM study between November 1, 1995 and April 1, 2000. Criteria for inclusion were previously untreated multiple myeloma stage II and III, WHO performance 0-3 and absence of severe cardiac, pulmonary, neurologic or metabolic disease. All patients gave written informed consent. The study was performed according to the Helsinki agreement. After registration patients received induction therapy with 3-4 courses of VAD, which was administered as rapid infusion (18).

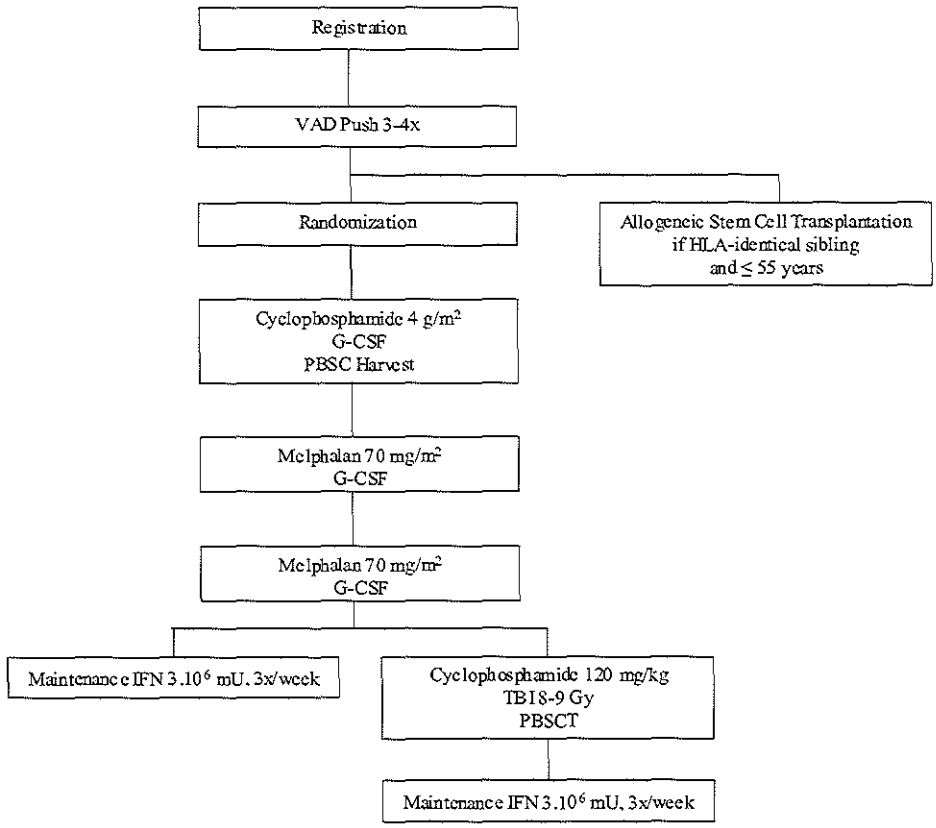

Figure 1. Outline of the study

After VAD, patients with WHO performance 3 or 4, severe cardiac/pulmonary disease, inadequate liver function and/or serum creatinine ≥ 177 µmol/l after IDM went off protocol treatment. HLA-typing of patients under 56 years of age was performed in the

first three months of treatment. Patients who had an HLA-identical donor could be allocated to undergo allo-SCT. The majority of these patients received further treatment with one or two courses of IDM before allo-SCT (see below: pretransplant therapies). Patients under 56 years without an HLA-identical donor and patients over 55 years were randomized after VAD to one of the 2 treatment arms irrespective their response. In Arm A, patients were to receive 2 cycles of IDM followed by maintenance therapy with IFN, 3 x 10^6 IU, thrice weekly until progression or relapse. In arm B, patients were to receive 2 cycles of IDM followed by myeloid-ablative treatment with cyclophosphamide 120 mg/m^2 (Cy) and total body irradiation (TBI) followed by autologous peripheral blood stem cell transplantation and maintenance therapy with IFN. In almost all randomized patients, peripheral blood stem cells were collected after remission induction with VAD following cyclophosphamide 4 g/m^2 and G-CSF. The outline of the HOVON 24 MM study is shown in Figure 1.

In February 2001 a first analysis was performed to present preliminary results to the Dutch National Health Council. In order to have complete data of the patients in that analysis (all patients should be in follow up or still be treated with IFN maintenance), it was decided to restrict the analysis to the 379 eligible patients who were registered before August 1, 1999. In November 2001 the results of these patients were updated using longer follow up data.

A total of 268 patients was randomized after VAD, but 7 of them were not eligible for randomization (4 due to renal insufficiency and 3 patients had an allogeneic donor which was not yet known at the date of randomization, as HLA family typing had not been completed yet). Fifty-four patients were planned to receive an allo-SCT, and the other 57 patients were not eligible for randomization for other reasons. Ultimately 56 patients received an allo-SCT (including 3 patients who were randomized at first; these are not the 3 patients mentioned before). Their results are compared to a control group of 73 patients who received 1 or 2 cycles of IDM, followed by auto-SCT and IFN maintenance, or IFN maintenance alone.

Eligibility criteria to start with auto-SCT or IFN were WHO performance 0-2, absence of severe cardiac, pulmonary, neurologic and psychiatric disease, adequate liver function, serum creatinine ≥ 177 µmol/l and responsive disease after the last IDM. Two patients in the control group still had no response after their last IDM; nevertheless they remained in the analysis. Moreover, patients in the control group had to be younger than 56 years at registration (the upper limit for allo-SCT) and they had to be registered in a center that subjected their patients to allo-SCT. Patient characteristics of both groups (allo-SCT vs. auto/IFN) are summarized in Table 1.

The reasons why the remaining 250 patients are not included in any of the 2 groups are that they were 56 years or older (n=172), were registered in hospitals that have not referred any patient for allo-SCT (n=51), went already off protocol treatment after VAD (n=13) or after cyclophosphamide priming (n=1). The remaining 13 patients received one (n=6) or two (n=7) cycles of IDM but did not proceed to auto-SCT or IFN, 10 due to excessive toxicity or rapidly progressive disease and 3 because they had no response after IDM.

Table 1. Patient characteristics

Characteristics	Allo-SCT (n=56)	Auto-SCT/IFN (n=73)	P-value
Sex			
Male	37	43	0.41
Female	19	30	
Age			
Median	48	50	0.28
Range	31-56	31-55	
Stage (Salmon & Durie)			
IIA	9	18	0.10
IIB	-	3	
IIIA	40	49	
IIIB	7	3	
WHO performance status			
0 - 1	41	55	0.81
2 - 3	14	17	
Unknown	1	1	
β_2-microglobulin (mg/l)			
0-3	23	39	0.33
>3	24	28	
Unknown	9	6	
Hemoglobin (mmol/l)			
≤ 6.21	21	25	0.70
> 6.21	35	48	
LDH			
Normal	50	55	0.34
Elevated	6	11	
Unknown	-	7	
M-protein heavy chain			
Ig A	9	20	0.08
Ig G	39	33	
Ig D	2	2	
LCD	4	11	
Non-secretor	2	6	
Plasma cells in BM (%) at diagnosis			
≤ 50	36	43	0.83
> 50	20	22	
Unknown	-	8	

Pretransplant therapies and myelo-ablative regimens in patients who underwent allo-SCT

The induction therapy consisted of 3-7 cycles of VAD only in 7 patients and of 3-4 cycles of VAD followed by 1 (n=26) or 2 cycles (n=23) of IDM in 49 patients. Inclusion criteria for allo-SCT, auto-SCT and IFN maintenance were identical with the exception that refractory disease was no exclusion criterion for allo-SCT. The pretransplant preparative regimen consisted of cyclophosphamide + TBI in 50 and cyclophosphamide + TBI + idarubicin (50 mg/m^2) in 5 patients. One patient received cyclophosphamide 120 mg/kg iv. and busulphan 16 mg/kg orally as conditioning. The schedule for TBI varied between different institutions. In 40 patients the total dose was 12 Gy, 1 patient received 10 Gy and 14 patients received 9 Gy. In 6 patients total lung dose is not known. Lungshielding was performed in all patients patients reducing lung dose to 6.0-8.5 Gy. Thirty-seven patients received a bone marrow graft and 18 patients received a peripheral blood stem cell transplant, which was harvested after 4 days stimulation with G-CSF 10 μg/kg s.c. One patient received a combined bone marrow and blood stem cell graft. All patients received their transplant from siblings, in 55 cases completely HLA-DR matched and in one case with 1 HLA locus mismatch.

Graft-versus-host-disease (GVHD) prophylaxis and evaluation.

Forty-eight patients received a partial T-cell depleted graft containing 1 to 7 x 10^5 T cells/kg. These patients received prophylactic immunosuppression consisting of cyclosporin only. In 7 patients in vitro T cell depletion was performed with campath (20). Acute and chronic GVHD were evaluated according to standard criteria (21).

CMV monitoring and treatment

CMV seropositive patients were monitored by the IEA assay once a week as described before (22). Pre-emptive ganciclovir therapy was initiated (5 mg/kg intravenously twice daily) if 4 or more positive leukocytes were identified or in case of GVHD grade II-IV for which high dose corticosteroids were prescribed. CMV disease was diagnosed as described before (19).

Response criteria

Complete remission was defined as complete disappearance of myeloma proteins from blood and/or urine as determined by immunofixation and normalization of the bone marrow including absence of monoclonal plasma cells assessed by immunophenotyping. Partial remission was defined as a decrease of more than 50% of myeloma proteins in the peripheral blood, a decrease in urinary light chains to less than 0.2 g/24 hours, combined with improvement of myeloma related symptoms like bone pain, anemia and hypercalcemia. Relapse from CR was defined as reappearance of myeloma proteins in serum and/or urine and/or recurrence of bone marrow infiltration. Progression from PR was defined as doubling of measurable myeloma proteins in 2 samples at least 4 weeks apart and/or doubling of bone marrow infiltration or progression of myeloma related symptoms.

Endpoints and statistical analysis

The data were analyzed as of November 15, 2001. Endpoints included response rate, progression free survival, time to progression and overall survival.

Progression free survival (PFS) was determined from transplantation or start IFN, whichever applicable, until progression/relapse or death, whichever came first.

Time to progression (TTP) was measured from either transplantation or start IFN until progression/relapse; patients with no date of progression specified who died of MM were considered to have progression at the date of death. Patients who died from other causes were censored at the date of death.

Overall survival (OS) was calculated from transplantation or start IFN until death. Patients still alive at the date of last contact were censored.

PFS, TTP and OS were estimated by the Kaplan-Meier method. Kaplan-Meier curves were generated to illustrate survival between the two subpopulations, and the logrank test was used to compare the survival curves.

Patient characteristics between the allogeneic transplanted patients and patients in the control group were compared using Pearson's chi-squared test or Fisher's exact test in case of discrete variables, whichever appropriate, or the Wilcoxon rank-sum test in case of continuous variables.

All reported P-values are two-sided and a significance level $\alpha = 0.05$ was used.

Results

Toxicity of allo-SCT

GVHD. Acute GVHD grade I was present in 20 patients, GVHD grade II was present in 18 patients and 7 patients had GVHD III-IV. Chronic GVHD could be evaluated in 47 patients. Limited chronic GVHD occurred in 5 patients and extensive in 12 patients.

Infections after transplantation

Infections WHO 2-4 occurred in 44 patients. Eleven patients received prophylactic treatment with ganciclovir or foscarnet. CMV reactivation was recorded in 15 patients. No CMV disease was reported.

Table 2. Treatment related mortality of allo-SCT

	Number of patients
Interstitial pneumonitis	5
Infections	7
GVHD	3
TTP	1
EBV lymphoma	1
VOD	1
Cardiomyopathy	1
Total	19

GVHD=graft-versus-host-disease;TTP=thrombotic thrombocytopenic purpura; EBV=Ebstein-Barr virus; VOD=veno-occlusive disease

Outcome of Allo-SCT

Response rate

Forty-seven patients were in remission before allo-SCT including 6 patients with a CR and 41 patients with a PR. Nine patients were refractory at the time of transplant. In 51 patients response could be evaluated 3 months after transplantation. The overall response after allo-SCT was 89% (50/56) including 18% (10/56) of patients with a CR. Six of 9 refractory patients responded (PR) to allo-SCT.

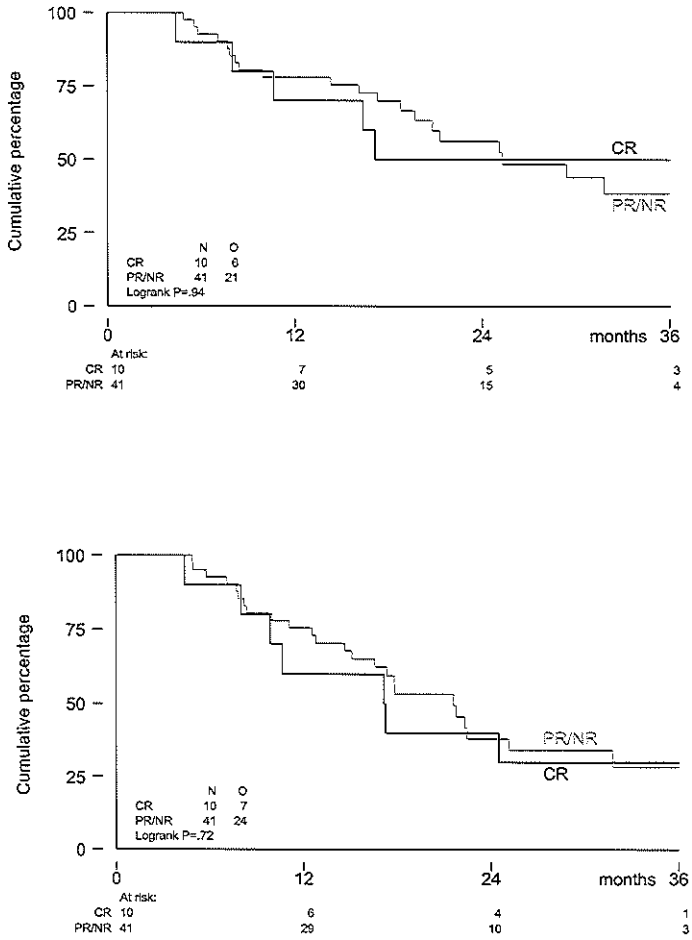

Figure 2. Kaplan-Meier survival curves of overall survival (fig 2A) and progression free survival (fig 2B) of patients treated with allogeneic stem cell transplantation. Response was determined 3 months after transplantation.

Survival

The median follow up of the 24 patients still alive is 29 months (range: 9-54 months); 32 patients have died. Eleven (20%) patients died from progressive MM, 19 (34%) patients from TRM and 2 (3%) patients from other causes (Table 2). Median progression free survival post-transplant was 18 months. Median overall survival was 25 months from transplantation and 30 months from start of initial therapy. Patients who were in CR 3 months after transplantation had no significantly longer OS or PFS compared to patients who were in PR or NR, see Figure 2.

Allo-SCT versus auto-SCT/IFN maintenance

The results of the patients who received an allo-SCT were compared to those of the patients in the control group.

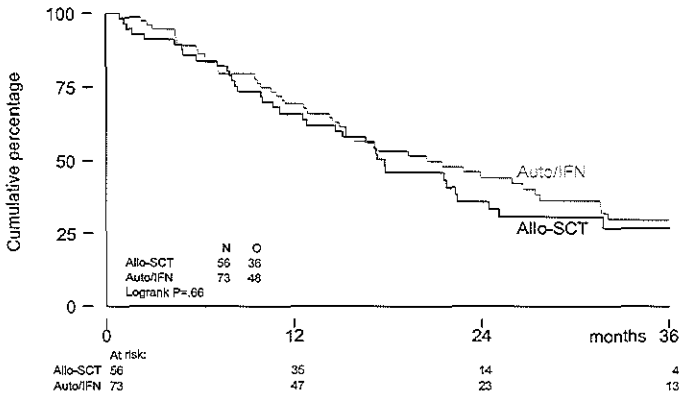

Figure 3. Kaplan-Meier survival curves of progression free survival. Progression free survival curves were determined from transplantation or start interferon (IFN).

Progression free survival

The median PFS in the allo-SCT group was 18 months versus 21 months in the control group (P=0.66, see Figure 3). The results hardly changed when the analysis was restricted to those patients who were at least in PR before SCT or start IFN: median 18 months in the allo-SCT group versus 22 months in the control group (P=0.52).

Time to progression

TTP tended to be somewhat better in the allo-SCT patients, though the median TTP was almost the same for both groups with 25 months in the allo-patients and 22 months in the control group (P=0.08). The Kaplan-Meier curves for the two groups are in Figure 4.

Overall survival

OS from SCT/start IFN for the allo-SCT patients versus the control group is shown in Figure 5. The median OS in the allo-group was 25 months and significantly shorter than the 47 months in the patients who received an autologous transplantation and/or IFN

maintenance (P<0.001). Even if the analysis was repeated without the non-responsive patients, the median survival times remained the same (P=0.001).

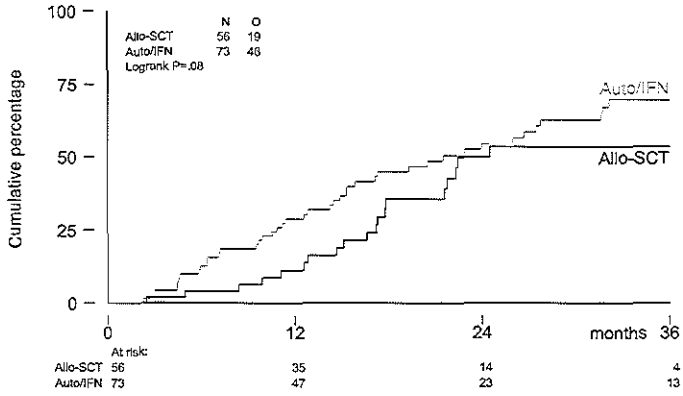

Figure 4. Kaplan-Meier estimates of time to progression. Time to progression was measured either from transplantation or start IFN, whichever applicable, until progression/relapse

Discussion

This is the first prospective study in which the results of allo-SCT in previously untreated multiple myeloma have been evaluated with other treatment options including auto-SCT and IFN maintenance. Patients up to 55 years of age participating in the HOVON 24 MM study with an HLA identical family donor could be allocated to allo-SCT. Allo-SCT was performed after induction therapy with VAD or after VAD and IDM and a comparison was made with an age-matched group of patients who had no HLA-identical family donor, who were registered in a hospital that referred for allo-SCT and who had received auto-SCT or IFN. Patient characteristics in both groups were comparable. Results show that the outcome with respect to overall survival is inferior after allo-SCT. Median survival after allo-SCT is 25 months or 30 months since start of therapy. In contrast, patients who had no HLA-identical donor survived median 47 months since auto-SCT or start of IFN maintenance. Not unexpected, an important reason for the inferior outcome after allo-SCT is the TRM (34%). In this study TRM is somewhat lower than usually reported for myeloma in the relapse setting but higher than TRM of T-cell depleted allo-SCT in other hematological malignancies (11,13,17,23-25). Excessive toxicity continues to be a major problem in MM even when applied in patients with a good performance status transplanted at a median interval of 8 months after diagnosis. The second reason for the inferior outcome is that the high TRM is not compensated for by a lower relapse rate, at least in the first 2 years following allo-SCT. Time to progression after allo-SCT tended to be somewhat longer but was not statistically different from IFN maintenance or auto-SCT. This indicates that either there is no important graft-versus-myeloma (GVM) effect induced after allo-SCT as part of front line therapy or it does not affect the relapse rate sufficiently enough to detect an effect in this group of patients. It may also be that, like in the retrospective EBMT study, the GVM effect becomes only obvious after a

prolonged follow-up (17). This lack of GVM may be due to the fact that a partial T-cell depleted graft is used in this study and the outcome may be different for patients receiving a full allograft. In a previous EBMT analysis on prognostic factors however the outcome of patients receiving full or T-cell depleted allografts were identical (13).

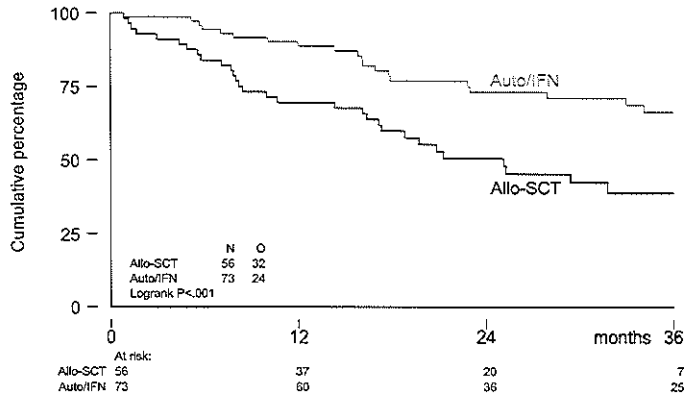

Figure 5. Kaplan-Meier estimates over overall survival. Overall survival was determined from transplantation or start IFN until death

Also in patients with low-grade lymphomas and acute leukemias PFS and OS after partial T-cell depleted allo-SCT or non-T cell depleted allo-SCT are comparable (26). Only in patients with CML the relapse rate is significantly higher after T-cell depletion (26). A recent update of the EBMT registry showed a remarkable improvement in outcome after allo-SCT for multiple myeloma over the last decade mainly due to the reduction of TRM (27). A plateau in the survival curve was not obvious although the majority of these patients received full allografts. In this study no comparison was made with patients receiving intensive treatment alone.

Well known prognostic factors for myeloma and for outcome after allo-SCT like acute or chronic GVHD, stem cell source, CMV status of patient and donor had no impact on PFS and OS (results not shown), but this may well be due to the fact that our group is too small (22,28,29). However it may also indicate that multiple myeloma itself is a strong negative prognostic factor for allo-SCT. A comparison with a matched group of allo-SCT patients with other hematological malignancies might confirm this assumption.

Our results indicate that there is no indication for standard allo-SCT as part of front-line therapy for myeloma due to the combination of a high TRM and an obvious lack of GVM. Alternative approaches like nonmyelo-ablative stem cell transplantation are now being explored including patients with a deletion of chromosome 13 or with refractory and relapsed disease (29-31). Important in those approaches may be the introduction of prophylactic donor lymphocyte infusions as this strategy has been shown to be highly effective in relapsed patients after allo-SCT, especially in patients with a low tumor burden after response to reinduction therapy (9).

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CHAPTER 6

Feasibility and efficacy of melphalan 140 mg/m^2 divided over 2 gifts without stem cell rescue for remission induction of previously untreated multiple myeloma

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Submitted

Summary

We explored prospectively the feasibility and efficacy of melphalan 140 mg/m^2 divided over 2 doses of 70 mg/m^2 (intermediate dose melphalan (IDM)) without stem cell rescue in 254 patients with multiple myeloma after initial induction treatment with VAD. The objective was to achieve a high response rate at the cost of minimal toxicity while avoiding stem cell rescue and possible tumor cell infusion.

While IDM I was administered to 254 patients, 206 patients received the second cycle of IDM. The initial response rate after VAD has been 70% (3% complete response (CR)). The response rate improved to 84% after the IDM I (CR: 7%) and to 89% after of IDM II (CR: 13%). Non-hematological side effects CTC grade 3 to 4 were observed in 9% and 5% of the patients after IDM I and IDM II, respectively. WHO grade 3 to 4 infections occurred in 13% of the patients after IDM I and IDM II. Treatment related mortality was < 1%. Because of delayed recovery rescue stem cells had to be reinfused in 8 patients after IDM I and 8 patients after IDM II.

In conclusion, 2 cycles of IDM without stem cell rescue is feasible and effective in previous untreated patients with multiple myeloma and can be applied in the outpatient setting.

Introduction

With the introduction of melphalan and prednisone the median survival of patients with stage II/III multiple myeloma has improved from 17 to 30-36 months but complete remissions remain rare (1-4). The introduction of the VAD regimen (vincristine, adriamycine and dexamethasone) significantly improved the response rate, which is generally rapidly achieved, but did not result in prolonged survival (5). In an attempt to improve the long-term survival McElwain and Powles were the first to explore the feasibility of dose-intensification, using high-dose melphalan administration (HDM) (6). After melphalan 140 mg/m^2 given intravenously as a single treatment, the overall response rate was 76 to 84%, with approximately 30% of previously untreated patients achieving a complete remission. However, the median duration of remissions obtained with HDM was only 16 to 19 months (7-10). The toxicity was severe and consisted of sustained myelosuppression and extensive mucositis. For this reason most centers now employ HDM only with stem cell rescue (11-13). We have first explored the possibility to subdivide HDM into two cycles of melphalan 70 mg/m^2 (IDM) in short intervals with the objective to maintain the anti-myeloma activity of HDM while reducing the toxicity. Eighteen of 21 previously untreated patients responded, 14 patients (67%) achieved a partial remission and 4 patients (18%) achieved a complete remission, with acceptable toxicity and moderate bone marrow suppression. None of the patients required stem cell rescue because of prolonged marrow hypoplasia (14). These results prompted to investigate IDM as part of the induction regimen in untreated patients. We have explored the feasibility and efficacy of successive treatment with 2 cycles of intermediate dose melphalan 70 mg/m^2 without stem cell rescue in patients with multiple myeloma who had received induction treatment with VAD. The objective was to achieve a high response rate of induction therapy at the cost of minimal toxicity, while avoiding the need for stem cell rescue.

Patients and methods

All patients were prospectively included in the treatment protocol from November 1995 to April 2000. Patients had stage II and III A/B disease according to the Salmon and Durie criteria and were under 66 years of age (15). Patients with WHO performance 4 were excluded. Severe cardiac, pulmonary, neurologic, liver and metabolic disease was not permitted. All patients gave written informed consent. The study was conducted according to the Helsinki agreement. Patient characteristics are shown in Table 1.

Study protocol

VAD

Patients were initially treated with 3 or 4 cycles of VAD for remission induction. This regimen consisted of vincristine 0.4 mg and doxorubicin 9 mg/m^2, both administered by rapid intravenous infusion in 30 minutes in 100 cc NaCl 0.9% via a peripheral intravenous catheter for 4 consecutive days in the outpatient clinic as described before (16). Dexamethasone was given orally at a standard dose of 40 mg on days 1-4 and in addition on days 9-12 and 17-20 during uneven cycles of VAD. The cycles were repeated every 4 weeks. Antibacterial and antifungal prophylaxis was given according to local guidelines.

Peripheral blood stem cell harvest

Peripheral blood stem cells were collected after the administration of cyclophosphamide 4 g/m^2 and recombinant human granulocyte colony-stimulating factor (G-CSF, filgrastim, Amgen Thousand Oaks, Ca) for possible later use. A minimum harvest of 5 x 10^6 CD34$^+$ cells/kg and/or 10 x 10^4 CFU-GM/kg was required. This allowed rescue treatment in case of prolonged aplasia after IDM. In case of insufficient stem cell collection after cyclophosphamide, the procedure could be repeated after IDM 1 or bone marrow cells were harvested (2 x 10^8 nucleated cells).

Intermediate dose melphalan

Two cycles of intermediate dose melphalan (IDM, 70 mg/m^2) were administered without stem cell rescue at 6 weeks after cyclophosphamide. Treatment was administered in the outpatient clinic. The maximum allowed interval between the 2 cycles of IDM was 8 weeks. IDM was given as a slow bolus injection (30 minutes) with hyperhydration and forced diuresis if necessary. Anti-emetics were given before the infusion of IDM. Antibacterial and antifungal prophylaxis was given for the duration of hypoplasia. Recombinant G-CSF (filgrastim, 300 μg/day subcutaneously for patients ≤ 75 kg, in all other patients 480 μg/day) was started on day + 4 after melphalan and continued until the neutrophil count was recovered to ≥ 1.0 x 10^9/l. Prophylactic platelet transfusions were given when the platelet count dropped below 10 x 10^9/l. A second cycle of IDM followed by G-CSF was administered 6-8 weeks after the first cycle except when excessive (bone marrow) toxicity occurred after the first cycle (neutrophils ≤ 0.5 x 10^9/l at day 30 and/or platelets ≤ 50 x10^9/l at day 42). In case the patient had insufficient recovery of the blood cells at day 42, reinfusion of the peripheral stem cell graft was allowed as rescue treatment.

Evaluation of response

Complete response (CR) was defined as a complete disappearance of M-protein in serum and/or 10 times concentrated urine by immunofixation analysis and less than 5% plasma cells with no abnormal morphology in bone marrow smears. Monoclonal plasma cells had to be absent by immunofluorescence staining. Partial response (PR) was defined as a minimum decrease of 50% of M-protein in serum and/or urine or more than 50% reduction of bone marrow infiltration in case of non-secretory myeloma.

Criteria of toxicity

The grading of toxicity was done by using the World Health Organization Common Toxicity Criteria CTC version 1984. Side effects with CTC grade 2 or more were documented. The World Health Organization Criteria were used for the grading of infections.

Statistical analysis

The data were analyzed as of November 19, 2001. The endpoints included response rate and hematological recovery.

Table 1. Patient characteristics at time of diagnosis

Characteristics	All registered patients (n=379)	Patients planned to receive IDM (n=261)
Age in years (median, range)	55 (31-65)	55 (32-65)
Male/female	235 (62%)/144 (38%)	155 (59%)/106 (41%)
WHO performance:		
0 – 1	297	215
2 – 3	78	44
missing data	4	2
Stage (Salmon & Durie)		
IIA	75	59
IIB	5	2
IIIA	259	182
IIIB	40	18
Monoclonal protein		
IgA	98	74
IgG	215	148
IgD	9	6
LcD	42	22
Non-secreting myeloma	15	11
Hemoglobin ≤ 6.21 mmol/l	136	88
Serum calcium >2.65 mmol/l	61	36
Serum LDH > ULN	55	37
Serum β_2-microglobulin		
0 – 1 mg/l	6	4
1 – 3 mg/l	149	113
> 3 mg/l	170	109
Bone marrow plasma cells		
≤ 50 %	221	154
> 50 %	125	83
missing data	33	24
Number of skeletal lesions		
0	75	55
1 – 2	59	41
≥ 3	243	165

Hematological recovery was calculated from the start of each cycle of IDM until the patient had recovered. Patients who died without recovery were censored at the date of death. Recovery was also determined from the day the blood value dropped below the specified levels and estimated by the Kaplan-Meier method.

Results

Treatment feasibility

Three hundred and seventy-nine patients were included in the protocol. Patient characteristics are listed in Table 1. Of these 359 patients actually received 3 cycles (n=318) or 4 cycles of VAD (n=41). One hundred and eighteen went off study because of allogeneic transplantation (n=54), death (n=16), progression (n=10) or other (n=38). Two hundred and fifty-eight patients received cyclophosphamide and G-CSF for stem cell harvest. Peripheral stem cells were collected in 248 patients. The median number of $CD34^+$ cells in the autograft was 8.6 x 10^6/kg (range: 0-76.7 x 10^6/kg). In 242 patients the graft was considered adequate.

Table 2. PR and/or CR reached on protocol

		PR and CR	CR
VAD	(n=261)	70%	3%
IDM I	(n=254)	84%	7%
IDM II	(n=206)	89%	13%

PR=partial remission; CR=complete remission; VAD=vincristine, doxorubicin, dexamethasone; IDM=intermediate dose melphalan

Two hundred and sixty-one patients were planned to receive IDM. The first cycle of IDM was actually administered to 254 patients and 206 patients received the second cycle of IDM. Seventy-nine percent of all patients who were planned to be treated with two cycles of IDM actually received these 2 cycles. In 7 patients IDM was not given because of progressive disease (n=2), excessive toxicity (n=3) or refusal (n=2). Forty-eight patients received just one cycle of IDM because of progressive disease (n=8), excessive toxicity or poor performance (n=31), refusal (n=2), misunderstanding of the physician (n=5) and allogeneic transplantation after one cycle of IDM (n=2).

Response rate

The overall response rate (PR and CR) of all 379 registered patients on VAD was 63%. Nine patients achieved a CR (2%) and 229 patients (60%) a PR. Twelve patient died before response evaluation, 30% of the patients had no response and 2% had progressive disease. The response rate on VAD of the patients who were planned to receive IDM was 70% (3% CR).

The first cycle of IDM was given in 254 patients and the response rate improved on an intention-to-treat basis to 84%. Eighteen patients achieved a CR (7%) and 200 patients a PR (77%). Two hundred and six patients received a second cycle of IDM. The overall response after the second cycle of IDM was 89%, of which 35 patients had a CR (13%) and 198 patients a PR (76%). PR and CR rates are shown in Table 2.

Toxicity

During the VAD chemotherapy 26% of the patients experienced CTC grade 2 and 14% of the patients grade 3 and 4 side effects, of which hyperglycemia and neurotoxicity were the most prominent.

CTC grade 2 to 4 toxicity was observed in 38% of the patients who received the first cycle of IDM. In 9% of the patients this was grade 3 or 4 toxicity. Of the 206 patients who received IDM II, 31% experienced toxicity CTC grade 2 to 4 of which CTC grade 3or 4 side effects in 5% of the patients. Nausea and vomiting were present in 10% and 7% of the patients after the first cycle of IDM and 11% and 8% after the second cycle of

Table 3. Non-hematologic toxicity CTC 2-4 grade after intermediate dose melphalan (IDM) cycles I and II (%)

	Treatment period	
	IDM cycle I (n=254)	IDM cycle II (n=206)
Liver	2	1
Mucositis	9	6
Nausea	11	11
Vomiting	7	8
Diarrhea	4	3
Renal	2	1
Fever	4	3
Allergy	2	2
Cutaneous	6	3
Cardiac	2	1
Neurologic	2	2
Other	11	11
Maximum CTC 2	28	26
Maximum CTC 3	7	4
Maximum CTC 4	2	1

IDM. Mucositis grade 2 to 4 was present in 9% of the patients after IDM I and 6% after IDM II. Infections WHO grade 2 were present in 18% of the patients who received the first cycle of IDM and in 17% of patients who received the second cycle of IDM. Thirteen percent of the patients had grade 3 or 4 infections after the first cycle of IDM and 13% of the patients after the second cycle of IDM. Grade 4 infections occurred in 3 patients (1%) after the first cycle of IDM and in 3 other patients (1%) after the second cycle of IDM.

Bacteremia, pulmonary infection and infection of the ear-nose-throat region, mucosa and skin were the most common infections seen. Two patients died after the first cycle of IDM and one patient after the second cycle of IDM from treatment related mortality.

Table 4. Infections WHO grade 2-4 after intermediate dose melphalan (IDM) cycles I and II in precentages

	Treatment period	
	IDM cycle I (n=254)	IDM cycle II (n=206)
Septicemia	9	8
Lung	6	4
Ear/nose/throat	7	5
GI tract	2	1
GU tract	2	1
Mucosa	5	2
Skin	7	9
Fever	8	10
Other	2	1
Maximum WHO 2	18	17
Maximum WHO 3	12	12
Maximum WHO 4	1	1

GI tract=gastro-intestinal tract; GU tract=genito-urithral tract

The non-hematological side effects and incidence of infections in percentages of the patients per treatment period are presented in Tables 3 and 4. Sites of infection and causative agents of these infections are listed in Table 5.

Hematological recovery
After start of the first cycle of IDM, white blood cell (WBC) counts reached a level of ≥ 1.0 x 10^9/l after a median of 20 days (range: 0-61 days) and neutrophil counts reached 0.5 x 10^9/l at a median of 20 days (range: 0-69 days). WBC counts were below 1.0 x 10^9/l for a median duration of 10 days. Platelet counts reached a level of ≥ 20 x 10^9/l after a median of 22 days (range: 0-61 days). Platelets were below 20 x 10^9/l for a median duration of 10 days.
Hematological recovery after start of the second cycle of IDM was for white blood cells not different from the first cycle: WBC counts reached a level of ≥ 1.0 x 10^9/l after a median duration of 19 days (range: 0-49 days), neutrophil counts reached 0.5 x 10^9/l after a median of 20 days (range: 0-107 days). Platelet recovery was slightly slower: platelet counts reached 20 x 10^9/l after a median of 27 days (range: 0-126 days). White blood cell counts were below 1.0 x 10^9/l for a median duration of 10 days and platelets were below 20 x 10^9/l for a median duration of 14 days. Median hematological recovery is shown in Table 6. Because of prolonged bone marrow aplasia, stem cells had to be reinfused in 8 patients after the first cycle of IDM and in 8 patients after the second cycle of IDM.

Table 5. Number of causative agents of infections

Site	Agent of infection	Treatment period	
		IDM cycle I	IDM cycle II
Septicemia	Gram-positive	12	8
	Gram-negative	7	6
	Fungi	1	1
	Combined	4	2
Lung	Gram-positive	1	-
	Gram-negative	2	1
	Fungi	4	1
	Combined	1	1
	Unknown	7	5
Ear/nose/throat	Gram-positive	3	3
	Gram-negative	1	1
	Fungi	2	3
	Virus	4	-
	Combined	-	1
	Unknown	8	2
GI tract	Gram-positive	1	-
	Gram-negative	1	1
	Fungi	1	-
	Protozoa	-	1
	Unknown	1	-
GU tract	Gram-negative	4	1
	Unknown	1	1
Mucosa	Gram-negative	1	-
	Fungi	5	1
	Virus	2	-
	Combined	1	1
	Unknown	3	2
Skin	Gram-positive	-	2
	Gram-negative	1	-
	Fungi	-	1
	Virus	13	13
	Unknown	3	3
Other	Gram-positive	1	-
	Gram-negative	1	-
	Virus	-	1
	Unknown	3	1

GI tract=gastro-intestinal tract; GU tract=genito-urithral tract

Discussion

McElwain and Powles were the first to use high-dose Melphalan 140 mg/m^2 (HDM) without stem cell support in an attempt to induce more CR's and thereby to improve survival (6). These high response rates have encouraged further exploration of HDM in previously untreated and refractory patients (Table 7). With 140 mg/m^2 melphalan the overall response rate in previously untreated patients is 76-84% with a CR rate of 27-46% (7-10). A maximum progression free survival of 19 months is observed and the overall survival with HDM is 36 to 47 months. Even in refractory patients the overall response rate is 44-66% with 7-21% complete remissions although the progression free survival and overall survival are short (3-6 months and 3-17 months, respectively) (7,9,17). These high remission rates can be achieved at the cost of severe toxicity. Myelo-suppression is severe especially in refractory patients and the treatment related mortality (TRM) is high (7-19%) mainly due to overwhelming septicemia and hemorrhage. Up to 55% of patients have a period of documented bacteremia (9). Nausea, vomiting, mucositis and diarrhea are the most frequent recorded non-hematological side effects. However, Cunningham et al. described an improvement of the quality of life in 89% of the patients in terms of bone pain and in 92% in terms of performance status (10).

Table 6. Median hematological recovery in days (range) after intermediate dose melphalan (IDM) cycles I and II

	Treatment period	
	IDM cycle I	IDM cycle II
WBC > 1.0 x 10^9 /l	20 (0-61)	19 (0-49)
ANC > 0.5 x 10^9 /l	20 (0-69)	20 (0-107)
ANC > 1.0 x 10^9 /l	22 (0-69)	22 (0-107)
Platelets > 20 x 10^9 /l	22 (0-61)	27 (0-126)
Platelets > 50 x 10^9 /l	28 (0-90)	36 (0-189)

IDM=intermediate dose melphalan; WBC=White Blood Cell Count; ANC=Absolute Neutrophil Count

Several attempts have been made to reduce the excessive morbidity and mortality associated with HDM by using growth factors such as granulocyte-macrophage colony-stimulating factor (GM-CSF) and granulocyte colony-stimulating factor (G-CSF) (11,18). Barlogie et al. tried to reduce the toxicity and duration of myelosuppression in 23 patients with refractory multiple myeloma with GM-CSF administered after melphalan 100 mg/m^2 (19). Nausea, vomiting and diarrhea were seen in 8 patients. Nine patients aged over 50 years with a long history of prior therapy required 63 days for the recovery of platelets above 50 x 10^9/l and 31 days for recovery of neutrophils above 0.5 x 10^9/l. When compared to this group, 14 patients without these adverse characteristics had more rapid recovery (26 days and 21 days, respectively). In historic controls who were not treated with GM-CSF after HDM, recovery always exceeded 5 weeks and TRM was 9% (11).
Moreau et al. also reported a significant reduction of duration of neutropenia while administering GM-CSF after melphalan 140 mg/m^2 in previously untreated patients compared to HDM alone (neutropenia median 23.5 vs. 29 days, P=0.05) but the infectious toxicity and TRM remained high (12%) (18).

Table 7. High-dose Melphalan (Mel) without Stem Cell Support in Multiple Myeloma

Author	Number of patients	Regimen	Response CR+PR (CR)	Median Recovery of Neutrophils (ANC) or White Blood Cells (WBC) x 10^9/l, in days	Median Recovery of Platelets (Pl) x 10^9/l, in days	Treatment Related Mortality	Median survival in months
Mc Elwain (6)	5 PU	Mel 140 mg/m^2	100% (60%)	ANC < 1.0: 27	-	0%	PFS 10 ; OS 18
	4 PT	Mel 140 mg/m^2	100% (25%)	ANC < 1.0: 45.7	-	0%	PFS 9 ; OS 12
Selby (7)	41 PU	Mel 140 mg/m^2	78% (27%)	WBC > 1.0: 28	Pl > 25: 24	19%	PFS 19
	15 RF	Mel 140 mg/m^2	66% (13%)	WBC > 1.0: 42	Pl > 25: 37	13%	PFS 6
Barlogie (17)	43 RF	Mel 70-100 mg/m^2	44% (7%)	ANC > 0.5: 28-35	Pl > 50: 28-35	19%	PFS 3 ; OS 5
Lokhorst (8)	13 PU	Mel 140 mg/m^2	84% (46%)	ANC > 0.5: 30	Pl > 30: 31	15%	OS 36+
Harousseau (9)	53 PU	Mel 140 mg/m^2	76% (28%)	ANC < 0.5: 23	Pl < ?: 17	10%	OS 37
	44 PT/RF	Mel 140 mg/m^2	66% (20.5%)	ANC < 0.5: 30	Pl < ?: 32	7%	OS 17
Cunningham (10)	63 PU	Mel 140 mg/m^2	82% (32%)	-	-	14%	PFS 18 ; OS 47
Barlogie (19)	23 RF	Mel 100 mg/m^2 + GM-CSF	75% reduction in 39% of pat.	ANC > 0.5:25	Pl > 50: 32	9%	PFS 7 ; OS 10
Moreau (18)	102 PU	Mel 140 mg/m^2					
		+ GM-CSF (n=69)	52%	ANC < 0.5: 24	-	11.5%	-
		- GM-CSF (n=33)	57%	ANC < 0.5: 29	-	6%	-
Lokhorst (29)	62 PU	Mel 70 mg/m^2 x 2	85% (10%)	ANC ≤ 0.5: 8	Pl < 20:6	0%	-

PU=previously untreated; PT=previously treated; RF=refractory; GM-CSF=Granulocyte-Macrophage Colony-Stimulating Factor; PFS=progression free survival; OS=overall survival

These studies indicate that myelosuppression caused by HDM can be reduced by using growth factors in patients with an adequate stem cell reserve (19).
Alternatively the duration of myelosuppression after HDM can be reduced by autologous stem cell rescue but this includes the risk of myeloma cell infusion (Table 8). Using HDM with autologous bone marrow in previously untreated patients the overall response is 74-98% with 50-75% CR's and time to recovery is shortened, while there is no significant TRM during the period of aplasia (20-22). The median overall survival was more than 36 months in these studies. Autologous peripheral blood stem cells are now the most widely used source of stem cells, as time to recovery is shorter when compared to autologous bone marrow. In addition peripheral blood stem cells can be easily obtained and blood derived stem cell harvests may have a lower rate of tumor contamination (22,23). The use of melphalan 200 mg/m^2 with peripheral blood stem cell rescue results in a high response rate of 75% to 97% with a short period of myelosuppression and is associated with a low TRM (12,13,22,24,25). Even in elderly patients (55-75 years) using stem cell support repeated doses of melphalan 100 mg/m^2 with stem cell support is feasible and effective (response 88% after 3 cycles) with limited toxicity and no TRM (26). When melphalan 200 mg/m^2 with stem cell rescue is used as first regimen in double transplantation programs a second transplantation is possible in 65-71% of patients with low TRM (24,25). This large number of studies shows that high-dose melphalan without stem cell support is highly effective in multiple myeloma even in refractory patients but that it is associated with extensive toxicity. Measures to reduce the duration of myelosuppression, such as hemopoietic growth factors or stem cell rescue may reduce the morbidity and TRM related to HDM.
We have explored the possibility to subdivide HDM 140 mg/m^2 into 2 separate dosages of melphalan 70 mg/m^2 iv. without stem cell rescue after induction with VAD in newly diagnosed multiple myeloma patients. The goal of this study was to reduce the toxicity but to maintain the anti-myeloma activity of HDM. A second objective of this approach was to eliminate the risk of tumor cell infusion. Although the relevance of malignant plasma cells in stem cell grafts for relapse is still under discussion, the contribution of infused myeloma cells for relapse might be avoided with this approach (27,28). In order to reduce the duration of myelosuppression, G-CSF was given after each cycle of IDM until leucocyte recovery. With the use of antibacterial and antifungal prophylaxis and prophylactic platelet transfusions when necessary during the period of aplasia after IDM patients could be treated in the outpatient clinic without hospitalization. Repeated cycles of IDM with G-CSF were effective and well tolerated by this group of previously untreated patients. The response rate after induction with VAD increased to 89% after two cycles of IDM, which is comparable to the results of HDM alone (12,13).
The duration of myelosuppresion after IDM was acceptable. Neutropenia $< 0.5 \times 10^9$/l was observed for 10 days and recovery to levels $> 0.5 \times 10^9$/l occurred after median 20 days. Platelets recovered to 20×10^9/l in 22 days after the first cycle of IDM and were below this level for 10 days. Platelet recovery was slightly slower after the second cycle of IDM (27 days and below 20×10^9 /l for 14 days). Rescue with stem cells because of prolonged bone marrow aplasia was necessary in only 8 patients after the first cycle of IDM and 8 patients after the second cycle of IDM. Hematological recovery was more rapid when compared to HDM without support of stem cells or hematopoietic growth

Table 8 High-dose Melphalan (Mel) with Stem Cell Support in Multiple Myeloma

Author	Number of patients	Regimen	Response CR+PR (CR)	Median Recovery: Neutrophils (ANC) or White Blood Cells (WBC) x 10^9/l, in days	Median Recovery: Platelets (Pl) x 10^9/l, in days	Treatment Related Mortality	Median survival in months
Gore (21)	50 PU	Mel 140 mg/m^2					
		+ ABMT (n=28) or	74% (50%)	WBC < 1.0: 20	Pl > 20: 34	0%	OS 3 years: 80%
		- ABMT (n=11)	95% (64%)	WBC < 1.0: 30	Pl > 20: 27		
Cunningham (20)	53 PU	Mel 200 mg/m^2 + ABMT	98% (75%)	WBC < 1: 12	Pl < 25: 13	0%	PFS 20 ; OS 31+
Rajc (22)	63 PU	Mel 200 mg/m^2					3 years:
		+ ABMT (n=26)	92% (84%)	ANC > 0.5: 22	Pl > 50: 33		PFS 54%; OS 77%
		+ PBSCT (n=37)	97% (70%)	ANC > 0.5: 19	Pl > 50: 19		PFS 58%; OS 85%
Vesole (30)	72 PT	Mel 200 mg/m^2 + PBSCT (n=56)	65% (29%)	-	-	7%	PFS 11 ; OS 19
Barlogie (24)	231 PU	Mel 200 mg/m^2	75% (26%)	ANC ≤ 0.5: 7	Pl < 50: 7	1%	-
		+ PBSCT (n=195)	86% (32%)				
Palumbo (26)	71 PU	Mel 100 mg/m^2 x 3 + PBSCT (n=71,n=68,n=63)	88% (47%) after 3 x Mel	ANC < 0.5: 5-4-4	Pl < 25: 2-2-1	0%	EFS 34 ; OS 56+
						1%	EFS 27
Lenloff (12)	214 PU	Mel 200 mg/m^2 + PBSCT	75%				4 years OS 61%
Cavo (25)	81 PU	Mel 200 mg/m^2 + PBSCT	CR 22%	-	-	6%	EFS 22 4 years OS 65.5%
Moreau (13)	142 PU	Mel 200 mg/m^2 + PBSCT	-	neutropenia 10 days	thrombocytopenia 8 days	0%	EFS 21 ; 45 months OS 65.5%

PU=previously untreated; PT=previously treated; RF=refractory; ABMT=autologous bone marrow transplantation; PBSCT=peripheral blood stem cell transplantation; PFS=progression free survival; EFS=event-free survival; OS=overall survival

factors and comparable to recovery after HDM with autologous stem cell rescue.(8-10,24)

Nausea and vomiting were the most prominent side effects but were in general manageable. Severe infections could be prevented in the majority of patients by the use of antibacterial and antifungal prophylaxis. The TRM was less than 1% which is low when compared to the reported mortality of HDM without autologous stem cell support of more than 10% and comparable with the TRM of HDM with stem cell reinfusion (0-7%) in previously untreated patients (8,9,12,13,24). We conclude that pretreatment of VAD followed by 2 cycles of IDM with G-CSF support but without stem cell support is feasible and effective in previously untreated patients with multiple myeloma. The toxicity is acceptable and treatment related mortality is low, which makes it an effective induction regimen that can be applied in an outpatient clinic setting. Only a minority of patients still needs stem cell support with this approach. Therefore, it may be especially useful for patients who cannot be treated with high-dose melphalan.

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CHAPTER 7

Abnormal chromosome 1p/q is highly associated with chromosome 13/13q deletions and is an adverse prognostic factor for outcome of high-dose therapy in patients with multiple myeloma

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Submitted

Summary

We studied the prognostic value of chromosomal abnormalities in untreated multiple myeloma patients who entered into a prospective randomized multicenter study for intensified treatment. 453 patients with stage II/III A/B disease under the age of 66 years were registered in the clinical study. Cytogenetic analysis was performed at diagnosis in 154 patients and was successful in 128/154 patients (83%). An abnormal karyotype was observed in 57/128 (45%) of the patients. In 28 of 57 patients (49%) abnormalities of chromosome 1p/q were found. There was a strong association between chromosome 1p/q abnormalities and a partial or complete deletion of chromosome 13 (n=25, P<0.001). Univariate analysis showed that chromosome 1p/q abnormalities but not chromosome 13 aberrations were of adverse prognosis for event-free survival (EFS). Adverse prognostic factors for time to progression (TTP) included complex cytogenetic abnormalities, hypodiploidy, chromosome 1p/q abnormalities, deletions of chromosome 13 and 13q and chromosome 6q abnormalities. Complex abnormalities, hypodiploidy, chromosome 1p/q abnormalities and chromosome 13 abnormalities were also adverse cytogenetic prognostic factors for overall survival (OS). By multivariate analysis an abnormal chromosome 1p/q was the only adverse prognostic factor for EFS, TTP and OS. In conclusion, chromosome 13 abnormalities and chromosome 1p/q abnormalities are highly associated, and risk factors for poor outcome after intensive therapy in multiple myeloma.

Introduction

Multiple myeloma is characterized by the clonal proliferation of plasma cells in the bone marrow, which produce a monoclonal heavy and/or light chain immunoglobulin (M-protein). This disorder accounts for 10% of all hematological malignancies.
Using flow cytometry for DNA content analysis a high incidence of genetic abnormalities have been detected in myeloma plasma cells (1). However with conventional karyotyping an abnormal karyotype is found in only 20 to 60% of patients (2-6). Cytogenetic studies using conventional karyotyping are often difficult because of the paucity of analyzable metaphases due to the low proliferation rate of the tumor cells in vitro. Where successful, multiple and complex cytogenetic abnormalities are often found (7). Using fluorescence in situ hybridization (FISH) chromosomal abnormalities can be detected in up to 97% of patients (8-11). The application of these techniques in a clinical setting has demonstrated that certain chromosomal abnormalities may have prognostic significance. A survival advantage was found in patients with hyperdiploidy as compared with patients with diploidy (12). Retrospective analyses have shown that partial or complete deletions of chromosome 13 are associated with poor prognosis in patients with multiple myeloma who had been treated with conventional chemotherapy or high dose therapy with autologous stem cell rescue (6,9,13-15). However, other retrospective studies have failed to detect chromosomal abnormalities with prognostic significance (3,4). In this study we have prospectively investigated the karyotype of 154 previously untreated patients who were included into a randomized phase III study for intensified treatment. The goal of this analysis was to evaluate the prognostic value of chromosome abnormalities, which were present at diagnosis for response to treatment and survival.

Patients and methods

Patients

Four hundred and fifty-three patients under the age of 66 years with stage II and III A/B multiple myeloma according to the Salmon and Durie classification were registered in a prospective randomized multicenter study for intensified treatment following remission induction treatment (16). In this study three to four cycles of rapid intravenous vincristine, adriamycine and dexamethasone (VAD) were given as standard remission induction treatment to all patients as described previously (17). Patients with an HLA-identical sibling under the age of 56 years were eligible for allogeneic bone marrow transplantation. Otherwise patients were randomized to receive either melphalan 140 mg/m^2 divided in 2 cycles of intermediate dose melphalan (IDM, 70 mg/m^2) with granulocyte colony-stimulating factor (G-CSF) or the same intensive regimen followed by cyclophosphamide and total body irradiation (TBI) as conditioning regimen and autologous peripheral blood stem cell rescue. Peripheral blood stem cells were harvested before intensive treatment after cyclophosphamide 4 g/m^2 and G-CSF. Randomized patients received interferon-α-2a as maintenance therapy.

Response criteria

Partial response (PR) was defined as a 50% or more reduction of M-protein in serum or urine or > 50% reduction of bone marrow infiltration (in non-secretory myeloma). Complete response (CR) was defined as both no M-protein measurable in serum and 10 times concentrated urine by immunofixation analysis and < 5% plasma cells with normal

Table 1. Clinical characteristics of 128 patients successfully studied by cytogenetic analysis

Characteristics	Normal karyotype (n=71)	Abnormal karyotype (n=57)	All patients (n=128)
Sex			(P=0.30)
Male/Female	41/30	38/19	79/49
Age at registration in years			(P=0.10)
Median (range)	53 (33-65)	54 (39-65)	54 (33-65)
Stage (Salmon & Durie)			(P=0.27)
IIA	19	8	27
IIB	2	1	3
IIIA	44	40	84
IIIB	6	8	14
WHO performance status			(P=0.97)
0-1	55	44	99
2-4	16	13	29
Calcium (mmol/l)			(P=0.48)
≤ 2.65	65	50	115
> 2.65	6	7	13
Serum β_2-microglobulin (mg/l)			(P=0.03)
0-3	36	16	52
> 3	29	31	60
Unknown	6	10	16
LDH (ULN=upper limit of normal)			(P=0.23)
≤ ULN	59	40	99
> ULN	10	12	22
Unknown	2	5	7
M-protein			(P=0.58)
IgA	11	14	25
IgG	46	32	78
IgD	2	1	3
Kappa/lambda	8	7	15
Unknown	4	3	7
Plasma cells in BM smear (%)			(P=0.02)
≤ 50	49	26	75
> 50	21	27	48
Unknown	1	4	5

morphology in bone marrow smears. These plasma cells had to be polyclonal by immunofluorescence staining. Relapse from CR was defined as recurrence of monoclonal plasma cells in the bone marrow or recurrence of M-protein in serum and/or urine measured by immunofixation. Progression from PR was defined as a doubling of M-protein on 2 consecutive measurements or an increase of M-protein with deterioration of clinical condition.

Cytogenetic study

Chromosome analysis of fresh bone marrow samples taken at diagnosis from the posterior iliac crest were carried out by members of the NWCGC at different clinical genetics centers in The Netherlands. Patients had given informed consent for the use of a bone marrow aspirate for these studies. Samples were collected in a sterile heparinized syringe and cultured in RPMI with 6-10% serum and in Iscove medium containing interleukin-4 and/or interleukin-6 and harvested after 24 and 96 hours according to standard cytogenetic techniques. In 7 cases interleukin-3 and/or granulocyte colony-stimulating factor and granulocyte-macrophage colony-stimulating factor were used instead. The metaphase preparations were stained for RFA-, QFQ-, or GTG-banding and karyotypes were described according to the international nomenclature (18). Where possible a minimum of 20 cells was analyzed. The presence of a clonal abnormality was defined as two metaphases with the same numerical or structural abnormality or as three metaphases missing the same chromosome. When only one abnormal metaphase was found, the clonality of the abnormalities observed had to be confirmed by FISH before they could be considered clonal. Those cases with a normal karyotype where less than 20 cells were analyzed were considered a failure.

Statistical analysis

The data were analyzed as of November 19, 2001. Endpoints included response rate, event-free survival, time to progression and overall survival.

Event-free survival (EFS) was calculated from the start of VAD until the moment no response had been attained after IDM or until progression/relapse after previous response or death without progression, whichever came first. Patients who had still no response after IDM were considered failure at one day. Time to progression (TTP) was determined from the start of VAD until progression/relapse or until death from multiple myeloma. Patients without progression who died from other causes were censored at the date of death. Overall survival (OS) was calculated from the date of the start of VAD until death. Patients still alive were censored at the date of last contact. EFS, TTP and OS were estimated by the Kaplan-Meier method.

Only those clonal chromosomal abnormalities found in at least 5 patients were included into the analysis of prognostic factors. These were structural abnormalities of chromosome 1p/q, 6q, 11q, 14q32, deletions and monosomy of chromosome 13, trisomies of chromosome 3, 5, 7, 9, 11, 15, 19, loss of a sex chromosome (X/Y) and complex (> 3) abnormalities.

The clinical characteristics of those patients with an apparently normal karyotype (NN) or those with an abnormal karyotype (AN/AA) were compared using Pearson's chi-squared test or Fisher's exact test, in case of discrete variables, or the Wilcoxon rank-sum test in case of continuous variables.

Table 2. Cytogenetic findings in 57 patients with multiple myeloma studied at diagnosis

Pat. No.	Karyotype (ISCN, 1995)
1	54-57,XX,+3,+5,+der(5)t(5;11)(q35;q13)[2],+9,+11[1],+15,add(16)(q24),+18[2],+19, +19,+21,+21[cp3]/46,XX[7]
2	50,XY,+11,+15,inc[cp5]/46,XY[32]
3	41-46,X,-Y[5],+del(1)(p12p21),i(1)(q10)[3],t(1;8)(p12;q24),add(6)(q12), add(7)(p12)[3],del(7)(p12)[2],-8,add(12)(p13),del(12)(p11)[2],-13,-14, +1-5mar[cp6]/46,XY[6]
4	50-51,X,-Y,+3,add(6)(q?16),add(8)(p1),+9,+11,-12,-14,+15,+15,+19, ?add(21)(p11),+2mar[cp11]/ 46,XY[9]
5	45,X,-X,-1,+3,?4,del(6)(q23q25),add(7)(p22),add(9)(p11),del(9)(p21),add(10)(q23), del(10)(p11p13),add(11)(q25),-12,add(14)(q32),-16,del(16)(q22),add(18)(q11),+19, -21, +der(?)t(?;1)(?;q11),+der(?)t(?;12)(?;p11)[15]/90,idemx2[4]/46,XX[1]
6	45,X,-Y[8]/46,XY[51]
7	44~47,XX,-4,add(6)(q?25),+?del(7)(q22),-10,del(12)(p12),-13,add(14)(q32), -16,+6~8mar,inc[14]/46,XX[1]
8	42-44,X,-Y,+del(1)(p32),-5[2],-13,-14[2],-18,-21[3],-22,+mar1,+1-3mar[cp4]/ 46,XY[6]
9	54-55,X,-Y,del(1)(p22p3?2),+del(1)(p22p3?2)[12],+del(3)(q2?5q29),+4,+5, +9,+11,+15,+15[13],+19,+21[cp14]/46,XY[14]
10	53,XX,der(1)t(1;11)(p13;q11),der(4)t(1;4)(p13;q35),+5,+9,add(13)(q2?1),+15,+19, +21,+21,+mar1[5]/53,idem,-mar1,+mar2[4]/46,XX[11]
11	55,X,-X,+3,+5,+7,+9,+9,+11,+15,+15,+19,+21,+21,-22[7]/46,XX[13]
12	54,XX,-1,+3,+?4,+add(6)(q2?),+7,-8,+9,-13,+15,+15,+19,+21,+2mar [1]*/46,XX, del(6)(q2?2)[3]/46,XX[68]
13	44,XX,add(5)(q35),-8,-13,-14,+18[3]/46,XX[27]
14	46,XX,?20[16]
15	46,XY,t(1;14)(p22 ~31;q3?2)[2]/46,XY[29]
16	57-58,XY,t(1;11;15)(q2?4;q1?3;q2?2),+2,+del(3)(p21p26)[3],+5, +der(6;19)(p10;p10),+7,+9,+9,+11,+11,+15,+15,-18,+20,+21[cp4]/ 57-59,XY,t(1;11;15)(q2?4;q1?3;q2?2),+2[2], +del(3)(p11p24)[5],+5,+7,+9,+9,+11, +11,+15,+15,-18,add(19)(q13)[4],+20,+21[cp6]/abnormal,inc[4]/46,XY[20]
17	44,X,-Y,+1, +add(1)(p13),dic(1;3)(p13;p26),add(2)(q?),del(5)(q32?),del(6)(q21),-13, -14,add(16)(q24)[20]
18	49-58,XY,+2,+3,+5,+9,+11,+11,+del(13)(q1?2q2?2),+15,+15,add(17)(p1?3), +19,+21[cp20]/113,idem x2[1]/46,XY[5]
19	45,X,-Y,+del(3)(q2?3),-?4,del(6)(q21),-8,-10,der(10)t(1;10)(q12;q26)x2,-13, -18,+mar1,+mar2[6]/88,XX,-Y,-Y,-1,del(3)(q23),+del(3)(q23),-4,del(4)(q2?7), del(6)(q21),del(6)(q21),-8,-9,der(10)t(1;10)(q21;q26)x3,-11,-13,-13,-14, -16,-18,-18,+8mar[13]/46,XY[5]
20	54-56,X,-Y,+X[3],del(1)(p11p21)[3],+2,+3,+5,+del(11)(q24),+15,+18[3],+19,+19, +mar1[3],+mar2[1],+mar3[1][cp4]/46,XY[6]

Pat.No	Karyotype (ISCN, 1995)
21	46-52,X,-X,add(1)(p11),+5[6],+6,+9,+11,-13,+15,+15[5][cp7]/53-56, XX,add(1)(p11),+add(1)(q1?2),+2,+5,+del(6)(q16q25)[2],+7,+9, +11,+15[2],+15[2],-16,+19,+21,+mar[cp3]/60-65,XX,add(1)(p11), +del(1)(q32)[2],+2,+3,+5,+6,+6,+7,+9,+11,+15,+15,+19,+21[cp3]/56,XX,add(1)(p11) ,+del(1)(q11),+5,+6,+7,+9,+11,+15,+15,+19,+21[2]/46,XY[8]
22	44,XY,del(1)(p2?),t(4;7)(q2?;q22),del(5)(p1?),del(6)(q2?), -10,der(16)t(10;16)(q1;q2),-22[cp2]/46,XY [18]
23	48,XY,-6,+?9,+?11,-15,-16,+?21,+2mar[2]/46,XY[30]
24	47,X,-Y,+del(1)(p1?2p36),+5,add(8)(q2?4),+9,-13,+19,-22[6]/46,XY[27]
25	43,XX,add(1)(p21),+3,-4,-5,der(7)t(1;7)(q12;p22),del(9)?(q22q32),-10,-12,-13, -22,+2mar[8]/ 46,XX[12]
26	55,XY,+3,+5,+9,+11,+15,+15,+19,+20,+21[2]/46,XY[18]
27	45,X,-X,-7,-8,inv(11)(p15q13),-12,add(14)(q32),+3mar[6]/45,idem,-6,+12[5]/ 46,idem,+11,-inv(11),+mar[2]/45,idem,-10,+11[2]/46,XX[4]
28	54,XY,+?2,+3,+14,add(14)(q32),+16,+20,+21,+2mar,inc[13]/46,XY[15]
29	52,XY,+?3,+?5,del(6)(q?),+del(6)(q?),+add(19)(?p),inc[cp2]/45,X,-Y[4]/46,XY[14]
30	43,X,-X,+1,der(1;16)(p10;q10),-6,add(7)(p21~22),der(8)t(6;8)(p21;p2?2), der(12)t(12;13)(q1?5;q2?1),-13,-14,add(14)(q32),+der(?)t(?;6)(?;p1)[cp17]/46,XX[7]
31	52,XY,+5,der(6)t(1;6)(?q24;q22),+9,+11,-16,+19,+22,+2mar[6]/46,XY[14]
32	46,XY,der(3)t(3;3)(p2?6;q12),del(6)(q12q21)[1]/46,idem,der(7)t(7;10)(q34~35;q21), del(10)(q21),t(11;14)(q13;q32)[7]/45,idem,add(8)(p22),add(9)(q34), -15,der(22)t(15;?;22)(q15;?;q11.2)[6]/46,XY[10]
33	44,XX,-1,-5,-9,?add(11)(p),del(12)(p),-13,-14,del(20)(q),-22,+4mar[1]*/46,XX[36]
34	53,X,-Y,+2,der(3;8)(q10;q10),+5,+7,+add(9)(q34),add(14)(q32),+15,+15,+19,+19, add(20)(q13)[4]/53,idem,+?der(3),-der(3;8),-8[3]/46,XY[27]
35	44,X,-Y,del(8)(p21),del(10)(p13),-13, der(16)t(1;16)(q10;p10),add(18)(q23)[34]46,XY[3]
36	51,XY,+4,+8,+9,+11,+11,-12,-13,+18,+2mar[cp3]46,XY[17]
37	45,X,-Y[4]/46,XY[6]
38	48,XX,+2mar[4]/46,XX[16]
39	50,X,-Y,-1,+5,+5,der(6)t(6;15)(p2?2;q15),+7,+9,-13,der(16)t(1;16)(q21;q12),-17,+19, +add(21)(q22),+der(?)t(?;1)(?;p31),+mar1[11]/52,X,-Y,-1,t(2;8)(p11.2;q24),+5,+5, +7,+9,+11,-13,der(16)t(1;16)(q21;q12),+19,+21,+der(?)t(?;1)(?;p31), +mar2[2]/46,XY[7]
40	76-78,XX,-X,del(1)(p?2p3?),+add(3)(q24),der(4)t(1;4)(q11;q35), +der(4)t(1;4)(q11;q35),der(6)t(1;6)(q11;q15),+der(6)t(1;6)(q11;q15),add(7)(q11),+der (10)t(3;10)(q23;p14),+add(11)(q11),- 13,add(14)(p11),+15,+17,+18,+19,+21[cp8]/46,XX[24]
41	46,XY,?7[10]/43,XY,add(1)(q32),add(3)(q2?8),add(6)(p21),?7,-13,-14,-21,-22, +der(?)t(?;1)(?;q12)[1]*/46,XY[21]
42	50,X,add(X)(p22),add(2)(p25),+3,?add(5)(q13),+9,del(11)(p12p15),+11,add(12)(p12) ,?del(15)(q25q26),+15,?add(16)(q12),-17,+19[3]/46,XX[22]
43	44,X,-X,dup(1)(q21q32),del(6)(q?),-13,add(18)(q23)[3]/46,XX[7]

Pat.No.	Karyotype (ISCN, 1995)
44	54,X,-X,+4,+5,+7,+9,+11,+15,+15,+19,+21[8]/106,XX,-X,-X,+4,+4,+5, +5,+9,+9,+11,+11,+15,+15,+15,+15,+19,+19,+21,+21[1]/46,XX[24]
45	45,XY,der(11)t(11;14)(q13;q32)?add(14)(q32),der(14)t(11;14)(q13;q32), -22,+mar1[2],+mar2[2] [4]/45,X,-Y[7]/46,XY[20]
46	45,X,-Y[5]/46,XY[27]
47	53-55,XY,+3,+4,+6,+7,+9,+11,+13,+14,+15,+19,+mar[cp6]/46,XY[25]
48	44,X,-Y,t(1;20)(p10;q10),-13,der(14)t(7;14)(q21~q22;q2?4), +21,-22[6]/46,XY[21]
49	44,X,-Y,add(1)(q?),der(1)del(1)(p2?2p3?2)(?q),-5,add(8)(p11),t(11;14)(q1?3;q32), add(12)(q2?4),add(14)(q3?2),16?p,add(17)(p1?2)[2]/42-43,X,-Y, der(1)add(1)(p?)add(1)(q?),-3,add(4)(p1?6),-5,add(6)(p2?),del(8)(p11), t(11;14)(q1?3;q32),add(17)(p1?2),-20,-22,inc[cp2]/42,X,-Y, der(1)?add(1)(p?)del(1)(q2?2q2?5),add(8)(p11),t(11;14)(q1?3;q32), -13,add(17)(p1?2),-20,-21[1]/45,X,-Y[3]/46,XY[23]
50	54,X,-Y,+5,del(6)(q2?),+del(6)(q2?),+7,+9,+add(11)(q24),+15,-16, -17,+18,+19,+3mar[19]/46,XY[25]
51	55,X,-X,der(1)ins(1;?)(q12;?)t(1;14)(q43;q11),+3,del(4)(p1?4),+5, +der(6)t(6;15)(q21;q21),+7,-8,+9,+del(11)(q22q23),-14,+15, -16,?der(17)t(X;17)(q13;p13),+18,+19,+der(?)t(?;4)(?;q12), +der(?)t(?;14)(?;q1),+mar1,+mar2[3]/55,idem,-5,+mar3[2]/56,X, -X,+3,del(4)(p1?4),+5,+der(6)t(6;15)(q21;q21),+7,-8,+9,+del(11)(q22q23), +14,+15,-16,der(16)t(1;16)(q12;q1?2),?der(17)t(X;17)(q13;p13)x2,+18,+19, +der(?)t(?;4)(?;q12),+mar1[5]/46,XX[10]
52	54~56,XY,+3,+5,+7,+9,+9,+11,+15,+15,+19,+19[cp7]/46,XY[13]
53	57,XX,+1,psu dic(1;16)(p13;p13.3),+2,+3,?add(4)(q31),+add(5)(q3?1),+7, +9,+add(11)(q2?3),+15,+15,+19,+19,+21[19]/46,XY[5]
54	44-45,X,-X,der(5)t(1;5)(q11;p15),del(8)(p2?),add(11)(q1?3), -13,der(17)t(1;17)(q1?1;q25),+mar[cp2]/82-83,XX,-X,-X,der(5)t(1;5)(q11;p15)x2, add(6)(q1?),-8,del(8)(p2?),-11,add(11)(q1?3)x2,-12,-13,-13,-16, der(17)t(1;17)(q1?1;q25)x2,+2mar[cp4]/46,XX[26]
55	41-44,X,-Y,add(4)(p1?),del(8)(p2?1),der(12)t(1;12)(q11;q24),- 13,der(14)t(13;14)(q11;p11)del(13)(q11q21),?21[cp9]/46,XY[26]
56	46,XY,add(9)(p11)[5]/44,XY,del(8)(p21),del(12)?(p11p13), -13,der(14)t(14;22)(q32;q11.2),der(16)t(13;16)(q12;p13),add(17)(q2?5),-22[3]/ 43,XY,-4,del(8)(p21),-13,der(14)t(14;22)(q32;q11.2), der(16)t(13;16)(q12;p13),add(17)(q2?5),der(18)t(4;18)(q11;q22),-22[4]/46,XY[21]
57	43,X,-Y,i(1)(q10),t(3;6)(p21;p21),-4,-13[16]/46,XY[4]

* Clonality confirmed by FISH

Spearman's Rank correlation was calculated for each pair of chromosomal abnormalities in patients with an abnormal karyotype to see whether some abnormalities were associated.

Univariate Cox regression analysis was performed to determine whether there was a difference in survival between the different subgroups. The variables that were significant in the univariate analysis were also included in a multivariate Cox regression using a

step-down method, i.e. the multivariate Cox regression started with all variables that were significant in the univariate analysis, and the variable with the largest P-value was then removed, until all remaining variables had a P-value ≤ 0.05. All reported P-values are two-sided and a significance level $\alpha = 0.05$ was used.

Results

From November 1995 until April 2000 453 patients were registered in the clinical study. Twelve patients were found not to be eligible. Cytogenetic analysis was performed on 154 out of 453 patients and of these 128 (83%) were successfully karyotyped according to our criteria. The analysis was restricted to this group of 128 patients. There was no significant difference in the clinical parameters for patients with a normal or abnormal karyotype except for bone marrow infiltration by plasma cells and β_2-microglobulin serum levels at diagnosis (Table 1).

Cytogenetic analysis

A normal karyotype was found in 71/128 (55%) of the patients studied. Chromosomal abnormalities were detected in 57/128 (45%) of the patients and the complete karyotypes of these are shown in Table 2. Table 3 shows this data broken down by ploidy and the presence of specific cytogenetic variation.

Table 3. Type of cytogenetic variation found in 57 patients with an abnormal karyotype

Cytogenetic Abnormality	No. patients
Pseudodipoloid	6
Hypodiploid	14
Hyperdiploid 47-50	4
Hyperdiploid 51-60	23
Not classifiable	10
Complex abnormalities (> 3)	42
14q32 (including t(11;14)(q13;q32)	10 (3)
11q	11
1p/q	28
6q	19
+3	19
+5	19
+7	12
+9	22
+11	18
+15	21
+19	23
-13	23
-13 or 13q-	25
-Y or –X	30

Twenty-seven patients had hyperdiploidy (47-60) (47%), 14 patients had hypodiploid karyotypes (25%) and 6 patients were pseudodiploid (11%). Ten patients were not classifiable or showed combinations of ploidy (18%). Complex abnormalities (> 3 abnormalities) were found in 42 of 57 patients (74%). The most frequently occurring numerical aberrations were trisomies of chromosome 3, 5, 7, 9, 11, 15, 19 and loss of a sex chromosome (X/Y), with the X only being lost in female patients. There was a strong association between trisomies of chromosome 3, 5, 7, 9, 11, 15 and 19.
In 28 of 57 patients chromosome 1p/q abnormalities were found (49%). Abnormalities of 1q21 were present in 3 patients and of 1q12 in another 3 patients. Monosomy 13 or deletions of 13q were present in 25 patients (44%). There was a strong association between chromosome 1p/q abnormalities and those of chromosome 13 (P<0.001); nineteen patients had both abnormalities. Abnormalities of 6q were present in 19 patients (33%). Ten patients showed a breakpoint at 14q32 (18%), three of these having the translocation t(11;14)(q13;q32). No other known specific translocations involving 14q32 were found. Other abnormalities of 11q were found in a total of 11/57 patients (19%).

Table 4. Univariate Cox regression analysis of risk factors for event-free survival

Risk factor	Univariate analysis		
	HR	95% CI	P-value
Abnormal karyotype	1.3	0.8-2.1	0.32
Hypodiploidy	1.7	0.8-3.6	0.17
1p/q abnormalities	1.9	1.1-3.4	0.02
-13/13q-	1.6	0.9-2.8	0.15
Complex abnormalities	1.6	1.0-2.7	0.06

Response
Univariate analysis showed that none of the chromosomal abnormalities was a statistically significant prognostic factor for partial response after VAD or IDM, nor for a complete remission after VAD, IDM and myelo-ablative treatment with stem cell rescue or interferon-α-2a (data not shown).

Event-free survival, time to progression and overall survival
The median follow up of the 90 patients still alive was 25 months (range: 4-69 months).
The results of the univariate analysis of prognostic factors for event-free survival are shown in Table 4.
An abnormality of chromosome 1p/q was the only adverse prognostic cytogenetic abnormality for EFS (P=0.02). Together with elevated LDH, an abnormal chromosome 1p/q was also the only cytogenetic variable that remained significant in the multivariate analysis when all significant variables were included into the step-down procedure.
Hypodiploidy, complex abnormalities, chromosome 1p/q abnormalities, chromosome 13 and 13q deletions (all P<0.01) and chromosome 6q abnormalities (P<0.05) were associated with a worse TTP (Table 5). An abnormal chromosome 1p/q was the only cytogenetic abnormality with adverse prognostic significance in the multivariate analysis.

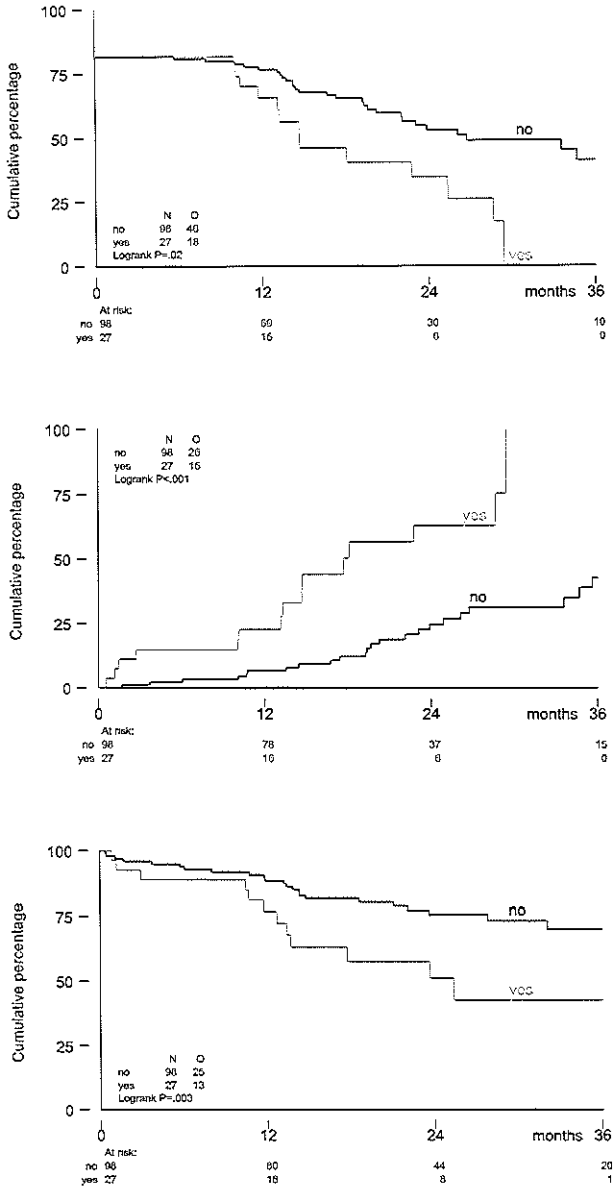

Figure 1. Kaplan-Meier curves of event-free survival (upper figure), time to progression (middle figure) and overall survival (lower figure) in the presence and absence of chromosome 1p/q abnormalities

cells and the poor growth in vitro. Cytokine stimulation of cultures with myeloma growth factors, such as interleukin-6 or interleukin-4, can increase the detection rate of cytogenetic abnormalities (4,19,20).

In the present study we found an abnormal karyotype in 45% of previously untreated patients with stage II or III multiple myeloma. A high frequency of multiple and complex chromosomal abnormalities was present as has been described previously (2,5,21-23). Our study confirms earlier reports that the outcome of patients with abnormal cytogenetics receiving high dose therapy is inferior to that of patients without abnormalities (6,23).

The most striking chromosome abnormality with the respect to outcome of therapy is a deletion of chromosome 13 or 13q, which was found in 44% of our patients (6,9,13-15). Using conventional cytogenetics, abnormalities of chromosome 13 have been observed in 36 to 47% of patients with an abnormal karyotype at diagnosis (4,6,21). With interphase FISH this percentage varies between 33% and 86% of newly diagnosed patients depending on the number of probes used (9,24-28). We found time to progression and overall survival but not event-free survival to be significantly shorter in patients with deletions of chromosome 13 or 13q.

In the present study however, it was demonstrated that deletions of chromosome 13 or 13q are highly associated with chromosome 1p/q abnormalities. The chromosome 1p/q variation was predictive of a poor outcome with regard to EFS, TTP and OS. In the multivariate analysis an abnormal chromosome 1p/q but not chromosome 13 and 13q deletions was a cytogenetic adverse prognostic factor for event-free survival, time to progression and overall survival. To our knowledge this observation has not been previously reported. Structural aberrations of chromosome 1 are common in multiple myeloma, being found in up to 40% of patients with abnormal karyotypes (2,29). A variety of partner chromosomes may be involved in translocations with the long arm of chromosome 1 (5). The reported high frequencies of the breakpoints 1p12 and 1q21 were not confirmed in our homogeneous group of untreated patients (30). Duplications and translocations of 1q are widely reported in neoplasm and are associated with progression of malignancies, including that of B-cell clones (30,31). The high incidence of abnormalities involving chromosome 1q may be due to an instability of the highly decondensed pericentromeric heterochromatin region, which may facilitate the formation of unstable translocations. The clonal evolution of cells with these abnormalities suggests that these abnormalities contribute to a proliferation advantage (29).

In addition to abnormal chromosomes 1 and 13 the most frequent numerical abnormalities were concurrent trisomies of chromosome 3, 5, 7, 9, 11, 15 and 19, associated with a hyperdiploid karyotype, as is known from previous studies (4,5,22,32). There was a strong association between these trisomies. The striking cosegregation of trisomies of chromosome 5, 7, 9, 11, 15 and 19 was also reported by Sawyer et al. This suggests a consistent pattern of cytogenetic progression in a subgroup of multiple myeloma (5). In contrast to other studies the overall survival of our hyperdiploid patients was not significantly better when compared to diploid patients (12). In our study hypodiploidy predicted for shorter time to progression and overall survival confirming recent studies (33).

In conclusion, our studies confirm that chromosomal studies at diagnosis are by now indispensable in the clinical evaluation of patients with multiple myeloma. We found that

complex abnormalities, total or partial deletions of chromosome 13 and 1p/q abnormalities are associated with poor outcome after intensive therapy in multiple myeloma. In addition, our study demonstrates that an abnormal chromosome 1p/q and a deletion of chromosome 13/13q are highly associated. However, it remains the critical therefore to be elucidated which of these 2 chromosomal abnormalities plays the critical role in terms of gene loss or gene activation, which ultimately leads to aggressive multiple myeloma. For patients with specific cytogenetics new treatment strategies may be required to improve the poor outcome after intensive therapy.

Acknowledgements
The authors like to thank the participating members and centers of the NWKGC responsible for the cytogenetic analysis: Academic Medical Center, Amsterdam, W. Kroes, C. Mellink; Academic Hospital Groningen, Groningen, E. van den Berg-de Ruiter; Leiden University Medical Center, Leiden, S. Bhola; Institution for Clinical Genetics Limburg, Maastricht, M. Stevens-Kroef; University Medical Center St. Radboud, Nijmegen, D. Olde Weghuis, A. Geurts van Kessel; Erasmus Medical Center, Rotterdam, R. Slater, H.B. Beverloo; University Medical Center Utrecht, Utrecht, P. Poddighe

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CHAPTER 8

High-dose therapy and quality of life in newly diagnosed multiple myeloma

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Submitted

Summary
We studied the effect of high-dose therapy on quality of life (QoL) in newly diagnosed patients with multiple myeloma. Patients under the age of 66 years with stage II/III disease were randomized after remission induction with VAD to receive either intensified chemotherapy consisting of 2 cycles of intermediate dose melphalan (IDM 70 mg/m^2) or the same regimen followed by myelo-ablative treatment. Patients with an HLA-identical sibling under the age of 56 were candidates for allogeneic stem cell transplantation. 453 patients entered the clinical study and 186 patients participated in the QoL life study for which EuroQoL-5D and EORTC QLQ-C30 questionnaires were used. During intensified chemotherapy global QoL improved and there was a significant reduction of a number of complaints, especially pain. During the first year of follow-up patients had a significantly higher level of physical, role and social functioning and a better QoL after intensified chemotherapy than after myelo-ablative treatment. After myelo-ablative treatment patients had more complaints of fatigue, pain, loss of appetite, nausea and vomiting, diminished sexual interest, shivers, thirst, weakness of arms and legs and change of taste. During the first 3 months of follow-up allogeneic transplanted patients had a significant lower level of physical, role, cognitive and social functioning when compared to myelo-ablative treatment with autologous stem cell rescue. Because neither myelo-ablative treatment with autologous stem cell rescue added intensified treatment, nor allo-SCT did result in a better event-free and overall survival, whereas QoL was significantly reduced, these regimens are not preferable for first-line treatment in multiple myeloma.

Introduction

Multiple myeloma (MM) is a malignant plasma cell disorder with an abundant proliferation of monoclonal plasma cells in the bone marrow, which produce an abnormal monoclonal immunoglobulin (M-component). MM is characterized by profound morbidity due to pain caused by skeletal destruction, osteoporosis and spontaneous fractures. The often present anemia due to bone marrow infiltration further increases fatigue and deteriorates physical functioning. Recurrent bacterial infections occur due to the suppression of normal immunoglobulin synthesis. The profound morbidity in the majority of patients with MM has considerable repercussions on the quality of life (QoL). With the introduction of conventional chemotherapy the median survival of patients improved from 17 months to 30 months (1). In recent years more intensive regimens have been explored in phase I and phase II studies which have resulted in higher response rates in poor risk MM at the cost of substantial toxicity and morbidity (2-4). The traditional end-points in most of these studies on high-dose therapy in MM have been response rate, event-free survival, progression free survival and overall survival. In addition, there has been growing interest in the evaluation of the effects of different therapeutic regimens on the quality of life in these patients.

The interest in the QoL of cancer patients led to the development of several cancer specific questionnaires on health-related QoL of which the standardized European Organisation for Research and Treatment of Cancer Core Quality of Life Questionnaire (EORTC QLQ C-30) is the most widely used in Europe (5).

In 1995 the Dutch HOVON group initiated a prospective multicenter randomized phase III study to compare intensified chemotherapy followed by myelo-ablative therapy and autologous stem cell rescue with intensified chemotherapy alone in newly diagnosed patients with MM (HOVON 24 MM). Patients under the age of 56 years with an HLA-identical sibling were candidates for allogeneic stem cell transplantation (allo-SCT), and were excluded from randomization.

Because of the differences with regard to the intensity of the treatment, quality of life measurements were integrated in the study in order to evaluate the impact of the different regimens on patients' quality of life. First, the changes of QoL during induction therapy and intensified chemotherapy were evaluated. Furthermore, the impact of the addition of myelo-ablative treatment with autologous stem cell rescue to intensified treatment on QoL was studied. Finally, the QoL after myelo-ablative treatment with autologous stem cell rescue was compared with QoL after allo-SCT.

Patients and methods

Patients

Patients under the age of 66 years with previously untreated MM and stage II and III A/B disease according to the Salmon and Durie criteria were eligible for the clinical study (6).

Study protocol

All registered patients were treated with 3 to 4 cycles of VAD (vincristine, adriamycin and dexamethasone) for remission induction (7). After remission induction irrespective of the response to VAD patients were randomized to receive either intensified chemotherapy or intensified chemotherapy followed by myelo-ablative therapy and autologous stem cell rescue. Patients under the age of 56 years with an HLA-identical sibling were candidates

for an allo-SCT, and were excluded from randomization. In all other patients autologous peripheral stem cells were harvested after high-dose cyclophosphamide iv. (4 g/m^2) and granulocyte colony-stimulating factor s.c. (G-CSF) four to six weeks after the last cycle of VAD. Thereafter melphalan 140 mg/m^2 was administered divided into 2 cycles of intermediate dose melphalan (IDM, 70 mg/m^2) with G-CSF, but without stem cell rescue at a maximal interval of 8 weeks. Patients randomized for myelo-ablative treatment received cyclophosphamide and total body irradiation as conditioning regimen followed by autologous peripheral blood stem cell rescue. Interferon-α-2a (IFN) 3 x 10^6 IU three times a week was started as maintenance therapy only in responsive patients in both treatment groups until progression or relapse. Eligibility criteria, response criteria and definitions of event-free and overall survival are presented elsewhere. The outline of the study is shown in Figure 1.

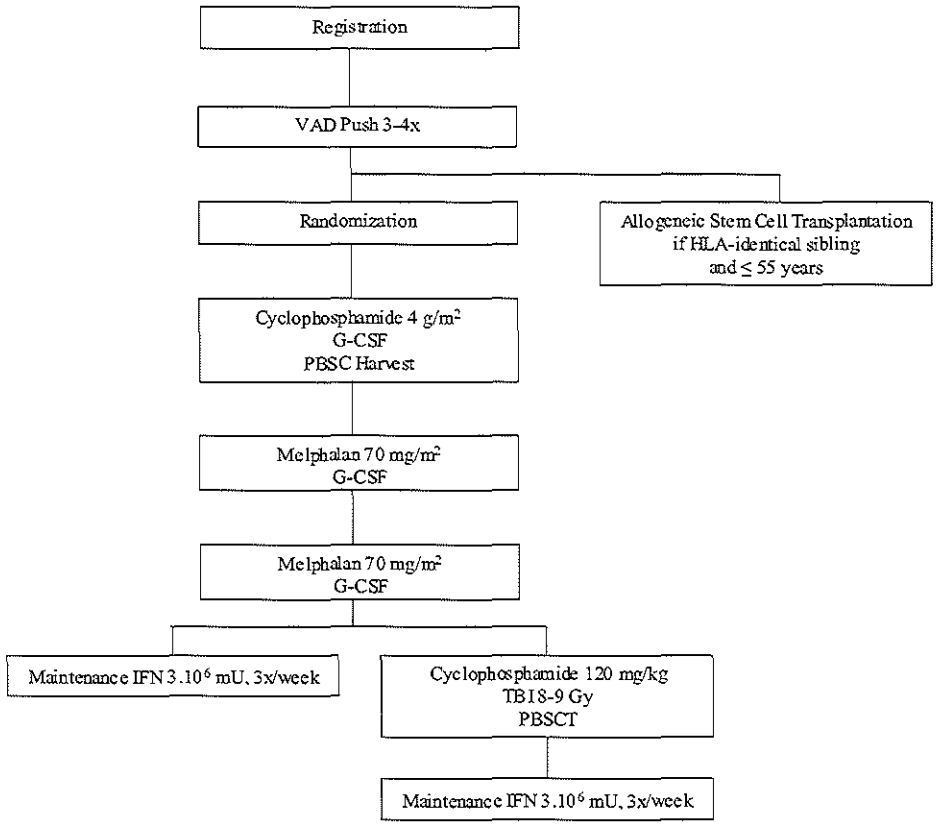

Figure 1. Outline of the study

QoL questionnaires

In this study two validated questionnaires were used: the EORTC QLQ-C30 questionnaire and the EuroQoL-5D instrument.

The EORTC QLQ-C30 is a cancer specific, multidimensional QoL core questionnaire (5). It contains 30 items relevant to a broad spectrum of cancer patients, is easy to complete for the patient and is available in many different languages including Dutch. The questionnaire incorporates five functional scales (physical, role, emotional, cognitive and social), three symptom scales (fatigue, nausea/vomiting and pain), a global health status/QoL scale and a number of single items (dyspnea, appetite loss, sleep disturbances, constipation and diarrhea). In addition, the financial impact of the disease and treatment is asked for. Scores for each scale in the EORTC QLQ-C30 questionnaire were calculated as suggested by the EORTC study Group on QoL. All scale and item scores were linearly transformed so that all scales ranged from 0 to 100. For the five functional scales and the single global health status/QoL scale, higher scale scores represent higher levels of functioning. For the three symptom scales and the single items higher scores represent higher levels of symptoms or more problems. Differences of at least 10 points on the scale from 0 to 100 were considered clinically relevant as was used in earlier Nordic studies on MM (8,9). Differences of less than 10 points were considered not to be clinically relevant and were therefore not analyzed statistically.

In the recent years the EORTC developed several disease specific modules to be used in conjunction with the EORTC QLQ-C30. At the start of this study such a module was not yet available for MM, so we added several MM specific symptom and treatment related side effect items to the EORTC QLQ-C30 questionnaire. The relevance of these items was tested in a small subset of patients but was not validated on a larger scale.

The EuroQoL-5D is a generic instrument which measures health related quality of life in 5 dimensions: mobility, self-care, usual activities, pain/discomfort and anxiety/depression (10). It was included in this study with the purpose to obtain utilities. These utilities represent preferences for different health states and make it possible to calculate quality adjusted life years (QALYs). QALYs combine the two dimensions of health, health status and life duration, into one single index. The preference weights used in this study have been obtained from a sample of the general population of the United Kingdom by using the Time Trade Off method (TTO) (11).

Data collection procedures

During therapy patients were asked to complete a questionnaire prior to start of treatment with VAD, at time of evaluation after VAD therapy, and at time of evaluation after the last cycle of IDM. Furthermore at 3, 6, 9, 12, 18 and 24 months after start of IFN in the intensified chemotherapy group, after myelo-ablative therapy with autologous stem cell rescue or after allogeneic transplantation questionnaires were given to the patients (see Figure 1). The measurement of QoL prior to start of treatment was the starting situation for the evaluation of the development of QoL during remission induction therapy and intensified chemotherapy thereafter. The measurements of QoL after IDM were used as baseline for the comparison between the randomized patients during follow-up. All QoL measurements were coupled to the clinical evaluations of therapy. QoL measurements were stopped at time of progression or relapse.

QoL measurements started on April 1, 1997. At that moment 133 patients were already included in the clinical study. All patients included after April 1, 1997 were asked by their physician to participate in the QoL study. In each of the participating hospitals a physician, a research nurse or a data manager was responsible for the execution of the QoL study and the distribution of questionnaires to patients at the right moment of evaluation. All patients gave written informed consent.

Statistical analysis

For statistical significance P-values < 0.05 (two-sided) were considered necessary. For the analysis of differences in QoL in time the paired Student's t-test was used. Differences between groups were non-parametrically tested using the Mann-Whitney test.

Results

Characteristics of participants and non-participants

From December 1995 to April 2000, 453 patients were registered in the clinical study. The first analysis of the clinical study was performed in 379 patients. The first QoL analysis included 186 patients: 169 of patients registered in the clinical study after April 1, 1997 and 17 patients who were included before this date. Of the 261 patients registered in the clinical study after April 1997, 92 patients were not included in the QoL life study because of ineligibility for the clinical study (n=7), incomplete data for the clinical analysis (n=9), refusal (n=30), treatment outside the Netherlands (n=15), problems with the Dutch language (n=3), logistic reasons (n=4), missing questionnaire before start of therapy and off study before IDM (n=22) and physicians refusal (n=2).

Characteristics of the 186 participants in the QoL study were compared with the characteristics of the total study population (n=379). Age and sex were not different. Characteristics of prognostic relevance in the clinical study for outcome, i.e., stage of disease, number of skeletal lesions, LDH and β_2-microglobine at diagnosis were also comparable. The complete remission (CR) rate on protocol among patients participating in the QoL analysis was the same as among the whole study population (18%), but the PR and CR rate was significantly higher (91% vs. 82%, P<0.01).

Results of the clinical study

The overall response after VAD in the randomized patients was 70%. After completion of the protocol treatment the overall response rate was 88% in patients randomized to the intensified chemotherapy group and 95% in patients randomized to myelo-ablative therapy. The CR rate was significantly higher after myelo-ablative therapy (29% vs. 13%, P=0.002). The median follow-up was 33 months from randomization (range 8-65 months). There was no difference in the event-free survival (EFS) calculated from randomization between the 2 groups, being median 21 months after intensified chemotherapy and 22 months after myelo-ablative therapy (P=0.28). The overall survival (OS) from randomization was also similar between the 2 treatment groups (median 50 months in the intensified chemotherapy group vs. median 47 months in the myelo-ablative therapy group, P=0.41).

The overall response after allo-SCT was 89% including 18% of patients with a CR. Overall survival from date of allo-SCT was 25 months.

QoL during intensified chemotherapy

For the description of changes in QoL during remission induction therapy and intensified therapy 343 completed questionnaires were available: 116 prior to VAD, 116 after VAD and 111 after IDM. These numbers correspond to a response rate to questionnaires of 90%, 88% and 81%, respectively. The response rate is calculated as the proportion of returned questionnaires of all questionnaires that had to be completed at each time point according to the protocol. Questionnaires of patients who underwent allogeneic transplantation were excluded from this analysis, because the treatment of those patients was different: they did not receive high-dose cyclophosphamide followed by autologous peripheral stem cell harvest and they mainly received only one cycle of IDM.

Four of five functional dimensions and global QoL as measured by the EORTC QLQ-C30 significantly improved after IDM when compared to prior to VAD. On a scale from 0 to 100, the total increase was 15 points for physical functioning, 20 points for role and emotional functioning, 14 points for social functioning and 16 points for global QoL (all $P<0.01$, Figure 2). The largest improvement was already accomplished after VAD.

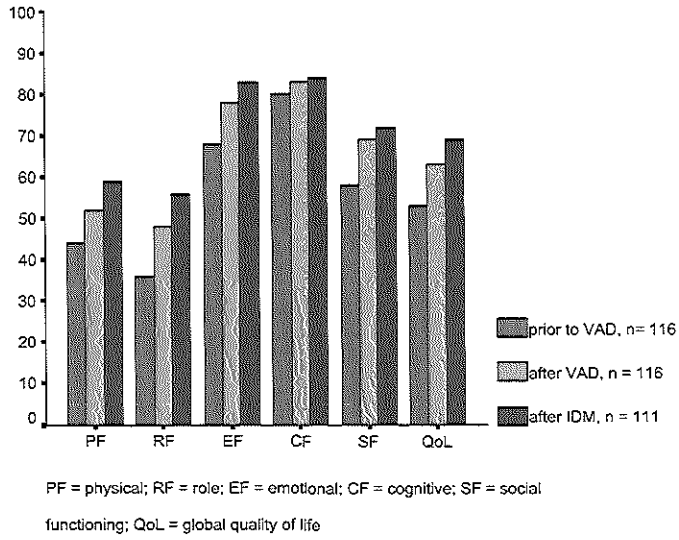

Figure 2. Changes in mean scores of functioning scales and global quality of life during intensive treatment: higher number represents better levels of functioning

The most frequent symptoms prior to treatment were pain, fatigue and sleep disturbances. Also after IDM these symptoms were the most frequent but significantly less prominent (pain, $P<0.01$; fatigue and sleep disturbances, $P<0.05$). The development of the mean scores of all symptoms of the EORTC QLQ C-30 questionnaire during treatment is shown in Figure 3.

Specific disease and treatment related symptoms and complaints were added to the EORTC QLQ C-30 questionnaire. Of these, dizziness, palpitations, fever, hematomas,

soreness of the mouth, shivers, headache and bleeding of gums and nose were scarcely seen. Complaints of back pain and bone pain and diminished sexual interest were the most frequent complaints prior to treatment as well as after the last cycle of IDM. The severity of back and bone pain diminished significantly during the period of treatment ($P<0.01$). Chest pain was present before VAD but was almost absent after IDM ($P<0.01$) as was the same for thirst ($P<0.01$).

Utilities as measured by the EuroQol-5D improved from 0.40 prior to VAD to 0.57 after VAD to 0.65 after IDM.

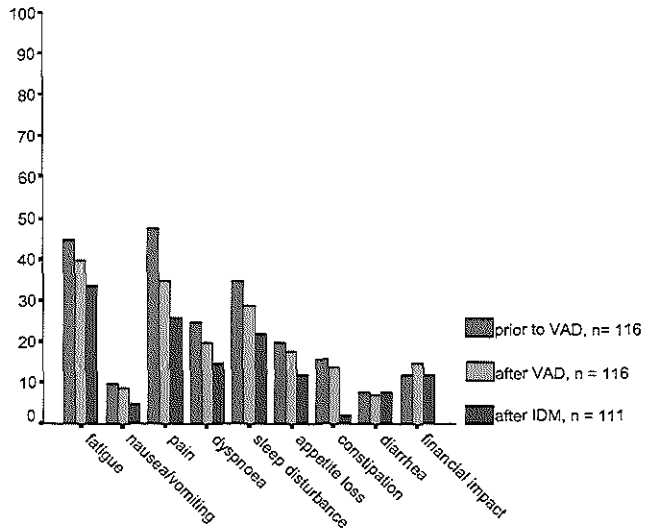

Figure 3. Changes of the mean symptom scores of the EORTC QLQ C-30 questionnaire during intensive treatment: higher number represents higher levels of symptoms

QoL during follow-up

The analysis of QoL during follow-up describes the similarities and differences of the quality of remission after the different treatment modalities. There were no differences in QoL between the treatment groups at baseline (= evaluation after the last IDM) except for significantly more financial problems in the allogeneic transplantation group. QoL was measured during 2 years of follow-up. Because the study was not completed yet and patients were off study at the time of relapse or progression, the number of observations during follow-up at 18 and 24 months was very low and are therefore not included in this analysis.

The QoL after intensified therapy (48 patients) was compared with the Qol after myelo-ablative treatment with autologous stem cell rescue (58 patients). The latter was also compared with the QoL after allo-SCT (28 patients). During follow-up the response rate to the questionnaires at 3, 6, 9 and 12 months was 80%, 78%, 67% and 80% respectively.

QoL after myelo-ablative therapy with autologous stem cell rescue compared to intensified chemotherapy alone

During the first year follow-up the global QoL, physical, role and social functioning were significantly worse in the myelo-ablative therapy group when compared to intensified chemotherapy alone. There was no difference in emotional and cognitive functioning between these 2 treatment groups. The mean scores for global QoL and the functional dimensions are shown in Figure 4.

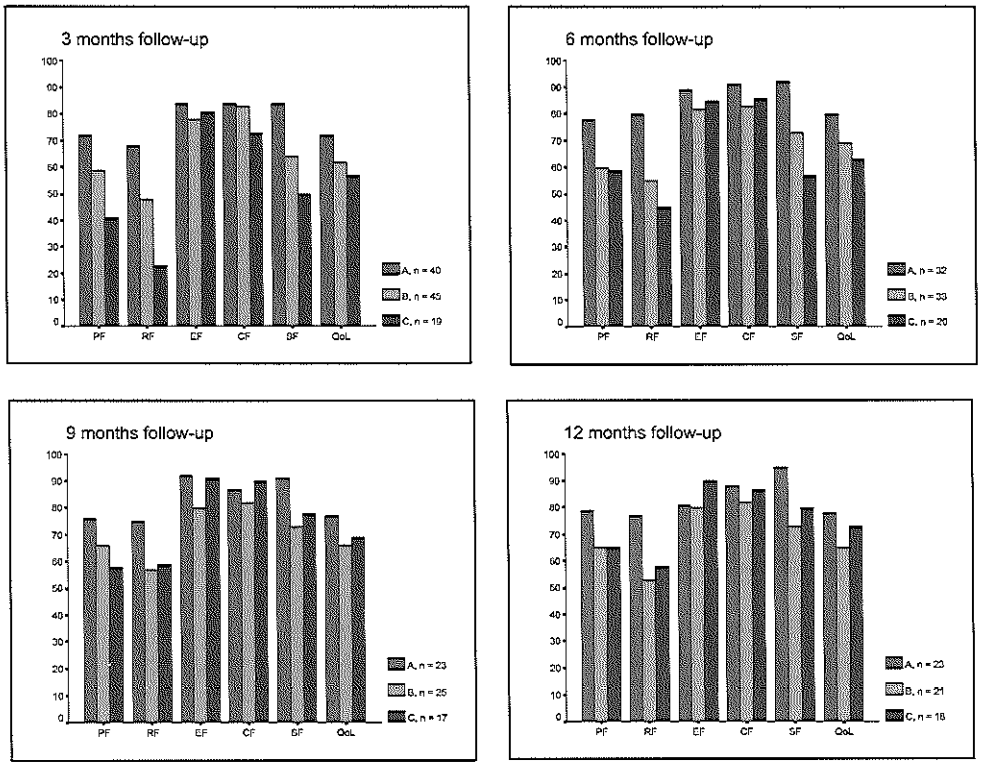

Figure 4. Mean functioning scores during follow-up after intensified chemotherapy (A), myelo-ablative therapy with autologous stem cell rescue (B) or allogeneic transplantation (C). Higher scores represent better functioning: PF=physical, RF=role, EF=emotional, CF=cognitive, SF=social functioning; QoL=global quality of life

Five of the 9 symptoms measured with the EORTC QLQ C-30 were different in the first year of follow-up between these treatment groups. Fatigue was a prominent symptom. In the intensified chemotherapy group as opposed to patients in the myelo-ablative treatment group an improvement of fatigue was seen during follow-up. During the first 3 months of follow-up patients in the myelo-ablative therapy group had significantly more complaints of nausea and vomiting than patients in the intensified chemotherapy group ($P<0.01$). From 6 to 12 months follow-up patients in the myelo-ablative therapy group had more complaints of pain ($P<0.05$). Loss of appetite was significantly more frequent in the

myelo-ablative therapy group up to 12 months of follow-up (P<0.01). Constipation, diarrhea and dyspnea were not frequently seen. Patients in the myelo-ablative therapy group had significantly more financial problems during the first year of follow-up. The mean scores of symptoms during follow-up are shown in Figure 5.
Of the disease and treatment related symptoms dizziness, palpitations, fever, hematomas, pain in the oral region, shivers, headache and hemorrhage of gums and nose were scarcely seen. Diminished sexual interest was the most important complaint during the first year in all patients. At 3 months significant fewer patients in the intensified chemotherapy group complained of thirst, change of taste, diminished sexual interest and shivers (all P<0.01) when compared to the myelo-ablative treatment group. Patients in the intensified chemotherapy group had significantly more hematomas (P<0.05). At 6 months patients in the intensified chemotherapy group still had significantly fewer complaints of thirst (P<0.01) and change of taste (P<0.01) than after myelo-ablative therapy. At 9 to 12 months this pattern was still present, but also less weakness and less complaints of shivers were present (P<0.05).

The utilities for the health states as measured by the EuroQol are lower for patients in the myelo-ablative group: at 3 months 0.77 versus 0.59 (P<0.01), at 6 months 0.81 versus 0.66 (P<0.01), at 9 months 0.79 versus 0.68 (P=0.11) and at 12 months 0.81 versus 0.63 (P<0.05).

QoL after myelo-ablative therapy with autologous stem cell rescue compared to allo-SCT
During follow-up the global QoL was not different after myelo-ablative therapy with autologous stem cell rescue as compared to allo-SCT. However, physical, role, cognitive and social functioning were significantly worse after allo-SCT at 3 months follow-up. There was no difference in emotional functioning. From 6 to 12 months follow-up, global QoL and all functioning scores were comparable in both groups. Mean scores are shown in Figure 4.
Considering the 9 symptoms measured with the EORTC QLQ C-30, patients after allo-SCT had less complaints of pain at 12 months of follow-up when compared to patients after myelo-ablative therapy with autologous stem cell rescue (P<0.05). In both treatment groups fatigue and appetite loss were prominent symptoms. In allogeneic transplanted patients as opposed to patients in the myelo-ablative treatment group an improvement of both complaints was seen during follow-up. After 3 months of follow-up both treatment groups complained of nausea and vomiting. Constipation, diarrhea and dyspnea were not frequently seen. Allogeneic transplanted patients and patients in the myelo-ablative therapy group had both financial problems during the first year of follow-up. The mean symptom scores during follow-up are shown in Figure 5.
Of the disease and treatment related symptoms dizziness, palpitations, fever, hematomas, pain in the oral region, shivers, headache and hemorrhage of gums and nose were scarcely seen. Diminished sexual interest was the most important complaint during the first year of follow-up in all patients. Complaints of thirst, diminished sexual interest and shivers were comparable between both treatment groups. More patients had a changed

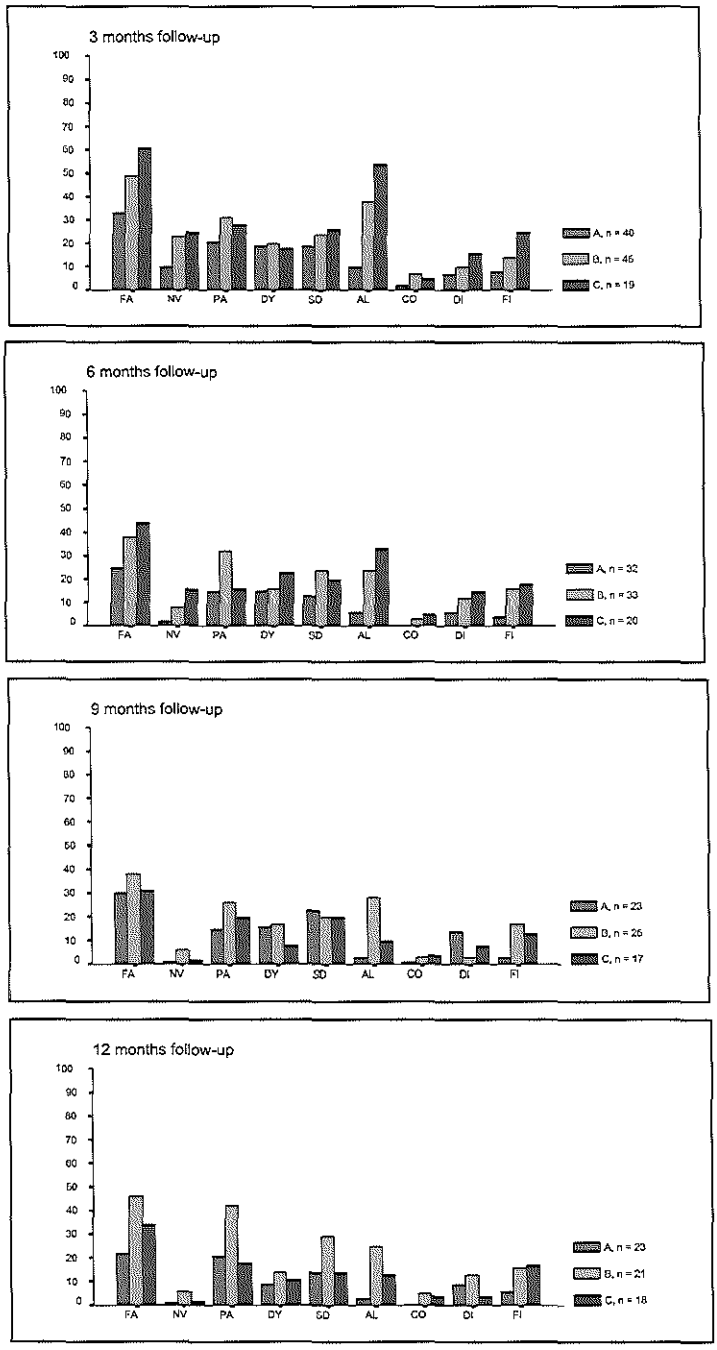

Figure 5. Mean symptom scores during follow-up after intensified chemotherapy (A), myelo-ablative therapy with autologous stem cell rescue (B) or allogeneic transplantation (C). Higher scores represent higher levels of symptoms. FA=fatigue, NV=nausea/vomiting, PA=pain, DY=dyspnea, SD=sleep disturbance, AL=appetite loss, CO=constipation, DI=diarrhea, FI=financial impact

taste after allo-SCT ($P<0.05$). Significantly less allogeneic transplanted patients complained of pain in their arms ($P<0.01$) but more patients complained of weakness ($P<0.05$) at 3 months.

Discussion

In this randomized trial the effect of intensive therapy on the QoL of newly diagnosed patients with MM was studied. It is the first large-scale longitudinal QoL study in MM in The Netherlands. Eighty-five percent of patients who were eligible for the QoL study participated, which is comparable with the participation of patients with MM in a large Scandinavian study (9,12). These participants were representative for the whole study population with regard to age, sex and characteristics of prognostic relevance for survival. Only the overall response rate was different from the response rate in the whole study population (91% vs. 82%, $P<0.01$).

The response to the questionnaires was 81% to 90% during the period of chemotherapy and 67% to 80% during follow-up. In Nordic QoL studies in patients with different types of malignancies the response rate was 62% to 99% and it was dependent on the time after inclusion. The response rate on the questionnaires dropped during follow-up mainly due to progression of the disease (13). In the present study the response rate was not influenced by progression of the disease as the QoL study was stopped at time of relapse or progression. The reasons for missing questionnaires were logistic problems and refusal of the patients. If non-response is selective, the results might be under- or overestimated. We have no reasons to assume that the non-response to questionnaires in our study was selective. First, all patients in the quality of life study were in clinical remission. Second, non-response was equal in all treatment groups.

In this first analysis, we did not calculate quality adjusted life years. However, utilities are reported because in the literature little is known about utilities in patients with multiple myeloma. The values established in this study might be used as input for modeling studies, e.g. cost-utility studies.

Treatment with VAD for remission induction can induce rapid responses (7). This QoL study shows that the objective response to VAD treatment is associated with a significant improvement of subjective well-being. There is a distinct improvement in complaints related to clinical symptoms often present in patients with MM. Pain, especially pain in the back and bone pain decreased, which was reflected by a significant improvement in functioning on several dimensions and a higher valuation for global QoL. Important is that this improvement in functioning and QoL continued through the 2 cycles of IDM, which are considered to be more intensive and toxic than the induction treatment with VAD (14). Although the QoL was not measured in the period of acute toxicity of the IDM cycles, the QoL measured 6 to 8 weeks after IDM was better than before start of IDM. If IDM influenced QoL in a negative way, this was in the majority of patients only during a brief period of time.

In comparison with the situation prior to start of treatment with VAD, there was a significant improvement of physical, role, emotional and social functioning after the intensification of treatment with IDM. This improvement was related to the decrease of general and MM specific symptoms such as pain, fatigue, sleep disturbances, thirst, chest pain, pain in the back and bone pain. We conclude that intensive therapy is well tolerated by the high-risk patients who entered the clinical study and that it significantly improves

QoL. However, it is possible that the absolute level of improvement in QoL during intensive therapy for the whole study group is somewhat overestimated. Patients with early treatment failure were underrepresented in the group of patients that participated in the QoL study. Those patients never reached at least a partial remission and it is reasonable to assume that their average quality of life is lower compared to patients who did reach a remission.

Additional value of myelo-ablative therapy with autologous stem cell rescue to intensified chemotherapy was not found in the clinical study. The event-free survival and overall survival did not improve with myelo-ablative therapy. Besides, the QoL study showed that the subjective experienced quality of remission was significantly lower after myelo-ablative therapy during the first year of follow-up when compared to intensified chemotherapy alone. There was a lower level of physical, role and social functioning and complaints of nausea, pain, fatigue, loss of appetite, diminished sexual interest, thirst, change of taste and financial problems were significantly more frequent after myelo-ablative therapy. Allogeneic transplanted patients had a significantly lower level of physical, role, cognitive and social functioning when compared to patients after myelo-ablative treatment with autologous stem cell rescue. At 3 months follow-up they had more complaints of fatigue, weakness in arms and legs and loss of appetite. From 6 months of follow-up their functioning was the same as patients treated with myelo-ablative therapy with autologous stem cell transplantation.

All reported symptoms are well known side effects of bone marrow or peripheral stem cell transplantation (15-23). Reports in the literature about the time to recovery from these side effects are conflicting. In three studies improvement in QoL in a mixed group of patients who were evaluated after autologous and allogeneic transplantation at 12 months to 3-5 years was observed (16,19,21). No relation between QoL and time since transplantation was noted in two studies in patients after autologous and allogeneic transplantation with a follow-up of 6-24 months, 25-48 months or more than 48 months after transplantation (17,23). In one study, in allogeneic transplanted patients the quality of life at one year after transplantation was comparable to the QoL before transplantation, while in autologous transplanted patients the QoL one year after transplantation was significantly better when compared to the situation before transplantation (18). In that study, the explanation for this finding was not the type of transplantation but the poor QoL of the autologous transplanted patients before transplantation due to differences in pre-treatment and status of disease. It is difficult to draw conclusions from these studies. First, none were prospective randomized studies. Some of them were cross-sectional (16,17,19,21). Others had a retrospective design (23). The major disadvantage of cross-sectional studies is selection bias of patients. Only the longest living patients can participate in these types of studies. Secondly, in the mentioned studies patients with different kinds of malignancies participated. In some studies both patients with solid tumors and hematological malignancies participated (17,19,23). In other studies patients with distinct hematological malignancies were included (16,18,21). Patients with MM are not altogether comparable with these patient groups because of the poor prognosis with regard to long-term event free and overall survival although treated with high-dose therapy and because of the persisting disease related morbidity after treatment. Vertebral fractures are very common in patients with MM and this is reflected by pain in the back and bone pain even at 1 year follow-up in patients in remission after intensified

chemotherapy, myelo-ablative therapy with autologous stem cell rescue and allogeneic transplantation.
A temporary decrease in QoL after myelo-ablative therapy or allogeneic transplantation is only acceptable when these therapies result in a long-term positive effect like a longer event-free or overall survival. In the clinical study additional myelo-ablative therapy did not improve outcome. The outcome of allo-SCT as first-line treatment was even worse.
In conclusion, intensified chemotherapy is subjectively well tolerated and improves quality of life. However, the addition of myelo-ablative therapy with autologous stem cell rescue or allo-SCT resulted in reduced QoL during the first year of follow-up, whereas clinical outcome was not improved. These are therefore not preferable regimens for first-line treatment in multiple myeloma.

Acknowledgements
The authors thank Monique Steijaert and Petra Westveer, data-managers of the HOVON Data Center and Aat van Klooster of the Institute for Medical Technology Assessment.

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CHAPTER 9

Cost analysis comparing intensified chemotherapy alone to myelo-ablative therapy followed by autologous stem cell rescue in newly diagnosed patients with stage II/III multiple myeloma: a prospective randomized phase III study

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Submitted

Summary

In a prospective randomized phase III study in patients $\leq$ 65 years with previously untreated multiple myeloma (MM), intensified chemotherapy was compared to the same treatment followed by myelo-ablative therapy with autologous stem cell rescue. An economic evaluation, based on 100 of 379 patients included in the clinical analysis, was included to find out if the anticipated clinical benefits would be justified by the anticipated higher costs.

Costs of the chemotherapy and stem cell collection as applicated to all patients amounted to €35,525. Subsequently, by randomization 35/100 patients received interferon-α-2a maintenance treatment (IFN, 3 x 10^6 units thrice weekly) after intensified chemotherapy, while 37 patients proceeded to myelo-ablative treatment with autologous stem cell rescue, followed by IFN. From the start of the randomized treatment up to three years after randomization, mean costs of intensified chemotherapy were significanly lower (€ 26,458; 95% CI = [21,679, 31,236] than the costs of myelo-ablative treatment (€43,864; 95% CI = [34,244, 53,485]. However, event-free and overall survival were similar.

Intensified chemotherapy is regarded the standard therapy for younger patients with previously untreated MM. We conclude that, considering the beneficial effect of this treatment, the cost-effectiveness of myeloma therapy is not favoured by applying a next course of myelo-ablative treatment.

Introduction

Melphalan based chemotherapy regimens have been the standard treatment for multiple myeloma (MM) for more than 30 years (1-3). With conventional chemotherapy, the median survival is around 30 to 36 months and the 10-year survival rate is less than 5% (4-6)

The exploration of the feasibility to administer high-dose chemotherapy for MM with the aim to overcome resistance to conventional alkylating agent containing chemotherapy has led to the introduction of high-dose chemotherapy and the concept of additional support by autologous stem cell rescue (7,8). Since then, one randomized study has been published which showed a superiority of myelo-ablative treatment followed by autologous bone marrow transplantation over conventional chemotherapy. However, this study has been criticized for its limited number of patients and for the relatively poor response in conventionally treated patients (9,10).

Along the lines of this concept, the Dutch-Belgian Hemato-Oncology Cooperative Study Group (HOVON) started a prospective multicenter randomized phase III study to evaluate the efficacy of intensified chemotherapy followed by myelo-ablative therapy with autologous stem cell rescue as compared to intensified chemotherapy only (HOVON MM-24 study). The clinical results of this study have been reported elsewhere (Segeren, et al, submitted). The study included an economic evaluation in order to examine whether the anticipated clinical benefits would justify the additional costs of myelo-ablative treatment. As to this moment, high-dose therapy has become a standard treatment for multiple myeloma, while a cost-effectiveness evaluation has never been pursued in a prospective randomized phase III study (11). We present the results of this economic evaluation in detail as related to the clinical outcome of the study.

Patients and Methods

Patients

This cost analysis was part of a prospective multicenter randomized phase III clinical study of the HOVON. Patients under 66 years of age with previously untreated multiple myeloma, and stage II or III A/B disease according to Salmon and Durie were eligible for the study. Exclusion criteria were: WHO performance score 4, severe cardiac, pulmonary, neurologic or metabolic disease, inadequate liver function (bilirubin $\geq$ 2.5 times normal), prior malignant disease except for non-melanoma skin tumours or stage 0 cervical carcinoma, and prior extensive radiation therapy involving the myelum precluding total body irradiation.

From earlier costs analyses, we calculated the patient numbers to be sufficient to obtain reliable conclusions. One hundred patients, matched for age and gender, were included. Response or survival were unknown at the time study participants were selected for the cost analysis. The following criteria were consequently applied from the first patient onwards to select 100 study participants for the cost analysis: patients from both university and local hospitals, and within each hospital patients in both study groups (to prevent cost differences from being caused by hospital specific differences). The median follow-up from the randomization date until the scheduled date of the analysis had to be at least two years.

Treatment
Treatment costs were aimed to be calculated up to maximally three years after randomization. For the cost analysis, this time interval was divided into the following phases.

Phase 1: VAD remission induction therapy (P1VAD)
The start date of this phase was day 1 of the first VAD cycle. Patients were treated with 3-4 cycles of the VAD remission induction chemotherapy (vincristine iv. 0.4 mg days 1-4; doxorubicin iv. 9 mg/m^2 days 1-4; dexamethasone p.o. 40 mg days 1-4, days 9-12 and days 17-20 at cycles 1 and 3, or only days 1-4 at cycles 2 and 4), repeated at 28 days intervals (12). After VAD, patients were randomized to undergo either intensified chemotherapy only or intensified chemotherapy followed by myelo-ablative therapy with autologous stem cell rescue (auto-SCT). Patients ≤ 55 years of age with an HLA-identical sibling were candidates for allogeneic stem cell transplantation and were therefore not randomized.

Phase 2: Cyclophosphamide and autologous stem cell collection (P2CYCLO)
The start date of this phase was the date of hospitalization for cyclophosphamide administration. Four to 6 weeks after the final VAD cycle, peripheral blood stem cells were collected from all randomized patients (also in the intensified chemotherapy only group, to be used in case of progression or relapse), after they had been administered cyclophosphamide (iv. 4 g/m^2 at day 1; granulocyte colony-stimulation factor G-CSF s.c. 300 μg to 480 μg, starting at day 5 until the last day of leukapheresis). A minimum of 2.5 x 10^6 CD34$^+$ cells/kg was required to proceed to myelo-ablative treatment and autologous stem cell reinfusion.

Phase 3: Intermediate dose melphalan (P3IDM)
The start date of this phase was day 1 of the first IDM cycle. Two cycles of intermediate dose melphalan (IDM, 70 mg/m^2 iv. each cycle) were administered as described before, at a maximum interval of 8 weeks (13). G-CSF was started on the fourth day after the melphalan infusion (300 μg to 480 μg) until the neutrophil count reached a level of ≥ 1.0 x 10^9/l.

Phase 4: Randomized treatment group
The start date of this phase was the first date of interferon-α-2a administration or the day of hospitalization for myelo-ablative treatment. Patients included in the myelo-ablative treatment group who had reached at least a partial remission and an adequate stem cell harvest were given cyclophosphamide 60 mg/kg given on 2 consecutive days, followed by total body irradiation 1 x 9 Gy, lung dose 8 Gy and autologous stem cell reinfusion (≥ 2.5 x 10^6/kg CD34$^+$ cells).
Interferon-α-2a (IFN) maintenance treatment (3 x 10^6 units thrice weekly) was scheduled to start 60-90 days after the second IDM cycle (intensified chemotherapy group) or 60-90 days after stem cell reinfusion (myelo-ablative treatment group) until relapse or progression in patients who had at least a partial remission, WHO performance score 0-2, absence of severe organ dysfunction, platelet count > 50 x 10^9/l, and neutrophil count > 1.0 x 10^9/l.

For the cost analysis, this phase ended three years after the date of randomization or death, if earlier. If patients were alive at December 31, 2000 without having reached this endpoint, they were censored at December 31, 2000 for pragmatical reasons.

Costs

The medical consumption of the patients was determined on the basis of case registry forms, patient files and records from the hospital information systems. Average unit costs were determined for the most important items within the medical consumption, reflecting full hospital costs, including overhead costs (14,15). To determine these unit costs (Table 1), we applied the micro-costing method. This method is based on a detailed inventory and measurement of resources consumed, e.g. materials and disposables used and time spent by nursing staff (16). The valuation of the resources and overhead costs was based on financial data from two university hospitals and six local hospitals (1998 level) to prevent costs from being different due to the type of hospital. Some financial data (marked by * in Table 1) were weighted for their origin: 66% of the final unit costs were based on financial data from the university hospitals, and 34% on financial data from the local hospitals, according to the relative distribution of all study participants to university hospitals and local hospitals, respectively.

Diagnostic tests and other procedures were multiplied by Dutch charges, as these are proper approximations of the actual unit costs (15). Costs of medication were based on Dutch 1998 wholesale prices (17). The hospital perspective was applied, but costs of medication used by the patient at home as part of the assessed treatments were also calculated (18).

Table 1. Unit costs (in Euro). The unit cost of a Hematology University Hospital day has been applied to all hospitalizations related to treatments that were only performed in university hospitals or specialized cancer centers (cyclophosphamide, leukapheresis, and stem cell reinfusion). The day care treatment unit cost was applied to all occasions at which patients had chemotherapy, blood components, or intravenous biphosphonates administered during a session in the outpatient clinic. Nutrition only included regular meals (costs of parenteral nutrition were calculated separately). * = weighting factor 66:34 for university and local hospitals applied

Unit costs	P	E	A	Total
Hematology Regular Hospital day*	190	49	102	**341**
Hematology University Hospital day	198	54	116	**368**
Intensive Care Unit Hospital day*	594	170	258	**1,022**
Hematology Dept. Outpatient visit*	64	9	10	**83**
Hematology Dept. Day Care Treatment*	54	37	68	**159**
Stem cell harvesting (Leukapheresis)	210	214	106	**530**
Stem cell freezing following harvesting	231	458	172	**861**
Stem cell defrosting preceding reinfusion	119	18	48	**185**
Radiotherapy Megavolt Session	125	17	50	**192**

P=Personnel, including specialist, nursing, administrative personnel; E=Equipment, materials, nutrition, laundry, cleaning; A=Accommodation and overhead.

Table 4. Mean values of the most important indicators of medical resource use and mean costs (Euro) of the remission induction chemotherapy (P1VAD), cyclophosphamide and leukapheresis (P2CYCLO) and intermediate dose melphalan courses (P3IDM) of all patients originally included in the cost analysis (n=90)

Resource use indicators	P1VAD	P2CYCLO	P3IDM	Total
Phase duration (days)	99.54	49.17	130.71	279.42
Hematology hospital days	8.20	6.91	12.20	27.31
Intensive Care Unit days	0.00	0.02	0.43	0.46
Day care treatments	9.13	1.07	6.18	16.37
Hematology outpatient visits	3.98	2.79	9.18	15.94
Days parenteral nutrition	0.41	0.04	0.44	0.90
Cost categories	**P1VAD**	**P2CYCLO**	**P3IDM**	**Total**
Hematology hospital days	2,804	2,510	4,174	9,488
Intensive Care Unit days	0	23	443	466
Day care treatments	1,442	169	976	2,587
Hematology outpatient visits	330	231	762	1,323
Outpatient visits other dept.'s	101	45	134	280
Parenteral nutrition	39	4	42	86
Blood components	133	285	2,699	3,117
Radiotherapy	203	0	17	220
Leukapheresis	0	2,040	15	2,056
Laboratory diagnostics	1,296	914	1,680	3,891
Radiology diagnostics	300	137	327	764
Other imaging diagnostics	39	20	68	127
Microbiology diagnostics	50	102	157	309
Pathology diagnostics	63	18	58	138
Other medical consumption	13	27	38	78
Cytostatics	1,020	92	191	1,303
G-CSF	0	1,680	4,694	6,374
Antifungal/antibacterial prophylaxis	353	222	496	1,071
Bisphosphonates	77	30	101	208
Antibiotics	163	152	645	960
Other medication	357	140	169	666
Total costs (Euro's)	**8,784**	**8,843**	**17,898**	**35,525**

G-CSF=granulocyte colony-stimulation factor

received the remission induction chemotherapy during hospitalizations. Most of the patients had their chemotherapy administered on an outpatient basis (all costs except for the cytostatics included in "day care treatments"). The P1VAD phase cost €8,784 on average (95% CI = [7,618, 9,949]).
During the P2CYCLO phase patients were hospitalized for 6.91 days which were virtually all scheduled admissions for administration of the cyclophosphamide chemotherapy and the leukapheresis procedure(s). This phase lasted 49.17 days and cost €8,843 (95% CI = [8,044, 9,642]). Of these costs, 23.1% were made on behalf of the procedural costs of the leukapheresis.
The P3IDM phase lasted 130.71 days, during which the patients were hospitalized for 12.20 days. Most of these hospitalizations were scheduled admissions for melphalan administration, although unscheduled admissions for chemotherapy induced fever (frequently combined with neutropenia) or general malaise also occurred frequently. This phase cost €17,898 (95% CI = [15,390, 20,405]) of which G-CSF costs and hospitalization costs were the main determinants.
In total, the P1VAD, P2CYCLO, and P3IDM phases cost €35,525.

Costs of intensified chemotherapy and myelo-ablative treatment
This phase lasted 814 days (intensified chemotherapy group; 95% CI 749-880) and 713 days (myelo-ablative treatment group; 95% CI = [613, 814]) on average (P=0.79). Mean costs (Table 5) of patients who received intensified chemotherapy only were significantly lower (€26,458; 95% CI = [21,679, 31,236]) than the costs of patients who additionaly underwent myelo-ablative chemotherapy with autologous stem cell rescue (€43,864; 95% CI = [34,244, 53,485]). This was mainly due to a difference in the mean number of hospital days (intensified chemotherapy 14.80; 95% CI = [7.36, 22.24] vs. myelo-ablative treatment 51.57; 95% CI = [38.61, 64.53]). In the myelo-ablative treatment group, the hospitalization for the treatment itself (myelo-ablative chemotherapy, total body irradiation, and autologous stem cell reinfusion) lasted 28.35 days on average (median 25; range 10-123), implying that these patients had also 8 additional unscheduled hospital days than the patients who underwent intensified chemotherapy only. Almost all other cost categories, particularly Intensive Care Unit hospital days, blood components, microbiology diagnostics, antifungal and antibacterial prophylaxis, and therapeutic antibiotics also showed higher values in the myelo-ablative treatment group.
The costs of IFN were higher in the intensified chemotherapy group, as these patients used IFN longer than the myelo-ablative treatment patients during the assessed time interval. In the intensified chemotherapy group, the costs of day care treatments and bisphosphonates were also higher.

Discussion
We analyzed the costs of intensified chemotherapy versus intensified chemotherapy followed by myelo-ablative chemotherapy with autologous stem cell rescue for newly diagnosed multiple myeloma (MM) in a prospective multicenter randomized phase III study.

Table 5. Mean values (median; range) of the most important indicators of medical resource use and mean costs (Euro) of the intensified chemotherapy group (n=35) and the myelo-ablative treatment group (n=37)

Resource use indicators	Intensified chemotherapy	Myelo-ablative treatment	P
Phase duration (days)	814.06 (865.00; 165-1.231)	713.27 (844.00; 37-998)	0.79
Hematology hospital days	14.80 (0; 0-95)	51.57 (38; 22-187)	0.00
Intensive Care Unit days	0.23 (0.00; 0-8)	1.86 (0.00; 0-33)	0.03
Day care treatments	10.06 (5.00; 0-45)	4.14 (0.00; 0-30)	0.03
Hematology outpatient visits	22.91 (20.00; 0-62)	21.68 (21.00; 0-44)	0.96
Days parenteral nutrition	2.26 (0.00; 0-54)	2.89 (0.00; 0-26)	0.05

Cost categories	Intensified chemotherapy	Myelo-ablative treatment	P
Hematology hospital days	5,127 (0; 0-32,490)	18,403 (13,672; 8,096-66,294)	0.00
Intensive Care Unit days	234 (0; 0-8,184)	1,908 (0; 0-33,759)	0.03
Day care treatments	1,589 (790; 0-7,110)	653 (0; 0-4,740)	0.03
Hematology outpatient visits	1,902 (1,660; 0-5,146)	1,799 (1,743; 0-3,652)	0.10
Outpatient visits other dept.'s	167 (83; 0-1,113)	768 (498; 0-4,283)	0.00
Parenteral nutrition	214 (0; 0-5,130)	275 (0; 0-2,470)	0.05
Blood components	970 (0; 0-11,639)	2,333 (1,467; 0-18,935)	0.00
Radiotherapy	620 (0; 0-4,032)	560 (384; 384-3,072)	0.01
Leukapheresis	397 (0; 0-2,782)	301 (0; 0-1,391)	0.64
Stem cell graft defrosting	26 (0; 0-185)	185 (185; 185-185)	0.00
Laboratory diagnostics	3,706 (3,072; 488-10,417)	5,430 (4,500; 314-24,993)	0.06
Radiology diagnostics	1,072 (955; 0-3,356)	1,477 (944; 0-8,024)	0.73
Other imaging diagnostics	94 (0; 0-699)	342 (72; 0-3,320)	0.00
Microbiology diagnostics	237 (0; 0-1,936)	959 (662; 0-6,591)	0.00
Pathology diagnostics	76 (0; 0-567)	234 (0; 0-1,296)	0.22
Other medical consumption	65 (0; 0-775)	188 (0; 0-2,225)	0.08
Cytostatics	598 (0; 0-2,435)	194 (105; 0-1,803)	0.42
G-CSF	107 (0; 0-3,414)	47 (0; 0-1,707)	0.58
Interferon-α-2a	5,833 (5,794; 467-12,574)	3,356 (2,509; 0-11,028)	0.00
Antifungal/antibacterial prophylaxis	302 (0; 0-2,908)	676 (447; 0-2,921)	0.00
Bisphosphonates	1,794 (549; 0-6,894)	578 (91; 0-4,936)	0.01
Antibiotics	373 (3; 0-3,480)	2,074 (1,357; 112-19,374)	0.00
Other medication	954 (260; 0-9,739)	1,122 (807; 121-3,732)	0.00
Total costs (Euro's)	**26,458 (23,595; 7,274-64,931)**	**43,864 (35,255; 19,790-180,358)**	**0.00**

The first part of the study was the same for all patients and consisted of VAD remission induction chemotherapy, cyclophosphamide followed by leukapheresis, and two intermediate dose melphalan cycles. This amounted to mean total costs of €35,525. Subsequently, the intensified chemotherapy group was treated with interferon-α-2a (IFN) maintenance treatment only costing €26,458 on average. The mean costs of the group which underwent myelo-ablative chemotherapy and autologous stem cell reinfusion followed by IFN maintenance treatment, were significantly higher. Up to maximally three years after randomization, the costs within the myelo-ablative treatment group were €43,864 on average. The higher costs in this group were caused by the hospitalization for myelo-ablative treatment, during which the hematologic recovery necessitated a more frequent administration of blood components and antibiotics. Also, more unscheduled additional admissions were registered in these patients. However in the intensified chemotherapy group, the costs of bisphosphonates and day care treatments were higher, which we expect to be due to the longer IFN use in this group. Intravenous bisphosphonates were administered at the day care department, which explains the higher costs of using these facilities in the intensified chemotherapy group.

Although the baseline characteristics were similar in both the intensified chemotherapy and the myelo-ablative treatment groups and equal to the characteristics of all patients included in the clinical study, significantly more patients in our myelo-ablative treatment group died before the scheduled endpoint. This is not consistent with the results of the clinical analysis, in which the overall survival was similar between both groups. The myelo-ablative treatment group in this cost analysis may therefore have generated a lower resource use. On the contrary in the intensified chemotherapy group, more patients experienced progression or relapse, which may have had an increasing influence on their resource use. These observations imply that the "real world" cost differences between both groups may be even larger than was observed in this analysis.

A factor that may have negatively influenced the cost of myelo-ablative therapy is the application of total body irradiation (TBI) as part of the conditioning regimen. In most protocols high-dose melphalan is nowadays used as the conditioning regimen, since TBI has been shown to result in more severe mucositis and treatment related mortality than high-dose melphalan alone (19,20).

Since its introduction, the costs of autologous stem cell transplantations have been significantly reduced by harvesting stem cells from the peripheral blood instead of from the bone marrow, which leads to more rapid hematologic recovery and early discharge (21,22). However, the costs generated by patients using IFN maintenance treatment following intensified chemotherapy can also be further reduced. For example, we observed a more frequent and prolonged use of bisphosphonates in these patients, necessitating a higher occupation of the daycare treatment department. Coyte et al showed that the latter costs can be reduced greatly if the medication is administered at home using a portable and disposable intravenous administration device (23). In addition, the adequate duration of bisphosphonate administration has to be validated in a prospective study. For the near future, we expect the costs of an additional consolidation therapy consisting of myelo-ablative treatment to remain substantially higher when compared to patients who undergo intensified chemotherapy only.

There have been no earlier publications on costs of high-dose chemotherapy followed by stem cell rescue for MM in the context of a prospective randomized study. Henon et al.

compared costs of high-dose therapy with stem cell rescue to conventional treatment in an uncontrolled study, but the patient numbers were low and the comparability of the patient groups has been questioned (24,25). They considered the costs of the high-dose therapy and stem cell rescue to be reasonable given the treatment's capability of improving survival. Sampson et al. have provided some cost estimates but it is not clear how these were calculated and moreover, these originated from one specific university hospital (11). For the latter, they are not comparable to our unit costs, which were based on two university hospitals and six local hospitals with the aim to enlarge the representativeness for the "every day" treatment costs. On the basis of the British study and the French randomized study by Attal et al., it has been suggested that the cost-effectiveness ratio of high-dose therapy and stem cell rescue is within 'acceptable' limits (10). Similar conclusions were drawn by others (26). However, these conclusions were all drawn from comparisons of high-dose chemotherapy followed by stem cell rescue with conventional chemotherapy.

In the present study however, the current standard treatment of intensified chemotherapy was compared with the same schedule followed by myelo-ablative treatment supported by stem cell rescue. In this comparison it was found that the costs of the latter treatment were 1.7 times higher than the costs of the former treatment. No differences between both treatments in terms of event-free survival, progression free survival or overall survival were shown. High-dose chemotherapy is currently offered to patients under and even above the age of 70 who are stable or in objective response following induction chemotherapy (27,28) Our results show that it may be not cost-effective if such myelo-ablative treatment and stem cell reinfusion is applied after patients have already received intensified chemotherapy.

Acknowledgements

We are very grateful to A. van Klooster-Rutteman, K.M. Goor, M.T. Groot, and M. Sari from the institute for Medical Technology Assessment for their help in the data collection. We would like to thank the following hospitals for their kind cooperation in performing the cost analysis: Eemland Hospital, Amersfoort, M.H.H. Kramer, S. Wittebol; Hospital de Baronie, Breda, O.J.L. Loosveld; Leyenburg Hospital, Den Haag, P.W. Wijermans; Medical Spectrum Twente, Enschede, M.R. Schaafsma; Beatrix Hospital, Gorinchem, P. van Vliet; University Hospital Groningen, E. Vellenga; Hospital Midden-Twente, Hengelo, H. Dankbaar; Medical Center Leeuwarden, Leeuwarden, P. Joosten; Leiden University Medical Center, W.E. Fibbe; Antonius Hospital, Nieuwegein, D.H. Biesma; Franciscus Hospital, Roosendaal, D.J. de Gooyer, J.T.P. Janssen; Erasmus Medical Center, Rotterdam, J.J. Cornelissen, C.M. Segeren, P. Sonneveld; Haven Hospital, Rotterdam, A.G.C. Bauer; Ikazia Hospital, Rotterdam, M.G.A. Baggen; Medical Center Rijnmond-Zuid, Rotterdam, A.A. van Houten; Hospital Zeeuws Vlaanderen, Terneuzen, T. Hoyset; Diaconessenhuis, Utrecht, H.D. Eggink; University Medical Center Utrecht, H.M. Lokhorst; Walcheren Hospital, Vlissingen, L.G.M. Kerkhofs; Hofpoort Hospital, Woerden, J. Holleman; Isala Klinieken, Zwolle, M. van Marwijk Kooy; HOVON Data Center, Rotterdam, M.M.C. Steijaert, P.H.M. Westveer.

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CHAPTER 10

General discussion and summary

General discussion

Traditionally chemotherapy with alkylating agents has been the treatment of choice for multiple myeloma with a median overall survival of 30 months. Alternative chemotherapy regimens such as VAD failed to show a survival superiority over melphalan/prednisone. With VAD the response rate was higher and more rapid than with other combination regimens but this did not translate into an improvement of survival.

Similar to acute myeloid leukemia dose intensification was attempted to improve the poor probability of long-term survival. With the introduction of high-dose melphalan it became possible to overcome resistance to conventional chemotherapy at the price of severe bone marrow toxicity associated with significant treatment related mortality. Later studies demonstrated that chemotherapy associated morbidity and mortality could be diminished if a rescue procedure with autologous stem cells was used. Initially bone marrow was used as the source of stem cells while more recently reinfusion of peripheral blood stem cells has been introduced. Up to now one randomized study was published, which showed superiority of high-dose therapy with autologous stem cell support over conventional chemotherapy. The benefit of high-dose therapy with stem cell support for prolonged survival is currently evaluated in several prospective studies in younger patients.

The essential question of this thesis has been how intensive patients should be treated and which subgroups benefit from intensified treatment with or without stem cell support.

The use of autologous stem cells as rescue after high-dose therapy includes the risk of infusion of tumor cells while the clinical relevance of the presence of malignant plasma cells in autografts for the risk of relapse is still under discussion and studies on positive selection of CD34 positive cells or purging of autografts have not demonstrated less relapses, it remains likely that reinfused myeloma cells from the graft contribute to relapse.

In a pilot study of the HOVON cooperative group we had challenged the hypothesis that melphalan 140 mg/m^2 split in 2 gifts would not require stem cell support. Indeed the majority of patients completed this phase of chemotherapy with normal hemopoietic recovery. In the HOVON-24 randomized study we again decided to split high-dose melphalan 140 mg/m^2 for the first intensive therapy step into two administrations of melphalan 70 mg/m^2 (intermediate dose melphalan, IDM) in order to avoid the need of stem cell rescue and tumor cell re-infusion. This decision was based on the fact that "maximal" chemotherapy without the risk of prolonged myelosuppression and/or the necessity to reinfuse stem cells had never been seriously investigated in multiple myeloma. It was demonstrated that the latter could be accomplished safely.

The second step in this study addressed the question whether a second treatment cycle consisting of myelo-ablative therapy with autologous stem cell rescue could further improve the clinical outcome. In several international studies, the idea of maximal therapy has been translated in a concept of repeated myelo-ablative treatment. This so-called double autologous hematopoietic stem cell transplantation has never been prospectively evaluated against single myelo-ablation plus maximal conservative treatment. Therefore, the role of double myelo-ablative treatment remains uncertain. Although the comparison of the "tandem program" at Little Rock of double autologous transplantation with historical controls was in favor of double transplantation, several investigators have failed to observe superiority of double repeated intensive therapy over

single intensive therapy. As an example in our study, myelo-ablative treatment did not have an optimal anti-myeloma effect since these patients continued to relapse. This observation was confirmed in the Italian multicenter study. We have explored the use of melphalan dose escalation, while avoiding myelo-ablation and potential tumor reinfusion. With this approach the risk of serious clinical infections was avoided by limiting hypoplasia with the help of G-CSF and prophylactic antibiotics to the minimum. Ultimately, the treatment related mortality was neglectable and the quality of life improved throughout the treatment. There is now international agreement that patients up to 65 years with multiple myeloma should be treated with intensive therapy in order to achieve a longer survival. However, cure is not reached and for that reason the most effective less toxic regimen should be preferred. In this study repeated intensive treatment including myelo-ablative therapy was not better than intensified chemotherapy alone. Therefore single intensive treatment should be the treatment of choice preferable performed within a short time period. At present, high-dose melphalan 200 mg/m^2 with stem cell support is considered the most effective option to achieve this goal. Total body irradiation containing regimens are considered to be more toxic resulting in a decrease of quality of life. There is no support for double autologous stem cell transplantation as standard treatment in patients with multiple myeloma. The benefit of this expensive option has not yet been proven and it does not result in a better quality of life. It remains to be assessed if certain subgroups of patients with poor prognostic characteristics like a high β_2-microglobulin or deletion of chromosome 13 or chromosome 1p/q abnormalities may benefit from repeated cycles of intensive treatment. There may also be a role for a repetition of intensive treatment with stem cell support at the time of relapse in patients who are likely to respond. The main problem of this disease however in spite of intensive treatment and autologous transplant, remains the inevitable relapse. Ultimately, all patients will die from relapse or complications.

Some patients may be cured from multiple myeloma using allogeneic stem cell transplantation although the role of this treatment option remains controversial. Most myeloma patients are ineligible because of older age or lack of an HLA-identical sibling. The advantage of allogeneic transplantation is the fact that a non-contaminated graft can be used and that a graft-versus-myeloma effect may induce a lasting remission. The treatment related mortality of allogeneic transplantation in multiple myeloma is significant in particular in older patients and in patients at relapse or refractory disease. In our study patients received an allogeneic transplantation after induction therapy at diagnosis using a partial T-cell depleted graft in order to limit graft-versus-host-disease. It was the first study in which the effect of allogeneic stem cell transplantation was prospectively evaluated. The results show, that the outcome was not better when compared to intensive therapy with or without myelo-ablation, at least partly due to the treatment related mortality. However, also the progression free survival was not better with allogeneic transplantation, and even patients in complete remission continued to relapse. These data indicate that while there may be a limited graft-versus-myeloma effect of partially T-cell depleted allogeneic stem cell transplantation, it is not sufficient to prevent relapse in the upfront setting. Therefore, the results of our study do not support a role for allogeneic transplantation as the treatment of choice in multiple myeloma. Recently it was shown that donor lymphocyte infusions induce long remissions in relapsed patients. Therefore alternative approaches using nonmyelo-ablative stem cell

transplantation with possible less toxicity or prophylactic donor lymphocyte infusion with likely more graft-versus-myeloma effects may be a better alternative and these procedures need to be further explored. If such an approach has to be restricted to younger high risk patients or can also be applied in older patients needs to be awaited.

At present multiple myeloma remains an incurable disease. Although dose escalation has significantly improved the response rate and the survival, the relapse rate is unacceptably high. Other treatment modalities are needed to improve the long-term outcome of patients with multiple myeloma.

With the accomplishments in the molecular pathogenesis of multiple myeloma, new target based treatment modalities will come forward. These modalities may not only target the tumor cell itself but also be directed against factors in the microenvironment that promote tumor cell growth and survival. Recently it was found that thalidomide has a substantial antitumor effect in advanced and refractory multiple myeloma. Multiple myeloma is accompanied by an increased bone marrow vascularisation and thalidomide may inhibit angiogenesis and myeloma growth. The role of thalidomide during remission induction or maintenance therapy will be explored and the potency of new antiangiogenic derivatives (ImiD's) needs furthest investigation. The role of proteasome inhibitors in the treatment of multiple myeloma seems promising. As more potent bisphosponates become available, their possible anti-tumor effect may become more relevant. Maintenance therapy with intensive chemotherapy may also improve the outcome after high-dose therapy by prolonging the relapse free survival.

With all these new treatment modalities, the classic staging system according to Salmon and Durie does not suffice anymore. New staging systems including adverse prognostic variables like high β_2-microglobulin, chromosomal aberrations like chromosome 13 or chromosome 1p/q abnormalities or microvascular density will be developed in order to define subgroups, which may benefit from different therapeutic approaches.

Summary

Multiple myeloma (MM) is a malignant plasma cell disorder characterized by an abundant proliferation of clonal plasma cells which produce monoclonal heavy and/or light chain immunoglobulines. This disorder accounts for 10% of hematological malignancies. The most common clinical features in MM consist of bone pain due to osteolytic lesions or osteoporosis and symptoms related to compromised normal hematopoiesis. Suppression of normal immunoglobulin synthesis leads to recurrent bacterial infections.

Multiple myeloma is still an incurable disease. With the introduction of melphalan and prednison the median overall survival improved to 30 months. Several other combination regimens have been used to improve outcome but were not superior to melphalan/prednisone. With the introduction of high-dose melphalan it became possible to overcome resistance to conventional chemotherapy but at the cost of high toxicity. Since then high-dose therapy with autologous stem cell rescue has been extensively used in phase I and II trials. Even further dose intensification with double transplantation has been used in an attempt to improve response rate and overall survival. However, randomized studies are needed to evaluate who may benefit from these different treatment modalities.

In 1995 a prospective, randomized, multicenter study addressing the issue of high-dose therapy in previously untreated multiple myeloma was initiated by the Dutch-Belgian Hemato-Oncology Cooperative Study Group, which was completed in 2000. The trial and its outcome have resulted in this comprehensive thesis on high-dose therapy in multiple myeloma.

In chapter 1 the biology of multiple myeloma is discussed. The development of multiple myeloma is a multistep process with multiple oncogenic events eventually leading to aggressive disease. Chromosomal translocations and gene activations play an important role in the development of multiple myeloma. Multiple and complex karyotypes have been described in multiple myeloma. Some chromosomal aberrations such as chromosome 13 and 13q deletions are associated with poor outcome after conventional and high-dose chemotherapy.

Until recently the prognosis of multiple myeloma was exclusively based on clinical variables and on the Salmon and Durie staging system. In chapter 1 the relevant prognostic factors which reflect characteristics of the malignant clone such as cytogenetics, plasma cell morphology, plasma cell labeling index and specific multiple myeloma antigens and characteristics reflecting tumor burden such as β_2-microglobulin are discussed.

Because of the poor outcome of multiple myeloma more intensive treatment regimen have been developed and explored in the last two decades. Conventional chemotherapy regimens are discussed in chapter 1. The results of more intensive regimens such as high-dose melphalan with or without autologous bone marrow or peripheral stem cell rescue, double autologous stem cell transplantation and allogeneic stem cell transplantation are summarized. Time of transplant, different conditioning regimens and the influence of contaminated graft are discussed.

The combination of vincristine, doxorubicine and dexamethasone (VAD) is known to induce rapid and marked responses in both newly diagnosed patients as in refractory and relapsed patients. This regimen is normally administered as a continuous infusion for 4

days via an indwelling catheter. In chapter 2 the clinical response after the developed VAD as rapid infusion for remission induction prior to high-dose therapy is described. This new way of administration was as effective as continuous infusion and could be applied as an outpatient regimen.

Glucocorticoids as used in the VAD regimen are the mainstay in the treatment of B-cell malignancies such as multiple myeloma but eventually all patients become resistant to glucocorticoids. We wondered if the earlier described splice variant of the glucocorticoid receptor, glucocorticoid receptor δ, would play a role in the occurrence of resistance. In chapter 3 is described that the glucocorticoid receptor δ was expressed at considerable amount in multiple myeloma. Transient transfection experiments in multiple myeloma cell lines led to increased activity of the glucocorticoid receptor α, the active form of the glucocorticoid receptor, suggesting a role of glucocorticoid receptor δ in glucocorticoid sensitivity.

In chapter 4 the clinical results of the randomized study are described. Patients were randomized after remission induction with the VAD regimen to receive either intensified chemotherapy consisting of high-dose melphalan (140 mg/m^2) divided in 2 cycles of intermediate dose melphalan (IDM, 70 mg/m^2) or the same regimen followed by myelo-ablative treatment. Further intensification with myelo-ablative therapy after intensified chemotherapy did result in a higher complete remission rate and a longer time to progression but this was not reflected by a better event-free survival and overall survival, suggesting that further intensification of therapy is not warranted at this stage of the disease.

Patients under the age of 56 years with an HLA-identical sibling were candidates for allogeneic stem cell transplantation (allo-SCT). In chapter 5 the outcome of allo-SCT of these patients is compared with matched patients who received intensified treatment with or without myelo-ablative therapy and autologous stem cell rescue. Overall survival after allogeneic transplantation was inferior to the overall survival after intensified therapy due to the combination of high treatment related mortality and the obvious lack of a graft-versus-myeloma effect. Our results indicate that there is by now no indication for standard allogeneic transplantation upfront in multiple myeloma.

High-dose melphalan (140 mg/m^2) was divided in 2 cycles of IDM without stem cell rescue. The objective was to maintain the anti-myeloma effect of induction therapy while reducing toxicity, and thus to avoid the need of stem cell reinfusion. In chapter 6 is described that this regimen is feasible and effective in the majority of patients. The outcome was comparable with the outcome after high-dose melphalan. The toxicity was acceptable, only a small number of patients needed autologous stem cell rescue because of persistent myelo-suppression and treatment related mortality was low. This regimen can therefore be applied in the outpatient setting.

In a subset of patients conventional cytogenetic analysis was performed at diagnosis to evaluate the prognostic relevance of chromosomal abnormalities for outcome after high-dose therapy as is reported in chapter 7. An abnormal karyotype was found in almost half of the patients. Of the observed chromosomal abnormalities the presence of complex chromosomal abnormalities, chromosome 13 and 13q deletions and chromosome 1p/q abnormalities predicted for poor overall survival. Chromosome 1p/q abnormalities are known to be present in a high percentage of patients with multiple myeloma but were never found related to prognosis before.

In chapter 8 the influence if high-dose chemotherapy on quality of life is evaluated. Intensified chemotherapy for remission induction resulted in an improvement of global quality of life and functioning and there was a reduction of a number of prominent complaints, especially pain. The quality of life during the first year of follow-up was significantly better in patients treated with intensified chemotherapy when compared to intensified chemotherapy followed by myelo-ablative treatment with autologous stem cell rescue or allo-SCT. Given the outcome of the clinical study, the beneficial additional role of a second high dose of myelo-ablative therapy with autologous or allogeneic stem cell rescue is disputed.

In chapter 9 a cost analysis comparing intensified chemotherapy alone with intensified chemotherapy followed by myelo-ablative therapy with autologous stem cell shows the latter being far more expensive due to an higher amount of hospitalization days, the use of more blood components and antifungal and antibacterial medication.

Samenvatting

Het multiple myeloom is een maligne plasmacel ziekte die gekenmerkt wordt door een overmatige proliferatie van monoklonale plasmacellen in het beenmerg die een monoklonaal zware of lichte keten immunoglobuline produceren. Deze ziekte vormt ongeveer 10% van de hematologische maligniteiten. De meest voorkomende klinische verschijnselen van het multipel myeloom bestaan uit botpijnen als gevolg van osteolytische haarden, botbreuken of osteoporosis en symptomen als gevolg van een gecompromitteerde normale hematopoiese. Door onderdrukking van de normale immunoglobuline synthese ontstaan recidiverende bacteriële infecties.

Het multipel myeloom is tot op heden een niet te genezen ziekte. Met de introductie van de combinatie van melfalan en prednison verbeterde de mediane overleving naar 30 maanden. Met verschillende andere combinatie chemotherapie schema's is daarna geprobeerd dit resultaat te verbeteren maar deze bleken niet beter te zijn dan melfalan en prednison. Met hoge-dosis melfalan bleek het mogelijk te zijn om resistentie tegen conventionele chemotherapie te overwinnen maar ten koste van veel toxiciteit. Sindsdien is hoge-dosis chemotherapie met autologe stamcel reinfusie uitvoerig gebruikt in fase I en II trials. Zelfs verdere dosis intensificatie middels dubbele stamceltransplantaties is inmiddels toegepast in een poging om het respons percentage en de overleving te verbeteren. Gerandomiseerde studies zijn nodig om te evalueren wie voordeel heeft bij de verschillende behandelingsmogelijkheden.

In 1995 startte de Stichting Hemato-Oncologie voor Volwassenen Nederland een prospectieve gerandomiseerde multicenter studie naar de rol van hoge-dosis therapie bij tevoren onbehandelde patienten met het multipel myeloom resulterend in dit omvattend proefschrift over hoge-dosis therapie bij het multiple myeloom.

In hoofdstuk 1 wordt de biologie van het multipel myeloom beschreven. Het ontstaan van het multiple myeloom is een multi-stap proces met multipele oncogene gebeurtenissen welke uiteindelijk leiden tot een agressief ziekteproces. Chromosomale translocaties en gen activaties spelen een belangrijke rol in de ontwikkeling van het multipel myeloom. Multiple complexe karyotypes van chromosomen zijn beschreven bij het multipel myeloom. Sommige chromosomale afwijkingen zoals chromosoom 13 en 13q deleties zijn geassocieerd met een sombere prognose na conventionele en hoge-dosis chemotherapie.

Tot recent was de prognose van het multipel myeloom alleen gebaseerd op klinische parameters en Salmon & Durie stagerings criteria. In hoofdstuk 1 worden andere relevante prognostische factoren besproken zoals cytogenetica, plasmacel morfologie, plasmacel labeling index, specifieke multiple myeloom antigenen en kenmerken die een afspiegeling vormen van de tumor last zoals β_2-microglobuline.

Als gevolg van de sombere prognose van het multiple myeloom zijn meer intensieve behandelings schema's geëxploreerd in de afgelopen 2 decennia. Conventionele chemotherapie schema's worden besproken in hoofdstuk 1. De resultaten van meer intensive behandeling schema's zoals hoge-dosis melfalan met of zonder autologe beenmerg of perifere bloed stamcel reinfusie, dubbele autologe stamceltransplantaties en allogene stamcel transplantaties zijn samengevat. Tijdstip van transplantatie, verschillende conditionering schema's en de rol van een met maligne plasmacellen gecontamineerd transplantaat worden bediscussieerd.

De combinatie van vincristine, doxorubicine en dexamethason (VAD) resulteert in een snelle en goede respons zowel bij onbehandelde patiënten als bij refractaire patiënten en patiënten met een recidief. Dit schema wordt normaliter gegeven als een 4 daags continue infuus via een centraal veneuze katheter. De toediening van VAD via een kortdurende infusie zoals in hoofdstuk 2 wordt beschreven als remissie inductie schema voorafgaande aan hoge-dosis therapie bleek even effectief als via continue infusie en kon poliklinisch worden toegediend.
Glucocorticosteroiden zoals in het VAD schema worden gebruikt vormen het belangrijkste bestanddeel van behandelingsschema's voor B-cel maligniteiten zoals het multiple myeloom maar uiteindelijk ontstaat er bij bijna alle patiënten resistentie tegen glucocorticosteroiden. Wij vroegen ons af of de eerder beschreven splijt variant van de glucocorticosteroid receptor, glucocorticosteroid receptor δ, een rol speelt bij in het ontstaan van resistentie.
In hoofdstuk 3 wordt beschreven dat er een aanzienlijke expressie van glucocorticosteroid receptor δ bestaat bij het multiple myeloom.
Kortstondige transfecties in verschillende cel typen en myeloom cellijnen verhogen de activiteit van de glucocorticosteroid receptor α, de actieve vorm van de glucocorticoid receptor, hetgeen suggereert dat de glucocorticosteroid receptor δ een rol speelt bij glucocorticosteroid gevoeligheid.
In hoofdstuk 4 worden de klinische resultaten van de gerandomiseerde studie beschreven. Patiënten werden gerandomiseerd na remissie inductie met het VAD schema voor ofwel intensieve therapie bestaande uit hoge-dosis melfalan (140 mg/m^2) verdeeld over 2 cycli intermediate dosis melfalan (IDM, 70 mg/m^2) ofwel hetzelfde regime gevolgd door myelo-ablatieve therapie. Verdere intensificatie met myelo-ablatieve therapie na intensieve therapie resulteert in een hoger respons percentage en een langere tijd tot progressie maar niet in een langere overleving hetgeen suggereert dat verdere intensificatie niet aan te bevelen is in dit stadium.
Patiënten jonger dan 56 jaar met een HLA-identiek familielid kwamen in aanmerking voor een allogene stamceltransplantatie (allo-SCT). In hoofdstuk 5 worden de resultaten van de allogene stamceltransplantaties vergeleken met vergelijkbare patiënten die werden behandeld met intensieve therapie met of zonder myelo-ablatieve therapie en autologe stamcel reinfusie. De overleving na een allogene stamceltransplantatie was inferieur aan de overleving na intensieve therapie als gevolg van een combinatie van een hoge behandelings gerelateerde mortaliteit en de afwezigheid van een graft-versus-myeloma effect. Deze bevindingen geven aan dat er op dit moment geen plaats is voor een ongemodificeerde allogene transplantatie als eerste behandeling bij het multiple myeloom.
Hoge-dosis melfalan (140 mg/m^2) werd verdeeld over 2 cycli van 70 mg/m^2 zonder stamcel reinfusie. Het doel hiervan was het anti-myeloom effect van de inductie behandeling te behouden met minimale toxiciteit en het reinfunderen van stamcellen te vermijden. In hoofdstuk 6 wordt beschreven dat dit mogelijk en effectief is bij de meerderheid van de patiënten. Het resultaat is vergelijkbaar met de uitkomst na hoge-dosis melfalan. De toxiciteit is acceptabel, slechts een klein aantal patiënten hadden reinfusie van stamcellen nodig als gevolg van langdurige beenmerg depressie en de mortaliteit was laag. Deze behandeling kan daardoor poliklinisch worden gegeven.

Bij een deel van de patiënten werd bij diagnose conventioneel cytogenetisch onderzoek gedaan om de prognostische waarde van chromosoom afwijkingen voor de uitkomst na hoge-dosis therapie te evalueren. Dit wordt beschreven in hoofdstuk 7. Bij de helft van de patiënten werden chromosomale afwijkingen gevonden. De aanwezigheid van complexe chromosomale afwijkingen, deleties van chromosome 13 en 13q en afwijkingen van chromosoom 1 bleken van voorspellende waarde te zijn voor overleving. Afwijkingen van chromosoom 1 zijn aanwezig bij een groot percentage patiënten met multipel myeloom maar waren niet eerder gerelateerd met prognose.
In hoofdstuk 8 wordt de invloed van hoge-dosis therapie op de kwaliteit van leven van de patiënten geëvalueerd. Intensieve chemotherapie voor remissie inductie resulteerde in een verbetering van de kwaliteit van leven en functioneren en een vermindering van klachten met name pijnklachten. De kwaliteit van leven gedurende eerste jaar van follow-up was significant beter na behandeling met intensieve therapie dan na myelo-ablatieve therapie met stamcel reinfusie of allogene stamceltransplantatie. Gezien de uitkomst van de klinische studie, staat het nut van additionele behandeling met myelo-ablatieve therapie met autologe stamcel reinfusion of allogene stamceltransplantatie ter discussie.
Een kosten analyse waarin intensieve chemotherapie wordt vergeleken met intensieve chemotherapie gevolgd door myelo-ablatieve therapie met autologe stamcel reinfusie is beschreven in hoofdstuk 9. De kosten na myelo-ablative therapie zijn veel hoger als gevolg van een hoger aantal ziekenhuis opname dagen en het toedienen van meer bloedproducten, antischimmel medicatie en antibiotica.

List of abbreviations

ABMT	autologous stem cell transplantation
Allo-SCT	allogeneic stem cell transplantation
β_2M	β_2-microglobulin
Ca	calcium
CR	complete remission
CRP	C-reactive protein
DLI	donor leukocyte infusion
ECOG	Eastern Cooperative Oncology Group
EFS	event-free survival
FISH	fluorescence in situ hybridization
GC	glucocorticoid
G-CSF	granulocyte colony-stimulating factor
GM-CSF	granulocyte-macrophage colony-stimulating factor
GR	glucocorticoid receptor
GVHD	graft-versus-host-disease
GVM	graft-versus-myeloma
Hb	hemoglobin
HDM	high-dose melphalan
HDT	high-dose therapy
HOVON	Dutch-Belgian Hemato-Oncology Cooperative Study Group
IDM	intermediate dose melphalan
IFN	interferon
IL-6	interleukin-6
LDH	lactate dehydrogenase
M-component	monoclonal immunoglobulin
Mel	melphalan
MGUS	monoclonal gammopathy of unknown significance
MM	multiple myeloma
MP	melphalan/prednisone
PBSCT	peripheral blood stem cell transplantation
PCLI	plasma cell labeling index
PD	progressive disease
PFS	progression free survival
PR	partial remission
QoL	quality of life
OS	overall survival
TBI	total body irradiation
TRM	treatment related mortality
TTP	time to progression
VAD	vincristine- doxorubicin-dexamethasone
WHO	World Health Organization

Participating hospitals in the HOVON 24 multiple myeloma study

Gasthuisberg Hospital, Leuven, G.E.G. Verhoef, M. Delforge; Eemland Hospital, Amersfoort, M.H.H. Kramer, S. Wittebol; Academic Medical Center, Amsterdam, M.H.J. van Oers; Antoni van Leeuwenhoek Hospital, Amsterdam, J.W. Baars; Onze Lieve Vrouwe Gasthuis, Amsterdam, K.J. Roozendaal; Slotervaart Hospital, Amsterdam, J.W. Mulder; Gooi-Noord Hospital, Blaricum, H.P. Muller; Hospital de Baronie, Breda, O.J.L. Loosveld; Atrium Medical Center, Brunssum, P. Voogt; Bosch Medical Center, Den Bosch, H.A.M. Sinnige; Leyenburg Hospital, Den Haag, P.W. Wijermans; Albert Schweitzer Hospital, Dordrecht, J.Ph.H.B. Sybesma, H.W.A. Berenschot; Medical Spectrum Twente, Enschede, M.R. Schaafsma; St. Anna Hospital, Geldrop, A.E.M. Smals; Groene Hart Hospital, Gouda, K.J. Heering; Academic Hospital Groningen, E. Vellenga; Hospital Kennemer Gasthuis, Haarlem, P.W.G. van der Linden; Spaarne Hospital, Haarlem, A.B. Arntzenius; Atrium Medical Center, Heerlen, P. Voogt; Medical Center Leeuwarden, Leeuwarden, P. Joosten; Leiden University Medical Center, W.E. Fibbe; Rijnland Hospital, Leiderdorp, M. Boekhout; Academic Hospital Maastricht, Maastricht, H.C. Schouten; St. Antonius Hospital, Nieuwegein, D.H. Biesma; University Medical Center St. Radboud, Nijmegen, A.J. Croockewit, R.A.P. Raymakers; Waterland hospital, Purmerend, H.J. Blomberg; Franciscus Hospital, Roosendaal, D.J. de Gooyer, J.T.P. Janssen; Erasmus Medical Center, Rotterdam, J.J. Cornelissen, C.M. Segeren, P. Sonneveld; Sint Franciscus Gasthuis, Rotterdam, H.C.T. van Zaanen, J.G. Pegels; Haven Hospital, Rotterdam, A.G.C. Bauer; Ikazia Hospital, Rotterdam, M.G.A. Baggen; Medical Center Rijnmond-Zuid, Rotterdam, A.A. van Houten; Vlietland Hospital, Schiedam, J.J. Braun; Maasland Hospital, Sittard, H.N.L.M. Bron; Ruwaard van Putten Hospital, Spijkenisse, M.H. Silbermann; Hospital Zeeuws Vlaanderen, Terneuzen, T. Hoyset; Diaconessenhuis, Utrecht, H.D. Eggink; University Medical Center Utrecht, Utrecht, H.M. Lokhorst; Hospital St. Jansgasthuis, Weert, P.J. de Haan; Hofpoort Hospital, Woerden, J. Holleman; Isala Klinieken, Zwolle, M. van Marwijk Kooy

Curriculum vitae

Christine Segeren werd geboren op 7 september 1961 te Geleen. In 1979 behaalde zij het diploma Gymnasium-β aan de scholengemeenschap Sint Michiel te Geleen. In ditzelfde jaar begon zij met de studie geneeskunde aan de Katholieke Universiteit te Nijmegen. Na het arts examen in 1988 begon zij met de opleiding tot internist in het Sint Lucas ziekenhuis te Amsterdam (opleider Dr. H.B. Schreuder). Deze opleiding werd vanaf 1991 vervolgd in het Academisch Ziekenhuis der Vrije Universiteit (opleider Prof.dr. J. van der Meer). In juli 1993 werd zij als internist geregistreerd. In dit jaar begon ze met het aandachtsgebied hematologie in de Dr. Daniel den Hoed Kliniek te Rotterdam (opleider prof.dr. B. Löwenberg). Na de registratie als internist-hematoloog bleef zij aldaar werkzaam als Chef de Clinique tot 1997. In deze periode verrichtte zij onderzoek onder leiding van Prof.dr. I.P. Touw naar de aanwezigheid van oplosbare G-CSF receptoren in plasma. Van april 1997 tot augustus 2001 was zij werkzaam als staflid hematologie in het Academisch Ziekenhuis Rotterdam, locatie Dijkzigt. In deze periode coördineerde zij onder leiding van Prof.dr. P. Sonneveld en Dr. H.M. Lokhorst de HOVON 24 ontwikkelingsgeneeskunde studie "myelo-ablative chemo-/radiotherapy and autologous stem cell transplantation as compared to only chemotherapy in patients with multiple myeloma". Dit onderzoek vormde de basis van dit proefschrift. In samenwerking met de afdeling Endocrinologie en Reproductie van het Academisch Ziekenhuis Rotterdam (Prof.dr. S.W.J. Lamberts) en de Erasmus Universiteit Rotterdam werd in deze periode onderzoek gedaan naar de rol en functie van glucocorticoid receptoren in hematologische maligniteiten. Sinds augustus 2001 is zij als staflid verbonden aan de afdeling Klinische Oncologie van het Leids Universitair Medisch Centrum (Prof.dr. J.W.R. Nortier).

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Nawoord

Een proefschrift komt tot stand dankzij intensieve samenwerking en de steun van vele mensen, waarvoor dank.

Allereerst wil ik alle patiënten en hun behandelend artsen bedanken voor hun vertrouwen en deelname aan deze klinische studie. Zonder hen zou het nooit mogelijk zijn geweest om de rol van intensieve behandeling bij het multipel myeloom zo nauwgezet te kunnen evalueren.

Prof.dr. B. Löwenberg, Bob, het was een voorrecht om de afgelopen jaren op de afdeling hematologie werkzaam te zijn. Dank voor de mogelijkheid die je hebt geboden om dit onderzoek te doen en het af te ronden tot een promotie.

Dr. H.M. Lokhorst, Henk, heel veel dank voor het vertrouwen dat je mij hebt gegeven om de ontwikkelingsgeneeskunde studie te mogen coördineren en uitwerken. Onze werkbesprekingen waren altijd een collegiaal en intellectueel genoegen. Ik hoop dat je je nog heel lang blijft inzetten voor patiënten met het multipel myeloom en nog tot vele goede en enthousiaste ideeën komt.

Prof.dr. S.W.J. Lamberts, Steven, de samenwerking van endocrinologen en hematologen in een nog eens echt translationeel onderzoek was bijzonder. Hartelijk dank voor het kritisch lezen van dit proefschrift. Het is jammer dat je tijdens de verdediging niet met een verrassende vraag aanwezig kan zijn.

Prof.dr. P.C. Huijgens, Peter, was ik niet door jou getipt dan was ik nooit hematoloog geworden in Rotterdam. Dank voor het kritisch doorlezen van dit proefschrift over een onderwerp wat je na aan het hart ligt.

Prof.dr. J.W. Oosterhuis, bedankt voor de beoordeling van dit proefschrift.

Ronnie van der Holt, wat is een studie zonder statisticus. Hoewel wij als klinicus en statisticus wel eens in een Babylonische spraak verwarring belandden, kwamen we altijd tot een goede oplossing. Ik ben er trots op dat je mijn paranimf wilde zijn.

Petra Westveer en Monique Steijaert, in de loop van de jaren hebben we samen 176.125 data op patiënten registratie formulieren bekeken en gecontroleerd. Op zeer nauwgezette wijze werd door jullie al het voorwerk gedaan.

Locale datamanagers en datamanagers van de integrale kanker centra, dank voor het zorgvuldig aanleveren van de benodigde gegevens voor de uitwerking van dit onderzoek.

Ivon Buijt, Michel van Agthoven, Aat van Klooster en Carin Uyl-de Groot, door jullie enthousiasme en inzet bleek het mogelijk om de behandeling van het multipel myeloom op een zeer veelzijdige wijze te onderzoeken. De gevolgen voor de kwaliteit van leven en de vraag van de kosten van een intensieve behandeling zijn door jullie op een uitstekende wijze uitgewerkt. Hoeveel telefoontjes en kilometers er nodig zijn geweest om deze vragen te beantwoorden moeten jullie me nog maar eens influisteren.

Rosalyn Slater, Pino Poddighe, Berna Beverloo en Jose van Kessel, in het begin van de studie stond cytogenetisch onderzoek bij het multipel myeloom nog in de kinderschoenen. Dankzij jullie enthousiasme kon in de loop van het onderzoek ook het cytogenetisch onderzoek worden opgestart. De betekenis daarvan is inmiddels gebleken. Hartelijk bedankt voor de bijzondere samenwerking.

Jan-Willem Koper en Pieter de Lange, de rol van de delta receptor bij hematologische maligniteiten is toch niet geheel opgehelderd. Misschien kan in de toekomst de goede samenwerking worden uitgebouwd tot Napels om tot een antwoord te komen op deze vraag.

Erik Wiemer, Tineke Buitenweg-de Jong en alle andere medewerkers van de MDR groep, het was een genoegen om gedurende een periode deel uit te maken van jullie groep. Ik heb grote bewondering voor het geduld en de precisie waarmee het basale onderzoek door jullie wordt gedaan. Gelukkig kwamen wij tot ontdekking dat voor translationeel onderzoek dokters en onderzoekers nodig zijn.
Jan van Kapel, vele malen heb je me gewaarschuwd: je vingers zijn te snel. Maar, als er weer eens een PC probleem ontstond dan stond jij altijd weer klaar, ook in het weekend.
Alle medeauteurs van de publicaties wil ik bedanken voor hun opbouwend commentaar waardoor ieder manuscript weer tot een betere versie kon worden gebracht.
Oud-collega's van de afdeling hematologie van het AZR, thans Erasmus MC: aan onze samenwerking gedurende al die jaren bewaar ik zeer warme herinneringen. Ik wil jullie bedanken voor de medewerking die ik heb gekregen om onderzoek te doen. Ook als oncoloog blijf ik nog dagelijks op de hoogte van jullie reilen en zeilen.
Willemijn Sizoo, Ton Hagenbeek, Mars van 't Veer, Jan Cornelissen en andere collega's, van jullie leerde ik het vak. Wat was ik trots toen jullie mij vroegen om als chef de clinique te blijven. Het was een goede tijd in de Daniel.
Jeanette Doorduijn, jij en ik weten als geen ander hoe het is om een OG studie te coördineren, succes met jouw laatste loodjes. Frank Leebeek, jarenlang mijn kamergenoot en een luisterend oor als het even niet mee zat. Elly Lugtenburg, collega en vriendin, Kerstmis en Oud en Nieuw hebben wij op bijzondere wijze gevierd.
Verpleegkundigen van B2, A2, 5-Zuid, poli-assistentes en secretariaat van de afdeling hematologie, jullie vormen samen een prachtige afdeling.
Ineke Vogel en Petra Landman, dank voor de zorg voor al de tabellen en Jeanne Vlasveld voor de secretariële ondersteuning.
Ivo Touw,de eerste kennismaking met het lab verliep via jou. Het kwantificeren van de G-CSF receptor is uiteindelijk niet gelukt maar het was hele goede en leerzame periode.Van jou heb ik ook geleerd dat het goed is om af en toe je frustratie te laten blijken.
Ineke Kersten, dat intensieve zorg nazorg behoeft is mede door jouw inzet een gegeven geworden.
Collega's van de afdeling klinische oncologie in het LUMC, dank voor de tijd die jullie hebben gegeven om dit proefschrift af te ronden, er komt nu gelukkig ruimte om mij verder te gaan verdiepen in dit mooie vakgebied en medisch oncoloog te worden.
Vriendinnen en vrienden, er komt gelukkig weer meer tijd voor gezellige dingen.
Sonja Zweegman, Zon, je bent al jaren mijn steun en toeverlaat in goede en minder goede tijden. Ik ben heel blij met jou als paranimf aan mijn zijde bij deze mijlpaal.
Mijn ouders, jullie hebben af en toe met verwondering mijn loopbaan en levensloop gevolgd. Ik wil jullie bedanken voor de mogelijkheden en de steun die jullie mij hebben gegeven.
Pieter, mijn ware promotor, jouw liefde voor het myeloom onderzoek sloeg al snel als een vonk over. Het was bijzonder om de afgelopen jaren hieraan gezamenlijk te werken. Jij gaat alleen verder met het myeloom onderzoek, maar wij gaan samen verder.