Ka Lung Wu

NEW GENETIC INSIGHTS and THERAPY IN MULTIPLE MYELOMA

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NIEUWE INZICHTEN IN DE CYTOGENETICA
EN BEHANDELING VAN HET MULTIPEL MYELOOM

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Het is niet moeilijk het goede te herkennen,
maar wel het in daden om te zetten
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Voor mijn ouders

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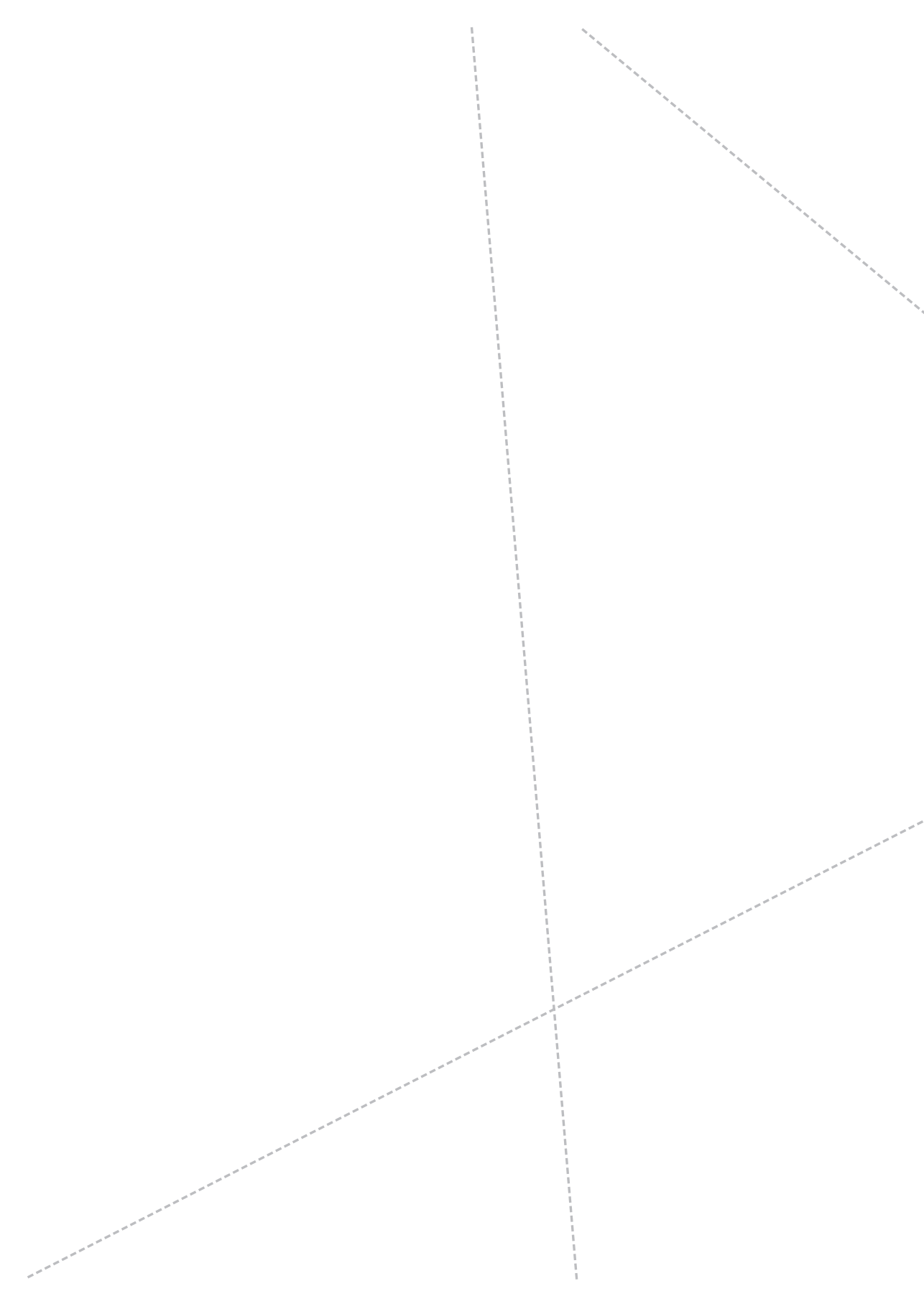

Chapter 1

INTRODUCTION AND OUTLINE OF THE THESIS

Adapted from *Clin Lymphoma Myeloma. 2005 Sep;6(2):96-101*

1.1
MULTIPLE MYELOMA

Multiple myeloma is a malignant plasma cell disorder characterized by the accumulation of abnormal plasma cells in bone marrow. These plasma cells produce a monoclonal immunoglobulin (M-protein), which is usually IgG (50%), IgA (30%) or light chain (15%) and occasionally IgD, IgM or IgE. Multiple myeloma accounts for approximately 1% of all malignancies and 10% of hematological cancers. The median age of diagnosis is 70 years. The annual incidence in the Netherlands is 8 per 100.000 and the incidence increases progressively with age.[1] The clinical symptoms of multiple myeloma are caused by skeletal destruction and by plasma cell infiltration of bone marrow, which results in a compromised normal hematopoiesis. Furthermore, a high level of M-protein can cause hyperviscosity, renal failure and neuropathy. Suppression of normal immunoglobulin production leads to a high incidence of infectious events.[2]

The diagnosis of multiple myeloma is based on the presence of M-protein, bone marrow plasmacytosis and lytic bone lesions. Several diagnostic systems have been proposed for the diagnosis and classification of multiple myeloma.[3-5] The criteria used are very similar with only slight differences in parameter combinations or the threshold used. Nevertheless, these slight differences may lead to different diagnosis depending on which system is used.[6] Recently, the International Myeloma Working Group agreed on new consensus criteria for the classification of multiple myeloma and other gammopathy.[7] In this classification, the concept of end-organ damage was introduced to distinguish between monoclonal gammopathy of undetermined significance (MGUS), asymptomatic myeloma and symptomatic myeloma. (Table 1)

MGUS is a clinically benign precursor of multiple myeloma. MGUS is found in 3% of persons older than 50 years and in about 5% of those older than 70 years.[8] Patients with MGUS are asymptomatic and treatment is not required. However, approximately one-fourth of the MGUS will ultimately evolve into multiple myeloma, malignant lymphoproliferative disorders or amlyoidosis.[9] There are no well-established predictors of malignant transformation of MGUS.[10-12]

Asymptomatic myeloma (smouldering multiple myeloma) is diagnosed when the patients fulfilled the diagnostic criteria of multiple myeloma with absence of end-organ damage or clinical symptoms. These patients should not be treated since the disease can remain stable for a long period of time without treatment.[13]

TABLE 1. Criteria for the classification of monoclonal gammopathies, multiple myeloma and related disorders according to International Myeloma Working Group[7]

Monoclonal gammopathy of undetermined significance
- M-protein in serum < 30 g/l
- Bone marrow clonal plasma cells < 10% and low level of plasma cell infiltration in a trephine biopsy
- No evidence of other B-cell proliferative disorders
- No related organ or tissue impairment* (no end organ damage, including bone lesions)

Asymptomatic myeloma ('smouldering multiple myeloma')
- M-protein in serum ≥ 30 g/l
  and/or
- Bone marrow clonal plasma cells ≥ 10%
- No related organ or tissue impairment (no end organ damage, including bone lesions) or symptoms

Symptomatic multiple myeloma
- M-protein in serum and/or urine
- Bone marrow (clonal) plasma cells or plasmacytoma
- Related organ or tissue impairment (end organ damage, including bone lesions)

Non-secretory myeloma
- No M-protein in serum and/or urine with immunofixation
- Bone marrow clonal plasmacytosis ≥ 105 or plasmacytoma
- Related organ or tissue impairment (end organ damage, including bone lesions)

Solitary plasmacytoma of bone
- M-protein in serum and/or urine
- Single area of bone destruction due to clonal plasma cells
- Bone marrow not consistent with multiple myeloma
- Normal skeletal survey (and MRI of spine and pelvis if done)
- No related organ or tissue impairment (no end organ damage other than solitary bone lesion)

Extramedullary plasmacytoma
- No M-protein in serum and/or urine
- Extramedullary tumour of clonal plasma cells
- Normal bone marrow
- Normal skeletal survey
- No related organ or tissue impairment (end organ damage, including bone lesions)

Multiple solitary plasmacytomas (± recurrent)
- No M-protein in serum and/or urine
- More than one localized area of bone destruction or extramedullary tumour of clonal plasma cells which may be recurrent
- Normal bone marrow
- Normal skeletal survey and MRI of spine and pelvis if done
- No related organ or tissue impairment (no end organ damage other than the localized bone lesions)

* Myeloma-related organ or tissue impairment (end organ damage) is defined as:
- Calcium levels increased: serum calcium > 0.25 mmol/l above upper limit of normal or > 2.75 mmol/l
- Renal insufficiency: creatinine > 173 mmol/l
- Anaemia: hemoglobin 2 g/dl below lower limit of normal or hemoglobin < 10 g/dl
- Bone lesions: lytic lesions or osteoporosis with compression fractures (MRI or CT may clarify)
- Other; symptomatic hyperviscosity, amyloidosis, recurrent bacterial infections (≥ 2 episodes in 12 months)

The majority of myeloma patients require treatment because of symptomatic disease at presentation. Without treatment the median survival is only 7 months.[14] With the introduction of melphalan and prednisone in 1969 and high-dose chemotherapy with autologous stem cell transplantation in the late 1980s, the overall survival has improved significantly.[15-18] However the disease remains incurable and the majority of patients will develop progressive disease within several years.

1.2 PROGNOSTIC FACTORS IN MULTIPLE MYELOMA

The survival duration of patients with multiple myeloma ranges from a few months to many years.[19] This heterogeneity relates mainly to specific characteristics of the disease itself and of the host. The identification of prognostic factors is critical in order to obtain more individualized information about disease outcome and to adapt the patients treatment according to the risk groups. Furthermore, the assessment of the prognostic factors is important for comparison of patients groups in different clinical trials.

In recent years, new potential prognostic factors have been identified and they need to be evaluated in the context of novel therapeutic strategies.

1.2.1 **Genetic factors of the malignant clone**

Cytogenetics are emerging as one of the most important prognostic factors in multiple myeloma. (Table 2) The Group of the University of Arkansas has shown that either partial or complete deletion of chromosome 13 is associated with poor prognosis after high-dose chemotherapy.[20] Other groups have confirmed this observation in patients treated with conventional or high-dose chemotherapy, in which deletion of chromosome 13 was analyzed by fluorescent in situ hybridization (FISH).[21-25] With metaphase cytogenetics, deletion of chromosome 13 can be found in approximately 15% of the newly diagnosed patients[20,26,27] and by using interphase FISH in 40-50% of the patients.[21,22,24] In the majority of patients with chromosome 13 abnormality, large proportions of 13q arm are deleted or even monosomy 13.[28] The molecular consequence of chromosome 13 deletion is not clear and still under investigation.

Translocations involving the immunoglobulin heavy chain locus (14q32) are present in approximately 75% of the newly diagnosed and in more than 85% of the patients with plasma cell leukemia.[29] The translocation partners of 14q32 are quite heterogeneous with 4p16.3, 11q13 and 16q23 being most frequently involved. The t(4;14)(p16.3;q32) and t(14;16)(q32;q23) are associated with poor prognosis after high-dose chemotherapy.[30-32]

The t(4;14)(p16.3;q32) is present in approximately 20% of the patients by using interphase FISH.[33] The translocation results in expression of multiple myeloma SET domain (mmset) and/or fibroblast growth factor receptor 3 (fgfr3), which promotes myeloma cell proliferation and prevents apoptosis.[34] The t(14;16)(q32;q23) is present in 10% of the patients and results in expression of c-maf.[32,35] Other chromosomal abnormalities associated with poor prognosis are 17p13 deletion (p53)[31,36] and translocations involving c-myc (8q24).[37]

The t(11:14)(q13;q32) and hyperdiploid karyotype are chromosomal abnormalities associated with a favorable prognosis. The t(11:14)(q13;q32) is detected in 20% of the patients and results in expression of cyclin D1.[23,30,38] Hyperdiploid karyotype is observed in 40-50% of the patients with multiple myeloma. The majority of these patients have a chromosome pattern, which consists of the combination trisomies of chromosomes 5, 7, 9, 11, 15, 19 and 21, and low prevalence of chromosome 13 deletions. Several studies have shown that hyperdiploid-myeloma patients have a better prognosis than nonhyperdiploid-myeloma patients.[39-42]

The introduction of microarray technology has made it possible to analyze the global gene profiles of normal and malignant plasma cell populations. Recent data have shown that gene expression profiling represents a valuable tool for identifying myeloma specific genetic aberrations[43] and it can define distinct clinico-pathogenetic subgroups of myeloma patients.[44] Recently, using gene expression profiling has revealed that gain of 1q21 leads to activation of CKS1B, a regulator of p27Kip1, which is associated with a poor prognosis after high-dose chemotherapy.[45] Comparison of expression profiles from myeloma patients with and without bone lesions has identified specific gene markers which are predictive of bone disease.[46,47]

TABLE 2. Chromosome abnormalities and survival after high-dose therapy

Chromosome	Gene	%	Impact	Median survival (with vs. without)	Reference
13q deletion	Rb-1?	50	unfavorable	35 vs. 51 months	Fonseca[23]
t(4;14)(p16;q32)	mmset/fgfr3	20	unfavorable	19 vs. 44 months	Gertz[31]
t(14;16)(q32;q23)	c-maf	10	unfavorable	14 vs. 45 months	Dewald[32]
17p13 deletion	p53	10	unfavorable	15 vs. 39 months	Gertz[31]
t(11;14)(q13;q32)	cyclin D1	20	favorable	41 vs. 26 months	Soverini[38]
1q21 gain	CKS1B?	-	unfavorable	-	Shaughnessy[45]

1.2.2 **Prognostic factors associated with tumor burden**

The tumor burden can be assessed by means of the Durie and Salmon staging system, which was specifically obtained from mathematical models for evaluation of tumor mass.[48] Multiple myeloma was divided in three tumor burden groups, which correlated with survival.

Several biochemical markers including interleukin-6, lactate dehydrogenase, calcium, albumin, β_2-microglobulin, and C-reactive protein have been used to discriminate prognostic subgroups.[49-51] The most important prognostic marker is the β_2-microglobulin. β_2-microglobulin is a low molecular weight light-chain of the class I histocompatibility antigen and is synthesized by all nucleated cells. High tumor burden and renal failure leads to elevated serum concentrations of β_2-microglobulin.

Recently, a new staging system has been proposed. The International Staging System (ISS) was obtained from statistical analysis of potential prognostic factors in a large international data set of symptomatic myeloma patients. Based on two simple biochemical parameters, serum β_2-microglobulin and albumin concentration, multiple myeloma is divided in three stages in which the median survival varies from 29 to 62 months. (Table 3)

TABLE 3. International Staging System for multiple myeloma[52]

Stage	Criteria	Median survival (months)
I	serum β_2-microglobulin < 3.5 mg/l and serum albumin ≥ 35 g/l	62
II	serum β_2-microglobulin < 3.5 mg/l but serum albumin < 35 g/l or serum β_2-microglobulin 3.5 - < 5.5 mg/l	44
III	serum β_2-microglobulin ≥ 5.5 mg/l	29

1.2.3 **Host factors**

Among the host factors, the favorable influence of a good performance status and of young age are well established.[53,54] The characterization of inherited genetic variation has become of interest because of its effect on tumor response and side effects of chemotherapy. Single nucleotide polymorphisms within important genes can alter the function of the gene. A number of studies have explored the genetic polymorphisms in tumor necrosis factor (TNF). These studies have shown that polymorphisms in regulating the expression of TNF may influence the outcome after therapy in multiple myeloma.[55,56] Furthermore, several studies have shown that polymorphisms in enzymes that are involved in the metabolism and detoxification of chemotherapeutic agents can influence the outcome after chemotherapy in myeloma and other cancers.[57-60]

1.3 TREATMENT OF MULTIPLE MYELOMA

1.3.1 **Conventional chemotherapy**

Melphalan and prednisone (MP) has been the mainstay of treatment of multiple myeloma for many years. In newly diagnosed patients, the response rate was 50-60% with a complete response rate of less than 5%. The median overall survival was 30 months.[15,16] Various combination regimens have been used in an attempt to improve the outcome obtained with MP. In 1998 the Myeloma Trialists Collaborative Group published a meta-analysis of 6.633 patients treated in 27 randomized trials in which combination chemotherapy and MP were compared.[61] Combination chemotherapy had a significant higher response rate as compared to MP (60% vs. 53.3%). However, there was no difference in median overall survival between both regimens (29 vs. 29 months).

The vincristine, adriamycin and dexamethasone regimen (VAD) was first introduced in the early 1980s.[62] The VAD regimen was first used in myeloma patients who were refractory to alkylating agents. In relapsed and refractory patients, the response rate was 50-70%.[63,64] Subsequently, studies were performed in newly diagnosed patients and the response rate to VAD was 60-80 % with a complete response rate that varied from 5% to 25%.[65-67] The wide range of response rate could be explained by differences in patient characteristics and criteria used to define response in these studies. The Myeloma Subcommittee of the European Group for Blood and Marrow Transplant (EBMT) has defined criteria for evaluation of disease response and progression.[68] When these criteria are applied, the complete response rate to VAD is less then 10%. The remissions are not durable (median 18 months)[65] and there is no long-term survival advantage of VAD over MP.[61]

High-dose steroids, particularly high-dose dexamethasone, have significant activity in myeloma. Several studies have reported on the efficacy of single-agent dexamethasone for treatment of myeloma, especially for relapse and refractory disease.[69,70] Using a more dose-intensive schedule of dexamethasone may induce a higher response rate but also significant toxicity in relapsed or refractory patients.[71]

1.3.2 **High-dose chemotherapy**

The first dose escalating study of chemotherapy was initiated by McElwain and Powles in 1983.[72] The dose of melphalan could be escalated up to 140 mg/m^2 without growth factor or stem cell support. All patients responded to treatment with a complete response rate of approximately 30%. The availability of growth factors and autologous stem cell support encouraged the introduction of myeloablative high-dose chemotherapy.

High-dose chemotherapy with autologous stem cell transplantation was first performed in patients with relapsed and refractory disease.[73,74] The response rate (complete and partial response) was 60-80% with a median overall survival of 25 months.

In newly diagnosed patients, high-dose chemotherapy with autologous stem-cell transplantation is performed as intensification or consolidation treatment after induction therapy. Randomized phase III studies were performed[75-78] and showed that high-dose chemotherapy as compared to conventional dose chemotherapy resulted in superior complete response rate, an improved event-free survival and overall survival.[17,18] (Table 4)

TABLE 4. Randomized studies comparing conventional dose chemotherapy (CC) and high-dose chemotherapy (HDT)

Study	No	Median follow-up	(n) CR (%)		Median EFS#		Median OS#	
			CC	HDT	CC	HDT	CC	HDT
IFM90[17]	200	7 years	5*	22*	18*	28*	44*	57*
MRC7[18]	407	42 months	8*	44*	20*	32*	42*	54*
Italian MMSG[75]	194	39 months	6*	24*	16	28	42	NR
PETHEMA[76]	190	56 months	11*	30*	33	42	66	61
MAG91[77]	190	10 years	NE	NE	19	25	48	48
US Intergroup[78]	516	76 months	15	17	22	22	48	48

(n) CR, (near) complete response; # Event-free survival (EFS) and Overall survival (OS) in months; NR, not reac hed; NE, not evaluable; * significant p-value; IMF, Intergroupe Francophone du Myélome; MRC, Medical Research Council; MMSG, Multiple Myeloma Study Group; PETHEMA, Programa para el Estudio de laTerapéutica en Hemopatia Maligna; MAG, Myélome Auto Greffe

There is increasing evidence that a complete response is necessary for a durable response after transplantation.[79-81] In an attempt to further improve the complete response rate, a more aggressive induction therapy and/or performing tandem transplantation was investigated.[82-91] (Table 5) The Intergroupe Francophone du Myélome (IFM) conducted a randomized study in myeloma patients with VAD induction therapy, high-dose chemotherapy followed by either one or two autologous stem cell transplantations.[84] A complete or a very good partial response was achieved by 42% of the patients in the single-transplant group and 50% of the patients in the double-transplant group. The survival rates at 7 years were 11% in the single-transplant group and 43% in the double-transplant group. Patients who had at least a very good partial response after the first transplantation did not benefit from the second transplantation.

In a single center non-randomized study, 88 myeloma patients received two autologous stem cell transplantations.[83] The complete response rate after the first transplantation was 30% and after the second transplantation 48%. The event-free survival and overall survival at 5 years were 28% and 55%, respectively. However, no differences in outcome

after complete response were found between patients after the first transplantation and those who had a second transplantation. When these outcomes were compared with those of a control group with a single transplantation, patients with a double transplantation had improved event-free and overall survival.

These observations illustrate the current hypothesis that high-dose chemotherapy should be regarded as consolidation treatment in responding patients, rather than as salvage treatment after failed remission induction. Patients who fail to achieve a very good partial response, a second transplantation may improve the event-free survival, however, the overall survival does not reach a plateau and ultimately relapses occur.

TABLE 5. Randomized studies comparing single vs. double high-dose chemotherapy

Study	No	Median follow-up	(n) CR (%)		Median EFS#		Median OS#	
			single	double	single	double	single	double
IFM94[84]	399	75 months	42	50	25*	30*	48*	58*
MAG95[85]	227	-	39	37	31	33	49*	73*
HOVON24[86]	441	56 months	13*	28*	20	22	50	55
Bologna96[87]	226	57 months	35*	48*	22*	35*	59*	73*
GMMG-HD2[88]	261	-	65	67	23	NR	-	-

(n) CR, (near) complete response; # Event-free survival (EFS) and Overall survival (OS) in months; NR, not reached; * significant p-value; IMF, Intergroupe Francophone du Myélome; MAG, Myélome Auto Greffe; HOVON, Dutch-Belgian Hemato-Oncology Cooperative Study Group; GMMG, German Multiple Myeloma Group

1.3.3 **Allogeneic stem cell transplantation**

The advantages of allogeneic stem cell transplantations are a graft that is not contaminated with tumor cells and the graft-versus-myeloma effect. However, only 5-10% of the patients are candidates for allogeneic transplantation when age and availability of a HLA-matched sibling donor are taken into consideration.

The early studies of allogeneic stem cell transplantation using myeloablative conditioning showed a complete response rate of 50% at 6 months and approximately 60% at 2 years.[92,93] The relapse rate was 20% at 2 years and no plateau was reached. The treatment related mortality, however, was relatively high approximately 40% at 6 months and 50% at 2 years. Since 1994, the treatment related mortality has reduced significantly to 30% at 2 years. In case-matched retrospective[94] and prospective comparisons[95,96] the overall survival of allogeneic stem cell transplantation has been inferior to those of autologous stem cell transplantation.

Recently, nonmyeloablative or reduced intensity conditioning (RIC) transplantation has been performed. The idea is to reduce transplantation related toxicity and rely more

on the immune effect of the graft versus myeloma.[97] Recent studies have reported encouraging results using the approach of tandem autologous followed by RIC transplantation from HLA-matched sibling in high-risk myeloma patients.[96,98-101] The treatment related mortality was reduced from 30% to 10%. The response rate was approximately 80% with a complete response rate of 60%. However, prospective comparisons of double autologous transplantation with tandem autologous followed by RIC transplantation showed similar overall survival in both groups at 2 years.[101] Studies with a longer follow up are needed to determine the role of tandem autologous followed by RIC transplantation as treatment of multiple myeloma.

1.3.4. **Novel therapeutic agents**

1.3.4.1 THALIDOMIDE

In 1999, thalidomide was introduced as a new therapeutic agent in the treatment of multiple myeloma. The rationale for the use of thalidomide was based on studies showing increased bone marrow microvascularity in multiple myeloma[102] and the observation that thalidomide had anti-angiogenic activity in animal models.[103] In-vitro and in-vivo studies have shown that thalidomide targets myeloma cells and the surrounding bone marrow through several different mechanisms.[104] The first clinical trial with thalidomide was conducted by Singhal et al. in patients with relapsed or refractory multiple myeloma.[105,106] The response rate was 30%. The event-free and overall survivals at 2 years were 20% and 48%, respectively. Several studies have confirmed the efficacy of thalidomide alone and in combination with dexamethasone or chemotherapy for the treatment of myeloma patients with relapsed and refractory disease.[107-114] (Table 6)

Subsequently, thalidomide was investigated in newly diagnosed patients. Three studies reported on the combination thalidomide and dexamethasone.[115-117] In these studies, thalidomide was administered at a daily dose of 200 mg orally and dexamethasone at an intermittent dose of 40 mg orally. Thalidomide and dexamethasone was given for up to 4 months, because their primary end-points were response rate and stem cell collection for patients eligible for high-dose chemotherapy. Objective responses were observed in 63% to 72% of patients, with a complete response rate of approximately 10%. A sufficient number of stem cells could be collected.

There are no randomized studies comparing thalidomide and dexamethasone, and VAD like regimens. However, a matched case-control study in 200 patients by Cavo et al.[118] reported a significantly higher response rate with thalidomide and dexamethasone as compared to VAD (76% vs. 52%). The complete response rate was 10% and 8%, respectively.

These studies indicate that the thalidomide and dexamethasone is a relatively safe and effective induction regimen that does not impair stem cell collection. Thalidomide and dexamethasone produce similar or better response rates as compared to VAD. Therefore, thalidomide and dexamethasone is considered as a suitable oral alternative for the VAD regimen.

Several studies have assessed the activity of thalidomide and dexamethasone with anthracycline-containing chemotherapy. Zervas et al.[119] treated 39 patients with the combination thalidomide, vincristine, liposomal doxorubicin and dexamethasone for up to 4 months. The response rate was 74%. Similar response rate was reported by Schütt et al.[120] In both studies, a sufficient number of stem cells could be collected.

The joint HOVON-50/GMMG-HD3 study compared VAD with thalidomide, adriamycin and dexamethasone (TAD) as pretransplantation induction treatment in newly diagnosed patients.[121] Interim analysis of the first 406 patients showed a response rate of 63% in the VAD group and 80% in the TAD group.[122] The complete response rate was 3% and 7%, respectively. However, the response rates after transplantation were similar in both groups (90%) with a complete response rate of 13% and 19%, respectively.

The largest randomized study with thalidomide and chemotherapy has been conducted at the University of Arkansas. A total of 668 patients were treated with subsequently chemotherapy, double autologous stem cell transplantation and followed by maintenance therapy (Total Therapy 2).[90] Patients were randomized up-front to receive thalidomide. At a median follow-up of 42 months, patients treated with thalidomide had a significant higher complete response rate (43% vs. 62%) and 5-years event-free survival (40% vs. 56%). However, no difference was observed in the 5-years overall survival (68% vs. 63%). The relapses in the thalidomide group appeared to be more drug-resistant than the relapses in the control group.

Recently, Palumbo et al.[123] published the results of a randomized study comparing melphalan 4 mg/m^2 d1-7, prednisone 40 mg/m^2 d1-7 and thalidomide 100 mg/d (MPT) and MP in elderly patients who were ineligible for high-dose chemotherapy. MPT resulted in a significant higher response rate (76% vs. 48%), a superior 2-years event-free survival (54% vs. 27%) and 3-years overall survival (80% vs. 64%). Peripheral neuropathy, deep-vein thrombosis and gastro-intestinal toxicity occurred more frequently in the MPT group than in the MP group.

Thalidomide is being investigated in the maintenance setting for its effect on the duration of response after high-dose chemotherapy and autologous stem cell transplantation.[124] The IFM99-2 has 780 patients enrolled from IFM99. These patients were randomly assigned to no maintenance treatment, maintenance with pamidronate alone or maintenance with

thalidomide and pamidronate. Thalidomide increased the overall survival compared with the other two groups. The 4-years probability of survival was 77% in the no maintenance group, 74% in the pamidronate group and 87% in the thalidomide and pamidronate group ($p < 0.4$).

Thalidomide has several adverse side effects that restrict its use in myeloma treatment, including somnolence, constipation, fatigue and neuropathy. Neuropathy is a treatment-limiting disorder that affects the majority of patients who receive thalidomide, and could restrict long-term use of the drug. Venous thromboembolism occurs in 1–3% of patients receiving thalidomide alone, and occurs more frequently when thalidomide is combined with dexamethasone (10–15%) or another cytotoxic drug (10–25%).[125] However, the increased risk of venous thromboembolism can be reduced by prophylactic anticoagulation.[126,127]

TABLE 6. Thalidomide treatment in advanced and newly diagnosed multiple myeloma

Reference	Regimen	No	Disease status	(n)CR (%)	CR+PR (%)
Singhal[105,106]	Thal 200-800mg/d	169	relapse	2	24
Neben[110]	Thal 100-400mg/d	83	relapse	1	20
Anagnostopoulos[111]	Thal 200-600mg/d Dex 40mg d1-5/2wks	47	relapse	13	47
Hovenga[113]	Thal 100mg/d Cyclophosphamide100mg/d	38	relapse	15	84
Garcia-Sanz[112]	Thal 200-800mg/d Cyclophosphamide 50mg/g D 40mg d1-4/3wks	71	relapse	2	57
Rajkumar[128]	Thal 200-800mg/d	31	newly	0	34
Rajkumar[117]	Thal 200mg/d Dex 40mg d1-4,9-12,17-20/4wks	103	newly◊	4	63*
	vs. Dex 40mg d1-4,9-12,17-20/4wks	104		0	41*
Goldschmidt[122]	Thal 200mg/d Adriamycin 9mg/m^2 d1 Dex 40mg d1-4,9-12,17-20/4wks	208	newly◊	7	80*
	vs. Vincristine 0.4mg d1 Adriamycin 9mg/m^2 d1 Dex 40mg d1-4,9-12,17-20/4wks	198		3	63*
Palumbo[123]	Melphalan 4mg/m^2 d1-7 Prednisone 40mg/m^2 d1-7/4wks Thal 100 mg/d (MPT)	176	newly**	16	76*
	vs. Melphalan 4mg/m^2 d1-7 Prednisone 40mg/m^2 d1-7/4wks	164		2	47*

(n) CR, (near) complete response; PR, partial response; Thal, thalidomide; Dex, dexamethasone; newly, newly diagnosed patients; * significant p-value; ◊ pretransplant induction regimen; ** ineligible for high-dose therapy

1.3.4.2 LENALIDOMIDE

Lenalidomide is an analog of thalidomide that has demonstrated significantly more potent preclinical activity compared with thalidomide.[129] Lenalidomide is non-teratogenic and has a different toxic profile to that of thalidomide. In phase I dose escalating studies of patients with relapsed or refractory myeloma, myelosuppression was the dose-limiting toxicity and 25 mg a day was established as the maximum tolerated dose.[129,130]

A subsequent multicenter phase II study in 100 patients reported a response rate of 24%. Approximately one third of the patients who did not respond to lenalidomide alone, an additional responses was achieved when dexamethasone was added to the regimen.[131]

Two randomized phase III studies have compared lenalidomide and dexamethasone to placebo and dexamethasone in patients with relapsed or refractory disease.[132] Lenalidomide was administered at a dose of 25 mg/d for 21 days every 4 weeks and dexamethasone 40 mg on day 1-4, 9-12 and 17-20. Interim analyses of both studies showed a higher response rate and improved time to progression in favour of the lenalidomide and dexamethasone group.

Recently, Rajkumar et al investigated lenalidomide in combination with dexamethasone in 34 patients with newly diagnosed multiple myeloma.[133] The response rate was 91% with a (near) complete response rate of 38%. After 4 cycles of therapy, 15 patients proceeded to stem cell collection. An adequate number of stem cell was obtained in all patients.

The most common adverse side effect of lenalidomide was myelosuppression.[132,133] However, myelosuppression was minimal in newly diagnosed patients, probable reflecting the better bone marrow reserves. Unlike thalidomide, lenalidomide has almost no sedative and constipating effect and only occasional neurotoxic side effect. Lenalidomide combined with dexamethasone has an increased risk for venous thromboembolisms (10%), and concomitant administration of erythropoietin may further increase the risk of thrombosis.[134,135] Prophylactic antithrombotic medication including aspirin is recommended.

1.3.4.3 BORTEZOMIB

Bortezomib is a novel proteasome inhibitor, which is highly active in patients with multiple myeloma. In dose escalating phase I studies of patients with hematological malignancies showed that a twice-weekly dosing schedule of bortezomib, which allowed recovery of proteasome activity towards baseline resulted in a manageable toxicity profile.[136]

A phase II study in 202 patients with relapsed or refractory multiple myeloma treated with 1.3 mg/m^2 bortezomib twice-weekly for 2 weeks, followed by 1 week without treatment,

for up to eight cycles, demonstrated an response rate of 35%.[137] The median overall survival was 16 months, with a median duration of response of 12 months.

A smaller, randomized study in 54 patients, comparing 1.0 mg/m^2 or 1.3 mg/m^2 bortezomib according the same schedule confirmed the activity of bortezomib.[138] In both studies some responses occurred after addition of dexamethasone in patients with no or a suboptimal response to bortezomib alone. Chromosome 13 deletion and elevated β_2-microglobulin, generally considered as poor prognostic factors were not predictive of poor outcome in patients treated with bortezomib.[139]

A subsequent international, multicenter phase III study in 669 patients who had a relapse after 1-3 prior therapies were randomized to receive bortezomib or high-dose dexamethasone.[140] Bortezomib demonstrated to be superior to high-dose dexamethasone in terms of response rate (38% vs. 18%), time to progression (6.2 months vs. 3.5 months) and 1-year survival (80% vs. 66%).

Based on preclinical findings of synergistic anti-myeloma activity with other agents, bortezomib-based combination regimens are under clinical investigation. Preliminary data from studies of bortezomib alone[141] or in combination with dexamethasone[138], liposomal doxorubicin[142], melphalan and prednisone[143,144], thalidomide and dexamethasone[145] or cyclophosphamide and prednisone[146] indicate encouraging activity with manageable toxicities in advanced and newly-diagnosed myeloma patients.

Several studies have accessed bortezomib-based regimens as pretransplantation induction treatment. Bortezomib and dexamethasone[147] or bortezomib, adriamycin and dexamethasone[148] showed to be promising regimens with high complete response rates (25%) and no stem cell toxicity. (Table 7)

Peripheral neuropathy and thrombocytopenia are the most clinically significant adverse events.[137,149] Peripheral neuropathy occurs in approximately 35% of patients.[150] The neuropathy is reversible in majority of the cases after dose reduction or discontinuation of bortezomib. Thrombocytopenia with bortezomib is transient and probable due to a reversible effect on megakaryocytic function rather than direct cytotoxic effect on megakaryocytes or their progenitors.[151] Bortezomib alone or in combination with dexamethasone and/or chemotherapy is not associated with an increased risk for venous thromboembolisms.

TABLE 7. Bortezomib treatment in advanced and newly diagnosed multiple myeloma

Reference	Regimen	No.	Disease status	(n)CR (%)	CR+PR (%)
Richardson[137,152]	Bort 1.3mg/m^2 d1,4,8,11/3wks	193	relapse	10	27
Jagannath[138]	Bort 1.0mg/m^2 d1,4,8,11/3wks	27	relapse	11	30
	vs.				
	Bort 1.3mg/m^2 d1,4,8,11/3wks	26		4	38
Richardson[140]	Bort 1.0mg/m^2 d1,4,8,11/3wks	333	relapse	13	38*
	vs.				
	Dex 40mg d1-4,9-12,17-20/4wks	336		2	18*
Orlowski[142]	Bort 0.9-1.5mg/m^2 d1,4,8,11 Doxil 30mg/m^2 d4/3wks	22	relapse	36	73
Zangari[145]	Bort 1.0-1.3mg/m^2 d1,4,8,11 Thal 50-200mg/d Dex 20mg d1,2,4,5,8,9,11,12/3wks	85	relapse	16	55
Weber[115]	Bort 1.3mg/m^2 d1,4,8,11 ± Dex 40mg d1,2,4,5,8,9,11,12/3wks	32	newly◊	25	88
Oakervee[148]	Bort 1.3mg/m^2 d1,4,8,11 Adriamycin 0-9mg/m^2 d1-4 Dex 40mg d1-4,9-12,17-20/3wks	21	newly◊	24	95
Mateos[143]	Bort 1.0-1.3mg/m^2 d1,4,8,11,22, 25,29,32 Melphalan 9mg/m^2 d1-4 Prednisone 60mg/m^2 d1-4/6wks	53	newly♣	8	72

(n) CR, (near) complete response; PR, partial response; Bort, bortezomib; Dex, dexamethasone; newly, newly diagnosed patients; * significant p-value; ◊ pretransplant induction regimen; ♣ ineligible for high-dose therapy

1.3.4.4 ARSENIC TRIOXIDE

Arsenic trioxide is an agent of recent interest because of its impressive activity in patients with acute promyelocytic leukemia.[153,154] In preclinical studies, arsenic trioxide targets myeloma cells and the bone marrow environment through several mechanisms.[155,156] The precise mechanism of action, however, is unknown.

The initial phase II study of 14 myeloma patients was the first to demonstrate efficacy. Arsenic trioxide was administered in a daily dosing schedule (0.15 mg/kg/d for 60 days) similar to that used for acute promyelocytic leukemia.[157] In this heavily pre-treated group, two patients had at least 50% reduction and one patient had at least 25% reduction in M-protein level. Responses were obtained after approximately 2 months of therapy and lasted at least 6 weeks.

Another phase II study evaluated a less frequent but higher dose of arsenic trioxide (0.25 mg/kg/d for 5 days a week for 2 weeks, every 4 weeks).[158] Eight of 24 patients (33%) had responded with at least 25% reduction in M-protein level. The median time to response was 67.5 days, with a median duration of response of 130 days.

In-vitro studies showed that ascorbic acid increased the cytotoxic effect of arsenic trioxide on myeloma cells.[159] A phase I/II study evaluated the safety and efficacy of arsenic trioxide in combination with ascorbic acid in patients with relapsed or refractory multiple myeloma.[160] Addition of ascorbic acid did not increase the toxicity of arsenic trioxide. Two of the six patients had at least 25% reduction of M-protein level. It is not yet known whether ascorbic acid enhances the clinical effect of arsenic trioxide in myeloma.

Studies are underway to investigate the use of arsenic trioxide with or without ascorbic acid and in various combinations with other agents, such as dexamethasone[161,162], melphalan[163], and bortezomib.[164]

1.4
AIMS AND OUTLINE OF THE THESIS

Multiple myeloma is an incurable disease. With conventional dose chemotherapy, the median survival is approximately 3 years. High-dose chemotherapy with autologous stem cell transplantation has improved the response rate and median survival. The majority of the patients, however, will develop progressive disease within several years.

In recent years, new therapeutic options have become available. The challenge is to identify patient subgroups that will benefit most from the different therapies and to determine how these new therapeutic options could be combined and incorporated into the overall management of multiple myeloma.

This thesis describes several approaches to value the prognostics relevance of cytogenetics in multiple myeloma and to identify patients who may benefit from novel therapies.

In chapter 2, the evolution of chromosomal abnormalities during disease progression is described. In chapter 3, the prognostic relevance of chromosomal abnormalities for outcome of high-dose chemotherapy in multiple myeloma is discussed. The impact of the novel therapeutic agents such as thalidomide, arsenic trioxide, and bortezomib in relapsed or refractory myeloma are discussed in chapter 4, chapter 5 and chapter 6, respectively. In chapter 7, the pleomorphic presentation of cutaneous adverse lesions associated with bortezomib is described. Finally, chapter 8 describes the predictive factor of graft versus host for response after donor lymphocyte infusions.

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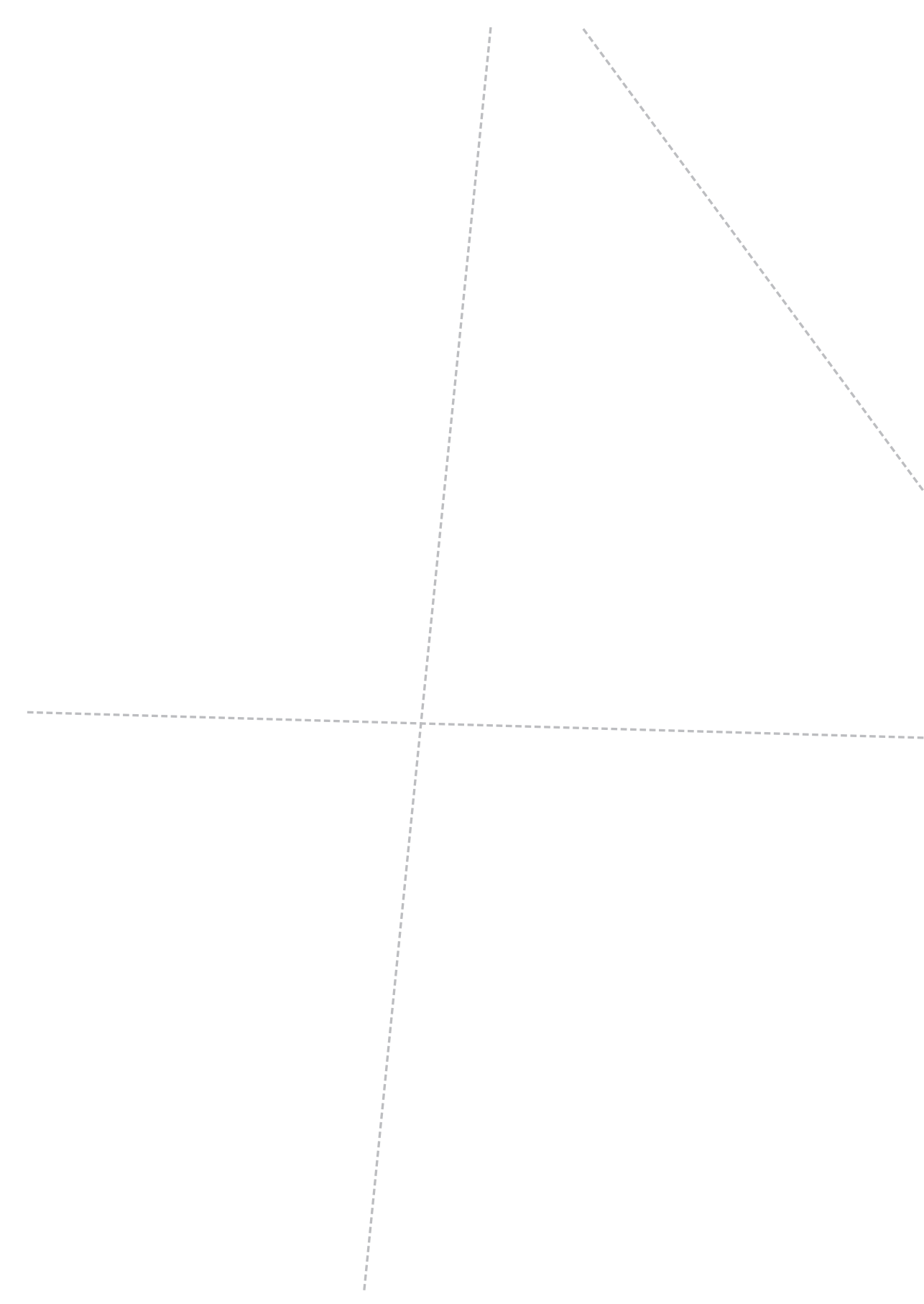

Chapter 2

SEQUENTIAL ANALYSIS OF CHROMOSOME ABERRATIONS IN MULTIPLE MYELOMA DURING DISEASE PROGRESSION

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(submitted)

ABSTRACT

Several chromosomal aberrations have been associated with molecular pathogenesis and classification of multiple myeloma. It is not known if the expression of abnormal karyotypes is consistent in patients during disease progression. Here we report on sequential analysis of conventional cytogenetics as well as fluorescence in situ hybridization (FISH) data of 79 bone marrow specimens from 38 myeloma patients longitudinally followed. We determined and characterized the development of additional chromosomal aberrations during progressive disease. Overall, an abnormal karyotype was found in 49% of the samples by using conventional cytogenetics and in 69% by FISH. Among the patients with an abnormal conventional karyotype, 44% had a hyperdiploid subtype of myeloma. Progressive disease was correlated with an increased complexity of genetic abnormalities, which consisted in the majority of structural aberrations acquired in the later stages of disease. Using conventional cytogenetics, rearrangements of chromosome 1 were the most common structural abnormality (15%). These rearrangements consisted in majority of unbalanced translocations of both 1p and 1q although no specific locus was predominantly affected. Second in frequency were the structural aberrations of chromosomes 8 and 17 (7%). The frequency of del(13q) by FISH was 40% and did not increase in later stages of the disease, suggesting that del(13q) is not a genetic event associated with disease progression. Change of ploidy category during disease progression occurred in a minority of the cases.

This study supports the notion that cytogenetic abnormalities in multiple myeloma are not random. Particular chromosomal alterations are associated with disease progression while others show a stable pattern during the course of the disease.

INTRODUCTION

Multiple myeloma (MM) is characterized by the uncontrolled proliferation and accumulation of monoclonal plasma cells in the bone marrow. The clinical presentation and symptomatology of MM is heterogeneous and the survival ranges from a few months to many years.[1]

Chromosomal analysis of the malignant plasma cells has revealed a wide variety of numerical and structural abnormalities. Recurrent chromosomal aberrations have been detected at presentation in all stages of plasma cell tumors[2] and they have been used to define a multistep model of the transition from a normal plasma cell through MGUS to MM.[3;4] Translocations of the heavy-chain locus (IgH) and deletions of chromosome 13q (del(13q)) are constant genetic events in a substantial portion of the patients. Primary IgH translocations are detected in approximately 60% of the MM patients.[5] These translocations are considered as initiating events in the pathogenesis of MM and seem to be associated with the nonhyperploid type of MM.[4;6] Secondary IgH translocations involving c-myc or other chromosome partners are mostly non-recurrent and can occur at any time during disease progression.[7-9] The role of del(13q) in the progression of MM is currently unclear.[2]

Evolution of the chromosomal abnormalities and the related genomic instability during disease progression in MM is poorly characterized. Our knowledge from conventional cytogenetic studies in MM is hampered by technical restrictions, i.e., the low percentage of plasma cells present in the bone marrow aspirate, the low mitotic index of the plasma cells and the predominance of a normal karyotype, mostly originating from normal hematopoietic components.[10;11] An abnormal karyotype is detected in 40% or less of all patients.[12-14] In those patients having an abnormal karyotype, the frequency and extent of karyotypic abnormalities show a correlation with the disease stage, e.g. approximately 20% abnormal karyotype in stage I, 60% in stage III and 80% for extramedullary tumor.[3]

An alternative technique of fluorescence in situ hybridization (FISH) analysis allows the detection of chromosomal abnormalities in interphase nuclei and cryptic translocations in both metaphase and interphase cells. Using FISH, an abnormal karyotype is detected in almost all patients.[15] However, a major disadvantage is that the obtained information is restricted to the specific chromosome sites or genes under investigation, depending on the chromosome probes used.

In the present study, we wanted to investigate if chromosomal abnormalities in MM change during disease progression using a combined approach of both conventional cytogenetic banding techniques and FISH analysis in sequential bone marrow samples.

MATERIAL AND METHODS

Patients and samples

All myeloma patients with sequential bone marrow samples for which conventional cytogenetic and/or FISH data were available were included in this single center study. A total of 79 samples were obtained from 38 patients. The median age of the patients at the time of first sample submission was 58 years (range, 44–87 years). The diagnosis of MM was based on the diagnostic criteria of Durie and Salmon.[16] The monoclonal protein was IgG in 25 patients, IgA in 8, while 4 patients expressed only light chain and 1 patient had a non-secreting myeloma.

Thirty-eight initial samples were submitted for cytogenetic analysis either at diagnosis (30 samples) or shortly after diagnosis (8 samples) and 41 follow-up samples were obtained at the moment of first or second disease progression (32 samples) or later during the disease process (9 samples). The patients characteristics and samples are shown in table 1.

TABLE 1. Patients characteristics and samples

Number of patients	38
Median age at time of first sample submission (range)	58 years (44-87)
Male/female	24/14
M-protein subtype	
- IgG	26
- IgA	7
- Light chain	4
- Non-secretory	1
Number of samples	79
Initial samples	38
- at diagnosis	30
- 1st relapse	3
- 2nd relapse	4
- 3rd relapse or higher	1
Follow-up samples	41
- 1st relapse	19
- 2nd relapse	13
- 3rd relapse or higher	9

Conventional cytogenetics

Bone marrow cells from each sample were cultured in RPMI with 6-10% serum and in serum free Iscove medium containing interleukin-4 and interleukin-6 and harvested after 96 hours according to standard cytogenetic protocols. The metaphase preparations were analyzed using both RBA- and QFQ-banding. The chromosome aberrations were described according to the ISCN (1995).[17] When possible, a minimum of 20 cells were analyzed. The presence of a clonal abnormality was defined according to ISCN. When only one abnormal metaphase was found, the clonality of the abnormalities observed had to be confirmed by FISH.

Ploidy categories were defined as hypodiploid (up to 45 chromosomes), diploid or pseudodiploid (46 chromosomes), hyperdiploid (47 to 74 chromosomes), and near tetraploid (≥ 75 chromosomes).

FISH analysis

Nuclei from bone marrow samples obtained from cytogenetic investigation were used for interphase FISH analysis. Del(13q) was analyzed using RB-1 (13q14) and D13S319 (13q14.3) probes. Numerical aberrations of chromosome 9 and 11, indicative for the presence of a hyperdiploid clone, were demonstrated using probes for the centromeric regions (cep 9 and cep 11, respectively). Rearrangements of 14q32 were demonstrated using an IgH dual-color break-apart probe. All probes are commercially available (Vysis, IL, USA). The probes were hybridized according to manufacturer's instructions. Slides were counterstained with 4'6-Diamino-2-Phenyl Indol (DAPI). For each hybridization a minimum of 200 interphase cells were scored, as well as 5-10 metaphases if present. Images were captured using an epifluorescence microscope (Axioplan 2, Zeiss, Sliedrecht, the Netherlands) and MacProbe software (version 4.3, Applied Imaging, Newcastle upon Tyne, UK). The cut-off values for the probe were 3% for RB-1, 3.5% for D13S319, 1.5% for cep 9, 1.2% for cep 11 and 3% for IgH rearrangement.

RESULTS

Conventional cytogenetics and ploidy category

Seventy-nine samples from 38 patients were analyzed and the mean interval between two samples was 26 months (range, 6-77). Fifteen of 38 initial (39%) and 21 of 41 follow-up samples (51%) showed an abnormal karyotype. The other samples did not reveal cytogenetic aberrations using conventional banding techniques. The observed abnormal karyotypes were in most cases complex aberrations of which the origin of 19 marker chromosomes in the initial and 46 marker chromosomes in the follow-up samples could not be identified.

Of the 36 samples with an abnormal karyotype, 15 were hyperdiploid (44%) and 21 were non-hyperdiploid (56%). The non-hyperdiploid samples showed hypodiploidy in 13, pseudodiploidy in 6, and near tetraploidy in 2 samples.

Numerical and structural aberrations by conventional cytogenetics

Analysis of the sequential samples showed an increased number of chromosomal abnormalities in the follow-up samples compared to the initial samples. (Figure 1) The mean number of aberrations per sample with karyotypic abnormalities increased from 8 (range, 1-38) in the initial samples to 15 (range, 2-36) in the follow-up samples. The increase was relatively greater for structural (n= 24 → 117) as compared to numerical aberrations (n= 75 → 143), respectively. The chromosomes involved in these aberrations are shown in figure 2.

FIGURE 1. Number of chromosomal abnormalities in 36 sequential samples with an abnormal conventional karyotype

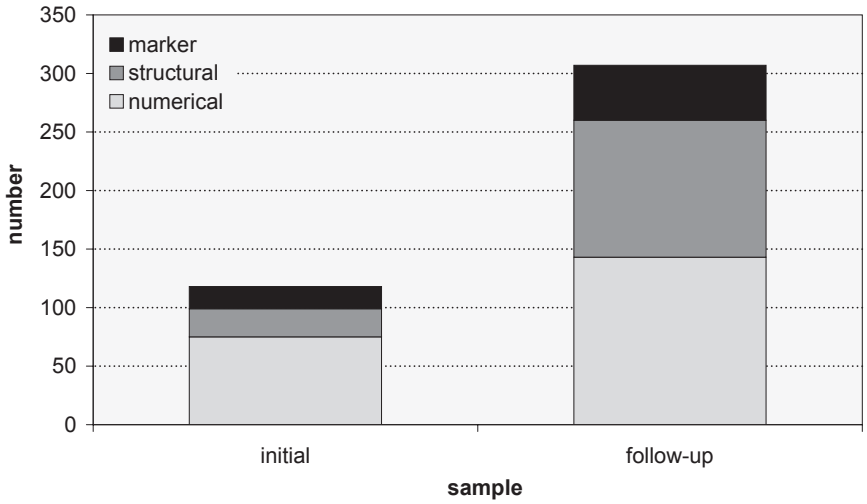
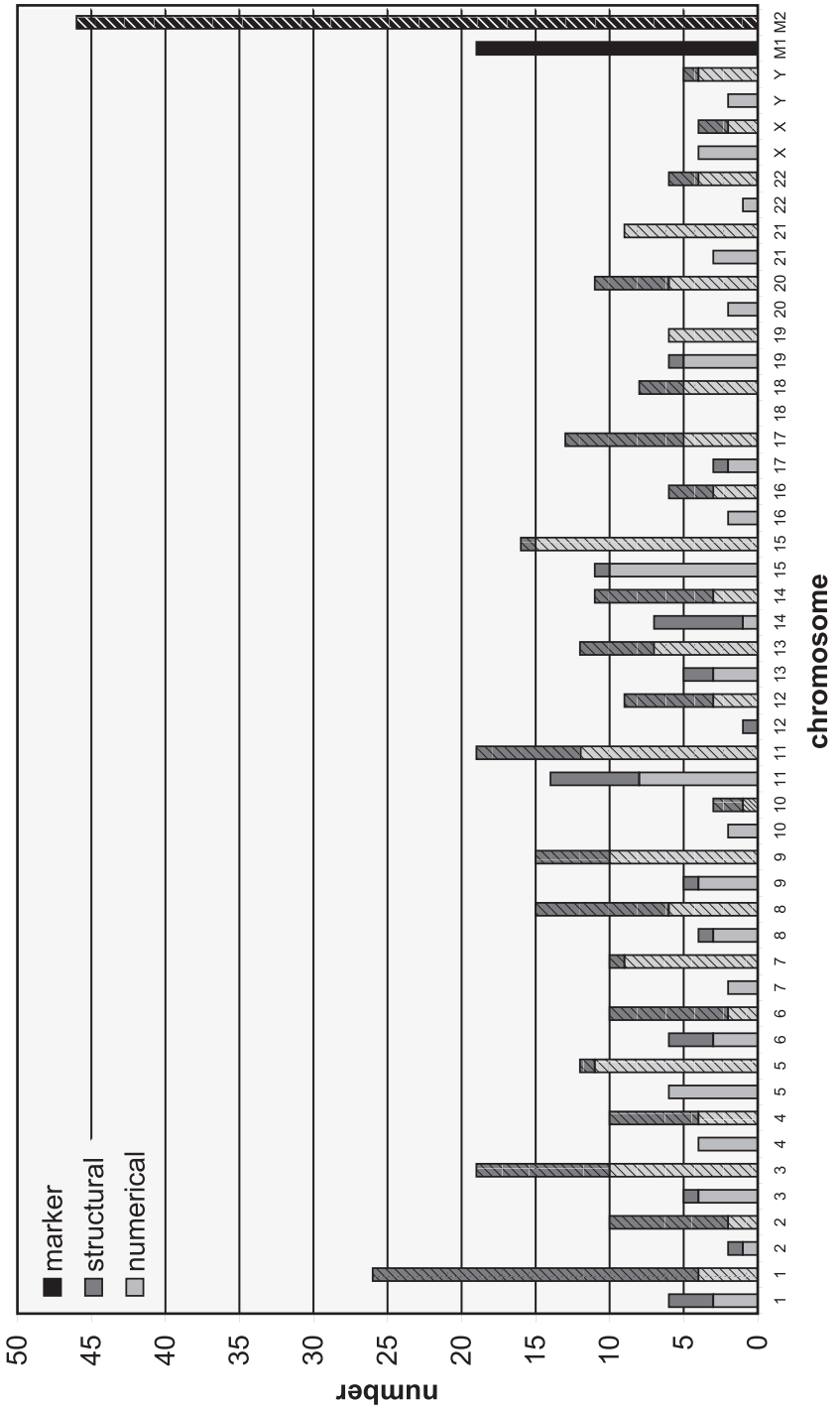


FIGURE 2. Distribution of chromosomal abnormalities by chromosome number in initial and follow-up samples (striped bars).



Among the numerical aberrations, trisomies (53%) and monosomies (47%) occurred in the same frequencies. Trisomies of chromosomes 5, 9, 11, 15 and 19, and monosomies of chromosomes 8 and 13 were the most commonly observed numerical abnormalities. Monosomy of chromosome 13 was identified in 3/15 initial (20%) and 7/21 follow-up samples (33%) with an abnormal karyotype.

The most commonly observed structural abnormality were aberrations of chromosome 1 (n=25) (15%). These aberrations were detected in 2/15 initial and 10/21 follow-up samples with an abnormal karyotype. Both arms of chromosome 1 were involved in the rearrangement and no specific locus was predominantly affected. The rearrangements of chromosome 1 consisted of unbalanced translocations, involving of 1p (n=4) or 1q (n=6), additional material of unknown origin on 1p (n=5) or 1q (n= 2), interstitial deletion in the 1p (n=2) or 1q (n= 1), dicentric chromosome (n=2), pseudodicentric chromosome (n=1), duplication (n=1) and isochromosome of the long arm of chromosome 10 (n=1).

One patient had a 'jumping translocation' of the long arm of chromosome 1, which was involved in dup(1), der(9)t(1;9) and der(14;?;1) in the same clone. The interstitial deletions in 1p had the breakpoint at 1p12 and 1p13. Structural aberrations of chromosome 1 resulted in partial gain of chromosome 1q in 5 cases.

Structural aberrations of chromosome 8 (n=10) (6%) and chromosome 17 (n=9) (6%) were seen less frequently. The structural aberrations of chromosome 8 were detected in 1 initial sample and 9 follow-up samples. The majority of these rearrangement resulted from unbalanced translocations. Structural aberrations of chromosome 17 were observed in 1 initial and 8 follow-up samples and consisted of additional material from unknown origin on 17q (n=2), unbalanced translocations with breakpoint at 17q (n=2), and a deletion on 17q12 (n=1). Nine samples showed structural aberrations of chromosome 14, of which 8 had aberrations of the 14q, including 2 samples with a breakpoint at 14q32; t(11;14)(q13;q32) and der(14)t(11:14)(q13;q32).

FISH-analysis

FISH analysis was performed on 24 initial samples and 37 follow-up samples obtained from 38 patients. Abnormalities were found in 16 initial (70%) and 26 follow-up samples (74%).

An abnormal signal for chromosomes 9, 11 and 13 was observed in 45%, 43% and 43% of the initial samples and 49%, 45% and 45% of the follow-up samples, respectively.

FISH analysis as compared to conventional cytogenetics showed a higher prevalence of abnormalities in chromosomes 9 (47% vs. 38%), 11 (45% vs. 44%) and 13 (45% vs. 29%).

Rearrangements of IgH (14q32-translocations) were observed in 1/3 initial (33%) and 11/17 follow-up FISH samples (65%). The translocation partners of 14q32 were 4p16.3 (n=2), 11q13 (n=2), or unknown (n=8).

Trisomy of both chromosome 9 and 11, suggestive of a hyperdiploid subtype, was observed in 20/61 samples (33%). The hyperdiploid subtype was confirmed by conventional cytogenetics in 7/20 samples.

Change of chromosome aberrations per patient and disease progression

Using conventional cytogenetics, 12 patients had karyotypic abnormalities in both initial and follow-up samples. Two of these patients (17%) changed of ploidy category during disease progression, one from hypodiploidy to hyperdiploidy and one from hyperdiploidy to hypodiploidy.

Eight patients were identified who developed additional genetic abnormalities of the original clone, indicating clonal evolution. The karyotypes of these patients are shown in table 2. All patients had an increase in numerical and/or structural abnormalities. The numerical abnormalities observed included both gains and losses of chromosomes. Monosomy of chromosome 17 was observed in 1 patient. Four patients developed structural aberrations of chromosome 8 involving the q-arm. Two patients had unbalanced translocations resulted in amplification of part of 1q. A specific clonal chromosome abnormality for disease progression was not observed.

Twenty-three patients had both initial and follow-up samples available for FISH analysis. Gains or losses of chromosomes 9 and/or 11 in the sequential samples, which were indicative for change of ploidy category, were observed in 5 patients (22%). Change of del(13q) status was observed in 6 patients (23%).

TABLE 2. Clonal evolution in 8 patients

No	Time	Conventional cytogenetic karyotype
1	diagnosis	88-97,XXYY,-1,-1,-4,-6,-8,-10,-11,-13,-17,-20,+mar1[2],+mar2x2[2],+6-10mar,+r,inc [cp4] /46,XY[18]
	1st relapse	46-49,XY,?der(1)(q),?der(3)[3],-4,?der(5)[3],der(6)t(1;6)(q1?2;q1?5),-8,?der(9)(p), ?der(12)(q),+15,?der(16)(p),add(17)(p1?2)[5],+19,-22,+mar1[5],+mar2,+mar3, +1-2mar[3],1-2dmin[3][cp9]/ 88-89,XXYY,?der(1)(q),-3,-4,-4,?der(5)[2],der(6)t(1;6)(q1?2;1?5)x2,-8,-8,-14,+15,+15, add(17)(p1?2)x2,+19,-22,-22,+mar1x2,+mar2x2,+mar3[2],+1-4mar[cp3]/45,XY,-7[7]/ 46,XY,-7,+mar[1]/46,XY[4]
2	diagnosis	49-58,XY,+2,+3,+5,+9,+11,+11,+del(13)(q1?q2?2),+15,+15,add(17)(p1?3),+19,+21[20]/ 46,XY[5]
	2nd relapse	55-57,XY,add(1)(q2?1)[4],add(2)(p11),+der(2)t(2;9)(q37;q11),+3,add(3)(p?21)[8], ?der(4)[6],+5,der(8;14)(q10;q10)[8],+9[6],+11,+11,+del(13)(q1?2q2?2),+15,+15, add(17)(p1?3),+19,+21,+21[5],+mar1[2],+mar2[4][cp11]/46,XY[17]
	3rd relapse	57-58,XY,add(2)(p11),+der(2)t(2;9)(q37;q11),+3,add(3)(p?21),add(4)(q2?8)[2],+5,+7[2], der(8;14)(q10;q10),+9[2],+11,+del(11)(q23),+del(13)(q1?1q2?2),+15,+15, add(17)(p1?3)[2],+19[3],+21,+21,+mar2[3][cp6]/46,XY[20]
3	diagnosis	54,X,-X,+4,+5,+7,+9,+11,+15,+15,+19,+21[8]/ 46,XX[24]
	1st relapse	54,X,-X,+4,+5,+7,+9,+11,+15,+15,+19,+21[4]/53,idem,der(8)t(8;20)(q12;q11),-20[5]/ 46,XX[22]
4	diagnosis	46,XY[34]
	1st relapse	32,X,-Y,+3,del(5)(q1?3q3?3),+7,+9,+11,+15,+18,+21,+del(22)(q12q13)[4]/ 32,idem,t(3;6)(q2?4;q?16)[9]/64,idemx2[7]/64,idemx2,t(3;6)(q2?4;q?16)[3]/46,XY[9]
5	diagnosis	46,XY[64]
	1st relapse	52-55,X,-Y[3],add(Y)(q1?2)[2],+add(1)(p11)[7],+add(1)(p11)[4],del(1)(q10)[2],+3[2], +5[2],+7[5],+9[2],+12[2],+add(12)(q24)[2],-13,+15,+17[5],+17[3],+18[3],+20[2], +21[5],+0-12mar[cp10]/46,XY[44]
	3rd relapse	52-53,X,-Y,add(1)(p11),+add(1)(p11)[5],+der(1;8)(q10;q?13)[4],+6[2],+7[8],-8[3], +12[4],+add(12)(q24)[4],-13[8],+15[7],+17,-18[3],add(20)(q?13),+add(20)(q?13)[5], +21[6],+2-6mar[cp11]/89-95,idemx2[3]/46,XY[7]
6	diagnosis	43-45,X,-X[5],-1,-3,-3[3],-4,-4[3],-5,-6[3],add(6)(p21)[4],add(6)(q23)[4],-8[5], ?add(9)(p21),-10[5],-11,add(11)(q21)[4],add(12)(q?24)[3],del(13)(q21q31)[3],-14[3], add(14)(q?)[3],-15[3],-16[4],+19[3],-22[5],+9-17mar[ ]/46,XX,?add(9)(p21)
	1st relapse	41-44,X,-X,-1,add(2)(p?),-3,add(3)(q21),-4,?der(4;22)(q10;q10),-5,add(6)(p21), add(6)(q23),-7,add(7)(p2?2),add(8)(q2?3),?der(10),-11,der(11)del(11)(p12)add(11)(q22), add(12)(q24),del(13)(q22q31),-14,add(14)(q2?),-15,der(15),add(16)(q21),-17,+3-6mar, inc[cp22]/46,XX[2]
7	diagnosis	45,X,-Y[29]*/46,XY[3]
	1st relapse	45,X,-Y[14]/45,X,-Y,-11,+mar[3]/46,XY[3]
8	diagnosis	46,XX,t(11;14)(q13;q32)[4]/46,XX[10]
	1st relapse	46,XX,add(6)(q23),t(11;14)(q13;q23)[4]/46,XX[17]

* Considered as myeloma-derived clone as FISH showed numerical aberrations in the same sample for chromosome 9 and 11.

DISCUSSION

In this study, we analyzed the sequential evolution of chromosomal abnormalities in plasma cells from 38 patients with multiple myeloma. An increased number of genetic abnormalities was observed at relapse or at disease progression, reflecting the genomic instability of the tumor cells.

In our analysis, structural aberrations occurred more frequently in later stages of the disease when compared to numerical aberrations. Aberrations of chromosome 1 were the most commonly observed structural abnormalities and both 1p and 1q were involved in these aberrations. No specific locus on chromosome 1 was predominantly affected by gains, losses or unbalanced translocations.

Our data are consistent with previous reports on the high prevalence of chromosome 1 aberrations in newly diagnosed and relapsed multiple myeloma patients based on conventional cytogenetic banding techniques[3;18;19], comparative genomic hybridization (CGH)[20] and FISH[21;22] In particular, rearrangements of chromosome 1 resulting in gains of 1q are recurrent findings. These rearrangements result from a variety of modes of whole-arm and jumping translocations.[23] Many breakpoints are involved ranging from 1q10→ 1q44.[19;20] However, Le Baccon et al. showed that band 1q21 is most frequently broken in multiple myeloma and non-Hodgkin lymphoma.[24] Deletion of 1p is a common aberration in multiple myeloma. The smallest region of overlap found in the majority of the cases covered the region 1p21→1p22.[19;20]

The molecular consequences of these aberrations are poorly understood. Previous studies have suggested that either CKS1B, BCL-9, IRTA or PDZK1 may be the key gene over-expressed in 1q amplified cases.[25-28]

Several studies have demonstrated that both gains of 1q and losses of 1p are associated with an unfavorable prognosis.[29-31] A recent study using interphase FISH showed that gains of 1q21 are associated with poor survival after high-dose chemotherapy.[32] Furthermore, the prevalence of 1q21 amplifications increased following progression from MGUS to multiple myeloma and may be associated with disease progression.[32]

Two major genetic subtypes of MM have been proposed: the hyperdiploid subtype characterized by multiple trisomies and low prevalence of del(13q) and the non-hyperdiploid subtype characterized by IgH translocations and del(13q).[6;33;34] The frequencies of IgH translocations like t(4;14), t(11;14) and t(14;16) in MGUS are similar to those in MM, suggesting these are primary oncogenetic events.[35;36]

Our study confirms that the ploidy category remained stable during disease progression, indicating that the underlying mechanisms leading to hyperdiploidy is probable also a primary oncogenetic event.[37]

Partial or complete del(13q), independent of the mode of detection, represents one of the most important prognostic parameters for survival.[38-40] Using conventional cytogenetics, del(13q) has been detected in 15-20% and by interphase FISH with two probes in 50% of the patients. A similar prevalence of del(13q) was observed in our patients. Overall, the frequency did not increase in later stages of the disease, suggesting that del(13q) is not a genetic event associated with disease progression.

In conclusion, cytogenetic abnormalities in multiple myeloma are not random. Progressive disease is often correlated with increasing complexity of cytogenetic karyotype consisting mainly of structural aberrations. Aberrations of chromosome 1 are common in multiple myeloma. In particular, unbalanced translocations of chromosome 1 have been delineated as genetic event associated with progressive disease and unfavourable prognosis

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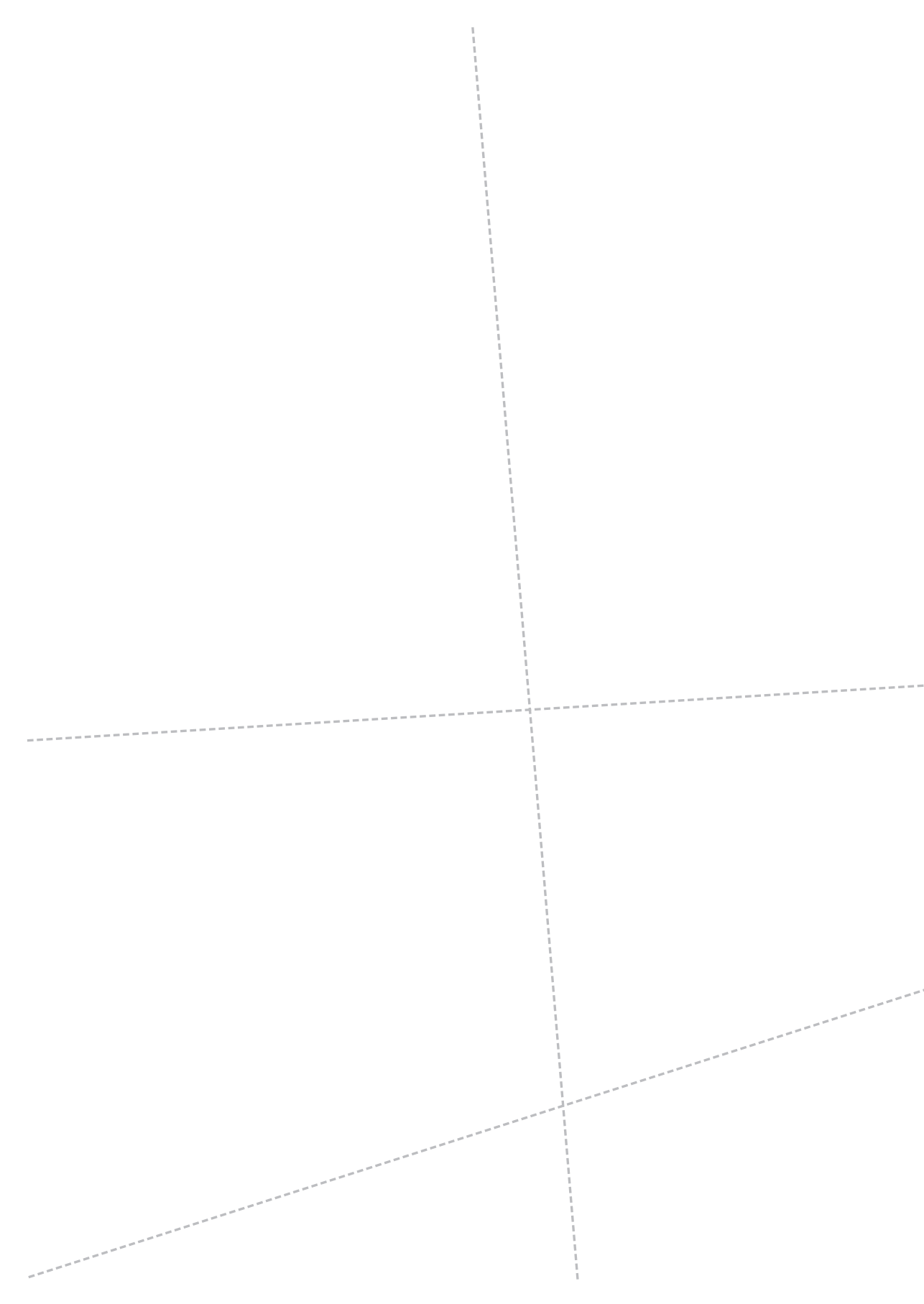

Chapter 3

ABNORMALITIES OF CHROMOSOME 1P/Q ARE HIGHLY ASSOCIATED WITH CHROMOSOME 13/13Q DELETIONS AND ARE AN ADVERSE PROGNOSTIC FACTOR FOR THE OUTCOME OF HIGH-DOSE CHEMOTHERAPY IN PATIENTS WITH MULTIPLE MYELOMA

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ABSTRACT

We studied the prognostic value of chromosomal abnormalities in untreated multiple myeloma patients who were registered into a prospective randomized multicentre phase 3 study for intensified treatment (HOVON24). A total of 453 patients with stage II and III A/B disease and under the age of 66 years were registered in the clinical study. Cytogenetic analysis was introduced as a standard diagnostic assay in 1998. It was performed at diagnosis in 160 patients and was successful in 137/160 patients (86%). An abnormal karyotype was observed in 53/137 (39%) of the patients. Abnormalities of chromosome 1p and 1q were found in 19 (36% of patients with an abnormal karyotype) and 21 patients (40%). There was a strong association between chromosome 1p and/or 1q abnormalities and deletion of chromosome 13 or 13q (n = 27, p <0.001). Patients with karyotypic abnormalities had a significant shorter OS than the patients with normal karyotypes. Complex abnormalities, hypodiploidy, chromosome 1p abnormalities, chromosome 1q abnormalities, and chromosome 13 abnormalities were associated with inferior overall survival on univariate analysis, as well as after adjustment for other prognostic factors.

In conclusion, chromosome 13 abnormalities and chromosome 1p and/or 1q abnormalities are highly associated, and are 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). Multiple myeloma accounts for approximately 10% of all hematological malignancies. The survival duration of patients with newly diagnosed multiple myeloma ranges from a few months to many years.[1]

Cytogenetics are emerging as one of the most important prognostic factors in multiple myeloma. Cytogenetic analysis using conventional chromosome banding can detect an abnormal karyotype in only 30-40% of patients.[2;3] Conventional cytogenetics are hampered by technical restrictions, i.e., the low percentage of plasma cells present in the bone marrow biopsy, the low mitotic index of the plasma cells and the predominance of a normal karyotype, mostly originating from normal haematopoietic components. Where successful, multiple and complex cytogenetic abnormalities are often found.[4] Using fluorescence in situ hybridization (FISH), chromosomal abnormalities can be detected in almost all patients.[5-8] However, a major disadvantage of FISH is that the obtained information is restricted to the specific chromosome sites or genes under investigation, depending on the chromosome probes used.

The application of these techniques in a clinical setting has demonstrated that certain chromosome abnormalities have prognostic significance. Hypodiploidy[9-12] and chromosome 13 abnormalities[6,13-16] are negative prognostic predictors for myeloma patients receiving either conventional chemotherapy or high-dose chemotherapy followed by autologous stem cell transplantation. Other recurrent chromosome abnormalities such as t(4:14), t(14;16) and deletion of 17q13 are associated with poor prognosis.[17,18]

In this study, we have prospectively investigated the karyotypes of 160 patients with newly-diagnosed multiple myeloma 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 at diagnosis for response and survival after high-dose chemotherapy. The study was closed for accrual in 2000 and the presented data are the result of a late analysis as of November 2006.

PATIENTS AND METHODS

Patients and treatment

From November 1995 until April 2000, 453 patients under the age of 66 years with stage II and III A/B multiple myeloma according to the Salmon & Durie classification[19] were registered in a prospective randomized multicenter study for intensified treatment following remission induction treatment. The study regimens and response criteria have been described in detail.[20] In short, the patients were treated with 3 to 4 cycles of vincristine, adriamycin, dexamethason (VAD) for remission induction. Patients were randomized to receive either one cycle of high-dose melphalan (2 administrations of 70 mg/m^2) or the same intensive regimen followed by cyclophosphamide plus Total Body Irradiation with autologous peripheral blood stem cell transplantation. Randomized patients received interferon-α-2a as maintenance therapy.

Cytogenetic study

Cytogenetic analysis was introduced in this trial as a standard diagnostic assay in 1998. 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. The karyotypes were described according to the ISCN (1995).[21] 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. Cases with a normal karyotype where less than 20 cells were analyzed were considered failures. Deletion or addition of the sex chromosome (X/Y) as sole chromosomal abnormality were not considered as a myeloma-derived karyotype.

Statistical analysis

The data were analyzed as of November 2006. The endpoints included response rate and overall survival (OS). OS was calculated from the date of the start of VAD until death. Patients still alive were censored at the date of last contact. The OS was 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 chromosomes 1p, 1q, 6q, 11q, 14q32, deletions and monosomy of chromosome 13, trisomies of chromosomes 3, 5, 7, 9, 11, 15, 19 and complex (> 3) abnormalities.

The clinical characteristics of patients with an apparently normal karyotype (NN) or those with an abnormal karyotype (AN/AA) were compared using Pearsons chi-squared test or Fishers exact test, in case of discrete variables, or the Wilcoxon rank-sum test in case of continuous variables. Spearmans rank correlation was calculated for each pair of chromosomal abnormalities to see whether some abnormalities were associated.

Univariate Cox regression analysis on cytogenetic abnormalities was performed to determine whether there was a difference in survival between the different subgroups. The cytogenetic aberrations that were statistically significant in the univariate analysis were also analyzed with adjustment for other prognostic factors, i.e. Salmon & Durie stage (2 vs 3) and serum β2-microglobulin. All reported p-values are two-sided and a significance level $\alpha = 0.05$ was used.

RESULTS

Cytogenetic analysis was performed at diagnosis in 160 patients and was successful according to predefined criteria in 137 patients (86%), to which the analysis was restricted. There were no significant differences between the pre-study characteristics of the patients with a normal or abnormal karyotype except for bone marrow infiltration by plasma cells at diagnosis, which was higher among patients showing abnormal karyotypes. (Table 1)

TABLE 1. Characteristics of 137 patients with successful cytogenetic analysis

Characteristics	**Normal karyotype n = 84**	**Abnormal Karyotype n = 53**	**All patients n = 137**
Sex			(P =0 .42)
Male	48	34	82
Female	36	19	55
Age at registration (years)			(*P* = 0.22)
Median	54	55	54
Range	33-65	39-65	33-65
Stage Salmon & Durie			(*P* = 0.45)
IIA	23	9	32
IIB	1	1	2
IIIA	53	36	89
IIIB	7	7	14
WHO performance status			(*P* = 0.68)
0-1	63	42	105
2-4	21	11	32
Calcium (mmol/l)			(*P* = 0.14)
Median	2.3	2.4	2.3
Range	1.1-4.0	1.9-4.0	1.1-4.0
Serum β2-microglobulin (mg/l)			(*P* =0.05)
Median	2.9	3.6	3.4
Range	0.4-18.4	1.8-22.8	0.4-22.8
M-protein			(*P* = 0.67)
IgA	13	12	25
IgG	55	31	86
IgD	2	1	3
Kappa/lambda	8	7	15
Unknown	6	2	8
Plasma cells in BM smear (%)			(*P* <0.01)
Median	30.0	50.6	39.6
Range	0.5- 100	0.0- 100	0.0- 100

Cytogenetic analysis

An abnormal karyotype was found in 53 of 137 (39%) patients. The karyotypes of these patients are shown in table 2. The most frequently observed genetic abnormalities are presented in table 3. Twenty-eight patients (51%) had hyperdiploid karyotypes (47-60), 12 patients (23%) had hypodiploid karyotypes and 4 patients (8%) were pseudodiploid. Eight patients (15%) were not classifiable or showed combinations of ploidy. Complex (> 3) abnormalities were present in 51 of 53 patients (96%). The most frequently observed numerical aberrations were trisomies of chromosomes 3, 5, 7, 9, 11, 15 and 19. There was a strong association between trisomies of chromosome 3, 5, 7, 9, 11, 15 and 19.

TABLE 2. Karyotypes of 53 patients with newly diagnosed multiple myeloma

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	44~47,XX,-4,add(6)(q?25),+?del(7)(q22),-10,del(12)(p12),-13,add(14)(q32),-16,+6~8mar, inc[cp14]/46,XX[1]
7	42-44,X,-Y,+del(1)(p32),-5[2],-13,-14[2],-18,-21[3],-22,+mar1,+1-3mar[cp4]/46,XY[6]
8	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]
9	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]
10	55,X,-X,+3,+5,+7,+9,+9,+11,+15,+15,+19,+21,+21,-22[7]/46,XX[13]
11	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]
12	44,XX,add(5)(q35),-8,-13,-14,+18[3]/46,XX[27]
13	46,XY,t(1;14)(p22 ~31;q3?2)[2]/46,XY[29]
14	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]
15	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]
16	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]
17	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]

18 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]

19 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]

20 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]

21 48,XY,-6,+?9,+?11,-15,-16,+?21,+2mar[2]/46,XY[30]

22 47,X,-Y,+del(1)(p1?2p36),+5,add(8)(q2?4),+9,-13,+19,-22[6]/46,XY[27]

23 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]

24 55,XY,+3,+5,+9,+11,+15,+15,+19,+20,+21[2]/46,XY[18]

25 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]

26 54,XY,+?2,+3,+14,add(14)(q32),+16,+20,+21,+2mar,inc[13]/46,XY[15]

27 52,XY,+?3,+?5,del(6)(q?),+del(6)(q?),+add(19)(?p),inc[cp2]/45,X,-Y[4]/46,XY[14]

28 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]

29 52,XY,+5,der(6)t(1;6)(?q24;q22),+9,+11,-16,+19,+22,+2mar[6]/46,XY[14]

30 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]

31 44,XX,-1,-5,-9,?add(11)(p),del(12)(p),-13,-14,del(20)(q),-22,+4mar[1]*/46,XX[36]

32 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]

33 44,X,-Y,del(8)(p21),del(10)(p13),-13,der(16)t(1;16)(q10;p10),add(18)(q23)[34]46,XY[3]

34 51,XY,+4,+8,+9,+11,+11,-12,-13,+18,+2mar[cp3]46,XY[17]

35 48,XX,+2mar[4]/46,XX[16]

36 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]

37 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]

38 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]

39 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]

40 44,X,-X,dup(1)(q21q32),del(6)(q?),-13,add(18)(q23)[3]/46,XX[7]

41 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]

42 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]

43 53-55,XY,+3,+4,+6,+7,+9,+11,+13,+14,+15,+19,+mar[cp6]/46,XY[25]

44 44,X,-Y,t(1;20)(p10;q10),-13,der(14)t(7;14)(q21~q22;q2?4), +21,-22[6]/46,XY[21]

45 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]

46 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]

47 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]

48 54~56,XY,+3,+5,+7,+9,+9,+11,+15,+15,+19,+19[cp7]/46,XY[13]

49 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]

50 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]

51 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]

52 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]

53 43,X,-Y,i(1)(q10),t(3;6)(p21;p21),-4,-13[16]/46,XY[4]

* Clonality confirmed by FISH

TABLE 3. Type and distribution of the most frequently observed genetic aberrations in 53 patients with an abnormal karyotype

Cytogenetic abnormality	No. patients (%)
Pseudodiploid	4 (8)
Hypodiploid	12 (23)
Hyperdiploid 47-50	4 (8)
Hyperdiploid 51-60	24 (45)
Not classifiable	9 (17)
Complex abnormalities (>3)	51 (96)
14q32	10 (19)
11q	11 (21)
1p	20 (38)
1q	22 (42)
6q	18 (34)
+3	20 (38)
+5	19 (36)
+7	13 (25)
+9	25 (47)
+11	22 (42)
+15	23 (43)
+19	24 (45)
-13	25 (47)
-13 or 13q-	27 (51)

Abnormalities of chromosomes 1p and 1q were found in 19 (36%) and 21 patients (40%), respectively. Seven patients had (partial) gains of 1q. Many breakpoints were involved in these aberrations, which ranged from 1p10→1p32 and 1q10→1q43. Three patients had a breakpoint at 1q21. Monosomy 13 or deletions of 13q were present in 27 patients (51%). There was a strong association between chromosome 1p and/or 1q abnormalities and those of chromosome 13 ($p < 0.001$); nineteen patients had both abnormalities. Abnormalities of 6q were present in 18 patients (34%). Ten patients had a breakpoint at 14q32 (19%) in which three had the translocation t(11;14)(q13;q32). No other known specific translocations involving 14q32 were found. Other abnormalities of 11q were found in 11 of 53 patients (21%).

Response

Univariate analysis showed that no specific chromosomal abnormality was a statistically significant prognostic factor for partial or complete response after VAD, high-dose melphalan, myeloablative treatment with stem cell transplantation or interferon-α-2a (data not shown).

Overall survival

The median follow up of the 39 patients still alive was 92 months (range, 67 -130 months). Patients with karyotypic abnormalities had a significant shorter median OS than the patients with normal karyotypes (38 vs 78 months, $p<0.001$). Specific chromosome abnormalities associated with shorter median OS on univariate analysis were 1p abnormality (16 vs 62 months, $p<0.001$), 1q abnormality (22 vs 62 months, $p<0.001$), and -13 or 13q- (37 vs 64 months, $p<0.001$). Complex abnormalities (38 vs 70 months, $p<0.001$) and hypodiploidy (18 vs 59 months, $p<0.001$) were also associated with poor prognosis. When adjusted for Salmon & Durie stage (2 vs 3) and serum β2-microglobulin, these variables remained highly significant. These results of the univariate and adjusted analyses are summarized in table 4.

Kaplan-Meier survival curves of OS in the presence of chromosome 1p/q or of deletions of chromosome 13 or 13q are shown in figure 1 and figure 2, respectively. Due to the association between abnormalities of chromosome 1p/q and deletions of chromosome 13/13q, separate survival curves are shown for patients without, with one, or with both of 1p/q and 13/13q abnormalities. (Fig 3) The outcome of the patients with both 1p/q and 13/13q was even worse than patients with only one of these aberrations.

TABLE 4. Univariate and adjusted Cox regression analysis of risk factors for overall survival

	Univariate analysis			Adjusted analysis		
Risk factor	**HR**	**95% CI**	**P-value**	**HR**	**95% CI**	**P-value**
Abnormal karyotype	2.9	1.9-4.4	< 0.001	2.7	1.8-4.1	< 0.001
Hypodiploid	3.3	1.8-6.0	< 0.001	3.2	1.7-5.9	< 0.001
1p abnormalities	3.2	2.0-5.3	< 0.001	2.5	1.5-4.3	< 0.001
1q abnormalities	2.7	1.6-4.4	< 0.001	2.2	1.3-3.6	0.002
1p/q abnormalities	3.2	2.0-5.1	< 0.001	2.5	1.6-4.1	< 0.001
-13/13q-	2.6	1.6-4.0	< 0.001	2.4	1.5-3.8	< 0.001
Complex abnormalities	2.8	1.8-4.2	< 0.001	2.5	1.7-3.8	< 0.001

FIGURE 1. Kaplan-Meier curves of overall survival in the presence and absence of chromosome 1p/q abnormalities

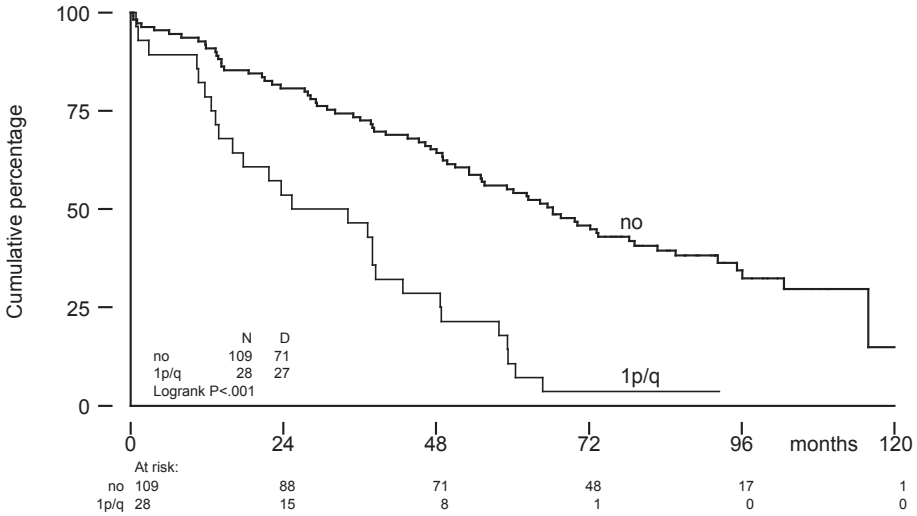


FIGURE 2. Kaplan-Meier curves of overall survival in the presence and absence of monosomy 13

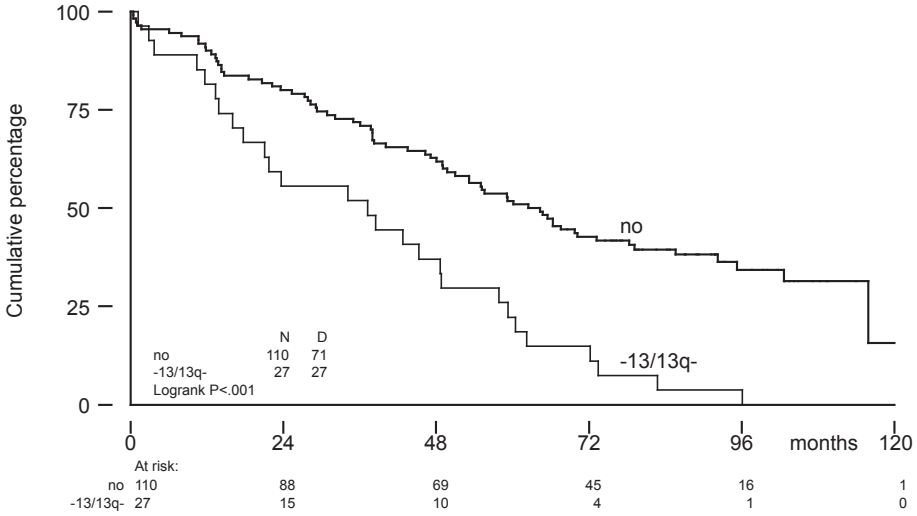
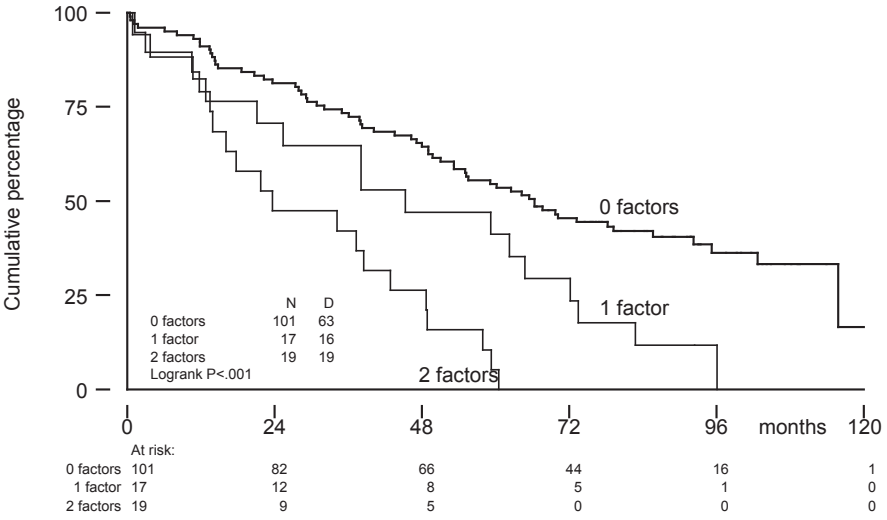


FIGURE 3. Kaplan-Meier curves of overall survival in the presence of chromosome 1p/q and/or –13/13q abnormalities. 0 factors indicates neither 1p/q nor –13/13q-; 1 factor, either 1p/q or –13/13q-; and 2 factors, both 1p/q and –13/13q-



DISCUSSION

During the past years, considerable progress has been made in identifying specific chromosomal abnormalities and their role in the biology and prognosis of multiple myeloma.

In our study, an abnormal karyotype was found in 43% of patients with newly diagnosed stage II or III multiple myeloma. A high frequency of multiple and complex chromosomal abnormalities was present as has been described previously.[2,3,22] Our original study report like others showed that the outcome of patients with abnormal cytogenetics receiving high-dose chemotherapy was inferior to that of patients without abnormalities.[20,23,24]

The most frequently reported chromosome abnormality with respect to outcome of therapy is deletion of chromosome 13 or 13q detected by conventional cytogenetics.[16,18,25] Using conventional cytogenetics, chromosome 13 abnormalities have been observed in approximately 15% of the newly diagnosed patients.[2,13,26] With interphase FISH this percentage varies between 33% and 86% of patients depending on the number of probes used.[6,27-29] Our data are consistent with previous reports that deletion(s) of chromosome 13 or 13q by conventional cytogenetics is associated with a significantly shorter OS in patients with newly diagnosed multiple myeloma.

In our study, deletions of chromosome 13 or 13q are highly associated with chromosome 1p and/or 1q abnormalities. Chromosome 1p and/or 1q abnormalities, as well as deletions of chromosome 13 and 13q, were predictive of a poor OS after high-dose chemotherapy both in univariate analysis as well as after adjustment for other prognostic factors.

Structural aberrations of chromosome 1 are common in multiple myeloma, being found in up to 40% of patients with abnormal karyotypes.[30-32] In particular, rearrangements of chromosome resulting in gains of 1q are recurrent findings. These abnormalities result from a variety of modes of whole-arm and jumping translocations, which may be due to instability of the highly decondensed pericentromeric heterochromatin region.[33,34] Duplications and translocations of 1q were extensively reported in many neoplasm and are associated with progression of malignancies, including that of B-cell clones.[35-37] The clonal evolution of cells with these abnormalities suggests that these abnormalities contribute to a proliferation advantage.[33] The reported high frequency of breakpoint at 1q21 could not be confirmed in this homogeneous group of previously untreated patients.[35] A recent study using interphase FISH showed that 1q21 amplifications in multiple myeloma are associated with poor survival after high-dose chemotherapy.[38] Furthermore, the prevalence of 1q21 amplifications increased following progression from MGUS to multiple myeloma and may be associated with disease progression. The question, however, remains whether 1q21 amplification is a cause or a consequence of disease progression. The molecular implications of 1q21 amplifications are unclear.[38-43]

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, which were associated with a hyperdiploid karyotype, as is known from previous studies.[2,3,44] The overall survival of hyperdiploid patients in the present study was not significantly better when compared to diploid patients.[9,11] While hypodiploid karyotype was predictive for shorter overall survival as was also observed in other studies.[10,12]

In conclusion, this study confirms that chromosomal studies at diagnosis are by now indispensable in the clinical evaluation of patients with multiple myeloma. We found that hypodiploidy, complex abnormalities, (partial) deletions of chromosome 13, chromosome 1p and 1q abnormalities are associated with poor outcome after intensive therapy in multiple myeloma. In addition, our study demonstrated that an abnormal chromosome 1p/q and a deletion of chromosome 13/13q were highly associated. However, it remains to be determined 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 genetic abnormalities new treatment strategies may be required to improve the poor outcome after intensive therapy.

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APPPENDIX

Participating hospitals in this 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; Hospital de Baronie, Breda, O.J.L. Loosveld; Leyenburg Hospital, Den Haag, P.W. Wijermans; Medical Spectrum Twente, Enschede, M.R. Schaafsma; Academic Hospital Groningen, E. Vellenga; Atrium Medical Center, Heerlen, P. Voogt; Medical Center Leeuwarden, Leeuwarden, P. Joosten; Leiden University Medical Center, W.E. Fibbe; 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; Medical Center Rijnmond-Zuid, Rotterdam, A.A. van Houten; Vlietland Hospital, Schiedam, J.J. Braun; Ruwaard van Putten Hospital, Spijkenisse, M.H. Silbermann; Diaconessenhuis, Utrecht, H.D. Eggink; University Medical Center Utrecht, Utrecht, H.M. Lokhorst; Hofpoort Hospital, Woerden, J. Holleman; Isala Klinieken, Zwolle, M. van Marwijk Kooy

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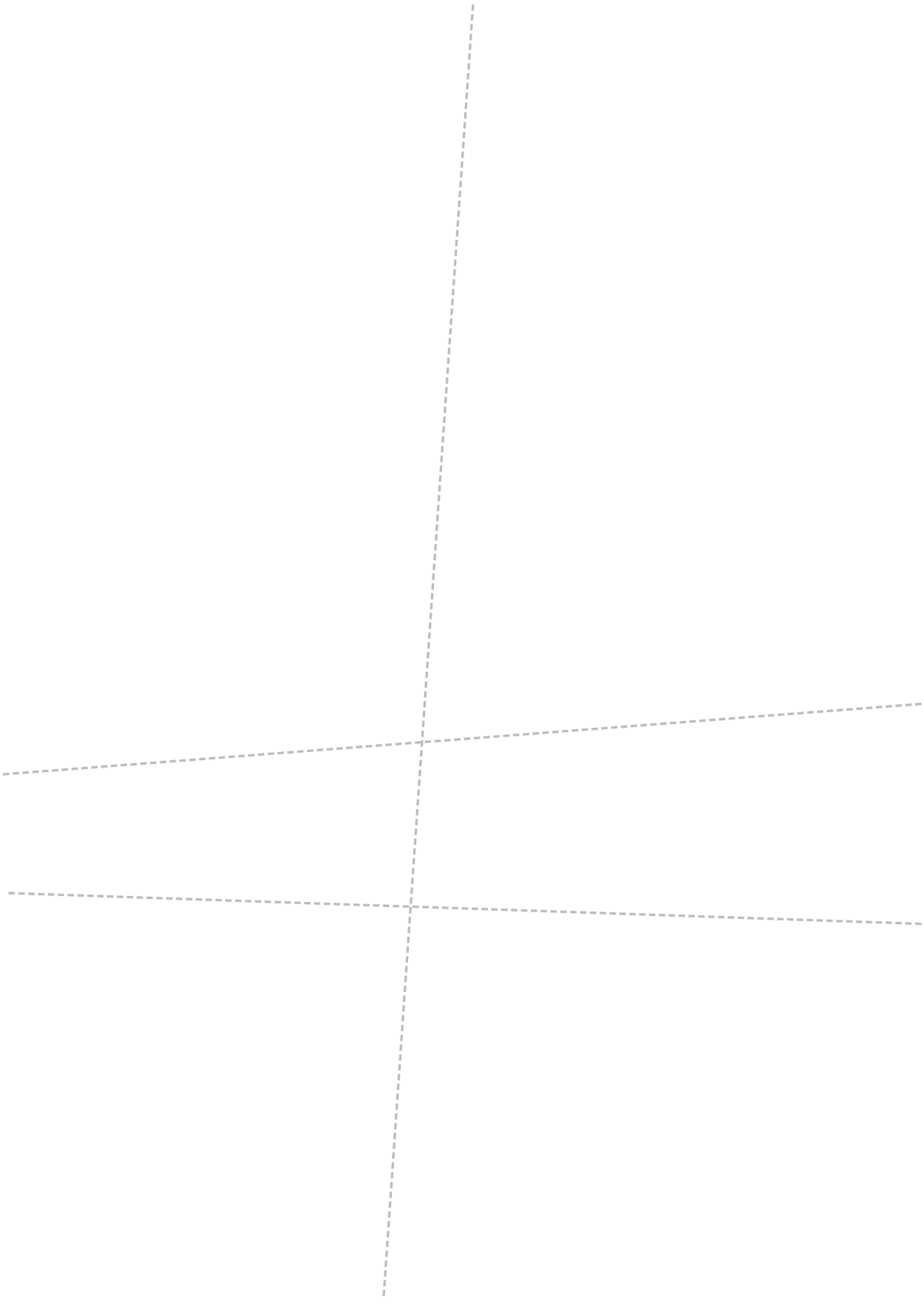

Chapter 4

ANALYSIS OF EFFICACY AND TOXICITY OF THALIDOMIDE IN 122 PATIENTS WITH MULTIPLE MYELOMA: RESPONSE OF SOFT-TISSUE PLASMACYTOMAS

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ABSTRACT

We evaluated the efficacy and toxicity of thalidomide in patients with relapsed and/or refractory multiple myeloma with a long term follow-up. A total of 122 patients were included in this retrospective analysis. The data were obtained by case research at five regional hematological centers in the period from May 1999 – July 2003. All patients had previously been treated with one or more lines of chemotherapy. The starting dose and dose escalation of thalidomide was according the local hospital regulations. At time of analysis, 52 patients were alive and the median follow-up was 17.5 months (range, 1-42 months). The response to thalidomide was complete in 5 patients, partial in 41 patients and minimal in 10 patients, giving an overall response of 46%. The mean time to response was 8 weeks. Thirty-nine patients had a stable disease and 27 patients had progressive disease at 12 weeks. The progression-free survival was 10 months and the median overall survival was 15 months. Eight of 15 patients with soft tissue plasmacytoma had a response to thalidomide. However, the duration of response in these patients was short compared with that of those without extramedullary involvement. Important clinical side effects included somnolence, constipation, peripheral polyneuropathy, dizziness and skin rash. The most clinical relevant chronic side effect was peripheral polyneuropathy. In 11 patients (9%) peripheral polyneuropathy was the reason to discontinue treatment.

In conclusion, thalidomide is an effective treatment in patients with relapsed and/or refractory multiple myeloma. It can induce marked and durable responses in some of the patients. The acute toxicity is acceptable, but chronic peripheral polyneuropathy is a dose-limiting side effect.

INTRODUCTION

Multiple myeloma is a malignant plasma cell disorder characterized by a proliferation of monoclonal plasma cells in bone marrow and production of a homogeneous heavy and/or light chain immunoglobulin (M-protein), which can be detected in the serum and/or the urine. Multiple myeloma accounts for approximately 1% of all malignancies and 10% of haematological cancers.

Melphalan combined with prednisone has been the treatment of choice for several decades. With melphalan and prednisone a response rate up to 60% was achieved and a median overall survival of 30 months.[1] Many trials with other combination of conventional chemotherapy have been performed, but these did not result in improved outcome.[2] High dose chemotherapy and autologous stem cell transplantation has improved the response rate and the overall survival, when compared with conventional chemotherapy.[3;4] However the disease remains incurable and the majority of the patients develop progressive disease within several years.

Treatment options for patients with relapsed or refractory disease are limited. The cumulative toxicity of melphalan and cyclophosphamide is associated with significant myelosuppression that prohibits the use of effective alkylating agents. Re-induction therapy with vincristine, adriamycin and dexamethasone (VAD) results in a response rate of 65% in relapsing patients and 32% in refractory patients, but such responses are usually short lived.[5;6] High-dose dexamethasone alone is also effective in the treatment of advanced multiple myeloma. For refractory or relapsing patients the response rate is similar or slightly inferior to VAD.[5]

In recent years, thalidomide has been introduced successfully in the treatment of multiple myeloma. The rationale for the use of thalidomide is based on studies showing increased bone marrow microvascularity in myeloma[7] and the observation that thalidomide inhibits angiogenesis in animal models.[8] The first clinical trial with thalidomide for patients with refractory multiple myeloma was conducted by Singhal et al.[9] Eighty-four patients were treated with thalidomide at a starting dose of 200 mg daily, and the dose was increased by 200 mg every two weeks until a dose of 800 mg daily was reached. Twenty-seven patients (32%) had clinical response, defined as at least 25% reduction in monoclonal protein. The median follow up was 14.5 months. No correlation between response on thalidomide and bone marrow microvascularity was observed. In an update of the initial report, 169 patients were enrolled and 37% had at least 25% reduction in monoclonal protein.[10] Two-year event-free and overall survival rates were 20% and 48%, respectively.

The anti-myeloma effect of thalidomide has been confirmed by other smaller studies with a limited duration of follow-up.[11-15] We here report the retrospective analysis of the efficacy and toxicity of thalidomide in multiple myeloma patients with a long term follow-up.

PATIENTS AND METHODS

Patients and treatment

All patients (n=122) who had been treated with thalidomide for relapsed and/or refractory multiple myeloma between May 1999 and July 2003 at five cancer centers in the Netherlands were included in the analysis. The clinical and treatment data of the patients were obtained by means of case research. Thalidomide in 100 mg tablets was supplied by Grünenthal Chemie (Aachen, Germany). The starting dose and dose escalation of thalidomide was according the local hospital regulations. All patients gave written informed consent before the start of thalidomide therapy.

All patients had received one or more treatments with chemotherapy. The median number of previous treatments was 3 (range, 1-5). The median time from diagnosis of multiple myeloma to start thalidomide was 48 months (range, 4-240 months). Seventy-nine patients were treated with conventional chemotherapy, 35 patients had received high-dose melphalan with autologous hematopoietic stem cell support and 8 patients had received allogeneic stem cell transplantation. Fifteen patients had a soft tissue plasmacytoma. The patients characteristics and details of previous chemotherapy are summarized in table 1.

At onset of thalidomide treatment all patients had active disease. Fourteen patients had renal failure with serum creatinine levels > 177 umol/l and 32 patients had serum LDH level above normal limit values. The median serum β2-microglobulin was 4 mg/l (range, 1-40 mg/l) and the median serum albumin level was 36 g/l (range, 23-48 g/l).

TABLE 1. Patients characteristics

Number of patients	122
Median age in years (range)	61 (37-85)
Male/female	72/50
M-protein subtype	
- IgG	74
- IgA	34
- Light chain	10
- Non-secretory	4
Number of patients with soft tissue plasmacytoma	15
Median serum creatinine level in µmol/l (range)	91 (39-520)
Median serum albumin g/l (range)	36 (23-48)
Median serum β_2-microglobulin in mg/l (range)	4 (1-40)
Number of prior treatment regimens (%)	
- 1	25 (20%)
- 2	30 (25%)
- 3	44 (36%)
- 4 or more	23 (19%)
Autologous stem cell transplantation (%)	35 (29%)
Allogeneic stem cell transplantation (%)	8 (7%)

Assessment of efficacy and toxicity

Response evaluation was based on the criteria of the European Bone Marrow Transplantation Group.[16] Patients who discontinued treatment before a response could be assessed were considered to have had no response to treatment. Complete response (CR) was defined as (1) disappearance of serum or urine M-protein; (2) bone marrow plasmacytosis < 5%; (3) no increase in size or number of lytic bone lesions; and (4) disappearance of soft-tissue plasmacytomas. Partial response (PR) required (1) a reduction of serum M-protein by ≥ 50%; (2) a reduction in 24 h urinary light chain excretion either > 90% or to < 200 mg; (3) in non-secretory myeloma ≥ 50% reduction in bone marrow plasmacytosis; (4) no increase in size or number of lytic bone lesions; and (5) a decrease ≥ 50% in the size of soft-tissue plasmacytomas. Minimal response (MR) was defined as (1) a reduction of serum M-protein by 25-49%; (2) 50-89% reduction in 24h urinary light chain excretion; (3) in non-secretory myeloma 25-49% reduction in bone marrow plasmacytosis; (4) no increase in the size or number of lytic bone lesions; and (5) a decrease of 25-49% in size of soft-tissue plasmacytomas. The disease was considered stable (SD) when the M-protein changes was < 25%; progressive disease (PD) was defined as (1) increase of M-protein or urinary light chain by 25%; (2) an increase of bone marrow plasmacytosis by > 25%; (3) an increase in size of existing bone lesions or soft-tissue plasmacytomas; and (4) development of new bone lesions or soft-tissue plasmacytomas.

Toxicity incidence was estimated and summarized using the frequency and descriptive techniques. The National Cancer Institute Common Toxicity Criteria (version 2.0) were used to grade the non-hematological toxicity.

Statistical analysis

The time to response was defined as the time from initial administration of thalidomide to the first evidence of response. Overall survival (OS) was measured from the date of start of thalidomide treatment until death. Patients still alive at the time of analysis were censored at the last follow-up date. Progression-free survival (PFS) was calculated for all patients who had a clinical response from the date of response until death, progression or end of follow-up, whichever came first. The Kaplan-Meier method was used to estimate overall survival and progression-free survival. The following patients characteristics at the start of thalidomide were included in the analysis of prognostic factors: age, gender, M-protein subtype, number of prior treatment regimens, creatinine, albumin, LDH and β2-microglobulin. Pearsons chi-squared test and Fishers exact test, whichever appropriate, and logistic regression were used to determine an association between clinical features at start treatment and the response to thalidomide.

RESULTS

Response and survival

At the time of analysis, 52 of the 122 patients were still alive. The median follow-up from the start of thalidomide treatment was 17.5 months (range, 1-42 months). A clinical response was observed in 56 patients (46%), i.e. complete response in 5, partial response in 41 and minimal response in 10 patients. Thirty-nine patients (32%) had a stable disease and 27 (22%) had progressive disease. Among the 56 responders, 14 patients were prior refractory and 22 patients had received high-dose chemotherapy. The mean time to clinical response was 8 weeks. Improvement of performance score, blood count and blood transfusion requirements were slower. In 8 of the 15 patients a reduction in size of soft tissue plasmacytoma was observed. Among 56 patients with a clinical response, 40 patients showed disease progression. The PFS was 10 months and the median OS was 15 months (Figure 1). In one patient, age 71 who achieved a CR on thalidomide, the treatment was discontinued on day 763 because of polyneuropathy. Six months after discontinuation the patient still had a CR. Analysis of variables such as age, gender, M-protein subtype, number of previous treatment, creatinine, albumin, LDH and serum β_2-microglobulin did not show any statistical significant difference in PFS and OS.

Toxicity

The main clinical side effects were somnolence, constipation, peripheral polyneuropathy, dizziness, skin rash and edema (Figure 2). The onset of symptoms varied between 1 days and 8 months. Somnolence and constipation occurred in most of the patients within 10 days and clinical symptoms of peripheral polyneuropathy 2 - 8 months after starting treatment. The majority of the side effects were low grade. Somnolence was a common side effect and patients were advised to take the drug at the evening. Another common side effect was constipation. To overcome this problem, patients used extra dietary fibres and laxatives. Thirty-six patients had complaints of polyneuropathy. Seven patients developed a skin rash, which required additional treatment. Dose reduction of thalidomide was observed in 43 patients (35%) and thalidomide was discontinued in 13 (11%) for reasons of intolerance: 9 polyneuropathy, 3 dizziness and 1 depression. The majority of the symptoms improved or resolved after dose reduction or discontinuation of thalidomide. The most disabling chronic side effect was the peripheral polyneuropathy. Thromboembolic complications were reported in a total of 7 patients (6%): 4 deep vein thrombosis and 3 pulmonary emboli.

The starting dose of thalidomide was 100 mg/day in 24 patients, 200 mg/day in 96 patients and 400 mg/day in 2 patients. Dose escalation was performed in 67 patients. The median daily dose of thalidomide was 200 mg (range, 50-800 mg) and the 3-months cumulative dose was 18 grams.

FIGURE 1. Overall survival (OS) and progression-free survival (PFS)

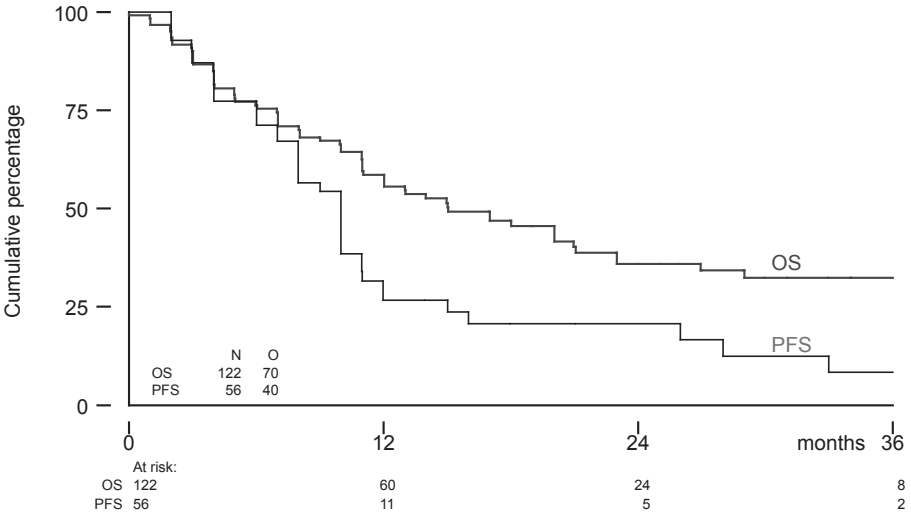
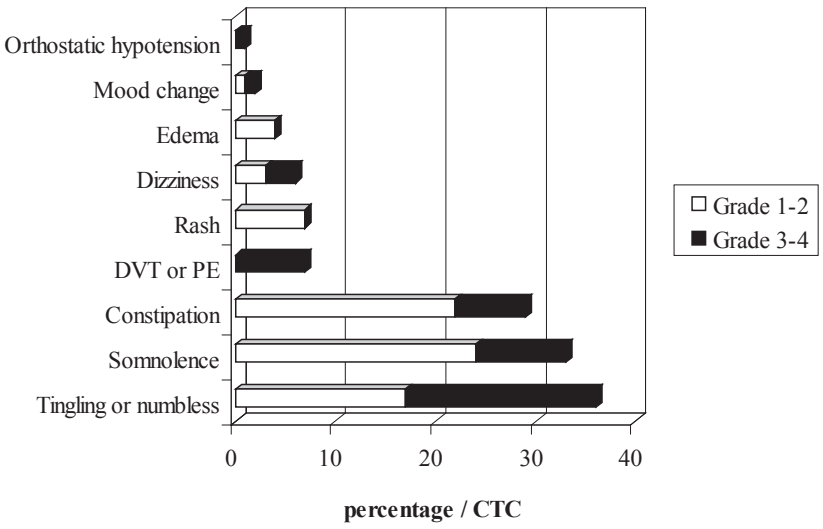


FIGURE 2. Observed toxicity of thalidomide



DISCUSSION

In our analysis of 122 patients with a median follow up of 17.5 months, 56 patients had a clinical response (46%) with at least ≥ 25 % M-protein reduction in serum and/or urine. Our data support those of other studies, which have reported response rates of 20-60%.[4,11-15]

The first sign of early response to treatment was observed after 4 weeks of treatment, while the latest response was at 18 weeks and the median time to response was 8 weeks. Therefore, re-induction therapy with thalidomide should be continued for at least 2-3 months in order to assess the response properly. If beyond this duration no clinical response is observed, the patient is probable a non-responder to thalidomide as a single agent. Thalidomide combined with dexamethasone may however have a synergistic anti-myeloma effect in a proportion of patients who are resistant to thalidomide alone.[17-20]

A possible association has been reported between the presence of soft-tissue plasmacytoma and a poor response to thalidomide treatment.[21,22] In contrast, other authors reported successful thalidomide treatment of extramedullary myeloma lesions.[23,24] In our series, we had 8 patients with soft-tissue plasmacytoma who responded to thalidomide. However, the duration of response in these patients was short compared with that of those without extramedullary involvement.

The minimal effective dose of thalidomide remains to be determined. No phase I trials have formally evaluated the maximum tolerated dose of thalidomide. There is some evidence for a thalidomide dose-response effect.[11,14] Barlogie et al. reported that patients with a cumulative 3-months thalidomide dosage of more than 42 grams have a higher response rate and a superior 2-year survival than counterparts who received less than 42 grams.[10] However, a number of studies have shown efficacy of thalidomide with doses as low as 50 mg daily.[25-29]

Thalidomide is associated with a variety of side effects. The teratogenicity of thalidomide is well known and its use in pregnant women is absolutely contraindicated. Frequently reported side effects are somnolence or fatigue, constipation, skin rash and peripheral polyneuropathy.[30] Thalidomide was first introduced as a sedative. Therefore somnolence is to be an expected common side effect. By taking thalidomide at the evening, symptoms of somnolence can be minimized. Constipation is another common side effect and patients are advised to use prophylactic laxatives to overcome this problem. The severity of somnolence, fatigue and constipation may be dose-related. Minor to moderate skin eruptions may occur up to 46% of the patients taken thalidomide alone.[31]

Thalidomide can cause peripheral polyneuropathy. The clinical manifestations of thalidomide-induced neuropathy are a slowly progressive symmetrical paresthesias and/

or numbness of the distal extremities.[32] The neuropathy can be irreversible if thalidomide is not promptly withdrawn. The prevalence of thalidomide-induced neuropathy has been variously estimated, from less than 1% in patients treated for lepra reactions[33] to 15-40 % in patients treated for multiple myeloma.[4,9,11] Potential host-related risk factors for developing thalidomide neuropathy are the patients age, cumulative doses of thalidomide, prior neuropathy and underlying disease status.

In our analysis and other studies, the observed incidence of deep venous thrombosis was from 2% to 6% when thalidomide was used as a single agent.[34] The risk of thrombosis is higher when thalidomide is combined with chemotherapy. Incidence rates of thrombosis up to 28% have been observed when thalidomide was given with anthracyclines.[34-36] For this reason, prophylactic low molecular weight heparin is now advised in the ongoing phase III trial comparing the efficacy of vincristine, doxorubicin and dexamethasone (VAD) versus thalidomide, doxorubicin and dexamethasone (TAD) as induction therapy for newly diagnosed multiple myeloma patients.[37]

The mechanism of thalidomide against multiple myeloma is pluriform.[38] Apart from its anti-angiogenic activity[8,39], thalidomide may have a direct effect on the myeloma cell and/ or bone marrow stroma cell by inducing free radical-mediated oxidative DNA damage.[40] Thalidomide also modulates the expression of cell adhesion molecules and so interfere with the stimulatory interaction between myeloma cells and the bone marrow stroma cells.[41] Thalidomide also interfere with the production of TNF-α[42], increases the in vivo production of IL-10[43], and enhances cell-mediated immunity by direct co-stimulation of T-lymphocytes.[44]

In conclusion, thalidomide can induce additional response (46%) in patients with relapsed or refractory multiple myeloma. Thalidomide was overall well tolerated at doses of up to daily 200 mg and had acceptable short-term toxicities. Peripheral polyneuropathy was the most important reason to discontinue treatment. Further studies are needed to determine to optimal thalidomide dose and duration.

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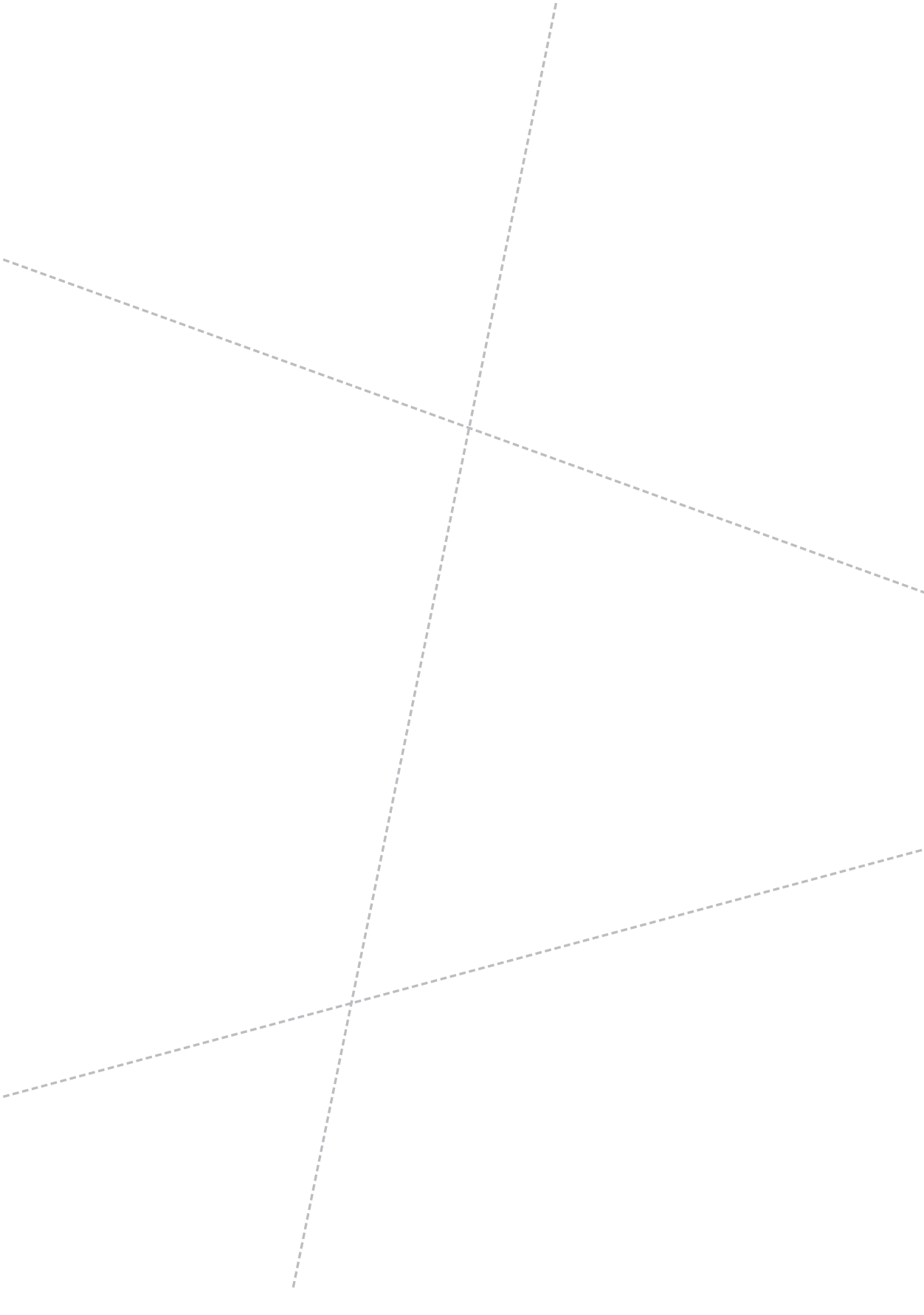

Chapter 5

PHASE II MULTI-CENTER STUDY OF ARSENIC TRIOXIDE, ASCORBIC ACID AND DEXAMETHASONE IN PATIENTS WITH RELAPSED OR REFRACTORY MULTIPLE MYELOMA

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ABSTRACT

We explored the combination of arsenic trioxide with ascorbic acid and dexamethasone in advanced multiple myeloma. Arsenic trioxide is associated with inter-individual variation in toxicity, which may be related to genetic polymorphisms in drug metabolizing enzymes.

Arsenic trioxide, ascorbic acid and dexamethasone were administered during 3 weeks of each 4-week cycle. Patients who achieved a clinical response after the fourth cycle continued to receive 2 additional treatment cycles. Twenty patients were included in this phase II multi-center study. At the time of analysis, eleven patients (55%) were alive with a median follow-up of 9 months. A clinical response was observed in eight of the twenty patients (40%), consisting of partial in two patients and minimal response in six patients. The median duration of response was 5 months. The progression-free survival was 4 months and the median overall survival was 11 months. The most common adverse events were peripheral edema (40%), fatigue (35%), dyspnoea (30%), herpes zoster (20%) and neuropathy (20%). Severe adverse events included neutropenia (15%), thrombocytopenia (10%), hepatic toxicity (15%) and bacterial infections (30%). Genetic polymorphisms showed no difference in toxicity based on the active wild type or inactive mutant genotype for ABCB1, GSTP1, GSTM1 or GSTT1.

In conclusion, combination therapy with arsenic trioxide, ascorbic acid and dexamethasone has moderate efficacy and significant toxicity in patients with advanced multiple myeloma.

INTRODUCTION

Multiple myeloma (MM) is a hematological malignancy characterized by proliferation of monoclonal plasma cells in bone marrow, production of monoclonal immunoglobulins (M-protein) and lytic bone lesions.[1] Despite recent therapeutic advances, MM remains an incurable disease. The current standard of first-line treatment with high-dose therapy followed by autologous stem cell transplantation has prolonged the median survival to 5 years, as compared to 3 years with conventional chemotherapy.[2;3] The introduction of novel agents, targeting not only myeloma cells but also the bone marrow microenvironment has expanded the therapeutic options. Non-cytotoxic treatment regimens with thalidomide or bortezomib alone and/or in combination with dexamethasone can produce robust responses in a substantial proportion of patients with relapsed or refractory disease.[4-7] However, the response durations are limited and all patients will relapse and become resistant to therapy.

Arsenic trioxide is an agent of recent interest because of its impressive activity in patients with acute promyelocytic leukaemia (APL).[8] Subsequent preclinical studies also showed activity in MM. The precise mechanism of action is unknown. Arsenic trioxide is active through three major mechanisms: (1) mitochondrial membrane depolarization and activation of downstream apoptotic pathways through the generation of reactive oxygen species[9-13] (2) specific activation of pro-apoptotic pathways[14;15] and (3) specific activation of immunologic mechanisms.[16] Unlike dexamethasone-induced apoptosis which can be inhibited by exogenous interleukine-6, arsenic trioxide-induced apoptosis is not overcome by interleukin-6.[17]

In vitro studies showed that arsenic trioxide-induced apoptosis was enhanced by reactive oxygen species and reduced by glutathione. The addition of ascorbic acid, a glutathione-depleting agent, increased the cytotoxic effect of arsenic trioxide on myeloma cells.[18;19]

There is considerable inter-individual variation in susceptibility to arsenic trioxide induced toxicity, which is probably related to differences in metabolism.[20] Genetic polymorphisms in drug metabolizing enzymes may have an impact on the pharmacokinetics and toxicity profile of arsenic trioxide. The glutathione-S-transferases superfamily plays an important role in the detoxification of the cell by catalyzing the conjugation of a variety of endogenous compounds and xenobiotics with glutathione. Polymorphisms in the GSTP1, GSTT1 and GSTM1 subclasses are very frequent among individuals. Homozygous mutations of GSTP1 (1578 A>G) lead to inactivation of the enzyme. Genetic polymorphisms of GSTM1 and GSTT1 have been described to be associated with complete deletion of enzyme activity. The ABC transmembrane drug transport protein MDR-1 (ABCB1) is responsible for transporting various endogenous and exogenous substrates out of the cell. The role of genetic polymorphisms of the ABCB1 (3435 C>T) is disputed.

Clinical trials showed that arsenic trioxide used as single agent had acceptable toxicity and promising activity in patients with advanced MM.[21-23] Based on these results, we initiated an international phase II trial with the combination arsenic trioxide, ascorbic acid and dexamethasone in patients with relapsed and refractory MM. The rationale for combining arsenic trioxide with ascorbic acid and dexamethasone was based on different mechanisms of action and the potential synergism that was observed in in-vitro models.

PATIENTS AND METHODS

Patients

Patients aged 18 years and older with a confirmed diagnosis of MM stage II or III according to the Salmon & Durie criteria[24], who had received at least 1 prior treatment regimen and required treatment because of recurrent or progressive disease were included in the study. Other criteria for entry were measurable disease and performance status of 0-3 according to the classification of the World Health Organisation (WHO).

Exclusion criteria included the following: severe cardiac dysfunction (New York Heart Association classification grade 2-4); a corrected QT-interval duration > 500 msec on electrocardiogram in the presence of normal serum potassium and magnesium; pregnancy or lactating women; hepatic dysfunction (serum bilirubin > 30umol/l or transaminases > 3 times normal level) unless related to MM; pulmonary dysfunction; systemic amyloidosis; active, uncontrolled infection; intolerance of arsenic trioxide and the use of investigational agents.

The study was designed as an investigator sponsored, prospective, multi-center, open-label study. The Medical Ethics Committee at each participating institution approved the study and the study was conducted in accordance with the Declaration of Helsinki. All patients provided written informed consent before entering the study.

Study treatment

Arsenic trioxide (Trisenox®, Cell Therapeutics, Inc., Seattle, WA, USA), ascorbic acid and dexamethasone were administered in 4-weekly cycles. In the first cycle, during the first week, a loading-dose with arsenic trioxide was administered as an intravenous infusion over 1-2 hours at a dose of 0.25 mg/kg per day for 5 days. Ascorbic acid 1000 mg was administered as an intravenous infusion over 15 minutes within 30 minutes after each arsenic trioxide infusion and dexamethasone was taken orally at a dose of 40 mg per

day for 5 days (Monday through Friday). During the second and third week, the patients received a maintenance dose of arsenic trioxide at 0.25 mg/kg intravenously given twice-weekly to which ascorbic acid 1000 mg intravenously and dexamethasone 20 mg orally were added. The fourth week was a rest period.

In the second, third and fourth cycles, the patients received a maintenance dose of twice weekly arsenic trioxide at a dose 0.25 mg/kg intravenously with ascorbic acid 1000 mg intravenously and dexamethasone 20 mg orally. The fourth week was a rest period.

Patients were scheduled to receive a minimum of four treatment cycles. Patients who achieved at least a minimal response within cycles 1-4, continued to receive two additional treatment cycles. If a Common Toxicity Criteria grade 4 haematological or any grade 3-4 non-hematological toxicity considered being treatment-related was experienced, the protocol treatment was held. For non-haematological toxicity, the treatment was held until the toxicity returned to grade 2 or better and for haematological toxicity until the toxicity returned to grade 3 or the hemoglobin, ANC and platelet count returned to baseline values. If the toxicity did not resolve within one month, as defined above, the treatment was discontinued. Dose reduction of arsenic trioxide was not allowed. During the use of high-dose dexamethasone, prophylactic antibiotics e.g., cotrimoxazol 2 x 960 mg per day and fluconazol 1 x 200 mg per day were administered.

Assessment of toxicity and efficacy

At each visit during treatment, a physical examination was performed in each patient. Hematology and chemistry profiles were assessed weekly and an electrocardiogram was obtained. The National Cancer Institute Common Toxicity Criteria (version 2.0) were used to grade the non-haematological toxicity.

Response evaluation was based on the criteria of the European Bone Marrow Transplantation Group.[25] Patients who discontinued treatment before a response could be assessed were considered to have had no response to treatment. Complete response (CR) was defined as (1) disappearance of serum or urine M-protein; (2) bone marrow plasmacytosis < 5%; (3) no increase in size or number of lytic bone lesions; and (4) disappearance of soft-tissue plasmacytomas. Partial response (PR) required (1) a reduction of serum M-protein by ≥ 50%; (2) a reduction in 24 hours urinary light chain excretion by either > 90% or to < 200 mg; (3) in non-secretory myeloma ≥ 50% reduction in bone marrow plasmacytosis; (4) no increase in size or number of lytic bone lesions; and (5) a decrease ≥ 50% in the size of soft-tissue plasmacytomas. Minimal response (MR) was defined as (1) a reduction of serum M-protein by 25-49%; (2) 50-89% reduction in 24 hours urinary light chain excretion; (3) in non-secretory myeloma 25-49% reduction in bone marrow plasmacytosis;

(4) no increase in the size or number of lytic bone lesions; and (5) a decrease of 25-49% in size of soft-tissue plasmacytomas. The disease was considered stable (SD) when the M-protein changes was < 25%. Progressive disease (PD) was defined as (1) an increase of M-protein or urinary light chain by > 25%; (2) an increase of bone marrow plasmacytosis by > 25%; (3) an increase in size of existing bone lesions or soft-tissue plasmacytomas; and (4) development of new bone lesions or soft-tissue plasmacytomas.

Genetic polymorphisms

Genomic DNA from peripheral blood was used for analysis of genetic polymorphisms of glutathione-S-transferases GSTP1, GSTM1 and GSTT1, and the ABCB1. A previously published polymerase chain reaction and restriction digestion protocol was used to genotype the ABCB1 (3435 C>T).[26] The GSTP1 genotyping was performed using a PCR-restriction fragment length polymorphism. Briefly, a PCR reaction was conducted in a 50 μl volume containing 50 ng of genomic DNA, 25 mM dNTP's, 10 × PCR buffer, 2 mM $MgCl_2$, 10% DMSO, and 2 U Taq DNA polymerase. For the forward primer (5' CCCACTGAGGTTACGTAGTT 3') as well as the reverse primer (5' GAAGCCCTTTCTTTGTTCAG 3') 20 pmol was used. The PCR conditions were: 5 min at 95°C; 42 cycles of 1 min at 95°C, 1 min at 48°C, 1 min at 72°C; and finally 10 min 72°C. The PCR product was digested with 5 U of *BsmA I* at 55°C for 1 h. The fragments that resulted after digestion were analyzed on a 3% agarose gel stained with ethidiumbromide. After electropheresis the genotype was determined by analysis of the bands on the gel; 296 bp and 118 bp homozygous for the wild type genotype; 222 bp, 118 bp and 74 bp for the homozygous mutant genotype; and fragments of 296 bp, 222 bp and 118 bp and 74 for the heterogenous genotype.

The GSTT1 and GSTM1 genotypes were determined using a multiplex PCR. The PCR was performed in a 50 μl volume containing 10 ng of genomic DNA, 10 × PCR buffer II, 2 nM $MgCl_2$, 25 mM of dNTPs, 1.25 U of AmplitaqGold polymerase and 5% DMSO. For the forward primers (GSTT1, 5' CTGGTCCTCACATCTCCTTAG 3'; GSTM1, 5' TCCCTGAAAAGCTAAAGCTCTA 3'; XIAP: 5' AGTGGTAGTCCTGTTTCAGCATCA 3') and the reverse primers (GSTT1, 5' TCACCGGATCATGGCCAGCA 3'; GSTM1, 5' GTTGGGCTCAAATATACGGTGG 3'; XIAP, 5' CCGCACGGTATCTCCTTCA 3') 20 pmol was used. As an internal control, we used primers for the XIAP gene. PCR conditions were; 10 min at 95°C, 45 cycli of 15 min at 95°C, 30 min at 60°C, and finally 30 min at 60°C. Analysis was performed on a 2% agarose gel stained with ethidiumbromide. The fragments were 451 bp for the presence of the GSTT1 and 215 bp for the presence of GSTM1. A fragment of 75 bp, produced by primers for the XIAP gene had to be present if the PCR reaction was successfully completed.

Statistical analysis

The primary goal of this trial was to obtain an estimate of the response rate, along with a 95% confidence interval (CI). Secondary endpoints were toxicity, progression-free survival (PFS) and overall survival (OS). PFS was calculated from the date of start of treatment until progression of death, whichever came first. Patients still alive without progression at the date of last contact were censored. OS was measured from the date of start of treatment until death from any cause. Patients still alive at the end of follow-up were then censored. The duration of response of the responding patients were measured from the date of response to progression or death. The Kaplan-Meier method was used to estimate PFS and OS.

RESULTS

Patients characteristics

During the 24 months study period, 20 patients with relapsed and/or refractory MM were included and they were treated according to protocol with arsenic trioxide, ascorbic acid and dexamethasone. The median age at study entry was 65 years (range, 41–76 years); 11 patients had IgG, 6 patients IgA and 3 patients had light-chain MM. All patients had received one or more lines of treatments with chemotherapy. The median number of prior treatments was 4 (range, 1-8). Eleven patients had received high-dose dexamethasone, 17 patients had been treated with thalidomide and 9 patients had received high-dose melphalan with autologous stem cell support. Table 1 summarizes the characteristics of the patients and details of previous treatments.

Of the 20 patients, 14 completed 2 or more cycles of therapy: 2 cycles (n= 2), 3 cycles (n=2), 4 cycles (n=6), 5 cycles (n=1) and 6 cycles (n=3). The median interval between the cycles was 28 days. Early discontinuation of treatment before the fourth cycle occurred in 10 of 20 patients (50%) because of toxicity in 3 and progressive disease in 7 patients.

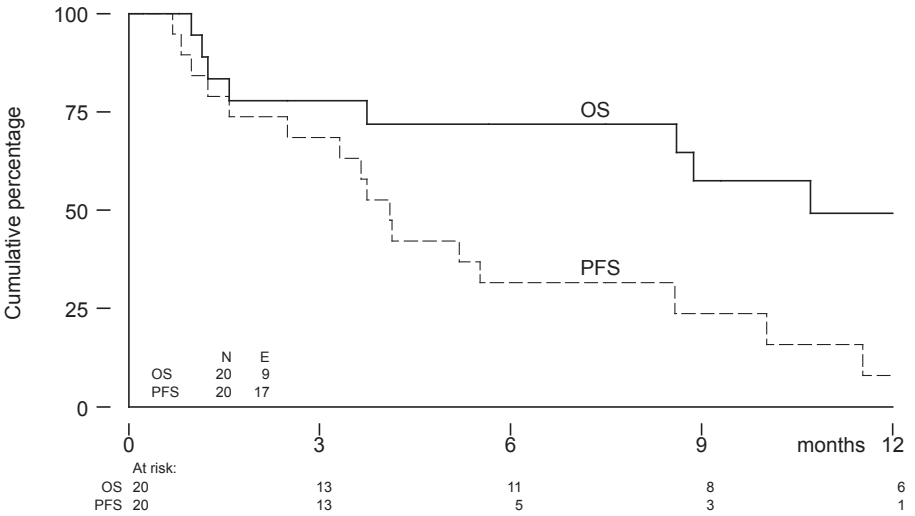
Response

At the time of analysis, 11 patients (55%) were still alive. The median follow-up from start of treatment was 9 months (range, 1-20 months). A clinical response was observed in 8 of the 20 patients (40%), including PR in 2 and MR in 6 patients. The median duration of response was 5 months (range, 0->10 months). The median progression-free survival was 4 months and the median overall survival was 11 months. (Figure 1)

TABLE 1. Patients characteristics

Number of patients	20
Median age in years (range)	65 (41-76)
Male/female	16/4
M-protein subtype	
- IgG	11
- IgA	6
- Light chain	3
WHO performance score	
- 0-1	17
- 2-3	3
Median serum albumin at inclusion in g/l (range)	36 (28-47)
Median serum β_2-microglobulin in mg/l (range)	3.0 (1.2-6.8)
Median number of previous treatment (range)	4 (1-8)
Cytogenetic abnormalities: n /total n (%)	
- deletion of chromosome 13	7/11 (64%)
Prior autologous stem cell transplantation (%)	9 (45%)
Prior high-dose dexamethasone (%)	11 (55%)
Prior thalidomide (%)	17 (85%)

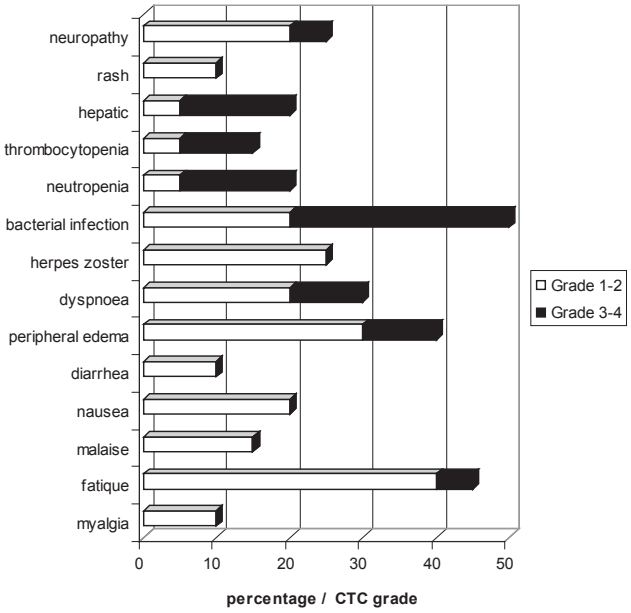
FIGURE 1. Overall survival (OS) and progression free survival (PFS)



Toxicity

The most common adverse events observed with this combination were bacterial infections (n=10), peripheral edema (n=8), fatigue (n=7), dyspnoea (n= 6), reactivation of herpes zoster (n=5), neuropathy (n=5), neutropenia (n=4), thrombocytopenia (n=3) and malaise (n=3) (Figure 2). The majority of the adverse events were low grade (CTC grade 1-2). One patient had a venous thromboembolic event. QT-interval prolongation was observed in one patient and did not require any specific intervention. Grade 3 and 4 adverse events included bacterial infections (n=6), neutropenia (n=3), hepatic toxicity (n=3) and thrombocytopenia (n=2).

FIGURE 2. Adverse events according to Common Toxicity Criteria (CTC)



Genetic polymorphisms

The results of the PCR analyses of ABCB1 (3435 C>T), GSTP1 (1578 A>G) single nucleotide polymorphisms, GSTT1 gene deletion and GSTM1 gene deletion, and the association between these genotypes with toxicity and response are summarized in table 2. Sixteen patients experienced any toxicity of grade 3-4. Hepatic toxicity was observed in three patients and none of them had a homozygous deletion of the GSTM1 gene.

TABLE 2. Genetic polymorphism, toxicity and response to arsenic trioxide

Polymorphism	N	Active genotype, (n)	T	R	Inactive genotype, (n)	T	R
ABCB1 (3435 C>T)	19	CC (1) + CT (14)	10	5	TT (4)	3	3
GSTP1 (1578 A>G)	18	AA (7) + AG (6)	9	5	GG (5)	3	3
GSTT1	19	T+ (11)	7	5	T0 (8)	5	3
GSTM1	19	M+(10)	8	4	M0 (9)	4	4

T, toxicity (CTC grade 3-4); R, response (≥ 50% reduction of M-protein)

DISCUSSION

Clinical trials with arsenic trioxide used as single agent in patients with relapsed or refractory MM have shown a response rate of approximately 25% with acceptable toxicity. To enhance the efficacy of arsenic trioxide, combinations of arsenic trioxide with or without ascorbic acid and various conventional chemotherapeutic agents have been explored. Using such a combination in our study, 8 of 20 patients (40%) had a clinical response as defined by a greater than 25% reduction in M-protein level, with median response duration of 5 months. These results were inferior as compared to other studies in which arsenic trioxide were combined with ascorbic acid and traditional chemotherapeutic agents.

Two other studies also have explored the combination of arsenic trioxide, ascorbic acid and high-dose dexamethasone in patients with relapsed or refractory disease. In one study, the combination was evaluated in a 12-week schedule.[27] Response evaluation after the first cycle showed a clinical response in 2 of 12 evaluable patients (17 %), including PR in 1 and MR in 1 patient. Another study evaluated this combination in a 15-week schedule.[28] Eight of 20 patients had a clinical response (40%) including (near) CR in 3 and PR in 5 patients. Both regimens reported to be well tolerated.

Similarly, a regimen consisting of melphalan, arsenic trioxide and ascorbic acid (MAC) has been evaluated.[29] In this pilot study, the patients received arsenic trioxide with ascorbic acid after each infusion plus oral melphalan at a dose of 0.1 mg/kg per day for 4 days every 4-6 weeks. All 10 patients responded to therapy. At the time of the analysis, 6 patients had a sustained response including 2 patients with continuing response for more than a year. Overall, the MAC therapy was generally well tolerated with only minor treatment delays due to thrombocytopenia and neutropenia.

In our study, therapy with arsenic trioxide, ascorbic acid and dexamethasone was associated with significant toxicity. Despite prophylactic antibiotics, severe bacterial infections were frequently observed which may be related to the use of high-dose dexamethasone in this heavily pre-treated population.

Genetic polymorphisms in the arsenic methylation pathway may have a role in its toxicity.[30;31] Mathews et. al. reported that homozygous mutant polymorphism of MTHFR 1298 (CC) and GSTM1 null genotype was associated with an increased risk of hepatotoxicity.[32] In our study, 3 patients developed hepatic enzyme disorders and they all had the GSTM1 wild type genotype. While the number of observations in this phase II trial was small, no difference in toxicity was observed among the patients based on the active wild type or inactive mutant genotype for ABCB1, GSTP1, GSTM1 or GSTT1.

Prolongation of the QT-interval and arrhythmias have been observed during arsenic trioxide treatment in patients with APL.[33] In our study, prolongation of the QT-interval was uncommon which may be related to the intermittent dosing schedule of arsenic trioxide.

Arsenic trioxide is predominately excreted through the kidneys. Pharmacokinetic studies conducted in APL patients receiving 10 mg per day of arsenic trioxide by a 4 hours intravenous infusion showed that plasma concentration of arsenic trioxide peaked at 5.54 to 7.30 μmol/l, following which the drug was rapidly eliminated.[34] Continuous administration did not alter the pharmacokinetic pattern and did not result in alteration of plasma concentration. Pharmacokinetic studies in MM patients receiving continuous arsenic trioxide at a dose of 0.15 mg/kg per day by a 3 hours infusion for a maximum of 56 days showed that the mean residual plasma concentration was 1.11 ± 0.16 μmol/l after 2 weeks.[22] Interestingly, in 2 patients who were treated with an intermittent schedule, the residual concentration of arsenic trioxide increased in subsequent cycles suggesting accumulation of arsenic trioxide. The most effective concentration of arsenic trioxide is undetermined. In vitro studies have shown that a concentration of 1 μmol/l was sufficient to induce apoptosis in plasma cells.[17]

Several schedules have been used in clinical trials. Continuous dosage of arsenic trioxide established for the treatment of APL was used in the treatment of MM in the earliest studies.[21;22] In our study, an intermittent dosing strategy was explored by using a loading dose of arsenic trioxide for 5 days followed by a twice-weekly maintenance dose. However, there is a clear need for further exploration of dose-response data for arsenic trioxide in multiple myeloma.

In conclusion, combination therapy with arsenic trioxide, ascorbic acid and dexamethasone is feasible, but has moderate efficacy and significant toxicity in heavily pre-treated patients with advanced MM. Further evaluation of arsenic trioxide using different dosing regimens and combinations with traditional chemotherapeutic agents or with novel therapeutic agents such as thalidomide and bortezomib are warranted.

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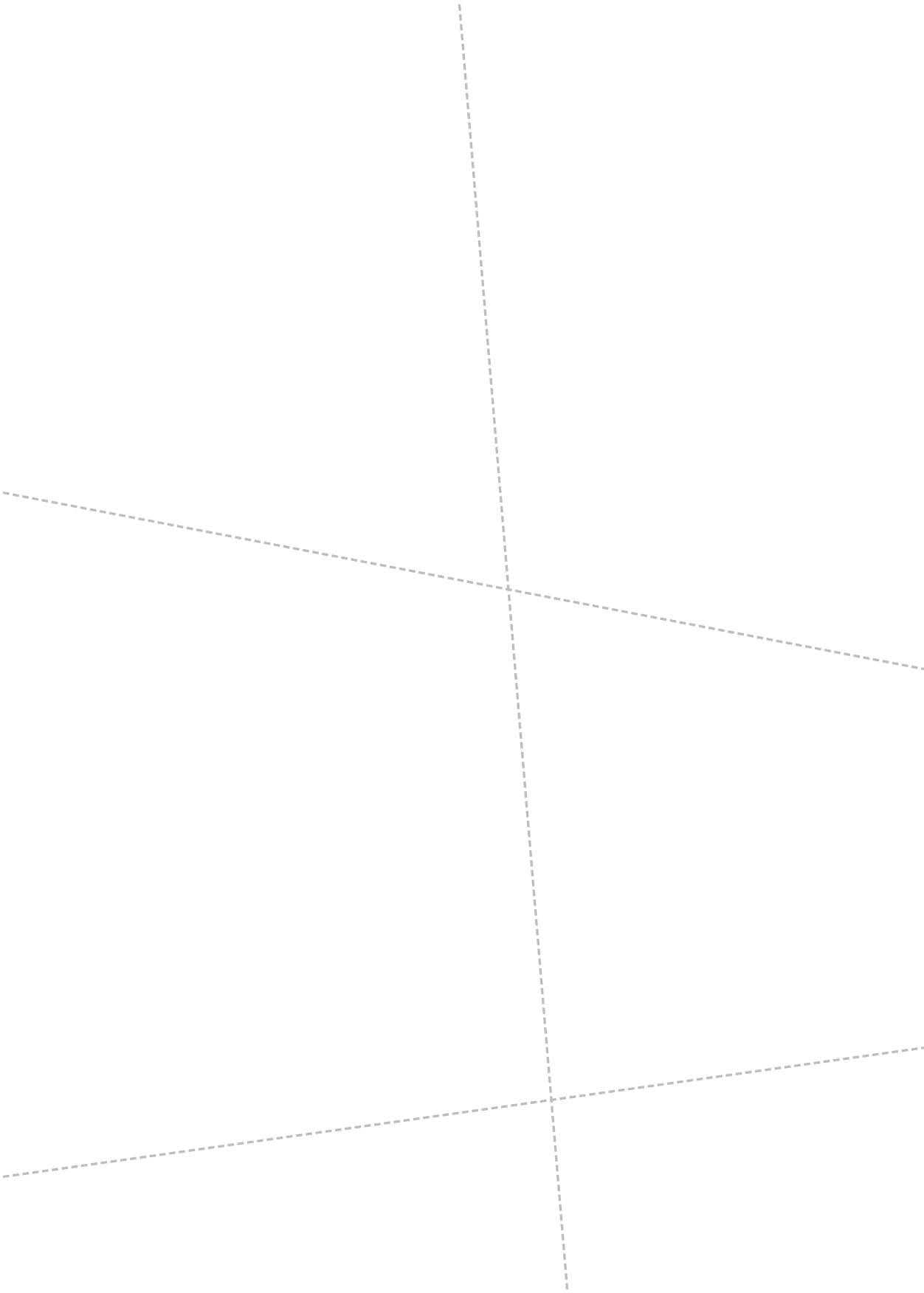

Chapter 6

ANALYSIS OF THE EFFICACY AND TOXICITY OF BORTEZOMIB FOR TREATMENT OF RELAPSED OR REFRACTORY MULTIPLE MYELOMA: PRACTICE AND EXPERIENCE BEYOND THE CONTEXT OF A CLINICAL TRIAL

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ABSTRACT

We evaluated the efficacy and toxicity of the novel proteasome inhibitor bortezomib in patients with multiple myeloma beyond the context of a clinical trial. A total of 50 patients with relapsed or refractory multiple myeloma who were treated with bortezomib during an observational period of 2 years were included in this prospective observational study. The data were obtained by case research. Bortezomib was administrated intravenously at 1.3 mg/m^2 twice weekly for two weeks in a 3-weeks cycle. At time of analysis, 32 patients were alive and the median follow-up was 7 months (range, 2-26 months). A clinical response was observed in 23 patients (46%), including complete response in 2 patients, partial response in 15 and minimal response in 6 patients. The median duration of response was 9 months. The median overall survival was 15 months, with a progression-free survival of 7 months. The most common observed toxicities were gastrointestinal symptoms, thrombocytopenia, neutropenia, herpes zoster, fatigue and peripheral neuropathy. Dose reduction was performed in 36% of the patients. Herpes zoster was observed in 12 % of the patients. Peripheral neuropathy was the most common and disabling side effect, which was the reason to discontinue treatment in 12% of the patients.

In conclusion, bortezomib can induce marked and durable response in a considerable number of patients with acceptable toxicity. The median duration of response was inferior compared to other clinical trials, which may be related to the more frequently executed dose reduction by local physicians.

INTRODUCTION

Multiple myeloma accounts for approximately 1% of all malignancies and 10% of hematological cancers. Although conventional and high-dose chemotherapy with autologous stem cell support can prolong remission and survival, multiple myeloma remains an incurable disease with a median survival of 3 - 4 years.[1] Recent advances in molecular genetics have provided the means to identify tumor specific pathways that regulate myeloma cell growth and survival. The development of small molecules that block a critical pathway within the myeloma cell or the bone marrow microenvironment provides new opportunities for therapeutic intervention.

Bortezomib (Velcade®), a novel dipeptide boronic acid, is a selective inhibitor of the 26S proteasome and interferes directly with the proteolytic activity of the 20S core particle.[2] The 26S proteasome is a multicatalytic enzyme found in the cytoplasm of all eukaryotic cells. It plays a central role in the degradation of regulatory proteins that control the cell cycle and survival pathways.[3]

Preclinical studies suggested that inhibition of the proteasome could be effective in patients with multiple myeloma. In myeloma cell lines and in xenograft mouse models, bortezomib has a potent activity. The cytotoxic effect of bortezomib may involve several distinct mechanisms, including inhibition of cell growth signaling pathways, induction of apoptosis and inhibition of expression of cellular adhesion molecules.[3-8] In addition, bortezomib modulates the secretion of cytokines and growth factors by bone marrow stromal cells.[3,9] These anti-tumor effects are partly mediated by inhibition of degradation of the inhibitor protein IκB, thereby preventing the translocation of NF-κB to the nucleus.

In a phase I study in patients with advanced hematological malignancies, bortezomib showed activity in patients with multiple myeloma.[10] The safety and efficacy of bortezomib has subsequently been assessed in two phase II studies of patients with relapsed or refractory multiple myeloma.[11,12] In both studies, bortezomib induced clinically significant responses with manageable toxicity. A phase II study (SUMMIT) in 202 myeloma patients treated with 1.3 mg/m^2 bortezomib intravenously for 2 weeks, followed by 1 week without treatment, for up to eight cycles, demonstrated an overall response rate of 35%.[12] The median overall survival was 16 months, with a median duration of response of 12 months. A smaller, randomized study (CREST) in 54 patients, comparing 1.0 mg/m^2 or 1.3 mg/m^2 bortezomib according the same schedule confirmed the activity of bortezomib.[11] In both studies some responses occurred after addition of dexamethasone in patients with no or a suboptimal response to bortezomib alone.

These clinical data of the efficacy and toxicity of bortezomib as treatment for myeloma patients are restricted to prospective phase II studies in expert myeloma centers.

We here report a prospective analysis of the efficacy and toxicity of bortezomib in patients with relapsed and/or refractory multiple myeloma who were treated in community centers in a compassionate need program.

PATIENTS AND METHODS

Patients and treatment

All myeloma patients (n=50) who were treated with bortezomib during a 2 years observational period were included in the analysis. The clinical and treatment data of these patients were obtained by means of case research. The patients received up to eight 3-weekly treatment cycles of bortezomib. Within each treatment cycle, bortezomib 1.3 mg/m^2 was administered as an intravenous bolus twice weekly on days 1, 4, 8 and 11 followed by a 10 days rest period. Treatment was withheld in case of any grade ≥ 3 non-hematological toxicity or grade 4 hematological toxicity considered being drug related. Treatment was resumed at a dose of 1.0 mg/m^2 after resolution of the non-hematological toxicity to grade 2 or better and for hematological toxicity to an absolute neutrophil count ≥ 0.5 x 10^9/L and platelet count ≥ 20 x 10^9/L. Further dose reduction to 0.7 mg/m^2 was allowed, but lower doses were not permitted.

All patients had active disease and received previous treatments with chemotherapy. The median number of previous treatment was 3 (range 1-5), 29 patients were treated with high-dose melphalan with autologous stem cell support and 8 patients had received allogeneic stem cell transplantation. The patients characteristics are summarized in table 1.

Assessment of efficacy and toxicity

Response evaluation was based on the criteria of the European Bone Marrow Transplantation Group.[13] Patients who discontinued treatment before a response could be assessed were considered to have had no response to treatment. Complete response (CR) was defined as (1) disappearance of serum or urine M-protein; (2) bone marrow plasmacytosis < 5%; (3) no increase in size or number of lytic bone lesions; and (4) disappearance of soft-tissue plasmacytomas. Partial response (PR) required (1) a reduction of serum M-protein by ≥ 50%; (2) a reduction in 24 h urinary light chain excretion either > 90% or to < 200 mg; (3) in non-secretory myeloma ≥ 50% reduction in bone marrow plasmacytosis; (4) no increase in size or number of lytic bone lesions; and (5) a decrease ≥ 50% in the size of soft-tissue plasmacytomas. Minimal response (MR) was defined as (1) a reduction of serum M-protein by 25-49%; (2) 50-89% reduction in 24h urinary light chain excretion;

(3) in non-secretory myeloma 25-49% reduction in bone marrow plasmacytosis; (4) no increase in the size or number of lytic bone lesions; and (5) a decrease of 25-49% in size of soft-tissue plasmacytomas. The disease was considered stable (SD) when the M-protein changes was < 25%; progressive disease (PD) was defined as (1) increase of M-protein or urinary light chain by 25%; (2) an increase of bone marrow plasmacytosis by > 25%; (3) an increase in size of existing bone lesions or soft-tissue plasmacytomas; and (4) development of new bone lesions or soft-tissue plasmacytomas.

Toxicity incidence was estimated and summarized using the frequency and descriptive techniques. The National Cancer Institute Common Toxicity Criteria (version 2.0) were used to grade the non-hematological toxicity.

TABLE 1. Patients characteristics

Number of patients	50
Median age in years (range)	59 (37–87)
Male/female	37/13
M-protein subtype	
- IgG	33
- IgA	10
- Light chains	6
- non secretory	1
Type light chain: kappa/lambda	30/19
Salmon-Durie Stage II/III	16/34
Cytogenetic abnormalities: n /total n (%)	20/30 (66%)
- deletion of chromosome 13	14/20 (70%)
Median serum β_2-microglobulin in mg/l (range)	4 (2-13)
Median serum albumin in g/l (range)	38 (25-48)
Number of previous treatment regimens (%)	
- 1	5 (10%)
- 2	13 (26%)
- 3	20 (40%)
- 4 or more	12 (24%)
Dexamethasone treatment (%)	15 (30%)
Thalidomide treatment (%)	40 (80%)
Autologous stem cell transplantation (%)	29 (58%)
Allogeneic stem cell transplantation (%)	8 (16%)

RESULTS

Response and survival

Between November 2002 and December 2004, 50 patients with relapsed or refractory multiple myeloma were treated with bortezomib at referral hospitals in the Netherlands.

Thirty-four patients (68%) completed at least 4 treatment cycles and 19 patients (38%) received 8 cycles. Discontinuation of treatment before eight cycles occurred in 15 patients (30%) because of progressive disease, in 12 patients (24%) because of toxicity, in 1 on patients' request and 1 patient proceeded to allogeneic stem cell transplantation.

At time of analysis, 33 patients (66%) were still alive. The median follow-up from start of bortezomib treatment was 7 months (range, 2-26 months). A clinical response was observed in 23 patients (46%), including complete response in 2 patients, partial response in 15 and minimal response in 6 patients. Median time to response was 6 weeks and the median duration of response was 9 months. Blood counts generally improved as soon as disease response was achieved. The median progression-free survival was 7 months and the median overall survival was 15 months. (Figure 1)

Response to bortezomib occurred in 5 of the 15 patients with a complete or partial deletion of chromosome 13, which is associated with a poor outcome with conventional and high-dose chemotherapy. Univariate and multivariate analysis of variables such as number of previous treatment regimens, previous treatment with thalidomide or dexamethasone, deletion of chromosome 13, β2- microglobulin and albumin did not show any statistically significant differences in PFS and OS. This could be partly due to the small sample size involved.

Seven patients who had no response after two treatment cycles of bortezomib alone continued treatment and received oral dexamethasone (20 mg) on the day of and the day after following each bortezomib dose. One of these patients had an additional minimal response on the combination therapy. This patient was previously refractory to corticosteroid treatment.

Toxicity

The observed toxicities were almost similar to those previously reported.[11,12] The most common toxicities were diarrhea, anorexia, nausea, vomiting, thrombocytopenia, neutropenia, fatigue and peripheral neuropathy. (Figure 2) The majority of the gastrointestinal toxicities were low grades (CTC grade 1-2) and manageable with routine

support. Six patients had a herpes zoster (12%) and 3 patients (6%) developed a skin rash. Observed severe toxicities (CTC grade 3-4) were peripheral neuropathy (20%), thrombocytopenia (18%), diarrhea (6%) and neutropenia (6%).

Dose reduction was performed in 18 patients (36%) and bortezomib was discontinued in 12 patients (24%) because of the side effects: 6 peripheral neuropathy, 4 gastrointestinal symptoms, 1 skin rash and 1 orthostatic hypotension.

FIGURE 1. Overall survival and progression-free survival

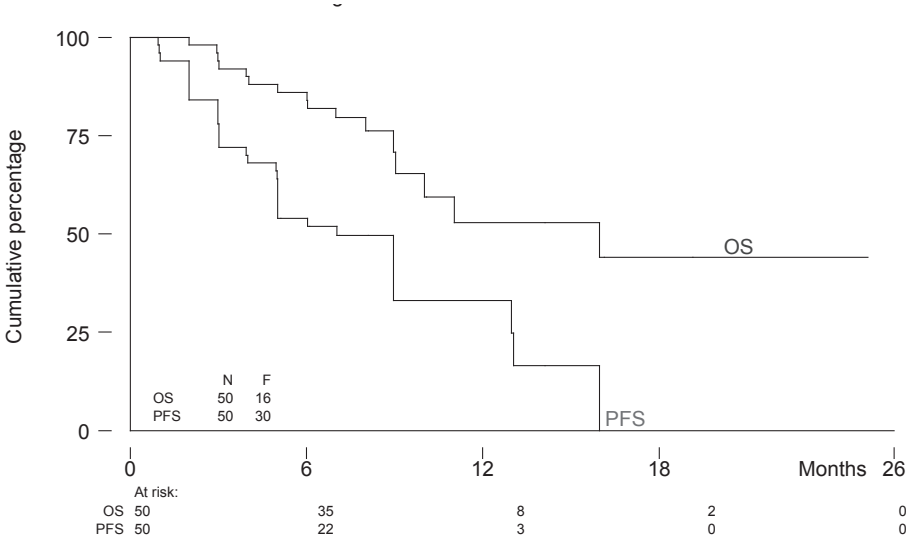
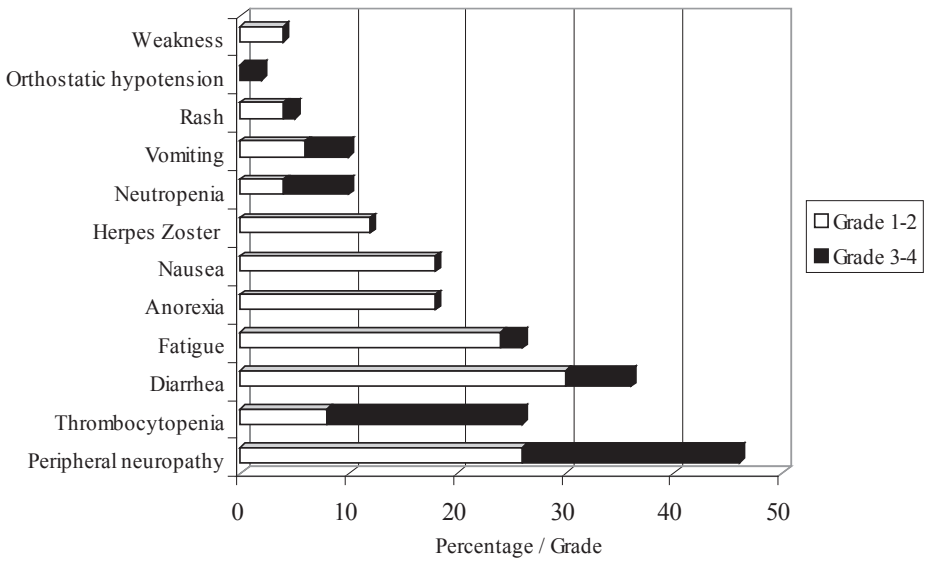


FIGURE 2. Observed toxicity of bortezomib



DISCUSSION

Bortezomib is a novel potent, selective inhibitor of the proteasome. Bortezomib has been approved at 1.3 mg/m^2 twice weekly for two weeks in a 3-weeks cycle for treatment of patients with relapsed and/or refractory multiple myeloma. In our analysis of 50 heavily pre-treated patients, 23 patients (46%) had a clinical response with ≥ 25% reduction of M-protein in serum and/or urine. Our data support those of 2 phase II studies, CREST and SUMMIT, which have reported response rate with bortezomib alone of 35-50%.[11,12]

Bortezomib combined with dexamethasone may have a synergistic anti-myeloma effect. In the phase II studies, some additional responses occurred after addition of dexamethasone to bortezomib. In the SUMMIT study, 74 patients received dexamethasone with bortezomib after having no or suboptimal response with bortezomib alone and 13 of these (18%) had an improved response. In the CREST study, patients in the bortezomib 1.3 mg/m^2 group had an overall response rate of 50% and with addition of dexamethasone the overall response was 62%. In our study, 1 of 7 patients (14%) had an additional response with the combination therapy. It is, however, difficult to determine the contribution of each individual agent to the response. Further investigations on the possibility of synergy between bortezomib and dexamethasone or chemotherapy are warranted.

The median duration of response on treatment with bortezomib alone was 9 months. In the CREST study, the median duration of response was 9.5 months and 13.7 months in the 1.0 mg/m^2 and 1.3 mg/m^2 groups, respectively, while in the SUMMIT study the median response duration was 12 months. A possible explanation for the less favorable duration of response may be that in our series dose reduction was executed more frequently by local physicians when compared with SUMMIT and CREST, which were conducted in multiple myeloma expert centers.

Overall, the most common observed toxicities were diarrhea, anorexia, nausea, vomiting, thrombocytopenia, neutropenia, fatigue and peripheral neuropathy. Most of the side effects were manageable with routine support and reversible with appropriate and temporary dose reduction.

Peripheral neuropathy was the most clinical significant and severe side effect during continued treatment. The majority of the patients who developed severe peripheral neuropathy had been heavily pre-treated with neurotoxic agents such as thalidomide and/or vincristine and had symptoms of neuropathy at baseline. The clinical manifestations of bortezomib-induced neuropathy were symmetrical hypoesthesia or paraesthesia of the distal extremities. New or worsening symptoms of neuropathy were observed in approximately 45 % of the patients.

Thrombocytopenia was commonly observed and developed primarily in patients with a low baseline platelet count. The thrombocytopenia was transient, with recovery within the 10 days rest period in each treatment cycle and was not associated with bleeding complications. The incidence of severe neutropenia was relatively low and not associated with severe bacterial infections.

A remarkable observation in our series was the high incidence of herpes zoster. Six patients developed a herpes zoster infection during treatment with bortezomib (n=5) or in combination with dexamethasone (n=1). Herpes zoster results from reactivation of a latent infection with varicella zoster virus from the dorsal root or cranial nerve ganglia and it is commonly observed among those who have an impaired cell-mediated immunity. The transcription factor NF-κB has been demonstrated to play a pivotal role in cytokine signaling and the generation of cell-mediated immune response in numerous models.[14,15] Therefore, inhibition of the NF-κB may increase the risk of reactivation of the varicella zoster virus. Prophylactic antiviral medication should be considered in predisposed patients who receive bortezomib.

In conclusion, the novel proteasome inhibitor bortezomib is an effective treatment in patients with relapsed and/or refractory multiple myeloma. Bortezomib can induce marked and durable response in a considerable number of patients. Bortezomib was overall well tolerated and the toxicity was acceptable. This survey shows that ongoing clinical studies with bortezomib alone and in combination with other agents are needed to assess the efficacy and toxicity in multiple myeloma and a range of other cancers. The clinical practice in community hospitals to apply dose-reduction in patients with treatment related toxicity without adherence to well defined guidelines may jeopardize the outcome of the efficacy of bortezomib in multiple myeloma.

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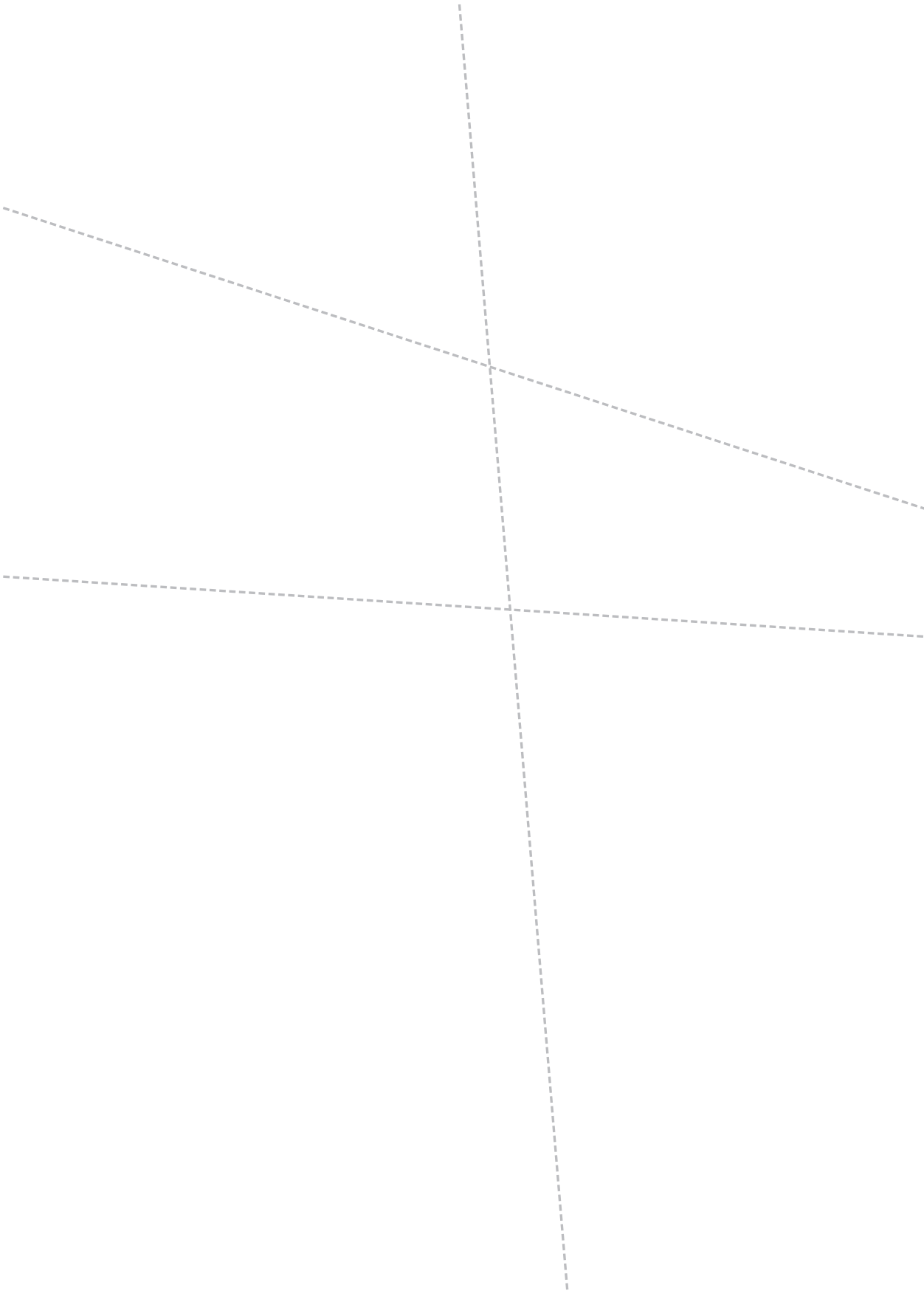

Chapter 7

PLEOMORPHIC PRESENTATION OF CUTANEOUS LESIONS ASSOCIATED WITH THE PROTEASOME INHIBITOR BORTEZOMIB IN PATIENTS WITH MULTIPLE MYELOMA

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ABSTRACT

The clinical course and histologic findings of cutaneous adverse reactions associated with bortezomib treatment are presented in this study. Bortezomib (VELCADE) is a proteasome inhibitor, which is used as a novel anti-cancer drug in the treatment of multiple myeloma. We have conducted an observational analysis of 47 myeloma patients who were treated with bortezomib in 3 prospective clinical trials. Cutaneous adverse reactions were observed in 6 patients (13%). Five patients presented with sharply demarcated erythematous plaques or nodules on the trunk and one patient had generalized morbilliform erythema with ulcerations and fever. The time between the first bortezomib dose and occurrence of the cutaneous eruptions was at least 30 days. The cutaneous eruptions usually resolve within a few days. One patient withdrew from further treatment due to severe cutaneous toxicity. The histologic findings ranged from perivascular dermatitis to interstitial and interface dermatitis corresponding with clinically more extensive lesions.

INTRODUCTION

Multiple myeloma is an incurable plasma cell malignancy characterized by a proliferation of monoclonal plasma cells in the bone marrow and production of a homogeneous immunoglobulin (M-protein).[1] Multiple myeloma accounts for approximately 1% of all malignancies and the median survival is 3 years.

Proteasome inhibitors represent novel anti-cancer drugs. They interact with the proteasome-ubiquitin pathway.[2] Bortezomib is a selective, reversible inhibitor of the proteasome, which is a multicatalytic enzyme complex found in the cytoplasm of all eukaryotic cells. The proteasome plays a role in the degradation of ubiquitinated proteins, which are predominately proteins involved in the regulation of cell cycle control.[3] The anti-tumor effect of bortezomib is partly mediated by inhibition of degradation of the inhibitor protein IκB, thereby preventing activation of the transcription factor Nuclear Factor κB (NF-κB).

Bortezomib is the first proteasome inhibitor that has entered clinical trials. Studies have demonstrated a substantial therapeutic activity of bortezomib in myeloma patients combined with limited toxicity.[4;5] In US and Europe, bortezomib has recently been approved for the treatment of relapsed and refractory multiple myeloma patients. The toxic effects of bortezomib are usually mild and consist of gastrointestinal symptoms, peripheral neuropathy and thrombocytopenia. Less known are the cutaneous adverse effects. We here report on the incidence and clinical presentation of bortezomib-induced cutaneous adverse reaction in myeloma patients.

PATIENTS AND METHODS

An observational analysis was conducted on patients with bortezomib-induced skin toxicity from 3 prospective randomized clinical trials in multiple myeloma. In the APEX (Assessment of Proteasome inhibition for Extending remissions) study, relapsed or refractory patients were randomized to receive high-dose dexamethasone or bortezomib.[6] In the DOXIL study, relapsed patients were randomized to receive bortezomib and DOXIL® (liposomal doxorubicin) or bortezomib monotherapy. Finally, the ongoing joint Dutch/Belgian/German (HOVON-65/GMMG-HD4) study compared vincristine, adriamycin and dexamethasone (VAD) with bortezomib, adriamycin and dexamethasone (PAD) as initial therapy followed by high-dose therapy with transplantation in newly diagnosed patients. Between November 2002 and August 2005, approximately 90 patients were included in these trials of which 47 received bortezomib. Bortezomib was administered at a standard dose of 1.3mg/m^2, as an intravenous bolus on days 1, 4, 8 and 11 of each

3-week cycle. Thirty-two patients had received bortezomib as monotherapy, 7 patients in combination with liposomal doxorubicin, and 8 patients in combination with adriamycin and dexamethasone.

Bortezomib treatment was withheld in case of any grade ≥ 3 non-hematological toxicity or grade 4 hematological toxicity which considered being drug related. The treatment was resumed according to protocol recommendations at a dose of 1.0 mg/m^2 after resolution of the non-hematological toxicity to grade 2 or less. The National Cancer Institute Common Toxicity Criteria (CTC version 3.0) were used to grade the non-hematological toxicity.

A total of 6 patients (13%) with bortezomib-induced skin toxicity were identified. Five patients received bortezomib as monotherapy and 1 patient in combination with liposomal doxorubicin**.** None of the 6 patients received additional medication, for e.g. antibiotics during bortezomib treatment**.** The clinical and histologic findings were reviewed. The characteristics of the patients, clinical presentation and histopathologic findings are summarized in table 1.

TABLE 1. Patients characteristics, clinical presentation and histopathologic findings

No	Sex/ age	Therapy	Skin lesions	Histopathologic finding	Grade (CTC)	Time of onset*
1	M/ 60y	Bor	Morbilliform exanthema and ulcerations on trunk	Perivascular dermatitis, mainly lymfocytic with some neutrophils	2/3	50
2	M/ 56y	Bor	Erythematous nodules on trunk & neck	Extensive interface, perivascular and periadnexal dermatitis, with spongiosis, apoptotic keratinocytes and some nuclear dust in the dermis	1	65
3	M/ 67y	Bor + Dox	Erythematous nodules on trunk	Interface, perivascular, periadnexal and interstitial dermatitis with spongiosis and discrete collagen necrosis in the dermis	1	37
4	M/ 70y	Bor	Erythematous papules on chest & neck	Perivascular dermatitis, mainly lymfocytic.	1	33
5	M/ 58y	Bor	Erythematous plaques on trunk & limbs	Minimal interface, but extensive perivascular and periadnexal dermatitis with minimal spongiosis and nuclear dust in the dermis	1	30
6	M/ 41y	Bor	Erythematous nodules on trunk & neck & face	Interface, extensive perivascular and periadnexal dermatitis with spongiosis, and apoptotic keratinocytes	1	43

Bor = bortezomib, Dox = liposomal doxorubicin, CTC = Common Toxicity Criteria
* Time of onset in days after first bortezomib administration

RESULTS

Patients characteristics

Six patients with bortezomib-induced skin lesions were identified. The mean age was 59 years (range, 41-70). All 6 patients had relapsed disease and had received one or more previous treatments with chemotherapy. The median number of prior treatments was 2 (range, 1-4).

Medical history and disease course

Five of the 6 patients had similar cutaneous lesions. These consisted of erythematous nodules or plaques, most of which measured 0.5 – 2 cm and were distributed on the trunk, neck and limbs. (Figure 1) The lesions were not associated with fever or pruritus. One patient developed generalized morbilliform exanthema with ulcerations on the trunk and fever up to 38.5^{o}C. All patients demonstrated no other manifestation of multi-organ involvement. The time between the first bortezomib administration and the cutaneous lesions varied from 30 – 65 days. The eruptions usually resolved in 4-7 days following treatment with low dose prednisone and/or antihistamines.

One patient discontinued treatment due to the severity of cutaneous lesions and lack of response and two patients because of progressive disease. Three patients received low dose prednisone (10 mg) before each bortezomib infusion in the next cycles. The cutaneous lesions did not reappear in 2 patients and recurred less extensively in 1 patient.

Histopathologic findings

A skin biopsy was performed in all 6 patients. The histopathologic findings can be categorized as a perivascular dermatitis (case 1 and 4) type of lesion and a more extensive lesion (case 2, 3, 5 and 6) consisting of an interface dermatitis with a varying degree of spongiosis and apoptosis of keratinocytes and interstitial dermatitis with or without signs of discrete damage to the vessels (nuclear dust) or collagen (discrete collagen necrosis). (Figures 2 and 3) However, full blown vasculitis with vascular wall necrosis, neutrophilic infiltrates and microthrombi was not observed. In contrast with the usual histology observed with skin biopsy of drug reactions, only a few eosinophilic granulocytes were found.

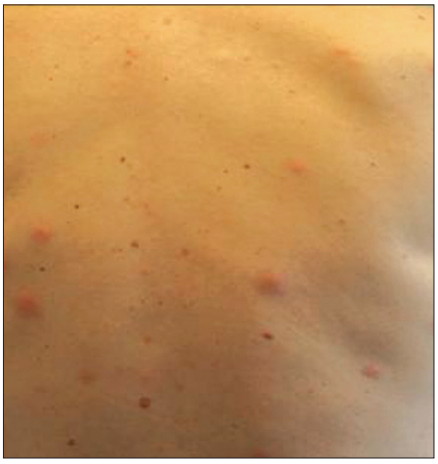

FIGURE 1. Multiple sharply, marketed, erythematous nodules on the trunk.

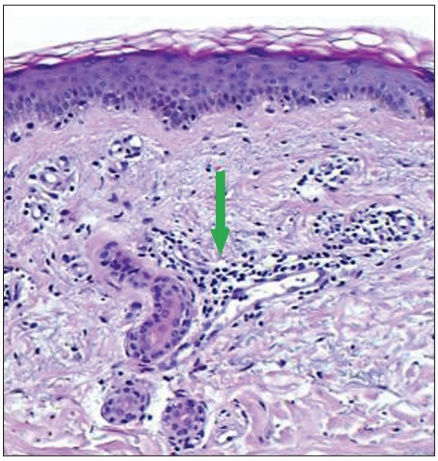

FIGURE 2. Skin biopsy of case 1. Note the perivascular infiltrate. HE staining, original objective lens magnification 20x.

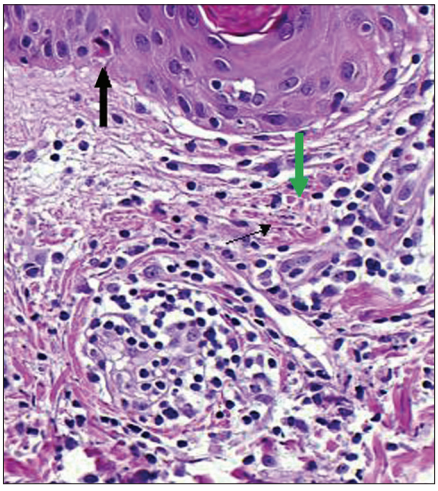

FIGURE 3. Skin biopsy of case 2. Note the apoptotic keratinocyte (black arrow), the discrete collagen necrosis at the infiltrate (green arrow), partly surrounding the superficial bloodvessels. There is also nuclear dust. HE staining, original objective lens magnification 20x.

DISCUSSION

Bortezomib is a novel selective proteasome inhibitor with activity in relapsed or refractory myeloma. Ongoing clinical trials with proteasome inhibition by bortezomib have also shown activity in non-Hodgkin lymphoma and non-small cell lung carcinoma.

The side effects of bortezomib are usually mild to moderate. The most common toxicities are diarrhea, nausea, fatigue, constipation, peripheral neuropathy, and thrombocytopenia.[5,6]

Recently, two case reports of bortezomib induced skin lesions have been published.[7,8] One patient developed generalized purpuric rash and the skin biopsy revealed a leukocytoclastic vasculitis. Another patient developed painful erythematous and edematous plaques, associated with fever and malaise. The skin biopsy confirmed the clinical diagnosis of Sweet syndrome.

In our study, skin toxicity was observed in 6 of the 47 patients (13%). The incidence of the skin toxicity is comparable with reports from clinical trials in multiple myeloma, non-Hodgkin lymphoma and solid malignancies.[5,6,9-12]

The clinical presentation, however, may be highly variable. The skin lesions usually occur during the second, third or fourth treatment cycle and resolve within a few days following treatment.

In our study, the histopathologic findings can be roughly categorized as a perivascular dermatitis type of lesion and a more extensive lesion with a varying degree of interface and interstitial dermatitis. They apparently form both ends of a spectrum, although this remains to be supported more firmly by additional studies.

A recent report suggests that the development of rash is a possible surrogate marker of response.[13] In our study, 3 of the 6 patients had a response to bortezomib.

The differential diagnosis of exanthematous drug reactions includes viral eruptions. Reactivation of a latent infection with varicella zoster is frequently observed in myeloma patients who receive bortezomib.[6,14] NF-κB plays a pivotal role in cytokine signalling and the generation of cell-mediated immune response. Therefore, inhibition of NF-κB may increase the risk of reactivation of the virus.[15,16]

We conclude that skin manifestations of bortezomib toxicity may be variable and highly pleomorphic. The underlying mechanism is an inflammatory response that may be caused by (1) delayed hypersensitivity, (2) cell-mediated immune responses, (3) vascular damage, and (4) direct toxic reaction by chemotherapeutic agent. The unique proteasome inhibitory effect of bortezomib may be associated with unexpected effects in tissues such as the skin and other organs.

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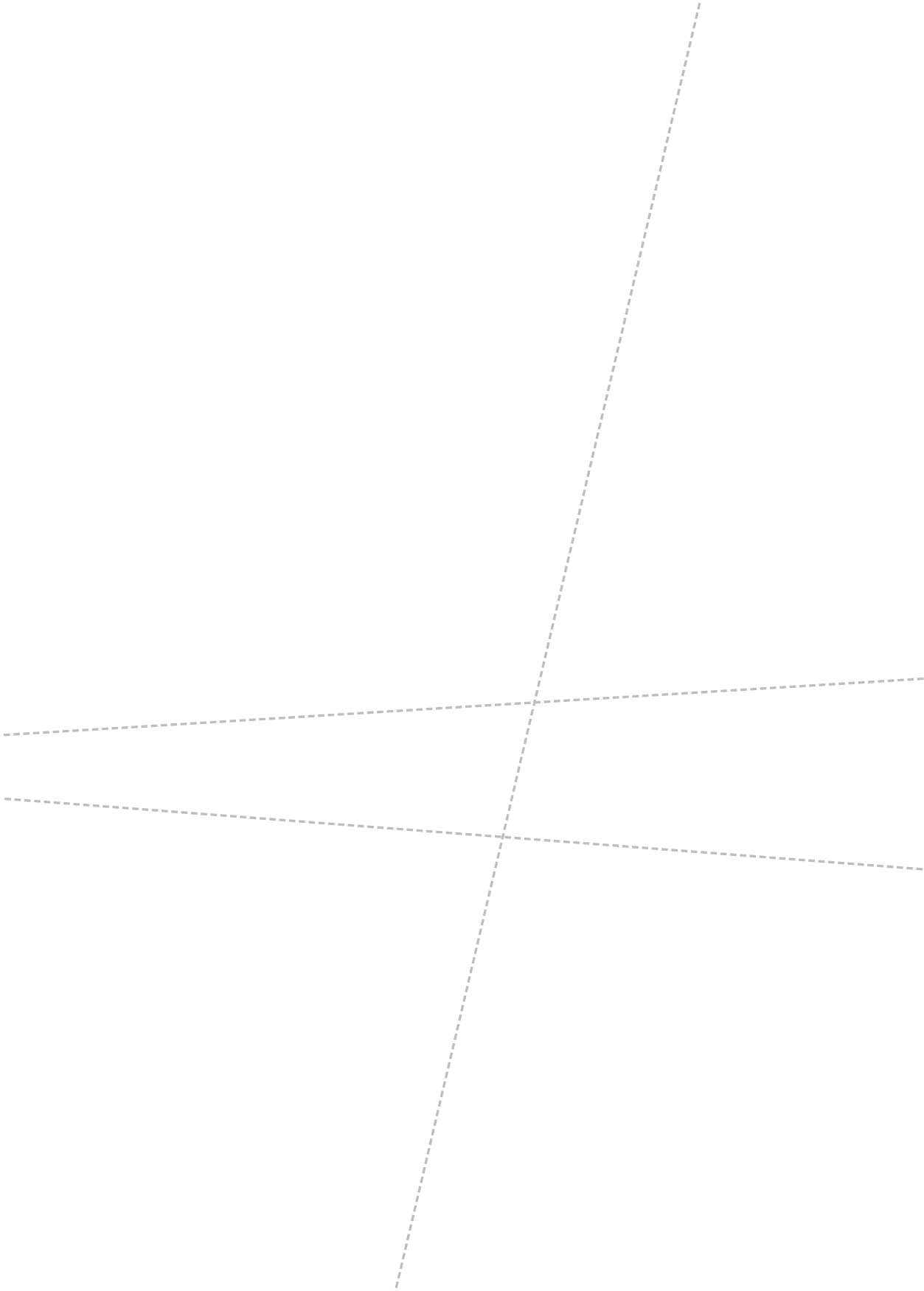

Chapter 8

THE OCCURRENCE OF GRAFT-VERSUS-HOST DISEASE IS THE MAJOR PREDICTIVE FACTOR FOR RESPONSE TO DONOR LYMPHOCYTE INFUSIONS IN MULTIPLE MYELOMA

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ABSTRACT

The graft-versus-myeloma (GVM) effect of donor lymphocyte infusions (DLIs) is well established. We now report the outcome of DLI in 54 patients with relapsed myeloma following allogeneic transplantation. Twenty-eight patients (52%) responded, 19 patients (35%) with a partial response and 9 patients (17%) with a complete response. The progression-free and overall survival were 19 and 23 months, respectively. We found that acute and chronic graft-versus-host disease (GVHD) observed in 57% and 47% of patients, respectively, following DLI were the strongest predictors for response. This suggests that targets for GVHD and GVM are identical. In a subgroup analysis, deletion of chromosome 13, as determined by double-color fluorescence in situ hybridization (FISH), had no impact on outcome, indicating that these patients are candidates for early allogeneic transplantation followed by DLI, in case of insufficient response.

INTRODUCTION

Donor lymphocyte infusions (DLIs) can induce remissions in patients with multiple myeloma who relapse after allogeneic stem cell transplantation (allo-SCT).[1;2] Response rates between 30% and 50% have been reported.[3;4] In a previous study with a limited number of patients, those with chemosensitive disease receiving a high T-cell dose seemed to have the best chance for response and prolonged survival.[5] We recently updated the results of DLI in a larger group of patients and analyzed prognostic factors for response and survival.

PATIENTS AND METHODS

Four transplantation centers in The Netherlands participated in the study of DLI administered for treatment of relapsed myeloma after allo-SCT. Approval was obtained from the participating centers institutional review boards (University Medical Centers of Amsterdam, Rotterdam, Nijmegen, and Utrecht). Informed consent was provided by all patients according to the Declaration of Helsinki. Fifty-four patients, median age 52 years (range, 34-68 years), were included, 50 patients with a relapse following myeloablative partially T-cell–depleted allo-SCT[6] and 4 patients following non–T-cell–depleted myeloablative allo-SCT. The first patients were conditioned with cyclophosphamide (120 mg/kg) and total body irradiation (TBI; 12 Gy) with lung shielding, and the latter patients were conditioned with low-dose TBI (2 Gy) and fludarabine (90 mg/m2).[7] Patients received a total of 95 DLI courses (range, 1-7 courses) for a median of 20 months (range, 4-90 months) following transplantation. T-cell dose of DLI varied between 1 x 10^6 and 5 x 10^8 cells/kg. In the vast majority of patients, the starting dose was 1 x 10^7 T-cells/kg. If after a minimum observation period of 3 months there was no response and no signs of graft-versus-host disease (GVHD), patients received a second course with 1 x 10^8 T-cells/kg. A further dose escalation could be performed in the event of no response/GVHD following this second DLI. Forty patients received reinduction therapy before DLI that consisted of vincristine, adriamycin, dexamethasone (VAD; n = 32), dexamethasone as monotherapy (n = 7), or melphalan (70 mg/m2) as monotherapy. Responses to reinduction therapy and to DLI were assessed according to the criteria of the European Group for Blood and Marrow Transplantation (EBMT).[8] A partial response (PR) was defined as decrease in myeloma proteins of at least 50% and improvement in myeloma-related signs/symptoms; a complete response (CR) was defined as absence of myeloma proteins as determined (at least twice, 3 weeks apart) by immunofixation of serum and urine and less than 5% plasma cells in the marrow. An ongoing response in patients already in PR was defined as additional decrease in myeloma proteins of at least 50% or achievement of CR.

RESULTS AND DISCUSSION

Eighteen of 40 patients treated with reinduction therapy achieved a PR: 14 of 32 patients after VAD, 3 of 7 patients after monotherapy with dexamethasone, and 1 of 1 patient after melphalan intravenously. Eleven of the 18 responding patients showed an ongoing response after DLI, including 7 patients with an additional 50% or higher reduction of tumor load and 4 patients who achieved a CR. Eight of 22 patients refractory to chemotherapy achieved a PR after DLI. Nine of 14 patients who did not receive reinduction treatment responded, including 4 patients with a PR and 5 patients with a CR. Altogether, 28 patients (52%) responded to DLI, 19 (35%) patients with a partial response and 9 (17%) with a complete response. This high response rate may be due to the fact that patients initially received T-cell–depleted transplants. Seventeen patients responded after the first course and 11 patients after dose escalation. Thirteen patients relapsed from DLI. Four of 5 relapsed patients responded again to a new course of DLI. Progression-free survival was a median of 19 months (range, 3-116+ months) and overall survival was a median of 23 months (range, 2-118+ months). (Figure 1A-B) Acute GVHD[9,10] occurred in 31 patients (57%): 6 patients (11%) with grade I, 14 patients (26%) with grade II, and 11 patients (20%) with grades III-IV. Eighty percent of patients with GVHD grades II-IV responded to DLI, including 20% with a CR, whereas 33% of patients with GVHD grades 0-I responded, including 12% with CR. Chronic GVHD[9,10] occurred in 25 patients (47%), limited GVHD in 9 patients (17%), and extensive GVHD in 16 patients (30%). Seventy-three percent of patients with chronic GVHD responded to DLI, including 9% with CR, whereas 37% of patients without chronic GVHD responded to DLI, including 22% with CR. Three patients (5%) died from toxicity: 2 patients from GVHD and 1 patient from septicemia.

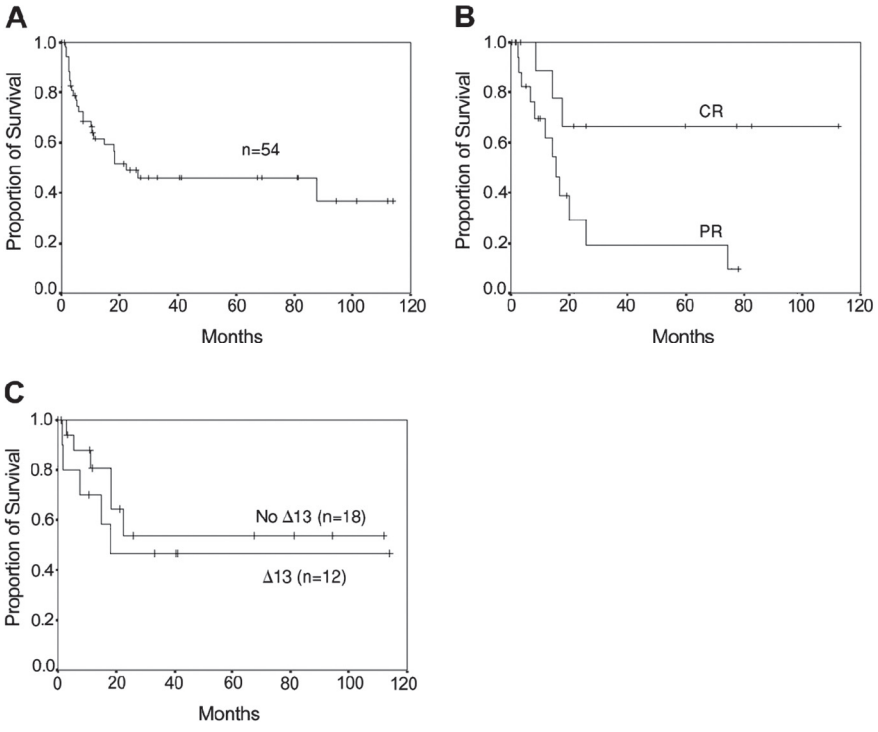
TABLE 1. Predictive factors for response to DLI, univariate

Factors	P-value
Time interval between previous Tx and DLI	0.941
GVHD after previous Tx	0.767
Induction therapy given	0.529
Response to reinduction therapy	0.05
T-cell dose of DLI	0.378
Chimerism of peripheral blood cells, T/non T	1.0
Acute GVHD grades II–IV following DLI	< 0.0001
Chronic GVHD following DLI	< 0.0001
Deletion of chromosome 13, FISH*	1.0

The Mann-Whitney U test and the Fisher exact test were used in case of continuous variables and categorical variables, respectively. Tx indicates transplantation. *Determined in 29 patients in diagnostic samples

In previous studies with a limited number of patients, chemosensitive disease and T-cell dose were significantly associated with response to DLI.[5] In this study, which included a larger number of patients, it appeared that only the occurrence of acute GVHD grades II-IV and chronic GVHD and, to a lesser extent, response to reinduction therapy had an impact on response. (Table 1) This observation strongly suggests that like leukemia[11], the targets for the cytotoxic donor (T) cells are minor histocompatibility antigens (mHa's) expressed on both recipient normal and myeloma plasma cells. We recently reported that donor-derived cytotoxic T-cell clones isolated from the peripheral blood of a myeloma patient responding to nonmyeloablative allo-SCT recognized both autologous normal B and malignant myeloma cells but not donor-derived B cells.[12] It cannot be excluded however that non–antigen-specific mechanisms in association with GVHD, such as cytokines[13] or tumor-specific antigens, are involved as well in graft-versus myeloma (GVM). GVM may occur without GVHD and recently it was shown that in patients achieving a complete response to DLI this was associated with high antibody responses to highly expressed myeloma-associated antigens.[14] One may speculate that although GVHD and GVM are strongly associated, both reactions are mediated by different T-cell populations triggered simultaneously. Functional studies of such T-cell populations after DLI or allo-SCT are

FIGURE 1. Survival curves. (A) Overall survival after DLI. (B) Progression-free survival of patients responding to DLI. (C) Overall survival by deletion of chromosome 13 (A13) as determined by FISH.



needed to define which target antigens are involved in GVM. Other factors including T-cell dose, time interval between transplantation and DLI, GVHD after previous transplantation, and chimerism had no impact on response. (Table 1)

Our study confirms the potential of the GVM effect of DLI to induce responses. However remissions were not long lasting, especially not in patients with a PR. Only 6 patients are in sustained CR more than 2 years following DLI. A better long-term outcome may be achieved when post-DLI (maintenance) treatment is introduced with immune-modulating drugs like interferon or thalidomide. Interestingly, the deletion of chromosome 13 did not influence response and outcome of DLI. (Figure 1 C) We could determine this retrospectively by doublecolor fluorescence in situ hybridization (FISH) in the stored diagnostic bone marrow samples in 29 patients.[15] However this must be confirmed prospectively in more patients, including those with cytogenetic-determined deletion of chromosome 13.

In conclusion, DLI is an effective treatment for myeloma patients with relapsed disease at the cost of acute and chronic GVHD in a substantial number of patients. The strong association between GVHD and GVM suggests that in both phenomena the same effector cells and target antigens are involved. Encouraging is the observation that GVM may overcome the adverse effect of deletion of chromosome 13.[16;17] This entails that high-risk patients may be candidates for early donor stem cell transplantation followed by DLI in case of insufficient response.

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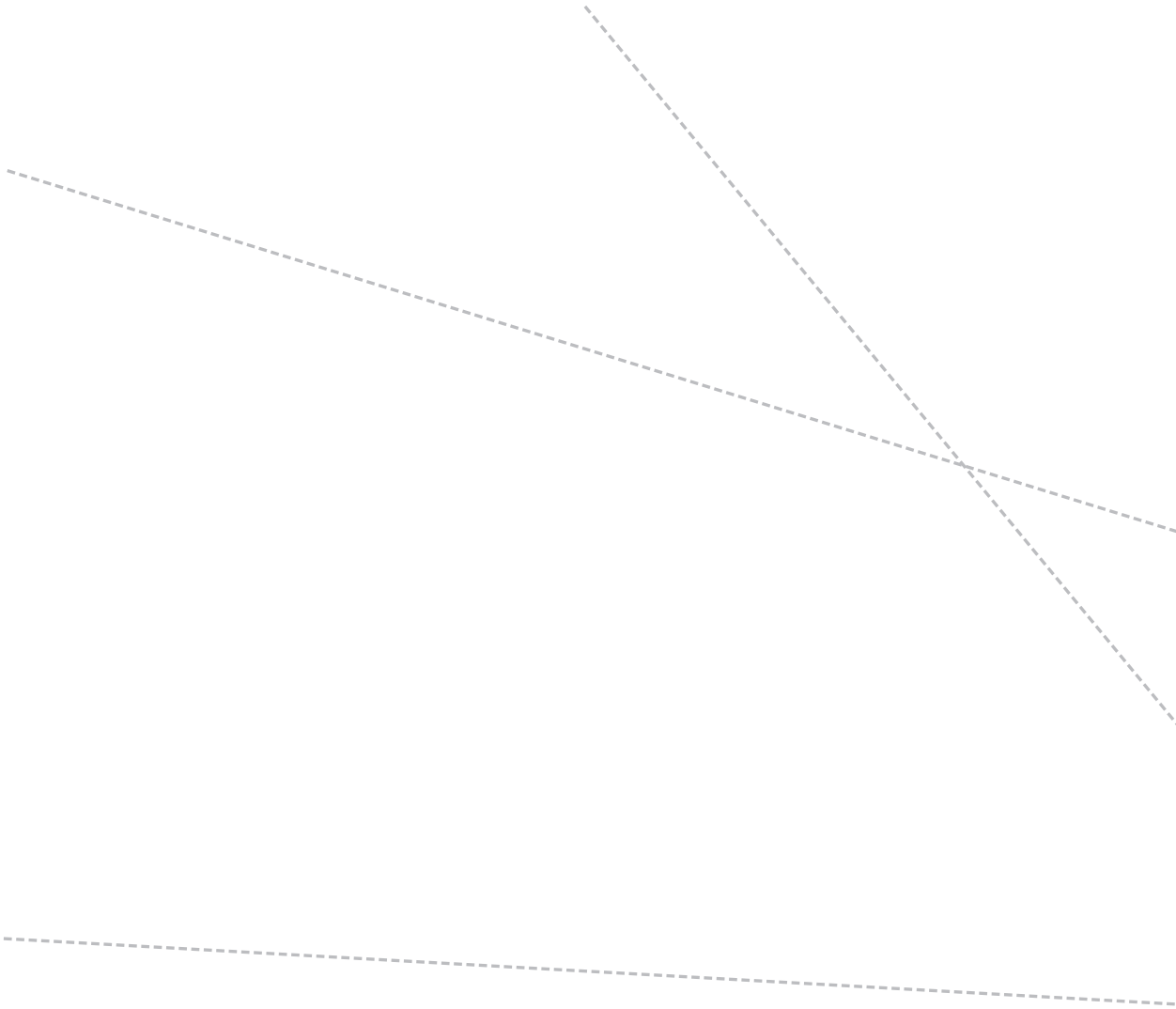

Chapter 9

GENERAL DISCUSSION AND FUTURE PERSPECTIVES

SUMMARY / SAMENVATTING

GENERAL DISCUSSION AND FUTURE PERSPECTIVES

In the last decade, several significant advances in myeloma therapy have occurred with the pace of change accelerated with the introduction of new anti-myeloma agents. The approach to the treatment of multiple myeloma has become more complex with an array of therapeutic options, including autologous stem cell transplantation, non-myeloablative allogeneic transplantation, and new therapeutic agents such as thalidomide, bortezomib, and lenalidomide.

High-dose chemotherapy followed by autologous stem-cell transplantation has emerged as the most effective approach to achieve high complete response rates and thereby improve the long-term overall survival. The majority of the patients, however, will develop progressive disease within several years. For patients who are not suitable candidates for high-dose chemotherapy due to advanced age or poor performance status conventional chemotherapy has remained the most common treatment with a median overall survival of 3 years. Treatment options for patients with relapsed or refractory disease are limited. The major obstacle to successful treatment of these patients is the development of drug-resistant disease.

The analysis of genetic rearrangements in monoclonal gammopathy of undetermined significance (MGUS) and multiple myeloma by the use of cytogenetics and molecular cytogenetics has contributed significantly to our understanding of the development of clonal plasma cell disorders. Modern techniques such as fluorescence in situ hybridization (FISH) and comparative genomic hybridization (CGH) have revealed chromosomal abnormalities in virtually all patients with multiple myeloma and most patients with MGUS.

The first objective of this thesis is to study the prognostic relevance of chromosomal abnormalities in multiple myeloma. Genetic abnormalities in multiple myeloma are complex and variable, which makes it difficult to correlate them with clinical outcome. Molecular cytogenetics have identified specific chromosomal abnormalities in distinct groups of patients with different natural histories. Specific IgH translocations such as the t(4;14) and t(14;16), chromosome 13 deletions by metaphase cytogenetics and loss of 17p13 by FISH are well established poor prognostic factors and may be of major interest for patient stratification in clinical trials**.** However, the prognostic relevance of most chromosomal abnormalities is unknown. New markers such as chromosome 1p/q abnormalities have to be evaluated in prospective clinical trials.

The second objective of the thesis is to identify patients who may benefit from novel therapies. New therapeutic agents such as thalidomide, bortezomib and lenalidomide have altered the management of multiple myeloma. These agents have expanded the

therapeutic options in relapsed or refractory myeloma. Strategies of novel combination regimens consisting of conventional and new agents have significantly improved the response rates and overall survival of these patients. However, the optimal sequence of applying these agents and the most synergistic combination regimens in upfront treatment as well as relapse need to be determined. Response rates to thalidomide, bortezomib, and lenalidomide have not been directly compared. Furthermore, differences in patients' selection and response criteria employed in clinical trials make comparative assessments difficult.

The position of new agents in relation to high-dose chemotherapy followed by autologous stem cell transplantation and their impact on the number (single vs double) and the timing of transplantation (upfront vs relapse) is as yet unclear. The introduction of new agents as induction therapy has improved the complete response rate significantly. The effect of the improved induction regimens on the results of the whole treatment strategy and the long-term outcome will have to be evaluated in prospective randomized trials.

As there is no plateau of the survival curves after high-dose chemotherapy, some form of maintenance therapy is necessary to control minimal residual disease after transplantation. Studies with interferon-α as maintenance therapy have not shown any meaningful clinical benefit. Thalidomide is the first agent that has shown promising effects in the maintenance setting.

Allogeneic stem cell transplantation provides the potential for long-term survival in a small proportion of patients. However, the application of allogeneic stem cell transplantation has been limited by significant treatment related mortality in particular older patients and in patients with relapsed or refractory disease. Non-myeloablative conditioning regimens have been developed to reduce the toxicity while maintaining the allogeneic graft-versus-myeloma effect. The initial results of this approach are encouraging and support the feasibility and efficacy of non-myeloablative allogeneic transplantation. Nevertheless, several issues need to be addressed regarding this strategy. These include the type of induction chemotherapy, the optimal timing to transplant, the optimal non-myeloablative conditioning regimen, and defining the patient population that may benefit of this strategy.

Gene expression profiling and proteomics are powerful tools for improving the understanding of the molecular pathogenesis of myeloma and to discover novel prognostic markers and key targets for therapy of the disease. Novel therapies targeting not only specific intracellular pathways but also the interaction of the myeloma clone with the bone marrow microenvironment, growth factors and cytokines are being developed.

Future treatment strategies will focus on the exploration of effective combination regimens that incorporate new agents in induction therapy and maintenance. The primary goal is to achieve stringent complete responses and develop effective long-term maintenance. The challenge will be to identify patient subgroups that will benefit most from different agents and to incorporate targeted therapies into the management on the basis of an increased understanding of the biology of multiple myeloma.

SUMMARY

Multiple myeloma is a malignant plasma cell disorder characterized by the accumulation of monoclonal plasma cells in bone marrow. Multiple myeloma accounts for approximately 1% of all malignancies and 10% of hematological cancers. The clinical symptoms are caused by skeletal destruction and by plasma cell infiltration of the bone marrow, which results in a compromised normal hematopoiesis. Despite recent advances in myeloma therapy, the disease remains incurable with a median survival of 3 years after conventional chemotherapy and 5 years after high-dose chemotherapy followed by autologous stem cell transplantation.

In **chapter 1** the literature is reviewed on relevant prognostic factors, which include genetic factors of the malignant clone and biochemical markers that reflect tumor burden.

Conventional chemotherapy is unable to achieve long-term disease control in myeloma. Improvements of outcome in recent years are the results of other approaches, including high-dose chemotherapy followed by autologous stem cell transplantation and non-myeloablative allogeneic stem cell transplantation, which are discussed in this chapter. Novel agents such as thalidomide, bortezomib, arsenic trioxide and lenalidomide target not only the malignant plasma cells but also their myeloma-host interaction and bone marrow microenvironment. These agents used alone and in combinations with conventional or other novel agents, are being explored in different stages of the disease.

In **chapter 2** the evolution of chromosomal abnormalities in multiple myeloma is described. Sequential conventional cytogenetic as well as fluorescence in situ hybridization (FISH) analysis were performed on bone marrow specimens from 38 myeloma patients during disease progression. This study supports the notion that cytogenetic abnormalities in multiple myeloma are not random. Disease progression was correlated with an increased complexity of genetic abnormalities, which consisted in the majority of structural aberrations acquired in the later stages of disease. Specific chromosomal alterations such as chromosome 1p/q aberrations were associated with disease progression while others e.g. chromosome 13q deletions showed a stable pattern during the course of the disease.

In **chapter 3** the prognostic relevance of chromosomal abnormalities for outcome after high-dose chemotherapy is reported. Conventional cytogenetic analysis was performed in 160 patients at diagnosis. Of the observed chromosomal abnormalities the presence of complex abnormalities (>3), hypodiploidy, chromosome 1p/q abnormalities, and chromosome 13q deletions were predictive for poor overall survival.

In **chapter 4** the results of thalidomide as treatment of multiple myeloma are presented. The rationale for the use of thalidomide was based on studies showing increased bone marrow microvascularity in myeloma and the observation that thalidomide inhibits angiogenesis and myeloma growth. In this retrospective analysis of 122 myeloma patients

with relapsed and/or refractory disease, response to thalidomide was observed in 56 patients (46%) including complete response in 5 patients, partial response in 41 patients and minimal response in 10 patients. The progression-free survival was 10 months. Eight of 15 patients with soft tissue plasmacytoma had a response to thalidomide. However, the duration of response in these patients was short compared with that of those without extramedullary involvement. Common side effects were somnolence, constipation, rash, and polyneuropathy. Chronic peripheral polyneuropathy was the dose-limiting side effect. It was concluded that thalidomide can induce marked and durable response in myeloma patients. Thalidomide up to a daily dose of 200 mg was well tolerated.

The results of a prospective, multicenter phase II trial with arsenic trioxide, ascorbic acid and dexamethasone are reported in **chapter 5**. In vitro studies of arsenic trioxide showed activity in myeloma cells and ascorbic acid could enhance the cytotoxic effect. Response to arsenic trioxide, ascorbic acid and dexamethasone was observed in 8/20 patients (40%), including partial response in 2 patients and minimal response in 6 patients. The progression-free survival was only 4 months. Severe adverse events were reported which included neutropenia, thrombocytopenia, hepatic toxicity and bacterial infections. The results of this study showed that arsenic trioxide, ascorbic acid and dexamethasone has moderate efficacy and significant toxicity.

Bortezomib is a potent and selective proteasome inhibitor. Clinical trials in expert centers have demonstrated a substantial therapeutic activity in myeloma patients combined with limited toxicity. In **chapter 6** the results are presented of a prospective analysis of the efficacy and toxicity of bortezomib in myeloma patients with relapsed and/or refractory disease who were treated in a compassionate need program. A clinical response was observed in 23/50 patients (46%), including complete response in 2 patients, partial response in 15 and minimal response in 6 patients. The progression-free survival was 7 months. The most common observed toxicities included gastrointestinal symptoms, thrombocytopenia, neutropenia, herpes zoster, fatigue, and peripheral neuropathy, and were overall well tolerated. A remarkable observation in our series was the high incidence of herpes zoster (12%). This study confirms the efficacy and limited toxicity of bortezomib in multiple myeloma.

Chapter 7 describes the pleomorphic presentation and histopathologic findings of cutaneous lesions associated with bortezomib.

In **chapter 8** the outcome of donor lymphocyte infusions (DLI) in patients with relapsed myeloma following allogeneic transplantation is presented. Response to DLI was observed in 28/54 patients (52%) including complete response in 9 patients and partial response in 19 patients. Progression-free and overall survival were 19 and 23 months, respectively. The occurrence of graft-versus-host disease is the major predictive factor for response to DLI. Deletion of chromosome 13 by FISH had no impact on outcome, indicating that these patients are candidates for early allogeneic transplantation followed by DLI, in case of insufficient response.

SAMENVATTING

Het multiple myeloom is een maligne plasmacelaandoening die gekenmerkt wordt door accumulatie van monoklonale plasmacellen in het beenmerg. Het multipel myeloom vormt ongeveer 1% van alle maligniteiten en 10% van de hematologische maligniteiten. De klinische verschijnselen ontstaan als gevolg van botdestructie en plasmacel infiltratie in het beenmerg waardoor de normale bloedaanmaak wordt onderdrukt. Ondanks recente vorderingen in de behandeling is genezing van het multipel myeloom tot op heden niet mogelijk. De mediane overleving met conventionele chemotherapie bedraagt 3 jaar en met hoge-dosis chemotherapie gevolgd door autologe stamceltransplantatie 5 jaar.

In **hoofdstuk 1** wordt een overzicht gegeven van relevante prognostische factoren zoals tumorcytogenetica en kenmerken die een afspiegeling vormen van de tumormassa. De behandeling van het multipel myeloom is aan het veranderen. Met conventionele chemotherapie kan geen langdurige remissie worden bereikt. Een meer intensieve benadering van de ziekte met hoge-dosis chemotherapie en autologe stamceltransplantatie of niet-myeloablatieve stamceltransplantatie heeft geleid tot betere behandelingsresultaten. Nieuwe behandelingsmodaliteiten zoals thalidomide, bortezomib, arseentrioxide en lenalidomide doen hun intrede in de kliniek. Deze geneesmiddelen onderscheiden zich van de traditionele cytostatica doordat ze niet alleen direct de tumorcel doden maar ook de interactie verstoren tussen tumorcel en beenmergstromacel en tussen tumorcel en gastheer. Deze geneesmiddelen, alleen of in combinatie met chemotherapie, worden in verschillende stadia van de ziekte onderzocht.

In **hoofdstuk 2** worden de conventioneel cytogenetische en fluorescentie in situ hybridisatie (FISH) data gepresenteerd van het beenmerg van 38 patiënten met multipel myeloom die longitudinaal werden vervolgd. Deze studie ondersteunt het concept dat chromosomale afwijkingen in myeloomcellen niet willekeurig zijn. Ziekteprogressie gaat gepaard met een toegenomen complexiteit van chromosomale afwijkingen die voornamelijk bestonden uit structurele afwijkingen, verworven in een laat stadium van de ziekte. Specifieke chromosomale afwijkingen zoals aan chromosoom 1p/q waren geassocieerd met ziekteprogressie. Andere chromosomale afwijkingen zoals verlies van chromosoom 13q toonden een stabiel patroon toonde tijdens ziekteprogressie.

In **hoofdstuk 3** wordt de prognostische waarde van chromosomale afwijkingen voor de uitkomst na hoge-dosis chemotherapie besproken. Bij 160 patiënten werd bij diagnose conventioneel cytogenetisch onderzoek gedaan. Bij ongeveer de helft van de patiënten werden chromosomale afwijkingen gevonden. De aanwezigheid van complexe chromosomale afwijkingen (>3), hypodiploid karyotype, chromosoom 1p/q afwijkingen en verlies van chromosoom 13q waren voorspellend voor een ongunstige overleving.

In **hoofdstuk 4** worden de resultaten van thalidomide als behandeling van het multipel myeloom gepresenteerd. De onderliggende gedachte voor het gebruik van thalidomide was gebaseerd op studies die aantoonden dat de vaatdichtheid in beenmerg is toegenomen bij multipel myeloom en dat thalidomide vaatnieuwvorming kan remmen. In deze retrospectieve studie bij 122 patiënten met recidief en/of refractaire ziekte hadden 56 patiënten (46%) een respons op thalidomide, waarvan compleet bij 5 patiënten, partieel bij 41 patiënten en minimaal bij 10 patiënten. De progressie vrije overleving was 10 maanden. Ook patiënten met een weke delen plasmacytoom respondeerden op thalidomide. Echter, de responsduur bij deze patiënten was kort in vergelijking met patiënten zonder extramedullaire lokalisatie. De meest voorkomende bijwerkingen waren slaperigheid, constipatie, huiduitslag en polyneuropathie. Polyneuropathie was de meest voorkomende ernstige bijwerking bij langdurig gebruik. De eindconclusie van deze studie was dat thalidomide duurzame remissies kan induceren bij patiënten met multipel myeloom in een vergevorderd stadium. Thalidomide tot een dosering van 200 mg per dag wordt goed verdragen.

De resultaten van een prospectieve, multicenter fase II studie met arseentrioxide, ascorbinezuur en dexamethason worden beschreven in **hoofdstuk 5**. Uit preklinisch onderzoek is gebleken dat arseentrioxide een tumorremmend effect heeft in myeloomcellijnen. Ascorbinezuur kan het cytotoxische effect versterken. Twintig patiënten met recidief en/of refractaire ziekte werden behandeld met arseentrioxide, ascorbinezuur en dexamethason. Acht patiënten (40%) hadden een respons waarvan partieel in 2 patiënten en minimaal in 6 patiënten. De progressie vrije overleving was slechts 4 maanden. Neutropenie, trombocytopenie, levertoxiciteit en bacteriële infecties waren de meest voorkomende ernstige bijwerkingen. De eindconclusie was dat behandeling met arseentrioxide, ascorbinezuur en dexamethason matig effectief is met veel ernstige bijwerkingen.

Bortezomib is een potente en selectieve remmer van het proteasoom. Klinische studies met bortezomib bij het multipel myeloom zijn beperkt tot gespecialiseerde centra. In **hoofdstuk 6** worden de resultaten gepresenteerd van een prospectieve studie naar de effectiviteit en toxiciteit van bortezomib bij patiënten met recidief en/of refractaire ziekte die in een compassionate use programma met dit middel worden behandeld. Drieëntwintig van de 50 patiënten (46%) hadden een respons op bortezomib, waarvan compleet in 2 patiënten, partieel in 15 en minimaal in 6 patiënten. De progressie vrije overleving was 7 maanden. Veel voorkomende bijwerkingen waren maagdarmklachten, trombocytopenie, neutropenie, moeheid en polyneuropathie. Opvallend was de relatieve hoge incidentie van herpes zoster reactivatie (12%).

Hoofdstuk 7 beschrijft de klinische presentatie en de histopathologisch bevindingen van cutane laesies geassocieerd met het gebruik van bortezomib.

In **hoofdstuk 8** worden de resultaten besproken van donor lymfocyten infusie in patiënten met recidief multipel myeloom na allogene stamceltransplantatie. Achtentwintig van de 54 patiënten (52%) hadden een respons waarvan compleet in 9 patiënten en partieel in 19 patiënten. De progressie vrije en totale overleving was 19 en 23 maanden, respectievelijk. Het optreden van 'graft-verus-host ziekte' was de belangrijkste voorspellende factor voor respons op donor lymfocyten infusie. Verlies van chromosoom 13 gemeten met FISH had geen invloed op de uitkomst van donor lymfocyten infusie. Bij deze subgroep van patiënten dient allogene stamceltransplantatie te worden overwogen met de mogelijkheid tot het geven van donor lymfocyten infusie indien er sprake is van suboptimale respons.

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CURRICULUM VITAE

De auteur van dit proefschrift werd geboren op 30 december 1965 te Hong Kong. Na het behalen van het VWO-diploma aan het Gemeentelijke Scholengemeenschap Oost in Groningen werd in 1985 gestart met de studie geneeskunde aan de Rijksuniversiteit Groningen. Het docteraal examen werd behaald in 1989 en het arts examen in 1992. Aansluitend werkte hij ruim 2 jaar als arts-assistent op de afdeling Interne Geneeskunde van het Sophia Ziekenhuis te Zwolle, thans Isala Kliniek (opleider dr. T. Tjabbes).

In 1994 begon hij de opleiding tot internist in het Twenteborg Ziekenhuis te Almelo (opleider dr. J. Wolthuis). Deze opleiding werd vanaf 1996 vervolgd in het Medisch Spectrum Twente te Enschede (opleider dr. D. Richel, later dr. B. Hylkema). In augustus 2000 werd hij als internist geregistreerd. Hierna kon hij de opleiding in het aandachtsgebied hematologie starten in de Dr. Daniel den Hoed Kliniek te Rotterdam (opleider Prof.dr. B. Löwenberg). Na de registratie als internist-hematoloog bleef hij aldaar werkzaam als staflid hematologie. Van januari 2003 tot oktober 2006 was hij werkzaam als staflid hematologie in Erasmus MC-centrumlokatie. In deze periode kwam het proefschrift grotendeels tot stand.

De auteur is thans werkzaam als internist-hematoloog in ZNA Stuivenberg te Antwerpen.

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LIST OF ABBREVIATIONS

Allo-SCT	allogeneic stem cell transplantation
CR	complete response
del(13q)	deletion of chromosome 13q
DLI	donor lymphocyte infusion
EBMT	European Group for Blood and Marrow Transplantation
EFS	event-free survival
FISH	fluorescence in situ hybridization
GVHD	graft-versus-host disease
GVM	graft-verus-myeloma
HOVON	Dutch-Belgian Hemato-Oncology Cooperative Study Group
IMF	International Myeloma Foundation
ISCN	international system for human cytogenetic nomenclature
ISS	International Staging System
MGUS	monoclonal gammopathy of undetermined significance
MM	multiple myeloma
MP	melphalan and prednisone
M-protein	monoclonal immunoglobulin
MR	minimal response
OS	overall survival
PD	progressive disease
PFS	progression-free survival
PR	partial response
RIC	reduced intensity (nonmyeloablative) conditioning
SD	stable disease
VAD	vincristine, adriamycin and dexamethasone