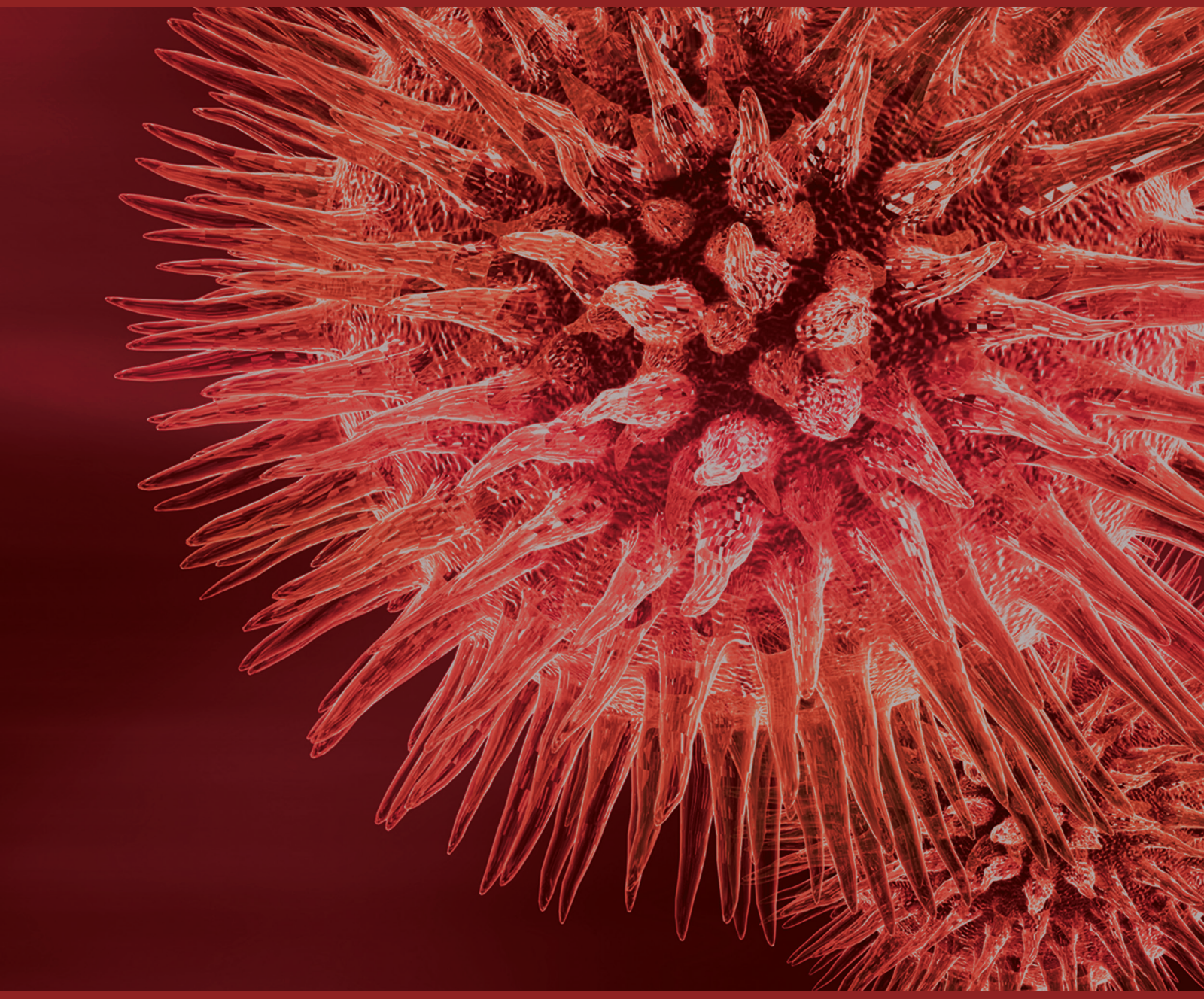
BioMed Research International

Plasma Cell Neoplasms: Genetics, Pathobiology, and New Therapeutic Strategies

Guest Editors: Dong Soon Lee, Wee-Joo Chng, and Kazuyuki Shimizu



Plasma Cell Neoplasms: Genetics, Pathobiology, and New Therapeutic Strategies

BioMed Research International

Plasma Cell Neoplasms: Genetics, Pathobiology, and New Therapeutic Strategies

Guest Editors: Dong Soon Lee, Wee-Joo Chng, and Kazuyuki Shimizu

Contents

Hindawi Publishing Corporation
BioMed Research International
Volume 2014, Article ID 941820, 2 pages
http://dx.doi.org/10.1155/2014/941820

Editorial

Plasma Cell Neoplasms: Genetics, Pathobiology, and New Therapeutic Strategies

Dong Soon Lee,[1] Wee-Joo Chng,[2] and Kazuyuki Shimizu[3]

[1]*Department of Laboratory Medicine, Seoul National University College of Medicine, Seoul 110-744, Republic of Korea*
[2]*Cancer Science Institute of Singapore, NUS, Singapore*
[3]*Department of Hematology, Tokai Central Hospital, Japan*

Correspondence should be addressed to Dong Soon Lee; soonlee@snu.ac.kr

Received 2 October 2014; Accepted 2 October 2014; Published 28 December 2014

Plasma cell neoplasms are phenotypically heterogeneous disorder with broad spectrum, including plasma cell myeloma, monoclonal gammopathy of unknown significance, and amyloidosis. Each plasma cell neoplasm is defined by diagnostic criteria and, accordingly, therapeutic strategies are developed. Scientists often have difficulties in discovering genetics of plasma cell neoplasms due to problem of sorting plasma cells. Deep sequencing utilizing next generation sequencing or single cell genomics are expected to break through this barrier, providing novel tools to characterize the plasma cell neoplasms.

In this special issue on plasma cell neoplasms, the accepted articles can be divided into 4 categories: (i) genetic features of plasma cell myeloma, (ii) new prognostic factors, (iii) new therapeutic strategies, and (iv) features of Waldenstrom's macroglobulinemia.

(i) Genetic Features of Plasma Cell Myeloma. W.-J. Chng et al. reviewed changes of microRNA, *p53* gene, and MMSET in myeloma. They specially focused on the evidences of multilayer heterogeneity coexisting in the same patient, clinical parameters, genetic level, cell differentiation level, and clonal heterogeneity. Revealing this heterogeneity will break through challenges in terms of treatment, prognostication, and monitoring of treatment. Y. Koh et al. described the establishment of 2 cell lines from both myeloma bone marrow and plasmacytoma from a single patient and, interestingly, their biologic natures from one patient were different, which also shows a slice of the heterogeneity of myeloma. A multicenter cytogenetic study using Agilent 44 K aCGH microarrays in Czech also showed large genomic heterogeneity in MM cases and revealed copy number alterations in almost all cases.

(ii) New Prognostic Factors. Many prognostic parameters were suggested in each special situation such as before peripheral stem cell mobilization, in infected patients, in patients with bone involvement, in complete remission group, in patients without chromosome abnormalities, and in elderly patients. Impact of RBC index, expression of myeloid antigens on plasma cells, plasma cell assessment before peripheral blood stem cell mobilization, impact of comorbidity, serum parathyroid hormone, hyperglycemia, and plasma level of osteopontin on clinical outcome were presented. C.-K. Min et al. suggested a significant impact of <5% BMPCs in patients who did not achieve immunofixation electrophoresis negativity and in patients with myeloma undergoing autologous stem cell transplantation.

(iii) New Therapeutic Strategies. K. Shimizu et al. suggested that ASCT could also be a mainstay in the initial treatment of elderly MM patients, and its indication should be evaluated based on performance status and the presence of complications and/or comorbidities of each elderly patient with MM. H. J. Kim et al. suggested an alternative one-hour IV infusion mode of injection after evaluation of the response rate and incidence of bortezomib induced peripheral neuropathy. F. Raimondo summarized new insights into salvage therapy for relapsed/refractory MM as emerging from recent clinical trials exploring the activity of bendamustine, new generation proteasome inhibitors, novel IMiDs, monoclonal antibodies, and drugs interfering with growth pathways.

(iv) Waldenstrom's Macroglobulinemia. H. S. Lee et al. reported the higher overall response rate in patients treated with novel agent combined chemotherapy, compared to conventional chemotherapy alone, and in patients with Waldenstrom's macroglobulinemia. S. Y. Kim et al. reported an association of MYD88 L265P mutation and the presence of 6q deletion.

Dong Soon Lee
Wee-Joo Chng
Kazuyuki Shimizu

Hindawi Publishing Corporation
BioMed Research International
Volume 2014, Article ID 510408, 8 pages
http://dx.doi.org/10.1155/2014/510408

Research Article

Establishment of Cell Lines from Both Myeloma Bone Marrow and Plasmacytoma: SNU_MM1393_BM and SNU_MM1393_SC from a Single Patient

Youngil Koh,[1] Woo-June Jung,[2] Kwang-Sung Ahn,[2,3] and Sung-Soo Yoon[1,2]

[1] *Department of Internal Medicine, Seoul National University Hospital, 101 Daehak-ro, Jongno-Ku, Seoul 110-744, Republic of Korea*
[2] *Cancer Research Institute, Seoul National University College of Medicine, 103 Daehak-ro, Jongno-Ku, Seoul 110-799, Republic of Korea*
[3] *Functional Genome Institute, PDXen Biosystem Inc., 437 Dongil-ro, Kwangjin-Ku, Seoul 143-901, Republic of Korea*

Correspondence should be addressed to Sung-Soo Yoon; ssysmc@snu.ac.kr

Received 15 April 2014; Accepted 30 June 2014; Published 12 August 2014

Academic Editor: Dong Soon Lee

Purpose. We tried to establish clinically relevant human myeloma cell lines that can contribute to the understanding of multiple myeloma (MM). *Materials and Methods.* Mononuclear cells obtained from MM patient's bone marrow were injected via tail vein in an NRG/SCID mouse. Fourteen weeks after the injection, tumor developed at subcutis of the mouse. The engraftment of MM cells into mouse bone marrow (BM) was also observed. We separated and cultured cells from subcutis and BM. *Results.* After the separation and culture of cells from subcutis and BM, we established two cell lines originating from a single patient (SNU_MM1393_BM and SNU_MM1393_SC). Karyotype of the two newly established MM cell lines showed tetraploidy which is different from the karyotype of the patient (diploidy) indicating clonal evolution. In contrast to SNU_MM1393_BM, cell proliferation of SNU_MM1393_SC was IL-6 independent. SNU_MM1393_BM and SNU_MM1393_SC showed high degree of resistance against bortezomib compared to U266 cell line. SNU_MM1393_BM had the greater lethality compared to SNU_MM1393_SC. *Conclusion.* Two cell lines harboring different site tropisms established from a single patient showed differences in cytokine response and lethality. Our newly established cell lines could be used as a tool to understand the biology of multiple myeloma.

1. Introduction

Multiple genetic and microenvironmental changes [1] lead to the transformation of postgerminal center B cells into malignant neoplasm. Multiple myeloma is a malignant B cell disorder characterized by proliferation of atypical plasma cells in bone marrow [2] with or without the presence of monoclonal immunoglobulin protein in serum and/or urine [3]. Over the past decades, several effective treatment strategies have been developed for multiple myeloma [2]. These include high-dose chemotherapy supported with autologous peripheral blood stem cells, proteasome inhibitors [4], and immunomodulatory drugs such as thalidomide and lenalidomide [5]. However, despite these advances, multiple myeloma is still thought to be an incurable disease. And researchers are vigorously on the way to understand the biology of myeloma in order to improve the clinical outcome of myeloma patients.

By the way, multiple myeloma has correlation with plasmacytoma, which is a mass of plasma cells found outside of bone marrow [6] that needs medical intervention with radiotherapy [7] or chemotherapy. While multiple myeloma frequently accompanies plasmacytoma at the time of diagnosis, plasmacytoma precedes multiple myeloma in some cases. The disease entity called primary extramedullary plasmacytoma exists in 4% of plasma cell tumors [8, 9], and approximately 40–50% of patients with solitary plasmacytoma will develop multiple myeloma [10]. Hence, plasmacytoma is an early form or an accompanying disease of myeloma, and the data regarding the "clinical behavior" of plasmacytoma are quite accumulated. However, not much is known about the cellular biology of plasmacytoma per se. For example, cell lines established from plasmacytoma are not abundant with less than 10 cell lines with evident plasmacytoma available at ATCC (https://www.atcc.org/).

Many clinicians are curious about the adequate treatment strategy of plasmacytoma [11]. And these clinical problems can be answered with the study focusing on plasmacytoma. Considering the tropism of plasmacytoma, cell clone in bone marrow in myeloma and a cell clone in plasmacytoma would have difference. We think focusing on that difference is a key factor for understanding plasmacytoma biology.

For this aspect, we present, in this study, the establishment of two human multiple myeloma cell lines, called SNU_MM1393_BM and SNU_MM1393_SC from a patient with aggressive multiple myeloma using an animal model. SNU_MM1393_BM cell line is derived from bone marrow of a mouse, and SNU_MM1393_SC is derived from a subcutaneous plasmacytoma. Here, we characterized phenotypic, genetic, and functional properties of these cell lines. Also, we further investigated the response to cytokines and chemotherapeutic agents of these cell lines.

2. Materials and Methods

2.1. Case History. In February 2012, a 63-year-old male patient visited Seoul National University Hospital for back pain and tingling sense on the trunk below nipple. He was diagnosed as multiple myeloma with spinal cord compression due to osseous plasmacytoma on the third thoracic vertebrae. His disease stage was 3 by Durie-Salmon staging and 2 by International Staging System. Karyotype of this patient was normal, but fluorescent in situ hybridization (FISH) revealed that the disease had trisomy 9, *RB1* deletion, trisomy 1q, *IgH* rearrangement, and *IgH* 3 copies. His myeloma cell secreted monoclonal protein of immunoglobulin A, kappa chain. He received radiotherapy for vertebral plasmacytoma. After radiotherapy, four cycles of thalidomide/dexamethasone chemotherapy were given to the patient, which yielded in partial response (PR). Stem cell collection was performed in preparation for autologous stem cell transplantation after PR to thalidomide/dexamethasone chemotherapy. We used bone marrow cells at the time of stem cell collection for this experiment. The karyotype was sustained to be normal by the time of this stem cell collection. He is still in PR after autologous stem cell transplantation with high-dose melphalan conditioning with progression free survival time of 8.3 months. He did not receive either bortezomib or panobinostat.

2.2. Cell Culture and Establishment of Cell Line. Bone marrow specimens were obtained from a patient diagnosed with multiple myeloma under a protocol approved by the Seoul National University Hospital Institutional Review Board. Mononuclear cells were separated by Ficoll-Hypaque density sedimentation. Eight-week-old NRG/SCID mice were injected intravenously via the dorsal tail vein (i.v.) with 1×10^6 mononuclear cells suspended in a total volume of 300-microliter PBS. After 14 weeks, bone marrow specimens were obtained from mice, and isolated bone marrow cells were cultured in RPMI-1640 medium (Gibco-BRL, Gaithersburg, MD, USA) supplemented with 10% heat-inactivated fetal bovine serum, penicillin (100 U/mL), and streptomycin (100 g/mL) (GIBCO, Grand Island, NY, USA). They were cultured in a highly humidified atmosphere of 5% CO_2 and 95% air at 37°C. The medium was exchanged every 3-4 days depending on the rate of cell growth.

2.3. Histopathology and Cytogenetic Analysis. Histological sections of bone marrow from NOD/SCID mice were prepared and stained with haematoxylin and eosin (H&E) using standard methods. Cell morphology was examined using light microscopy.

Metaphase chromosome spreads from peripheral blood and the cell line were prepared and G-banded according to standard procedures. The karyotype was described according to the International System for Human Cytogenetic Nomenclature (ISCN) (2009) [12].

2.4. Cell Proliferation Assay. Cell proliferation assay was performed using Cell Counting Kit-8 (Dojindo Laboratories, Kumamoto, Japan) according to the manufacturer's instructions. Means and standard deviations were generated from three independent experiments. Absorbance values were normalized to the values obtained from control group to determine the value for % of survival.

2.5. Western Blot Analysis. The cells were treated with indicated reagents for the indicated time periods, washed once in ice-cold phosphate buffered saline (PBS), and resuspended in lysis buffer (20 mM MOPS (pH 7.0), 2 mM EGTA, 5 mM EDTA, 30 mM sodium fluoride, 60 mM b-glycerophosphate (pH 7.2), 20 mM sodium pyrophosphate, 1 mM sodium, orthovanadate, 1% Triton X-100, 1 mM PMSF, aprotinin, leupeptin, and pepstatin 1 mg/mL). The protein concentration of lysate was measured, 30 μg of whole cell protein extracts was boiled for 5 min, and the proteins were resolved in 10% SDS-polyacrylamide gel electrophoresis and electrotransferred to polyvinylidene difluoride membranes (Millipore, Bedford, MA, USA). The membranes were blocked in Tris-buffered saline containing 0.05% Tween 20 and 5% nonfat dry milk for 1 h at room temperature and incubated with the appropriate primary antibody for 2 h. Immunoreactive proteins were detected using horseradish peroxidase-conjugated secondary antibody (Jackson ImmunoResearch Laboratories Inc., PA, USA) and enhanced chemiluminescence reagents (Amersham Pharmacia Biotech, Piscataway, NJ, USA).

2.6. FACS Analysis. Cells (1×10^6 cells/mL) were incubated with 20 μL of phycoerythrin-conjugated anti-CD138 and anti-CD45, respectively (Becton Dickinson, San Jose, CA, USA) for 30 min at 4°C, washed, and then fixed with 2% paraformaldehyde. Then, the samples were analyzed by FACSCalibur flow cytometer (Becton Dickinson) and inbuilt software.

2.7. Drug Response Evaluation. Cells were treated with various concentrations of bortezomib and panobinostat, respectively, for 72 hours. After the treatment, cell proliferation assay was performed to examine their growth inhibitory

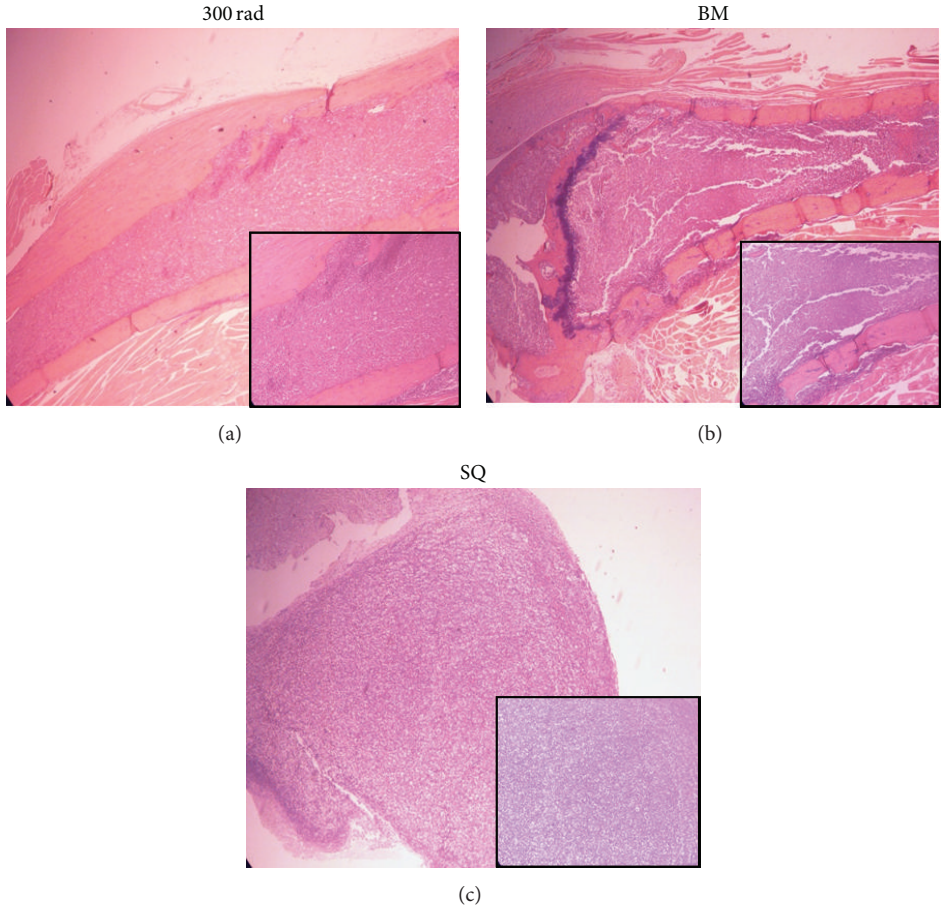


Figure 1: Representative appearances of histopathologic hematoxylin and eosin (H&E) staining of the irradiated bone marrow (a), bone marrow of the leg with cancer cell infiltration (b), and tumors at subcutis (c).

effects. As a control experiment, we used U266 cells and U266_SC cells.

2.8. Use of Commercially Available Cell Lines. We used commercially available cell lines which were bought from DSMZ (http://www.dsmz.de/) as a control experiment in our study. The used cell lines include U266, U266_SC, IM9, and RPMI8226 cell lines.

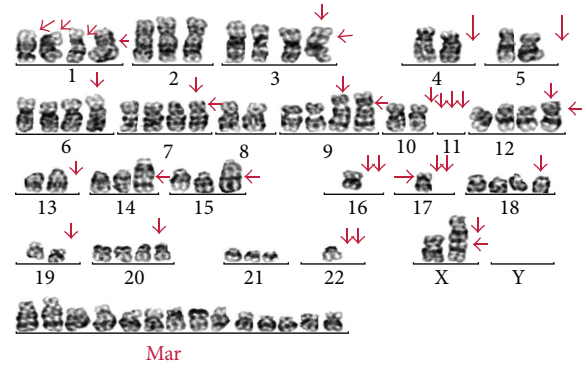
3. Results

3.1. Establishment and Cytogenetic Characterization of SNU_MM1393_BM Cell Line and SNU_MM1393_SC Cell Line. Fourteen weeks after the injection of patient's mononuclear cell to NRG/SCID mouse, we found that myeloma cell grew at a subcutis. We also observed myeloma cells engrafted into bone marrow (H&E stain) (Figure 1). Myeloma cells obtained from both sites had undergone *ex vivo* culture and those cells successfully grew in RPMI-1680 with 10X FBS. They have reached over 20 population doublings through culture crisis and have been growing rapidly compared to the established myeloma cell lines. Doubling time was approximately 48 hours. A karyotype of the patient at diagnosis was displayed as normal which was in a diploidy. Interestingly, when cytogenetic analysis was performed for the newly established cell lines, the karyotype of SNU_MM1393_BM and that of SNU_MM1393_SC were near-tetraploidy (Figure 2). Hence, karyotypes of newly established multiple myeloma cell lines were different from those of original patient at diagnosis. Moreover, there was a difference in karyotypes between SNU_MM1393_BM and SNU_MM1393_SC. Chromosome 13 loss was noted in both cell lines, but there was a difference in copy numbers. That is, while SNU_MM1393_BM had 2 copies loss in chromosome 13, SNU_MM1393_SC cell line had 3 copies loss. Also, differences were noted in chromosome 6 and chromosome 18.

As shown in Figure 3, the expression patterns of CD138 and CD45 by FACS analysis signified that both cell lines are of lymphoid origin. Also, light Giemsa stain revealed that the established cell lines have large, round nuclei, 1-2 nuclei/cell, and basophilic cytoplasm as previously mentioned [13].

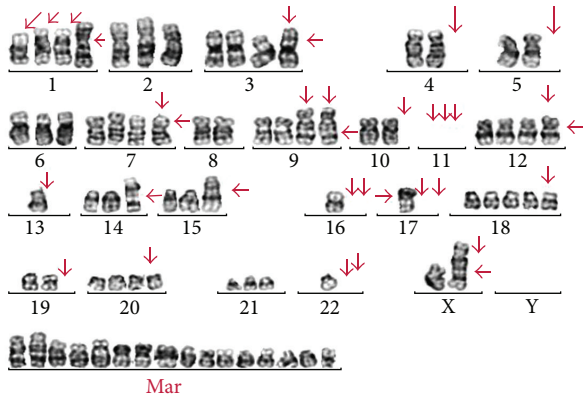
3.2. IL-6 Mediated Cell Signaling Pathway Was Differentially Regulated between the Two Cell Lines. When we evaluated the cytokine response in these cell lines, differential response was noted between the two cell lines. We used cytokine interleukin-6 (IL-6) and soluble IL-6 receptor (sIL-6R), which is well known to regulate the biologic behaviors of myeloma cells in the progression of multiple myeloma. It is well known that sIL-6R potentiates the IL-6 mediated

Karyotype: 74~76, X, der(X)t(X; 1)(q28; q21), add(1)(q11), der(1; 8)(q10; q10), der(1; 19)(q10; p10), +der(1; 19), add(3)(p26), −4, −5, +6, +add(7)(p15), der(9)t(9; 11)(q34; q13), +der(9)add(9)(p22)t(9; 11)(q34; q13), −10, −11, −11, −11, +add(12)(p13), −13, der(14)t(11; 14)(q13; q32), add(15)(q26), −16, −16, −17, −17, add(17)(p11.2), +18, +20, −22, −22, +14~16mar[cp10]



(a)

Karyotype: 74~76, X, der(X)t(X; 1)(q28; q21), add(1)(q11), der(1; 8)(q10; q10), der(1; 19)(q10; p10), +der(1; 19), add(3)(p26), −4, −5, +6, +add(7)(p15), der(9)t(9; 11)(q34; q13), +der(9)add(9)(p22)t(9; 11)(q34; q13), −10, −11, −11, −11, +add(12)(p13), −13, der(14)t(11; 14)(q13; q32), add(15)(q26), −16, −16, −17, −17, add(17)(p11.2), +18, +20, −22, −22, +14~16mar[cp10]



(b)

Figure 2: Karyotype of SNU_MM1393_BM (a) and SNU_MM1393_SC (b) cell line using G-banding.

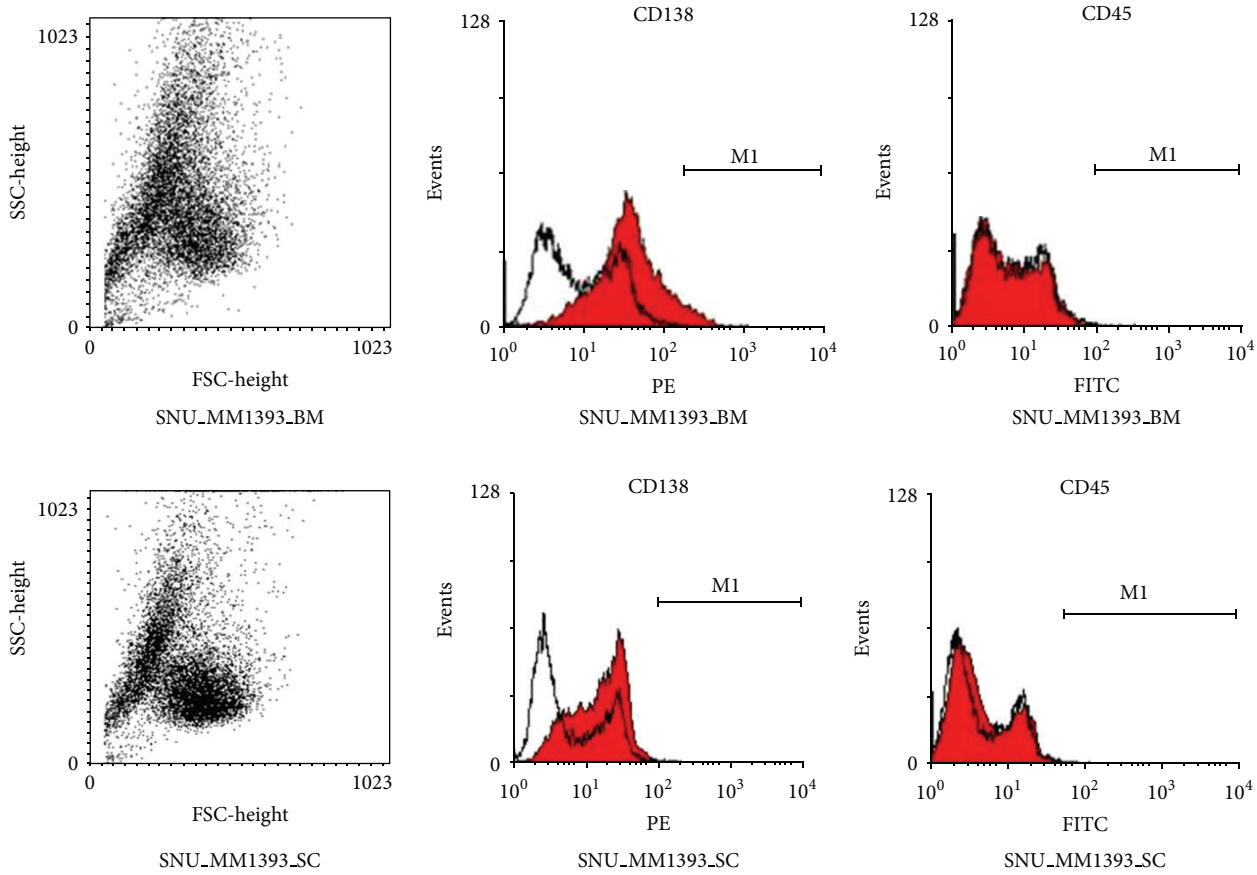


Figure 3: Flow cytometry analysis of SNU_MM1393_BM and SNU_MM1393_SC cell lines. Expression of CD138 and CD45 of both cell lines was analyzed using fluorescein-activated cell sorting (FACS).

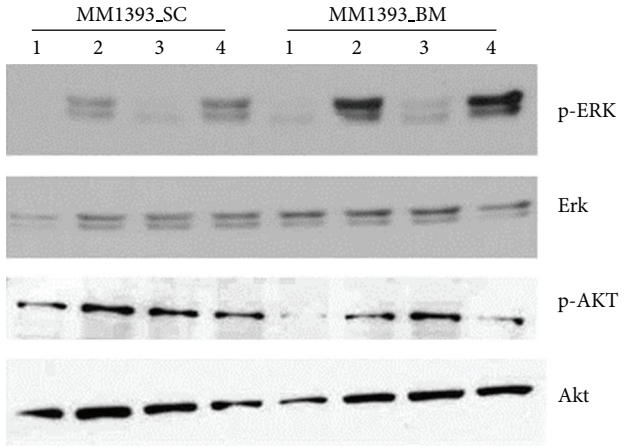


Figure 4: Western blot showing response to IL-6 in SNU_MM1393_BM and SNU_MM1393_SC. Whole cell lysates obtained from both cell lines were treated with IL-6, sIL-6R, and combined IL-6 and sIL-6R for 30 min (1: control, 2: IL-6 5 ng/mL, 3: sIL-6R 25 ng/mL, and 4: IL-6 5 ng/mL + sIL-6R 25 ng/mL).

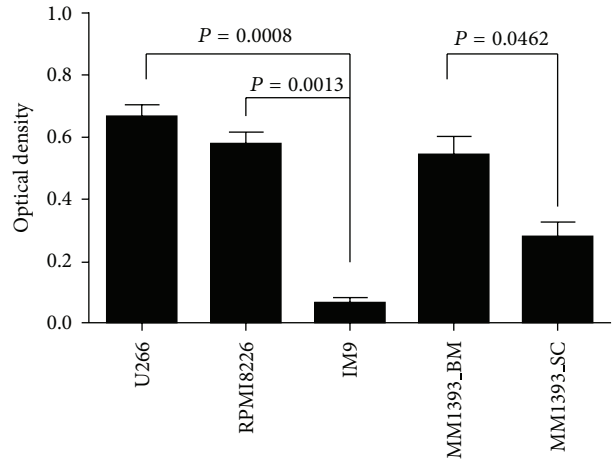


Figure 5: Production of IL-6 from SNU_MM1393_BM and SNU_MM1393_SC, U266, RPMI8226, and IM9.

signaling [14]. Based on these, we treated myeloma cells with IL-6 and sIL-6R together to determine the activation of IL-6 mediated signaling pathway. Incubation of multiple myeloma cells with 5 nM IL-6, 25 nM sIL-6R, and combined treatment of IL-6 and sIL-6R (5 nM and 25 nM) for 30 min, respectively, was performed.

In myeloma cells established from bone marrow of a mouse (SNU_MM1393_BM), phosphorylated form of Erk (p-ERK) was increased when treated with IL-6 only and combination of IL-6 and sIL-6R, but phosphorylated form of Akt (p-AKT) was not. On the other hand, in myeloma cells established from subcutaneous plasmacytoma (SNU_MM1393_SC), the induction of p-ERK was not as evident as in SNU_MM1393_BM. Increase in p-AKT was not evident in SNU_MM1393_SC either (Figure 4).

Since IL-6 acts in an autocrine manner [15], we measured the amount of IL-6 in culture soup of U266, RPMI8226, IM9, SNU_MM1393_BM, and SNU_MM1393_SC, respectively, after those cells were cultured with 1% FBS for 72 hours. Result of SNU_MM1393_BM was similar to that of U266 and RPMI8226, suggesting that it is an IL-6 dependent cell line. However, SNU_MM1393_SC and IM9 released only a small amount of IL-6 (Figure 5). And any changes in the amount of sIL-6R in both cell lines were not observed.

3.3. Drug Response of Newly Established Cell Lines. We tested the cytotoxic effect of proteasome inhibitor bortezomib and pan-HDAC inhibitor panobinostat in these two cell lines. Both SNU_MM1393_SC and SNU_MM1393_BM were more sensitive to panobinostat when compared to U266 ($P < 0.05$). Meanwhile, SNU_MM1393_BM seemed to be more sensitive to panobinostat compared to SNU_MM1393_SC ($P > 0.05$). However, when compared to well-known myeloma cell lines (U266 and U266_SC), SNU_MM1393_SC and SNU_MM1393_BM were more resistant to bortezomib (P value < 0.05) (Figure 6).

3.4. Determination of Tumorigenicity of the Established Two Cell Lines. To determine the tumorigenicity of these cells, we reinjected these *ex vivo* cultured cells via tail vein into NRG/SCID mouse. We used three kinds of cells: (1) *ex vivo* cultured myeloma cells of a patient's bone marrow, (2) cells from SNU_MM1393_BM, and (3) cells from SNU_MM1393_SC. When this was performed, we found tumor growth at both bone marrow and subcutis with *ex vivo* cultured myeloma cells. However, when cells from SNU_MM1393_BM were injected into a mouse, growth at subcutis was not noted. Only engraftment at bone marrow was observed. Lastly, when SNU_MM1393_SC cells were injected into a mouse, a mouse died in 7 weeks after injection. Tumor at subcutis was not noted at the time of death.

4. Discussion

It is known that the establishment of multiple myeloma cell line is notoriously difficult [16]. Here, we report two newly established cell lines from a single patient with multiple myeloma. Newly established cell lines secreted the kappa light chain, which was the similar to myeloma cells at diagnosis of our patient. Cell-surface markers of established cell lines exhibit positive expression of CD138, CD45, and CD34 as shown in multiple myeloma cells from a patient at diagnosis. Given these observations, we consider two newly established cell lines (SNU_MM1393_BM and SNU_MM1393_SC) that have originated from the donor patient's primary myeloma cells. Also, in a separate study, we performed whole exome sequencing of these two cell lines to reveal genetic difference between these two cell lines. In the study, genomic signature of these two cell lines was almost the same except for some single nucleotide variants and copy number variations, which implies that these two cell lines are from a single patient.

We observed several interesting findings during the establishment of these cell lines. First, there was a difference between patient's original myeloma cells and established cell lines. While patient's karyotype was a diploidy, karyotype of

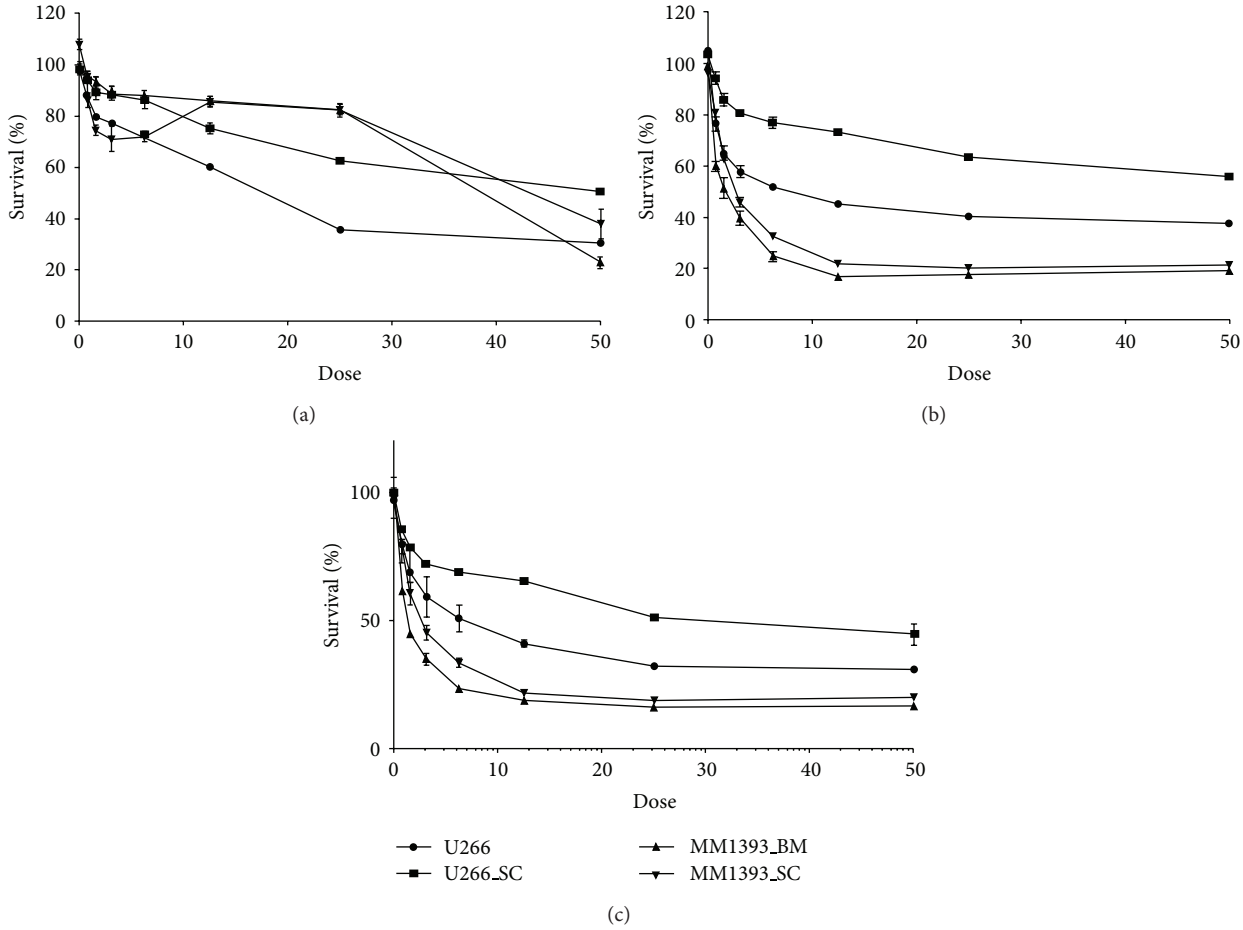


Figure 6: Growth inhibitory effect of bortezomib (a), panobinostat (b), and combination of bortezomib and panobinostat (c) in newly established MM cell lines. The data shows the mean ± standard error of three independent experiments.

SNU_MM1393 was tetraploidy. This reflects a clonal evolution of a myeloma cell during cell line establishment. In fact, there are reports regarding the clonal evolution to near-tetraploidy both *in vivo* and *ex vivo*. As an *in vivo* report, Yuan et al. [17] reported a case of female patient who became lenalidomide refractory after one year of treatment with lenalidomide/steroid. The patient experienced clonal evolution to near-tetraploidy with chromosome 13 loss. As an *ex vivo* data, Balsas et al. [18] reported that RPMI8226 cell line which became resistant to bortezomib after serial cultivation evolved to near-tetraploidy. Hence, we assume that clonal evolution to near-tetraploidy is related to drug resistance and aggressive tumor biology. We assume that change of a host from human to NRG/SCID mouse in our experiment would act as a tremendous stress to myeloma cell, and the cell from our patient would evolve to near-tetraploidy. In fact, tetraploidy is frequently observed in myeloma patients at the time of diagnosis [19]. In a cohort at Seoul National University Hospital which consists of 80 myeloma patients, tetraploidy at diagnosis was noted in 3 patients. One interesting report by Koren-Michowitz et al. is that 13q deletion and IgH abnormalities which have a prognostic value in multiple myeloma might have some correlation with near-tetraploidy [20]. We think that tumor biology associated with near-tetraploidy needs further research.

From the above, we conjecture that aggressive myeloma cells might have been selected through cell line establishment using animal model. And, as expected, SNU_MM1393_SC had greater lethality than original myeloma cells of the patient. Mouse injected with SNU_MM1393_SC died within 7 weeks, while mouse injected with the original myeloma cells survived more than 14 weeks. One interesting finding in this experiment (regarding the tumorigenicity of established cell line) is that SNU_MM1393_BM was not as lethal as SNU_MM1393_SC. This coincides with the results that SNU_MM1393_SC showed more prominent resistance to bortezomib and SNU_MM1393_SC had more chromosome 13 loss which is a well-known negative prognostic marker in multiple myeloma [21]. Various experiments using these two cell lines are underway in our laboratory, and detailed clinicopathologic information including pharmacologic profile of these cell lines will be reported soon.

Second, there were differences between SNU_MM1393_BM and SNU_MM1393_SC in various aspects (Table 1). In an

Table 1: Comparison of SNU_MM1393_BM and SNU_MM1393_SC cell lines.

	Chromosome 13 loss	IL-6 dependence	Bortezomib resistance	Panobinostat resistance	Plasmacytoma generation	Tumor lethality
Original tumor	None	Unknown	Unknown	Unknown	+	−
SNU_MM1393_BM	2 copies	+	+	−	−	−
SNU_MM1393_SC	3 copies	−	++	−	−	+

NA: not applicable.

experiment regarding response to cytokine, these differences were dramatic. Various cytokines, especially IL-6 and sIL-6R, directly affect the biologic behaviors of multiple myeloma cells [14, 22], and the response to IL-6 in myeloma cells is very important. Because IL-6 is known to act in an autocrine manner [15], the induction of IL-6 and sIL-6R needs to be analyzed in each myeloma cell line. And, when this was analyzed, degree of IL-6 induction was different between SNU_MM1393_BM and SNU_MM1393_SC, with low IL-6 induction level in SNU_MM1393_SC. The similar results were found in coculture analysis. These findings suggested that myeloma cells growing at soft tissue were not dependent on IL-6 mediated cell signaling. And we think that aggressive biology of SNU_MM1393_SC is related to cell growth potential independent of IL-6 signaling. However, this conjecture needs further research for verification.

Lastly, interesting findings were when drug response was investigated in these cell lines. Most importantly, newly established cell lines showed relative resistance to bortezomib compared to U266. This is an unexpected phenomenon, because the myeloma cell of our patient is bortezomib naïve. Considering relative indolent clinical course of our patient, it is unusual that a myeloma clone of our patient is resistant to bortezomib. When used with dexamethasone, response rate of bortezomib in treatment-naïve myeloma patients is 88%. Hence, we conjecture that this resistance to bortezomib shown in our newly established cell line would originate from clonal evolution during cell line establishment. We suggest that biologic behaviors of myeloma cells could be altered in the course of clonal expansion and this alteration would contribute to the chemotherapeutic resistance.

Whether the above findings could be generalized to myeloma cells in bone marrow and plasmacytoma is questionable. Not many researches have been performed regarding the comparison of bone marrow myeloma cells and plasmacytoma, and we think our study results should be recognized as exploratory ones. Comparison with future researches focusing on plasmacytoma biology is necessary to adapt our findings to clinical practices. We are also willing to supply our established cell lines to other researchers for further studies.

5. Conclusion

Two cell lines harboring different site tropisms established from a single patient showed differences in cytokine response. Our newly established cell lines could be used as a tool to understand the biology of multiple myeloma and its chemotherapeutic responses.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

Acknowledgments

This work was supported by the National Research Foundation of Korea (NRF) Grant funded by the Korean Government (MSIP) (no. 2011-0029703) and the Grant of Korea Health Technology R&D Project through the Korea Health Industry Development Institute (KHIDI), funded by the Ministry of Health & Welfare, Republic of Korea (Grant no. HI14C0072).

References

[1] K. Podar, D. Chauhan, and K. C. Anderson, "Bone marrow microenvironment and the identification of new targets for myeloma therapy," *Leukemia*, vol. 23, no. 1, pp. 10–24, 2009.

[2] M. A. Dimopoulos and E. Terpos, "Multiple myeloma," *Annals of Oncology*, vol. 21, no. 7, pp. vii143–vii150, 2010.

[3] A. Mahindra, T. Hideshima, and K. C. Anderson, "Multiple myeloma: biology of the disease," *Blood Reviews*, vol. 24, supplement 1, pp. S5–S11, 2010.

[4] P. Moreau, P. G. Richardson, M. Cavo et al., "Proteasome inhibitors in multiple myeloma: 10 years later," *Blood*, vol. 120, no. 5, pp. 947–959, 2012.

[5] A. Larocca and A. Palumbo, "Evolving paradigms in the treatment of newly diagnosed multiple myeloma," *JNCCN: Journal of the National Comprehensive Cancer Network*, vol. 9, no. 10, pp. 1186–1196, 2011.

[6] K. Bink, E. Haralambieva, M. Kremer et al., "Primary extramedullary plasmacytoma: similarities with and differences from multiple myeloma revealed by interphase cytogenetics," *Haematologica*, vol. 93, no. 4, pp. 623–626, 2008.

[7] M. A. Dimopoulos, J. Goldstein, L. Fuller, K. Delasalle, and R. Alexanian, "Curability of solitary bone plasmacytoma," *Journal of Clinical Oncology*, vol. 10, no. 4, pp. 587–590, 1992.

[8] P. Galieni, M. Cavo, A. Pulsoni et al., "Clinical outcome of extramedullary plasmacytoma," *Haematologica*, vol. 85, no. 1, pp. 47–51, 2000.

[9] C. Alexiou, R. J. Kau, H. Dietzfelbinger et al., "Extramedullary plasmacytoma: tumor occurrence and therapeutic concepts," *Cancer*, vol. 85, no. 11, pp. 2305–2314, 1999.

[10] R. Jyothirmayi, V. P. Gangadharan, M. K. Nair, and B. Rajan, "Radiotherapy in the treatment of solitary plasmacytoma," *British Journal of Radiology*, vol. 70, pp. 511–516, 1997.

[11] J. Bladé, C. Fernández de Larrea, L. Rosiñol, M. T. Cibeira, R. Jiménez, and R. Powles, "Soft-tissue plasmacytomas in multiple

myeloma: incidence, mechanisms of extramedullary spread, and treatment approach," *Journal of Clinical Oncology*, vol. 29, no. 28, pp. 3805–3812, 2011.

[12] L. G. Shaffer, M. L. Slovak, and L. J. Campbell, "International Standing Committee on Human Cytogenetic Nomenclature," in *Proceedings of the International System for Human Cytogenetic Nomenclature*, Basel, Switzerland, 2009.

[13] A. I. C. C. Baba, *Comparative Oncology*, The Publishing House of the Romanian Academy, Bucharest, Romania, 2007.

[14] V. M. Lauta, "A review of the cytokine network in multiple myeloma: diagnostic, prognostic, and therapeutic implications," *Cancer*, vol. 97, no. 10, pp. 2440–2452, 2003.

[15] U. H. Weidle, S. Klostermann, D. Eggle, and A. Krüger, "Interleukin 6/interleukin 6 receptor interaction and its role as a therapeutic target for treatment of cachexia and cancer," *Cancer Genomics and Proteomics*, vol. 7, no. 6, pp. 287–302, 2010.

[16] H. G. Drexler and Y. Matsuo, "Malignant hematopoietic cell lines: in vitro models for the study of multiple myeloma and plasma cell leukemia," *Leukemia Research*, vol. 24, no. 8, pp. 681–703, 2000.

[17] J. Yuan, R. Shah, A. Kulharya, and C. Ustun, "Near-tetraploidy clone can evolve from a hyperdiploidy clone and cause resistance to lenalidomide and bortezomib in a multiple myeloma patient," *Leukemia Research*, vol. 34, no. 7, pp. 954–957, 2010.

[18] P. Balsas, P. Galán-Malo, I. Marzo, and J. Naval, "Bortezomib resistance in a myeloma cell line is associated to PSMβ5 overexpression and polyploidy," *Leukemia Research*, vol. 36, no. 2, pp. 212–218, 2012.

[19] T. Nilsson, M. Höglund, S. Lenhoff et al., "A pooled analysis of karyotypic patterns, breakpoints and imbalances in 783 cytogenetically abnormal multiple myelomas reveals frequently involved chromosome segments as well as significant age- and sex-related differences," *British Journal of Haematology*, vol. 120, no. 6, pp. 960–969, 2003.

[20] M. Koren-Michowitz, I. Hardan, J. Berghoff et al., "Chromosome 13q deletion and IgH abnormalities may be both masked by near-tetraploidy in a high proportion of multiple myeloma patients: a combined morphology and I-FISH analysis," *Cancer Letters*, vol. 255, no. 2, pp. 307–314, 2007.

[21] A. K. Stewart and R. Fonseca, "Prognostic and therapeutic significance of myeloma genetics and gene expression profiling," *Journal of Clinical Oncology*, vol. 23, no. 26, pp. 6339–6344, 2005.

[22] K. Bommert, R. C. Bargou, and T. Stühmer, "Signalling and survival pathways in multiple myeloma," *European Journal of Cancer*, vol. 42, no. 11, pp. 1574–1580, 2006.

Hindawi Publishing Corporation
BioMed Research International
Volume 2014, Article ID 437852, 11 pages
http://dx.doi.org/10.1155/2014/437852

Research Article

Comparison of the Freiburg and Charlson Comorbidity Indices in Predicting Overall Survival in Elderly Patients with Newly Diagnosed Multiple Myeloma

Sung Min Kim, Moon Jin Kim, Hyun Ae Jung, Kihyun Kim, Seok Jin Kim, Jun Ho Jang, Won Seog Kim, and Chul Won Jung

Division of Hematology-Oncology, Department of Medicine, Samsung Medical Center, School of Medicine, Sungkyunkwan University, 81 Irwon-ro, Gangnam-gu, Seoul 135-710, Republic of Korea

Correspondence should be addressed to Kihyun Kim; kihyunkimk@gmail.com

Received 25 March 2014; Revised 29 May 2014; Accepted 18 June 2014; Published 10 July 2014

Academic Editor: Dong Soon Lee

Multiple myeloma occurs primarily in elderly patients. Considering the high prevalence of comorbidities, comorbidity is an important issue for the management of myeloma. However, the impact of comorbidity on clinical outcomes has not been fully investigated. We retrospectively analyzed patients with newly diagnosed myeloma. Comorbidities were assessed based on the Charlson comorbidity index (CCI) and the Freiburg comorbidity index (FCI). The CCI is a summary measure of 19 comorbid conditions. FCI is determined by performance status, renal impairment, and lung disease. This study included 127 patients with a median age of 71 years. Approximately half of the patients had additional disorders at the time of diagnosis, and diabetes mellitus was the most frequent diagnosis (18.9%). The most significant factors for prognosis among patient-related conditions were a history of solid cancer and performance status (ECOG ≥ 2). The FCI score was divided into 3 groups (0, 1, and 2-3), and the CCI score was divided into 2 groups (2-3 and ≥ 4). FCI was a strong prognostic tool for OS ($P > 0.001$) and predicted clinical outcome better than CCI ($P = 0.059$). In conclusion, FCI was more useful than CCI in predicting overall survival in elderly patients with myeloma.

1. Introduction

Multiple myeloma (MM) is a hematologic malignancy of plasma cells that results in bone destruction, marrow failure, and renal impairment. The median age at the time of diagnosis is 70 years, with 36% of patients younger than 65 years, 27% aged 65 to 74 years, and 37% older than 75 years [1]. Considering the increasing life expectancy of the general population, the number of geriatric patients affected by MM is expected to increase over time.

Risk stratification of myeloma using the international staging system (ISS) and host factors such as age, performance status, and comorbidities are thought to be important for determining prognosis and choosing treatment options [2–4]. In 2011, Palumbo et al. suggested that appropriate screening for age (>75 years) and vulnerability, in addition to assessment of cardiac, pulmonary, renal, hepatic, and neurological functions, at the start of therapy allows treatment strategies to be individualized and drug doses to be tailored to improve tolerability and optimize efficacy [5]. Their study emphasized that elderly MM patients are more susceptible to side effects and are often unable to tolerate full drug doses. For these patients, lower-dose intensity regimens improve the safety profile and thus optimize the treatment outcome. However, this study was not based specifically on MM because there were few data on the impact of vulnerability on outcomes in MM patients [5]. Nonetheless, in general, several studies have reported problems related to comorbidity and cancer treatment in elderly patients [6–10].

Kleber et al. developed the Freiburg comorbidity index (FCI) to assess patient-related conditions as a risk factor for MM. The FCI is composed of three comorbidity factors: renal impairment, moderate to severe lung disease, and performance status. Interestingly, the FCI showed strong clinical relevance for overall survival (OS) and progression-free survival (PFS). Moreover, compared with other comorbidity indices,

such as the Charlson comorbidity index (CCI), hematopoietic cell transplantation-specific comorbidity index (HCT-CI), Kaplan-Feinstein (KF), and Satariano index (SI), FCI was better able to stratify risk in patients with MM [11].

Although CCI is a widely used tool for assessing comorbidity in malignancy, this comorbidity index is complicated and difficult to apply. Moreover, there is no proven cut-off value that divides patients into low- or high-risk groups. As a result, several studies have determined their own cut-off values [11–16]. For example, Offidani et al. suggested a vulnerability score consisting of performance status and comorbidity score of CCI 0 or ≥1 [15].

Because of the increased incidence of multiple myeloma with aging and the fact that elderly patients have more comorbidity than younger patients, in the present study, we assessed comorbidities at diagnosis, the impact of host factors on OS, and compared CCI and FCI as prognostic factors in newly diagnosed elderly patients with MM.

2. Methods

2.1. Study Design. This study was a retrospective, single-center case series. OS was calculated as the time from diagnosis to death from any cause. Adverse events were graded according to the National Cancer Institute Common Toxicity Criteria (NCI-CTC) version 4.0. This study was reviewed and approved by the Institutional Review Board.

2.2. Patients. A total of 127 consecutive patients aged 65 years and older who were newly diagnosed with symptomatic MM at the Samsung Medical Center, Seoul, South Korea, between January 1, 1999, and June 30, 2011, met the inclusion criteria for this study. We excluded patients with amyloidosis, those who were suitable for autologous stem cell transplantation (ASCT), and those who were lost to follow-up within 6 months from the time of diagnosis due to any cause except death. The last follow-up date was March 31, 2013.

2.3. Charlson Comorbidity Index (CCI). The CCI (Table 1) is a summary measure of 19 comorbid conditions that are each weighted from 1 to 6 based on disease severity. This measure provides a total score ranging from 0 to 37 [17]. Information on comorbidity was extracted from a detailed review of each patient's medical records and laboratory values at the time of diagnosis. In the original study, MM was included in the classification of lymphoma for convenience. In this study, lymphoma was defined, as in the original study, except that the definition did not include myeloma.

In addition, according to the original study, each decade of age over 40 would add 1 point to the risk value (i.e., 50 years = +1 point), and the age point would be added to the score of the comorbidity index. In this study, we used the method described by Kleber et al., which adds the age point to the CCI score [11].

2.4. Freiburg Comorbidity Index (FCI). Renal impairment is defined as estimated glomerular filtration rate (eGFR) ≤30 mL/min/1.73 m^2, based on the modification of diet in renal disease (MDRD) study equation [18]. Poor performance status is based on a Karnofsky performance status (KPS) score ≤70. Moderate or severe lung disease is defined in the same manner as in the CCI [11]. Each of the variables contributes 1 point and the FCI is a summation of these points; thus, the FCI value ranges from 0 to 3.

2.5. Statistical Analysis. Numerical variables are summarized by median and range and categorical variables are described by count and relative frequency (%) of subjects in each category. Comparison of the distribution of categorical variables in the different groups was performed with either Fisher's exact test or the χ^2 test. OS was estimated using the Kaplan-Meier methodology. Uni- and multivariate Cox regression analyses were applied to assess factors affecting OS. Components with a P value less than 0.05 in univariate analysis were included in the subsequent multivariate analysis.

These analyses were performed using PASW statistics 18.0.0 (WinWrap, IBM, New York, USA). Null hypotheses of no difference were rejected if P values were less than 0.05.

3. Results

3.1. Patient Characteristics. During the study period, a total of 159 patients aged 65 years or older were newly diagnosed with symptomatic MM. Among them, 22 patients were excluded because of a combined diagnosis of amyloidosis, early follow-up loss, or ASCT. Therefore, data from 127 patients were included in the analysis.

Table 2 shows baseline characteristics of patients at the time of diagnosis. The median overall survival of all patients was 34.1 months, and the median follow-up duration for the surviving patients was 46 months. The median age of the patients was 71 years and 26.8% of the patients were aged 75 years or older. Performance status was evaluated by ECOG status. In FCI, performance status was assessed by KPS, and, by definition, ECOG grade 2 is interchangeable with KPS grade 70 [19–21].

The prevalence of comorbidity at the time of diagnosis was 48.8% (Table 3). The most frequent comorbid condition was diabetes without end organ damage (n = 24, 18.9%). The median CCI score, including age points, was 3 (range: 2 to 13). The CCI score was divided into two groups based on the median score; CCI scores of 2-3 were classified as the low CCI score group, and CCI scores ≥4 were classified as the high CCI score group. In this study, 53 and 74 patients belonged to the low and high CCI score groups, respectively. When using the FCI classification, 59, 54, and 12 patients belonged to the 0, 1, and 2-3 score groups, respectively.

3.2. Host Factors and Comorbidity Indices as Prognostic Factors. We analyzed the impact of host factors, such as age, sex, performance status, and each of the comorbidities on OS (Table 4). The results showed that all of the factors constituting FCI, such as performance status, chronic lung disease, and eGFR, were significant for OS. In addition, any tumor, metastatic solid tumor, cerebrovascular disease, and ISS each had statistical significance. When multivariate analysis was conducted with these factors, only performance

Table 1: Definitions of the Charlson and Freiburg comorbidity indices.

Weight	Condition	Definition
		Charlson comorbidity index
1	Myocardial infarct	Hospitalization and electrocardiographic and/or enzyme change
	Congestive heart failure	Exertional or paroxysmal nocturnal dyspnea and responded symptomatically (or on physical examination) to digitalis, diuretics, or afterload reducing agents
	Peripheral vascular disease	Intermittent claudication or prior bypass for arterial insufficiency; gangrene or acute arterial insufficiency; untreated thoracic or abdominal aneurysm (≥6 cm)
	Cerebrovascular disease	Cerebrovascular accident with minor or no residual and transient ischemic attacks
	Dementia	Chronic cognitive deficit
	Chronic pulmonary disease	*Moderate*: dyspneic with slight activity, with or without treatment, and dyspneic with moderate activity despite treatment; *Severe*: dyspneic at rest, despite treatment, requires constant oxygen; CO_2 retention and a baseline PO_2 below 50 torr
	Connective tissue disease	SLE, PM, MCTD, polymyalgia rheumatic, and moderate to severe RA
	Ulcer disease	Required treatment for ulcer disease, including bleeding from ulcers
	Mild liver disease	Cirrhosis without portal hypertension or chronic hepatitis
	Diabetes	*Mild*: treated with insulin or oral hypoglycemics, but not with diet alone. *Moderate*: previous hospitalizations for ketoacidosis, hyperosmolar coma, or/and those with juvenile onset or brittle diabetics
2	Hemiplegia	Dense hemiplegia or paraplegia, as a result of either a cerebrovascular accident or other conditions
	Moderate or severe renal disease	*Severe*: on dialysis, had a transplant, and with uremia. *Moderate*: serum creatinine > 3 mg%
	Diabetes with end organ damage	*Severe*: with retinopathy, neuropathy, or nephropathy
	Any tumor	Solid tumors without documented metastases, but initially treated in the last 5 years
	Leukemia	AML, CML, ALL, CLL, and PV
	Lymphoma	HD, lymphosarcoma, WM, myeloma, and other lymphomas
3	Moderate or severe liver disease	*Severe*: cirrhosis, portal hypertension, and a history of variceal bleeding. *Moderate*: cirrhosis with portal hypertension, but without history of variceal bleeding
6	Metastatic solid tumor	Metastatic solid tumors
	AIDS	Define or probable AIDS (i.e., AIDS related complex)
		Freiburg comorbidity index
1	Renal impairment	$eGFR_{MDRD} \leq 30$ mL/min/1.73 m^2
	Performance status	Karnofsky performance status (KPS) score ≤ 70
	Moderate or severe lung disease	Same as CCI

Abbreviations: SLE, systemic lupus erythematous; PM, polymyositis; MCTD, mixed connective tissue disease; RA, rheumatoid arthritis; AML, acute myelogenous leukemia; CML, chronic myelogenous leukemia; ALL, acute lymphocytic leukemia; CLL, acute lymphocytic leukemia; PV, polycythemia vera; HD, Hodgkin disease; WM, Waldenstrom's macroglobulinemia; AIDS, acquired immune deficiency syndrome; eGFR, estimated glomerular filtration rate; MDRD, modification of diet in renal disease; CCI, Charlson comorbidity index.

status and previous cancer history (regardless of metastasis) remained significant.

Figures 1, 2, 3, and 4 show Kaplan-Meier survival curves for both comorbidity indices. FCI effectively predicted the OS of the three different groups ($P < 0.001$). The median survival times were 55.0 months, 29.5 months, and 19.5 months for FCI scores of 0, 1, and 2-3, respectively. Although the P value was not statistically significant, CCI also distinguished between the two score groups for OS (44.8 months versus 34.7 months, $P = 0.059$). However, OS based on CCI without age points did not demonstrate clinical relevance ($P = 0.147$). In contrast, FCI was significant in subgroup analysis for age groups (65–74 and ≥75 years; $P < 0.001$ and 0.04, resp.)

3.3. Serious Adverse Events. We defined serious adverse events as grade ≥4 for hematologic adverse events and grade ≥3 for nonhematologic adverse events, according to NCI-CTC version 4.0. The most frequent serious adverse event was infection ($n = 35$, 30.0%) followed by neutropenia and anemia ($n = 15$, 12.7% for both). Grade ≥3 nonhematologic adverse events occurred in 50% of patients, whereas grade ≥4 hematologic adverse events occurred in 22.9% of patients.

Table 2: Baseline characteristics (n = 127).

Characteristics	N (%)	Median (range)
Age, years		71 (65–92)
65–74	93 (73.2%)	
≥75	34 (26.8%)	
Sex		
Male	62 (48.8%)	
Female	65 (51.2%)	
ECOG		
0-1	74 (58.3%)	
≥2	52 (40.9%)	
Unknown	1 (0.8%)	
Durie-Salmon stage		
1	10 (7.9%)	
2	28 (22.0%)	
3	89 (70.1%)	
International staging system		
1	23 (18.1%)	
2	51 (40.2%)	
3	47 (37.0%)	
Unknown	6 (4.7%)	
Lytic bone lesion		
Yes	112 (88.2%)	
No	14 (11.0%)	
Hemoglobin (g/dL)		9.66 (5.20–16.30)
<10	75 (59.1%)	
≥10	52 (40.9%)	
Platelets ($\times 10^9$/L)		195 (44–484)
<100	11 (8.7%)	
≥100	117 (91.3%)	
Plasma cells in bone marrow (%)		43.88 (1.10–100)
≥40	58 (45.7%)	
<40	65 (51.2%)	
Serum calcium (mg/dL)		9.35 (7.00–15.70)
>11.5	11 (8.7%)	
≤11.5	115 (90.6%)	
Serum albumin (mg/dL)		3.28 (1.70–4.80)
≤3.5	85 (66.9%)	
>3.5	42 (33.1%)	
eGFR (mL/min/1.73 m^2)		66.60 (5.90–170.5)
>30	112 (88.2%)	
≤30	15 (11.8%)	
Serum LD		
>UNL	27 (21.3%)	
≤UNL	74 (58.3%)	
Initial chemotherapy regimen		
Conventional (CP, MP, and others)	92 (78.0%)	
Novel agents (imid, bortezomib-based)	26 (22.0%)	
Treatment		
Chemotherapy	118 (92.9%)	
No chemotherapy	9 (7.1%)	

ECOG, eastern cooperative oncology group; eGFR, estimated glomerular filtration rate; LD, lactate dehydrogenase; UNL, upper normal limit; CP, cyclophosphamide and prednisolone; MP, melphalan and prednisolone.

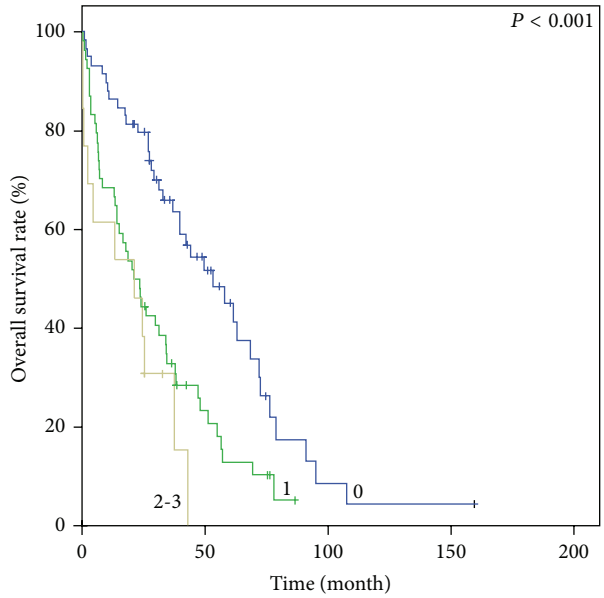


Figure 1: Kaplan-Meier survival curves of comorbidity index score groups. OS according to FCI.

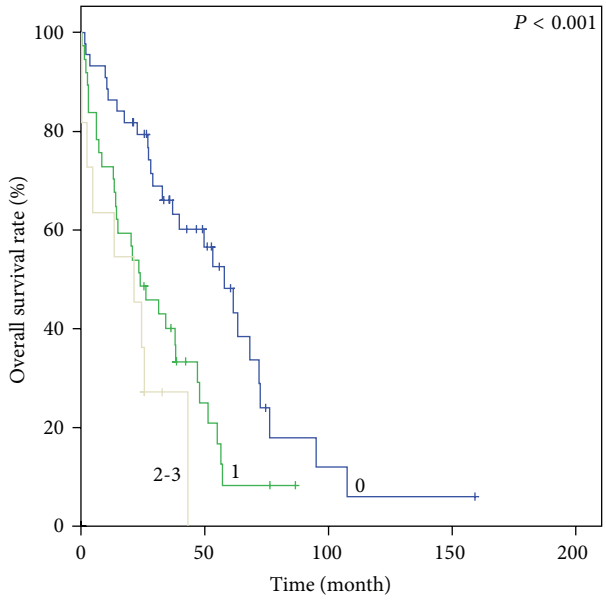


Figure 2: Kaplan-Meier survival curves of comorbidity index score groups. OS according to FCI in patients aged 65–74 years.

Grade 5 adverse events due to any cause occurred in 6.8% of the patients, as shown in Table 5.

4. Discussion

This study assessed comorbidities at diagnosis of MM, the impact of host factors on overall survival, and compared CCI and FCI as prognostic factors in newly diagnosed elderly patients.

Univariate analysis revealed that performance status, ISS, and several comorbid conditions such as chronic lung disease, azotemia (eGFR < 30 mL/min/1.73 m^2), presence of any tumor, metastatic solid tumor, and cerebrovascular disease were significant factors. However, azotemia as defined by CCI (serum creatinine ≥ 3 mg/mL) was not a prognostic factor. In multivariate analysis, azotemia, as defined by impaired eGFR or chronic lung disease, was not shown to

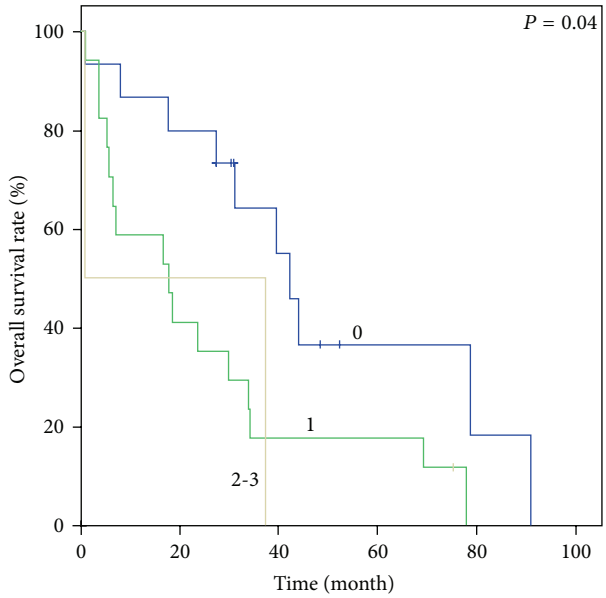


Figure 3: Kaplan-Meier survival curves of comorbidity index score groups. OS according to FCI in patients aged ≥75 years.

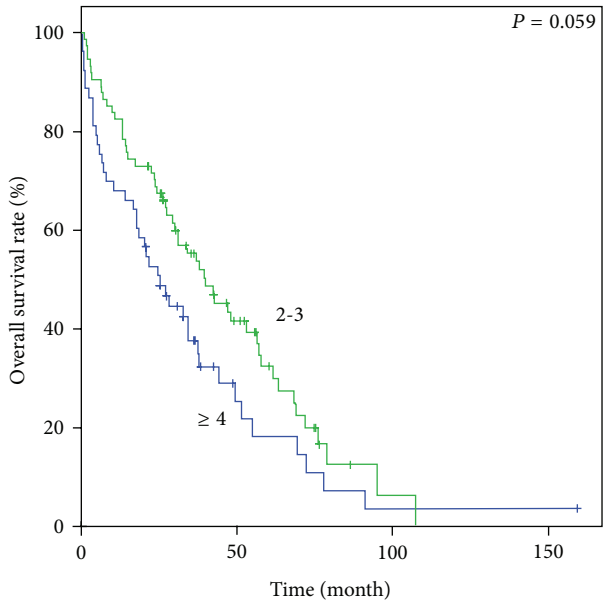


Figure 4: Kaplan-Meier survival curves of comorbidity index score groups. OS according to CCI.

be a significant risk factor in our study. In contrast, a history of cancer, regardless of whether metastasis occurred, was the strongest prognostic factor for elderly patients with myeloma. Unfortunately, use of novel agents over conventional drugs did not significantly improve OS, although this might reflect the relatively short period of use of novel agents.

Although two components of FCI-renal impairment and moderate or severe lung disease failed to demonstrate significance in multivariate analysis, when we compared both comorbidity indices and overall survival, the FCI showed a greater ability to separate OS among the three score groups ($P < 0.001$). The CCI score including age points was not statistically significant but was still valuable and superior to the CCI without age points. As briefly mentioned above, the CCI score without age points did not discriminate for OS.

FCI provides a clear definition of each component and all three components were statistically significant, at least in univariate analysis. In contrast, CCI is more subjective and

Table 3: Prevalence of comorbidities according to the Charlson and Freiburg comorbidity indices and patient distribution according to comorbidity indices.

(a) Prevalence of comorbidities according to the Charlson comorbidity index.

Comorbidity	Yes	No
Myocardial infarct	7 (5.5%)	120 (94.5%)
Congestive heart failure	7 (5.5%)	120 (94.5%)
Peripheral vascular disease	0 (0%)	127 (100%)
Cerebrovascular disease	6 (4.7%)	121 (95.3%)
Dementia	0 (0%)	127 (100%)
Chronic lung disease	14 (11.0%)	113 (89.0%)
Connective tissue disease	2 (1.6%)	125 (98.4%)
Ulcer disease	8 (6.3%)	119 (93.7%)
Mild liver disease	2 (1.6%)	125 (98.4%)
DM	24 (18.9%)	103 (81.1%)
Hemiplegia	6 (4.7%)	121 (95.3%)
Moderate to severe renal disease	1 (0.8%)	126 (99.2%)
DM with end organ damage	0 (0%)	127 (100%)
Any tumor	8 (6.3%)	119 (93.7%)
Leukemia	0 (0%)	127 (100%)
Lymphoma	0 (0%)	127 (100%)
Moderate to severe liver disease	2 (1.6%)	125 (98.4%)
Metastatic solid tumor	2 (1.6%)	125 (98.4%)
AIDS	0 (0%)	127 (100%)

DM, diabetes mellitus; AIDS, acquired immune deficiency syndrome.

(b) Prevalence of comorbidities according to the Freiburg comorbidity index.

Component	Yes	No
Renal impairment (eGFR $\leq$ 30 mL/min/1.73 m^2)	15 (11.8%)	112 (88.2%)
Performance status (KPS $\leq$ 70)	52 (40.9%)	74 (58.3%)
Moderate or severe lung disease	14 (11.0%)	113 (89.0%)

eGFR, estimated glomerular filtration rate; KPS, Karnofsky performance status.

(c) Patient distribution according to comorbidity indices.

(I) CCI total (comorbidity scores without age points)	
0	65 (51.2%)
1	37 (29.1%)
2	12 (9.4%)
3	5 (3.9%)
4	6 (4.7%)
6	1 (0.8%)
8	1 (0.8%)
(II) CCI total (comorbidity scores with age points)	
2	25 (19.7%)
3	49 (38.6%)
4	31 (24.4%)
5	9 (7.1%)
6	5 (3.9%)
7	6 (4.7%)
9	1 (0.8%)
13	1 (0.8%)
(III) CCI score group (with age points)	
Low (2-3)	53 (41.7%)
High ($\geq$4)	74 (58.3%)
(IV) FCI	
0	59 (46.5%)
1	54 (425%)
2	12 (9.4%)
3	1 (0.8%)
Unknown	1 (0.8%)

CCI, Charlson comorbidity index; FCI, Freiburg comorbidity index.

only 4 among 19 conditions were significant. Most importantly, at the present time, CCI does not have any standard cut-off value. Various studies have divided CCI scores into groups of 0, 1-2, and $\geq$3; 0, 1, and $\geq$2; or 0 and $\geq$1. Some studies included an age point, but others did not [11–14]. These variations might explain why FCI is more predictable than CCI.

Moreover, FCI is also very simple to apply. FCI consists of performance status, moderate or severe lung disease, and azotemia, and each of these factors is worth 1 point. The FCI score is, therefore, a simple summation of these three factors. In contrast, CCI consists of 19 comorbid conditions, and, within the same disease, scores are weighted based on severity ranging from 1 to 6 points. In addition, an age point is calculated and added to the CCI score. Comorbidity definitions frequently use a symptomatic grade.

In this study, all 118 patients who were treated with chemotherapy received a full dose of chemotherapeutic agents as scheduled. Interestingly, the profile for serious adverse events showed that treatment was relatively safe and adverse events were easily controllable. In fact, since the approval of various novel agents, clinical outcomes such as survival and toxicity profiles have improved in transplant-ineligible elderly patients with multiple myeloma [22–27]. Thus, we propose that the full dose of chemotherapy might be tolerated, regardless of the presence of comorbid conditions, even though this is against the recommendation that chemotherapy dose reduction is required for patients 75 years or older or those with cardiac, pulmonary, hepatic, renal, or neurologic dysfunctions [5]. Furthermore, this finding might be important evidence for preventing chemotherapy dose reduction because of physician bias.

There are some limitations in this study. First, the follow-up duration was short and the sample size was small. Second, this is a retrospective single center study. Third, there were no patients with peripheral vascular disease, dementia, DM with end organ damage, or AIDS. Despite these limitations, this study successfully applied the FCI and the CCI to newly

Table 4: Univariate and multivariate Cox's regression analysis for overall survival.

	Univariate analysis			Multivariate analysis		
	P value	HR	95% CI	*P* value	HR	95% CI
ECOG	0.002	1.951	1.287–2.958	0.009	1.890	1.176–3.038
0-1						
≥2						
Chronic lung disease	0.028	1.941	1.073–3.510	0.300	1.425	0.730–2.778
Yes						
No						
eGFR (mL/min/1.73 m^2)	0.012	2.139	1.183–3.869	0.228	1.515	0.771–2.976
>30						
≤30						
Any tumor	0.001	3.513	1.678–7.356	0.003	3.717	1.617–8.554
Yes						
No						
Metastatic solid tumor	<0.001	44.034	8.449–229.485	<0.001	85.847	14.628–503.822
Yes						
No						
Cerebrovascular disease	0.016	3.064	1.228–7.641	0.210	1.855	0.706–4.887
Yes						
No						
International staging system	0.024			0.250		
1						
2						
3						
Age, years	0.339	1.242	0.796–1.938			
65–74						
≥75						
Sex	0.692	0.920	0.610–1.388			
Male						
Female						
Durie-Salmon stage	0.242					
1						
2						
3						
Myocardial infarct	0.146	1.783	0.818–3.884			
Yes						
No						
Congestive heart failure	0.581	1.329	0.484–3.644			
Yes						
No						
Connective tissue disease	0.302	0.047	0.000–15.543			
Yes						
No						
Ulcer disease	0.196	0.571	0.244–1.334			
Yes						
No						
Mild liver disease	0.262	2.246	0.545–9.249			
Yes						
No						
DM	0.256	0.718	0.405–1.272			

Table 4: Continued.

	Univariate analysis			Multivariate analysis		
	P value	HR	95% CI	*P* value	HR	95% CI
Yes						
No						
Hemiplegia	0.081	0.353	0.110–1.135			
Yes						
No						
Moderate to severe renal disease	1.000	1.000	0.000–4.271*E*9			
Yes						
No						
Moderate-severe liver disease	0.302	2.100	0.514–8.583			
Yes						
No						
Initial chemotherapy regimen	0.844	1.058	0.603–1.858			
Conventional agents						
Novel agent						
CCI	0.061	0.677	0.450–1.018			
2-3						
≥4						
FCI	<0.001					
0						
1						
2-3						

HR, hazard ratio; CI, confidence interval; ECOG, eastern cooperative oncology group; eGFR, estimated glomerular filtration rate; DM, diabetes mellitus; CCI, Charlson comorbidity index; FCI, Freiburg comorbidity index.

Table 5: Serious adverse events (AEs).

	N (%)	
	Grade 0–3	Grade 4-5
Hematologic AE		
Anemia	103 (87.3%)	15 (12.7%)
Neutropenia	103 (87.3%)	15 (12.7%)
Thrombocytopenia	114 (92.2%)	4 (7.8%)
Febrile neutropenia	113 (90.8%)	5 (9.2%)
	Grade 0–2	Grade 3–5
Nonhematologic AE		
Infection	83 (70.0%)	35 (30.0%)
Diarrhea/constipation	105 (89.0%)	13 (11.0%)
Fatigue	117 (90.7%)	11 (9.3%)
Sensory neuropathy	108 (91.5%)	10 (8.5%)
Nausea/vomiting	111 (94.1%)	7 (5.9%)
Azotemia	112 (94.9%)	6 (5.1%)
Grade ≥4 hematologic AE	27 (22.9%)	
Grade ≥3 nonhematologic AE	59 (50.0%)	
Grade 5 AE	8 (6.8%)	

diagnosed elderly multiple myeloma patients and revealed the superiority of FCI to CCI in predicting OS.

5. Conclusions

In this study, approximately 50% of elderly patients with newly diagnosed multiple myeloma had at least one comorbid disease at the time of diagnosis. Among host factors tested, performance status and a history of malignancy were the most important prognostic factors. The Freiburg comorbidity index is very simple to use and predicts overall survival better than the Charlson comorbidity index.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

References

[1] S. Y. Kristinsson, O. Landgren, P. W. Dickman, Å. R. Derolf, and M. Björkholm, "Patterns of survival in multiple myeloma: a population-based study of patients diagnosed in Sweden from 1973 to 2003," *Journal of Clinical Oncology*, vol. 25, no. 15, pp. 1993–1999, 2007.

[2] M. Kleber, G. Ihorst, B. Deschler et al., "Detection of renal impairment as one specific comorbidity factor in multiple myeloma: multicenter study in 198 consecutive patients," *European Journal of Haematology*, vol. 83, no. 6, pp. 519–527, 2009.

[3] M. Kleber, G. Ihorst, J. Udi, B. Koch, R. Wsch, and M. Engelhardt, "Prognostic risk factor evaluation in patients with relapsed or refractory multiple myeloma receiving lenalidomide treatment: analysis of renal function by eGFR and of additional comorbidities by comorbidity appraisal," *Clinical Lymphoma, Myeloma & Leukemia*, vol. 12, no. 1, pp. 38–48, 2012.

[4] E. Eleftherakis-Papapiakovou, E. Kastritis, M. Roussou et al., "Renal impairment is not an independent adverse prognostic factor in patients with multiple myeloma treated upfront with novel agent-based regimens," *Leukemia & lymphoma*, vol. 52, no. 12, pp. 2299–2303, 2011.

[5] A. Palumbo, S. Bringhen, H. Ludwig et al., "Personalized therapy in multiple myeloma according to patient age and vulnerability: a report of the European Myeloma Network (EMN)," *Blood*, vol. 118, no. 17, pp. 4519–4529, 2011.

[6] A. Nanda, M. Chen, M. H. Braccioforte, B. J. Moran, and A. V. D'Amico, "Hormonal therapy use for prostate cancer and mortality in men with coronary artery disease-induced congestive heart failure or myocardial infarction," *The Journal of the American Medical Association*, vol. 302, no. 8, pp. 866–873, 2009.

[7] J. A. Meyerhardt, P. J. Catalano, D. G. Haller et al., "Impact of diabetes mellitus on outcomes in patients with colon cancer," *Journal of Clinical Oncology*, vol. 21, no. 3, pp. 433–440, 2003.

[8] P. J. Goodwin, M. Ennis, K. I. Pritchard et al., "Fasting insulin and outcome in early-stage breast cancer: results of a prospective cohort study," *Journal of Clinical Oncology*, vol. 20, no. 1, pp. 42–51, 2002.

[9] G. R. Simon, M. Extermann, A. Chiappori et al., "Phase 2 trial of docetaxel and gefitinib in the first-line treatment of patients with advanced nonsmall-cell lung cancer (NSCLC) who are 70 years of age or older," *Cancer*, vol. 112, no. 9, pp. 2021–2029, 2008.

[10] F. Gay and A. Palumbo, "Management of older patients with multiple myeloma," *Blood Reviews*, vol. 25, no. 2, pp. 65–73, 2011.

[11] M. Kleber, G. Ihorst, M. Terhorst et al., "Comorbidity as a prognostic variable in multiple myeloma: comparative evaluation of common comorbidity scores and use of a novel MM-comorbidity score," *Blood Cancer Journal*, vol. 1, no. 9, article e35, 2011.

[12] T. S. Field, J. L. F. Bosco, M. N. Prout et al., "Age, comorbidity, and breast cancer severity: impact on receipt of definitive local therapy and rate of recurrence among older women with early-stage breast cancer," *Journal of the American College of Surgeons*, vol. 213, no. 6, pp. 757–765, 2011.

[13] M. L. Sorror, M. B. Maris, B. Storer et al., "Comparing morbidity and mortality of HLA-matched unrelated donor hematopoietic cell transplantation after nonmyeloablative and myeloablative conditioning: influence of pretransplantation comorbidities," *Blood*, vol. 104, no. 4, pp. 961–968, 2004.

[14] L. Labonté, T. Iqbal, M. A. Zaidi et al., "Utility of comorbidity assessment in predicting transplantation-related toxicity following autologous hematopoietic stem cell transplantation for multiple myeloma," *Biology of Blood and Marrow Transplantation*, vol. 14, no. 9, pp. 1039–1044, 2008.

[15] M. Offidani, L. Corvatta, C. Polloni et al., "Assessment of vulnerability measures and their effect on survival in a real-life population of multiple myeloma patients registered at marche region multiple myeloma registry," *Clinical Lymphoma, Myeloma and Leukemia*, vol. 12, no. 6, pp. 423–432, 2012.

[16] W. R. Sperr, F. Wimazal, M. Kundi et al., "Comorbidity as prognostic variable in MDS: comparative evaluation of the HCT-CI and CCI in a core dataset of 419 patients of the Austrian MDS study group," *Annals of Oncology*, vol. 21, no. 1, Article ID mdp258, pp. 114–119, 2010.

[17] M. E. Charlson, P. Pompei, K. A. Ales, and C. R. MacKenzie, "A new method of classifying prognostic comorbidity in longitudinal studies: development and validation," *Journal of Chronic Diseases*, vol. 40, no. 5, pp. 373–383, 1987.

[18] A. S. Levey, L. A. Stevens, C. H. Schmid et al., "A new equation to estimate glomerular filtration rate," *Annals of Internal Medicine*, vol. 150, no. 9, pp. 604–612, 2009.

[19] I. de Kock, M. Mirhosseini, F. Lau et al., "Conversion of Karnofsky Performance Status (KPS) and Eastern Cooperative Oncology Group Performance Status (ECOG) to Palliative Performance Scale (PPS), and the interchangeability of PPS and KPS in prognostic tools," *Journal of Palliative Care*, vol. 29, no. 3, pp. 163–169, 2013.

[20] C. Ma, S. Bandukwala, D. Burman et al., "Interconversion of three measures of performance status: an empirical analysis," *European Journal of Cancer*, vol. 46, no. 18, pp. 3175–3183, 2010.

[21] M. M. Oken, R. H. Creech, and D. C. Tormey, "Toxicology and response criteria of the Eastern Cooperative Oncology Group," *The American Journal of Clinical Oncology: Cancer Clinical Trials*, vol. 5, no. 6, pp. 649–655, 1982.

[22] S. A. Ataergin, T. Kindwall-Keller, N. A. Berger, and H. M. Lazarus, "New generation pharmacotherapy in elderly multiple myeloma patients," *Expert Opinion on Pharmacotherapy*, vol. 10, no. 1, pp. 81–98, 2009.

[23] A. Palumbo, F. Davies, M. Kropff et al., "Consensus guidelines for the optimal management of adverse events in newly diagnosed, transplant-ineligible patients receiving melphalan and prednisone in combination with thalidomide (MPT) for the treatment of multiple myeloma," *Annals of Hematology*, vol. 89, no. 8, pp. 803–811, 2010.

[24] A. A. Chanan-Khan, S. Lonial, D. Weber et al., "Lenalidomide in combination with dexamethasone improves survival and time-to-progression in patients ≥65 years old with relapsed or refractory multiple myeloma," *International Journal of Hematology*, vol. 96, no. 2, pp. 254–262, 2012.

[25] J.-L. Harousseau and M. Dreyling, "Multiple myeloma: ESMO clinical practice guidelines for diagnosis, treatment and follow-up," *Annals of Oncology*, vol. 21, supplement 5, pp. v155–v157, 2010.

[26] A. Palumbo, M. Attal, and M. Roussel, "Shifts in the therapeutic paradigm for patients newly diagnosed with multiple myeloma: Maintenance therapy and overall survival," *Clinical Cancer Research*, vol. 17, no. 6, pp. 1253–1263, 2011.

[27] G. Barosi, G. Merlini, A. Billio et al., "SIE, SIES, GITMO evidence-based guidelines on novel agents (thalidomide, bortezomib, and lenalidomide) in the treatment of multiple myeloma," *Annals of Hematology*, vol. 91, no. 6, pp. 875–888, 2012.

Hindawi Publishing Corporation
BioMed Research International
Volume 2014, Article ID 232546, 12 pages
http://dx.doi.org/10.1155/2014/232546

Review Article

Implications of Heterogeneity in Multiple Myeloma

Sanjay de Mel,[1] Su Hong Lim,[1] Moon Ley Tung,[1] and Wee-Joo Chng[1,2,3]

[1] *Department of Haematology-Oncology, National University Cancer Institute of Singapore, Singapore*
[2] *Cancer Science Institute of Singapore, National University of Singapore, Singapore*
[3] *National University Health System, NUHS Tower Block, Level 7, 1E Lower Kent Ridge Road, Singapore 119228*

Correspondence should be addressed to Wee-Joo Chng; wee_joo_chng@nuhs.edu.sg

Received 27 March 2014; Accepted 2 May 2014; Published 2 July 2014

Academic Editor: Dong Soon Lee

Multiple myeloma is the second most common hematologic malignancy in the world. Despite improvement in outcome, the disease is still incurable for most patients. However, not all myeloma are the same. With the same treatment, some patients can have very long survival whereas others can have very short survival. This suggests that there is underlying heterogeneity in myeloma. Studies over the years have revealed multiple layers of heterogeneity. First, clinical parameters such as age and tumor burden could significantly affect outcome. At the genetic level, there are also significant heterogeneity ranging for chromosome numbers, genetic translocations, and genetic mutations. At the clonal level, there appears to be significant clonal heterogeneity with multiple clones coexisting in the same patient. At the cell differentiation level, there appears to be a hierarchy of clonally related cells that have different clonogenic potential and sensitivity to therapies. These levels of complexities present challenges in terms of treatment and prognostication as well as monitoring of treatment. However, if we can clearly delineate and dissect this heterogeneity, we may also be presented with unique opportunities for precision and personalized treatment of myeloma. Some proof of concepts of such approaches has been demonstrated.

1. Introduction

Multiple myeloma (MM) is the second most common haematologic malignancies in the world. It arises from clonal plasma cells that secrete monoclonal proteins that can be measured in the serum and urine for diagnosis and disease monitoring. The disease manifests through anemia, hypercalcaemia, renal impairment, and lytic bone lesions. Patients may present with bone fractures, renal failure, and hence significant morbidity [1]. All myelomas are probably preceded by a precursor asymptomatic state called monoclonal gammopathy of undetermined significance (MGUS) or smoldering myeloma (SMM) [2, 3]. The progression to symptomatic disease is most likely through clonal evolution and acquisition of additional genetic events [4]. In recent years, a number of new treatments have been approved for myeloma, including thalidomide, bortezomib, lenalidomide, liposomal doxorubicin, carfilzomib, and pomalidomide. The increase in therapeutic options and the potency of these drugs have greatly improved the survival of patient who now survives for a median of 8 years from diagnosis [5]. Despite this progress, MM is still generally an incurable disease. Drug resistance and disease refractoriness are the common terminal pathways leading to death. A key factor underlying the clinical and therapeutic challenge is multiple layer of heterogeneity that exists in myeloma.

2. Molecular Heterogeneity

Studies over the years have shown that the MM genome is complex. However some of the genetic abnormalities cluster together which may suggest cooperating events. In addition, many abnormalities may affect similar pathways suggesting that there are key pathways affected in MM that may be important for disease pathogenesis and may represent good therapeutic targets.

2.1. Ploidy. At the chromosome level, myeloma can be broadly classified into hyperdiploid (48–74 chromosomes) and nonhyperdiploid myeloma. The hyperdiploid myeloma is characterized by a unique pattern of trisomies affecting many

of the odd-numbered chromosomes such as chromosomes 3, 5, 7, 9, 11, 15, 19, and 21 [6]. The hyperdiploid and nonhyperdiploid dichotomy is an early event in myeloma pathogenesis as these patterns can be detected at the MGUS stage [7]. The reasons that this unique pattern of trisomies is seen and what triggers the acquisition of these trisomies are currently unknown.

2.2. Chromosome Gains and Losses. A number of chromosomal gains and deletions are common in multiple myeloma. These include deletion of chromosome 13, deletion of chromosome 17p13, deletion of chromosome 1p, and gain of chromosome 1q21. Chromosome 13 deletion is an early event that is also present in a substantial proportion of MGUS. Chromosome 17p13 deletion, chromosome 1p deletion, and gain of chromosome 1q21 on the other hand are most likely secondary events associated with disease progression, as they are rarely detected in MGUS [8]. Importantly, the critical gene(s) located on these chromosomes that may be of functional importance is not yet known. For chromosome 13, we previously showed that minimal deleted regions contain RB1 and NBEA and hence may be the implicated genes in this region [9]. In addition, miRNAs that are located within this region may also be relevant [10]. However, there is as yet no functional study that confirms their relevance to myeloma biology.

For chromosome 1q, a number of genes, such as CKS1B [11], BCL9 [12], MCL1 [13], PDZK1 [14], and MUC1 [15], have been implicated. However, it is still unclear whether one or more of these genes are relevant to myeloma biology. For chromosome 1p, we previously identified a region around 1p31-32, which is relevant for prognosis. Amongst the genes located within this locus, several have correlated expression with DNA copy number and may be functionally relevant, although there is no conclusive evidence so far [16]. For chromosome 17p13, the most likely candidate is *TP53*. *TP53* is within the minimally deleted region on chromosome 17p13 [17]. Furthermore, using sequencing in isolated plasma cells, p53 was mutated in 37% of patients with 17p13 deletion and none in those without [18]. However, 17p loss is almost always monoallelic and p53 mutation does not occur in most patients with 17p13 deletion, while methylation of the *TP53* promoter is relatively rare. This suggests that if p53 is the important gene, then it has to be acting in a haploinsufficient manner. We showed, using a panel of cell lines with different p53 abnormalities and using knockdown and overexpression studies, that the level of p53 affects its function and response to both genotoxic and nongenotoxic stress providing clear evidence that *TP53* is a haploinsufficieent tumor suppressor in MM (Teoh PJ et al. leukemia in press).

2.3. Translocations. These rearrangements juxtapose the strong promoters within the immunoglobulin heavy chain (IgH) gene locus to an oncogene and lead to very high expression of these oncogenes. A number of recurrent translocations have been identified in myeloma dysregulating a few classes of proteins, namely, FGFR3/MMSET, Cyclin D (Cyclin D1 and Cyclin D3), and MAFs [8] (Table 1). These

Table 1: Recurrent translocations involved in multiple myeloma.

Translocations	Gene deregulated	Frequency
t(4; 14) (p16; q32)	MMSET FGFR3	15%
t(11; 14) (q13; q32)	CCND1	16%
t(6; 14) (p21; q32)	CCND3	2%
t(12; 14) (p13; q32)	CCND2	<1%
t(14; 16) (q32; q23)	MAF	5%
t(14; 20) (q32; q11)	MAFB	2%
t(8; 14) (q24; q32)	MAFA	1%

are thought to be primary translocations as they already exist at the MGUS stage and are probably initiating events. In gene expression studies, these translocations drive cognate expression signatures that are dominant and are easily identified in gene expression studies. Besides these recurrent translocations, the IgH locus is also involved in nonrecurrent or what is sometimes called secondary translocations involving unknown partners. These are so called because they are thought to represent secondary events that may be important in disease progression. *MYC* is another gene that is recurrently rearranged. It was initially described as the archetypical genes involved in complex rearrangement in myeloma as a secondary event in myeloma progression. It was subsequently found that translocation of MYC can be detected in about 15% of newly diagnosed patient [19]. More recently, it was shown that the MYC pathway is activated in majority of myeloma patients and may be an important transforming event from MGUS to MM [20]. Using high resolution tiling arrays around the MYC locus, it was found that rearrangements of MYC are also common in newly diagnose myeloma and most of these rearrangements affect enhancers and superenhancers of MYC causing an increase in MYC expression [21].

2.4. Mutations. *RAS* was one of the commonly mutated genes in MM, detected in 20–45% of newly diagnosed myeloma patients depending on study cohort and methods used for mutation detection [6]. Both N-RAS and K-RAS can be mutated, with different studies showing different frequency of mutations in these genes. In a large ECOG study, mutations in *N-RAS* (about 70% of all *RAS* mutations) are more common than in *K-RAS* (about 30% of all RAS mutations) [22]. On the other hand, a study from the University of Arkansas found that the frequencies of *N-* and *K-RAS* mutations are quite similar [23]. Recently, sequencing studies in 203 MM patients found that *K-RAS* and *N-RAS* mutations are found in 23% and 20%, respectively [24]. RAS mutation is rare in MGUS [25]. This suggests that it is a potential transforming genetic factor and may also be involved in disease progression. *TP53*, a well-known tumor suppressor gene, is also mutated in myeloma although it is not very common. A large ECOG study using conformation sensitive gel electrophoresis found *TP53* mutated in 3% of cases [26]. More recently, mutation in *TP53* was found in 9% of cases using whole exome or whole genome sequencing. The use of deep sequencing has resulted

Table 2: Genes affected by recurrent mutations in multiple myeloma.

Gene	Frequency (%)
KRAS	23
NRAS	20
DIS3	11
FAM46C	11
TP53	8
BRAF	6
TRAF3	5
PRDM1	5
RB1	3
CYLD	2

in the identification of previously unknown genes that are recurrently mutated at a significant frequency in myeloma. These include *DIS3*, *FAM46C*, *BRAF*, *TRAF3*, *PRDM1*, and *RB1* (Table 2) [24].

2.5. Clustering. Amongst all these genetic chaos, some patterns and clustering around certain pathways exist. The recurrent primary translocations are predominantly seen in nonhyperdiploid myeloma. The primary events of translocations and hyperdiploidy seem to converge on the activation of one or more of the Cyclin D proteins [27].

Deletion 13 is also more common in nonhyperdiploid myeloma [28]. However, MYC rearrangements and activation occur more commonly in hyperdiploid myeloma. As MYC activation and chromosome 13 deletion are considered early events likely to play a role in disease transformation from MGUS to MM, it suggests that different genetic subtypes of myeloma may have different requirements for transformation. RAS mutations are not enriched according to ploidy but are significantly more common in tumors overexpressing Cyclin D1. More recent studies suggest that DIS3 mutations are significantly associated with nonhyperdiploid myeloma [24]. This suggests that there are specific combinations of genetic abnormalities that may collaborate during disease progression. On the other hand, secondary events such as IgH translocations involving unknown partners as well as chromosome 1 abnormalities and chromosome 17p13 deletions are distributed similarly across the main genetic subtypes.

Besides disease developmental pathways where there appears to be some preferential combination of abnormalities, overall, there are some pathways that are recurrently deregulated by different mechanisms in myeloma. The NFKB is affected through mutations, deletions, and amplifications of different genes including CYLD, TRAF3, TRAF2, and NFKB1A [24, 29, 30]. Cell cycle related genes are also commonly affected including CCND1, CCND2, CCND3, RB1, p16, and p18 [4, 23, 24]. Another commonly affected signaling pathway is the RAS-MAPK pathway with N-RAS, K-RAS, and BRAF recurrently mutated [24, 31]. Histone modifying enzymes, coagulation cascades, and the telomerase related pathways are also commonly affected (Table 3) [24, 31].

2.6. Clinical Implication. The clinical impact of these different genetic and molecular abnormalities is 3-fold. One, the different abnormalities has different prognostic relevance. Studies have consistently shown that deletion of 17p13 and the t(4;14) translocation whether detected by FISH or cytogenetics is an independent bad prognostic factor, although the adverse effect of t(4; 14) can be partially abrogated by velcade-based treatment [8, 32, 33]. Other abnormalities such as 1p21 amplification [34–36] and t(14; 16) [37–39] have also been commonly associated with poor outcome although this is not observed in every study. More recently a number of studies has shown that 1p deletion is independently and significantly associated with shorter survival, confirming earlier data from cytogenetic analysis [16, 40, 41]. Two, an understanding of the potential driving mutations in myeloma could lead to opportunities for precision medicine and targeted therapy. The recent description of a relapse patient with BRAF mutation responding spectacularly to BRAF inhibitor is one such proof of concept example [42]. It is therefore critical to identify driver mutations and pathways in myeloma so that patients can be matched to the most appropriate treatment. In this regard, treatment targeting the recurrently affect and functionally important pathway would be an important way forward. The cell cycle pathway, NFKB and RAS-MAPK pathways, would be good starting point. Third, some of these genetic mutations are predictors of response to drugs. In particular NFKB mutations, especially TRAF3 mutation, are associated with better response and progression free survival with bortezomib treatment [29]. More recently, mutation of NRAS but not KRAS has been shown to reduce myeloma sensitivity to bortezomib therapy [43].

3. Clinical Heterogeneity

3.1. Important Determinant of Outcomes. The variable outcome of patients with the same therapy suggests that there is underlying clinical heterogeneity. While some of this can be explained by the underlying biology of the tumor as described in the sections above, other factors such as host factors and extent of disease involvement are also important.

Age is an important prognostic factor. It impacts on the ability of patient to tolerate treatment and cope with disease complications. A large global study showed that there is progressive shortening of survival with increasing age even in patients treated with novel agents [44]. Conversely, a young age is an important independent factor associated with very long outcome [45].

A number of factors that may reflect underlying disease burden have been developed. The Durie-Salmon staging system combines a number of factors including renal function and number of bone lesions [46]. However, the system is cumbersome and lacks sensitivity. It has since been superceded by the International Staging System which is made up of 2 factors, beta-2 microblobulin, which reflects disease activity, and albumin, which reflects host fitness. The ISS is robustly derived and validated in large global cohorts and is easy to apply. These 2 factors emerged as the most significant and independent prognostic factors, trumping

Table 3: Pathways commonly affected by mutations.

Pathway	Mutated genes
Cell cycle pathway including G1-S phase transition and checkpoints	CCNA1, CCNB1, CCND1, CDK4, CDK6, CDK7, CDKN1B, CDKN2A, CDKN2C, RBL1, CDK4, PRB1, ABL1, ATM, ATR, CDK6, SKP2, TGFB1, TGFB2, TGFB3
TERT pathway	MAX, MYC, SP1, SP3, WT1
p38 MAPK pathway	ATF2, DAXX, GRB2, HMGN1, MAP2K6, MAP3K7, MAP3K9, MAPK14, MAX, MEF2A, MEF2D, MKNK1, MYC, PLA2G4A, RAC1, RIPK1, RPS6KA5, SHC1, TGFB1, TGFB2, TGFB3, TRAF2
Histone methyltransferase	KDM6A, MLL, MLL2, MLL3, NSD1, WHSC1, WHSC1L1
NFKB pathway	BIRC2, BIRC3, BTRC, CARD10, CARD11, CARD6, CARD8, CYLD, FBXW11, IKBIP, IKBKAP, IKBKB, IKBKE, IL1R1, IRAK1, MAP3K14, MAP3K7, MYD88, NFKB2, NFKBIB, NOD2, RELA, RIPK1, RIPK2, RIPK4, TLR4, TRAF2, TRAF3, TRAF3IP1
Clotting pathway	COL4A1, COL4A2, COL4A3, COL4A5, COL4A6, F11, F3, F5, F7, F8, FGA, FGG, TFPI

Table 4: GEP-based prognostic signatures.

GEP signature	Methods
UAMS 70-gene [49]	Derived by comparing expression of profiles of patients with top and bottom quartile of survival treated on total therapy II
IFM signature [50]	Derived by comparing expression of profiles of patients with good and poor outcome in IFM trials
Centrosome index [51]	Based on expression of constituents of the centrosome
HZD cell death signature [52]	Signature derived from genes homozygously deleted in myeloma as detected by array comparative genomic hybridization
IL6-HMCL signature [53]	13-gene signature from genes induced upon IL6 stimulation of human myeloma cells lines
Proliferation index [54]	Curated signature based on proliferation genes
EMC 92-gene signature [55]	92-gene signature based on differentially expressed genes between patient with good and poor outcome on HOVON trial
Chromosome instability genomic event count (CINGEC) signature [56]	Based on differentially expressed genes between patients with the top and bottom quartile of genomic instability score based on number of genetic abnormalities identified by array comparative genomic hybridization

more traditional prognostic factors such as blood counts, m-protein levels, bone marrow plasma cell involvement, renal function, calcium level, and the types of immunoglobulin [47]. The incorporation of ISS with high-risk genetics provides further refinement to the prognostic system [48].

Can we further refine this dissection of clinical heterogeneity to more accurately segregate patients with different response to treatment and outcome? Gene expression profiles are very strong predictors of outcome. There are a number of signatures that are independently associated with poor outcome (Table 4). However, the confusion surrounds which one is best, how best to use them, and why they hardly share any individual genes. We undertook a meta-analysis to look at these signatures individually and in combination to set up a framework for applying gene expression signature for the prognosis of patients in the clinical setting. We found that combinations of signatures are more powerful predictors of outcome than any individual signature and we develop a package, which will be available to all investigators to apply to their own data for real-time prospective validation (Chung TH et al. in press).

The next challenge will be to assess this GEP signature in combination with ISS and high-risk genetics in an international exercise along the lines of ISS to hopefully arrive at a unified prognostic system.

3.2. Therapeutic Implications. The ability to carefully dissect the risk of patients in relation to the different treatment regimen and strategy is important. While the path towards precision medicine in myeloma will be a long process as we have to develop the treatment to match to patients' different mutations and also importantly identify the critical mutations to target, what we already have now are very effective treatments that have benefitted a large number of patients. How best to use the current treatment in terms of optimizing benefit while minimizing unnecessary treatment and toxicity is therefore a highly relevant clinical questions. Based on currently available prognostic factors, patients can be stratified into 3 risk groups. While at present, there is no evidence that different treatment should be recommended for the different risk groups, it will be important for risk stratification to be incorporated into future trial design to ensure that we can optimize treatment according to risk groups [57].

4. Clonal Heterogeneity

Data from next generation sequencing studies has challenged the traditional dogma that clonal evolution in cancer occurs in a linear fashion through stepwise accumulation of mutations. Numerous studies now propose a branching pattern of

Table 5: Driver mutations that may be responsible for clonal evolution in MM.

Affected gene	Type of mutation	Normal function	Postulated role in disease evolution	Study
AFF1	Damaging	Histone methylation	Driver of Myelomagenesis (found at all disease time points)	Egan et al. 2012 [61]
RUNX2	Inactivating	Regulates osteopontin a bone matrix glycoprotein involved in cell survival	HR SMM to MM	Walker et al. 2014 [64]
BRCA2	Disrupted due to t(13; 21)	DNA repair	HR SMM to MM	Walker et al. 2014 [64]
UNC5D	Inactivating	Induces apoptosis, regulated by p53	HR SMM to MM	Walker et al. 2014 [64]
ZKSCAN3	Truncating	Possible effects on VEGF	PCL transformation	Egan et al. 2012 [61]
Rb1	Truncating	Key tumour suppressor gene	PCL transformation	Egan et al. 2012 [61]

VEGF = Vascular endothelial growth factor, PCL = Plasma cell leukaemia, and HR SMM = High risk smouldering multiple myeloma.

clonal evolution in keeping with Darwinian principles [58]. In myeloma it is no different. Furthermore, these studies suggest related clones with different composition of genetic abnormalities coexist.

4.1. Evidence for Clonal Heterogeneity and Evolution in MM. Recent studies using single nucleotide polymorphism (SNP) arrays and whole exome sequencing [59], array comparative genomic hybridization (aCGH) [60], and whole genome sequencing (WGS) [61] have clearly demonstrated the presence of multiple clonally related tumor cell populations within the same patient that has different composite of genetic aberrations.

In a clinical study using SNP arrays and whole exome sequencing on 67 MM patients, the percentage of subclonal mutations in each patient varied from 10% to 80% [59]. In a study using WGS on bone marrow samples obtained from a patient with MM at serial time points from diagnosis to demise, 15 mutations were found to be present at all time points, representing the mutational profile of the common ancestor clone. The samples taken at each relapse had unique mutations, some of which disappeared at the first relapse and returned at subsequent relapse [61]. These findings strongly support the hypothesis that clonal evolution occurred in this patient and contributed to her disease progression; it also raises the possibility of alternating clonal dominance that may be selected under treatment pressure.

Using aCGH in 28 patients with serial samples, Keats et al. identified three patterns of clonal evolution among the patients: loss of copy number abnormalities (CNA), gain of CNA, and both losses and gains of CAN, with increasing number of CNAs as the disease progress. There is also an association between increase CAN and high-risk genetic abnormalities. In a patient whom they have material to study in depth, they were able to identify four clones that showed alternating dominance at different time points. In addition, specific clones were selected for by proteasome inhibitor treatment and melphalan. Clonal dominance and selection by treatment is further demonstrated using a mouse model of myeloma [60].

4.2. Mechanisms of Clonal Evolution. Clonal evolution occurs due to driver mutations or larger scale genomic crises, which can be stimulated by tumour, host, environmental, and treatment-related factors. A number of driver mutations have been implicated in the clonal evolution of MM (Table 5). Chromothripsis is an example of a genomic crisis, which is characterized by spontaneous catastrophic chromosome breakage followed by reassembly resulting in significant loss of chromosomal material [62]. Features of chromothripsis were detected in 1.3% of patients in a study of 764 patients with newly diagnosed MM. Of the 10 patients with chromothripsis, five relapsed within one year and three died during that period [63]. This study suggests that chromothripsis is associated with rapid clinical progression and a poor prognosis that needs to be validated in further studies.

It has recently been shown by whole exome sequencing that clonal heterogeneity is also present at the asymptomatic MGUS and SMM stages. The investigators went on to demonstrate that the majority of subclones present at the MM stage are already present at the SMM stage. Genetic lesions that may trigger the transformation from SMM to MM were identified [64] (Table 5).

4.3. Clinical Implications of Clonal Heterogeneity. The emerging concept of clonal heterogeneity and alternating clonal dominance in myeloma has clear therapeutic implications. In the patient studied by Keats et al., the dominant clone detected during her first relapse had mutations leading to NFkB activation, this clone was effectively treated with carfilzomib. At her second relapse however the dominant clone was associated with a different mutational profile and she had an inferior response to bortezomib [60]. These findings suggest that we should have an understanding of the clonal composition of the tumor at each treatment phase and use this information to guide treatment. The challenge is to identify treatment modalities and combinations effective against the main functionally relevant genetic abnormalities in MM. While the current treatment armamentarium is expanding, it is still limited to a few classes of drugs. We need to expand this portfolio in a systematic manner to match treatment with mutational profiles. The use of patient-derived xenograft models and faithful myeloma mouse models such as the V∗K-MYC model [65] and the availability of an increasingly broad range of novel therapeutics should facilitate the generation of this knowledge.

5. Cellular Differentiation Heterogeneity

Evidence is emerging that the clonal cells that constitute myeloma are not homogenously of mature plasma cell phenotype. In fact, a hierarchy of precursor cells with different clonogenic potential, different gene expression, phenotype, and sensitivity to therapy may exist. Sitting at the apex of this hierarchy of clonally related cellular population is the putative clonogenic myeloma progenitor cells (MPC).

5.1. Evidence for the Existence of MPC. MM plasma cells are quiescent and have a low proliferative index. As a result, its tumorigenic potential and ability has been questioned [66]. It is therefore postulated that a precursor population might be responsible for disease initiation.

Early work by Hamburger and colleagues showed that bone marrow cells from MM patients were capable of in vitro colony formation [67]. Bakkus and coworkers later identified a population of mature B-cells in the peripheral blood and marrow of MM patients that were clonally related to MM plasma cells and hence postulated to be MPCs [68]. In a separate report, a patient with MM, who went on to developed B lymphoblastic leukaemia, has B lymphoblasts that share similar clonal immunoglobulin gene rearrangement as the malignant PCs detected at diagnosis. The leukaemic B-cells when grafted into NOD/SCID mice led to the development of lytic bone lesions. The B-cells identified in the bone marrow of these mice were CD45+, CD19+, CD20+, and CD138−. This study suggested that some of the circulating B-cells in the patient were MPCs capable of initiating MM in vivo [69].

More recently, Matsui et al. isolated CD138− mature B-cells from MM cell lines as well as clinical samples. This population had a greater capacity for colony formation in vitro and in vivo than CD138+ MM plasma cells and their expression of Ki 67 was greater [70]. They also showed that these MM forming mature B-cells had a CD19+, CD20+, and CD138− phenotype with light chain restriction, consistent with the phenotype of the putative MM clonogenic cells identified in earlier studies [71].

The expression of aldehyde dehydrogenase (ALDH) is characteristic of cancer stem cells [72]. Using ALDH as a marker, Reghunathan and coworkers identified a CD138− population constituting 2.5% of cells in a human MM cell line. Indeed, 45% of the CD138− population expresses ALDH compared to less than 1% of the CD138+ cells. These CD138−ALDH+ cells had superior colony forming capacity both in vitro and in vivo. They also have similar gene expression profiles compared to hematopoietic stem cells (HSC) and leukaemic stem cells (LSC). These data further support the existence of a clonogenic population in the CD138− fraction of MM [73].

5.2. Hierarchical Organization of MPC. Clonally related subpopulations of MPCs, sharing common IgH gene rearrangement, have been detected in bone marrow of MM patients. Amongst these immunophenotypically distinct subpopulations, the CD19+CD138− cells had a superior colony forming capacity compared to the CD138+ population [75]. Chaidos and colleagues analyzed bone marrow samples from 10 MM patients and identified four clonally related subpopulations of MPC. Using a mathematical growth model they showed that differentiation occurred in the following sequence: memory B-cell followed by plasmablast followed by preplasma cell and finally plasma cell. Importantly, they demonstrated that the CD138− dim plasma cells were also capable of reverting back to a preplasma cell phenotype [76]. A hierarchical organization of clonally related MPCs was furtherly demonstrated by studying the expression of XBP-1, a transcription factor important for the differentiation of plasma blasts into plasma cells. In this study, five clonally related subpopulations of MPCs in bone marrow samples of MM patients were identified with XBP-1 expression universally negative in the CD38−CD138− populations [77].

These studies strongly suggest the presence of a clonal hierarchy of MPCs consisting of phenotypically distinct subsets with a defined maturation sequence (see Table 6).

5.3. Gene Expression Differs between MPC and Mature Plasma Cells. Several studies have compared the gene expression profile of CD138− to CD138+ clonal myeloma cells. In 2 of these studies [73, 76], the polycomb repressor complex 2 (PRC2) related genes, such as EZH2, EED, and SUZ12, were upregulated in the CD138− subsets. The PRC genes promote histone methylation and reduced expression of their targets. The overexpression of PRC2 genes and subsequent reduced expression of target genes such as the cyclin dependent kinase inhibitors (such as p21) would increase the proliferative capacity of the CD138− MPCs. In one of these studies, other genes involved in epigenetic regulation of gene expression such as histone demethylases, histone acetyltransferases, and deacetylases also have altered expression. The resultant "epigenetic plasticity" may explain the bidirectional transition of MPCs observed in the study [76]. In another study, the RARα2 gene was found to be overexpressed in the CD138− subset. Overexpression of RARα2 resulted in the activation of the Wnt and Hedgehog pathways, increased expression of ALDH, expression of embryonic stem cell genes, and greater clonogenic potential in the MM cells. These effects were reversed upon silencing of RARα2 [78].

Two groups have also looked at the clinical relevance of gene expression signature derived from these clonogenic myeloma cells. Kassambara and colleagues focused on genes differentially expressed in clonogenic myeloma cells that are not related to proliferation or previous prognostic gene expression signatures. They identified 50 genes which were of prognostic significance in MM. Thirty-seven of these 50 genes were also found to be overexpressed in three human stem cell populations, pluripotent stem cells, hematopoietic stem cells, and mesenchymal stem cells. They went on to build a "stem cell score" based on the expression of these genes, which proved strongly prognostic in two independent patient cohorts [79]. In the study of Reghunathan and colleagues, the gene signature comprising genes differentially expressed in CD138− clonogenic population compared to the CD138+ population was associated with poorer outcome in stem cell transplant as well as velcade-treated MM patient

Table 6: Subtypes of MPC and their phenotypes described in the key publications.

Study	MPC subpopulation and phenotype				
Rasmussen 2000 [74]	*Early B cell* CD38+, CD19+	*More differentiated B cell* CD38−, CD19+			
Boucher et al. 2012 [75]	*LCR B-cells* CD138−/CD34+/CD19+	*More Differentiated B cells* CD138−/CD34−/CD19+	*Plasma cells* CD138+/CD34−/CD19−		
Chaidos et al. 2013 [76]	*Memory B cells* CD 19+, CD 138−, CD 38−	*Plasmablasts* CD 19+, CD 138−, CD38+	*Preplasma cells* CD 19−, CD 138−, CD38+, CD56+	*Plasma cells* CD 19−, 138dim or +, CD 56+, CD38	
Leung-Hagesteijn et al. 2013 [77]	*B cell* CD20+, CD38−, CD138−	*Activated B-cell* CD20low, CD38−, CD138−	*Preplasmablast* CD20−, CD38−, CD138−	*Plasmablast* CD20−, CD38+, CD138+/−	*Plasma cell* CD20−, CD38+, CD138+

LCR = Light chain restricted.

Table 7: Summary of differential gene expression between CD 138− and CD 138+ subsets. The list of genes is not exhaustive but includes selected genes of importance described in the studies.

Study	Gene	Differential expression	Function
Reghunathan et al. 2013 [73]	PRC2 related (EZH2, EED, SUZ12) PRC1 related (BMI1)	Upregulated in CD 138− subset.	Via histone methylation, reduces the expression of p21 and other CDK inhibitors, driving proliferation.
	BMP 2, BMP3, BMP4	Upregulated in CD 138+ subset	Promote differentiation of plasmablasts to mature plasma cells.
Yang et al. 2013 [78]	RARα2	Upregulated in CD 138− subset.	Increased ALDH expression, increased activity of WNT and Hedgehog pathway signaling as well as Cyclin D1.
	Oct 4, SOX 2, Nanog, Lin 28A	Upregulated in CD 138− subset	Genes expressed in pluripotent stem cells.

cohorts. Table 7 summarizes the important genes differentially expressed between MPC and mature PC.

5.4. Clinical Significance of MPC

5.4.1. Implications for Minimal Residual Disease (MRD) Assessment. Recent studies have shown the importance of MRD, as assessed by flow cytometry (FC) [80, 81], allele specific oligonucleotide (ASO) PCR [82], or PET imaging [83], in predicting early relapse and poorer outcome in patients who have achieve conventional complete remission as defined by the International Myeloma Working Group. While potentially clinically useful, each technique has its technical advantages and limitations but importantly they also assess different tumor cell populations of the disease. As a result, the recent insights into the hierarchical organization of clonally related myeloma precursors need to be taken into consideration when we choose the techniques to utilize. Both the FC and PCR methods only assess the bone marrow tumor cells as these tests are performed on bone marrow samples. Therefore, disease outside the bone marrow or even disease within the bone marrow, that is, not in the area sampled, may be missed for MRD assessment. This limitation is relevant as studies have shown that the CD138− clonogenic MPCs have propensity for extramedullary sites [76]. This is where whole body imaging such as PET-CT may be useful in identifying extramedullary disease. However, the sensitivity of PET-CT in terms of MRD assessment is still unclear. Comparing FC and PCR, there is also subtle yet important difference in the population they may detect. FC-based MRD assessment is based on the detection of the aberrant phenotype of myeloma plasma cells and is therefore predominantly detecting the plasma cell component and hence will miss the clonogenic MPCs. PCR on the other hand detects the clonal rearrangements of IgH gene and therefore will identify any clonally related cells, including the MPCs (Figure 1). The predominant limitation of the PCR method is that clone specific rearrangements can only be identified in only less than 50% of cases in MM. This limitation may be overcome by using sequencing-based method to detect IgH gene rearrangement, which can be applied to more than 90% of cases (Martinez-Lopez et al. blood *in press*). Due to the challenge of cellular heterogeneity in MM, it is likely that more than 1 technique will need to be used for comprehensive MRD assessment in the future. Prospective study should be conducted to correlate PET-CT with FC and PCR/sequencing-based methods and to develop clinically useful algorithm for the application of these techniques possibly in a step-wise manner.

5.5. The Role of MPC in Drug Resistance and Disease Relapse. CD138− clonogenic MPCs have been shown by a number of groups to be more resistant to drugs used for myeloma treatment such as lenalidomide, dexamethasone, and bortezomib. This is in part due to the increased expression of ABCG2/BCRP drug transporter as well as higher levels of

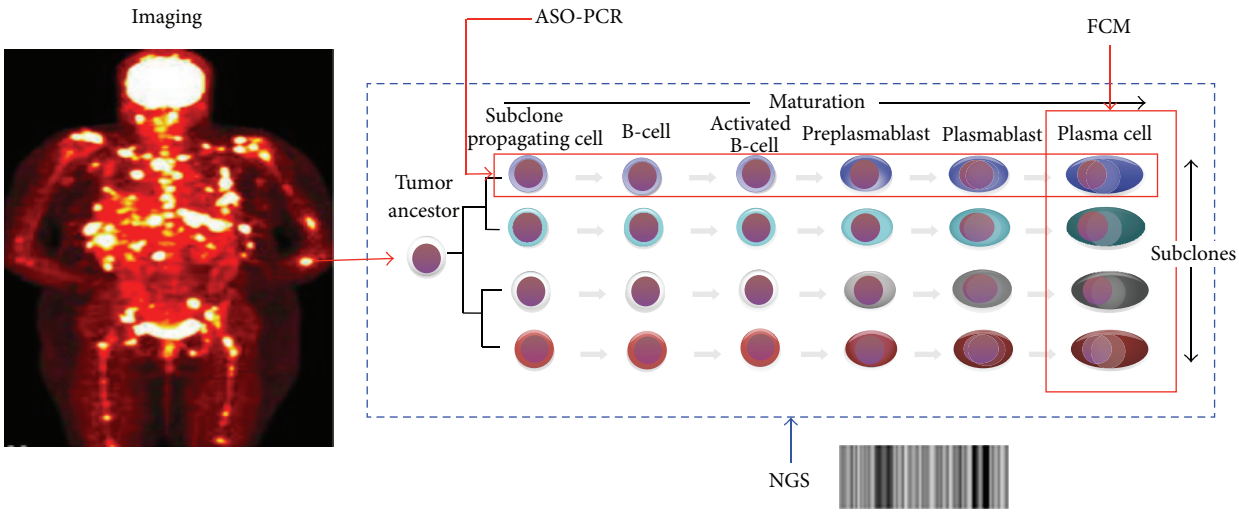


Figure 1: Implications of heterogeneity on MRD detection. MM involvement may be patchy and involve extramedullary sites. All these lesions may be detected by whole body imaging modality such as PET-CT scan. Within the individual lesions, 2 dimensions of heterogeneity may exist in the population of tumor cells. On one hand, there may be clonal heterogeneity where related clones with different genetic composition may coexist. On the other hand, clonally related progenitor populations at earlier stage of differentiation may exist. Flow cytometry can detect the plasma cell component but not the precursor population while ASO-PCR can detect all the clonal cells including the precursor population but its applicability is limited. The development of NGS methods may allow utility in larger population of patients.

ALDH [84]. The expression of these drug transporters may be induced by an increased expression of RARα2 [78]. More recently, through a series of elegant experiments, it was shown that in Velcade resistant patients, there is an increase in XBP1 low expressing MPCs which share common genetic changes as the myeloma plasma cells. These cells produce less immunoglobulin and have less endoplasmic reticulum (ER) stress and express less unfolded protein response (UPR) genes and are therefore more resistant to velcade, which induce cell death in plasma cells with high immunoglobulin production and ER stress by inhibiting the UPR, which requires an active proteasome [77].

MPCs have also been shown to rely on survival and proliferation pathways used by stem cells. Peacock and colleagues demonstrated that CD138− CD19+ B-cells from MM cell lines and patient samples had constitutive activation of the Hedgehog (Hh) signaling pathway evident by the increased expression of the smoothened (SMO) protein [85]. It is possible that MPCs rely on these to avoid cell death induced by agents that are active against mature plasma cells.

5.6. Targeted Therapy against MPCs. Signaling pathways and other lesions unique to the clonogenic MPCs may form the basis for targeted therapy against MPCs. The histone methyltransferase inhibitor DZNep inhibits PRC2 and was shown to be more effective in killing CD138− compared to CD138+ MM cells [73]. Inhibition of Hh pathway signaling by cyclopamine was shown to reduce the clonogenic capacity of the CD138−CD19+ MPC fraction in two MM cell lines [85]. A phase I trial using another Hh pathway antagonist GDC-0449 (Vimodegib) in patients with high risk MM postautologous stem cell transplant has been completed and results are awaited. Blockade of the JAG-NOTCH interaction using NOTCH-Fc chimeric molecules resulted in impaired self-renewal capacity in MM cell lines [86]. Targeting the NOTCH pathway using the NOTCH inhibitor R0490927 in combination with melphalan has also been investigated in a phase II clinical trial for which the results are awaited. Based on the identification of high RARα2 expression in the CD138− MPC, Yang and coworkers showed that al-trans retinoic acid (ATRA) preferentially induced apoptosis in the CD138− fraction [78]. Telomerase activity is required for the survival of normal stem cells. Brennan and colleagues treated MM stem cells from cell lines and clinical samples with the telomerase inhibitor imetelstat. They found that telomerase inhibition resulted in inhibition of clonogenic growth as well as reduced expression of genes expressed by stem cells [87]. Clinical trials using telomerase inhibitors in MM are awaited. The long-term remissions achieved by selected MM patients who survive allogeneic stem cell transplant suggest the presence of a graft versus myeloma stem cell effect [88]. This has led to investigation of cellular therapy modalities targeting MPCs. Swift and colleagues demonstrated that the natural killer (NK) cell lines KHYG2 and NK-92 were selectively toxic to the MPC fraction in vitro [89]. Clinical trials using NK cells in MM are in progress. The concepts are still in early phase of clinical development and results from early phase clinical trials are still pending (Table 8).

6. Conclusion

Multiple levels of heterogeneity exist in MM, providing tremendous clinical challenges in diagnosis, prognosis, treatment, and monitoring. The understanding of biological relevance of the heterogeneity at the molecular, clonal, and cellular level and how these relate to clinical heterogeneity

Table 8: Summary of agents in development which may be selectively toxic to MPC.

Drug/molecule	Mechanism of action	Phase/used in combination with other agents
DZNep inhibitor	Disruption of PRC2	Preclinical
Vimodegib (GDC-0449)	Hedgehog signaling inhibitor	Phase I/after auto SCT
R0490927	NOTCH signaling inhibitor	Phase II/melphalan
MK571	MRP3 inhibitor	Preclinical/bortezomib
ATRA	Induces degradation of RARα2	Preclinical
Imetelstat	Telomerase Inhibitor	Preclinical
NK cell therapy	Cellular cytotoxicity	Preclinical

SCT = Stem cell transplant.

will provide important mechanistic insights that will guide future development of diagnostic, prognostic, therapeutic, and monitoring modalities to further personalize treatment, improve treatment precision, and lengthen the survival of MM patients.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

References

[1] R. A. Kyle and S. V. Rajkumar, "Multiple myeloma," *Blood*, vol. 111, no. 6, pp. 2962–2972, 2008.

[2] B. M. Weiss, J. Abadie, P. Verma, R. S. Howard, and W. M. Kuehl, "A monoclonal gammopathy precedes multiple myeloma in most patients," *Blood*, vol. 113, no. 22, pp. 5418–5422, 2009.

[3] O. Landgren, R. A. Kyle, R. M. Pfeiffer et al., "Monoclonal gammopathy of undetermined significance (MGUS) consistently precedes multiple myeloma: a prospective study," *Blood*, vol. 113, no. 22, pp. 5412–5417, 2009.

[4] G. J. Morgan, B. A. Walker, and F. E. Davies, "The genetic architecture of multiple myeloma," *Nature Reviews Cancer*, vol. 12, no. 5, pp. 335–348, 2012.

[5] E. M. Ocio, P. G. Richardson, S. V. Rajkumar et al., "New drugs and novel mechanisms of action in multiple myeloma in 2013: a report from the International Myeloma Working Group (IMWG)," *Leukemia*, vol. 28, pp. 525–542, 2014.

[6] R. Fonseca, B. Barlogie, R. Bataille et al., "Genetics and cytogenetics of multiple myeloma: a workshop report," *Cancer Research*, vol. 64, no. 4, pp. 1546–1558, 2004.

[7] W. J. Chng, S. A. van Wier, G. J. Ahmann et al., "A validated FISH trisomy index demonstrates the hyperdiploid and non-hyperdiploid dichotomy in MGUS," *Blood*, vol. 106, no. 6, pp. 2156–2161, 2005.

[8] R. Fonseca, P. L. Bergsagel, J. Drach et al., "International Myeloma Working Group molecular classification of multiple myeloma: spotlight review," *Leukemia*, vol. 23, no. 12, pp. 2210–2221, 2009.

[9] J. O'Neal, F. Gao, A. Hassan et al., "Neurobeachin (NBEA) is a target of recurrent interstitial deletions at 13q13 in patients with MGUS and multiple myeloma," *Experimental Hematology*, vol. 37, no. 2, pp. 234–244, 2009.

[10] A. M. Roccaro, A. Sacco, B. Thompson et al., "MicroRNAs 15a and 16 regulate tumor proliferation in multiple myeloma," *Blood*, vol. 113, no. 26, pp. 6669–6680, 2009.

[11] F. Zhan, J. Sawyer, and S. Gupta, "Elevated expression of *CKS1B* at 1q21 is highly correlated with short survival in myeloma," *Blood*, vol. 104, abstract 77, 2004.

[12] T. G. Willis, I. R. Zalcberg, L. J. A. Coignet et al., "Molecular cloning of translocation t(1;14)(q21;q32) defines a novel gene (BCL9) at chromosome 1q21," *Blood*, vol. 91, no. 6, pp. 1873–1881, 1998.

[13] R. W. Craig, E. W. Jabs, P. Zhou et al., "Human and mouse chromosomal mapping of the myeloid cell leukemia-1 gene: MCL1 maps to human chromosome 1q21, a region that is frequently altered in preneoplastic and neoplastic disease," *Genomics*, vol. 23, no. 2, pp. 457–463, 1994.

[14] J. Inoue, T. Otsuki, A. Hirasawa et al., "Overexpression of PDZK1 within the 1q12-q22 amplicon is likely to be associated with drug-resistance phenotype in multiple myeloma," *American Journal of Pathology*, vol. 165, no. 1, pp. 71–81, 2004.

[15] V. G. Dyomin, N. Palanisamy, K. O. Lloyd et al., "MUC1 is activated in a B-cell lymphoma by the t(1;14)(q21;q32) translocation and is rearranged and amplified in B-cell lymphoma subsets," *Blood*, vol. 95, no. 8, pp. 2666–2671, 2000.

[16] W. J. Chng, M. A. Gertz, T.-H. Chung et al., "Correlation between array-comparative genomic hybridization-defined genomic gains and losses and survival: identification of 1p31-32 deletion as a prognostic factor in myeloma," *Leukemia*, vol. 24, no. 4, pp. 833–842, 2010.

[17] K. D. Boyd, F. M. Ross, W. J. Tapper et al., "The clinical impact and molecular biology of del(17p) in multiple myeloma treated with conventional or thalidomide-based therapy," *Genes Chromosomes and Cancer*, vol. 50, no. 10, pp. 765–774, 2011.

[18] L. Lodé, M. Eveillard, V. Trichet et al., "Mutations in TP53 are exclusively associated with del(17p) in multiple myeloma," *Haematologica*, vol. 95, no. 11, pp. 1973–1976, 2010.

[19] H. Avet-Loiseau, F. Gerson, F. Magrangeas, S. Minvielle, J.-L. Harousseau, and R. Bataille, "Rearrangements of the c-myc oncogene are present in 15% of primary human multiple myeloma tumors," *Blood*, vol. 98, no. 10, pp. 3082–3086, 2001.

[20] W.-J. Chng, G. F. Huang, T. H. Chung et al., "Clinical and biological implications of MYC activation: a common difference between MGUS and newly diagnosed multiple myeloma," *Leukemia*, vol. 25, no. 6, pp. 1026–1035, 2011.

[21] M. Affer, M. Chesi, and W. D. Chen, "Promiscuous rearrangements of the MYC locus hijack enhancers and super-enhancers

to dysregulate MYC expression in multiple myeloma," *Leukemia*, 2014.

[22] W. J. Chng, N. Gonzalez-Paz, T. Price-Troska et al., "Clinical and biological significance of RAS mutations in multiple myeloma," *Leukemia*, vol. 22, no. 12, pp. 2280–2284, 2008.

[23] W. J. Chng, O. Glebov, P. L. Bergsagel, and W. M. Kuehl, "Genetic events in the pathogenesis of multiple myeloma," *Best Practice and Research: Clinical Haematology*, vol. 20, no. 4, pp. 571–596, 2007.

[24] J. G. Lohr, P. Stojanov, S. L. Carter et al., "Widespread genetic heterogeneity in multiple myeloma: implications for targeted therapy," *Cancer Cell*, vol. 25, pp. 91–101, 2014.

[25] T. Rasmussen, M. Kuehl, M. Lodahl, H. E. Johnsen, and I. M. S. Dahl, "Possible roles for activating RAS mutations in the MGUS to MM transition and in the intramedullary to extramedullary transition in some plasma cell tumors," *Blood*, vol. 105, no. 1, pp. 317–323, 2005.

[26] W. J. Chng, T. Price-Troska, N. Gonzalez-Paz et al., "Clinical significance of TP53 mutation in myeloma [13]," *Leukemia*, vol. 21, no. 3, pp. 582–584, 2007.

[27] P. L. Bergsagel, W. M. Kuehl, F. Zhan, J. Sawyer, B. Barlogie, and J. Shaughnessy Jr., "Cyclin D dysregulation: an early and unifying pathogenic event in multiple myeloma," *Blood*, vol. 106, no. 1, pp. 296–303, 2005.

[28] R. Fonseca, C. S. Debes-Marun, E. B. Picken et al., "The recurrent IgH translocations are highly associated with nonhyperdiploid variant multiple myeloma," *Blood*, vol. 102, no. 7, pp. 2562–2567, 2003.

[29] J. J. Keats, R. Fonseca, M. Chesi et al., "Promiscuous mutations activate the noncanonical NF-κB pathway in multiple myeloma," *Cancer Cell*, vol. 12, no. 2, pp. 131–144, 2007.

[30] C. M. Annunziata, R. E. Davis, Y. Demchenko et al., "Frequent engagement of the classical and alternative NF-κB pathways by diverse genetic abnormalities in multiple myeloma," *Cancer Cell*, vol. 12, no. 2, pp. 115–130, 2007.

[31] M. A. Chapman, M. S. Lawrence, J. J. Keats et al., "Initial genome sequencing and analysis of multiple myeloma," *Nature*, vol. 471, no. 7339, pp. 467–472, 2011.

[32] N. C. Munshi, K. C. Anderson, P. L. Bergsagel et al., "Consensus recommendations for risk stratification in multiple myeloma: report of the International Myeloma Workshop Consensus Panel 2," *Blood*, vol. 117, no. 18, pp. 4696–4700, 2011.

[33] H. Avet-Loiseau, "Role of genetics in prognostication in myeloma," *Best Practice and Research: Clinical Haematology*, vol. 20, no. 4, pp. 625–635, 2007.

[34] I. Hanamura, J. P. Stewart, Y. Huang et al., "Frequent gain of chromosome band 1q21 in plasma-cell dyscrasias detected by fluorescence in situ hybridization: incidence increases from MGUS to relapsed myeloma and is related to prognosis and disease progression following tandem stem-cell transplantation," *Blood*, vol. 108, no. 5, pp. 1724–1732, 2006.

[35] R. Fonseca, S. A. van Wier, W. J. Chng et al., "Prognostic value of chromosome 1q21 gain by fluorescent in situ hybridization and increase CKS1B expression in myeloma," *Leukemia*, vol. 20, no. 11, pp. 2034–2040, 2006.

[36] H. Avet-Loiseau, M. Attal, P. Moreau et al., "Genetic abnormalities and survival in multiple myeloma: the experience of the Intergroupe Francophone du Myélome," *Blood*, vol. 109, no. 8, pp. 3489–3495, 2007.

[37] H. Avet-Loiseau, F. Malard, L. Campion et al., "Translocation t(14;16) and multiple myeloma: is it really an independent prognostic factor?" *Blood*, vol. 117, no. 6, pp. 2009–2011, 2011.

[38] K. D. Boyd, F. M. Ross, L. Chiecchio et al., "A novel prognostic model in myeloma based on co-segregating adverse FISH lesions and the ISS: analysis of patients treated in the MRC Myeloma IX trial," *Leukemia*, vol. 26, no. 2, pp. 349–355, 2012.

[39] R. Fonseca, E. Blood, M. Rue et al., "Clinical and biologic implications of recurrent genomic aberrations in myeloma," *Blood*, vol. 101, no. 11, pp. 4569–4575, 2003.

[40] B. Hebraud, X. Leleu, V. Lauwers-Cances et al., "Deletion of the 1p32 region is a major independent prognostic factor in young patients with myeloma: the IFM experience on 1195 patients," *Leukemia*, vol. 28, pp. 675–679, 2014.

[41] K. D. Boyd, F. M. Ross, B. A. Walker et al., "Mapping of chromosome 1p deletions in myeloma identifies FAM46C at 1p12 and CDKN2C at 1p32.3 as being genes in regions associated with adverse survival," *Clinical Cancer Research*, vol. 17, no. 24, pp. 7776–7784, 2011.

[42] M. Andrulis, N. Lehners, D. Capper et al., "Targeting the BRAF V600E mutation in multiple myeloma," *Cancer Discovery*, vol. 3, no. 8, pp. 862–869, 2013.

[43] G. Mulligan, D. I. Lichter, A. di Bacco et al., "Mutation of NRAS but not KRAS significantly reduces myeloma sensitivity to single-agent bortezomib therapy," *Blood*, vol. 123, pp. 632–639, 2014.

[44] H. Ludwig, V. Bolejack, J. Crowley et al., "Survival and years of life lost in different age cohorts of patients with multiple myeloma," *Journal of Clinical Oncology*, vol. 28, no. 9, pp. 1599–1605, 2010.

[45] H. Avet-Loiseau, M. Attal, L. Campion et al., "Long-term analysis of the ifm 99 trials for myeloma: cytogenetic abnormalities [t(4;14), del(17p), 1q gains] play a major role in defining long-term survival," *Journal of Clinical Oncology*, vol. 30, no. 16, pp. 1949–1952, 2012.

[46] B. G. M. Durie and S. E. Salmon, "A clinical staging system for multiple myeloma. Correlation of measured myeloma cell mass with presenting clinical features, response to treatment, and survival," *Cancer*, vol. 36, no. 3, pp. 842–854, 1975.

[47] P. R. Greipp, J. S. Miguel, B. G. M. Dune et al., "International staging system for multiple myeloma," *Journal of Clinical Oncology*, vol. 23, no. 15, pp. 3412–3420, 2005.

[48] H. Avet-Loiseau, B. G. M. Durie, M. Cavo et al., "Combining fluorescent in situ hybridization data with ISS staging improves risk assessment in myeloma: an International Myeloma Working Group collaborative project," *Leukemia*, vol. 27, no. 3, pp. 711–717, 2013.

[49] J. D. Shaughnessy Jr. and B. Barlogie, "Using genomics to identify high-risk myeloma after autologous stem cell transplantation," *Biology of Blood and Marrow Transplantation*, vol. 12, no. 1, pp. 77–80, 2006.

[50] O. Decaux, L. Lodé, F. Magrangeas et al., "Prediction of survival in multiple myeloma based on gene expression profiles reveals cell cycle and chromosomal instability signatures in high-risk patients and hyperdiploid signatures in low-risk patients: a study of the Intergroupe Francophone du Myélome," *Journal of Clinical Oncology*, vol. 26, no. 29, pp. 4798–4805, 2008.

[51] W. J. Chng, E. Braggio, G. Mulligan et al., "The centrosome index is a powerful prognostic marker in myeloma and identifies a cohort of patients that might benefit from aurora kinase inhibition," *Blood*, vol. 111, no. 3, pp. 1603–1609, 2008.

[52] N. J. Dickens, B. A. Walker, P. E. Leone et al., "Homozygous deletion mapping in myeloma samples identifies genes and an expression signature relevant to pathogenesis and outcome," *Clinical Cancer Research*, vol. 16, no. 6, pp. 1856–1864, 2010.

[53] J. Moreaux, B. Klein, R. Bataille et al., "A high-risk signature for patients with multiple myeloma established from the molecular classification of human myeloma cell lines," *Haematologica*, vol. 96, no. 4, pp. 574–582, 2011.

[54] D. Hose, T. Rème, T. Hielscher et al., "Proliferation is a central independent prognostic factor and target for personalized and risk-adapted treatment in multiple myeloma," *Haematologica*, vol. 96, no. 1, pp. 87–95, 2011.

[55] R. Kuiper, A. Broyl, Y. De Knegt et al., "A gene expression signature for high-risk multiple myeloma," *Leukemia*, vol. 26, no. 11, pp. 2406–2413, 2012.

[56] T.-H. Chung, G. Mulligan, R. Fonseca, and W. J. Chng, "A novel measure of chromosome instability can account for prognostic difference in multiple myeloma," *PLoS ONE*, vol. 8, no. 6, Article ID e66361, 2013.

[57] W. J. Chng, A. Dispenzieri, C. S. Chim et al., "IMWG consensus on risk stratification in multiple myeloma," *Leukemia*, vol. 28, pp. 269–277, 2014.

[58] L. R. Yates and P. J. Campbell, "Evolution of the cancer genome," *Nature Reviews Genetics*, vol. 13, no. 11, pp. 795–806, 2012.

[59] N. Bolli, H. Avet-Loiseau, D. C. Wedge et al., "Heterogeneity of genomic evolution and mutational profiles in multiple myeloma," *Nature Communications*, vol. 5, article 2997, 2014.

[60] J. J. Keats, M. Chesi, J. B. Egan et al., "Clonal competition with alternating dominance in multiple myeloma," *Blood*, vol. 120, no. 5, pp. 1067–1076, 2012.

[61] J. B. Egan, C.-X. Shi, W. Tembe et al., "Whole-genome sequencing of multiple myeloma from diagnosis to plasma cell leukemia reveals genomic initiating events, evolution, and clonal tides," *Blood*, vol. 120, no. 5, pp. 1060–1066, 2012.

[62] J. M. C. Tubio and X. Estivill, "Cancer: when catastrophe strikes a cell," *Nature*, vol. 470, no. 7335, pp. 476–477, 2011.

[63] F. Magrangeas, H. Avet-Loiseau, N. C. Munshi, and S. Minvielle, "Chromothripsis identifies a rare and aggressive entity among newly diagnosed multiple myeloma patients," *Blood*, vol. 118, no. 3, pp. 675–678, 2011.

[64] B. A. Walker, C. P. Wardell, L. Melchor et al., "Intraclonal heterogeneity is a critical early event in the development of myeloma and precedes the development of clinical symptoms," *Leukemia*, vol. 28, pp. 384–390, 2014.

[65] M. Chesi, D. F. Robbiani, M. Sebag et al., "AID-dependent activation of a MYC transgene induces multiple myeloma in a conditional mouse model of post-germinal center malignancies," *Cancer Cell*, vol. 13, no. 2, pp. 167–180, 2008.

[66] B. Drewinko, R. Alexanian, H. Boyer, B. Barlogie, and S. I. Rubinow, "The growth fraction of human myeloma cells," *Blood*, vol. 57, no. 2, pp. 333–338, 1981.

[67] A. Hamburger and S. S. Salmon, "Primary bioassay of human myeloma stem cells," *Journal of Clinical Investigation*, vol. 60, no. 4, pp. 846–854, 1977.

[68] M. H. C. Bakkus, I. van Riet, B. van Camp, and K. Thielemans, "Evidence that the clonogenic cell in multiple myeloma originates from a pre-switched but somatically mutated B cell," *British Journal of Haematology*, vol. 87, no. 1, pp. 68–74, 1994.

[69] L. M. Pilarski and A. R. Belch, "Clonotypic myeloma cells able to xenograft myeloma to nonobese diabetic severe combined immunodeficient mice copurify with CD34+ hematopoietic progenitors," *Clinical Cancer Research*, vol. 8, no. 10, pp. 3198–3204, 2002.

[70] W. Matsui, C. A. Huff, Q. Wang et al., "Characterization of clonogenic multiple myeloma cells," *Blood*, vol. 103, no. 6, pp. 2332–2336, 2004.

[71] J. Epstein, "Myeloma stem cell phenotype: implications for treatment," *Hematology/Oncology Clinics of North America*, vol. 11, no. 1, pp. 43–49, 1997.

[72] M. M. Gottesman, T. Fojo, and S. E. Bates, "Multidrug resistance in cancer: role of ATP-dependent transporters," *Nature Reviews Cancer*, vol. 2, no. 1, pp. 48–58, 2002.

[73] R. Reghunathan, C. Bi, S. C. Liu et al., "Clonogenic multiple myeloma cells have shared stemness signature assocuated with patient survival," *Oncotarget*, vol. 4, pp. 1230–1240, 2013.

[74] T. Rasmussen, L. Jensen, and H. E. Johnsen, "The clonal hierachy in multiple myeloma," *Acta Oncologica*, vol. 39, no. 7, pp. 765–770, 2000.

[75] K. Boucher, N. Parquet, R. Widen et al., "Stemness of B-cell progenitors in multiple myeloma bone marrow," *Clinical Cancer Research*, vol. 18, no. 22, pp. 6155–6168, 2012.

[76] A. Chaidos, C. P. Barnes, G. Cowan et al., "Clinical drug resistance linked to interconvertible phenotypic and functional states of tumor-propagating cells in multiple myeloma," *Blood*, vol. 121, no. 2, pp. 318–328, 2013.

[77] C. Leung-Hagesteijn, N. Erdmann, G. Cheung et al., "Xbp1s-negative tumor B cells and pre-plasmablasts mediate therapeutic proteasome inhibitor resistance in multiple myeloma," *Cancer Cell*, vol. 24, pp. 289–304, 2013.

[78] Y. Yang, J. Shi, G. Tolomelli et al., "RARalpha2 expression confers myeloma stem cell features," *Blood*, vol. 122, pp. 1437–1447, 2013.

[79] A. Kassambara, D. Hose, J. Moreaux et al., "Identification of pluripotent and adult stem cell genes unrelated to cell cycle and associated with poor prognosis in multiple myeloma," *PLoS ONE*, vol. 7, no. 7, Article ID e42161, 2012.

[80] B. Paiva, M.-B. Vidriales, J. Cerveró et al., "Multiparameter flow cytometric remission is the most relevant prognostic factor for multiple myeloma patients who undergo autologous stem cell transplantation," *Blood*, vol. 112, no. 10, pp. 4017–4023, 2008.

[81] A. C. Rawstron, J. A. Child, R. M. de Tute et al., "Minimal residual disease assessed by multiparameter flow cytometry in multiple myeloma: impact on outcome in the Medical Research Council Myeloma IX Study," *Journal of Clinical Oncology*, vol. 31, pp. 2540–2547, 2013.

[82] M. E. Sarasquete, R. García-Sanz, D. González et al., "Minimal residual disease monitoring in multiple myeloma: a comparison between allelic-specific oligonucleotide real-time quantitative polymerase chain reaction and flow cytometry," *Haematologica*, vol. 90, no. 10, pp. 1365–1372, 2005.

[83] E. Zamagni, F. Patriarca, C. Nanni et al., "Prognostic relevance of 18-F FDG PET/CT in newly diagnosed multiple myeloma

patients treated with up-front autologous transplantation," *Blood*, vol. 118, no. 23, pp. 5989–5995, 2011.

[84] W. Matsui, Q. Wang, J. P. Barber et al., "Clonogenic multiple myeloma progenitors, stem cell properties, and drug resistance," *Cancer Research*, vol. 68, no. 1, pp. 190–197, 2008.

[85] C. D. Peacock, Q. Wang, G. S. Gesell et al., "Hedgehog signaling maintains a tumor stem cell compartment in multiple myeloma," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 104, no. 10, pp. 4048–4053, 2007.

[86] D. Chiron, S. Maïga, G. Descamps et al., "Critical role of the NOTCH ligand JAG2 in self-renewal of myeloma cells," *Blood Cells, Molecules, and Diseases*, vol. 48, no. 4, pp. 247–253, 2012.

[87] S. K. Brennan, Q. Wang, R. Tressler et al., "Telomerase inhibition targets clonogenic multiple myeloma cells through telomere length-dependent and independent Mechanisms," *PLoS ONE*, vol. 5, no. 9, Article ID e12487, 2010.

[88] B. Björkstrand, P. Ljungman, H. Svensson et al., "Allogeneic bone marrow transplantation versus autologous stem cell transplantation in multiple myeloma: a retrospective case-matched study from the European Group for Blood and Marrow Transplantation," *Blood*, vol. 88, no. 12, pp. 4711–4718, 1996.

[89] B. E. Swift, B. A. Williams, Y. Kosaka et al., "Natural killer cell lines preferentially kill clonogenic multiple myeloma cells and decrease myeloma engraftment in a bioluminescent xenograft mouse model," *Haematologica*, vol. 97, no. 7, pp. 1020–1028, 2012.

Hindawi Publishing Corporation
BioMed Research International
Volume 2014, Article ID 636514, 5 pages
http://dx.doi.org/10.1155/2014/636514

Review Article

MMSET: Role and Therapeutic Opportunities in Multiple Myeloma

Zhigang Xie[1] and Wee Joo Chng[1,2,3]

[1] *Cancer Science Institute of Singapore, National University of Singapore, 14 Medical Drive, Singapore 117599*
[2] *Department of Medicine, Yong Loo Lin School of Medicine, National University of Singapore, 1E Kent Ridge Road, Singapore 119228*
[3] *Department of Haematology-Oncology, National University Cancer Institute of Singapore, National University Health System, Singapore 119228*

Correspondence should be addressed to Wee Joo Chng; mdccwj@nus.edu.sg

Received 17 March 2014; Accepted 16 June 2014; Published 1 July 2014

Academic Editor: Dong Soon Lee

Recurrent chromosomal translocations are central to the pathogenesis, diagnosis, and prognosis of hematologic malignancies. The translocation t(4; 14)(p16; q32) is one of the most common translocations in multiple myeloma (MM) and is associated with very poor prognosis. The t(4; 14) translocation leads to the simultaneous overexpression of two genes, *FGFR3* (fibroblast growth factor receptor 3) and *MMSET* (multiple myeloma SET domain), both of which have potential oncogenic activity. However, approximately 30% of t(4; 14) MM patients do not express FGFR3 and have poor prognosis irrespective of FGFR3 expression, whereas MMSET overexpression is universal in t(4; 14) cases. In this review, we provide an overview of recent findings regarding the oncogenic roles of MMSET in MM and its functions on histone methylation. We also highlight some of MMSET partners and its downstream signalling pathways and discuss the potential therapeutics targeting MMSET.

1. Introduction

Compelling data has emerged that epigenetic changes underlie a wide variety of pathologies, including cancer [1, 2]. Epigenetic regulation includes DNA methylation and covalent histone modifications. These processes may play an important role in the initiation and progression of many cancers, including the haematological malignancy multiple myeloma (MM).

MMSET (multiple myeloma SET domain), also known as Wolf-Hirschhorn syndrome candidate 1 (WHSC1) or nuclear receptor-binding SET domain 2 (NSD2), is a member of the NSD histone methyltransferase (HMT) family also including NSD1 and NSD3 [3–5]. The *MMSET* gene spans 120 kb, consists of 24 exons, and undergoes complex alternative splicing. Two major transcripts were identified: type I encodes a protein of 647 amino acids and type II encodes a protein of 1365 amino acids. Both proteins share a common amino terminus [6]. A third transcript initiated within a middle intron of *MMSET* encodes a protein named RE-IIBP [7] (Figure 1). Conserved domain architecture analysis indicated that MMSET is a multidomain protein containing a set domain (determining protein lysine methyltransferase activity), 2 PWWP (named for a conserved Pro-Trp-Trp-Pro motif) domains, a HMG (high mobility group) box, and 3 PHD (plant homeodomain) fingers (Figure 1). The PWWP, HMG, and PHD domains are responsible for nuclear localization, DNA-binding, and recognition of histone marks [8–11]. The importance of MMSET in malignancy was first highlighted by characterization of the t(4; 14) translocation in about 15% of MM, which fuses the *MMSET* gene to the immunoglobulin heavy-chain promoter/enhancer, leading to dramatic upregulation of MMSET expression [6, 12].

2. Oncogenic Role of MMSET in MM

Recurrent chromosomal translocations are central to the pathogenesis, diagnosis, and prognosis of hematologic malignancies. In the past decade, it has become apparent that approximately 50% of MM harbor recurrent translocations

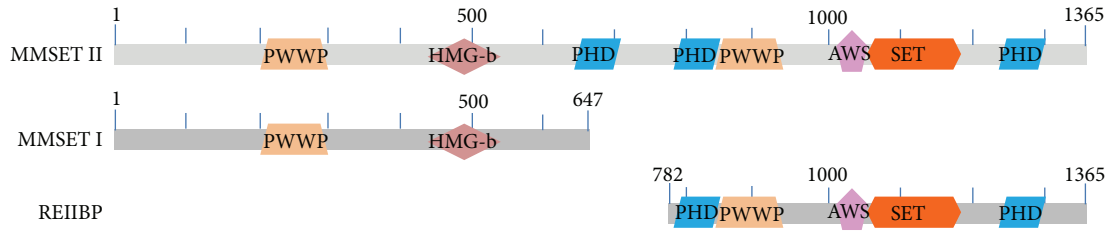


Figure 1: *Schematic primary structure of three major MMSET isoforms*. PWWP, named for a conserved Pro-Trp-Trp-Pro motif; HMG-b, high mobility group (HMG) box domain; PHD, plant homeodomain zinc finger; AWS, associated with SET domain; and SET, lysine methyltransferase catalytic domain. Conserved domain architecture was analyzed through http://blast.ncbi.nlm.nih.gov.

involving the immunoglobulin heavy chain (IgH) locus on chromosome 14q32 [13–15]. The translocation t(4; 14)(p16; q32) is one of the most common translocations in MM, affecting 15% of patients, and is associated with very poor prognosis [16]. The t(4; 14) translocation leads to the simultaneous overexpression of two genes, *FGFR3* (fibroblast growth factor receptor 3) and *MMSET*. FGFR3 has transforming activity *in vitro* and *in vivo*, but approximately 30% of t(4; 14) MM patients do not express FGFR3, whereas overexpression of MMSET isoforms is a universal feature of t(4; 14) cases [12, 17, 18]. Furthermore, the poor prognosis of t(4; 14) persists irrespective of FGFR3 expression [12]. These data suggest that MMSET may be the critical oncogene in this translocation. Downregulation of MMSET expression in MM cell lines indicated that t(4; 14) MM cells rely on MMSET expression for clonogenic growth and tumorigenicity *in vivo* [13]. These results provide the first direct evidence that MMSET is an oncogene and plays a significant role in t(4; 14) MM. Later several groups reported that depletion of MMSET could also inhibit proliferation and induce cell cycle arrest and apoptosis [19–22]. Consistent with MMSET knockdown results, wild type MMSET, but not the MMSET catalytic mutants, could restore proliferation and colony formation of t(4; 14) MM cells upon MMSET deletion. Furthermore, complementation of MMSET knockout cells with wild type MMSET instead of catalytic mutants could restore their tumorigenicity *in vivo* [10, 22]. These data further conformed that MMSET is an oncogene and its oncogenic role is dependent on its catalytic activity. Recent study has shown that MMSET mRNA level is also upregulated in 15 of 40 tumor types compared to their normal tissue counterparts. Furthermore, MMSET mRNA levels are associated with tumor aggressiveness or prognosis in several of these tumors [23]. Thus, in addition to t(4; 14) MM, MMSET may contribute to the development of other cancer types.

3. MMSET Function on Histone Methylation

Histones are the stage of diverse posttranslational modifications that ultimately regulates the gene transcription. Lysine methylation is one prominent feature of the posttranslational histone modifications in the regulation of chromatin structure and function. Lysine-HMTs target specific histone residues on H3 and H4 and can transfer one, two, or three methyl groups on specific lysines on the histone tails [24]. The MMSET protein contains AWS (associated with set) SET-PostSET domains that are highly conserved with yeast H3K36-specific methyltransferase Set2 [25]. It is first reported that the MMSET protein is a H4K20 methyltransferase with characteristics of a transcriptional corepressor [19]. Later, several reports suggested that MMSET could generate numerous different histone marks, including H3K4me2, H3K9me2, H3K27me3, H3K36me2, H3K36me3, and H4K20me2 [26–30]. Biologically, MMSET is reported to repress transcription through generation of H4K20me3 [19], H3K27me3 [27], or H3K36me3 [29], to enhance transcription through generation of H3K4me2 [26] and H3K36me2 [21], and to mediate accumulation of 53BP1 to DNA damage sites through generation of H4K20me2 [30]. Recently multiple independent biochemical and cellular approaches were used to investigate and resolve the discrepancies regarding MMSET enzymatic activity. It is suggested that the principal physiologic activity of MMSET at chromatin is dimethylation of H3K36, and in the process rules out generation of H3K36me3, H4K20me2, and several other putative methyl products of MMSET [22]. This conclusion is in agreement with a study reporting *in vitro* dimethylation activity at H3K36 by the three NSD family members NSD1, NSD2, and NSD3/WHSC1L1 [28].

4. MMSET Interacting Proteins and Downstream Targets

A preliminary step in understanding protein structure and function is to determine which proteins interact with each other, thereby identifying the relevant biological pathways. MMSET is a multidomain protein containing a catalytic set domain and other highly conserved domains, such as PWWP, PHD, and HMG-box, to mediate chromatin interaction and recognition of histone marks. Coprecipitation experiments indicated that MMSET could interact with histone modifiers HDAC1, HDAC2, and LSD1 [19, 31]. SET domain containing methyltransferases seems to be particularly sensitive to the sequence and posttranslational modifications surrounding the target lysine site [32]. Hence, the histone modification signature of MMSET is complex and might be mediated both by direct action of MMSET and by interplay with other histone modifiers. To determine the genes regulated by MMSET, Martinez-Garcia et al. profiled gene expression

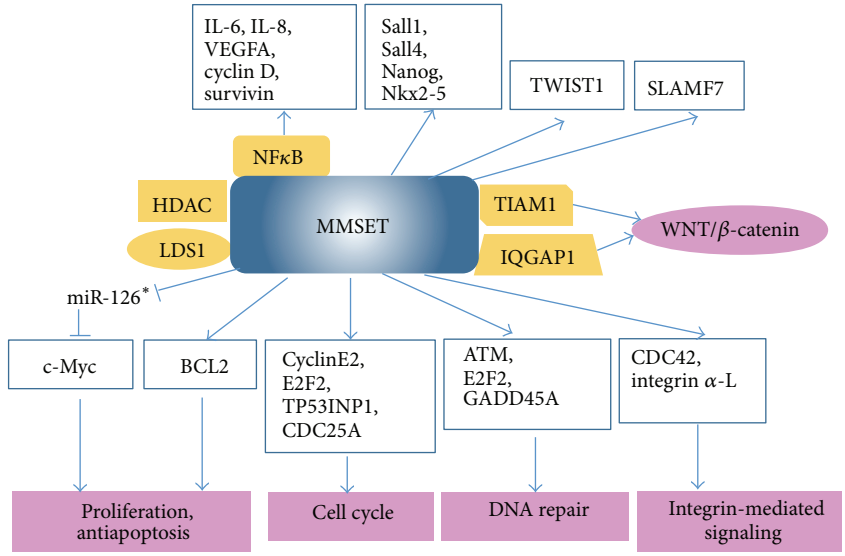


Figure 2: *MMSET interacting proteins and downstream targets.* MMSET interacts with its partners and activates oncogenic signalling pathways, which establish causal roles for MMSET in driving cancer initiation, development, and survival. Orange boxes: MMSET interacting proteins. White boxes: MMSET target genes.

in the loss-of-function (MMSET knockdown) and gain-of-function (MMSET reexpression in the MMSET knockout cell line) systems [21]. Signaling pathway analysis indicated that MMSET could regulate cell death and the p53 pathway (e.g., BAX, BCL2, and caspase 6), the cell cycle (cyclin E2, E2F2, TP53INP1, and CDC25A), genes for DNA repair (ATM, E2F2, and GADD45A), and integrin-mediated signaling (CDC42 and integrin alpha-L). These results are consistent with phenotypes of proliferation inhibition and apoptosis induction upon MMSET knockdown in t(4; 14) MM cells. Recently a microRNA (miRNA) profiling in t(4; 14) MM cells identified miR-126* as an MMSET-regulated miRNA, which could specifically target the 3′-untranslated region of c-Myc and inhibit its translation [33]. Moreover, the expression of miR-126* was sufficient to decrease the proliferation rate of t(4; 14) MM cells. Chromatin immunoprecipitation (ChIP) analysis showed that MMSET binds to the miR-126* promoter along with the KAP1 corepressor and histone deacetylases to repress miR-126* transcription. Through quantitative mass spectrometry analysis we found that overexpression of SLAMF7 (also known as CS1) was associated with MMSET overexpression in t(4; 14) MM cells [34]. Quantitative RT-PCR and ChIP analysis indicated that MMSET might regulate the transcription level of SLAMF7 and be an important functional element for *SLAMF7* promoter activity.

Nimura et al. found that MMSET is associated with the cell-type-specific transcription factors Sall1, Sall4, and Nanog in embryonic stem cells (ESCs) and Nkx2-5 in embryonic hearts [29]. These results suggested that MMSET functions together with developmental transcription factors to prevent the inappropriate transcription that can lead to various pathophysiologies. Immunoprecipitation combined with mass spectrometry analysis revealed IQGAP1 and TIAM1 as candidate interacting partners with MMSET, and these interactions were confirmed by coimmunoprecipitation [35]. IQGAP1 and TIAM1 are both involved in the WNT signaling pathway through interaction with β-catenin protein. Gene expression array and real-time PCR analysis indicated that the expression levels of CCND1, an established downstream target of the β-catenin/Tcf-4 complex, were reduced significantly upon MMSET knockdown. ChIP analysis showed that MMSET bounds the promoter region of CCND1 [35]. These results suggested that MMSET may regulate the WNT signaling pathway through interaction with β-catenin. Yang et al. reported that MMSET is a strong coactivator of NFκB by directly interacting with NFκB for activation of target genes, including those for interleukin-6 (IL-6), IL-8, vascular endothelial growth factor A (VEGFA), cyclin D, Bcl-2, and survivin, in castration-resistant prostate cancer (CRPC) cells [36]. They also found that MMSET is critical for cytokine-induced recruitment of NFκB and acetyltransferase p300 and histone hyperacetylation. Ezponda et al. reported that MMSET could activate TWIST1 to promote an epithelial-mesenchymal transition and invasion in prostate cancer [37]. Whether the aforementioned MMSET interacting proteins and downstream targets in ESCs and solid tumors play critical roles in t(4; 14) MM remains to be determined. The MMSET interacting proteins and downstream targets were summarized in Figure 2.

5. Potential Therapeutics Targeting MMSET or Its Downstream Signalling

MMSET overexpression is a universal feature of t(4; 14) MM. Furthermore, t(4; 14) MM cells rely on MMSET expression for proliferation, survival, and tumorigenicity *in vivo*. These findings highlight MMSET as an attractive target for the treatment of t(4; 14) MM. HMT activity of MMSET is essential for its oncogenic function, so it would seem straightforward to design small molecular inhibitors to target MMSET substrate

binding pocket. However, very few lead compounds have been published to selectively inhibit MMSET catalytic function. The development of MMSET inhibitors might be hampered by the lack of crystallographic structural information on enzyme-substrate complexes. Recently Cao et al. reported a novel strategy for selective targeting of HMT activity [38]. Instead of directly targeting the catalytic SET domain, they exploit the unique regulation of the MLL1 complex by WDR5 and target MLL1 complex assembly without affecting other MLL family HMTs. As a result, the compound MM-401 shows no inhibition for global H3K4me and little toxicity for normal cells. Transcriptome analyses also confirmed the remarkable selectivity of MM-401, which induces changes in gene expression that are highly correlative with MLL1 gene deletion. MMSET is multidomain protein and several of its partners have been identified, so it is a promising strategy to target MMSET complex assembly for inhibitor development. Numerous HDAC inhibitors are being developed, some of which are at clinical trial stages for MM therapy [39]. These HDAC inhibitors might be promising candidates to inhibit MMSET complex assembly, because MMSET associates with HDACs in a large complex [19, 27, 31].

Sequence-specific gene silencing with small interfering RNA (siRNA) has transformed basic science research, and the efficacy of siRNA therapeutics toward a variety of diseases is now being evaluated in preclinical and clinical trials [40]. The key therapeutic advantage of using siRNA lies in its ability to specifically and potently knock down the expression of disease-causing genes of known sequence. However, clinical use of siRNAs encounters one of the obstacles: delivery of siRNAs to the appropriate cells. Antibody-mediated delivery is an effective method of targeting siRNA to particular cells [41]. Our study showed that SLAMF7 overexpression in t(4; 14) MM was associated with MMSET expression [34]. Thus, it is potential to develop t(4; 14) MM targeted therapy by SLAMF7 antibody mediated MMSET siRNA delivery. This therapeutic strategy will achieve two levels of targeting for t(4; 14) MM: tumor cell selective delivery by the SLAMF7 antibody and gene pathway selectivity by the MMSET siRNA. Furthermore, it was found that, similar to the effects of knocking down MMSET, overexpression of miR-126* could inhibit proliferation of t(4; 14) MM cells *in vitro*. It will be appealing to test whether expression of miR-126* can cause therapeutic effects *in vivo*.

6. Conclusion

The outcome of MM patients has dramatically improved in recent years and this has been possible essentially due to the introduction of the new active agents thalidomide, bortezomib, and lenalidomide, autologous stem-cell transplantation (ASCT), and improvements in supportive care [42]. The median survival is in excess of 5 years. Nevertheless, MM is still considered an incurable disease in the vast majority of patients and the classical pattern of evolution of the disease is of subsequent responses/relapses, with each relapse generally being of shorter duration than the previous ones [43]. Therefore, additional potent therapeutic strategies are urgently needed. The translocation t(4; 14) is one of the most common translocations in MM and is associated with very poor prognosis. MMSET overexpression characterizes all t(4; 14) MM patients, and furthermore MMSET protein is required for t(4; 14) MM cell survival *in vitro* and *in vivo*. The key role of MMSET in t(4; 14) MM allows speculating that a specific pharmacological therapy, which targets this protein, may constitute a novel approach to the treatment of MM.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

Acknowledgments

This work was supported by the National Medical Research Council of Singapore (http://www.nmrc.gov.sg/) Grant NMRC/BNIG/2006/2013. Wee Joo Chng is supported by NMRC Clinician Scientist Investigator award. This work was partly supported by the National Research Foundation Singapore and the Singapore Ministry of Education under its Research Centres of Excellence initiative.

References

[1] M. Rodríguez-Paredes and M. Esteller, "Cancer epigenetics reaches mainstream oncology," *Nature Medicine*, vol. 17, no. 3, pp. 330–339, 2011.

[2] M. A. Dawson and T. Kouzarides, "Cancer epigenetics: from mechanism to therapy," *Cell*, vol. 150, no. 1, pp. 12–27, 2012.

[3] P. Angrand, F. Apiou, A. F. Stewart, B. Dutrillaux, R. Losson, and P. Chambon, "*NSD3*, a new SET domain-containing gene, maps to 8p12 and is amplified in human breast cancer cell lines," *Genomics*, vol. 74, no. 1, pp. 79–88, 2001.

[4] I. Stec, T. J. Wright, G. J. van Ommen et al., "WHSC1, a 90 kb SET domain-containing gene, expressed in early development and homologous to a Drosophila dysmorphy gene maps in the Wolf-Hirschhorn syndrome critical region and is fused to IgH in t(4;14) multiple myeloma," *Human Molecular Genetics*, vol. 7, pp. 1071–1082, 1998.

[5] N. Huang, E. vom Baur, J. Garnier et al., "Two distinct nuclear receptor interaction domains in NSD1, a novel SET protein that exhibits characteristics of both corepressors and coactivators," *The EMBO Journal*, vol. 17, no. 12, pp. 3398–3412, 1998.

[6] M. Chesi, E. Nardini, R. S. C. Lim, K. D. Smith, W. Michael Kuehl, and P. L. Bergsagel, "The t(4;14) translocation in myeloma dysregulates both FGFR3 and a novel gene, MMSET, resulting in IgH/MMSET hybrid transcripts," *Blood*, vol. 92, no. 9, pp. 3025–3034, 1998.

[7] C. G. Garlisi, A. S. Uss, H. Xiao et al., "A unique mRNA initiated within a middle intron of WHSC1/MMSET encodes a DNA binding protein that suppresses human IL-5 transcription," *American Journal of Respiratory Cell and Molecular Biology*, vol. 24, no. 1, pp. 90–98, 2001.

[8] P. V. Peña, F. Davrazou, X. Shi et al., "Molecular mechanism of histone H3K4me3 recognition by plant homeodomain of ING2," *Nature*, vol. 442, no. 7098, pp. 100–103, 2006.

[9] H. Wu, H. Zeng, R. Lam et al., "Structural and histone binding ability characterizations of human PWWP domains," *PLoS ONE*, vol. 6, no. 6, Article ID e18919, 2011.

[10] Z. Huang, H. Wu, S. Chuai et al., "NSD2 is recruited through its PHD domain to oncogenic gene loci to drive multiple myeloma," *Cancer Research*, vol. 73, pp. 6277–6288, 2013.

[11] M. Štros, D. Launholt, and K. D. Grasser, "The HMG-box: a versatile protein domain occurring in a wide variety of DNA-binding proteins," *Cellular and Molecular Life Sciences*, vol. 64, no. 19-20, pp. 2590–2606, 2007.

[12] J. J. Keats, C. A. Maxwell, B. J. Taylor et al., "Overexpression of transcripts originating from the MMSET locus characterizes all t(4;14)(p16;q32)-positive multiple myeloma patients," *Blood*, vol. 105, no. 10, pp. 4060–4069, 2005.

[13] J. Lauring, A. M. Abukhdeir, H. Konishi et al., "The multiple myeloma-associated MMSET gene contributes to cellular adhesion, clonogenic growth, and tumorigenicity," *Blood*, vol. 111, no. 2, pp. 856–864, 2008.

[14] P. L. Bergsagel and W. M. Kuehl, "Molecular pathogenesis and a consequent classification of multiple myeloma," *Journal of Clinical Oncology*, vol. 23, no. 26, pp. 6333–6338, 2005.

[15] P. L. Bergsagel and W. M. Kuehl, "Critical roles for immunoglobulin translocations and cyclin D dysregulation in multiple myeloma," *Immunological Reviews*, vol. 194, pp. 96–104, 2003.

[16] J. J. Keats, T. Reiman, A. R. Belch, and L. M. Pilarski, "Ten years and counting: so what do we know about t(4;14)(p16;q32) multiple myeloma," *Leukemia and Lymphoma*, vol. 47, no. 11, pp. 2289–2300, 2006.

[17] J. J. Keats, T. Reiman, C. A. Maxwell et al., "In multiple myeloma, t(4;14)(p16;q32) is an adverse prognostic factor irrespective of FGFR3 expression," *Blood*, vol. 101, no. 4, pp. 1520–1529, 2003.

[18] M. Santra, F. Zhan, E. Tian, B. Barlogie, and J. Shaughnessy Jr., "A subset of multiple myeloma harboring the t(4;14)(p16;q32) translocation lacks *FGFR3* expression but maintains an *IGH/MMSET* fusion transcript," *Blood*, vol. 101, no. 6, pp. 2374–2376, 2003.

[19] J. Marango, M. Shimoyama, H. Nishio et al., "The MMSET protein is a histone methyltransferase with characteristics of a transcriptional corepressor," *Blood*, vol. 111, no. 6, pp. 3145–3154, 2008.

[20] J. L. R. Brito, B. Walker, M. Jenner et al., "MMSET deregulation affects cell cycle progression and adhesion regulons in t(4;14) myeloma plasma cells," *Haematologica*, vol. 94, no. 1, pp. 78–86, 2009.

[21] E. Martinez-Garcia, R. Popovic, D. Min et al., "The MMSET histone methyl transferase switches global histone methylation and alters gene expression in t(4;14) multiple myeloma cells," *Blood*, vol. 117, no. 1, pp. 211–220, 2011.

[22] A. J. Kuo, P. Cheung, K. Chen et al., "NSD2 links dimethylation of histone H3 at lysine 36 to oncogenic programming," *Molecular Cell*, vol. 44, no. 4, pp. 609–620, 2011.

[23] A. Kassambara, B. Klein, and J. Moreaux, "MMSET is overexpressed in cancers: link with tumor aggressiveness," *Biochemical and Biophysical Research Communications*, vol. 379, no. 4, pp. 840–845, 2009.

[24] M. Morishita and E. di Luccio, "Cancers and the NSD family of histone lysine methyltransferases," *Biochimica et Biophysica Acta*, vol. 1816, no. 2, pp. 158–163, 2011.

[25] X. J. Sun, J. Wei, X. Y. Wu et al., "Identification and characterization of a novel human histone H3 lysine 36-specific methyltransferase," *Journal of Biological Chemistry*, vol. 280, no. 42, pp. 35261–35271, 2005.

[26] H. B. Kang, Y. Choi, J. M. Lee et al., "The histone methyltransferase, NSD2, enhances androgen receptor-mediated transcription," *FEBS Letters*, vol. 583, no. 12, pp. 1880–1886, 2009.

[27] J. Y. Kim, J. K. Hae, N. W. Choe et al., "Multiple myeloma-related WHSC1/MMSET isoform RE-IIBP is a histone methyltransferase with transcriptional repression activity," *Molecular and Cellular Biology*, vol. 28, no. 6, pp. 2023–2034, 2008.

[28] Y. Li, P. Trojer, C. Xu et al., "The target of the NSD family of histone lysine methyltransferases depends on the nature of the substrate," *The Journal of Biological Chemistry*, vol. 284, no. 49, pp. 34283–34295, 2009.

[29] K. Nimura, K. Ura, H. Shiratori et al., "A histone H3 lysine 36 trimethyltransferase links Nkx2-5 to Wolf-Hirschhorn syndrome," *Nature*, vol. 460, no. 7252, pp. 287–291, 2009.

[30] H. Pei, L. Zhang, K. Luo et al., "MMSET regulates histone H4K20 methylation and 53BP1 accumulation at DNA damage sites," *Nature*, vol. 470, no. 7332, pp. 124–128, 2011.

[31] K. Todoerti, D. Ronchetti, L. Agnelli et al., "Transcription repression activity is associated with the type I isoform of the *MMSET* gene involved in t(4;14) in multiple myeloma," *British Journal of Haematology*, vol. 131, no. 2, pp. 214–218, 2005.

[32] O. Binda, "On your histone mark, SET, methylate!," *Epigenetics*, vol. 8, no. 5, pp. 457–463, 2013.

[33] D. J. Min, T. Ezponda, M. K. Kim et al., "MMSET stimulates myeloma cell growth through microRNA-mediated modulation of c-MYC," *Leukemia*, vol. 27, no. 3, pp. 686–694, 2013.

[34] Z. Xie, J. Gunaratne, L. L. Cheong et al., "Plasma membrane proteomics identifies biomarkers associated with MMSET overexpression in T(4;14) multiple myeloma," *Oncotarget*, vol. 4, no. 7, pp. 1008–1018, 2013.

[35] G. Toyokawa, H. Cho, K. Masuda et al., "Histone lysine methyltransferase Wolf-Hirschhorn syndrome candidate 1 is involved in human carcinogenesis through regulation of the WNT pathway," *Neoplasia*, vol. 13, no. 10, pp. 887–898, 2011.

[36] P. Yang, L. Guo, Z. J. Duan et al., "Histone methyltransferase NSD2/MMSET mediates constitutive NF-kb signaling for cancer cell proliferation, survival, and tumor growth via a feed-forward loop," *Molecular and Cellular Biology*, vol. 32, no. 15, pp. 3121–3131, 2012.

[37] T. Ezponda, R. Popovic, M. Y. Shah et al., "The histone methyltransferase MMSET/WHSC1 activates TWIST1 to promote an epithelial-mesenchymal transition and invasive properties of prostate cancer," *Oncogene*, vol. 32, no. 23, pp. 2882–2890, 2013.

[38] F. Cao, E. C. Townsend, H. Karatas et al., "Targeting MLL1 H3K4 methyltransferase activity in mixed-lineage leukemia," *Molecular Cell*, vol. 53, no. 2, pp. 247–261, 2014.

[39] E. M. Smith, K. Boyd, and F. E. Davies, "The potential role of epigenetic therapy in multiple myeloma," *British Journal of Haematology*, vol. 148, no. 5, pp. 702–713, 2010.

[40] D. H. Kim and J. J. Rossi, "Strategies for silencing human disease using RNA interference," *Nature Reviews Genetics*, vol. 8, no. 3, pp. 173–184, 2007.

[41] K. F. Pirollo and E. H. Chang, "Targeted delivery of small interfering RNA: approaching effective cancer therapies," *Cancer Research*, vol. 68, no. 5, pp. 1247–1250, 2008.

[42] S. V. Rajkumar, "Treatment of multiple myeloma," *Nature Reviews Clinical Oncology*, vol. 8, no. 8, pp. 479–491, 2011.

[43] M. V. Mateos, E. M. Ocio, and J. F. San Miguel, "Novel generation of agents with proven clinical activity in multiple myeloma," *Seminars in Oncology*, vol. 40, pp. 618–633, 2013.

Hindawi Publishing Corporation
BioMed Research International
Volume 2014, Article ID 245247, 7 pages
http://dx.doi.org/10.1155/2014/245247

Clinical Study

Changes in Osteoblastic Activity in Patient Who Received Bortezomib as Second Line Treatment for Plasma Cell Myeloma: A Prospective Multicenter Study

Ki-Seong Eom,[1] Seok Jin Kim,[2] Je-Jung Lee,[3] Cheolwon Suh,[4] Jin Seok Kim,[5] Sung-Soo Yoon,[6] Byung Soo Kim,[7] Hye Jin Kang,[8] Young Jin Choi,[9] Chul Soo Kim,[10] Yang Soo Kim,[11] Jae-Yong Kwak,[12] Yoo Jin Kim,[1] Young Don Joo,[13] Yeung-Chul Mun,[14] Deog Yeon Jo,[15] Joon Seong Park,[16] Chi-Young Park,[17] Sung-Hyun Kim,[18] and Chang-Ki Min[1]

[1] *Division of Hematology, Department of Internal Medicine, Catholic Blood and Marrow Transplantation Center, Seoul St. Mary's Hospital, College of Medicine, The Catholic University of Korea, 222 Banpo-daero, Seocho-gu, Seoul 137-701, Republic of Korea*
[2] *Department of Medicine, Samsung Medical Center, Sungkyunkwan University School of Medicine, Seoul 135-710, Republic of Korea*
[3] *Department of Hematology-Oncology, Chonnam National University Medical School, Jeollanam-do 519-763, Republic of Korea*
[4] *Department of Oncology, Asan Medical Center, University of Ulsan College of Medicine, Seoul 138-736, Republic of Korea*
[5] *Department of Internal Medicine, Yonsei University College of Medicine 120-752, Republic of Korea*
[6] *Department of Internal Medicine, Seoul National University College of Medicine, Seoul 110-744, Republic of Korea*
[7] *Department of Medicine, Korea University Medical Center, Seoul 136-705, Republic of Korea*
[8] *Department of Internal Medicine, Korea Cancer Center Hospital, Seoul 139-706, Republic of Korea*
[9] *Department of Internal Medicine, Pusan National University School of Medicine, Busan 602-739, Republic of Korea*
[10] *Inha University Hospital, Incheon 400-711, Republic of Korea*
[11] *Departments of Hematology/Oncology and Internal Medicine, Kosin University Gospel Hospital, Busan 602-702, Republic of Korea*
[12] *Department of Internal Medicine, Chonbuk National University Hospital, Jeonju 561-712, Republic of Korea*
[13] *Department of Internal Medicine, Inje University College of Medicine, Busan 612-896, Republic of Korea*
[14] *Department of Internal Medicine, Ewha Womans University School of Medicine, Seoul 158-710, Republic of Korea*
[15] *Department of Internal Medicine, Chungnam National University College of Medicine, Daejeon 310-721, Republic of Korea*
[16] *Department of Hematology-Oncology, Ajou University Hospital, Suwon 443-380, Republic of Korea*
[17] *Department of Internal Medicine, Chosun University College of Medicine, Gwangju 501-717, Republic of Korea*
[18] *Department of Internal Medicine, Dong-A University College of Medicine, Busan 602-715, Republic of Korea*

Correspondence should be addressed to Chang-Ki Min; ckmin@catholic.ac.kr

Received 21 February 2014; Accepted 23 May 2014; Published 23 June 2014

Academic Editor: Dong Soon Lee

We conducted a prospective multicenter study identifying the role of bortezomib in patients with relapsed or refractory plasma cell myeloma (PCM) in bone resorption and formation via bone turnover markers. A total of 104 patients received at least 1 cycle of bortezomib. Most of them had advanced disease ($n = 89$). Among them, 75 patients completed 4 cycles of treatment. Most of the patients (81.7%) were treated in combination with steroid. After the 4th cycle treatment, 47 of 75 patients achieved CR, nCR, VGPR, and PR (64.4%), while 26 patients achieved less than PR (35.6%). The proportion of patients who achieved ≥ PR increased as patients received more treatment cycles, reaching 90% after the 8th cycle. DKK-1 levels decreased significantly posttreatment. Bone formation markers (bALP and OC) and osteoclast regulator such as sRANKL also decreased significantly. These findings were observed primarily in patients who received steroid and who had a longer disease duration. While sRANKL demonstrated significant reduction posttreatment, osteoprotegerin (OPG) level did not significantly change posttreatment, resulting in a decreased sRANKL/OPG ratio ($P = 0.037$). In conclusion, our clinical data suggest that treatment with bortezomib and steroid may rearrange the metabolic balance between osteoblast and osteoclast activities in PCM.

1. Introduction

Plasma cell myeloma (PCM) is a neoplasm of plasma cells characterized by the appearance of monoclonal immunoglobulin, that is, bone pain caused by osteolytic lesions and pathologic fracture, hypercalcemia, renal insufficiency, and anemia [1]. Prognosis of PCM is variable, with the survival ranging from several months to over 10 years.

Myeloma bone disease is the result of increased destruction of bone that cannot be compensated by new bone formation, which develops in approximately 80% of the patients. Myeloma cells activate osteoclasts through various osteoclast activating substances and suppress the activity of osteoblasts, causing an imbalance between bone resorption and formation. This imbalance induces myeloma-related bone problems, which are the most debilitating manifestation of the disease and have direct relationship with patient's quality of life. Therefore, controlling myeloma bone disease has been regarded to be an important goal of treatment. Currently, various types of bisphosphonate have been used for myeloma bone disease [2]. Bisphosphonates inhibit osteoclastic function which reduces bone resorption and bone pain, improve patient's performance, and preserve one's quality of life [3–5]. However, bisphosphonates are known to be associated with renal impairment [2] and an increase in the risk of osteonecrosis of the jaw [6]. Furthermore, these agents have been shown to have little impact on osteoblasts.

Bortezomib (Velcade) has been approved to treat PCM as it activates osteoblasts [7–13] and suppresses osteoclasts [7, 10, 11, 13–15], in addition to an antimyeloma effect, ultimately leading to bone formation [16–18]. Preclinical studies indicate that bortezomib induces mesenchyme stem cells to preferentially undergo osteoblastic differentiation, resulting in increased bone formation and rescue from bone loss [19]. Clinically, bortezomib containing treatment for relapsed or refractory PCM demonstrated an improvement in bone lesions on radiologic examination [20] with an association of direct bone anabolism. These studies indicate that bortezomib provides a differential advantage from other agents used for treatment of PCM. It should be preferentially considered as a treatment method as bone disease has significant impact on mortality and morbidity of the patients. Until now, there have been no other antimyeloma agents to have anabolic effect on bone. In clinical practice, however, most of treatment regimens contain steroid, which exerts differential effects on bone metabolism. As far as we know, there have not been much data exploring the role of bortezomib in combination with or without steroid specifically in bone metabolism. Therefore, we conducted a prospective multicenter study to identify the role of bortezomib along with steroid among patients with relapsed or refractory PCM in bone resorption and formation using bone turnover markers prior to and after treatment.

2. Patients and Methods

2.1. Patients. Eligible patients were at least 18 years old and treated with bortezomib as a second line treatment for relapsed or refractory PCM. All patients provided a written informed consent to blood sampling to measure serum bone markers before and after therapy. Exclusion criteria included hypersensitivity to bortezomib, inadequate organ function, and pregnancy. The study was approved by each institutional review board of the participation centers in accordance with the Declaration of Helsinki.

2.2. Study Design and Treatment. Between March 2008 and June 2009, this multicenter prospective study was conducted at 20 centers in the Republic of Korea to investigate the role of bortezomib in bone resorption and formation. The details on treatment schedule were given elsewhere [9]. Briefly, bortezomib was administered as intravenous bolus (1.3 mg/m^2 twice weekly in a 21-day cycle) in various combinations with other chemotherapeutic agents including steroid, thalidomide, or alkylating agents. If patients experienced grade 4 hematologic toxicities or nonhematologic toxicities ≥grade 3 other than peripheral neuropathy related to bortezomib, bortezomib was withheld until toxicity recovered to grade ≤1. Once the toxicity resolved, bortezomib was readministered with a reduced dose of 25% (1.3 mg/m^2 to 1.0 mg/m^2; 1.0 mg/m^2 to 0.7 mg/m^2).

The primary end point was changes in the levels of bone markers before and after 4 cycles of bortezomib infusion. Secondary end points included complete response (CR) rate, overall response rate, correlation between changes in bone markers and response rate, numeric rating scale (NRS) pain score, and safety profiles.

2.3. Assessment. Blood samples were obtained before and after bortezomib infusion at baseline, after 4 cycles, and after 5–8 cycles of treatment, respectively. Patients who received at least 4 cycles of bortezomib with blood samples from each bortezomib infusion were included for the evaluation. As an osteoclast regulator, soluble receptor activator of nuclear factor-κB ligand (sRANKL) and osteoprotegerin (OPG) were measured. Dickkopf-1 (DKK-1) was measured as an osteoblast inhibitor. Bone-specific alkaline phosphatase (bALP) and osteocalcin (OC) were measured as bone formation indices.

Assessment of response, relapse, and progression was based on serum and/or urine M-protein quantification, bone marrow evaluation, and skeletal survey using European Bone and Marrow Transplantation (EBMT) criteria [21] and International Myeloma Working Group (IMWG) criteria [22]. Pain was graded using NRS (numeric rating scale), and pain scale was measured before day 1 of each bortezomib cycle. The pain was regarded as mild, moderate, and severe if the visual analogue scale (VAS) was 0 to 3, 4 to 6, and 7 to 10, respectively.

2.4. Statistical Analysis. Differences between pre- and post-bortezomib values of the studied parameters were evaluated using the Wilcoxon rank sum test. Differences between pretreatment and posttreatment values within each group were analyzed. Results were considered statistically significant when $P < 0.05$.

3. Results

A total of 104 patients received cycle 1 bortezomib treatment (Table 1). Among them, 75 patients (72.1%) completed the 4th treatment cycle and 23 patients (22.1%) finished 8 cycles of treatment. The reasons for not completing the whole treatment cycle were the termination of the clinical study as decided by the investigators ($n = 18$), adverse drug reactions or death of any cause ($n = 16$), obtaining CR before the 8th treatment cycle ($n = 7$), withdrawal by patients ($n = 5$), loss of followup ($n = 3$), and other reasons ($n = 32$). The median of treatment cycles for all patients was 4.6 ± 2.2. The majority of the patients were stages II and III ($n = 89$, 85.6%) via the international staging system. The first line of treatment included vincristine + adriamycin + dexamethasone (VAD), melphalan + prednisolone (MP), and autologous stem cell transplantation (ASCT). Most of the patients received bortezomib in combination with other agents, with the most common one being steroid (81.7%).

3.1. Response to Bortezomib Treatment. Of 75 patients who finished the 4th cycle treatment, 64% of patients who finished the 4th cycle treatment achieved ≥PR, while the remaining 36% did not reach PR (Figure 1). Meanwhile, the proportion of patients who achieved ≥PR increased as patients received more treatment cycles, reaching 90% after the 8th cycle.

The median VAS for pain at baseline and after the 4th cycle was 3.1 ± 2.3 and 3.1 ± 2.2, respectively. Although there was no significant reduction in bone pain in terms of total VAS after cycle 4, the proportion of patients who reported severe bone pain (VAS ≥ 7) decreased from 11.5% to 6.7% after cycle 4. The number of skeletal lesions did not show significant changes (2.5 ± 1.9 at baseline, 2.5 ± 1.9 after cycle 4, and 3.3 ± 2.9 after cycle 8, resp.) after bortezomib treatment. Most of the patients experienced peripheral neuropathy with the progress of treatment, but the severity of peripheral neuropathy was grade I/II in most cases (58.7% after cycle 4 and 73.9% after cycle 8, resp.; Figure 2). The proportion of grade II neuropathy decreased while that of grade I neuropathy increased as treatment progressed.

3.2. Changes in Bone Markers. There were no significant differences in bone turnover markers (OC, bALP, DKK-1, sRANKL, OPG, and sRANKL/OPG ratio) at baseline according to gender and age except for OPG. OPG levels were significantly higher in patients aged ≥65 years ($P = 0.002$). Changes in the level of bone markers before treatment and posttreatment are shown in Table 2. sRANKL demonstrated a significant reduction posttreatment ($P = 0.011$), and OPG levels did not change significantly, resulting in a decreased sRANKL/OPG ratio ($P = 0.037$). Despite a significant decrease in levels of DKK-1 ($P = 0.035$), an osteoblastic inhibitor, after bortezomib treatment, the levels of OC and bALP were also significantly lower posttreatment ($P < 0.0001$ and 0.004, resp.). The inconsistency in changes of DKK-1 and OC/bALP may result from the long disease duration (relapsed and/or refractory status) and advanced disease status (stages III and IV, 85.6%). The type of agents used in combination with bortezomib also could be associated with the discordance. Therefore, we analyzed the changes in OC and bALP before and after bortezomib treatment according to disease duration and combination with corticosteroid. The levels of OC and bALP decreased more significantly in longer diagnosis-treatment period group (≥6 months, longer prevalence period group) than those in the shorter group (Table 3). Influences of steroid in combination with bortezomib on bone markers are also shown in Table 3. Serum OC and bALP levels decreased significantly in the

Table 1: Patient characteristics.

Total enrolled patients, *n* (%)	104
Cycle 1	104 (100.0)
Cycle 4	75 (72.1)
Cycles of bortezomib treatment, mean ± SD	4.6 ± 2.2
Sex, *n*, M/F (%)	56/48 (53.9/46.1)
Age at treatment, *n*, <65/≥65 (%)	67/37 (64.4/35.6)
ECOG performance status, *n* (%)	
0	23 (22.1)
1	56 (53.9)
2	23 (22.1)
3	2 (1.9)
4	0
Quantity of M-protein (g/L) in SPEP, median (range)	1.3 (0.0–6.7)
Hb ≤ 10.0 g/dL, *n*/median (%/range)	41/10.7 (39.4/6.9–15.3)
Platelet, median, *n* (range)	203.0 (30.0–760.0)
Creatinine, median (range)	0.98 (0.5–5.6)
Calcium (g/dL), median (range)	8.7 (6.5–11.1)
LDH > 472 IU/L, *n*/median (%/range)	20/350.0 (19.2/15.0–1452.0)
Albumin (g/L), median (range)	3.7 (2.4–6.4)
Stage, *n* (%) Durie-Salmon Staging System	
I	13 (12.5)
II	21 (20.2)
III	70 (67.3)
Stage, *n* (%) International Staging System	
I	15 (14.4)
II	44 (42.3)
III	45 (43.3)
Previous treatment, *n* (%)	
VAD	37 (35.6)
MP	30 (28.9)
Dexamethasone	29 (27.9)
ASCT	21 (20.2)
Thalidomide	19 (18.3)
Others	21 (20.2)

VAD: vincristine + Adriamycin + dexamethasone; MP: melphalan + prednisolone; ASCT: autologous stem cell transplantation.

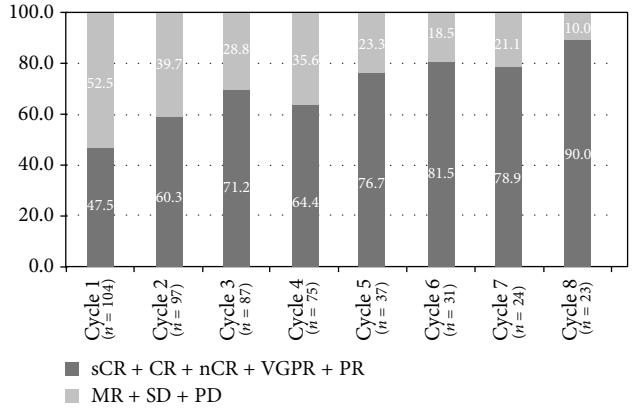


Figure 1: Response to bortezomib treatment.

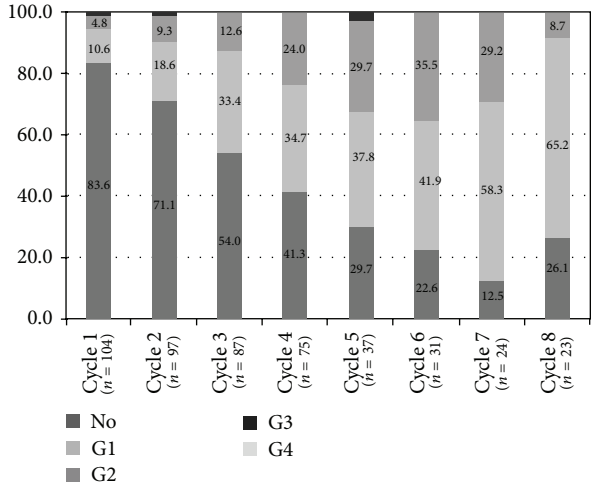


Figure 2: Peripheral neuropathy according to bortezomib treatment cycles.

steroid combination group ($P = 0.0002$ and 0.001, resp.), while the same bone marker changes were not significant in the bortezomib single-treatment group.

4. Discussion

There have been multiple studies of bortezomib, alone and in combination, in both previously untreated and relapsed/refractory PCM patients and its effect on bone remodeling markers [8, 10, 12, 13, 18, 23–26]. Most studies revealed that biomarkers of osteoblastic activity increased and the levels of osteoblast inhibitors decreased, while the levels of markers of osteoclastic activity have also been shown to decrease. Bortezomib treatment is associated with increased ALP and OC, while depressing sRANKL and OPG. Additionally, bortezomib was associated with a decrease in DKK-1 levels.

In our study, despite bortezomib treatment, the levels of biomarkers related to bone formation (bALP and OC) substantially decreased, especially in the patients with longer prevalence period and steroid combination. Most of the patients (81.7%) in our study were treated in combination with corticosteroid. Overall patterns in changes of bone markers (decreased levels in OC and bALP) were similar to those in steroid combination group, but these patterns were not observed in bortezomib single-treatment group and the shorter prevalence group. In general, the effects of steroid are represented by a reduction in bone formation markers and a trend towards an increase or no change in bone resorption markers, which implies that steroids have a dominant effect on bone formation rather than bone resorption [27]. As a result, corticosteroid has been known to depress serum OC level, mainly due to the reduction in the rate of bone formation [28]. bALP, an enzyme released from osteoblasts, decreased in patients receiving steroid due to the reduction in the number of osteoblasts. Therefore, the reduction seen in the level of bone formation-related markers, bALP and OC in our study, is thought to result from the dominant effect of corticosteroid over bortezomib on bone formation. In a similar context, patients with longer prevalence duration also have a probability of longer exposure to steroid at a higher dose, which could explain the similar pattern in biomarkers in that group. The fact that significantly low levels of OC and bALP were found in the longer prevalence group before bortezomib therapy could also explain the decreased level posttreatment in the group. Terpos et al. also showed that the combination of bortezomib with melphalan, dexamethasone, and intermittent thalidomide (VMDT) did not increase the bone formation markers (bALP and OC) although a reduction in DKK-1 levels was observed [12]. On the other hand, bortezomib was associated with a decrease in DKK-1 levels. Consistent with these results, our study showed decreased serum DKK-1 level posttreatment. However, patients in steroid combination group did not show statistical significance in DKK-1 level (data not shown). Decreased production of DKK-1 by bortezomib might be offset by increased production of DKK-1 by steroid. Generally, concentrations of osteoclast regulators such as sRANKL and OPG were shown to be reduced following treatment with bortezomib in previous reports [12]. In our study, sRANKL level diminished significantly, whereas there were no significant changes in OPG levels posttreatment, resulting in marked reduction in sRANKL/OPG ratio. The reasons for the inconsistency of results in our study remain unclear but may be explained by the predominant effect of steroid on bone formation rather than increased bone resorption. As for bone formation markers, such as OC and bALP, they appear to decrease significantly due to the predominant effect of steroid on bone formation, while the effect of bortezomib predominates for osteoclast regulators such as sRANKL.

As the treatment in our study also included combination with dexamethasone in most cases and some patients received thalidomide, it is difficult to interpret exactly the role of bortezomib in the bone markers. The effect of corticosteroid on biochemical markers of skeletal turnover can be varied according to a number of biases. These include the different effects on different markers; different steroids, exposure time, and amount of administered steroids; different routes of administration, evaluation of the effect on normal subjects (men versus women, fertile versus postmenopausal

Table 2: Changes in bone markers.

Variables	Pretreatment	Posttreatment	Difference†	P
n (%)	75 (100.0)	73 (97.3)	73 (97.3)	
Osteocalcin ECLIA (ng/mL)	17.7 ± 18.6	13.7 ± 16.0	4.0 ± 15.9	**<0.0001**
Bone ALP (U/L)	25.9 ± 16.2	21.8 ± 11.2	4.2 ± 14.3	**0.004**
DKK-1 (pmol/L)	136.4 ± 86.3	116.3 ± 58.8	20.2 ± 78.6	**0.035**
sRANKL (total, pmol/L)	75.1 ± 86.8	48.4 ± 47.2	28.1 ± 101.6	**0.011**
OPG (pmol/L)	3.9 ± 1.8	4.0 ± 1.9	−0.1 ± 1.1	0.464
sRANKL/OPG ratio	22.2 ± 34.4	13.5 ± 16.1	9.4 ± 36.6	**0.037**

ECLIA: electrochemiluminescence immunoassay.
†Pretreatment value − posttreatment value.

Table 3: Changes in bone markers according to the duration of the disease and steroid combination.

	Osteocalcin	P^*	Bone ALP	P^{**}
Disease duration				
<6 months				
Pretreatment	18.6 ± 13.9		29.3 ± 18.8	
Posttreatment	19.6 ± 24.3		25.7 ± 13.0	
Difference	1.2 ± 24.1	0.009	4.2 ± 17.0	0.050
P^*	0.058		0.088	
≥6 months				
Pretreatment	13.9 ± 19.6		23.4 ± 14.0	
Posttreatment	10.1 ± 9.7		18.0 ± 6.4	
Difference†	4.9 ± 13.3		5.5 ± 12.4	
P^*	**<0.0001**		**0.002**	
Steroid				
Combination				
Pretreatment	16.5 ± 18.0		27.3 ± 18.0	
Posttreatment	13.6 ± 15.6		22.0 ± 10.5	
Difference	4.2 ± 18.7		5.4 ± 15.7	
P^*	**0.0002**		**0.001**	
Without combination				
Pretreatment	15.5 ± 12.1		22.7 ± 9.3	
Posttreatment	20.5 ± 30.9		20.9 ± 13.2	
Difference	−2.8 ± 22.3		2.1 ± 9.0	
P^*	0.153		0.275	

*P value represents difference from pretreatment value to posttreatment value within each group.
**P value represents difference of treatment outcome by prevalence duration.
†Posttreatment value − pretreatment value.

subjects, or young versus adults) or on patients with disease; or the inability to control parameters (gonadal function, parathyroid function, vitamin D status, etc.) that *per se* influence turnover [27].

Overall, these clinical data suggest that the combination treatment with bortezomib and steroid could rearrange the metabolic balance between osteoblast and osteoclast activities in PCM and the effects of corticosteroid predominate in inhibiting bone formation. Bortezomib appears to dominate in inhibiting bone resorption, while the effect of steroid is minimal in the inhibition of bone resorption.

Conflict of Interests

The authors have no financial conflicts or other conflict of interests to declare.

Acknowledgment

This study was supported by Janssen Korea, Inc.

References

[1] E. Terpos, O. Sezer, P. Croucher, and M. Dimopoulos, "Myeloma bone disease and proteasome inhibition therapies," *Blood*, vol. 110, no. 4, pp. 1098–1104, 2007.

[2] E. Terpos, O. Sezer, P. I. Croucher et al., "The use of bisphosphonates in multiple myeloma: recommendations of an expert panel on behalf of the European Myeloma Network," *Annals of Oncology*, vol. 20, no. 8, pp. 1303–1317, 2009.

[3] J. R. Berenson, A. Lichtenstein, L. Porter et al., "Long-term pamidronate treatment of advanced multiple myeloma patients reduces skeletal events," *Journal of Clinical Oncology*, vol. 16, no. 2, pp. 593–602, 1998.

[4] J. R. Berenson, A. Lichtenstein, L. Porter et al., "Efficacy of pamidronate in reducing skeletal events in patients with advanced multiple myeloma," *The New England Journal of Medicine*, vol. 334, no. 8, pp. 488–493, 1996.

[5] P. Major, A. Lortholary, J. Hon et al., "Zoledronic acid is superior to pamidronate in the treatment of hypercalcemia of malignancy: a pooled analysis of two randomized, controlled clinical trials," *Journal of Clinical Oncology*, vol. 19, no. 2, pp. 558–567, 2001.

[6] K. Zervas, E. Verrou, Z. Teleioudis et al., "Incidence, risk factors and management of osteonecrosis of the jaw in patients with multiple myeloma: a single-centre experience in 303 patients," *British Journal of Haematology*, vol. 134, no. 6, pp. 620–623, 2006.

[7] N. Giuliani, F. Morandi, S. Tagliaferri et al., "The proteasome inhibitor bortezomib affects osteoblast differentiation in vitro and in vivo in multiple myeloma patients," *Blood*, vol. 110, no. 1, pp. 334–338, 2007.

[8] U. Heider, M. Kaiser, C. Müller et al., "Bortezomib increases osteoblast activity in myeloma patients irrespective of response to treatment," *European Journal of Haematology*, vol. 77, no. 3, pp. 233–238, 2006.

[9] C. Min, M. Lee, K. Eom et al., "Bortezomib in combination with conventional chemotherapeutic agents for multiple myeloma compared with bortezomib alone," *Japanese Journal of Clinical Oncology*, vol. 37, no. 12, pp. 961–968, 2007.

[10] T. Lund, K. Søe, N. Abildgaard et al., "First-line treatment with bortezomib rapidly stimulates both osteoblast activity and bone matrix deposition in patients with multiple myeloma, and stimulates osteoblast proliferation and differentiation in vitro," *European Journal of Haematology*, vol. 85, no. 4, pp. 290–299, 2010.

[11] E. Terpos, D. J. Heath, A. Rahemtulla et al., "Bortezomib reduces serum dickkopf-1 and receptor activator of nuclear factor-κB ligand concentrations and normalises indices of bone remodelling in patients with relapsed multiple myeloma," *British Journal of Haematology*, vol. 135, no. 5, pp. 688–692, 2006.

[12] E. Terpos, E. Kastritis, M. Roussou et al., "The combination of bortezomib, melphalan, dexamethasone and intermittent thalidomide is an effective regimen for relapsed/refractory myeloma and is associated with improvement of abnormal bone metabolism and angiogenesis," *Leukemia*, vol. 22, no. 12, pp. 2247–2256, 2008.

[13] G. L. Uy, R. Trivedi, S. Peles et al., "Bortezomib inhibits osteoclast activity in patients with multiple myeloma," *Clinical Lymphoma and Myeloma*, vol. 7, no. 9, pp. 587–589, 2007.

[14] P. Boissy, T. L. Andersen, T. Lund, K. Kupisiewicz, T. Plesner, and J. M. Delaissé, "Pulse treatment with the proteasome inhibitor bortezomib inhibits osteoclast resorptive activity in clinically relevant conditions," *Leukemia Research*, vol. 32, no. 11, pp. 1661–1668, 2008.

[15] I. von Metzler, H. Krebbel, M. Hecht et al., "Bortezomib inhibits human osteoclastogenesis," *Leukemia*, vol. 21, no. 9, pp. 2025–2034, 2007.

[16] B. O. Oyajobi, I. R. Garrett, A. Gupta et al., "Stimulation of new bone formation by the proteasome inhibitor, bortezomib: implications for myeloma bone disease," *British Journal of Haematology*, vol. 139, no. 3, pp. 434–438, 2007.

[17] A. Pennisi, X. Li, W. Ling, S. Khan, M. Zangari, and S. Yaccoby, "The proteasome Inhibitor, bortezomib suppresses primary myeloma and stimulates bone formation in myelomatous and nonmyelomatous bones in vivo," *American Journal of Hematology*, vol. 84, no. 1, pp. 6–14, 2009.

[18] E. Terpos, D. Christoulas, P. Kokkoris et al., "Increased bone mineral density in a subset of patients with relapsed multiple myeloma who received the combination of bortezomib, dexamethasone and zoledronic acid," *Annals of Oncology*, vol. 21, no. 7, pp. 1561–1562, 2010.

[19] S. Mukherjee, N. Raje, J. A. Schoonmaker et al., "Pharmacologic targeting of a stem/progenitor population in vivo is associated with enhanced bone regeneration in mice," *The Journal of Clinical Investigation*, vol. 118, no. 2, pp. 491–504, 2008.

[20] S. Ozaki, O. Tanaka, S. Fujii et al., "Therapy with bortezomib plus dexamethasone induces osteoblast activation in responsive patients with multiple myeloma," *International Journal of Hematology*, vol. 86, no. 2, pp. 180–185, 2007.

[21] J. Blade, D. Samson, D. Reece et al., "Criteria for evaluating disease response and progression in patients with multiple myeloma treated by high-dose therapy and haemopoietic stem cell transplantation," *British Journal of Haematology*, vol. 102, no. 5, pp. 1115–1123, 1998.

[22] B. G. M. Durie, J.-L. Harousseau, J. S. Miguel et al., "International uniform response criteria for multiple myeloma," *Leukemia*, vol. 20, no. 9, pp. 1467–1473, 2006.

[23] E. Terpos, D. Christoulas, E. Kastritis et al., "Consolidation therapy with bortezomib, thalidomide and dexamethasone (VTD) regimen after asct in myeloma patients who do not receive bisphosphonates reduces bone resorption and is associated with low incidence of skeletal related events (SREs)," *Haematologica*, vol. 96, supplement 1, p. 126, 2011.

[24] M. Delforge, E. Terpos, P. G. Richardson et al., "Fewer bone disease events, improvement in bone remodeling, and evidence of bone healing with bortezomib plus melphalan-prednisone vs. Melphalan-prednisone in the phase III VISTA trial in multiple myeloma," *European Journal of Haematology*, vol. 86, no. 5, pp. 372–384, 2011.

[25] M. Zangari, D. Esseltine, F. Cavallo et al., "Predictive value of alkaline phosphatase for response and time to progression in bortezomib-treated multiple myeloma patients," *American Journal of Hematology*, vol. 82, no. 9, pp. 831–833, 2007.

[26] U. Heider, M. Kaiser, M. Mieth et al., "Serum concentrations of DKK-1 decrease in patients with multiple myeloma responding to anti-myeloma treatment," *European Journal of Haematology*, vol. 82, no. 1, pp. 31–38, 2009.

[27] S. Minisola, R. del Fiacco, S. Piemonte et al., "Biochemical markers in glucocorticoid-induced osteoporosis," *Journal of Endocrinological Investigation*, vol. 31, supplement 7, pp. 28–32, 2008.

[28] I. R. Reid, G. E. Chapman, T. R. C. Fraser et al., "Low serum osteocalcin levels in glucocorticoid-treated asthmatics," *Journal of Clinical Endocrinology and Metabolism*, vol. 62, no. 2, pp. 379–383, 1986.

Hindawi Publishing Corporation
BioMed Research International
Volume 2014, Article ID 717919, 9 pages
http://dx.doi.org/10.1155/2014/717919

Review Article

p53 Abnormalities and Potential Therapeutic Targeting in Multiple Myeloma

P. J. Teoh[1,2] and W. J. Chng[1,2]

[1] *Department of Medicine, Yong Loo Lin School of Medicine, National University of Singapore, Singapore 119077*
[2] *Cancer Science Institute of Singapore, National University of Singapore, Singapore 117599*

Correspondence should be addressed to W. J. Chng; mdccwj@nus.edu.sg

Received 17 March 2014; Accepted 20 May 2014; Published 17 June 2014

Academic Editor: Dong Soon Lee

p53 abnormalities are regarded as an independent prognostic marker in multiple myeloma. Patients harbouring this genetic anomaly are commonly resistant to standard therapy. Thus, various p53 reactivating agents have been developed in order to restore its tumour suppressive abilities. Small molecular compounds, especially, have gained popularity in its efficacy against myeloma cells. For instance, promising preclinical results have steered both nutlin-3 and PRIMA-1 into phase I/II clinical trials. This review summarizes different modes of p53 inactivation in myeloma and highlights the current p53-based therapies that are being utilized in the clinic. Finally, we discuss the potential and promise that the novel small molecules possess for clinical application in improving the treatment outcome of myeloma.

1. Introduction

Located at the chromosome 17p13.1, *TP53* encodes for p53 tumor suppressor protein. Deemed the guardian of the genome, p53 safeguards the integrity of the genome and ensures that the tissue homeostasis is kept in check. Under normal physiological conditions, cellular p53 is expressed at low levels, thereby turning off the activity of p53 network. With stress induction, p53 is stabilized by means of posttranslational modifications, such as phosphorylation and acetylation [1, 2]. The subsequent accumulation of p53 in the nucleus ultimately results in the massive activation of the downstream signaling, whereby a range of diverse antiproliferation and proapoptotic genes are actively transcribed by the p53 transcription factor. These genes mediate tumor suppressive mechanisms such as the cell cycle arrest (p21, Gadd45, 14-3-3σ), senescence (p21), apoptosis (Bax, PUMA, Noxa), and inhibition of angiogenesis (TSP1, maspin) [1, 2].

Thirty years of intensive research on p53 have yielded significant understanding of its structure and basic functions. The high percentage of patients with p53 germline mutations succumbing to a wide range of cancer in Li-Fraumeni syndrome [3] is a testament of p53 being a critical tumor suppressor gene. Furthermore, the importance of p53 as a tumor suppressor is underscored by the fact that it is mutated in approximately 50% of human cancer [1, 2, 4, 5]. In contrast to all other types of human cancer, p53 abnormalities in hematological malignancies are uncommon events. This review summarizes the current knowledge about the p53 abnormalities in multiple myeloma (MM) and discusses the current and potential therapeutics targeting p53 abnormalities in this disease.

2. p53 Abnormalities in Multiple Myeloma

In MM, mutation of p53 gene is a rare occurrence at diagnosis; however, the incidence increases as the stage of disease advances, suggesting its essential role in disease progression [6–8]. Overall, p53 mutations were found to occur in about 3% of newly diagnosed patients [6–9]. The next generation sequencing methods that were recently employed into p53 mutational studies have also recapitulated low incidence rate of p53 mutations in MM [6, 7]. Nonetheless, it is often associated with poor prognosis and accounts for a significantly low survival rate [6, 8].

Deletion of chromosome 17p13 region, which contains the p53 gene locus, is a recurrent cytogenetic abnormality in MM and has been associated with less favorable outcome [6–11]. p53 deletion which was found to be predominantly monoallelic has a reported incidence rate ranging from 10% to 34% of the cases [6, 8, 12, 13]. In particular, this chromosomal abnormality was identified as one of the few factors that defined high risk and poor prognosis in MM [14]. In line with this, p53 deletion has been reported as an important factor associated with resistance to chemotherapy [15]. Furthermore, Chang et al. reported that myeloma patients with central nervous system involvement were found to have p53 deletion and this finding may suggest the association of this genetic abnormality with metastatic properties of myeloma cells [16]. Consistently, Elnenaei et al. and Billecke et al. also reported a higher percentage of patients with p53 deletions being in MM stage IIIb or having plasma cell leukemia, with advanced stage of organ infiltrations [15, 17]. Moreover, another recent study has also reported more rapid progression of MM to plasma cell leukemia in 17p13(del) cases as compared to patients without this abnormality [18]. Essentially, loss of p53 has also been reported to be important in the progression of MM which involved reprogramming of the hematopoietic progenitor cells to malignant plasma cells [19]. Therefore, these reports collectively highlighted the critical value of p53 deletion in the pathogenesis of MM.

Fifty percent of cancer harbours p53 mutations, while in the remaining 50%, the wild type p53 is deemed to lose its function via various mechanisms that affect the expression and activity of p53. The main inhibition mechanism of p53 has been described to be the amplification or overexpression of its negative regulator mouse double minute 2 homolog (MDM2). MDM2 is an E3 ubiquitin ligase which promotes proteasomal degradation of p53 as well as inhibiting the transactivation domain of the tumor suppressor protein [20–23]. Under normal physiological conditions, p53 is a labile protein with very short half-life ranging only from 5 to 30 minutes, owing to the incessant degradation by MDM2 [23]. Importantly, MDM2 itself is the product of a p53-inducible gene. Thus, the two molecules interact with each other through an autoregulatory negative feedback loop aimed at maintaining low cellular p53 levels in the absence of stress. Of importance, MDM2 has been found to be deregulated in various types of cancers, including MM [24–27]. Deregulation of MDM2 gene gives rise to the overexpression of its protein, thereby increasing the turnover rate of p53, keeping p53 level low at all times, and ultimately suppressing its tumor suppressive actions. In particular, overexpression of MDM2 was shown to be essential in promoting both the entry into cell cycle and tumor cell survival in myeloma cells [27].

MDM4, a homolog of MDM2, does not have E3 ubiquitin ligase activity but inactivates p53 by binding to and inhibiting the transactivation domain of p53. Due to its essential role in inactivating p53, MDM4 dysregulation in cancer has also been receiving important attention lately [24, 25]. In fact, MDM4 was said to enhance the E3 ligase activity of MDM2 and to increase p53 proteasomal degradation rate [21, 24]. This genetic abnormality is also relevant in the perspective of MM because amplification of chromosome 1q, a region at which the MDM4 gene resides, has been established as an independent and significant prognostic factor [28, 29]. Indeed, patients harbouring this abnormality are categorized in the subgroup of high risk MM [29].

On top of that, epigenetic regulation of *TP53* is also a subject of intense research of late. Deregulation of miRNAs in cancer is being rigorously explored and this has led to the hypothesis of the role of this group of noncoding genes in the pathogenesis of MM [30, 31]. miRNAs are a set of noncoding RNA sequence of 19 to 25 nucleotides that play a major role in regulating gene expression by degrading its target coding mRNA and by repressing protein translation through partial or complete base pairing to its complimentary sites on target mRNA [31]. miR-125b and miR-504 were described as bona fide negative regulators of p53 in human cell lines [32, 33]. Importantly in MM, studies on the miRNA regulation on p53 expression have identified that both miR-25 and miR-30d directly target the 3′-UTR of p53 mRNA and subsequently result in the decrease of p53 protein expression, depletion of the apoptosis response rate, and diminishment of cellular senescence [34]. Introduction of the inhibitors of miR-25 and miR-30d to a human myeloma cell line, NCI-H929, in turn increased the endogenous level of p53 protein, accompanied by the upregulation of proapoptotic gene PUMA and ultimately the increase of apoptosis [34].

In addition, epigenetic factors are also possible regulators of the expression and the activity of p53. For instance, the deregulation of p14ARF has been reported to be responsible in abolishing the integrity of the p53 pathway [35]. ARF has an essential role in downregulating the expression of MDM2, thereby reinstating the stability of p53 which then leads to the activation of its downstream pathway [2]. Hypermethylation of p14ARF has been described in various tumors [2, 36, 37] and more relevantly for this review, this epigenetic abnormality has been reported in MGUS and MM samples [38]. This finding reflects a situation where p14ARF hypermethylation occurs as an early event during the pathogenesis and development of MM.

Hypermethylation of the promoter region of *TP53* gene itself has also been demonstrated in human myeloma cell lines [39, 40]. Reversal of this epigenetic alteration by zebularine (DNA methyltransferase inhibitor) restored the expression of p53 in the cells followed by decreased cell viability and increased apoptosis [39, 40].

Collectively, these findings describe the diverse mechanisms of p53 inactivation in multiple myeloma.

3. p53 Reactivating Agents in Cancer and Myeloma

Due to the fact that p53 is the nexus of various tumor suppressive pathways, it is imperative to study the means of reactivating or restoring p53 functions in human cancer in order to revert or rescue cells from resistance towards standard chemotherapeutic treatments. In fact, many anticancer drugs induce apoptosis through multiple pathways that are at least in part dependent upon functional p53 activation. In the late 1980s and early 1990s, the introduction of wild type p53

gene into a variety of human tumor cells has shown induction of efficient growth inhibition and apoptosis [41]. In line with this, multiple efforts have been channeled into research for effective p53-based therapy. In fact, p53 gene therapy (Gendicine) has been approved as the standard treatment for a number of cancers in China [41].

In myeloma, a preclinical study demonstrated that adenovirus mediated delivery of wild type p53 could potently induce apoptosis in myeloma cells while sparing the normal hematopoietic cells and normal lymphocytes [42]. Furthermore, when p53 was ectopically reexpressed in human myeloma cell lines that are absent of p53 expression, a reduction in cell viability, with increased rate of apoptosis and cell cycle arrest, was observed [13]. These findings suggest that functional p53 pathways have a therapeutic effect on MM. Therefore, various drugs have been developed with this purpose of p53 pathway reactivation. The following section briefly describes the current p53-based antimyeloma therapies that are being administered in the clinic.

3.1. Current p53-Based Antimyeloma Therapy

3.1.1. Chemotherapy. The use of conventional or high-dose chemotherapy has been a long-standing approach to treat MM patients. First line therapy for eligible patients remains autologous stem cell transplantation following high-dose chemotherapy. This is often preceded by induction therapy to decrease tumor load by utilizing a combination of treatments that often include chemotherapeutic agents such as alkylating compounds [43]. In relapsed or chemoresistant cases, combination chemotherapy is used as salvage therapy [44]. The most commonly prescribed chemotherapeutic drugs are as follows: melphalan, dexamethasone, prednisone, and etoposide [44]. Chemotherapy is useful in MM and other cancers due to its efficiency in killing malignant cells via a genotoxic mechanism. The majority of traditional chemotherapy agents target fast-growing tumor cells based on the notion that cancer cells are rapidly dividing and, therefore, are more sensitive to drugs that affect DNA replication. By mechanism, chemotherapy drugs potently induce DNA damage in cancer cells, thereby activating the p53 pathway which ultimately manifests as cell death [1, 2]. The importance of p53 in executing cytotoxicity is attested by the finding that p53 mutation and deletion conferred chemoresistance and significantly unfavorable outcome [6, 15]. In view of the mechanism of action of these genotoxic drugs targeting highly dividing cells, normal host cells that are rapidly growing are also damaged in the treatment process.

3.1.2. Proteasome Inhibitors. Bortezomib was approved by the Food and Drug Administration (FDA) for MM treatment in 2008 [44]. Bortezomib belongs to a class of proteasome inhibitor. 26S proteasome is an enzyme complex located in the cytoplasm and nucleus of cells that degrades unneeded, damaged, or misfolded proteins that have been polyubiquitinated by E1, E2, and E3 ubiquitin ligases [45]. As mentioned in the earlier section, MDM2 is an E3 ubiquitin ligase that induces polyubiquitination of p53 protein and subsequently promotes its proteolytic degradation in the 26S proteasome complex, thereby keeping basal expression of p53 at bay under normal conditions [41, 46, 47]. In MM, overexpression of MDM2 will lead to a high production of polyubiquitinated p53 ready proteasomal degradation. In this instance, bortezomib was developed to rescue this mechanism by inhibiting and blocking the actions of proteasome, thus preventing p53 from being degraded [48–50]. Proteasome inhibition stabilizes p53 itself and its downstream targets such as p21 and Bax, resulting in halting of cell cycle progression and, ultimately, apoptosis [48–50]. Promising results arising from clinical trials have brought bortezomib into the clinic [51, 52]. The emergence of bortezomib represented a paradigm shift in the treatment of myeloma and has brought improved outcome and longer survival of MM patients [53]. Indeed, in MM patients, including both newly diagnosed and relapsed or refractory cases, bortezomib treatment demonstrated good efficacy with response rate (partial and complete response) ranging from 35% to as high as 80% [51–54]. However, like any other antimyeloma drug in the clinic, resistance towards bortezomib remains inevitable. Nearly a third of MM patients never respond to this drug treatment and those who responded initially developed resistance over time.

3.2. Potential Therapeutics Targeting p53. Despite the introduction of bortezomib shifting the paradigm of MM treatment, the disease remains incurable and resistance towards this remarkable drug still arises. Given this situation, novel therapeutics targeting the critical p53 pathway is of utmost importance in order to reactivate the tumor suppressor network to execute apoptosis, in the hope of improving the treatment outcome in MM. In view of this, novel p53-reactivating agents have been developed and these agents are relevant to the nature and pathology of MM. The following describes these p53-reactivating drugs and the potential they hold for clinical applications. Figure 1 depicts the mechanism of actions these drugs undertake in their course to reactivate the p53 pathway.

3.2.1. Inhibitors of p53-MDM2 Interaction. Due to the fact that newly diagnosed cases of MM are often presented with wild type p53, therapeutic induction of p53 is an attractive potential treatment strategy for this disease. The conventional way of inactivating wild type p53 is through deregulation/overexpression of MDM2, which inhibits the transcriptional activity of p53 as well as increasing the rate of the p53 degradation. In view of this, the development of drugs to reactivate wild type p53 has focused on developing small molecule inhibitors to the MDM2-p53 complex.

(i) Nutlin. The first reported and the most well studied MDM2 inhibitor is the nutlins [29, 41]. Nutlins are a group of cis-imidazole analogs with high affinity for the p53-binding pocket on the amino terminal of MDM2 [56]. Nutlin was shown to resemble three important residues (Phe19, Trp23, and Leu26) on the transactivation domain of p53 that are critical for MDM2 binding [56]. In other words, nutlin competitively displaces p53 from the binding on MDM2 and

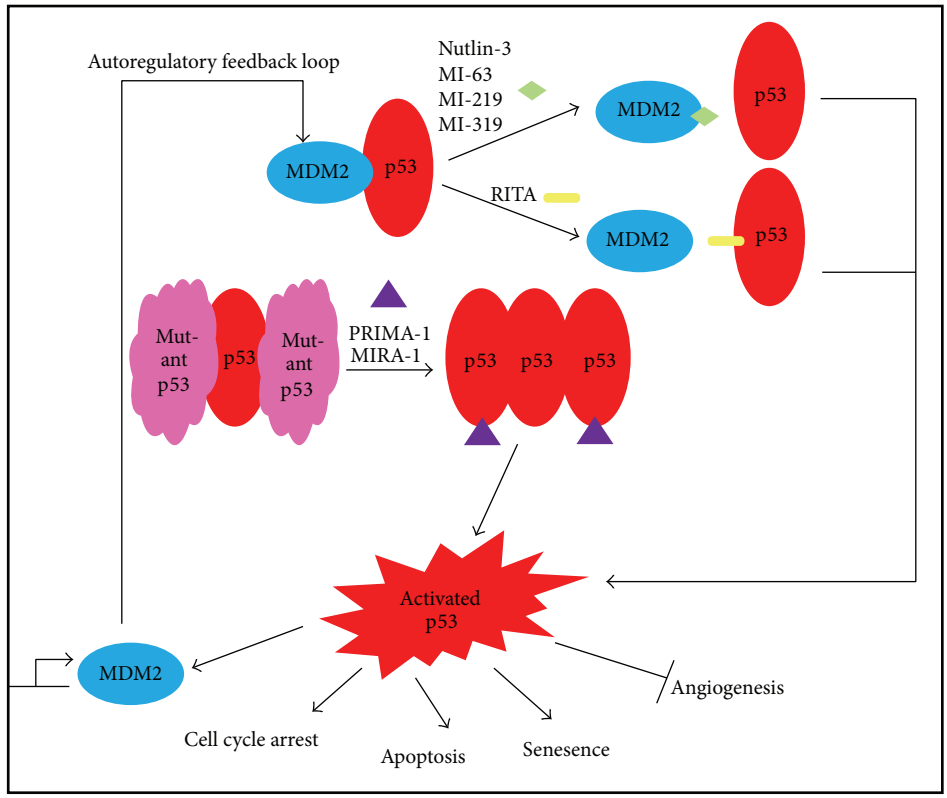


Figure 1: Schematic representation of mechanism of action of small molecules targeting p53 abnormalities. Simplified from [55].

effectively causes the stabilization of p53. Accumulation of p53 protein subsequently leads to its downstream pathway activation in cancer cells with wild type p53.

Because MDM2 inhibitors depend on p53 activation in cells expressing wild type p53, hematological malignancies that mostly retain wild type genotype of *TP53* are potential attractive targets for MDM2 inhibitor-based therapy. Nutlin-3 has been shown to be a potent inducer of apoptosis in cell lines deriving from hematological malignancies, including MM, ALL, AML, CLL, and Hodgkin's lymphoma [57, 58]. In MM, nutlin-3 demonstrated potent antimyeloma activity in MM cell lines, primary MM patient samples, as well as in MM cells grown in the bone marrow microenvironment [58, 59]. It was shown to reactivate the p53 pathway of the cells with wild type p53 by inducing the transcription of its downstream targets, p21 and MDM2, alongside the proapoptotic genes, PUMA, Bax, and Bak, which subsequently triggered cell death [58, 59]. This effect was observed specifically only in wild type but not in mutant p53 cells [58, 59]. The molecular mechanisms behind nutlin-induced apoptosis in MM were associated with both p53-transcription dependent and independent pathways [59]. Imperatively, nutlin was found to be lacking toxicity towards normal bone marrow hematopoietic cells [58]. In fact, this drug was demonstrated to have antigrowth instead of apoptosis-inducing effects on the normal hematopoietic stem cells [58]. This finding implicates the efficiency of nutlin acting as a nongenotoxic drug, killing myeloma cells while sparing normal host cells.

Furthermore, nutlin displayed wide synergy with various conventional chemotherapeutic drugs, namely, melphalan, etoposide, and velcade [58, 60, 61]. Taken together, these studies support the usage of nutlin as a novel treatment for MM either as a single agent or in combination with cytotoxic drugs. Nutlin could be utilized as a prechemotherapy agent in order to halt the growth of normal host cells, and subsequently standard and conventional chemotherapy drugs may be administered to induce cytotoxic death only to the cancerous cells. This strategy can be employed to minimize the chemotherapy-induced toxicity in normal dividing and growing cells, while concurrently tapering the high doses of cytotoxic chemotherapy.

As mentioned earlier, monoallelic deletion of p53 with the remaining allele being a wild type is a recurrent cytogenetic abnormality in MM patients and this group of patients often suffers from poor prognosis. It still remains inconclusive whether the single allele of wild type p53 in MM cells is actually still functional or is able to be reactivated by a nongenotoxic agent such as nutlin-3. Our lab has shown that, in WT/- cases with high p53 expression, nutlin-3 was able to induce a functional p53 pathway, but with a compromised activity compared to the WT/WT cells, whereas in WT/- cases with low or zero p53 expression, nutlin-3 showed no efficacy. The findings indicate a haploinsufficient activity of p53 in myeloma (P.J. Teoh et al. Leukemia in Press [62]).

The current evidence holds a lot of promise for nutlin-3 to be translated into the clinic as a treatment for MM

with wild type p53. However, it must be kept in mind that sporadic mutations of p53 could arise from selective pressure in the cells upon prolonged nutlin treatment, rendering a state of resistance towards the drug [63]. Furthermore, overexpression of MDM4 (MDM2 homolog), another potent p53 negative regulator, could also bring about nutlin resistance. This follows the mechanism by which the freed p53 from MDM2 control could very possibly be in turn bound and inactivated by the high levels of MDM4. In fact, cells overexpressing MDM4 showed a decreased nutlin efficacy of inducing p53 activity, whereas silencing of the former enhanced the efficiency of nutlin in inducing apoptosis [64]. Even though MDM4 overexpression in MM is rare, its locus on chromosome 1q is frequently amplified [28]; thus it would be important not to rule out the possibility of nutlin resistance arising from this genetic deregulation. These findings also suggest the importance of designing small-molecule inhibitors of the MDM4-p53 interaction, or preferably, a dual inhibitor of both MDM2-p53/MDM4-p53 interactions to completely reactivate p53.

(ii) RITA (Reactivation of p53 and Induction of Tumor Cell Apoptosis). RITA is a small molecule compound identified in a cell-based screen. It has a reversed mechanism from nutlin, whereby RITA binds to the amino terminal on p53 domain instead of on MDM2 protein. This binding causes conformational changes of p53 that reduces the p53-MDM2 interaction and hence decreases p53 ubiquitination which then leads to p53 accumulation, MDM2 downregulation, and p53-dependent apoptotic pathway induction [65]. Antimyeloma activity of RITA was first described in 2010 by Saha et al. by demonstrating that RITA also potently activates p53 pathway and showed efficient killing of myeloma cells with wild type p53, just like nutlin-3. Further validating the *in vitro* findings, mouse xenograft models of MM which were subjected to RITA treatment displayed tumor regression and lengthened survival [66].

The efficiency of RITA as an antimyeloma agent was further strengthened when RITA was found to be able to overcome resistance of MM cells towards MDM2 inhibitors such as nutlin-3 and MI-63 [67]. In this instance, RITA potently induced cell cycle arrest and apoptosis in resistant cells which were found to harbor p53 mutations after prolonged exposure to both nutlin-3 and MI-63 [67]. This piece of data suggests that RITA may very well possess a p53-independent role in exerting its antimyeloma activity. In line with this, another earlier study has reported a novel function of this compound in apoptotic signaling. Besides activating the p53 pathway, JNK signaling was also found to be induced upon RITA treatment, suggesting that this compound may function as a multitarget molecule [66]. Future investigations are needed to decipher this issue.

The clinical translation of RITA as an antimyeloma agent was further highlighted by its synergistic relationship with nutlin in inhibiting the growth and killing of MM cells [65].

(iii) Other Small Molecules Inhibitors of p53-MDM2. The importance of MDM2 inhibitors in hematological malignancies was emphasized when these compounds, MI-63, MI-219, and MI-319, showed preclinical efficacies [57]. Like nutlin, these MI compounds also bind to the p53 pocket on the surface of MDM2 only in cells with wild type p53 to reactivate the tumor suppressor pathway. MI-219 was shown to be effective in inducing apoptosis in p53 wild type cells of solid tumors originating from breast, colon, and prostate [41, 68]. The efficacy of MI-219 in hematological malignancy was evidenced when it was demonstrated to enhance the rate of MDM2 autoubiquitination, thereby increasing the degradation of this p53 negative regulator [69]. On the other hand, MI-319, which is a more potent derivative of MI-219, was found to be effective against another form of blood cancer, follicular lymphoma, with *in vitro* and *in vivo* evidence of reactivation of the p53 pathway [70]. These results suggest that the MI compounds could potentially be promising in myeloma although preclinical data is yet to be established.

3.2.2. Reactivation of p53 Mutants

(i) PRIMA-1 (p53 Reactivation and Induction of Massive Apoptosis). p53 mutations are often associated with resistance to chemotherapy treatment in cancer [6, 15]. These findings, together with the evidence that mutant p53 is often expressed at high levels, render mutant p53 as an important study target for cancer therapy. In view of this, p53 mutant reactivating agents have been developed. One such agent is the PRIMA-1. PRIMA-1 is a small molecule drug that reactivates mutant p53 by restoring its wild type conformation and transcriptional functions, consequently triggering massive apoptosis in tumor cells carrying mutant p53. Investigations into the molecular mechanism of the drug demonstrated that PRIMA-1 is converted into a by-product (methylene quinuclidinone) that forms adducts with thiols in mutant p53 [71]. This covalent modification of the mutant protein is sufficient to restore its binding ability to its transcriptional targets [71]. Since mutant p53 is often overexpressed in cancer cells, the restoration of wild type function in these high numbers of mutants ultimately triggers massive apoptosis, rendering this drug to be a highly effective anticancer strategy.

PRIMA-1 has been shown to have good efficacy against various types of solid cancer cells, namely, breast cancer [72], small cell lung carcinoma [73], and thyroid cancer [74]. This drug was able to reactivate the p53 pathway by inducing the transcription of various downstream targets (p21, MDM2, and Bax) and a consequent mutant-p53-dependent apoptosis [75]. It has also been shown that this drug has good antitumor effects at the *in vivo* level, whereby it potently inhibits the growth of tumor in human tumor xenograft model [73, 76]. PRIMA-1 was also shown to synergize with various chemotherapeutic agents to induce cancer cell death [77–79]. Due to its promising anticancer properties, PRIMA-1Met/APR246, a more potent derivative of the first generation drug, was developed and is currently in phase I/II clinical trials [80].

The potency of PRIMA-1 in hematological malignancies came to light when it was found to have an antileukemic

effect in CLL and AML cells [78, 81]. Interestingly, it was found to be more cytotoxic to AML cell with hemizygous p53 deletion [81]. However, there is still very little information reporting antimyeloma activity of PRIMA-1 until recently it was shown to induce apoptosis in several human myeloma cell lines tested, irrespective of their p53 status [79]. Further investigation demonstrated that PRIMA toxicity was actually mediated by p73 (the p53 subfamily member) and Noxa [79]. This interesting finding denotes that the drug could be a versatile agent in treating MM patients with or without p53 abnormalities. Preliminary studies in our lab have revealed a p53-independent mechanism of PRIMA-1 in myeloma cell lines, consistent with the findings reported by Saha et al. [79]. Importantly, we also found that cell lines with no p53 expression were particularly more sensitive to the drug and when the response was further probed and elucidated, we found that the activation of the endoplasmic reticulum stress pathway seems to be the mechanism behind PRIMA-induced apoptosis. In fact Lambert et al. once reported that treatment of human sarcoma cell lines by PRIMA-1 induces multiple signaling pathways that eventually converge on a common apoptosis route, and ER stress was noted to be increased in response to the drug treatment as well [82]. This interesting finding calls for a more in-depth study to explore the drug efficacy, including its functional and biochemical effects in myeloma.

(ii) MIRA-1. MIRA-1, structurally distinct from PRIMA-1, is a maleimide compound that targets mutant p53 with higher potency than PRIMA-1 [83]. MIRA-1 was described to shift the equilibrium between the native and unfolded conformation of p53 towards the native conformation, leading to restoration of p53-mediated transactivation of target genes and induction of apoptosis in a mutant-p53-dependent manner [83]. First investigation of MIRA-1 in multiple myeloma was conducted by Saha et al. whereby, with resemblance to PRIMA-1 treatment, MIRA-1 showed antimyeloma activity independently of p53 status [57].

3.2.3. Drug Combinations. Since drug resistance is ubiquitous in multiple myeloma, drug combination has been employed as a treatment regime to improve the treatment outcome. For instance, nutlin-3 has been shown to act in concert with various conventional chemotherapeutics to activate p53, in efforts to improve the treatment efficacy and reduce the collateral damage caused by chemotherapy. Nutlin-3 was reported to induce growth arrest in normal cells, and upon removal of the drug, cell cycle resumes [84]. As chemotherapy targets actively dividing cells, this mechanism of nutlin can be exploited to halt the growth of normal cells, preventing the toxicity caused by subsequent administration of chemotherapy. Table 1 summarizes the combination therapies that have been reported to be effective in killing myeloma cells. The nongenotoxic small molecular agents, nutlin-3 and PRIMA-1, were shown to exert synergistic effects with a wide variety of chemotherapeutics. This indicates potential therapeutic efficacy utilizing these small molecules in the treatment of chemorefractory myeloma patients.

Table 1: Synergistic response of small molecules with various anticancer agents.

Drug	Synergistic drug	Target	Reference
Nutlin	Melphalan	WT p53, MDM2	[58]
Nutlin	Etoposide	WT p53, MDM2	[58]
Nutlin	Bortezomib	WT p53, MDM2	[60, 61]
Nutlin	Lexatumumab	WT p53, MDM2, DR5	[85]
Nutlin	RITA	WT p53, MDM2	[65]
RITA	MI-63	WT p53, mutant p53	[67]
PRIMA-1	Dexamethasone	p53-independent	[79]
PRIMA-1	Doxorubicin	p53-independent	[79]

4. Conclusion and Future Directions

As p53 is the bridging point of apoptotic mechanisms, reactivation of the p53 pathway itself confers an excellent therapeutic approach in treating cancer. The current perspective points towards the importance of utilizing small molecular agents to reactivate the wild type p53 or to restore the transcriptional activities of the mutant p53. Even though each agent on its own was reported to show potent *in vitro* and *in vivo* antimyeloma effects, promising results arising from drug combinations suggest that combined use is a more attractive therapeutic option. The fact that p53-independent activities are involved in the drug mechanisms denotes that further investigations in this aspect are strongly called for so that we could fully utilize the versatility of the drug while maximizing its killing capacity. Of course, understanding the precise mechanism of action of these drugs would also aid in the future design of a novel and improved compound for the treatment of multiple myeloma.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

References

[1] K. H. Vousden and X. Lu, "Live or let die: the cell's response to p53," *Nature Reviews Cancer*, vol. 2, no. 8, pp. 594–604, 2002.

[2] B. Volgelstein, D. Lane, and A. J. Levine, "Surfing the p53 network," *Nature*, vol. 408, pp. 307–310, 2000.

[3] D. Malkin, F. P. Li, L. C. Strong et al., "Germ line p53 mutations in a familial syndrome of breast cancer, sarcomas, and other neoplasms," *Science*, vol. 250, no. 4985, pp. 1233–1238, 1990.

[4] A. M. Goh, C. R. Coffill, and D. P. Lane, "The role of mutant p53 in human cancer," *Journal of Pathology*, vol. 223, no. 2, pp. 116–126, 2011.

[5] P. A. J. Muller and K. H. Vousden, "P53 mutations in cancer," *Nature Cell Biology*, vol. 15, no. 1, pp. 2–8, 2013.

[6] W. J. Chng, T. Price-Troska, N. Gonzalez-Paz et al., "Clinical significance of TP53 mutation in myeloma," *Leukemia*, vol. 21, no. 3, pp. 582–584, 2007.

[7] L. Lodé, M. Eveillard, V. Trichet et al., "Mutations in TP53 are exclusively associated with del(17p) in multiple myeloma," *Haematologica*, vol. 95, no. 11, pp. 1973–1976, 2010.

[8] J. Drach, J. Ackermann, E. Fritz et al., "Presence of a p53 gene deletion in patients with multiple myeloma predicts for short survival after conventional-dose chemotherapy," *Blood*, vol. 92, no. 3, pp. 802–809, 1998.

[9] K. D. Boyd, F. M. Ross, W. J. Tapper et al., "The clinical impact and molecular biology of del(17p) in multiple myeloma treated with conventional or thalidomide-based therapy," *Genes Chromosomes and Cancer*, vol. 50, no. 10, pp. 765–774, 2011.

[10] R. Fonseca, E. Blood, M. Rue et al., "Clinical and biologic implications of recurrent genomic aberrations in myeloma," *Blood*, vol. 101, no. 11, pp. 4569–4575, 2003.

[11] M. H. Chen, C. X. Y. Qi, M. N. Saha, and H. Chang, "p53 nuclear expression correlates with hemizygous TP53 deletion and predicts an adverse outcome for patients with relapsed/refractory multiple myeloma treated with lenalidomide," *American Journal of Clinical Pathology*, vol. 137, no. 2, pp. 208–212, 2012.

[12] H. Chang, C. Qi, Q. L. Yi, D. Reece, and A. K. Stewart, "p53 gene deletion detected by fluorescence in situ hybridization is an adverse prognostic factor for patients with multiple myeloma following autologous stem cell transplantation," *Blood*, vol. 105, no. 1, pp. 358–360, 2005.

[13] W. Xiong, X. Wu, S. Starnes et al., "An analysis of the clinical and biologic significance of TP53 loss and the identification of potential novel transcriptional targets of TP53 in multiple myeloma," *Blood*, vol. 112, no. 10, pp. 4235–4246, 2008.

[14] T. Chou, "Multiple myeloma: recent progress in diagnosis and treatment," *Journal of Clinical and Experimental Hematopathology*, vol. 52, no. 3, pp. 149–159, 2012.

[15] M. O. Elnenaei, A. M. Gruszka-Westwood, R. A'Hern et al., "Gene abnormalities in multiple myeloma; the relevance of TP53, MDM2, and CDKN2A," *Haematologica*, vol. 88, no. 5, pp. 529–537, 2003.

[16] H. Chang, S. Sloan, D. Li, and A. K. Stewart, "Multiple myeloma involving central nervous system: high frequency of chromosome 17p13.1 (p53) deletions," *British Journal of Haematology*, vol. 127, no. 3, pp. 280–284, 2004.

[17] L. Billecke, E. M. M. Penas, A. M. May et al., "Similar incidences of TP53 deletions in extramedullary organ infiltrations, soft tissue and osteolyses of patients with multiple myeloma," *Anticancer Research*, vol. 32, no. 5, pp. 2031–2034, 2012.

[18] S. Mangiacavalli, L. Pochintesta, F. Cocito et al., "Correlation between burden of 17p13.1 alteration and rapid escape to plasma cell leukaemia in multiple myeloma," *British Journal of Haematology*, vol. 162, no. 4, pp. 555–558, 2013.

[19] C. Vicente-Dueñas, I. González-Herrero, M. B. G. Cenador, F. J. G. Criado, and I. Sánchez-García, "Loss of p53 exacerbates multiple myeloma phenotype by facilitating the reprogramming of hematopoietic stem/progenitor cells to malignant plasma cells by*MafB*," *Cell Cycle*, vol. 11, no. 20, pp. 3896–3900, 2012.

[20] J. Chung and M. S. Irwin, "Targeting the p53-family in cancer and chemosensitivity: triple threat," *Current Drug Targets*, vol. 11, no. 6, pp. 667–681, 2010.

[21] N. Allende-Vega and M. K. Saville, "Targeting the ubiquitin-proteasome system to activate wild-type p53 for cancer therapy," *Seminars in Cancer Biology*, vol. 20, no. 1, pp. 29–39, 2010.

[22] S. Haupt, M. Berger, Z. Goldberg, and Y. Haupt, "Apoptosis—the p53 network," *Journal of Cell Science*, vol. 116, no. 20, pp. 4077–4085, 2003.

[23] U. M. Moll and O. Petrenko, "The MDM2-p53 Interaction," *Molecular Cancer Research*, vol. 1, no. 14, pp. 1001–1008, 2003.

[24] M. Wade, Y. C. Li, and G. M. Wahl, "MDM2, MDMX and p53 in oncogenesis and cancer therapy," *Nature Reviews Cancer*, vol. 13, no. 2, pp. 83–96, 2013.

[25] F. Toledo and G. M. Wahl, "Regulating the p53 pathway: in vitro hypotheses, in vivo veritas," *Nature Reviews Cancer*, vol. 6, no. 12, pp. 909–923, 2006.

[26] B. Quesnel, C. Preudhomme, D. Oscier et al., "Over-expression of the MDM2 gene is found in some cases of haematological malignancies," *British Journal of Haematology*, vol. 88, no. 2, pp. 415–418, 1994.

[27] G. Teoh, M. Urashima, A. Ogata et al., "MDM2 protein overexpression promotes proliferation and survival of multiple myeloma cells," *Blood*, vol. 90, no. 5, pp. 1982–1992, 1997.

[28] W. J. Chng, O. Glebov, P. L. Bergsagel, and W. M. Kuehl, "Genetic events in the pathogenesis of multiple myeloma," *Best Practice and Research: Clinical Haematology*, vol. 20, no. 4, pp. 571–596, 2007.

[29] I. Hanamura, J. P. Stewart, Y. Huang et al., "Frequent gain of chromosome band 1q21 in plasma-cell dyscrasias detected by fluorescence in situ hybridization: incidence increases from MGUS to relapsed myeloma and is related to prognosis and disease progression following tandem stem-cell transplantation," *Blood*, vol. 108, no. 5, pp. 1724–1732, 2006.

[30] F. Pichiorri, S. S. Suh, M. Ladetto et al., "MicroRNAs regulate critical genes associated with multiple myeloma pathogenesis," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 105, no. 35, pp. 12885–12890, 2008.

[31] F. Pichiorri, L. de Luca, and R. I. Aqeilan, "MicroRNAs: new players in multiple myeloma," *Frontiers in Genetics*, vol. 2, article 22, 2011.

[32] W. Hu, C. S. Chan, R. Wu et al., "Negative regulation of tumor suppressor p53 by microRNA miR-504," *Molecular Cell*, vol. 38, no. 5, pp. 689–699, 2010.

[33] M. T. N. Le, C. Teh., N. Shyh-Chang et al., "MicroRNA-125b is a novel negative regulator of p53," *Genes and Development*, vol. 23, no. 7, pp. 862–876, 2009.

[34] M. Kumar, Z. Lu, A. A. L. Takwi et al., "Negative regulation of the tumor suppressor p53 gene by microRNAs," *Oncogene*, vol. 30, no. 7, pp. 843–853, 2011.

[35] S. Amir, A. H. Ma, X. B. Shi, L. Xue, H. J. Kung, and R. W. deVere White, "Oncomir miR-125b suppresses p14ARF to modulate p53-dependent and p53-independent apoptosis in prostate cancer," *PLoS ONE*, vol. 8, no. 4, Article ID e61064, 2013.

[36] N. E. Sharpless, "INK4a/ARF: a multifunctional tumor suppressor locus," *Mutation Research: Fundamental and Molecular Mechanisms of Mutagenesis*, vol. 576, no. 1-2, pp. 22–38, 2005.

[37] W. Lu, Y. Xie, Y. Ma, R. J. Matusik, and Z. Chen, "ARF represses androgen receptor transactivation in prostate cancer," *Molecular Endocrinology*, vol. 27, no. 4, pp. 635–648, 2013.

[38] C. Stanganelli, J. Arbelbide, D. B. Fantl, C. Corrado, and I. Slavutsky, "DNA methylation analysis of tumor suppressor genes in monoclonal gammopathy of undetermined significance," *Annals of Hematology*, vol. 89, no. 2, pp. 191–199, 2010.

[39] D. R. Hodge, B. Peng, J. C. Cherry et al., "Interleukin 6 supports the maintenance of p53 tumor suppressor gene promoter methylation," *Cancer Research*, vol. 65, no. 11, pp. 4673–4682, 2005.

[40] E. M. Hurt, S. B. Thomas, B. Peng, and W. L. Farrar, "Reversal of p53 epigenetic silencing in multiple myeloma permits apoptosis by a p53 activator," *Cancer Biology and Therapy*, vol. 5, no. 9, pp. 1154–1160, 2006.

[41] D. P. Lane, C. F. Cheok, and S. Lain, "p53-based cancer therapy," *Cold Spring Harbor Perspectives in Biology*, vol. 2, no. 9, Article ID a001222, 2010.

[42] Q. Liu and Y. Gazitt, "Adenovirus-mediated delivery of p53 results in substantial apoptosis to myeloma cells and is not cytotoxic to flow-sorted CD34+ hematopoietic progenitor cells and normal lymphocytes," *Experimental Hematology*, vol. 28, no. 12, pp. 1354–1362, 2000.

[43] D. Tan, W. J. Chng, T. Chou et al., "Management of multiple myeloma in Asia: resource-stratified guidelines," *The Lancet Oncology*, vol. 14, no. 12, pp. 70404–70402, 2013.

[44] R. Castelli, R. Gualtierotti, N. Orofino, A. Losurdo, S. Gandolfi, and M. Cugno, "Current and emerging treatment options for patients with relapsed myeloma," *Clinical Medicine Insights: Oncology*, vol. 7, pp. 209–219, 2013.

[45] C. Monneret, J. P. Buisson, and H. Magdelenat, "A new therapy with bortezomib, an oncologic medicinal product of the year 2004," *Annales Pharmaceutiques Francaises*, vol. 63, no. 5, pp. 343–349, 2005.

[46] C. M. Eischen and G. Lozano, "The Mdm network and its regulation of p53 activities: a rheostat of cancer risk," *Human Mutation*, vol. 35, no. 6, pp. 728–737, 2014.

[47] F. Toledo and G. M. Wahl, "MDM2 and MDM4: p53 regulators as targets in anticancer therapy," *International Journal of Biochemistry and Cell Biology*, vol. 39, no. 7-8, pp. 1476–1482, 2007.

[48] H. Matta and P. M. Chaudhary, "The proteasome inhibitor Bortezomib (PS-341) inhibits growth and induces apoptosis in primary effusion lymphoma cells," *Cancer Biology and Therapy*, vol. 4, no. 1, pp. 77–82, 2005.

[49] Y. H. Ling, L. Liebes, J. D. Jiang et al., "Mechanisms of proteasome inhibitor PS-341-induced G2-M-phase arrest and apoptosis in human non-small cell lung cancer cell lines," *Clinical Cancer Research*, vol. 9, no. 3, pp. 1145–1154, 2003.

[50] Y. H. Ling, L. Liebes, B. Ng et al., "PS-341, a novel proteasome inhibitor, induces Bcl-2 phosphorylation and cleavage in association with G2-M phase arrest and apoptosis," *Molecular Cancer Therapeutics*, vol. 1, no. 10, pp. 841–849, 2002.

[51] P. G. Richardson, B. Barlogie, J. Berenson et al., "A phase 2 study of Bortezomib in relapsed, refractory myeloma," *New England Journal of Medicine*, vol. 348, no. 26, pp. 2609–2617, 2003.

[52] S. Jagannath, B. Barlogie, J. Berenson et al., "A phase 2 study of two doses of bortezomib in relapsed or refractory myeloma," *British Journal of Haematology*, vol. 127, no. 2, pp. 165–172, 2004.

[53] S. K. Kumar, S. V. Rajkumar, A. Dispenzieri et al., "Improved survival in multiple myeloma and the impact of novel therapies," *Blood*, vol. 111, no. 5, pp. 2516–2520, 2008.

[54] J. B. Berenson, S. Jagannath, B. Barlogie et al., "Safety of prolonged therapy with bortezomib in relapsed or refractory multiple myeloma," *Cancer*, vol. 104, no. 10, pp. 2141–2148, 2005.

[55] M. N. Saha, J. Micallef, L. Qiu, and H. Chang, "Pharmacological activation of the p53 pathway in haematological malignancies," *Journal of Clinical Pathology*, vol. 63, no. 3, pp. 204–209, 2010.

[56] L. T. Vassilev, B. T. Vu, B. Graves et al., "In vivo activation of the p53 pathway by small-molecule antagonists of MDM2," *Science*, vol. 303, no. 5659, pp. 844–848, 2004.

[57] M. N. Saha, L. Qiu, and H. Chang, "Targeting p53 by small molecules in hematological malignancies," *Journal of Hematology and Oncology*, vol. 6, no. 1, article 23, 2013.

[58] T. Stühmer, M. Chatterjee, M. Hildebrandt et al., "Nongenotoxic activation of the p53 pathway as a therapeutic strategy for multiple myeloma," *Blood*, vol. 106, no. 10, pp. 3609–3617, 2005.

[59] M. N. Saha, H. Jiang, and H. Chang, "Molecular mechanisms of nutlin-induced apoptosis in multiple myeloma: evidence for p53-transcription-dependent and -independent pathways," *Cancer Biology and Therapy*, vol. 10, no. 6, pp. 567–578, 2010.

[60] M. N. Saha, J. Jayakar, and H. Chang, "Nutlin-3 and velcade synergistically induce cell cycle arrest and apoptosis in multiple myeloma through activation of p53 pathway," *Modern Pathology*, vol. 22, no. 1s, article 1287, 2009.

[61] M. N. Saha, H. Jiang, J. Jayakar, D. Reece, D. R. Branch, and H. Chang, "MDM2 antagonist nutlin plus proteasome inhibitor velcade combination displays a synergistic anti-myeloma activity," *Cancer Biology and Therapy*, vol. 9, no. 11, pp. 937–945, 2010.

[62] P. J. Teoh, T. H. Chung, S. Sebastian et al., "p53 haploinsufficiency and functional abnormalities in multiple myeloma," *Leukemia*, vol. 14, article 102, no. 10, 2014.

[63] M. H. Aziz, H. Shen, and C. G. Maki, "Acquisition of p53 mutations in response to the non-genotoxic p53 activator Nutlin-3," *Oncogene*, vol. 30, no. 46, pp. 4678–4686, 2011.

[64] J. T. Patton, L. D. Mayo, A. D. Singhi, A. V. Gudkov, G. R. Stark, and M. W. Jackson, "Levels of HdmX expression dictate the sensitivity of normal and transformed cells to Nutlin-3," *Cancer Research*, vol. 66, no. 6, pp. 3169–3176, 2006.

[65] M. N. Saha, H. Jiang, A. Mukai, and H. Chang, "RITA inhibits multiple myeloma cell growth through induction of p53-mediated caspase-dependent apoptosis and synergistically enhances nutlin-induced cytotoxic responses," *Molecular Cancer Therapeutics*, vol. 9, no. 11, pp. 3041–3051, 2010.

[66] M. N. Saha, H. Jiang, Y. Yang et al., "Targeting p53 via JNK pathway: a novel role of RITA for apoptotic signaling in multiple myeloma," *PLoS ONE*, vol. 7, no. 1, Article ID e30215, 2012.

[67] R. J. Jones, C. C. Bjorklund, V. Baladandayuthapani, D. J. Kuhn, and R. Z. Orlowski, "Drug resistance to inhibitors of the human double minute-2 E3 ligase is mediated by point mutations of p53, but can be overcome with the p53 targeting agent RITA," *Molecular Cancer Therapeutics*, vol. 11, no. 10, pp. 2243–2253, 2012.

[68] S. Shangary and S. Wang, "Targeting the MDM2-p53 interaction for cancer therapy," *Clinical Cancer Research*, vol. 14, no. 17, pp. 5318–5324, 2008.

[69] A. M. Sosin, A. M. Burger, A. Siddiqi, J. Abrams, R. M. Mohammad, and A. M. Al-Katib, "HDM2 antagonist MI-219 (spirooxindole), but not Nutlin-3 (cis-imidazoline), regulates p53 through enhanced HDM2 autoubiquitination and degradation in human malignant B-cell lymphomas," *Journal of Hematology and Oncology*, vol. 5, article 57, 2012.

[70] R. M. Mohammad, J. Wu, A. S. Azmi et al., "An MDM2 antagonist (MI-319) restores p53 functions and increases the life span of orally treated follicular lymphoma bearing animals," *Molecular Cancer*, vol. 8, article 115, 2009.

[71] J. M. R. Lambert, P. Gorzov, D. B. Veprintsev et al., "PRIMA-1 reactivates mutant p53 by covalent binding to the core domain," *Cancer Cell*, vol. 15, no. 5, pp. 376–388, 2009.

[72] Y. Liang, C. Besch-Williford, and S. M. Hyder, "PRIMA-1 inhibits growth of breast cancer cells by re-activating mutant p53 protein," *International Journal of Oncology*, vol. 35, no. 5, pp. 1015–1023, 2009.

[73] R. Zandi, G. Selivanova, C. L. Christensen, T. A. Gerds, B. M. Willumsen, and H. S. Poulsen, "PRIMA-1Met/APR-246 induces apoptosis and tumor growth delay in small cell lung cancer expressing mutant p53," *Clinical Cancer Research*, vol. 17, no. 9, pp. 2830–2841, 2011.

[74] R. L. Messina, M. Sanfilippo, V. Vella et al., "Reactivation of p53 mutants by p53 reactivation and induction of massive apoptosis in thyroid cancer cells," *International Journal of Cancer*, vol. 130, no. 10, pp. 2259–2270, 2012.

[75] V. J. N. Bykov, N. Issaeva, A. Shilov et al., "Restoration of the tumor suppressor function to mutant p53 by a low-molecular-weight compound," *Nature Medicine*, vol. 8, no. 3, pp. 282–288, 2002.

[76] N. Zache, J. M. R. Lambert, K. G. Wiman, and V. J. N. Bykov, "PRIMA-1MET inhibits growth of mouse tumors carrying mutant p53," *Cellular Oncology*, vol. 30, no. 5, pp. 411–418, 2008.

[77] V. J. N. Bykov, N. Zache, H. Stridh et al., "PRIMA-1MET synergizes with cisplatin to induce tumor cell apoptosis," *Oncogene*, vol. 24, no. 21, pp. 3484–3491, 2005.

[78] H. Nahi, S. Lehmann, L. Mollgard et al., "Effects of PRIMA-1 on chronic lymphocytic leukaemia cells with and without hemizygous p53 deletion," *British Journal of Haematology*, vol. 127, no. 3, pp. 285–291, 2004.

[79] M. N. Saha, H. Jiang, Y. Yang, D. Reece, and H. Chang, "PRIMA-1Met/APR-246 displays high antitumor activity in multiple myeloma by induction of p73 and Noxa," *Molecular Cancer Therapeutics*, vol. 12, no. 11, pp. 2331–2341, 2013.

[80] S. Lehmann, V. J. N. Bykov, D. Ali et al., "Targeting p53 in vivo: a first-in-human study with p53-targeting compound APR-246 in refractory hematologic malignancies and prostate cancer," *Journal of Clinical Oncology*, vol. 30, no. 29, pp. 3633–3639, 2012.

[81] H. Nahi, M. Merup, S. Lehmann et al., "PRIMA-1 induces apoptosis in acute myeloid leukaemia cells with p53 gene deletion," *British Journal of Haematology*, vol. 132, no. 2, pp. 230–236, 2006.

[82] J. M. R. Lambert, A. Moshfegh, P. Hainaut, K. G. Wiman, and V. J. N. Bykov, "Mutant p53 reactivation by PRIMA-1 MET induces multiple signaling pathways converging on apoptosis," *Oncogene*, vol. 29, no. 9, pp. 1329–1338, 2010.

[83] V. J. N. Bykov, N. Issaeva, N. Zache et al., "Reactivation of mutant p53 and induction of apoptosis in human tumor cells by maleimide analogs," *Journal of Biological Chemistry*, vol. 280, no. 34, pp. 30384–30391, 2005.

[84] C. F. Cheok, C. S. Verma, J. Baselga, and D. P. Lane, "Translating p53 into the clinic," *Nature Reviews Clinical Oncology*, vol. 8, no. 1, pp. 25–37, 2011.

[85] S. Surget, D. Chiron, P. Gomez-Bougie et al., "Cell death via DR5, but not DR4, is regulated by p53 in myeloma cells," *Cancer Research*, vol. 72, no. 17, pp. 4562–4573, 2012.

Hindawi Publishing Corporation
BioMed Research International
Volume 2014, Article ID 893243, 8 pages
http://dx.doi.org/10.1155/2014/893243

Research Article

Expression of Myeloid Antigen in Neoplastic Plasma Cells Is Related to Adverse Prognosis in Patients with Multiple Myeloma

Hyoeun Shim,[1] Joo Hee Ha,[1] Hyewon Lee,[2,3] Ji Yeon Sohn,[1] Hyun Ju Kim,[2] Hyeon-Seok Eom,[2] and Sun-Young Kong[1,4]

[1] *Department of Laboratory Medicine, Center for Diagnostic Oncology, Research Institute and Hospital, National Cancer Center, 323 Ilsan-ro, Ilsandong-gu, Goyang, Gyeonggi-do 410-769, Republic of Korea*
[2] *Hematologic-Oncology Clinic, Center for Specific Organs Cancer, Research Institute and Hospital, National Cancer Center, 323 Ilsan-ro, Ilsandong-gu, Goyang, Gyeonggi-do 410-769, Republic of Korea*
[3] *Hematologic Malignancy Branch, Research Institute and Hospital, National Cancer Center, 323 Ilsan-ro, Ilsandong-gu, Goyang-si, Gyeonggi-do 410-769, Republic of Korea*
[4] *Translational Epidemiology Research Branch, Research Institute and Hospital, National Cancer Center, 323 Ilsan-ro, Ilsandong-gu, Goyang-si, Gyeonggi-do 410-769, Republic of Korea*

Correspondence should be addressed to Hyeon-Seok Eom; hseom@ncc.re.kr and Sun-Young Kong; ksy@ncc.re.kr

Received 21 February 2014; Revised 28 April 2014; Accepted 8 May 2014; Published 4 June 2014

Academic Editor: Dong Soon Lee

We evaluated the association between the expression of myeloid antigens on neoplastic plasma cells and patient prognosis. The expression status of CD13, CD19, CD20, CD33, CD38, CD56, and CD117 was analyzed on myeloma cells from 55 newly diagnosed patients, including 36 men (65%), of median age 61 years (range: 38–78). Analyzed clinical characteristics and laboratory parameters were as follows: serum β2-microglobulin, lactate dehydrogenase, calcium, albumin, hemoglobin, serum creatinine concentrations, bone marrow histology, and cytogenetic findings. CD13+ and CD33+ were detected in 53% and 18%, respectively. Serum calcium ($P = 0.049$) and LDH ($P = 0.018$) concentrations were significantly higher and morphologic subtype of immature or plasmablastic was more frequent in CD33+ than in CD33− patients ($P = 0.022$). CD33 and CD13 expression demonstrate a potential prognostic impact and were associated with lower overall survival (OS; $P = 0.001$ and $P = 0.025$) in Kaplan-Meier analysis. Multivariate analysis showed that CD33 was independently prognostic of shorter progression free survival (PFS; $P = 0.037$) and OS ($P = 0.001$) with correction of clinical prognostic factors. This study showed that CD13 and CD33 expression associated with poor prognosis in patients with MM implicating the need of analysis of these markers in MM diagnosis.

1. Introduction

Flow cytometry (FCM) is widely used for the diagnosis and monitoring of hematological disorders, such as acute leukemias or lymphomas, in order to detect and characterize abnormal compartments or to enumerate rare events [1]. Flow cytometric analysis of neoplastic plasma cells in patients diagnosed with multiple myeloma (MM) can distinguish clonal cell populations and can be used to determine the numbers of neoplastic cells and to monitor residual disease during treatment [2].

In plasma cells, aberrant expression of CD56 and CD28 but lack of CD19 and CD27 showed the association with malignancy [3]. Downregulation of CD56 and a higher expression of CD44 have been associated with extramedullary spreading of malignant plasma cells [4, 5] and expression of CD28 has been related to disease activity [6, 7]. Though many studies have reported the associations between the expression of several antigens, including CD19, CD28, CD56, and CD117, and patient prognosis [8–10], no consensus has been reached regarding the expression status of antigens and their clinical relevance. Here we evaluated the impact of

antigen expression of neoplastic plasma cells on survival of patients diagnosed with MM.

2. Materials and Methods

Bone marrow (BM) aspiration samples were obtained from 55 patients newly diagnosed with MM from November 2007 to March 2013. Flow cytometric analyses perfomed in condition of plasma cells over 5% in the specimens. Whole erythrocyte-lysed BM samples were stained using the following four-color combinations of antibodies (FITC/PE/PerCP/APC): CD19/CD117/CD138/CD45, CD20/CD33/CD138/CD45, CD-38/CD13/CD138/CD45, -/CD56/CD138/CD45, and cyto-Kappa/cyto-Lambda/CD138/CD45. Antibody combinations were changed once from anti-CD38/CD13/CD138/CD45 to anti-CD38/CD28/CD138/CD45 during the study period. To assess antigens expression an aliquot of approximately 1×10^6 cells was labeled with preconjugated monoclonal antibodies in accordance with the manufacturer's recommendations (BD Biosciences, USA). The cells were then washed with phosphate buffered saline (PBS). For CD138 gating, at least 1×10^3 events per tube were acquired. Analyses were carried out using the FACS Diva software (BD Biosciences). Cells were also incubated with irrelevant isotype-matched antibodies to determine background fluorescence. Side scatter and high level expression of CD138 were used to gate each preparation of plasma cells. CD138 gated cells from patients with MM were retrospectively defined as neoplastic plasma cells when it was diagnosed as monoclonal gammopathy on serum and/or urine electrophoresis and light chain restriction on immunohistochemical staining of BM biopsy section. Positivity for antigen expression on flow cytometry was defined as staining of >20% of the cells.

Patient characteristics were retrospectively evaluated, including laboratory parameters including serum β2-microglobulin, calcium, albumin, hemoglobin, lactate dehydrogenase (LDH), serum creatinine concentrations, and immunoglobulin type of monoclonal protein. Fifty-five patients with MM were analyzed, 36 males (65%) and 19 females (35%), of median age 61 years (range: 38–78 years) (Table 1). BM histologic findings were classified as mature ($n = 39$), immature ($n = 9$), plasmablastic ($n = 2$), or pleomorphic ($n = 5$) myeloma cell types. Infiltration was categorized by interstitial ($n = 16$), focal ($n = 3$), or diffuse ($n = 36$) pattern. The FISH panels included *p53* (17p13), *Rb1* (13q14), *IGH/FGFR* t(4;14), and trisomy 1q (1q21). Cytogenetic abnormalities of t(4;14) or del(17p) were designated as high risk [11].

The initial treatment regimen consisted of including thalidomide and dexamethasone (57%), bortezomib (19%), combination of thalidomide and bortezomib (6%), lenalidomide (4%), and others (14%). Autologous peripheral blood stem cell transplantation (PBSCT) was performed in 33% of patients. Stage was classified by the international staging system and Durie-Salmon staging system [12, 13]. Risk group and disease progression were defined according to the International Myeloma Working Group (IMWG) risk stratification and response criteria for MM, respectively [14, 15].

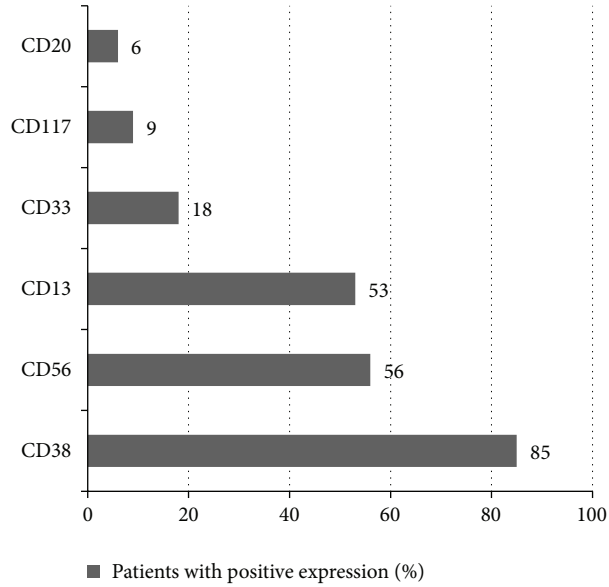


Figure 1: Frequency of antigen expression in patients newly diagnosed with multiple myeloma. CD56 and CD13 were the most common aberrant antigens in neoplastic plasma cells (56% and 53%, resp.), followed by CD33, CD117, and CD20. CD13 and CD33, the traditional myeloid markers, showed relatively high prevalence.

Progression-free survival (PFS) was calculated from the date of diagnosis to the date of relapse, disease progression, or death from any cause. Overall survival (OS) was calculated as the time from the date of diagnosis to death from any cause. PFS and OS were determined by the Kaplan-Meier method and log-rank test. Continuous variables were compared using independent t-tests or Mann-Whitney tests and categorical variables using Pearson chi-square or Fisher's exact tests. Multivariate analysis was performed using Cox regression analysis. Data were analyzed using SPSS 21 software (IBM Corp. 2012, IBM *SPSS Statistics, version 21.0,* Armonk, NY). This study was approved by the institutional review board of National Cancer Center of Korea (NCCNCS-13-774).

3. Results

The expression of CD38 was detected in 85% of cases (47 of 55) in CD138+ gated plasma cells. The expression of CD56, a marker involved in anchoring plasma cells to stromal structures, was found in 56% of cases (31 of 55). CD13 and CD33, the markers of myeloid lineage, were detected in 53% (20 of 38) and 18% (10 of 55) of cases, respectively. CD117, a tyrosine kinase receptor was detected in 9% (5 of 54). CD20, an antigen associated with the early stages of B-cell maturation, was detected in only 6% (3 of 55) of cases (Figure 1).

CD33 positivity was significantly associated with higher serum calcium ($P = 0.049$) and LDH ($P = 0.018$) concentrations (Table 2). Moreover, immature and plasmablastic cell type was more frequently observed in CD33+ than CD33–

Table 1: Clinical characteristics of the 55 patients with multiple myeloma.

Characteristics	Number (%) or median (range)
Number of patients	55
Age	61 (38–78)
Gender (male : female)	36 : 19 (65 : 35)
Durie-Salmon stage (I : II : III)	5 : 10 : 40 (9 : 18 : 73)
ISS stage (I : II : III)	22 : 18 : 15 (40 : 33 : 27)
Calcium (mg/dL)	9.1 (7.2–13.0)
Creatinine (mg/dL)	1.2 (0.7–3.9)
Albumin (mg/dL)	4.0 (2.3–4.9)
β2-Microglobulin (mg/dL)	3.8 (1.6–19.0)
Hemoglobin (g/dL)	10.3 (6.0–16.2)
Lactate dehydrogenase (U/L)	167 (79–1832)
C-reactive protein (mg/dL)	0.27 (0–10.01)
IgG : IgA : IgM : IgD : IgE : light* : biclonal	33 : 11 : 0 : 0 : 0 : 9 : 2 (60 : 20 : 0 : 0 : 0 : 16 : 4)
Kappa : Lambda (electrophoresis)	23 : 22† (51 : 49)
Plasma cell type	
Mature	39 (71)
Immature	9 (16)
Plasmablastic	2 (4)
Pleomorphic	5 (9)
Infiltration pattern	
Interstitial	16 (29)
Focal	3 (5)
Diffuse	36 (66)
Frequency of CD138-positive cells on biopsy‡	80 (10–100)
Cytogenetics (FISH)	
1q gain†	21/47 (45)
13q deletion†	19/47 (40)
t(4;14)†	8/48 (17)
17p deletion†	2/41 (5)

ISS: international staging system; FISH: fluorescent in situ hybridization; *light chain type; †absent values due to tests not done; the percentages are calculated based on the number of tests completed; ‡immunohistochemical stain on bone marrow biopsy.

patients ($P = 0.022$). CD13 expression did not show the association with clinical characteristics except infiltration pattern ($P = 0.046$). High risk cytogenetics, IMWG risk stratification, ISS stage, or Durie-Salmon stage has no significant difference in expression of myeloid antigens. Univariate analysis showed that CD13 positivity ($P = 0.008$), β2-microglobulin > 3.5 mg/dL ($P = 0.003$), and LDH > 202 U/L ($P = 0.007$) were significantly associated with shorter PFS. In addition, CD13 positivity ($P = 0.025$), CD33 positivity ($P = 0.001$), β2-microglobulin > 3.5 mg/dL ($P = 0.007$), and LDH > 202 U/L ($P < 0.001$) were significantly associated with shorter OS.

The prognostic indicators found to be significant in univariate analyses were included in multivariate analyses. CD33 positivity was the factor independently prognostic for OS (HR: 14.2, 95% CI: 3.3–61.8, $P < 0.001$). β2-Microglobulin > 3.5 mg/dL was another independent prognostic factor associated with PFS (HR: 6.93, 95% CI: 2.0–24.1, $P = 0.002$) (Table 3).

CD33 and CD13 expression were associated with lower OS ($P = 0.001$ and $P = 0.025$) at a median followup of 51 months. The estimated 2-year OS rate was significantly lower in CD33+ than in CD33− patients (38% versus 78%, $P = 0.046$) and CD13+ than in CD13− patients (55% versus 83%, $P = 0.046$). PFS was significantly shorter in CD13+ than CD13− patients ($P = 0.008$, Figure 2). Other antigens did not influence OS or PFS as follows: CD56 ($P = 0.252$, $P = 0.417$), CD117 ($P = 0.912$, $P = 0.975$), and CD20 ($P = 0.679$, $P = 0.253$).

The numbers of patients with CD13+/CD33+, CD13+/CD33−, CD13−/CD33+, and CD13−/CD33− groups were 3, 17, 3, and 15, respectively, and the CD13+/CD33+ group showed significantly shorter PFS and OS than other groups (Figure 3).

4. Discussion

This study showed myeloid antigens CD13 and CD33 were associated with poor prognosis in MM patients. Univariate analysis showed that both antigens were associated with

Table 2: Comparison of clinical data in groups positive and negative for CD33 and CD13.

Clinical parameters	CD33 Mean or number (%)			CD13 Mean or number (%)		
	Negative ($N = 44$)	Positive ($N = 10$)	P	Negative ($N = 18$)	Positive ($N = 20$)	P
Age	61.2	61.1	0.978	61.9	60.4	0.688
Calcium (mg/dL)	9.03	9.78	0.049	9.03	9.60	0.145
Creatinine (mg/dL)	1.36	1.28	0.710	1.32	1.50	0.434
Albumin (mg/dL)	3.81	3.55	0.270	3.66	3.91	0.243
β2-Microglobulin (mg/dL)	4.76	4.71	0.966	3.09	4.28	0.635
Hemoglobin (g/dL)	10.6	9.8	0.277	10.3	10.6	0.687
LDH (U/L)	172	369	0.018	140	302	0.078
Monoclonal heavy chain			0.793			0.454
IgG	24 (77)	7 (23)		12 (60)	8 (40)	
IgA	10 (91)	1 (9)		2 (29)	5 (71)	
IgD	3 (100)	0 (0)		1 (33)	2 (67)	
Light chain only	7 (29)	2 (71)		3 (38)	5 (52)	
Monoclonal light chain			0.603			0.207
Kappa	27 (82)	6 (18)		9 (39)	14 (61)	
Lambda	17 (81)	4 (9)		9 (60)	6 (40)	
BM aspirate plasma cell (%)	38	48	0.903	36	48	0.198
Plasma cell type			0.022			0.519
Mature	35 (90)	4 (10)		14 (54)	12 (46)	
Immature	4 (50)	4 (50)		2 (29)	5 (71)	
Plasmablastic	1 (50)	1 (50)		0 (0)	1 (100)	
Pleomorphic	4 (80)	1 (20)		2 (50)	2 (50)	
Infiltration pattern			0.487			0.046
Interstitial	14 (88)	2 (12)		5 (100)	0 (0)	
Focal	2 (67)	1 (33)		1 (50)	1 (50)	
Diffuse	28 (80)	7 (20)		12 (44)	15 (56)	
Cytogenetics (FISH)[‡]						
t(4;14)	7/40	1/4	0.566	3/18	4/19	0.532
1q amplification	14/39	4/7	0.258	6/18	9/19	0.297
13q deletion	14/39	4/7	0.258	5/18	8/19	0.286
17p deletion	2/33	0/7	0.677	0/15	2/15	0.241
Cytogenetic high risk group[¶]	9/35	1/7	0.461	3/16	6/16	0.217
International staging system			0.742			0.647
Stage I	19 (86)	3 (14)		6 (43)	8 (57)	
Stage II	14 (78)	4 (22)		6 (43)	8 (57)	
Stage III	11 (79)	3 (21)		6 (60)	4 (40)	
Durie-Salmon stage			0.753			0.766
Stage I	5 (100)	0 (0)		2 (67)	1 (33)	
Stage II	9 (82)	2 (18)		3 (38)	5 (62)	
Stage III	30 (77)	9 (23)		13 (48)	14 (52)	
IMWG risk			0.867			0.791
Low	8 (82)	1 (18)		3 (60)	2 (40)	
Standard	30 (79)	8 (21)		12 (46)	14 (54)	
High	6 (86)	1 (14)		3 (43)	4 (47)	

Table 2: Continued.

Clinical parameters	CD33 Mean or number (%)			CD13 Mean or number (%)		
	Negative ($N = 44$)	Positive ($N = 10$)	P	Negative ($N = 18$)	Positive ($N = 20$)	P
IMWG response			0.742			0.698
Complete response	8 (80)	2 (20)		4 (50)	4 (50)	
Very good partial response	6 (86)	1 (14)		2 (40)	3 (60)	
Partial response	10 (83)	2 (17)		5 (71)	2 (29)	
Stable disease	1 (50)	1 (50)		1 (100)	0 (0)	
Progressive disease	5 (71)	2 (29)		3 (43)	4 (57)	

BM: bone marrow; LDH: lactate dehydrogenase; IMWG: International Myeloma Working Group; ‡numbers of positive cases among FISH tests done; percentages were not written because meanings were different from that of other parameters; ❡including t(4;14) or del(17p).

Table 3: Multivariate regression analysis of factors significantly associated with PFS and OS.

Variables	PFS			OS		
	HR	95% CI	P	HR	95% CI	P
CD13+	3.46	0.8–14.8	0.093	2.77	0.4–17.7	0.283
CD33+	3.86	1.1–13.7	0.037	13.8	3.1–61.3	0.001
β2-Microglobulin > 3.5 mg/dL	6.93	2.0–24.1	0.002	4.02	1.0–16.7	0.055
LDH > 202 U/L	1.84	0.5–6.9	0.370	2.88	0.6–14.2	0.195
Age ≥ 65 years	0.40	1.1–0.1	0.076	1.50	0.5–4.6	0.481
t(4;14)	0.51	0.1–2.2	0.368	1.21	0.2–6.4	0.823

PFS: progression free survival; OS: overall survival; HR: hazard ratio; CI: confidence interval.

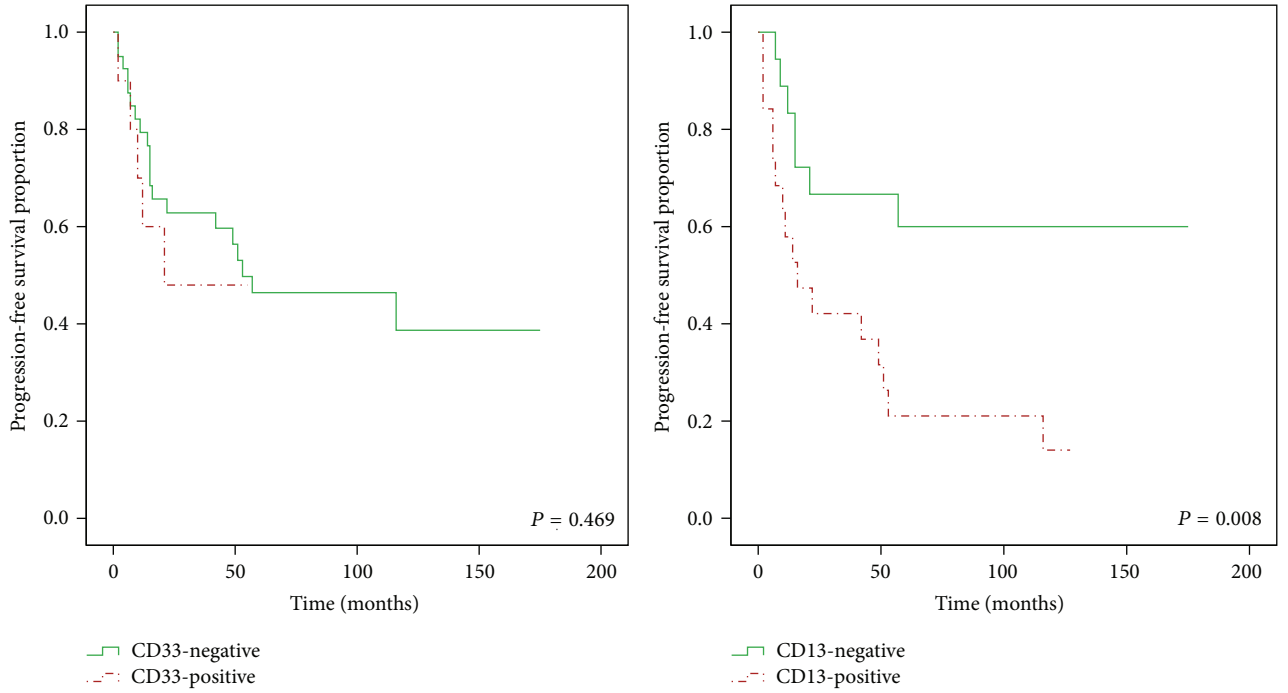
short OS; moreover multivariate analysis showed that CD33 expression was independent prognostic factor for poor prognosis. Both CD13+/CD33+ group showed significantly short OS and PFS and it suggests that expression of CD13 and CD33 has additive effect on unfavorable prognosis even though each group was not big enough to conclude. Though CD33 expression on plasma cells showed significant difference in OS, it did not show correlation with PFS. Since our study has limitation which included several treatment regimens, PFS which reflects more treatment response rather than biologic entity of myeloma did not reached the significant level.

With correlation of clinical parameters, the previous study has shown CD33 positivity was associated with higher serum LDH and β2-microglobulin concentrations and higher incidence rates of anemia or thrombocytopenia [16], and this study showed a significant association between CD33 positivity and higher serum LDH concentration ($P = 0.018$). For cytogenetic risk, there was the study showing higher incidence of t(4;14) in CD33-positive patients [17]; however, the association with t(4;14) was not observed in our study.

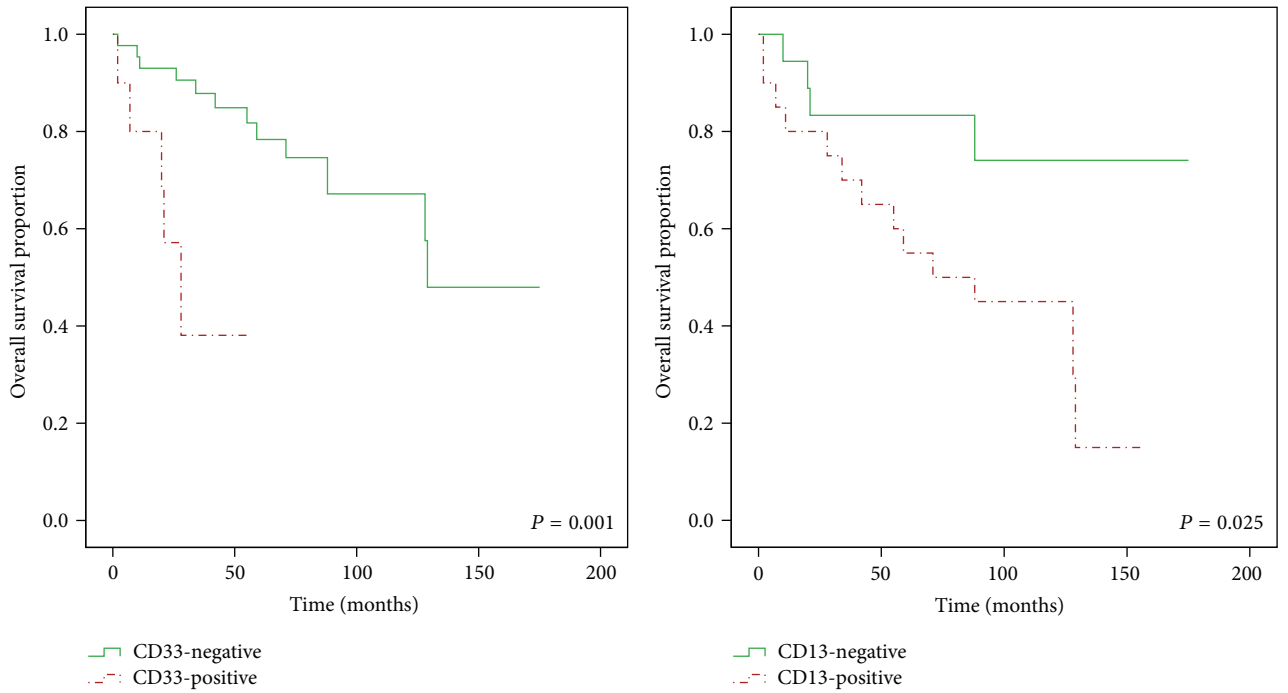
For mechanism of CD13 and CD33 in myeloma cells, there was no suggested pathway. The normal function of CD13 and CD33 in myeloid lineage is a zinc-dependent metalloproteinase anchored to cells as a type II transmembrane protein [18] and a sialic acid dependent cell adhesion molecule with a cytoplasmic tail bearing two tyrosine residues [19] which recruits Src homology-2 domain-containing tyrosine phosphatases [20]. These markers have been shown correlation with cancer in increased motility of lung cancer cells resulting in high invasiveness [21] and drug resistance and refractoriness with significantly lower 1-year survival rate in MM [16].

The clue why our study represented correlation with prognosis lied in plasma cell type and infiltration pattern. Morphologic subtype of MM plasma cells and infiltration pattern were reported as prognostic factors by the previous studies, which showed plasmablastic cells and diffuse infiltrations were associated with poor prognosis [22–24]. In the present study, immature and plasmablastic types of plasma cells were significantly associated with CD33 positivity. This implicated CD33+ myeloma associated with poorly differentiated neoplastic plasma cell type. Also CD13+ myeloma patients showed either focal or diffuse pattern of infiltration which suggests the association of antigen expression with infiltration characteristics.

For other antigen expressions, we found that 56% of patients were positive for CD56, 53% for CD13, 18% for CD33, 9% for CD117, and 6% for CD20. In comparison, previous studies have found that 60–75% of MM patients were positive for CD56, 18–35% for CD33, 32% for CD117, and 17–30% for CD20 [9, 17, 25–27]. These discrepancies in the antigen expression frequencies could result from the differences in the definition of neoplastic plasma cell; some studies exclude CD138+, CD19+, CD45+, CD27+, CD56−, and CD20− cells because they were regarded as normal plasma cells [8], but we included all CD138+ gated cells. The immunophenotypic definition of neoplastic plasma cells remains still unclear, because antigen expression profiles in normal or benign plasma cells are not uniform. Other



(a)



(b)

Figure 2: Kaplan-Meier analysis of (a) progression free survival (PFS) and (b) overall survival (OS) in groups of patients positive and negative for CD33 and CD13. CD33 expression demonstrates a potential prognostic impact and was associated with lower OS ($P = 0.001$). Patients with CD13 associated with significantly shorter PFS times ($P = 0.008$), not only lower OS ($P = 0.025$).

traditional myeloid markers have shown divergent impact in patients with MM. CD117, c-kit receptor, has been associated with good prognosis [3, 28] or not associated with prognosis [29–31]. The mechanism was explained as follows: CD117 expression might act as anchor molecule resulting in a decrease spread of plasma cells for good prognosis [28]. In this study, CD117+ patients did not display neither different disease characteristics nor a worse outcome. It might be due

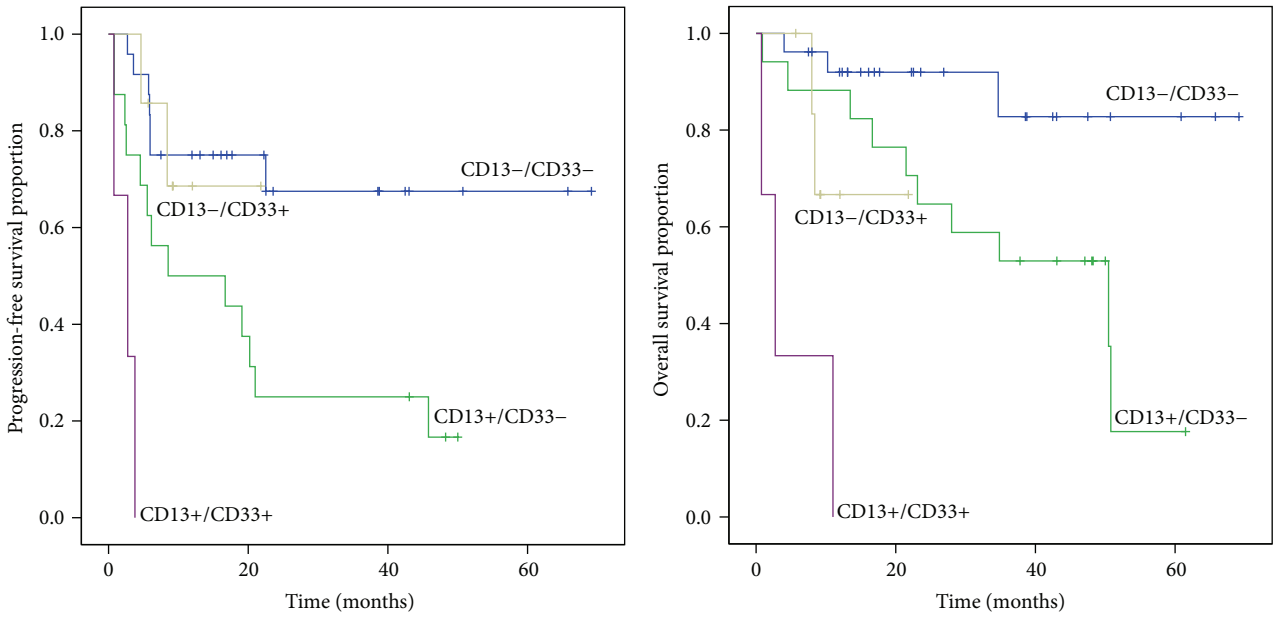


Figure 3: Kaplan-Meier analysis of (a) PFS and (b) OS in groups of patients with CD13−/CD33−, CD13−/CD33+, CD13+/CD33−, and CD13+/CD33+. The CD13+/CD33+ group showed significantly shorter PFS and OS than other groups: CD13−/CD33− group ($P < 0.001$ in PFS and OS), CD13+/CD33− group ($P = 0.013$ in PFS and $P < 0.001$ in OS), and CD13−/CD33+ group ($P = 0.001$ in PFS, $P = 0.049$ in OS). CD13+/CD33− group showed significantly shorter PFS and OS than CD13−/CD33− group ($P = 0.006$ in PFS, $P = 0.020$ in OS).

to low frequency of CD117 positivity in the present study, which could result from different destination of neoplastic plasma cells.

The major limitation of this study was the lack of homogenous treatment. However, CD33 expression was associated with significant short OS in both patients who underwent PBSCT ($n = 16$, $P < 0.001$) or who did not ($P = 0.046$). Thus, our findings implicate the need of analysis of these markers in MM diagnosis.

5. Conclusion

In conclusion, this study showed that the expression of CD13 and CD33 in neoplastic plasma cells from patients with MM was associated with poor prognosis independently of other prognostic factors. Further study is needed to clarify the role of these markers in MM pathogenesis.

Conflict of Interests

All authors declare that there is no conflict of interests regarding the publication of this paper.

Authors' Contribution

Hyoeun Shim and Joo Hee Ha equally contributed to this work.

References

[1] F. E. Craig and K. A. Foon, "Flow cytometric immunophenotyping for hematologic neoplasms," *Blood*, vol. 111, no. 8, pp. 3941–3967, 2008.

[2] B. Paiva, J. Almeida, M. Perez-Andres et al., "Utility of flow cytometry immunophenotyping in multiple myeloma and other clonal plasma cell-related disorders," *Cytometry B: Clinical Cytometry*, vol. 78, no. 4, pp. 239–252, 2010.

[3] R. Bataille, G. Jego, N. Robillard et al., "The phenotype of normal, reactive and malignant plasma cells. Identification of "many and multiple myelomas" and of new targets for myeloma therapy," *Haematologica*, vol. 91, no. 9, pp. 1234–1240, 2006.

[4] W. Eisterer, O. Bechter, W. Hilbe et al., "CD44 isoforms are differentially regulated in plasma cell dyscrasias and CD44v9 represents a new independent prognostic parameter in multiple myeloma," *Leukemia Research*, vol. 25, no. 12, pp. 1051–1057, 2001.

[5] C. Pellat-Deceunynck, S. Barille, G. Jego et al., "The absence of CD56 (NCAM) on malignant plasma cells is a hallmark of plasma cell leukemia and of a special subset of multiple myeloma," *Leukemia*, vol. 12, no. 12, pp. 1977–1982, 1998.

[6] N. Robillard, C. Pellat-Deceunynck, and R. Bataille, "Phenotypic characterization of the human myeloma cell growth fraction," *Blood*, vol. 105, no. 12, pp. 4845–4848, 2005.

[7] N. J. Bahlis, A. M. King, D. Kolonias et al., "CD28-mediated regulation of multiple myeloma cell proliferation and survival," *Blood*, vol. 109, no. 11, pp. 5002–5010, 2007.

[8] Y. U. Cho, C. J. Park, S. J. Park et al., "Immunophenotypic characterization and quantification of neoplastic bone marrow plasma cells by multiparametric flow cytometry and its clinical

significance in Korean myeloma patients," *Journal of Korean Medical Science*, vol. 28, no. 4, pp. 542–549, 2013.

[9] G. Mateo, M. A. Montalban, M.-B. Vidriales et al., "Prognostic value of immunophenotyping in multiple myeloma: a study by the PETHEMA/GEM cooperative study groups on patients uniformly treated with high-dose therapy," *Journal of Clinical Oncology*, vol. 26, no. 16, pp. 2737–2744, 2008.

[10] H. E. Johnsen, M. Bogsted, T. W. Klausen et al., "Multiparametric flow cytometry profiling of neoplastic plasma cells in multiple myeloma," *Cytometry B: Clinical Cytometry*, vol. 78, no. 5, pp. 338–347, 2010.

[11] R. A. Kyle and S. V. Rajkumar, "Criteria for diagnosis, staging, risk stratification and response assessment of multiple myeloma," *Leukemia*, vol. 23, no. 1, pp. 3–9, 2009.

[12] B. G. Durie and S. E. Salmon, "A clinical staging system for multiple myeloma. Correlation of measured myeloma cell mass with presenting clinical features, response to treatment, and survival," *Cancer*, vol. 36, no. 3, pp. 842–854, 1975.

[13] P. R. Greipp, J. S. Miguel, B. G. Dune et al., "International staging system for multiple myeloma," *Journal of Clinical Oncology*, vol. 23, no. 15, pp. 3412–3420, 2005.

[14] B. G. Durie, J.-L. Harousseau, J. S. Miguel et al., "International uniform response criteria for multiple myeloma," *Leukemia*, vol. 20, no. 9, pp. 1467–1473, 2006.

[15] W. J. Chng, A. Dispenzieri, C. S. Chim et al., "IMWG consensus on risk stratification in multiple myeloma," *Leukemia*, vol. 28, no. 2, pp. 269–277, 2014.

[16] N. Sahara, K. Ohnishi, T. Ono et al., "Clinicopathological and prognostic characteristics of CD33-positive multiple myeloma," *European Journal of Haematology*, vol. 77, no. 1, pp. 14–18, 2006.

[17] N. Robillard, S. Wuilleme, L. Lode, F. Magrangeas, S. Minvielle, and H. Avet-Loiseau, "CD33 is expressed on plasma cells of a significant number of myeloma patients, and may represent a therapeutic target," *Leukemia*, vol. 19, no. 11, pp. 2021–2022, 2005.

[18] D. Riemann, A. Kehlen, and J. Langner, "CD13—not just a marker in leukemia typing," *Immunology Today*, vol. 20, no. 2, pp. 83–88, 1999.

[19] S. D. Freeman, S. Kelm, E. K. Barber, and P. R. Crocker, "Characterization of CD33 as a new member of the sialoadhesin family of cellular interaction molecules," *Blood*, vol. 85, no. 8, pp. 2005–2012, 1995.

[20] S. P. Paul, L. S. Taylor, E. K. Stansbury, and D. W. McVicar, "Myeloid specific human CD33 is an inhibitory receptor with differential ITIM function in recruiting the phosphatases SHP-1 and SHP-2," *Blood*, vol. 96, no. 2, pp. 483–490, 2000.

[21] Y.-W. Chang, S.-C. Chen, E.-C. Cheng et al., "CD13 (aminopeptidase N) can associate with tumor-associated antigen L6 and enhance the motility of human lung cancer cells," *International Journal of Cancer*, vol. 116, no. 2, pp. 243–252, 2005.

[22] P. R. Greipp, N. M. Raymond, R. A. Kyle, and W. M. O'Fallon, "Multiple myeloma: significance of plasmablastic subtype in morphological classification," *Blood*, vol. 65, no. 2, pp. 305–310, 1985.

[23] A. Carter, I. Hocherman, S. Linn, Y. Cohen, and I. Tatarsky, "Prognostic significance of plasma cell morphology in multiple myeloma," *Cancer*, vol. 60, no. 5, pp. 1060–1065, 1987.

[24] R. Subramanian, D. Basu, and T. K. Dutta, "Prognostic significance of bone marrow histology in multiple myeloma," *Indian Journal of Cancer*, vol. 46, no. 1, pp. 40–45, 2009.

[25] J. Drach, C. Gattringer, and H. Huber, "Expression of the neural cell adhesion molecule (CD56) by human myeloma cells," *Clinical and Experimental Immunology*, vol. 83, no. 3, pp. 418–422, 1991.

[26] N. Robillard, H. Avet-Loiseau, R. Garand et al., "CD20 is associated with a small mature plasma cell morphology and t(11;14) in multiple myeloma," *Blood*, vol. 102, no. 3, pp. 1070–1071, 2003.

[27] A. M. Harrington, P. Hari, and S. H. Kroft, "Utility of CD56 immunohistochemical studies in follow-up of plasma cell myeloma," *American Journal of Clinical Pathology*, vol. 132, no. 1, pp. 60–66, 2009.

[28] M. Schmidt-Hieber, M. Perez-Andres, B. Paiva et al., "CD117 expression in gammopathies is associated with an altered maturation of the myeloid and lymphoid hematopoietic cell compartments and favorable disease features," *Haematologica*, vol. 96, no. 2, pp. 328–332, 2011.

[29] M. Ocqueteau, A. Orfao, R. Garcia-Sanz, J. Almeida, M. Gonzalez, and J. F. S. Miguel, "Expression of the CD117 antigen (C-Kit) on normal and myelomatous plasma cells," *British Journal of Haematology*, vol. 95, no. 3, pp. 489–493, 1996.

[30] M. Kraj, R. Poglod, J. Kopec-Szlezak, U. Sokolowska, J. Wozniak, and B. Kruk, "C-kit receptor (CD117) expression on plasma cells in monoclonal gammopathies," *Leukemia and Lymphoma*, vol. 45, no. 11, pp. 2281–2289, 2004.

[31] G. Pruneri, M. Ponzoni, A. J. M. Ferreri et al., "The prevalence and clinical implications of c-kit expression in plasma cell myeloma," *Histopathology*, vol. 48, no. 5, pp. 529–535, 2006.

Hindawi Publishing Corporation
BioMed Research International
Volume 2014, Article ID 237698, 6 pages
http://dx.doi.org/10.1155/2014/237698

Clinical Study

The Positive Effects of One-Hour Intravenous Administration of Bortezomib on Peripheral Neuropathy in Multiple Myeloma Patients

Joo Young Jung,[1] Ho Young Kim,[2] Boram Han,[2] Dae Ro Choi,[3] Dae Young Zang,[2] and Hyo Jung Kim[2]

[1] *Department of Internal Medicine, Dongtan Sacred Heart Hospital, Hwaseong-si 445-907, Republic of Korea*
[2] *Division of Hematology/Oncology, Department of Internal Medicine, Hallym University Sacred Heart Hospital, Hallym University College of Medicine, 22 Gwanpyeong-ro, 170 Beon-gil, Dongan-gu, Anyang-si 431-796, Republic of Korea*
[3] *Department of Internal Medicine, Chuncheon Sacred Heart Hospital, Chuncheon-si 200-704, Republic of Korea*

Correspondence should be addressed to Hyo Jung Kim; hemonc@hallym.or.kr

Received 24 April 2014; Revised 17 May 2014; Accepted 20 May 2014; Published 4 June 2014

Academic Editor: Dong Soon Lee

Bortezomib-induced peripheral neuropathy (BiPN) in multiple myeloma (MM) patients is a common and serious side effect. Currently, it has been reported that subcutaneous (SC) administration of bortezomib decreases the incidence of BiPN as compared to standard intravenous (IV) bolus injection without any differences in efficacy. However, there are reports of severe injection site reaction following SC administration of bortezomib. The aim of this study was to evaluate the response rate and incidence of BiPN following one-hour IV infusion of bortezomib. The data was retrospectively collected from MM patients who had been treated with IV administration of bortezomib for one hour. Twenty-three patients were evaluated (median age 72 years, 13 males). The median number of treatment cycles was 5 (range 2–10). The cumulative bortezomib dose was 26.0 mg/m^2 (14.3–66.3) and percent of actual per expected cumulative dose was 90% (50–100). The overall response (complete response plus partial response) rate was 65%. The incidence of BiPN was 57% ($n = 13$) and incidence of severe neuropathy was 4% ($n = 1$). One-hour IV infusion of bortezomib was an effective regimen for MM with reduced incidence of severe BiPN. This route of administration of bortezomib could be an alternative mode of delivery for patients with severe injection site reactions following SC administration.

1. Introduction

With respect to patient outcomes in multiple myeloma (MM), there have been significant improvements after the discovery of novel induction agents, including bortezomib [1, 2]. However, intravenous (IV) bolus injection of bortezomib, which is the standard mode of administration has limitations because of bortezomib-induced peripheral neuropathy (BiPN) [3, 4]. BiPN is one of the most common, often reversible, but significant side effects of bortezomib therapy, which leads to dose modification [3–6]. The typical form of neuropathy is largely sensory rather than motor and is in the feet rather than in the hands. Although the pathogenesis underlying BiPN is not precisely understood, most investigators agree that bortezomib induces sensory nerve injury in a dose-dependent manner. Furthermore, the most significant risk factor of the onset or aggravation of BiPN is preexisting peripheral neuropathy.

Until now, the management of BiPN has consisted of dose reduction or discontinuation of IV bolus bortezomib. Recent reports have demonstrated that the incidence and severity of BiPN in patients receiving subcutaneous (SC) bortezomib are significantly lower than those in patients treated with an IV bolus of the drug, with noninferior efficacy in MM [7, 8]. This decreased incidence and severity of BiPN in the SC bortezomib group could be potentially due to the lower maximum plasma drug concentration (C_{max}) and longer time to reach C_{max} (T_{max}) with the same area

under the concentration (AUC) when compared to IV bolus bortezomib.

SC bortezomib also has a different weak point, the injection site reaction (ISR), because bortezomib itself is one of the irritant drugs and induces an inflammatory reaction. In most cases, the SC bortezomib-related ISR is well tolerated, but cases of severe skin reactions including necrosis have been reported [8, 9]. Therefore, a new route of administration is necessary, particularly in patients with severe ISR following SC administration of bortezomib. SC administration of bortezomib could not be applied to Korean MM patients, because this route has not been previously allowed by the Korean health insurance system until recently. Meanwhile, our group had attempted to increase the infusion time of bortezomib to one hour, in order to maintain AUC, without abrupt change of concentration of bortezomib, while decreasing C_{max} and T_{max} of the drug in MM patients.

In this study, we retrospectively evaluated the response rate of the drug and incidence of BiPN following one-hour IV infusion of bortezomib.

2. Patients and Methods

This study was designed to retrospectively review the medical records of patients with MM, who were treated with bortezomib-based regimen between January 2009 and March 2014 at Hallym University Sacred Heart Hospital. The route of bortezomib administration was IV infusion for one hour. Bortezomib was reconstituted with 100 mL of normal saline. The data of patients with any regimen containing bortezomib, any line of treatment, and previous treatment with bortezomib were included. Patients excluded from the study were as follows: (1) patients with dementia or other neurologic deficits which prevent expression of subjective neurologic symptoms, (2) patients who received less than 2 cycles of chemotherapy and could not measure the treatment response, and to these patients BiPN should not be the reason of treatment cessation, and (3) patients diagnosed with peripheral neuropathy grade 3 or more according to the National Cancer Institute Common Terminology Criteria for Adverse Events (CTCAE) ver4.0 [10], before starting the bortezomib-based chemotherapeutic regimen.

The primary end points of this study were the incidence and severity of BiPN according to the CTCAE ver4.0. Assessment of CTCAE grades of BiPN was dependent on the subjective symptoms of patients, physical examination by attending physicians, and medication history. The secondary end points were the cumulative dose and duration of bortezomib, the best response rate, and survival. The expected cumulative dose of bortezomib was calculated based on the dose of the drug at VISTA trial [11] when patients were treated with velcade, melphalan, and prednisolone (VMP) regimen. The dosage was calculated based on 1.3 mg/m^2 at days 1, 4, 8, and 11 per one cycle for other bortezomib-containing regimens. The dose modification of bortezomib was performed according to the package insert of the drug [12] depending on the adverse events of patients. Disease response was assessed with criteria from the International Myeloma Working Group (IMWG) [13, 14]. However, not all patients with negative serum and urine protein electrophoresis and immunofixation results underwent bone marrow biopsy to confirm complete response (CR) in routine clinical practice. We incorporated near complete response (nCR) [14] as a response measurement. The reimbursement policy of Korean National Health Insurance Service supports bortezomib treatment only if the response is partial response (PR) or better at the end of four cycles; patients with minimal response (MR) or stable disease (SD) had to discontinue the bortezomib-based treatment. Therefore, we incorporated MR [15] to subdivide the response measurement, in order to know the status of disease at the time of drug cessation. We defined progression free survival (PFS) as the time from initiation of treatment to disease progression, relapse, or death from any cause or initiation of the subsequent chemotherapy, whichever came first. Overall survival was defined as the time from initiation of treatment to death from any cause.

Survival was analyzed using the Kaplan-Meier methods, and statistical analyses were performed with IBM SPSS (version 21). The Institute Review Board of Hallym University Sacred Heart Hospital approved the protocol of this retrospective study.

3. Results

Twenty-three Korean patients with MM were enrolled in this study. All patients had measureable disease. Patient characteristics and demographics are summarized in Table 1. Median age was 72 years. Eleven were newly diagnosed MM (NDMM) patients and treated with VMP regimen. Twelve were relapsed or refractory MM (RRMM) patients to previous lines of chemotherapy, and among them 3 patients had been exposed to bortezomib. Various bortezomib-containing regimens were administered to these patients (Table 2). The total cycles of chemotherapy regimens were 78 cycles, and the median number of cycles per patient was 5 cycles with the median treatment duration of 19 weeks. The median cumulative dose of bortezomib was 26 mg/m^2 (range 14–66 mg/m^2) and the percent intensity of actual per expected cumulative dose of each regimen was 90% (range 50–100%). Seven patients (31%) discontinued therapy because of disease progression. Four patients (17%) with MR or SD, at the end of the 4th cycle of chemotherapy, had to finish the current treatment.

The best overall response rate (ORR, from PR to CR) during treatment was 65% (Table 3), including 1 (4%), 2 (9%), and 6 (26%) patients with CR, nCR, or VGPR, respectively. And the best ORR of NDMM and RRMM group was 55% and 75%, respectively ($P = 0.4$). After a median follow-up of 16.3 months (interquartile range 6.0–21.0), 5 patients (21.7%) had died and median overall survival was not reached (Figure 1). Causes of death were infection ($n = 3$) and progression of MM ($n = 2$). Median PFS was 15.0 months (95% CI 10.2–19.8), and 1-year PFS rate was 54.4% (95% CI 31.9–76.9).

Table 4 shows the safety profiles of intravenous bortezomib infusion treatment. Five patients (22%) discontinued therapy because of adverse events, but peripheral neuropathy

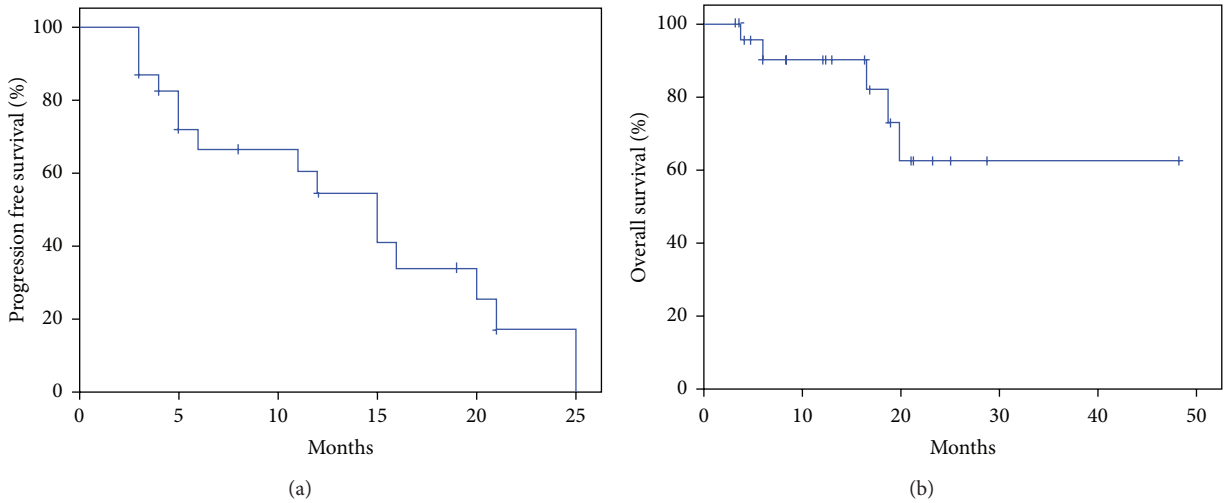


Figure 1: Progression free survival (a) and overall survival (b).

was not a cause for cessation of bortezomib therapy for any patient. Anorexia, peripheral sensory neuropathy, upper respiratory infection, and nausea were the most common adverse effects and few patients suffered grade 3 toxicities. However, hematology laboratory data showed 39%, 35%, 43%, and 26% of grade 3 or more results on hemoglobin, WBC, absolute neutrophil count, and platelet, respectively.

Four out of 23 patients had peripheral neuropathy with grade 1 ($n = 2$) or 2 ($n = 2$) before initiation of bortezomib treatment. The grade 2 neuropathy of a patient was exacerbated to grade 3 during bortezomib therapy, but it turned to grade 2 after cessation of bortezomib. There was no change on the severity of neuropathy of the other 3 patients during chemotherapy. Rates of peripheral neuropathy events of grades 1, 2, and 3 severity were 43.5% ($n = 10$), 8.7% ($n = 2$), and 4.3% ($n = 1$), respectively. Among 13 patients with peripheral neuropathy, 7 were NDMM patients with grade 1 ($n = 5$) or 2 ($n = 2$) severity. Six patients were RRMM patients with grade 1 ($n = 5$) or 3 ($n = 1$) severity. The estimated time to onset of peripheral neuropathy was 4.6 months (95% CI 1.1–8.0) from initiation of bortezomib treatment. There was no significant difference of this time between NDMM and RRMM patients (4.6 months, 95% CI 3.0–6.1 and 6.5 months, 95% CI 0–14.6, resp.). Cumulative dose of bortezomib at the first onset of peripheral neuropathy was 16.5 mg/m^2 (95% CI 9.2–23.6). There was no significant difference of cumulative doses at the end of bortezomib treatment between patients with and without peripheral neuropathy ($P = 0.13$).

4. Discussion

Bortezomib, a proteasome inhibitor, has improved the response and survival of MM patients [1, 2, 11]. This study showed that one-hour IV infusion of bortezomib was an effective regimen for MM with reduced incidence of severe BiPN. The patient population of this study was heterogeneous. Both NDMM and RRMM patients were evaluated with various bortezomib-containing chemotherapeutic regimens and schedules. Drug cessation was defined not only by response or adverse reactions but also by insurance guidelines. Under these circumstances, we decided to use the best overall response rate for measurement of effectiveness. Compared with 71% in the VISTA trial for NDMM patients [11] and 38% in the APEX trial for relapsed MM patients [16], the best overall response rate of our trial was 65%. Taken together, with 17% of patients ($n = 4$) who had to finish the drug to follow insurance guidelines, even with the response of MR or SD at the end of 4 cycles, one-hour IV infusion did not seem to affect the efficacy of bortezomib.

Several studies have reported that severe BiPN affects the quality of life of patients receiving bortezomib and could be a hurdle to continuation of the drug, even in patients with a good response [3–6]. The incidence of BiPN has been reported in up to 70% of patients, including grade ≥3 BiPN in up to 16%, increased with cumulative dose of standard twice-weekly 1.3 mg/m^2 dose schedule through IV bolus administration [5]. In the VISTA trial [11], the incidence of grades 1, 2, 3, and 4 sensory BiPN was 14%, 17%, 13%, and <1%, respectively, in NDMM patients on the VMP regimen. Moreau et al. [17] reported 70% of any grade BiPN in the bortezomib and dexamethasone treatment group, which was used for induction before autologous stem cell transplantation in NDMM patients. Furthermore, the rates of grade 2 or worse and grade 3 BiPN were 34% and 11%, respectively. Bringhen et al. [18] reported that the incidence of grade 3/4 BiPN was significantly decreased, yet not completely resolved, in the once-weekly scheduled bortezomib group compared with the twice-weekly group. Currently, the MMY-3021 trial [8] shows that SC administration is not inferior in efficacy to IV bolus administration with 1.3 mg/m^2 twice-weekly bortezomib in relapsed MM patients and significantly reduces the incidence of BiPN of any grade (38% versus 53%), ≥grade 2 (24% versus 41%), and ≥grade 3 (6% versus 16%). In our study, the incidence of BiPN was not reduced (57%); however, most cases of BiPN were grade 1 (44%) and did

Table 1: Patients baseline characteristics.

	$N = 23$
Age (years)	72 (43–80)
Sex (male/female)	13 (56)/10 (44)
Diabetes mellitus	6 (26)
Time since diagnosis of MM (months)	1.9 (0–64.4)
Previous lines of treatment	
0	11 (48)
One line	11 (48)
VAD	1
BTD followed by MPT	2
High dose dexamethasone	3
Thalidomide + dexamethasone	2
Lenalidomide + dexamethasone	2
Cyclophosphamide + prednisolone	1
Two lines	1 (4)
Melphalan + prednisolone → bortezomib	
MM type	
IgG	14 (61)
IgA	5 (22)
Light chain	4 (17)
ISS stage	
I	8 (35)
II	6 (26)
III	9 (39)
Beta-2-microglobulin (mg/L)	4.6 (2–17.5)
Albumin (g/dL)	3.5 (2.2–4.6)
GFR (mL/min/1.74 m^2)	74.3 (15.1–151.7)
Hemoglobin (g/L)	98 (64–130)
White blood cell ($\times 10^9$/L)	4.9 (1.3–8.5)
Platelet ($\times 10^9$/L)	171 (53–408)

Data are median (range) or number (%). GFR was calculated with MDRD equation. MM: multiple myeloma, VAD: vincristine + doxorubicine + dexamethasone, BTD: bortezomib + thalidomide + dexamethasone, MPT: melphalan + prednisolone + thalidomide, ISS: international staging system, and GFR: glomerular filtration rate.

Table 2: Treatment exposure.

	$N = 23$
Regimen	
VMP	13 (57)
VD	8 (35)
PAD	1 (4)
Bortezomib	1 (4)
Number of treatment cycles	5 (2–10)
Time on treatment (weeks)	19 (7–54)
Bortezomib cumulative dose (mg/m^2)	26.0 (14.3–66.3)
Bortezomib dose intensity (mg/m^2/cycle)	6.5 (2.6–10.4)
Bortezomib real/expected cumulative dose (%)	90 (50–100)
Cause of treatment cessation	
Progressive disease	7 (31)
Adverse events except peripheral neuropathy	5 (22)
Insurance reimbursement issue	4 (17)
On treatment	3 (13)
Patients refusal	3 (13)
Preplanned cycles	1 (4)

Data are median (range) or number (%). VMP: bortezomib, melphalan, and dexamethasone, VD: bortezomib and dexamethasone, and PAD: bortezomib, doxorubicin, and dexamethasone.

Table 3: The best response rate.

	$N = 23$	Rate (%)	Cumulative rate (%)
CR	1	4	4
nCR	2	9	13
VGPR	6	26	39
PR	6	26	65
Minimal response	3	13	78
Stable disease	3	13	91
Progressive disease	2	9	100

CR: complete response, nCR: near complete response, VGPR: very good partial response, and PR: partial response.

not require special management. And the rates of grade 2 or worse and grade 3 BiPN were low, only 13% and 4%, respectively, and BiPN did not necessitate cessation of the drug. In addition, cumulative dose of bortezomib was comparable to other prospective studies with the standard dose schedule. Therefore, in conjunction with dose or schedule modification and SC administration, one-hour infusion of bortezomib could be an additional option to reduce severe BiPN.

The MMY-3021 trial [8] also reported pharmacokinetic and pharmacodynamic evaluation of bortezomib administered through SC and IV bolus routes, and C_{max} was ten times lower in the SC group than in the IV bolus group, with longer T_{max} and similar AUC. Theoretically, one-hour IV infusion would decrease C_{max} and prolong T_{max}. Until now, there has been no conclusive evidence of the correlation between these pharmacokinetic results and the low incidence of BiPN. However, this could be a potential explanation for the decrease in BiPN with SC administration and the one-hour IV infusion method, and further evaluation is warranted.

The SC mode of delivery of bortezomib is now indicated for use in the clinic in the EU, USA, and recently in Korea. Barbee et al. [19] surveyed MM patients in the clinic regarding their preferences on the route of administration between SC and IV bolus. Interestingly, not all patients preferred SC. A quarter of the patients preferred IV bolus, and one patient switched from SC to IV. Their reason for favoring IV was mainly injection site reactions (ISRs) of SC administration, such as bruising and pain. The reported incidences of bortezomib-related ISRs of all grades were variable up to 39% [9]. Most common reaction was erythema and bruising, and ISRs were usually well tolerated, resolving completely. However, 1% of patients suffered from severe ISRs [8]. There was a case of necrotizing skin eruption on

Table 4: Adverse events.

	All grades		Grade ≥ 3	
Anorexia	15	(65%)	0	
Peripheral sensory neuropathy	13	(57%)	1	(4%)
Upper respiratory infection	11	(48%)	1	(4%)
Nausea	11	(48%)	0	
Fatigue	8	(35%)	0	
Gastrointestinal pain	7	(31%)	0	
Diarrhea	7	(31%)	1	(4%)
Skin rash	5	(22%)	0	
Dizziness	5	(22%)	0	
Constipation	5	(22%)	0	
Headache	4	(17%)	0	
Vomiting	3	(13%)	0	
Cramping	3	(13%)	0	
Myalgia	3	(13%)	0	
Insomnia	3	(13%)	0	
Neuralgia caused by herpes zoster	3	(13%)	0	
Fever	3	(13%)	0	
Dyspnea	3	(13%)	0	
Noncardiac chest pain	3	(13%)	0	
Hematology laboratory data				
Hemoglobin	11	(48%)	9	(39%)
White blood cell count	19	(83%)	8	(35%)
Absolute neutrophil count	17	(74%)	10	(43%)
Platelets	20	(87%)	6	(26%)

the whole abdomen after SC administration of bortezomib [9]. Cautious monitoring is crucial at each SC administration.

Limitations of this study include a small sample size, single-center data, and the retrospective nature. Matsuoka et al. [20] have presented a small retrospective study about the incidence and severity of BiPN in patients who had been treated with three-hour intravenous infusion of bortezomib. Incidences of grades 1 and 2 BiPN were 30% and 15% of patients, respectively, and there were no patients with grade 3/4 BiPN. These results are very similar to our data and appear to support our conclusion. However, to confirm these observations, prospective study with pharmacokinetic and pharmacodynamic evaluation is needed.

5. Conclusion

The current study showed that one-hour IV infusion of bortezomib-containing regimen was an effective treatment for multiple myeloma. This administration route did not reduce the incidence of bortezomib-induced peripheral neuropathy but seemed to decrease the severity of peripheral neuropathy. This route of administration of bortezomib could be a promising alternative to subcutaneous administration, particularly in patients with severe injection site reactions.

Conflict of Interests

The authors declare no conflict of interests regarding the publication of this paper.

Authors' Contribution

Hyo Jung Kim designed study concept; Ho Young Kim, Boram Han, Dae Young Zang, and Hyo Jung Kim performed the study; Joo Young Jung and Hyo Jung Kim wrote the manuscript; Joo Young Jung and Dae Ro Choi critically reviewed the study.

Acknowledgments

The authors thank Min Young Kang, CRA, for data collection and Nayoung Lee for her assistance with review of the paper in English. The authors also thank Professor Joy J. Goto for her valued comments on the paper.

References

[1] A. Palumbo and K. Anderson, "Multiple myeloma," *The New England Journal of Medicine*, vol. 364, no. 11, pp. 1046–1060, 2011.

[2] P. Moreau, P. G. Richardson, M. Cavo et al., "Proteasome inhibitors in multiple myeloma: 10 years later," *Blood*, vol. 120, no. 5, pp. 947–959, 2012.

[3] M. Delforge, J. Bladé, M. A. Dimopoulos et al., "Treatment-related peripheral neuropathy in multiple myeloma: the challenge continues," *The Lancet Oncology*, vol. 11, no. 11, pp. 1086–1095, 2010.

[4] P. G. Richardson, P. Sonneveld, M. W. Schuster et al., "Reversibility of symptomatic peripheral neuropathy with bortezomib in the phase III APEX trial in relapsed multiple myeloma: impact of a dose-modification guideline," *British Journal of Haematology*, vol. 144, no. 6, pp. 895–903, 2009.

[5] P. G. Richardson, M. Delforge, M. Beksac et al., "Management of treatment-emergent peripheral neuropathy in multiple myeloma," *Leukemia*, vol. 26, no. 4, pp. 595–608, 2012.

[6] M. A. Dimopoulos, M.-V. Mateos, P. G. Richardson et al., "Risk factors for, and reversibility of, peripheral neuropathy associated with bortezomib-melphalan-prednisone in newly diagnosed patients with multiple myeloma: subanalysis of the phase 3 VISTA study," *European Journal of Haematology*, vol. 86, no. 1, pp. 23–31, 2011.

[7] P. Moreau, V. Coiteux, C. Hulin et al., "Prospective comparison of subcutaneous versus intravenous administration of bortezomib in patients with multiple myeloma," *Haematologica*, vol. 93, no. 12, pp. 1908–1911, 2008.

[8] P. Moreau, H. Pylypenko, S. Grosicki et al., "Subcutaneous versus intravenous administration of bortezomib in patients with relapsed multiple myeloma: a randomised, phase 3, non-inferiority study," *The Lancet Oncology*, vol. 12, no. 5, pp. 431–440, 2011.

[9] K. M. Obeid, R. Ferrara, and M. Sharma, "Cutaneous lesion induced by a subcutaneous administration of bortezomib," *Clinical Lymphoma, Myeloma and Leukemia*, vol. 12, no. 4, pp. 284–286, 2012.

[10] N. C. Institute, *National Cancer Institute Cancer Therapy Evaluation Program Common Terminology Criteria for Adverse Events*, version 4.0, 2009.

[11] J. F. San Miguel, R. Schlag, N. K. Khuageva et al., "Bortezomib plus melphalan and prednisone for initial treatment of multiple myeloma," *The New England Journal of Medicine*, vol. 359, no. 9, pp. 906–917, 2008.

[12] L. Janssen Korea, *Package Insert of Bortezomib*, 2013.

[13] B. G. Durie, J. L. Harousseau, J. S. Miguel et al., "International uniform response criteria for multiple myeloma," *Leukemia*, vol. 20, no. 9, pp. 1467–1473, 2006.

[14] P. G. Richardson, B. Barlogie, J. Berenson et al., "A phase 2 study of Bortezomib in relapsed, refractory myeloma," *The New England Journal of Medicine*, vol. 348, no. 26, pp. 2609–2617, 2003.

[15] J. Blade, D. Samson, D. Reece et al., "Criteria for evaluating disease response and progression in patients with multiple myeloma treated by high-dose therapy and haemopoietic stem cell transplantation," *British Journal of Haematology*, vol. 102, no. 5, pp. 1115–1123, 1998.

[16] P. G. Richardson, P. Sonneveld, M. W. Schuster et al., "Bortezomib or high-dose dexamethasone for relapsed multiple myeloma," *The New England Journal of Medicine*, vol. 352, no. 24, pp. 2487–2498, 2005.

[17] P. Moreau, H. Avet-Loiseau, T. Facon et al., "Bortezomib plus dexamethasone versus reduced-dose bortezomib, thalidomide plus dexamethasone as induction treatment before autologous stem cell transplantation in newly diagnosed multiple myeloma," *Blood*, vol. 118, no. 22, pp. 5752–5758, 2011.

[18] S. Bringhen, A. Larocca, D. Rossi et al., "Efficacy and safety of once-weekly bortezomib in multiple myeloma patients," *Blood*, vol. 116, no. 23, pp. 4745–4753, 2010.

[19] M. S. Barbee, R. D. Harvey, S. Lonial et al., "Subcutaneous versus intravenous bortezomib: efficiency practice variables and patient preferences," *Annals of Pharmacotherapy*, vol. 47, no. 9, pp. 1136–1142, 2013.

[20] K. H. Matsuoka H, M. Sugita, and H. Yamaguchi, "Three hours intravenous administration of bortezomib in myeloma patients reduced severity of peripheral neuropathy induced by bortezomib," *Clinical Lymphoma, Myeloma & Leukemia*, vol. 13, supplement 1, p. S207, 2013.

Hindawi Publishing Corporation
BioMed Research International
Volume 2014, Article ID 253243, 7 pages
http://dx.doi.org/10.1155/2014/253243

Research Article

Clinical Factors Associated with Response or Survival after Chemotherapy in Patients with Waldenström Macroglobulinemia in Korea

Ho Sup Lee,[1] Kihyun Kim,[2] Dok Hyun Yoon,[3] Jin Seok Kim,[4] Soo-Mee Bang,[5] Jeong-Ok Lee,[5] Hyeon Seok Eom,[6] Hyewon Lee,[6] Inho Kim,[7] Won Sik Lee,[8] Sung Hwa Bae,[9] Se Hyung Kim,[10] Mark Hong Lee,[11] Young Rok Do,[12] Jae Hoon Lee,[13] Junshik Hong,[13] Ho-Jin Shin,[14] Ji Hyun Lee,[15] Yeung-Chul Mun,[16] and Chang-Ki Min[17]

[1] *Department of Internal Medicine, Kosin University College of Medicine, 262 Gamcheon-ro, Seo-gu, Busan 602-702, Republic of Korea*
[2] *Department of Internal Medicine, Samsung Medical Center, School of Medicine, Sungkyunkwan University, 81 Irwon-ro, Gangnam-gu, Seoul 135-710, Republic of Korea*
[3] *Department of Oncology, Asan Medical Center, University of Ulsan College of Medicine, 13 Gangdong-daero, Songpa-gu, Seoul 138-736, Republic of Korea*
[4] *Department of Internal Medicine, Yonsei University College of Medicine, 50-1 Yonsei-ro, Seodaemun-gu, Seoul 120-752, Republic of Korea*
[5] *Department of Internal Medicine, Seoul National University Bundang Hospital, 82 Gumi-ro, 173 Beon-gil, Bundang-gu, Seongnam-si, Gyeonggi-do 463-707, Republic of Korea*
[6] *Department of Internal Medicine, National Cancer Center of Korea, 323 Ilsan-ro, Ilsandong-gu, Goyang-si, Gyeonggi-do 410-769, Republic of Korea*
[7] *Department of Internal Medicine, Seoul National University Hospital, 101 Daehak-ro, Jongno-gu, Seoul 110-744, Republic of Korea*
[8] *Department of Internal Medicine, Busan Paik Hospital, 75 Bokji-ro, Busanjin-gu, Busan 614-735, Republic of Korea*
[9] *Department of Internal Medicine, Daegu Catholic University Medical Center, 33 Duryugongwon-ro 17-gil, Nam-gu, Daegu 705-718, Republic of Korea*
[10] *Department of Internal Medicine, Soonchunhyang University College of Medicine, Bucheon Hospital, 170 Jomaru-ro, Wonmi-gu, Bucheon-si, Gyeonggi-do 420-767, Republic of Korea*
[11] *Department of Internal Medicine, Konkuk University Hospital, 120-1 Neungdong-ro, Gwangjin-gu, Seoul 143-729, Republic of Korea*
[12] *Department of Internal Medicine, Keimyung University College of Medicine, 56 Dalseong-ro, Jung-gu, Daegu 700-712, Republic of Korea*
[13] *Department of Internal Medicine, Gachon University Gil Hospital, 21 Namdong-daero, 774 Beon-gil, Namdong-gu, Incheon 405-760, Republic of Korea*
[14] *Department of Internal Medicine, Pusan National University Hospital, 179 Gudeok-ro, Seo-gu, Busan 602-739, Republic of Korea*
[15] *Department of Internal Medicine, Dong-A University College of Medicine, 26 Daesingongwon-ro, Seo-gu, Busan 602-715, Republic of Korea*
[16] *Department of Internal Medicine, Ewha Womans University School of Medicine, 1071 Anyangcheon-ro, Yangcheon-gu, Seoul 158-710, Republic of Korea*
[17] *Division of Hematology, Department of Internal Medicine, Seoul St. Mary's Hospital, College of Medicine, The Catholic University of Korea, 222 Banpo-daero, Seocho-gu, Seoul 137-701, Republic of Korea*

Correspondence should be addressed to Chang-Ki Min; hemonhs@gmail.com

Received 27 March 2014; Revised 12 May 2014; Accepted 12 May 2014; Published 4 June 2014

Academic Editor: Dong Soon Lee

Waldenström's macroglobulinemia (WM) is a B-cell proliferative malignancy characterized by immunoglobulin M monoclonal gammopathy and bone marrow infiltration by lymphoplasmacytic cells. Clinical features and cytogenetics of WM in Asia including Republic of Korea remain unclear. Moreover, no study has reported treatment outcomes in patients with WM treated with novel agent combined with conventional chemotherapy. This study investigated clinical features and assessed treatment outcomes with novel agent and conventional chemotherapy in Republic of Korea. Data from all ($n = 71$) patients with newly diagnosed WM at 17 hospitals who received chemotherapy between January 2005 and December 2012 were collected retrospectively. The median age of patients was 66 years (range: 37–92 years) and male to female ratio was 5 : 1. Patients treated with novel agent combined chemotherapy displayed higher overall response rate (ORR) compared to conventional chemotherapy alone (92.9% versus 52.6%, $P = 0.006$). The 5-year overall survival rate was 62.6% (95% confidence interval: 34.73–111.07). Use of novel agents produced higher ORR but survival benefit was not apparent due to the small number of patients and short follow-up duration. Further studies are needed to confirm the efficacy of novel agents in patients with WM.

1. Introduction

The consensus group at the Second International Workshop on Waldenström's macroglobulinemia (WM) in 2002 redefined WM as a distinct clinicopathologic entity characterized by bone marrow infiltration by lymphoplasmacytic lymphoma (LPL) and immunoglobulin M (IgM) monoclonal gammopathy [1]. Diagnostic criteria for WM are IgM monoclonal gammopathy of any concentration, bone marrow infiltration by small lymphocytes showing plasmacytoid or plasma cell differentiation, intertrabecular pattern of bone marrow infiltration, and surface IgM^+, $CD5^{\pm}$, $CD10^-$, $CD19^+$, $CD20^+$, $CD22^+$, $CD23^-$, $CD25^+$, $CD27^+$, $FMC7^+$, $CD103^-$, and $CD138^-$ immunophenotype [2].

WM is a very rare lymphoid malignancy, with an overall incidence estimated at 0.35 for WM and 0.63 for LPL/WM per 100,000 person-years during 2001–2003, representing 1.2% or 2.1% of all non-Hodgkin's lymphomas in the United States Surveillance Epidemiology and End Results (SEER) cancer registries, respectively [3]. Between 1996 and 2003, the crude incidence of LPL/WM was 0.078 per 100,000 person-years in Japan (0.112 for men and 0.048 for women) and 0.032 per 100,000 person-years in Taiwan (0.042 for men and 0.021 for women) [4]. A previous nationwide survey of the incidence of lymphoma based on the REAL classification reported the incidence of LPL in Korea as 0.8%, with the exact incidence rate of WM/LPL not recognized [5]. The incidence rate of WM is lower in the Republic of Korea than those in the USA, which was documented to be about 0.3 per million person-years according to data of the National Cancer Information Center in the Republic of Korea.

The most common clinical manifestations are hepatomegaly (20%), splenomegaly (15%), and lymphadenopathy (15%) [6]. The most common presenting symptom is fatigue related to normochromic or normocytic anemia. The median hemoglobin value at diagnosis is 10 g/dL [7]. Patients with a disease-related hemoglobin level <10 g/dL, platelet count $<100 \times 10^9$/L, bulky adenopathy or organomegaly, symptomatic hyperviscosity, moderate to severe or advancing peripheral neuropathy on the basis of disease, symptomatic amyloidosis, cryoglobulinemia, or cold-agglutinin disease should be considered for therapy but asymptomatic patients should be observed [8]. Recently, the superior efficacy of chemotherapy combined with novel agent including rituximab, bendamustine, bortezomib, lenalidomide, and thalidomide than that of conventional chemotherapy has been established. However, little is known about the clinical features, epidemiology, and cytogenetics of WM in Asia including the Republic of Korea. Novel agent combined chemotherapy for patients with WM has been restricted in the Republic of Korea because of very low incidence and insurance coverage limitation.

Treatment outcomes in the Republic of Korea patients with WM treated by novel agent combined chemotherapy are unclear. This study is aimed at investigating the clinical features and assess the treatment outcomes of novel agent combined chemotherapy and conventional chemotherapy.

2. Materials and Methods

2.1. Patients. Data from 71 patients newly diagnosed with WM who received chemotherapy at 17 university hospitals in the Republic of Korea between January 2005 and December 2012 were collected retrospectively. All cases fulfilled the diagnostic criteria [1] and were confirmed as WM by hematopathologists and hematologists. The pretreatment evaluation included a physical examination with performance status evaluation, complete blood cell count with differential count, blood chemistry including lactase dehydrogenase (LDH), protein electrophoresis (PEP), IgM, free light chain kappa and lambda, bone marrow biopsy, chromosomal study, fluorescence in situ hybridization (FISH), and computed tomography (CT) of the chest, abdomen, and pelvis.

2.2. Treatment. All patients were treated with conventional chemotherapy or chemotherapy along with novel agent (rituximab, bortezomib, thalidomide, and bendamustine). Bendamustine is old chemotherapeutic agent but recently the roles of this drug were rediscovered by its efficacy and toxicities in indolent lymphoma including WM [9, 10]. Rituximab combined chemotherapy included rituximab, cyclophosphamide, vincristine, and prednisolone (R-CVP) and rituximab, cyclophosphamide, doxorubicin, vincristine, and prednisolone (R-CHOP). Bortezomib combined chemotherapy included bortezomib plus dexamethasone (VD). Thalidomide combined chemotherapy included thalidomide plus dexamethasone (TD) and thalidomide plus cyclophosphamide and dexamethasone (TCD). Bendamustine was used along with prednisolone. Conventional chemotherapy included chlorambucil, CVP, CHOP, melphalan plus prednisolone (MP), cyclophosphamide plus prednisolone (CP), fludarabine plus cyclophosphamide (FC), and fludarabine plus cyclophosphamide and mitoxantrone (FCM).

All patients were treated with one or more chemotherapeutic regimens. Patients who displayed progression or intolerance against previous chemotherapy were changed from chemotherapy to a salvage regimen. Introduction of novel agents was applied to four patients as first-line, three as second-line, five at third-line, and two at fourth-line chemotherapy.

2.3. Analysis. Overall response rates (ORR) of patients treated with conventional and novel therapy were estimated as the best response at first-line and applied periods. ORR were estimated by clinical para-meters in all patients including age, sex, hemoglobin levels, platelet counts, absolute lymphocyte counts (ALC), C-reactive protein (CRP), LDH, serum β2-microglobulin, serum albumin, hepatosplenomegaly, Eastern Cooperative Oncology Group (ECOG) performance status, presence of B symptoms, and hyperviscosity syndrome. Those clinical parameters and international staging system (ISS), International Prognostic Scoring System for Waldenstrom's Macroglobulinemia (ISSWM), and treatment modalities were estimated to find prognostic markers for survival. The treatment response was assessed according to the Sixth International Workshop on WM [11].

2.4. Statistical Analyses. We investigated independent prognostic factors associated with survival in above clinical and laboratory parameters. The definition of overall survival (OS) was calculated from the date of diagnosis to the date of death from disease-related cause or final follow-up date. Progression-free survival (PFS) was from the date of starting treatment (conventional chemotherapy or novel agent combined chemotherapy) to the date of disease progression, relapse, or death from disease-related cause. Associations between the clinical parameters and ORR were analyzed using the chi-square test. A multiple logistic regression analysis was used for multivariate analysis of independent prognostic factors for ORR. Survival probabilities were calculated according to the Kaplan-Meier method and compared using the log-rank test. The Cox proportional hazards regression model was used for multivariate analysis of independent prognostic factors for survival. Information about the baseline medical status and treatment modalities was collected from the medical records. Approval for these studies was obtained from the Institutional Review Board.

3. Results

3.1. Clinical and Laboratory Characteristics. The median age of the 71 patients was 66 years (range: 37–92 years) and the male to female ratio was 5 : 1 (Table 1). 25.4% and 38.0% of patients had clinical or radiological evidence of splenomegaly and of lymphadenopathy, respectively. 11.3% of patients had B symptoms before the initiation of treatment. Hyperviscosity and involvement of other organs were shown in 11.3% and 40.8% of patients, respectively. The median serum monoclonal protein level was 3.640 g/dL (range: 0.0183–10.795). The cytogenetic abnormalities identified included deletion of the long arm of chromosome 6 in two cases and absence of trisomy 4. Other cytogenetic abnormalities were identified in 11.3% of cases. Other clinical or laboratory characteristics are summarized in Table 1.

Table 1: Clinical and laboratory characteristics.

Characteristic	N (%) or median (range)
Patients	71
Age, years, median (range)	66 (37–92)
Gender	
Male	59 (83.1)
Female	12 (16.9)
Hemoglobin, g/dL, median (range)	9.6 (3.80–17.10)
Platelet count, $\times 10^9$/L, median (range)	213 (23–575)
ALC, $\times 10^9$/L, median (range)	1.70 (0.10–12.30)
CRP, mg/dL, median (range)	2.44 (0.05–23.80)
Serum β2-microglobulin, mg/L, median (range)	4.20 (1.34–30.00)
Serum albumin, g/dL, median (range)	3.2 (1.50–4.60)
LDH, IU/L, median (range)	261.0 (74.0–968.0)
BM lymphocyte, %, median (range)	14 (5–100)
Cytogenetic abnormalities, present (%)	8 (11.3%)
Serum monoclonal protein, mg/dL, median (range)	3640.0 (18.30–10795.0)
B symptom, present (%)	8 (11.3)
ECOG (%) ≥2	23 (32.4)
Hyperviscosity, present (%)	8 (11.3)
Lymphadenopathy, yes (%)	27 (38.0)
Extranodal involvement, yes (%)	29 (40.8)
Splenomegaly, yes (%)	18 (25.4)
Hepatomegaly, yes (%)	7 (9.9)
ISS (%)	
I	13 (18.3)
II	26 (36.6)
III	26 (36.6)
Unknown	6 (8.5)
Treatment regimen	
Novel agent combined chemotherapy	14 (19.7)
Conventional chemotherapy	57 (80.3)
Treatment	
Novel group	
R-combined CTx; R-CVP, R-CHOP	6 (8.5)
VD	5 (7.0)
TD	2 (2.8)
Bendamustine plus prednisolone	1 (1.4)
Conventional group	
Chlorambucil	25 (35.2)
CVP or CHOP	14 (19.7)
MP or CP	15 (21.1)
FC or FCM	3 (4.2)

ALC: absolute lymphocyte count; CRP: C-reactive protein; LDH: lactate dehydrogenase; BM: bone marrow; ECOG: Eastern Cooperative Oncology Group performance status; ISS: international staging system; R-combined CTx: rituximab combined chemotherapy; R-CVP: rituximab, cyclophosphamide, vincristine, and prednisolone; R-CHOP: rituximab, cyclophosphamide, doxorubicin, vincristine, and prednisolone; VD: bortezomib plus dexamethasone; TD: thalidomide plus dexamethasone; MP: melphalan plus prednisolone; CP: cyclophosphamide plus prednisolone; FC: fludarabine plus cyclophosphamide; FCM: fludarabine plus cyclophosphamide and mitoxantrone.

3.2. Treatment and Outcomes. The 71 patients were treated with novel agent combined chemotherapy or conventional chemotherapy. 25 patients were treated with chlorambucil with or without prednisone, 14 were treated with CVP or CHOP regimen, 15 were treated with the MP or CP regimen, and three were treated with FC or FCM as first-line therapy. Other patients were treated with novel agent combined chemotherapy. Six patients were treated with R-CVP, R-CHOP, five with VD, two with thalidomide plus dexamethasone, and one with bendamustine plus prednisolone (Table 1). Overall, an objective response (complete or partial response) and more than minimal response (MR) rates were documented in 53.5% and 69.0% of patients, respectively. The median follow-up was 22.97 months. The 5-year PFS and OS rates were 50.5% and 62.6% (95% confidence interval (95% CI): 48.32–81.41 and 34.73–111.07), respectively.

3.3. Analysis of Prognostic Factors for Response and Survival. Multiple parameters were analyzed for their possible prognostic impact on ORR and OS. Univariate analysis showed that the following factors were associated with higher ORR (Table 2): higher ALC ($<1.0 \times 10^9$/L versus $\geq 1.0 \times 10^9$/L; 20.0% versus 64.6%, $P = 0.069$), good ECOG performance status (<2 versus ≥2; 67.4 versus 43.5, $P = 0.057$), and novel agent combined chemotherapy versus conventional chemotherapy (92.9% versus 52.6%, $P = 0.006$). The following factors were associated with superior 5-year OS (Table 2): younger age (<65 years versus ≥65 years; 82.2% versus 36.8%, $P = 0.024$), good ECOG performance status (<2 versus ≥2; 72.6% versus 26.9%, $P = 0.004$), higher serum albumin levels (<3.5 g/dL versus ≥3.5 g/dL; 44.8% versus 84.1%, $P = 0.010$), lower risk international staging system (ISS I, II, and III; 85.7%, 84.8%, and 36.8%, resp.; $P = 0.004$), and novel agent combined chemotherapy versus conventional chemotherapy (100% versus 53.0%, $P = 0.067$). In the multivariate analysis, novel agent combined chemotherapy was an independent prognostic value for ORR ($P = 0.046$) and lower ISS was an independent prognostic value for OS ($P = 0.008$) (Table 3).

4. Discussion

The clinical manifestations and laboratory abnormalities associated with WM are related to direct tumor infiltration and to the amount and specific properties of monoclonal IgM. The most common symptoms are weakness and fatigue, usually secondary to anemia. Symptoms of weight loss, excessive sweating, and low-grade fever affect a quarter of patients. Hepatomegaly, splenomegaly, and lymphadenopathy each occur in 15%–30% of patients. Similarly, previous Korean studies reported the most common symptoms as anemia and thrombocytopenia, with other frequently expressed symptoms being (20–40%), hepatosplenomegaly (25–35%), and lymphadenopathy (25–40%) [12–14]. In this study, anemia was the most frequent symptom and thrombocytopenia was secondary frequent symptom with lymphadenopathy, hepatomegaly, and splenomegaly being expressed in 10–40% of the cases. However, hyperviscosity syndrome was documented only in eight cases, which was a relatively low incidence compared to western results [6]. The most common cytogenetic abnormality was deletion of the long arm of chromosome 6 (6q deletion) and trisomy 4 [15, 16]. However, there was only one report about cytogenetics of Korean WM patients, which documented a low rate of 6q deletion (10%) and no trisomy 4 [13]. Similar to a previous Korean study, this study documented a low rate of 6q deletion and other cytogenetic abnormalities.

In this study, novel agent combined chemotherapy was the only independent predictive factor for response rates, although higher ALC count and good performance status were also associated with higher ORR in univariate analysis. Many studies have been shown to improve response and survival rates in patients with WM. Gertz et al. presented meaningful results about efficacy of rituximab in patients with WM [17]. The efficacy of rituximab in WM has been amply described. Rituximab combined chemotherapy including R-CHOP, R-CVP, R-CP, R-F (fludarabine), R-cladribine, and R-CD (cyclophosphamide and dexamethasone) produces superior response rates to conventional chemotherapy [18–23]. Rituximab combined with thalidomide reportedly produced a 72% response rate, and rituximab combined with lenalidomide produced a 50% response rate [24, 25]. Bortezomib has high levels of activity in the management of relapsed WM with response rates ranging from 81% to 96% [26, 27]. In a prospective randomized study of bendamustine plus rituximab compared with R-CHOP in patients with WM, of whom 22 received bendamustine and rituximab and 19 received R-CHOP, the response rate was 95% in both groups, but median progression-free survival was significantly prolonged with bendamustine. The median progression-free survival for R-CHOP was 36 months in contrast to not being reached with bendamustine and rituximab ($P < 0.0001$) [28].

In our study, only lower risk ISS showed superior survival to those of higher risk ISS although younger age, good performance status, higher serum albumin levels, and novel agent combined chemotherapy were associated with longer OS in univariate analysis. Patients treated with novel agent combined chemotherapy especially did not show superior survival rates to conventional chemotherapy in spite of higher ORR in patients receiving novel agents. These results might be associated with small sample size, short follow-up duration, and the clinical features of WM (which seems to be indolent lymphoma). Prior studies have documented several prognostic factors for survival [29–33]. Age, anemia, leukopenia, thrombocytopenia, serum albumin levels, and β2-microglobulin values were linked to survival. However, these prognostic factors were not meaningful in this study. Very low incidence rates of WM and restriction of using novel agents because of the limitation of medical reimbursement in Korea might be reasons for the insufficient comparison between novel agent combined chemotherapy and conventional chemotherapy in this study.

5. Conclusions

Clinical features of Korean WM are similar to western WM, except for the low incidence of hyperviscosity syndrome. Response rates after chemotherapy were improved by

Table 2: Clinical and laboratory values associated with survival on univariate analysis.

Characteristic	ORR ≥ PR (%)	P	5-year PFS (%)	P	5-year OS (%)	P
Age, years						
<65	66.7	0.327	64.8	0.708	82.2	0.024
≥65	55.3		37.0		36.8	
Gender						
Male	64.4	0.197	46.8	0.530	65.9	0.130
Female	41.7		77.9		45.0	
BM lymphocyte, %						
<50	63.0	0.461	61.8	0.478	40.6	0.610
≥50	52.9		26.2		52.5	
Cytogenetic abnormalities						
Presence	75.0	0.466	64.3	0.599	50.0	0.444
Absence	58.7		50.9		42.4	
Hemoglobin, g/dL						
<11.5	61.3	0.732	54.3	0.140	60.2	0.700
≥11.5	55.6		0.0		87.5	
Platelet count, $\times 10^9$/L						
<100	69.2	0.479	0.0	0.049	61.9	0.124
≥100	58.6		60.6		63.0	
ALC, $\times 10^9$/L						
<1.0	20.0	0.069	33.3	0.611	40.0	0.224
≥1.0	64.6		54.3		64.6	
CRP, mg/dL						
<5	58.0	0.817	46.4	0.937	65.5	0.096
≥5	61.5		51.3		48.6	
Serum β2-microglobulin, mg/L						
<3	66.7	0.862	48.9	0.130	50.0	0.143
≥3	64.0		50.6		58.4	
Serum albumin, g/dL						
<3.5	58.5	0.683	43.1	0.712	44.8	0.010
≥3.5	63.3		64.4		84.1	
LDH, IU/L						
<450	59.7	0.688	50.1	0.849	61.8	0.403
≥450	66.7		65.6		72.9	
B symptom						
Presence	87.5	0.132	83.3	0.666	60.0	0.385
Absence	56.5		48.8		62.8	
ECOG, (%)						
0-1	67.4	0.057	68.7	0.012	72.6	0.004
≥2	43.5		18.1		26.9	
Hyperviscosity syndrome						
Presence	25.0	0.055	58.3	0.980	0.0	0.918
Absence	63.8		49.7		66.2	
Splenomegaly						
Presence	61.1	0.838	77.4	0.342	0.0	0.300
Absence	58.3		49.5		52.7	
Hepatomegaly						
Presence	57.1	0.884	40.0	0.246	28.6	0.913
Absence	60.0		42.5		45.1	
ISSWM (%)						
Low	57.1	0.567	66.7	0.912	50.0	0.380
Intermediate	58.8		58.7		58.0	
High	75.0		0.0		58.4	

Table 2: Continued.

Characteristic	ORR ≥ PR (%)	*P*	5-year PFS (%)	*P*	5-year OS (%)	*P*
ISS (%)						
I	61.5		48.7		85.7	
II	69.2	0.522	41.9	0.714	84.8	0.004
III	53.8		64.7		36.8	
Treatment regimen						
Novel agent combined chemotherapy	92.9	0.006	79.1	0.418	100.0	0.067
Conventional chemotherapy	52.6		46.3		53.0	

ORR: overall response rates; PR: partial response rates; 5-year PFS: 5-year progression-free survival rates; 5-year OS: 5-year overall survival rates; BM: bone marrow; ALC: absolute lymphocyte count; CRP: C-reactive protein; LDH: lactate dehydrogenase; ECOG: Eastern Cooperative Oncology Group performance status; ISSWM: International Prognostic Scoring System for Waldenstrom's Macroglobulinemia; ISS: international staging system.

Table 3: Multivariate analysis for response and survival.

Value	ORR			OS		
	RR	95% CI	*P* value	RR	95% CI	*P* value
Age, years						
<65						
≥65				1.021	0.350–2.980	0.970
ALC, $\times 10^9$/L						
<1.0						
≥1.0	0.362	0.060–2.193	0.369			
ECOG (%)						
0-1						
≥2	2.006	0.711–5.660	0.188	0.421	0.147–1.208	0.108
Serum albumin, g/dL						
<3.5						
≥3.5				1.123	0.264–4.772	0.875
ISS (%)						
I						
II				0.439	0.078–2.486	0.352
III				0.209	0.066–0.665	0.008
Treatment regimen						
Novel agent combined chemotherapy						
Conventional chemotherapy	5.048	1.032–24.702	0.046	0.368	0.075–1.803	0.217

ORR: overall response rates; OS: overall survival rates; RR: relative risk; 95% CI: 95% confidence interval; ALC: absolute lymphocyte count; ECOG: Eastern Cooperative Oncology Group performance status; ISS: international staging system.

introduction of novel agents such as rituximab, bortezomib, thalidomide, and bendamustine, although survival benefit was not shown. Independent prognostic factor for survival was high risk ISS in Korean WM. However, further study with more patients is needed to determine the efficacy of novel agent combined chemotherapy and to definitively identify the prognostic factors.

Conflict of Interests

The authors declare that they have no conflict of interests.

Authors' Contribution

Ho Sup Lee analyzed the clinical data and wrote the paper. Ho Sup Lee and Chang-Ki Min designed the study. Kihyun Kim, Dok Hyun Yoon, Jin Seok Kim, Soo-Mee Bang, Jeong-Ok Lee, Hyeon Seok Eom, Hyewon Lee, Inho Kim, Won Sik Lee, Sung Hwa Bae, Se Hyung Kim, Hong-Kee Lee, Young-Rok Do, Jae Hoon Lee, Junshik Hong, Ho-Jin Shin, Ji Hyun Lee, Yeung-Chul Mun, and Korean Multiple Myeloma Working Party (KMMWP) contributed to conception of the study and analyzed the clinical data. Chang-Ki Min was involved in revising the paper critically for intellectual content and gave final approval for the submission of the paper.

References

[1] R. G. Owen, S. P. Treon, A. Al-Katib et al., "Clinicopathological definition of Waldenstrom's macroglobulinemia: consensus panel recommendations from the Second International Workshop on Waldenstrom's Macroglobulinemia," *Seminars in Oncology*, vol. 30, no. 2, pp. 110–115, 2003.

[2] A. Vijay and M. A. Gertz, "Waldenström macroglobulinemia," *Blood*, vol. 109, no. 12, pp. 5096–5103, 2007.

[3] L. M. Morton, J. J. Turner, J. R. Cerhan et al., "Proposed classification of lymphoid neoplasms for epidemiologic research from the Pathology Working Group of the International Lymphoma Epidemiology Consortium (InterLymph)," *Blood*, vol. 110, no. 2, pp. 695–708, 2007.

[4] M. Iwanaga, C.-J. Chiang, M. Soda et al., "Incidence of lymphoplasmacytic lymphoma/Waldenström's macroglobulinaemia in Japan and Taiwan population-based cancer registries, 1996–2003," *International Journal of Cancer*, vol. 134, no. 1, pp. 174–180, 2014.

[5] Y. H. Ko, C. W. Kim, C. S. Park et al., "REAL classification of malignant lymphomas in the Republic of Korea: incidence of recently recognized entities and changes in clinicopathologic features. Hematolymphoreticular Study Group of the Korean Society of Pathologists. Revised European-American lymphoma," *Cancer*, vol. 83, no. 4, pp. 806–812, 1998.

[6] M. A. Dimopoulos and A. Anagnostopoulos, "Waldenström's macroglobulinemia," *Best Practice and Research: Clinical Haematology*, vol. 18, no. 4, pp. 747–765, 2005.

[7] M. Björkholm, E. Johansson, D. Papamichael et al., "Patterns of clinical presentation, treatment, and outcome in patients with Waldenstrom's macroglobulinemia: a two-institution study," *Seminars in Oncology*, vol. 30, no. 2, pp. 226–230, 2003.

[8] S. P. Treon, "How I treat Waldenström macroglobulinemia," *Blood*, vol. 114, no. 12, pp. 2375–2385, 2009.

[9] M. Merli, A. Ferrario, C. Basilico et al., "Novel agents in indolent lymphomas," *Therapeutic Advances in Hematology*, vol. 4, no. 2, pp. 133–148, 2013.

[10] M. J. Rummel and S. A. Gregory, "Bendamustine's emerging role in the management of lymphoid malignancies," *Seminars in Hematology*, vol. 48, no. 1, pp. S24–S36, 2011.

[11] S. P. Treon, G. Merlini, E. Morra, C. J. Patterson, and M. J. Stone, "Report from the sixth international workshop on Waldenström's macroglobulinemia," *Clinical Lymphoma, Myeloma and Leukemia*, vol. 11, no. 1, pp. 68–73, 2011.

[12] S.-M. Bang, S. R. Park, S. H. Park et al., "Clinical features of Waldenstrom macroglobulinemia in Korea," *Korean Journal of Internal Medicine*, vol. 19, no. 3, pp. 137–140, 2004.

[13] S.-M. Bang, J.-W. Seo, K. U. Park et al., "Molecular cytogenetic analysis of Korean patients with Waldenström macroglobulinemia," *Cancer Genetics and Cytogenetics*, vol. 197, no. 2, pp. 117–121, 2010.

[14] Y.-W. Won, S. J. Kim, K. Kim, Y. H. Ko, and W. S. Kim, "Clinical features and treatment outcomes of lymphoplasmacytic lymphoma: a single center experience in Korea," *Annals of Hematology*, vol. 89, no. 10, pp. 1011–1018, 2010.

[15] R. F. J. Schop, S. A. Van Wier, R. Xu et al., "6q deletion discriminates Waldenström macroglobulinemia from IgM monoclonal gammopathy of undetermined significance," *Cancer Genetics and Cytogenetics*, vol. 169, no. 2, pp. 150–153, 2006.

[16] C. Terré, F. Nguyen-Khac, C. Barin et al., "Trisomy 4, a new chromosomal abnormality in Waldenström's macroglobulinemia: a study of 39 cases," *Leukemia*, vol. 20, no. 9, pp. 1634–1636, 2006.

[17] M. A. Gertz, M. Rue, E. Blood, L. S. Kaminer, D. H. Vesole, and P. R. Greipp, "Multicenter phase 2 trial of rituximab for Waldenström macroglobulinemia (WM): an Eastern Cooperative Oncology Group Study (E3A98)," *Leukemia and Lymphoma*, vol. 45, no. 10, pp. 2047–2055, 2004.

[18] S. P. Treon, Z. Hunter, and A. R. Branagan, "CHOP plus rituximab therapy in Waldenström's macroglobulinemia," *Clinical Lymphoma*, vol. 5, no. 4, pp. 273–277, 2005.

[19] L. Ioakimidis, C. Patterson, Z. Hunter et al., "Comparative outcomes following CP-R, CVP-R, and CHOP-R in Waldenström's macroglobulinemia," *Clinical Lymphoma and Myeloma*, vol. 9, no. 1, pp. 62–66, 2009.

[20] M. A. Dimopoulos, A. Anagnostopoulos, M.-C. Kyrtsonis et al., "Primary treatment of Waldenström macroglobulinemia with dexamethasone, rituximab, and cyclophosphamide," *Journal of Clinical Oncology*, vol. 25, no. 22, pp. 3344–3349, 2007.

[21] S. P. Treon, A. R. Branagan, L. Ioakimidis et al., "Long-term outcomes to fludarabine and rituximab in Waldenström macroglobulinemia," *Blood*, vol. 113, no. 16, pp. 3673–3678, 2009.

[22] D. Laszlo, G. Andreola, L. Rigacci et al., "Rituximab and subcutaneous 2-chloro-2′-deoxyadenosine as therapy in untreated and relapsed Waldenström's macroglobulinemia," *Clinical Lymphoma, Myeloma and Leukemia*, vol. 11, no. 1, pp. 130–132, 2011.

[23] A. Tedeschi, G. Benevolo, M. Varettoni et al., "Fludarabine plus cyclophosphamide and rituximab in waldenstrom macroglobulinemia: an effective but myelosuppressive regimen to be offered to patients with advanced disease," *Cancer*, vol. 118, no. 2, pp. 434–443, 2012.

[24] S. P. Treon, J. D. Soumerai, A. R. Branagan et al., "Thalidomide and rituximab in Waldenstrom macroglobulinemia," *Blood*, vol. 112, no. 12, pp. 4452–4457, 2008.

[25] S. P. Treon, J. D. Soumerai, A. R. Branagan et al., "Lenalidomide and rituximab in Waldenstrom's macroglobulinemia," *Clinical Cancer Research*, vol. 15, no. 1, pp. 355–360, 2009.

[26] S. P. Treon, L. Ioakimidis, J. D. Soumerai et al., "Primary therapy of Waldenström macroglobulinemia with bortezomib, dexamethasone, and rituximab: WMCTG clinical trial 05-180," *Journal of Clinical Oncology*, vol. 27, no. 23, pp. 3830–3835, 2009.

[27] I. M. Ghobrial, F. Hong, S. Padmanabhan et al., "Phase II trial of weekly bortezomib in combination with rituximab in relapsed or relapsed and refractory Waldenström macroglobulinemia," *Journal of Clinical Oncology*, vol. 28, no. 8, pp. 1422–1428, 2010.

[28] M. J. Rummel, N. Niederle, G. Maschmeyer et al., "Bendamustine plus rituximab versus CHOP plus rituximab as first-line treatment for patients with indolent and mantle-cell lymphomas: an open-label, multicentre, randomised, phase 3 non-inferiority trial," *The Lancet*, vol. 381, no. 9873, pp. 1203–1210, 2013.

[29] M. A. Gertz, "Waldenström macroglobulinemia: 2013 update on diagnosis, risk stratification, and management," *The American Journal of Hematology*, vol. 88, no. 8, pp. 703–711, 2013.

[30] P. G. Gobbi, R. Bettini, C. Montecucco et al., "Study of prognosis in Waldenstrom's macroglobulinemia: a proposal for a simple binary classification with clinical and investigational utility," *Blood*, vol. 83, no. 10, pp. 2939–2945, 1994.

[31] P. Morel, M. Monconduit, D. Jacomy et al., "Prognostic factors in Waldenstrom macroglobulinemia: a report on 232 patients with the description of a new scoring system and its validation on 253 other patients," *Blood*, vol. 96, no. 3, pp. 852–858, 2000.

[32] P. Morel, A. Duhamel, P. Gobbi et al., "International prognostic scoring system for Waldenström macroglobulinemia," *Blood*, vol. 113, no. 18, pp. 4163–4170, 2009.

[33] R. García-Sanz, S. Montoto, A. Torrequebrada et al., "Waldenström macroglobulinaemia: presenting features and outcome in a series with 217 cases," *British Journal of Haematology*, vol. 115, no. 3, pp. 575–582, 2001.

Hindawi Publishing Corporation
BioMed Research International
Volume 2014, Article ID 513170, 6 pages
http://dx.doi.org/10.1155/2014/513170

Research Article

Plasma Levels of Osteopontin and Vascular Endothelial Growth Factor in Association with Clinical Features and Parameters of Tumor Burden in Patients with Multiple Myeloma

Toni Valković,[1] Emina Babarović,[2] Ksenija Lučin,[2] Sanja Štifter,[2] Merica Aralica,[3] Sanja Pećanić,[2] Irena Seili-Bekafigo,[4] Antica Duletić-Načinović,[1] Damir Nemet,[5] and Nives Jonjić[2]

[1] *Department of Hematology, Rijeka University Hospital Centre, Krešimirova 42, 51000 Rijeka, Croatia*
[2] *Department of Pathology, School of Medicine, University of Rijeka, Braće Branchetta 20, 51000 Rijeka, Croatia*
[3] *Department of Laboratory Medicine, Rijeka University Hospital Centre, Krešimirova 42, 51000 Rijeka, Croatia*
[4] *Department of Cytology, Rijeka University Hospital Centre, Krešimirova 42, 51000 Rijeka, Croatia*
[5] *Department of Hematology, Zagreb University Hospital Centre, Kišpatićeva 12, 10000 Zagreb, Croatia*

Correspondence should be addressed to Emina Babarović; esinozic@gmail.com

Received 12 February 2014; Revised 20 May 2014; Accepted 23 May 2014; Published 4 June 2014

Academic Editor: Dong Soon Lee

The aim of this pilot study was to determine the plasma levels of osteopontin (OPN) and vascular endothelial growth factor (VEGF) and find possible association between them and main clinical features and parameters of tumor burden in patient with multiple myeloma (MM). Plasma levels of OPN and VEGF were determined in 44 newly diagnosed MM patients and 24 healthy persons by ELISA method. These values were compared with the presence of anemia, renal dysfunction, and bone lesions as myeloma related clinical manifestations and with serum beta-2 microglobulin and Durie-Salmon clinical stage as prognosticators related to tumor mass. The value of OPN was significantly higher in MM patients with evident bone lesions ($P = 0.03$) and there was also a positive correlation with serum beta-2 microglobulin ($r = 0.366$; $P = 0.04$). Furthermore, patients with lower Durie-Salmon stage had significantly lower OPN and VEGF levels ($P = 0.05$; $P = 0.04$, resp.). Our preliminary results found positive association between plasma level of OPN, tumor burden, and bone destruction. Further analysis should provide information about the possible use of OPN as useful clinical biomarker for monitoring bone disease and tumor mass, as well as a prognostic factor, or a possible target for pharmacological intervention.

1. Introduction

Multiple myeloma (MM) is a common haematological neoplasm with heterogeneous clinical manifestations, course of disease, response to treatment, and survival [1]. This unpredictable biological behaviour is a consequence of remarkably interesting, complex, and still unclear biological interactions between neoplastic plasma cells and other components of bone marrow microenvironment. Despite the great improvement in antitumor and supportive therapy, MM still remains an incurable disease. Vascular endothelial growth factor (VEGF) is considered one of the most potent angiogenic promoters in many solid tumours [2–6]. According to previous studies, it plays an angiogenic and tumorigenic role in the pathophysiology of MM [7–9], but the significance of its plasma level is still not well recognized. VEGF is produced by malignant plasma cells, as well as various inflammatory and stromal cells, acting through autocrine and/or paracrine crosstalk via their VEGFR-1 and VEGFR-2 receptors [7, 8, 10, 11].

Osteopontin (OPN) is a multifunctional, acid-rich, noncollagenous glycol-phosphoprotein expressed in bone, which interacts with integrin and CD44 receptors [12–14]. The binding of OPN to these surface receptors can elicit extensive

changes in cell functions, such as enhanced mobility and adhesion, accelerated growth and division, prolongation of cell survival, and angiogenesis [15]. It is involved in a variety of physiological and pathological processes including inflammation, ischemia-reperfusion, bone resorption, atherosclerosis, and tumour progression [15]. In many types of human cancers the overexpression of OPN in tumour tissue or in blood has been associated with more advanced disease and recently it has been shown that OPN has the value as a clinical tumour progression marker [16]. It is well known that different stromal elements such as endothelial cells, macrophages, especially osteoclasts, lymphocytes, smooth muscle cells, and myeloma cells have potential to secrete OPN [17, 18]. There is a growing evidence for the role of OPN in the bone destruction and angiogenesis of MM [18–21].

However, until now, only a very few studies have partially explored associations between plasma levels of VEGF and OPN with some of the clinical features and parameters of tumor burden in myeloma patients [18–22]. In this pilot investigation we tried to detect possible association of plasma OPN and VEGF with myeloma related clinical manifestations such as anemia, renal dysfunction, and bone disease, as well as with serum beta-2 microglobulin and Durie-Salmon clinical stage which are prognosticators related to tumour burden.

2. Patients and Methods

2.1. Patients. We retrospectively analyzed 44 newly diagnosed, previously untreated myeloma patients (21 males, 23 females; median age of 69 years; age range 44–86 years) and 24 age-matched healthy individuals as a control group (12 males, 12 females; median age of 67 years; age range 35–83 years). Diagnoses were established at the Department of Hematology, Clinical Centre Rijeka, during the period from 2010 to 2012, according to the International Myeloma Working Group Criteria [23]. The control group consisted of healthy volunteers who were treated in the outpatient clinic hematology because of altered blood findings but none of them had any hematological disease. Patients with liver or renal impairment, current or previous other malignancies, infectious diseases, or incapability to consent were excluded from the control group. Blood samples were collected at the time of diagnosis from all MM patients, before the initiation of any antimyeloma treatment, including supportive treatment (e.g., bisphosphonate administration). All blood samples, collected from patients and controls, were aliquoted into separate vials, stored at −20°C, and assayed at the end of the study, in order to avoid interassay variability. Written informed consent was obtained from each patient and healthy volunteer prior to their inclusion in the study. The study was approved by the local ethics committee.

Patients were categorized according to Durie-Salmon clinical stage [24]. Because of the small number of cases in each Durie-Salmon stage group, we grouped clinical stages I and II together and compared them to stage III with the intent of separating patients with the largest tumor mass. The main characteristics of our patients are summarized in Table 1.

Table 1: Clinical features of patients with multiple myeloma (MM) and healthy volunteers who form the control group.

Clinical features	Patients with MM ($N = 44$)	Healthy control group ($N = 24$)
Age and sex distribution	Cases	Cases
Male	21	12
Female	23	12
Age (years)	Median 69	Median 67
	Range 44–86	Range 35–83
Durie-Salmon stage	Cases	
I	7	
II	8	
III	29	
Renal dysfunction	Cases	
Yes	11	
No	32	
Anemia	Cases	
Yes	31	
No	13	
Beta-2 microglobulin	Cases	
Normal	9	
Increased	33	
Bone disease	Cases	
Yes	32	
No	10	

Note: renal dysfunction = serum creatinine level above the upper limit of normal; anemia = haemoglobin value 20 g/L below the lower limit of normal; beta-2 microglobulin = normal values less than 2.5 μg/mL versus increased plasma values; and bone disease = presence of any lytic lesion or severe osteopenia with compressive fractures on standard radiographs of the bones.

The obtained plasma cytokine levels were correlated with main clinical manifestations in MM: anemia (hemoglobin 20 g/L below the lower limit of normal, defined as 138 g/L for men and 119 g/L for women), renal dysfunction (serum creatinine level above the upper limit of normal, defined as 117 μmol/L for men and 96 μmol/L for women), and bone disease (the presence of any lytic lesion or severe osteopenia with compressive fractures on standard radiographs of the bones). The values of OPN and VEGF were also correlated with serum beta-2 microglobulin and with Durie-Salmon clinical stage (stages I and II combined versus stage III) as a measure of tumor mass.

2.2. Measurement of Cytokines. The concentrations of OPN and VEGF were determined in plasma samples by enzyme-linked immunoassay (ELISA, Quantikine RD Systems, Minneapolis, MN, USA) according to the manufacturer's instructions. In brief, these assays employ the quantitative sandwich immunoassay technique. A monoclonal antibody specific for protein was precoated onto microplates. Standards and samples were pipetted into the wells. After binding and

Table 2: Comparison of measured plasma OPN and VEGF levels with clinical parameters in patients with MM.

	Durie-Salmon stage		P value	Bone lesions		P value	Anemia		P value	Renal dysfunction		P value
	I, II	III		Yes	No		Yes	No		Yes	No	
OPN (ng/mL)												
Median	5.65	8.9	**0.05**	7.4	2.8	**0.03**	7.6	6.6	0.29	12.6	7.05	0.13
Range	0.3–14.6	2.2–26.5		3.3–26.5	0.3–12.9		0.3–26.5	2.3–12.9		3.7–26.5	0.3–22.2	
VEGF (pg/mL)												
Median	35.9	60.15	**0.04**	48.1	55.75	0.79	57.4	47	0.43	46.9	59	0.51
Range	5.3–111.7	20.3–178.9		5.3–178.9	22.6–111.7		5.3–178.9	29.2–69.2		20.3–148.9	5.3–178.9	

washing, an enzyme-linked polyclonal antibody specific for each growth factor was added to each well. After a wash to remove any antibody-enzyme reagent, a substrate solution was added to the wells and color developed in proportion to the amount of each growth factor that was bound in the first step. The color development was stopped and optical density of each well was measured using the microplate reader set at 450 nm. The concentration of each specific growth factor in each plasma sample was calculated from standard curves and reported in pg/mL for VEGF and ng/mL for OPN.

2.3. Statistical Analysis. Statistical analyses were performed using MedCalc for Windows, version 12.2.1.0 (MedCalc Software, Ostend, Belgium). The distribution of data was tested for normality using the Kolmogorov-Smirnov test. The measures of central tendency for continuous data such as OPN and VEGF values were compared by Student's t-test or Mann-Whitney U test, depending on data distribution. The independent t-test and Mann-Whitney U test were used to assess whether continuous variables differed significantly between categories (patients with bone lesions versus patients without bone lesions, Durie-Salmon clinical stages I and II versus stage III, patients with anemia versus patients without anemia, etc.). Correlation between continuous variables was studied using Pearson correlation. Statistical differences with $P < 0.05$ were considered significant.

3. Results

Certain amounts of both cytokines, OPN and VEGF, were detected in plasma samples from all patients. In addition, OPN value was significantly higher in MM patients (median 6.5 ng/mL, range 0.3–21.7 ng/mL) in comparison with the control group (median 2.4 ng/mL, range 0.2–8.9 ng/mL; $P < 0.0001$; Figure 1). Such differences were not observed regarding VEGF (median 52.5 pg/mL, range 5.3–178.9 pg/mL in MM patients versus median 60.5 pg/mL, range 12.2–205.6 pg/mL in control group; $P = 0.67$).

In contrast with VEGF, plasma OPN levels were significantly higher in patients with evident bone lesions ($P = 0.03$; Figure 2). However, there were no statistically significant differences in levels of the analyzed cytokines in patients with anemia and renal insufficiency compared to those without these myeloma related complications. Further aim was to compare plasma levels of OPN and VEGF with parameters of tumor burden, and statistically significant differences among Durie-Salmon stages were observed. More specifically, patients in stages I and II had significantly lower plasma OPN ($P = 0.05$) and lower plasma VEGF ($P = 0.04$) values compared to patients in stage III of the disease. Furthermore, a significant positive correlation between plasma OPN concentration and serum beta-2 microglobulin level ($r = 0.366$; $P = 0.04$) was determined, while there was no correlation for VEGF plasma values. The results are summarized in Table 2.

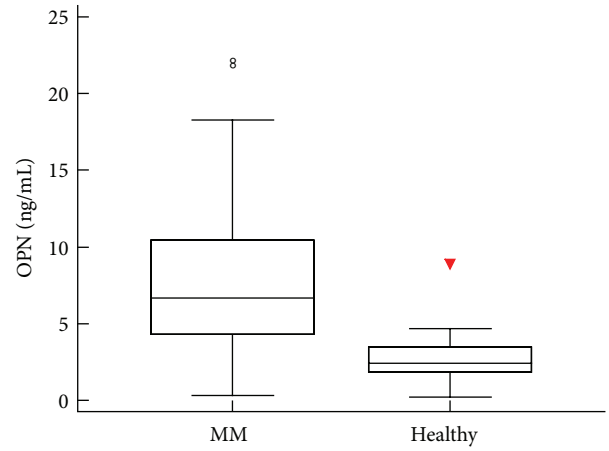


Figure 1: Comparison of plasma OPN levels between patients with MM and healthy volunteers who form the control group. The plasma concentrations of OPN were significantly higher in MM patients than in healthy volunteers ($P < 0.0001$, Mann-Whitney U test). The upper and lower borders of the box indicate the 75th and 25th percentiles, respectively, and the line in the box represents the median. The ends of the whiskers represent the minimum and maximum of all of the data excluding outliers. Outliers are plotted as individual points.

4. Discussion

There is a certain body of evidence demonstrating the involvement of VEGF and OPN in angiogenesis and bone disease during MM progression. However, little is known about the possible clinical significance of plasma OPN and VEGF regarding other aspects of disease. As we know, myeloma is a collection of related disorders rather than a single disease. For that reason, the present study was conducted with the purpose of correlating serum concentrations of these

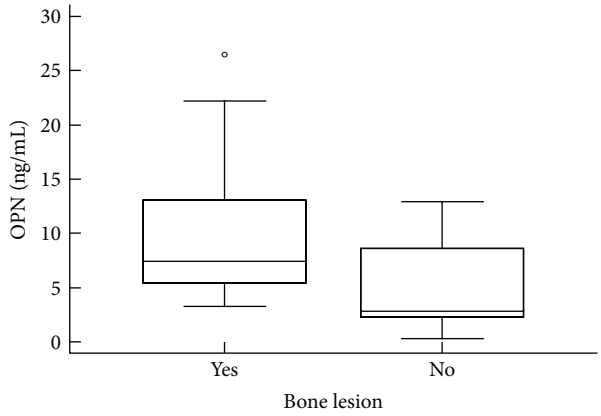


Figure 2: Comparison of plasma OPN levels between patients with bone disease and those without manifest bone lesions. The plasma concentrations of OPN were significantly higher in patients with bone disease ($P = 0.03$, Mann-Whitney U test). The upper and lower borders of the box indicate the 75th and 25th percentiles, respectively, and the line in the box represents the median. The ends of the whiskers represent the minimum and maximum of all of the data excluding outliers. Outliers are plotted as individual points.

cytokines with the most common presenting symptoms of MM (e.g., anemia, renal insufficiency, and bone disease), as well as with routine prognosticators beta-2 microglobulin and Durie-Salmon clinical stage, which reflect tumor burden.

The first result of this study revealed significantly higher plasma OPN in myeloma patients than in healthy controls. This finding agrees with some [25, 26], but not all, investigations [20, 27]. Furthermore, Saeki et al., Scudla et al., and Minarik et al. have observed differences in plasma OPN levels between monoclonal gammopathy of undetermined significance and MM [26, 28–30]. All of these results taken together implicate OPN in the biology of MM. Although several groups have demonstrated significantly increased plasma VEGF among myeloma patients in comparison with control groups [31–33], our current study and Sezer et al. [34] did not confirm this finding.

Our second finding revealed the associations of plasma concentrations of both VEGF and OPN with Durie-Salmon clinical stage. Plasma levels of both cytokines were significantly elevated in stage III compared with stages I and II. This positive association between cytokine values and Durie-Salmon clinical stage is not unexpected and is likely related to tumor burden. If that assumption is correct, the plasma levels of these cytokines (which can be produced by malignant plasma cells) might represent a measure of myeloma cell mass, not unlike the Durie-Salmon Staging System, which is based on a mathematical model for estimating the number of tumor cells. This result is in accordance with the finding that VEGF can act directly or indirectly as a growth factor for myeloma cells, which has been reported previously by some authors [7, 35, 36]. In addition, the present study demonstrated a significant positive association between OPN and another prognostic factor related to tumor burden and aggressive biological behavior of MM- beta-2 microglobulin, as previously described [20, 22]. However, we did not find the same association between VEGF and beta-2 microglobulin, which is intriguing and can be attributed to the relatively small number of cases included in this study. Still, it is possible that beta-2 microglobulin, in addition to myeloma cell mass and renal filtering capacity, might also reflect some other as-yet-unrecognized aspects of myeloma biology because, in contrast to Durie-Salmon clinical stage, it has retained prognostic value in the era of novel therapeutic agents.

We also observed significantly increased plasma OPN levels among myeloma patients with overt bone disease in comparison to those who had normal bone radiologic findings. Few groups have been able to demonstrate the same results [20, 22, 26]. Conversely, Robbiani and his group proposed that MM-derived OPN plays a critical role in bone disease by protecting bone from destruction [18]. Hence, the results of this study support the idea that OPN is an important pathophysiological factor in the biology of MM involved in bone disease. In addition, its positive association with parameters of tumor burden might suggest a role in the proliferation and survival of myeloma cells which should be explored in the future. All of this information affords us the opportunity to modulate its role in the tumor ecosystem through different pharmacological interventions.

According to our knowledge, only Dizdar et al. have investigated and proven a positive correlation between OPN and serum creatinine [22]. In contrast, we did not observe any relationship between plasma OPN level and renal impairment or anemia, which suggests that this multifunctional phosphoprotein is not relevantly involved in these aspects of MM. Moreover, our study reveals that plasma VEGF level was not associated with any of the main clinical features of MM or beta-2 microglobulin, a finding that is in concordance with the findings of other studies [32, 34]. Still, Di Raimondo et al. have observed that plasma VEGF levels correlate positively with beta-2 microglobulin, but not with osteolytic lesions, hemoglobin concentration, or creatinine concentration [9].

One question is whether plasma OPN (or VEGF) levels objectively reflect their concentrations in bone marrow, where the tumor is actually growing. Previous studies have shown that the concentrations of investigated angiogenic factors were higher in bone marrow but correlated significantly with plasma levels [9, 37].

Important limitations of present study are rather small sample size with missing clinical data for some patients, as well as its retrospective design, which limits any definite conclusions.

5. Conclusion

Our preliminary results support plasma OPN level as a possible marker of bone destruction. Although VEGF reportedly represents a critical angiogenic factor in the tumor microecosystem, the present study did not suggest that plasma VEGF level has similar clinical value to OPN. Furthermore, we did not observe associations between plasma levels of the investigated cytokines and other clinical features of MM, such as anemia or renal impairment. However, in the present pilot study, both cytokines were positively associated with

tumor burden. Further prospective analysis of a larger group of patients should provide definitive information about the possible role of OPN as a useful clinical biomarker for monitoring bone disease and tumor mass, as well as a prognostic factor during the course of MM.

Ethnical Approval

Ethnical approval was received from the Ethnics Committees of the Rijeka University Hospital Centre and School of Medicine, University of Rijeka.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper. The authors have no support from any organization for the submitted work; no financial relationships with any organization that might have an interest in the submitted work; or any other relationships or activities that could appear to have influenced the submitted work.

Acknowledgment

Funding was received from the Grant of Ministry of Science, Education and Sport, Project no. 062-0620095-0079.

References

[1] A. Palumbo and K. Anderson, "Multiple myeloma," *The New England Journal of Medicine*, vol. 364, no. 11, pp. 1046–1060, 2011.

[2] H. Yoshiji, D. E. Gomez, M. Shibuya, and U. P. Thorgeirsson, "Expression of vascular endothelial growth factor, its receptor, and other angiogenic factors in human breast cancer," *Cancer Research*, vol. 56, no. 9, pp. 2013–2016, 1996.

[3] M. Volm, R. Koomagi, and J. Mattern, "Prognostic value of vascular endothelial growth factor and its receptor Flt-1 in squamous cell lung cancer," *International Journal of Cancer*, vol. 74, pp. 64–68, 1997.

[4] T. A. Olson, D. Mohanraj, L. F. Carson, and S. Ramakrishnan, "Vascular permeability factor gene expression in normal and neoplastic human ovaries," *Cancer Research*, vol. 54, no. 1, pp. 276–280, 1994.

[5] L. M. Ellis, Y. Takahashi, C. J. Fenoglio, K. R. Cleary, C. D. Bucana, and D. B. Evans, "Vessel counts and vascular endothelial growth factor expression in pancreatic adenocarcinoma," *European Journal of Cancer*, vol. 34, no. 3, pp. 337–340, 1998.

[6] T. Valković, F. Dobrila, M. Melato, F. Sasso, C. Rizzardi, and N. Jonjić, "Correlation between vascular endothelial growth factor, angiogenesis, and tumor-associated macrophages in invasive ductal breast carcinoma," *Virchows Archiv*, vol. 440, no. 6, pp. 583–588, 2002.

[7] K. Podar, Y.-T. Tai, F. E. Davies et al., "Vascular endothelial growth factor triggers signaling cascades mediating multiple myeloma cell growth and migration," *Blood*, vol. 98, no. 2, pp. 428–435, 2001.

[8] A. Vacca, R. Ria, D. Ribatti et al., "A paracrine loop in the vascular endothelial growth factor pathway triggers tumor angiogenesis and growth in multiple myeloma," *Haematologica*, vol. 88, no. 2, pp. 176–185, 2003.

[9] F. Di Raimondo, M. P. Azzaro, G. A. Palumbo et al., "Angiogenic factors in multiple myeloma: higher levels in bone marrow than in peripheral blood," *Haematologica*, vol. 85, no. 8, pp. 800–805, 2000.

[10] W. T. Bellamy, L. Richter, Y. Frutiger, and T. M. Grogan, "Expression of vascular endothelial growth factor and its receptors in hematopoietic malignancies," *Cancer Research*, vol. 59, no. 3, pp. 728–733, 1999.

[11] B. Berse, L. F. Brown, L. Van de Water, H. F. Dvorak, and D. R. Senger, "Vascular permeability factor (vascular endothelial growth factor) gene is expressed differentially in normal tissues, macrophages, and tumors," *Molecular Biology of the Cell*, vol. 3, no. 2, pp. 211–220, 1992.

[12] A. Franzen and D. Heinegard, "Isolation and characterization of two sialoproteins present only in bone calcified matrix," *Biochemical Journal*, vol. 232, no. 3, pp. 715–724, 1985.

[13] L. Liaw, M. P. Skinner, E. W. Raines et al., "The adhesive and migratory effects of osteopontin are mediated via distinct cell surface integrins: role of $\alpha v\beta 3$ in smooth muscle cell migration to osteopontin in vitro," *Journal of Clinical Investigation*, vol. 95, no. 2, pp. 713–724, 1995.

[14] G. F. Weber, G. F. Weber, S. Ashkar et al., "Receptor-ligand interaction between CD44 and osteopontin (Eta-1)," *Science*, vol. 271, no. 5248, pp. 509–512, 1996.

[15] D. T. Denhardt, M. Noda, A. W. O'Regan, D. Pavlin, and J. S. Berman, "Osteopontin as a means to cope with environmental insults: regulation of inflammation, tissue remodeling, and cell survival," *Journal of Clinical Investigation*, vol. 107, no. 9, pp. 1055–1061, 2001.

[16] G. F. Weber, G. S. Lett, and N. C. Haubein, "Osteopontin is a marker for cancer aggressiveness and patient survival," *British Journal of Cancer*, vol. 103, no. 6, pp. 861–869, 2010.

[17] M. Mazzali, T. Kipari, V. Ophascharoensuk, J. A. Wesson, R. Johnson, and J. Hughes, "Osteopontin—a molecule for all seasons," *QJM*, vol. 95, no. 1, pp. 3–13, 2002.

[18] D. F. Robbiani, K. Colon, S. Ely, S. Ely, M. Chesi, and P. L. Bergsagel, "Osteopontin dysregulation and lytic bone lesions in multiple myeloma," *Hematological Oncology*, vol. 25, no. 1, pp. 16–20, 2007.

[19] S. Colla, F. Morandi, M. Lazzaretti et al., "Human myeloma cells express the bone regulating gene Runx2/Cbfa1 and produce osteopontin that is involved in angiogenesis in multiple myeloma patients," *Leukemia*, vol. 19, no. 12, pp. 2166–2176, 2005.

[20] A. Sfiridaki, S. Miyakis, C. Pappa et al., "Circulating osteopontin: a dual marker of bone destruction and angiogenesis in patients with multiple myeloma," *Journal of Hematology and Oncology*, vol. 4, article 22, 2011.

[21] Y. Tanaka, M. Abe, M. Hiasa et al., "Myeloma cell-osteoclast interaction enhances angiogenesis together with bone resorption: a role for vascular endothelial cell growth factor and osteopontin," *Clinical Cancer Research*, vol. 13, no. 3, pp. 816–823, 2007.

[22] O. Dizdar, I. Barista, U. Kalyoncu et al., "Biochemical markers of bone turnover in diagnosis of myeloma bone disease," *American Journal of Hematology*, vol. 82, no. 3, pp. 185–191, 2007.

[23] R. A. Kyle, J. A. Child, K. Anderson et al., "Criteria for the classification of monoclonal gammopathies, multiple myeloma and related disorders: a report of the International Myeloma Working Group," *British Journal of Haematology*, vol. 121, no. 5, pp. 749–757, 2003.

[24] B. G. M. Durie and S. E. Salmon, "A clinical staging system for multiple myeloma. Correlation of measured myeloma cell mass with presenting clinical features, response to treatment, and survival," *Cancer*, vol. 36, no. 3, pp. 842–854, 1975.

[25] T. Standal, H. Hjorth-Hansen, T. Rasmussen et al., "Osteopontin is an adhesive factor for myeloma cells and is found in increased levels in plasma from patients with multiple myeloma," *Haematologica*, vol. 89, no. 2, pp. 174–182, 2004.

[26] Y. Saeki, T. Mima, T. Ishii et al., "Enhanced production of osteopontin in multiple myeloma: clinical and pathogenic implications," *British Journal of Haematology*, vol. 123, no. 2, pp. 263–270, 2003.

[27] S. Y. Kang, J. J. Lee, and W. I. Lee, "Clinical significance of serum osteopontin in patients with multiple myeloma," *The Korean Journal of Laboratory Medicine*, vol. 27, no. 6, pp. 400–405, 2007.

[28] V. Scudla, M. Budikova, P. Petrova et al., "Analysis of serum levels of selected biological parameters in monocolonal gammopathy of undetermined significance and multiple myeloma," *Klinická Onkologie*, vol. 23, no. 3, pp. 171–181, 2010.

[29] V. Scudla, P. Petrova, J. Minarik, T. Pika, and J. Bacovsky, "Analysis of the serum levels of selected biological parameters in monoclonal gammopathy of undetermined significance and different stages of multiple myeloma," *Neoplasma*, vol. 58, no. 6, pp. 499–506, 2011.

[30] J. Minarik, T. Pika, J. Bacovsky, P. Petrova, K. Langova, and V. Scudla, "Prognostic value of hepatocyte growth factor, syndecan-1, and osteopontin in multiple myeloma and monoclonal gammopathy of undetermined significance," *The Scientific World Journal*, vol. 2012, Article ID 356128, 6 pages, 2012.

[31] T. Iwasaki, T. Hamano, A. Ogata, N. Hashimoto, M. Kitano, and E. Kakishita, "Clinical significance of vascular endothelial growth factor and hepatocyte growth factor in multiple myeloma," *British Journal of Haematology*, vol. 116, no. 4, pp. 796–802, 2002.

[32] N. Sato, Y. Hattori, D. Wenlin et al., "Elevated level of plasma basic fibroblast growth factor in multiple myeloma correlates with increased disease activity," *Japanese Journal of Cancer Research*, vol. 93, no. 4, pp. 459–466, 2002.

[33] E. Hatjiharissi, E. Terpos, M. Papaioannou et al., "The combination of intermediate doses of thalidomide and dexamethasone reduces bone marrow micro-vessel density but not serum levels of angiogenic cytokines in patients with refractory/relapsed multiple myeloma," *Hematological Oncology*, vol. 22, no. 4, pp. 159–168, 2004.

[34] O. Sezer, C. Jakob, J. Eucker et al., "Serum levels of the angiogenic cytokines basic fibroblast growth factor (bFGF), vascular endothelial growth factor (VEGF) and hepatocyte growth factor (HGF) in multiple myeloma," *European Journal of Haematology*, vol. 66, no. 2, pp. 83–88, 2001.

[35] B. Dankbar, T. Padró, R. Leo et al., "Vascular endothelial growth factor and interleukin-6 in paracrine tumor- stromal cell interactions in multiple myeloma," *Blood*, vol. 95, no. 8, pp. 2630–2636, 2000.

[36] W. M. Swelam and D. M. Al Tamimi, "Biological impact of vascular endothelial growth factor on vessel density and survival in multiple myeloma and plasmacytoma," *Pathology Research and Practice*, vol. 206, no. 11, pp. 753–759, 2010.

[37] N. F. Andersen, T. Standal, J. L. Nielsen et al., "Syndecan-1 and angiogenic cytokines in multiple myeloma: correlation with bone marrow angiogenesis and survival," *British Journal of Haematology*, vol. 128, no. 2, pp. 210–217, 2005.

Hindawi Publishing Corporation
BioMed Research International
Volume 2014, Article ID 521586, 12 pages
http://dx.doi.org/10.1155/2014/521586

Review Article

MicroRNA: Important Player in the Pathobiology of Multiple Myeloma

Chonglei Bi[1,2] and Wee Joo Chng[1,2,3]

[1] *Experimental Therapeutics, Cancer Science Institute of Singapore, No. 12-02, Centre for Translational Medicine, 14 Medical Drive, Singapore 117599*
[2] *Yong Loo Lin School of Medicine, NUHS Tower Block level 11, 1E Kent Ridge Road, Singapore 119228*
[3] *Department of Hematology-Oncology, National University Cancer Institute, National University Health System, 1E, Kent Ridge Road, Singapore 119228*

Correspondence should be addressed to Wee Joo Chng; mdccwj@nus.edu.sg

Received 26 March 2014; Accepted 19 May 2014; Published 3 June 2014

Academic Editor: Kazuyuki Shimizu

Recent studies have revealed a pivotal role played by a class of small, noncoding RNAs, microRNA (miRNA), in multiple myeloma (MM), a plasma cell (PC) malignancy causing significant morbidity and mortality. Deregulated miRNA expression in patient's PCs and plasma has been associated with tumor progression, molecular subtypes, clinical staging, prognosis, and drug response in MM. A number of important oncogenic and tumor suppressor miRNAs have been discovered to regulate important genes and pathways such as p53 and IL6-JAK-STAT signaling. miRNAs may also form complex regulatory circuitry with genetic and epigenetic machineries, the deregulation of which could lead to malignant transformation and progression. The translational potential of miRNAs in the clinic is being increasingly recognized that they could represent novel biomarkers and therapeutic targets. This review comprehensively summarizes current progress in delineating the roles of miRNAs in MM pathobiology and management.

1. Multiple Myeloma

Multiple myeloma (MM) is a tumor of antibody-secreting plasma cells (PCs) characterized by the clonal expansion and accumulation of monotypic PCs in the bone marrow (BM) [1]. It causes about 1% of neoplastic diseases and 13% of hematological malignancies [2]. Clinically, MM patients exhibit one or more symptoms including lytic bone disease, hypercalcemia, anemia, and compromised renal functions [3]. MM is always preceded by an asymptomatic premalignant stage called monoclonal gammopathy of undetermined significance (MGUS), which progresses to myeloma or related malignancies at a rate of 1% per year [4, 5]. Patients with MGUS are by definition symptom-free, but with measurable concentration of monoclonal protein or have an abnormality in serum-free light chain assay [6]. Although MM cells are strongly dependent on BM microenvironment, more aggressive tumors may extend to extramedullary sites. Extramedullary MM (EMM) can also present with a leukemic phase which can be classified as primary plasma cell leukemia (pPCL) if it arises *de novo*, or secondary PCL if preceded by intramedullary MM [7, 8]. Most of the human MM cell lines (HMCLs) are generated from EMM or PCL tumors [9]. The Durie-Salmon staging system which mainly reflects tumor burden was the first commonly used staging system for MM [10]. It has been superseded by the International Staging System (ISS), a 3-group classification based on two simple and routine laboratory tests widely available [11, 12].

MM is characterized by complex genetic and epigenetic abnormalities [13, 14]. Biologically, myeloma can be broadly divided into hyperdiploid and nonhyperdiploid categories, each consists of about half of MGUS and MM tumors. Hyperdiploid myeloma (H-MM) is characterized by multiple trisomies involving odd number chromosomes except chromosome 13 and a lower prevalence of primary translocation involving the immunoglobulin heavy chain (IgH) locus at 14q32, whereas nonhyperdiploid myeloma (NH-MM) is characterized by IgH translocations, most commonly t(4; 14) which translocates MMSET and FGFR3 at 4p16.3 to the IgH enhancers, t(11; 14) involving CCND1, and t(14; 16)

involving MAF [15, 16]. H-MM and NH-MM are observed in both MGUS and MM, suggesting that they are early oncogenic events although high-risk MM is more common in NH-MM cases [17]. Another early and perhaps unifying event in MGUS and MM is the upregulation of cyclin D genes independent of the H-MM and NH-MM dichotomy, although it does not appear to be associated with increased proliferation [8]. In addition, a number of secondary genetic lesions associated with disease progression and survival have been identified, including activating mutations in RAS and BRAF, increasing frequency of MYC overexpression in disease progression, chromosomal 13 deletion, chromosome 17p loss, and p53 abnormalities, chromosome 1 abnormalities, IL6-JAK-STAT3 and NFκB activation. Notably, the frequency of 17p loss increases as disease progresses and has been uniformly accepted as a marker for high-risk MM [18–27].

Studies have investigated the molecular basis for MM progression using gene expression profiling (GEP); although normal PCs have distinct gene expression profiles with MGUS and MM, at present it is still not possible to clearly distinguish MGUS from MM by GEP [28–32]. Studies have also looked at the association between gene expression patterns with molecular subtypes of MM [33–36]. It was shown that gene expression patterns are associated with primary IgH translocations and cyclin D gene expression. Various gene signatures associating with high risk have been proposed [33, 37–43], for instance the 70-gene signature (GEP70) developed by the University of Arkansas Medical Sciences. However, most of these signatures are not overlapping and have not been integrated into routine clinical care [6].

In addition, epigenetic deregulation was implicated in myelomagenesis. DNA methylation changes significantly during disease progression and could silence important tumor suppressor genes including SOCS1 [44–48]. Furthermore, the BM microenvironment also plays a crucial role in MM pathogenesis by promoting growth, survival, and drug resistance in MM cells. The adherence of MM cells to bone marrow stromal cells (BMSCs) increases the release of cytokines/growth factors, including IL-6 which activates the JAK-STAT signaling and promotes MM cell survival and proliferation [49–51].

However, despite enormous advances in understanding MM biology and emergence of novel therapeutics, the mechanism behind MM initiation and progression remains to be fully elucidated and the disease remains largely incurable [52]. Therefore, research effort on the elucidation of important role miRNA plays in MM may shed new light on MM pathobiology and identify novel biomarkers and therapeutic targets.

2. MicroRNAs

miRNAs are ~20-nucleotide genome-encoded RNAs highly conserved across different species and regulate most cellular processes [53]. As of 2013, more than 1800 miRNA precursors have been identified and deposited in the miRNA registry, miRBase. Each miRNA can target hundreds of different conserved or nonconserved genes. It has been estimated that a large proportion of the transcriptome (about 50% in humans) is subject to miRNA regulation [54, 55].

miRNAs are mostly transcribed by RNA polymerase II which generates long, capped, and polyadenylated precursors known as pri-miRNAs. Each pri-miRNA is subsequently processed by the microprocessor complex consisting of Drosha, a member of ribonuclease III enzyme family, and dsRNA-binding protein DGCR8/Pasha, resulting in a ~70-nucleotide precursor known as pre-miRNA which is actively exported by exportin 5 to the cytoplasm where it is cleaved near the terminal loop by another RNase III type endonuclease Dicer, generating a ~20-nucleotide miRNA duplex. Finally, the mature single-stranded miRNA product is loaded onto an Ago protein to form the effector complex called the RNA-induced silencing complex (RISC), and the other strand of the duplex is degraded. In RISC, the mature miRNA recognizes complementary sequence (usually in the 3′UTR region) of target to the seed sequence of miRNA (nucleotides 2–8 at 5′end), binds to the target mRNA, and regulates gene expression by translation repression or mRNA degradation depending on the degree of complementarity [54, 56, 57].

Deregulation of miRNAs has been associated with a plethora of human diseases including cancer. miRNAs regulate critical processes in tumor initiation and development by targeting oncogenes and/or tumor suppressor genes [58–60]. miRNA expression profiles have been shown to be able to classify human cancers with better accuracy than traditional GEP [61–64] and could serve as novel biomarkers for diagnosis, disease progression, and prognosis [61, 65–68]. Recently, miRNA deregulation has also been implicated in drug resistance in cancers including leukemia [69–71].

Deregulation of miRNA in cancer arises from both genomic and epigenetic changes [72]. Many human miRNA genes are located at cancer-associated fragile genomic locus that are subjected to frequent mutations [73–75]. Defect in miRNA biogenesis machinery is also shown to be affecting miRNA expression in cancer [76–78]. In addition, disruption of canonical miRNA/target binding sequence, for instance SNPs and altered splicing pattern of target mRNAs, may deregulate miRNA function and contribute to oncogenesis [79]. Recently, epigenetic aberrations, including DNA hypermethylation and/or histone modification, have emerged as a major cause in miRNA deregulation [80–82].

Increasing evidence suggests that miRNA deregulation is a hallmark of myeloma. This paper reviews the current literature on the roles miRNA play in MM pathobiology, prognosis, and therapy.

3. miRNA Deregulation in Different Stages of MM

As mentioned above, MM is characterized by multistep transformation and complex genomic aberrations both structurally and numerically. Many molecular subtypes of MM have been identified by GEP. Given that miRNA expression profile has the potential of improved accuracy over traditional GEP signatures and could represent novel biomarkers,

a number of studies have looked at miRNA expression profiles in different stages and molecular subtypes of MM.

In a pioneering paper, Pichiorri et al. performed global miRNA expression profiles in samples from 5 MGUS patients, 10 MM and 4 normal PCs, and identified miRNA deregulated in MM and MGUS relative to normal PCs, including upregulation of miRNAs with known oncogenic activity such as miR-21, miR-106b~25 cluster, miR-181a, miR-181b, miR-32, and miR 17–92 cluster [75, 83, 84]. Among these, miR-32 and miR 17–92 are unique to overt MM but not MGUS.

Roccaro et al. conducted miRNA expression profiling in PCs from 15 relapsed/refractory MM samples, 3 MM cell lines, and 4 healthy donors. Unsupervised analysis showed clear separation of MM subjects and normal, although MM samples and cell lines were not separated. miRNAs deregulated in relapsed/refractory MM including downregulation of miR-15a and miR-16. Functional studies showed that these two tumor suppressor miRNAs inhibit proliferation and growth of MM cells *in vitro* and *in vivo*. At the same time, miR-15a and miR-16 decreased MM cell-induced proangiogenic activity on endothelial cells by reducing VEGF secretion from MM cells. Both miRNAs inhibited MM cells in the presence of BMSCs [85].

In a study focusing on PCL, Lionetti et al. compared miRNA expression profile in 18 primary PCL (pPCL) with 39 myeloma samples. Unsupervised analysis revealed a fairly distinct miRNA expression profile for pPCL relative to MM, as all PCL cases were clustered together and formed a main cluster with a few MM cases, whereas 4 normal samples were clustered as a distinct group [86].

Zhou et al. profiled miRNA expression in PCs from 52 newly diagnosed MM cases and 2 healthy donors and observed an elevated total miRNA expression level in MM. 39 miRNAs were upregulated in MM, including miR-18, miR-92a, miR-181a, miR-181b, miR-221, miR-222, and miR-99a which were consistent with previous reports. Only 1 miRNA, miR-370, was downregulated [87]. Chi et al. performed miRNA expression profiling in PCs from 33 MM patients, 5 MGUS cases, 4 HMCLs, and 5 healthy donors. Unsupervised analysis showed separation of normal samples from the rest. However, MM, MGUS, and HMCL were not separated into distinct clusters. Differentially expressed miRNAs between MM and normal were partially consistent with other reports [88].

These studies have explored the potential use of miRNA to distinguish MGUS from MM. Although some differentially expressed miRNAs between PCs from MGUS and MM were identified, no robust miRNA signature able to distinguish MGUS and MM were established. In a profiling study involving MM and pPCL, miRNA expression-based unsupervised clustering separated MM and pPCL samples with moderate success. Interestingly, majority of these differentially expression miRNAs showed same trend (upregulation or downregulation) from healthy controls, through MM, to pPCL. It is therefore tempting to hypothesize that the degree of miRNA deregulation correlates with the extent of tumor progression.

4. miRNA Deregulation in Different Molecular Subtypes of MM

Lionetti et al. profiled miRNA expression in MM subjects representative of 5 Translocation/Cyclin (TC) subtypes defined previously [34]. Unsupervised clustering loosely classified the samples according to their TC group. TC4 (MMSET-FGFR3) samples tightly clustered as a single branch, with upregulation of miR-99b, miR-125a-5p, and let-7e which belong to a cluster at 19q13.33. All samples in the TC5 group (MAF or MAFB translocation) except one were also tightly clustered. Interestingly, miRNAs specifically upregulated in TC5 group include miR-99a, let-7c, and miR-125b-2 which belong to a paralogous miRNA cluster of the three in TC4 [89]. The upregulation of all or some members of the miR-99b, miR-125a-5p, and let-7e cluster in t(4; 14) MM was also observed in three other studies including the study on pPCL [86, 88, 90].

Gutiérrez et al. compared 60 MM patients with 5 healthy donors and identified downregulation of 11 miRNAs. Unsupervised analysis did not classify samples into clearly separated clusters according to molecular subtype, although the four samples with MAF translocations were tightly clustered [91]. In another analysis with overlapping samples, unsupervised analyses based on miRNA expression in MM identified unique clusters not associated with chromosomal abnormalities; one cluster is comprised of upregulated miRNAs including miR-21, members of the miR-17~92, and miR-106b~25 clusters, although the biological relevance of the clustering pattern remained elusive.

Studies have also looked at the association of miRNA deregulation and other genetic features of MM. Pichiorri et al. compared miRNA expression profile between HMCLs with wild-type (WT) TP53 and those with mutant TP53. Higher expression of miR-192, miR-194, and miR-215 in HMCLs were observed in HMCLs with WT TP53, as well as miR-34a which is a well-documented TP53 target. miR-192, miR-194, and miR-215 could also be induced by nutlin-3a treatment in MM cell harboring WT TP53 but not mutant TP53, suggesting that these miRNAs were regulated by p53. The authors went on to show that these miRNAs were transcriptionally activated by p53 and target MDM2, forming a positive feedback loop. These miRNAs exhibited anti-MM functions in a p53 dependent manner and could sensitize TP53 WT cells to MDM2 inhibitors [92].

Rio-Machin et al. examined miRNA expression profiles in hyperdiploid and nonhyperdiploid MM. Downregulation of specific miRNAs including miR-425, miR-152, and miR-24 was observed in hyperdiploid MM. Intriguingly, downregulation of these miRNAs was accompanied by a concomitant upregulation of their targets CCND1, TACC3, MAFB, FGFR3, and MYC, which were also the oncogenes upregulated by the most recurrent IgH translocations in nonhyperdiploid MM. This suggested that miRNA deregulation could be the mechanism behind cyclin D as a unifying feature in both nonhyperdiploid and hyperdiploid MM [93].

These studies have demonstrated that miRNA expression tends to correlate with molecular subtypes of MM, most notably with t(4; 14) and t(14; 16) translocations. Interestingly,

miR-99b, miR-125a, and let-7e which belong to a cluster at 19q13.33 were consistently associated with t(4; 14) in multiple studies. However the cause and effect relationships for these are still not clear.

5. miRNA Deregulation and Clinical Parameters in MM

A number of studies attempted to correlate miRNA with clinical parameters such as risk group and survival. Roccaro et al. identified a significant reduction of miR-15a level in MM patients in ISS II and III groups as compared to ISS I group, which was confirmed by another study [94], consistent with its function as a tumor suppressor. Meanwhile, miR-181a and miR-181b were expressed at higher levels in ISS II and III groups [85]. Zhou et al. showed that globally elevated miRNA expression was associated with higher GEP70 risk score and proliferation index, suggesting that high expression level of miRNA might confer an inferior clinical outcome. In addition, unsupervised clustering of miRNA expression profiles stratified patients according to risk, although no association was found with proliferation index [87]. Chi et al. identified differentially expressed miRNAs between light chain only MM and nonlight chain only MM, IgG and IgA-type MM, as well as patients with event-free survival (EFS, median follow-up = 20 months) and those who relapsed/died in this interval. These differentially expressed miRNAs were shown to have good prediction accuracy [88].

Wu et al. identified that higher expression of three miRNAs miR-886-5p, miR-17, and miR-18a was significantly associated with shorter overall survival of patients. It was noteworthy that miR-17 and miR-18a were members of the oncogenic miR-17~92 cluster. Furthermore, miR-886-5p and miR-17 formed a robust outcome classifier which could improve the ISS/FISH based risk stratification independent of previously validated GEP signatures [90].

In the pPCL study, Lionetti et al. identified 4 miRNAs (miR-106b, miR-497, miR-181b, and miR-181a*) upregulated in pPCL patients not responding to initial therapy consisting of lenalidomide and low-dose dexamethasone, compared to responders. Consistent with their oncogenic roles, miR-106b and miR-181b were already reported to be upregulated in MM cells compared to normal PCs [84]. Moreover, the expressions of miR-22 and miR-146a were identified to be associated with progression-free survival (PFS) while the expressions of miR-92a and miR-330-3p were identified to be associated with overall survival (OS) of pPCL patients, demonstrating their relevance in clinical prognostication in this aggressive form of plasma cell dyscrasia [86].

Besides miRNA expression, miRSNPs (SNPs in miRNA genes, miRNA processing machinery, or miRNA target genes) could affect the final level and function of miRNAs and could be clinically important. Two miRSNPs that had prognostic impact after autologous stem cell transplant (ASCT) were identified, one in the 3′UTR of a miRNA target gene, KRT81, another in XPO5, a crucial gene in the miRNA biogenesis pathway. Patients with different SNPs in either of these two miRSNPs showed significant difference in OS [95].

A number of recent studies have looked into circulating miRNAs for their potential as novel biomarkers. In one study comparing plasma miRNA profile between MM patients and healthy controls, six miRNAs (miR-148a, miR-181a, miR-20a, miR-221, miR-625, and miR-99b) were found upregulated in MM patients. Notably, miR-181a, miR-20a, miR-221, and miR-625 were identified previously to be unregulated in the plasma cells of MM or MGUS [84, 87, 88]. miR-99b was upregulated in t(4; 14) MM, consistent with previous reports [88–90]. Moreover, higher plasma levels of miR-20a and miR-148a were found to correlate with a shorter relapse-free survival [96].

Other deregulated serum/plasma circulating miRNAs have been identified, including miR-92a whose expression was lower in MM [97] and miR-29a which was expressed at a higher level in serum of MM patients [98]. miR-1308 and miR-720 could distinguish MGUS and MM patients from healthy controls [99]. Lower levels of miR-744 and let-7e were associated with shorter OS and remission [100].

These studies have linked miRNAs with clinical parameters, although the results are different from each other and no consistent miRNA-based biomarker is reported. Moreover, few studies have compared the usefulness of miRNA-based biomarkers with the current standard of care, except one study which identified a miRNA-based OS classifier that performed better than traditional ISS/FISH based method and outperformed existing GEP-based models in multivariate analysis [90]. Further validation of this prognostic signature in other cohort of patients is needed to ascertain its clinical utility. Similarly, while these studies demonstrated the feasibility of detecting miRNA in the serum and their potential clinical relevance, these findings needs to be further validated. Therefore, the exact clinical utility of measuring miRNA in serum is still unclear.

6. Interaction of miRNA and Current Therapeutic Agents in MM

In one of the earlier studies, Munker et al. studied miRNA expression profiles between MM cell lines with acquired resistance to doxorubicin or melphalan and the respective parental cells. Differentially expressed miRNAs include miR-21 and miR-181a/b, although their functional link to the resistance was not clear [101]. Wang et al. showed that adherence of MM cells to BMSCs upregulates miR-21 which resulted in decreased cytotoxicity to dexamethasone, doxorubicin, and bortezomib. Inhibition of miR-21 sensitized cells to dexamethasone and doxorubicin but not Bortezomib [102]. Tessel et al. identified a link between miR-130b and glucocorticoid resistance in MM, where miR-130b inhibited dexamethasone-induced apoptosis [103]. Similar result was observed in another study in which miR-125b was shown to attenuate dexamethasone-induced cell death in MM [104].

Hao et al. showed that the reduced sensitivity of MM cells to bortezomib and melphalan after coculture with BMSCs is at least partially due to inhibition of tumor suppressor miR-15a [105], while another miRNA, miR-29b, could sensitize MM cells to bortezomib-induced apoptosis and exerts anti-MM activity both in cultured MM cells and in MM xenografts

in mice. miR-29b showed wide variation of expression in MM and the expression was decreased with the presence of BMSCs, again demonstrating the critical role of BMSCs in promoting drug resistance and survival of MM cells. Notably, miR-29b mimic was able to overcome the protective role of BMSCs in an *in vivo* model [106].

Tian et al. identified miR-33b as an important mediator for the anti-MM function of MLN2238, a novel, orally active proteasome inhibitor. Inhibited in MM cells, miR-33b was upregulated by MLN2238, but not by other agents including dexamethasone, lenalidomide, and SAHA. Upregulation of miR-33b decreased MM cell viability, migration, and colony formation and increased apoptosis and sensitivity of MM cells to MLN2238 treatment. Notably, MLN2238 induced miR-33b even in the presence of BMSCs, and introduction of miR-33b partially blocked the protective effect of BMSCs on MM cells [107].

The evidence therefore suggests that a number of miRNA may be involved in therapeutic resistance mediated by stromal interaction. These may offer potential strategies to overcome drug resistance in myeloma. However, it is still not clear what the pathways affected by these miRNAs that may be critical in mediating drug resistance are.

7. The Interplay between miRNA and Epigenetics in MM

Aberrant miRNA expression or function in cancer can be attributed to various mechanisms involving both genomic and epigenetic aberrations. It has been observed in MM that miRNA expression could be disrupted by deregulation of miRNA host genes, copy number (CN) at miRNA-containing genomic locus [86, 89, 108, 109], abnormalities in miRNA biogenesis pathways [87], and abnormal activity of transcription factors [110]. However, it seems that the most important mechanism behind aberrant miRNA deregulation is epigenetic alterations, including abnormal DNA methylation and histone modifications [111–114]. Inactivation by methylation of all three members of miR-34 family tumor suppressor miRNAs was identified in MM [115–119]. Di Martino et al. provided a proof-of-principle that formulated that lipid emulsion delivery of synthetic miR-34a has therapeutic activity in preclinical, TP53 mutant xenograft models in MM [120]. Recently, the same group used a nanotechnology-based delivery system for miR-34a delivery and demonstrated similar anti-MM effect in tumor xenograft [119]. The promoter of the other two members of the miR-34 family, miR-34b and miR-34c, was not methylated in normal PCs, methylated in about 5.3% at MM diagnosed and increased frequency to more than half of relapsed/progressed MM patients. Functionally, restoration of miR-34b exhibited anti-MM activity *in vitro* [117]. Similar to miR-34a, downregulation of p53-inducible miR-192, miR-194, and miR-215 was attributed to promoter hypermethylation, which would impair the p53/MDM2 loop and favors MM development [92].

Another tumor suppressor, miR-203, was identified to be methylated at its promoter region in MM but not in normal PCs, and transfection of its precursor inhibited proliferation of MM cells [116]. Moreover, increasing frequency of promoter methylation in MM than MGUS for miR-129-2 was observed [118]. Aberrant DNA methylation could also explain the downregulation of other miRNAs in MM, including miR-214 which inhibited cell proliferation when overexpressed in MM cells. Consistent with the epigenetic silencing hypothesis, the level of miR-214 could be increased by treatment with DNA demethylating agent $5'$aza-$2'$-deoxycytidine [91, 121].

Besides aberrant DNA methylation, miRNA deregulation by histone modification had also been documented in MM. Min et al. showed that in t(4; 14) myeloma, repression of miR-126* expression, contributed to c-Myc upregulation and enhanced proliferation of MM cells. The downregulation of miR-126* was due to heterochromatin modification by MMSET [122].

It has been shown that miRNAs themselves can regulate the epigenetic machinery by directly targeting their enzymatic mediators such as DNMTs [123]. One such miRNA in MM is miR-29b, whose tumor suppressor property was earlier discussed. miR-29b targets *de novo* methyltransferases DNMT3A and DNMT3B mRNAs and reduces global DNA methylation in MM cells and therefore could restore expression of tumor suppressor genes silenced by hypermethylation such as SOCS1 [124].

Various mechanisms behind miRNA deregulation have been identified. Epigenetic aberrations, in particular abnormal DNA methylation at miRNA promoter regions, seem to be widespread and critical in silencing tumor suppressor miRNAs such as miRs-192, -194, -215, and miR-34 family. Our group has conducted genome-wide analysis of miRNAs silenced by DNA methylation and functionally studies miRNAs upregulated by demethylating treatment. Apart from known miRNAs that are epigenetically silenced, our study has revealed novel tumor suppressor miRNAs relevant in MM pathobiology (unpublished data). Again it highlights the importance of the epigenetic-miRNA regulatory network in MM.

8. miRNA and IL6-STAT3 Signaling in MM

The IL6-JAK-STAT axis is a major mediator of growth/survival promoting effect on MM conferred by BM microenvironment. Secreted by the BMSCs, IL6 binds to its receptor and activates JAK kinase, which in turn activates STAT3. The activated STAT3 translocates into the nucleus and activates transcription of genes that promote growth, proliferation, and survival of MM cells. The IL6-STAT3 signaling pathway is tightly controlled by SOCS proteins which binds to JAK and inhibits receptor phosphorylation and STAT3 activation [49–51]. However, SOCS1 is often silenced by promoter hypermethylation in MM, leading to enhanced IL6-STAT signaling [44, 45].

Studies have revealed roles of miRNAs as important regulators and mediators of this axis in MM. miR-21 is upregulated upon adherence of MM cells to BMSCs [102, 125]. It can be directly induced by STAT3 and contribute to the oncogenic potential of STAT3 [83]. At the same time, miR-21 can indirectly induce STAT3 by targeting PIAS3, a STAT3 inhibitor, forming a positive feedback loop [126]. miR-19

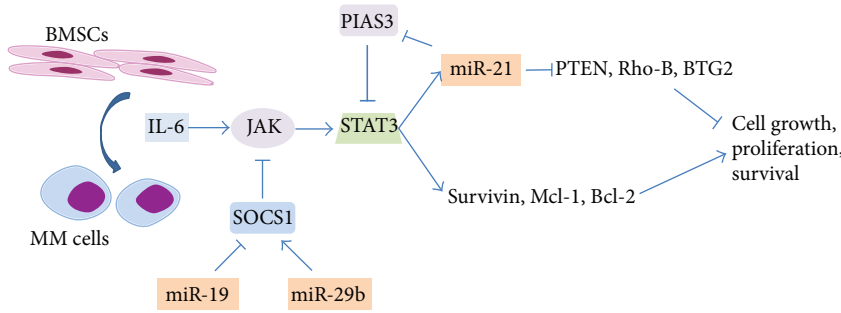


Figure 1: miRNA and IL6-STAT3 signaling in MM. miR-21 is upregulated upon adherence of MM cells to BMSCs. It can be directly induced by STAT3 and contribute to the oncogenic potential of STAT3. At the same time, miR-21 can indirectly induce STAT3 by targeting PIAS3, a STAT3 inhibitor, forming a positive feedback loop. miR-19 has been shown to promote STAT3 signalling by repressing SOCS1. miR-29b could demethylate SOCS1 by targeting DNMTs, leading to upregulation of SOCS1, and negatively regulates IL6-STAT3 signalling.

has been shown to promote STAT3 signaling by repressing SOCS1 [84]. Interestingly, miR-29b could demethylate SOCS1 by targeting DNMTs, leading to upregulation of SOCS1 levels and negatively regulates IL6-STAT3 signaling [45, 127] (Figure 1).

9. miRNA and p53 in MM

P53 mutation in newly diagnosed MM is rare and its frequency increases with disease progression. One copy loss of p53 by FISH has been uniformly recognized to be an adverse prognostic factor of MM [6]. It has been shown that p53 can be directly targeted by miR-125b, miR-25 and miR-30d [104, 128], and indirectly targeted by miR-106b~25 cluster, miR-32, and miR-181a which target PCAF, a positive regulator of p53 [84]. Upregulation of these miRNAs in MM was observed in multiple studies. On the other hand, p53 transcriptionally induces miRs-192, -194, and -215 which target MDM2 [92], and miR-34a which target SIRT1 [104]. Both pathways lead to upregulation of p53, forming two positive feedback loops. Deregulation of these miRNAs leads to compromised p53 tumor suppressor pathway and favors oncogenesis (Figure 2).

10. Key miRNAs with Therapeutic Potential in MM

MiRNA possesses promising therapeutic potential in cancer because it can target many important genes or pathways at the same time. A number of deregulated miRNAs are consistently identified and their important functions are demonstrated (see Table 1). Discussed below are some of the miRNAs that have demonstrated the most promising therapeutic potential.

10.1. MiR-29b and miR-21. MiR-29b has been shown to inhibit tumor growth in HMCLs and in mouse. It can also contribute to the antitumor activity of Bortezomib and potentiates Bortezomib-induced apoptosis when used together [106]. The tumor suppressor property of miR-29b may be partially explained by its inhibition on IL6-JAK-STAT3 signaling via targeting DNMTs and subsequent demethylation and activation of SOCS1 [124]. Moreover, it target proangiogenic factors including VEGFA, inhibits migration, and negatively regulates osteoclast activity which may alleviate lytic bone disease [127, 129, 130].

MiR-21 has been identified as a direct target of STAT3 that potentiates IL6-STAT3 signaling [83]. Upregulation of miR-21 upon adherence to BM has been shown for HMCLs and primary samples, which may be explained by enhanced IL6-STAT signaling. Targeting miR-21 inhibits *in vitro* and *in vivo* MM growth even in the context of BM and could synergize with chemotherapeutic agents dexamethasone and doxorubicin [102, 125].

10.2. miRs-192, -194, -215, and miR-34a. 17p deletions, mostly including TP53, have been unequivocally identified as a predictor for worse prognosis in MM. miRNAs have been implicated in p53 pathway. In particular, miRs-192, -194, -215, and miR-34a have been extensively studied. All these four miRNAs are direct transcriptional targets of p53, reversely; these miRNAs indirectly induce p53, forming two positive feedback loops and participating in the regulatory balance of p53. Promoter hypermethylation of these miRNAs has been found in MM and could lead to their silencing. Reexpressing miRs-192, 194, and 215 leads to downregulation of their direct target MDM2 and could sensitize TP53 WT cells to pharmacological inhibition of MDM2 *in vitro* and *in vivo*. In addition, miRs 192, -194, and -215 could inhibit migration and invasion of MM cells by targeting IGF1 and IGF1R [92]. miR-34a is an established tumor suppressor in cancer. In MM, miR-34 has demonstrated excellent antitumor activity in preclinical models. Transient and prolonged expression of miR-34a inhibited tumor growth both *in vitro* and *in vivo*. In mouse models, both intramural injection and systemic delivery of miR-34a in lipid particles inhibited tumor growth. Importantly, miR-34a could overcome the BM-dependent protective effect on MM cells, as demonstrated by a novel 3D system [119, 120]. It should be noted that most of the cells used in miR-34a study are TP53 mutant, suggesting that patients with p53 inactivation may in particular benefit from miR-34a replacement therapy.

10.3. miR-15a. MiR-15a has been closely associated with bone marrow microenvironment. The secretion of tumor suppressive miR-15a in exosomes by normal BM is reduced in tumor

Table 1: Selected miRNA deregulations in MM.

miRNA	Deregulation in MM (versus normal control unless specified)	Targets/function/clinical relevance	Association with clinical parameters
21	Upregulated in MM and MGUS [84, 88]; upregulated in primary PCL versus MM [86]	PIAS3 [126], PTEN [125], Rho-B [102, 125], BTG2 [125]; induced by STAT3 in response to IL-6 [83]	
221, 222	221: upregulated in MM [84, 87, 88]; 222: upregulated in MGUS [84, 88], MM [87, 88]; 221 and 222: upregulated in relapse/refractory MM [85]	p27Kip1, PUMA, PTEN and p57Kip2 [132]	
17-92 cluster (17, 18a, 19a, 19b-1, 20a, 92a)	Upregulated in MM but not in MGUS [84]; upregulated in MM [87, 88, 133]; Positively regulated by Myc [110]; lower plasma miR-92a level in MM than MGUS, SMM and normal.[97]	BIM, SOCS1 [84]; P21 [87]	Higher 92a was associated with shorter OS [86]; Higher 17, 20a, and 92-1 were associated with shorter PFS [110, 134]; higher 17 and 18a were associated with shorter OS [90]; Higher plasma miR-20a was associated with shorter relapse-free survival [96]
106b~25 cluster (106b, 93, 25)	Upregulated in MM and MGUS [84]; upregulated in MM [133]; miR-25 is overexpressed in MM [128]	PCAF [84]	miR-106b was correlated with treatment response [86]
181a/b	Upregulated in MM and MGUS [84]; upregulated in MM [85, 87, 88]	PCAF [84]	miR-181a* and miR-181b were correlated with treatment response [86]
25, 30d, 125b	Upregulated in MM [84, 87, 128]	P53 [128]	
32	Upregulated in MM not in MGUS [84]	PCAF [84]	
15a and 16-1	Decreased in relapsed/refractory MM [85]; decreased in MM [88]; decreased in patients with 13del as compared to those without [88]; expressed in MM independent of chr13 status [134, 135]	AKT3, rpS6, MAP-kinases, MAP3KIP, VEGF [85]	Decreased in patients with ISS stage III [94]; higher expression correlates with shorter PFS [134]
192, 194, 215	Downregulated in MM by promoter hypermethylation [92]	Activated by TP53 and targeted MDM2, IGF1, IGF1R [92]	
34 family	Downregulated in MM by promoter hypermethylation [115, 117]	BCL2, CDK6 and NOTCH1 [120]	
203	Downregulated in MM [91, 135]; decreased in MGUS and MM by promoter hypermethylation [116]	CREB1 [116]	
33b	Downregulated in MM [107]	Involved in MLN2238-induced apoptotic signaling in MM cells [107]	
29b	Wide variation of expression in MM and further decreased with the presence of BMSCs [106]	DNMT3A/B [124], CDK6 [106], MCL-1 [106, 129], Sp1 [106]; Targeted VEGFA, IL8; induced SOCS1 [127]	
425, 152, 24	Downregulated in hyperdiploid MM versus nonhyperdiploid MM [93]	CCND1, TACC3, MAFB, FGFR3, MYC [93]	
214	Downregulated in MM versus normal PCs [91], possibly by methylation [121]	PSMD10 [121]	
126*	Downregulated in t(4;14) MM cells; inhibited by MMSET by heterochromatin modification [122]	c-Myc [122]	

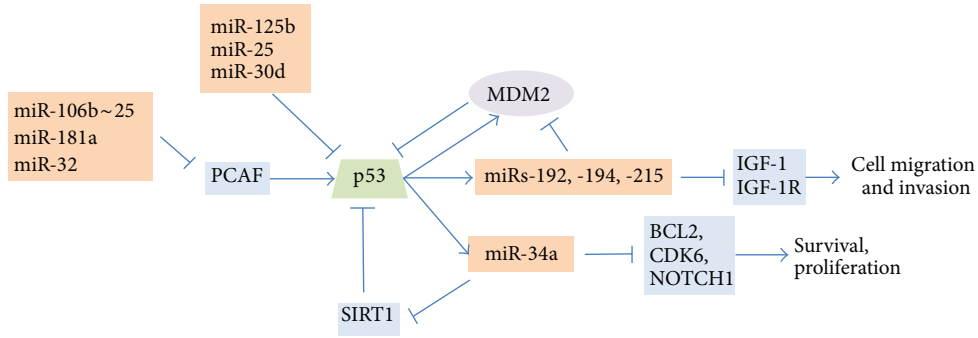


Figure 2: miRNA and p53 in MM. P53 can be directly targeted by miR-125b, miR-25, and miR-30d and indirectly targeted by miR-106b~25 cluster, miR-32, miR-181a which target PCAF, a positive regulator of p53. Upregulation of these miRNAs in MM were observed in multiple studies. On the other hand, p53 transcriptionally induces miRs-192, -194, and -215 which target MDM2 and miR-34a which target SIRT1. Both pathways lead to upregulation of p53, forming two positive feedback loops. Deregulation of these miRNAs leads to compromised p53 tumor suppressor pathway and favors oncogenesis.

microenvironment, producing a permissible environment for tumorigenesis and reduces sensitivity to bortezomib and melphalan [105, 131]. Restoring miR-15 inhibited AKT, NFκB activity, and VEGF and exerted antitumor effects even in the context of BM [85, 94].

11. Conclusion

In conclusion, miRNAs have emerged as important players in the pathobiology of MM and have potential in improving clinical practice. Future research should focus on the validation of miRNA signatures and the integration of validated signatures in clinical practice for better disease classification, prognostication, and prescription. At the same time, miRNAs with the most promising therapeutic potential should be moved into the pipeline of clinical development, as single agents or in combination with current therapy, guided by improved understanding of the disease.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

References

[1] J. C. Wee, G. L. Lee, N. Yusof, and B. M. F. Mow, "Targeted therapy in multiple myeloma," *Cancer Control*, vol. 12, no. 2, pp. 91–104, 2005.

[2] M. S. Raab, K. Podar, I. Breitkreutz, P. G. Richardson, and K. C. Anderson, "Multiple myeloma," *The Lancet*, vol. 374, no. 9686, pp. 324–339, 2009.

[3] S. Kumar, "Multiple myeloma—current issues and controversies," *Cancer Treatment Reviews*, vol. 36, supplement 2, pp. S3–S11, 2010.

[4] R. A. Kyle, T. M. Therneau, S. Vincent Rajkumar et al., "A long-term study of prognosis in monoclonal gammopathy of undetermined significance," *The New England Journal of Medicine*, vol. 346, no. 8, pp. 564–569, 2002.

[5] R. A. Kyle, T. M. Therneau, S. V. Rajkumar et al., "Prevalence of monoclonal gammopathy of undetermined significance," *The New England Journal of Medicine*, vol. 354, no. 13, pp. 1362–1369, 2006.

[6] R. Fonseca and J. Monge, "Myeloma: classification and risk assessment," *Seminars in Oncology*, vol. 40, no. 5, pp. 554–566, 2013.

[7] F. Albarracin and R. Fonseca, "Plasma cell leukemia," *Blood Reviews*, vol. 25, no. 3, pp. 107–112, 2011.

[8] M. Chesi and P. L. Bergsagel, "Molecular pathogenesis of multiple myeloma: basic and clinical updates," *International Journal of Hematology*, vol. 97, no. 3, pp. 313–323, 2013.

[9] H. G. Drexler and Y. Matsuo, "Malignant hematopoietic cell lines: in vitro models for the study of multiple myeloma and plasma cell leukemia," *Leukemia Research*, vol. 24, no. 8, pp. 681–703, 2000.

[10] B. G. M. Durie and S. E. Salmon, "A clinical staging system for multiple myeloma. Correlation of measured myeloma cell mass with presenting clinical features, response to treatment, and survival," *Cancer*, vol. 36, no. 3, pp. 842–854, 1975.

[11] P. R. Greipp, J. S. Miguel, B. G. M. Dune et al., "International staging system for multiple myeloma," *Journal of Clinical Oncology*, vol. 23, no. 15, pp. 3412–3420, 2005.

[12] W. J. Chng, A. Dispenzieri, C. S. Chim et al., "IMWG consensus on risk stratification in multiple myeloma," *Leukemia*, vol. 28, no. 2, pp. 269–277, 2014.

[13] W. J. Chng, O. Glebov, P. L. Bergsagel, and W. M. Kuehl, "Genetic events in the pathogenesis of multiple myeloma," *Best Practice and Research: Clinical Haematology*, vol. 20, no. 4, pp. 571–596, 2007.

[14] C. Bi and W. Chng, *Molecular Genetics of Multiple Myeloma*, John Wiley & Sons, New York, NY, USA, 2010.

[15] C. S. Debes-Marun, G. W. Dewald, S. Bryant et al., "Chromosome abnormalities clustering and its implications for pathogenesis and prognosis in myeloma," *Leukemia*, vol. 17, no. 2, pp. 427–436, 2003.

[16] R. Fonseca, C. S. Debes-Marun, E. B. Picken et al., "The recurrent IgH translocations are highly associated with nonhyperdiploid variant multiple myeloma," *Blood*, vol. 102, no. 7, pp. 2562–2567, 2003.

[17] R. Fonseca, P. L. Bergsagel, J. Drach et al., "International Myeloma Working Group molecular classification of multiple myeloma: spotlight review," *Leukemia*, vol. 23, no. 12, pp. 2210–2221, 2009.

[18] A. C. Bharti, S. Shishodia, J. M. Reuben et al., "Nuclear factor-κB and STAT3 are constitutively active in CD138$^+$ cells derived from multiple myeloma patients, and suppression of these transcription factors leads to apoptosis," *Blood*, vol. 103, no. 8, pp. 3175–3184, 2004.

[19] C. M. Annunziata, R. E. Davis, Y. Demchenko et al., "Frequent engagement of the classical and alternative NF-kappaB pathways by diverse genetic abnormalities in multiple myeloma," *Cancer Cell*, vol. 12, no. 2, pp. 115–130, 2007.

[20] J. J. Keats, R. Fonseca, M. Chesi et al., "Promiscuous mutations activate the noncanonical NF-kappaB pathway in multiple myeloma," *Cancer Cell*, vol. 12, no. 2, pp. 131–144, 2007.

[21] W. J. Chng, N. Gonzalez-Paz, T. Price-Troska et al., "Clinical and biological significance of RAS mutations in multiple myeloma," *Leukemia*, vol. 22, no. 12, pp. 2280–2284, 2008.

[22] A. Gabrea, M. L. Martelli, Y. Qi et al., "Secondary genomic rearrangements involving immunoglobulin or MYC loci show similar prevalences in hyperdiploid and nonhyperdiploid myeloma tumors," *Genes Chromosomes and Cancer*, vol. 47, no. 7, pp. 573–590, 2008.

[23] R. E. Tiedemann, N. Gonzalez-Paz, R. A. Kyle et al., "Genetic aberrations and survival in plasma cell leukemia," *Leukemia*, vol. 22, no. 5, pp. 1044–1052, 2008.

[24] L. Chiecchio, G. P. Dagrada, A. H. Ibrahim et al., "Timing of acquisition of deletion 13 in plasma cell dyscrasias is dependent on genetic context," *Haematologica*, vol. 94, no. 12, pp. 1708–1713, 2009.

[25] L. Lodé, M. Eveillard, V. Trichet et al., "Mutations in TP53 are exclusively associated with del(17p) in multiple myeloma," *Haematologica*, vol. 95, no. 11, pp. 1973–1976, 2010.

[26] K. D. Boyd, F. M. Ross, B. A. Walker et al., "Mapping of chromosome 1p deletions in myeloma identifies FAM46C at 1p12 and CDKN2C at 1p32.3 as being genes in regions associated with adverse survival," *Clinical Cancer Research*, vol. 17, no. 24, pp. 7776–7784, 2011.

[27] W. J. Chng, G. F. Huang, T. H. Chung et al., "Clinical and biological implications of MYC activation: a common difference between MGUS and newly diagnosed multiple myeloma," *Leukemia*, vol. 25, no. 6, pp. 1026–1035, 2011.

[28] F. Zhan, J. Hardin, B. Kordsmeier et al., "Global gene expression profiling of multiple myeloma, monoclonal gammopathy of undetermined significance, and normal bone marrow plasma cells," *Blood*, vol. 99, no. 5, pp. 1745–1757, 2002.

[29] F. E. Davies, A. M. Dring, C. Li et al., "Insights into the multistep transformation of MGUS to myeloma using microarray expression analysis," *Blood*, vol. 102, no. 13, pp. 4504–4511, 2003.

[30] F. Zhan, E. Tian, K. Bumm, R. Smith, B. Barlogie, and J. Shaughnessy Jr., "Gene expression profiling of human plasma cell differentiation and classification of multiple myeloma based on similarities to distinct stages of late-stage B-cell development," *Blood*, vol. 101, no. 3, pp. 1128–1140, 2003.

[31] F. Zhan, B. Barlogie, V. Arzoumanian et al., "Gene-expression signature of benign monoclonal gammopathy evident in multiple myeloma is linked to good prognosis," *Blood*, vol. 109, no. 4, pp. 1692–1700, 2007.

[32] A. Zingone and W. M. Kuehl, "Pathogenesis of monoclonal gammopathy of undetermined significance and progression to multiple myeloma," *Seminars in Hematology*, vol. 48, no. 1, pp. 4–12, 2011.

[33] P. L. Bergsagel, W. M. Kuehl, F. Zhan, J. Sawyer, B. Barlogie, and J. Shaughnessy Jr., "Cyclin D dysregulation: an early and unifying pathogenic event in multiple myeloma," *Blood*, vol. 106, no. 1, pp. 296–303, 2005.

[34] L. Agnelli, S. Bicciato, M. Mattioli et al., "Molecular classification of multiple myeloma: a distinct transcriptional profile characterizes patients expressing CCND1 and negative for 14q32 translocations," *Journal of Clinical Oncology*, vol. 23, no. 29, pp. 7296–7306, 2005.

[35] F. Zhan, Y. Huang, S. Colla et al., "The molecular classification of multiple myeloma," *Blood*, vol. 108, no. 6, pp. 2020–2028, 2006.

[36] A. Broyl, D. Hose, H. Lokhorst et al., "Gene expression profiling for molecular classification of multiple myeloma in newly diagnosed patients," *Blood*, vol. 116, no. 14, pp. 2543–2553, 2010.

[37] P. L. Bergsagel and W. M. Kuehl, "Molecular pathogenesis and a consequent classification of multiple myeloma," *Journal of Clinical Oncology*, vol. 23, no. 26, pp. 6333–6338, 2005.

[38] M. Mattioli, L. Agnelli, S. Fabris et al., "Gene expression profiling of plasma cell dyscrasias reveals molecular patterns associated with distinct IGH translocations in multiple myeloma," *Oncogene*, vol. 24, no. 15, pp. 2461–2473, 2005.

[39] D. R. Carrasco, G. Tonon, Y. Huang et al., "High-resolution genomic profiles define distinct clinico-pathogenetic subgroups of multiple myeloma patients," *Cancer Cell*, vol. 9, no. 4, pp. 313–325, 2006.

[40] J. D. Shaughnessy Jr., F. Zhan, B. E. Burington et al., "Avalidated gene expression model of high-risk multiple myeloma is defined by deregulated expression of genes mapping to chromosome 1," *Blood*, vol. 109, no. 6, pp. 2276–2284, 2007.

[41] W. J. Chng, S. Kumar, S. VanWier et al., "Molecular dissection of hyperdiploid multiple myeloma by gene expression profiling," *Cancer Research*, vol. 67, no. 7, pp. 2982–2989, 2007.

[42] W. J. Chng, E. Braggio, G. Mulligan et al., "The centrosome index is a powerful prognostic marker in myeloma and identifies a cohort of patients that might benefit from aurora kinase inhibition," *Blood*, vol. 111, no. 3, pp. 1603–1609, 2008.

[43] A. Anguiano, S. A. Tuchman, C. Acharya et al., "Gene expression profiles of tumor biology provide a novel approach to prognosis and may guide the selection of therapeutic targets in multiple myeloma," *Journal of Clinical Oncology*, vol. 27, no. 25, pp. 4197–4203, 2009.

[44] O. Galm, H. Yoshikawa, M. Esteller, R. Osieka, and J. G. Herman, "SOCS-1, a negative regulator of cytokine signaling, is frequently silenced by methylation in multiple myeloma," *Blood*, vol. 101, no. 7, pp. 2784–2788, 2003.

[45] C. S. Chim, T. K. Fung, W. C. Cheung, R. Liang, and Y. L. Kwong, "SOCS1 and SHP1 hypermethylation in multiple myeloma: implications for epigenetic activation of the Jak/STAT pathway," *Blood*, vol. 103, no. 12, pp. 4630–4635, 2004.

[46] C. Ribas, G. W. B. Colleoni, R. S. Felix et al., "p16 gene methylation lacks correlation with angiogenesis and prognosis in multiple myeloma," *Cancer Letters*, vol. 222, no. 2, pp. 247–254, 2005.

[47] F. de Carvalho, G. W. B. Colleoni, M. S. Sampaio Almeida, A. L. Carvalho, and A. L. Vettore, "TGFβR2 aberrant methylation is a potential prognostic marker and therapeutic target in multiple myeloma," *International Journal of Cancer*, vol. 125, no. 8, pp. 1985–1991, 2009.

[48] B. A. Walker, C. P. Wardell, L. Chiecchio et al., "Aberrant global methylation patterns affect the molecular pathogenesis and prognosis of multiple myeloma," *Blood*, vol. 117, no. 2, pp. 553–562, 2011.

[49] T. Hirano, K. Ishihara, and M. Hibi, "Roles of STAT3 in mediating the cell growth, differentiation and survival signals relayed through the IL-6 family of cytokine receptors," *Oncogene*, vol. 19, no. 21, pp. 2548–2556, 2000.

[50] K. Brocke-Heidrich, A. K. Kretzschmar, G. Pfeifer et al., "Interleukin-6-dependent gene expression profiles in multiple myeloma INA-6 cells reveal a Bcl-2 family-independent survival pathway closely associated with Stat3 activation," *Blood*, vol. 103, no. 1, pp. 242–251, 2004.

[51] K. Bommert, R. C. Bargou, and T. Stühmer, "Signalling and survival pathways in multiple myeloma," *European Journal of Cancer*, vol. 42, no. 11, pp. 1574–1580, 2006.

[52] R. A. Kyle and S. V. Rajkumar, "Multiple myeloma," *Blood*, vol. 111, no. 6, pp. 2962–2972, 2008.

[53] D. P. Bartel, "MicroRNAs: genomics, biogenesis, mechanism, and function," *Cell*, vol. 116, no. 2, pp. 281–297, 2004.

[54] J. Krol, I. Loedige, and W. Filipowicz, "The widespread regulation of microRNA biogenesis, function and decay," *Nature Reviews Genetics*, vol. 11, no. 9, pp. 597–610, 2010.

[55] A. Kozomara and S. Griffiths-Jones, "MiRBase: integrating microRNA annotation and deep-sequencing data," *Nucleic Acids Research*, vol. 39, no. 1, pp. D152–D157, 2011.

[56] V. N. Kim, J. Han, and M. C. Siomi, "Biogenesis of small RNAs in animals," *Nature Reviews Molecular Cell Biology*, vol. 10, no. 2, pp. 126–139, 2009.

[57] M. Inui, G. Martello, and S. Piccolo, "MicroRNA control of signal transduction," *Nature Reviews Molecular Cell Biology*, vol. 11, no. 4, pp. 252–263, 2010.

[58] L. Ma, J. Teruya-Feldstein, and R. A. Weinberg, "Tumour invasion and metastasis initiated by microRNA-10b in breast cancer," *Nature*, vol. 449, no. 7163, pp. 682–688, 2007.

[59] M. V. Iorio and C. M. Croce, "MicroRNAs in cancer: small molecules with a huge impact," *Journal of Clinical Oncology*, vol. 27, no. 34, pp. 5848–5856, 2009.

[60] R. Spizzo, M. S. Nicoloso, C. M. Croce, and G. A. Calin, "SnapShot: MicroRNAs in Cancer," *Cell*, vol. 137, no. 3, pp. 586–586.e1, 2009.

[61] G. A. Calin, C. G. Liu, C. Sevignani et al., "MicroRNA profiling reveals distinct signatures in B cell chronic lymphocytic leukemias," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 101, no. 32, pp. 11755–11760, 2004.

[62] J. Lu, G. Getz, E. A. Miska et al., "MicroRNA expression profiles classify human cancers," *Nature*, vol. 435, no. 7043, pp. 834–838, 2005.

[63] S. Volinia, G. A. Calin, C. G. Liu et al., "A microRNA expression signature of human solid tumors defines cancer gene targets," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 103, no. 7, pp. 2257–2261, 2006.

[64] S. Mi, J. Lu, M. Sun et al., "MicroRNA expression signatures accurately discriminate acute lymphoblastic leukemia from acute myeloid leukemia," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 104, no. 50, pp. 19971–19976, 2007.

[65] J. Takamizawa, H. Konishi, K. Yanagisawa et al., "Reduced expression of the let-7 microRNAs in human lung cancers in association with shortened postoperative survival," *Cancer Research*, vol. 64, no. 11, pp. 3753–3756, 2004.

[66] M. V. Iorio, M. Ferracin, C. G. Liu et al., "MicroRNA gene expression deregulation in human breast cancer," *Cancer Research*, vol. 65, no. 16, pp. 7065–7070, 2005.

[67] G. A. Calin, M. Ferracin, A. Cimmino et al., "A microRNA signature associated with prognosis and progression in chronic lymphocytic leukemia," *The New England Journal of Medicine*, vol. 353, no. 17, pp. 1793–1801, 2005.

[68] N. Yanaihara, N. Caplen, E. Bowman et al., "Unique microRNA molecular profiles in lung cancer diagnosis and prognosis," *Cancer Cell*, vol. 9, no. 3, pp. 189–198, 2006.

[69] X. Rao, G. di Leva, M. Li et al., "MicroRNA-221/222 confers breast cancer fulvestrant resistance by regulating multiple signaling pathways," *Oncogene*, vol. 30, no. 9, pp. 1082–1097, 2011.

[70] Z. Wang, Y. Li, A. Ahmad et al., "Pancreatic cancer: understanding and overcoming chemoresistance," *Nature Reviews. Gastroenterology and Hepatology*, vol. 8, no. 1, pp. 27–33, 2011.

[71] D. D. Feng, H. Zhang, P. Zhang et al., "Down-regulated miR-331-5p and miR-27a are associated with chemotherapy resistance and relapse in leukaemia," *Journal of Cellular and Molecular Medicine*, vol. 15, no. 10, pp. 2164–2175, 2011.

[72] A. Ventura and T. Jacks, "MicroRNAs and cancer: short RNAs go a long way," *Cell*, vol. 136, no. 4, pp. 586–591, 2009.

[73] G. A. Calin, C. D. Dumitru, M. Shimizu et al., "Frequent deletions and down-regulation of micro-RNA genes miR15 and miR16 at 13q14 in chronic lymphocytic leukemia," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 99, no. 24, pp. 15524–15529, 2002.

[74] G. A. Calin, C. Sevignani, C. D. Dumitru et al., "Human microRNA genes are frequently located at fragile sites and genomic regions involved in cancers," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 101, no. 9, pp. 2999–3004, 2004.

[75] L. He, J. M. Thomson, M. T. Hemann et al., "A microRNA polycistron as a potential human oncogene," *Nature*, vol. 435, no. 7043, pp. 828–833, 2005.

[76] M. S. Kumar, J. Lu, K. L. Mercer, T. R. Golub, and T. Jacks, "Impaired microRNA processing enhances cellular transformation and tumorigenesis," *Nature Genetics*, vol. 39, no. 5, pp. 673–677, 2007.

[77] B. Muralidhar, L. D. Goldstein, G. Ng et al., "Global microRNA profiles in cervical squamous cell carcinoma depend on Drosha expression levels," *Journal of Pathology*, vol. 212, no. 4, pp. 368–377, 2007.

[78] M. S. Kumar, R. E. Pester, C. Y. Chen et al., "Dicer1 functions as a haploinsufficient tumor suppressor," *Genes and Development*, vol. 23, no. 23, pp. 2700–2704, 2009.

[79] S. A. Melo and M. Esteller, "Dysregulation of microRNAs in cancer: Playing with fire," *FEBS Letters*, vol. 585, no. 13, pp. 2087–2099, 2011.

[80] Y. Saito, G. Liang, G. Egger et al., "Specific activation of microRNA-127 with downregulation of the proto-oncogene BCL6 by chromatin-modifying drugs in human cancer cells," *Cancer Cell*, vol. 9, no. 6, pp. 435–443, 2006.

[81] E. Bandres, X. Agirre, N. Bitarte et al., "Epigenetic regulation of microRNA expression in colorectal cancer," *International Journal of Cancer*, vol. 125, no. 11, pp. 2737–2743, 2009.

[82] E. Ballabio, T. Mitchell, M. S. van Kester et al., "MicroRNA expression in Sézary syndrome: identification, function, and diagnostic potential," *Blood*, vol. 116, no. 7, pp. 1105–1113, 2010.

[83] D. Löffler, K. Brocke-Heidrich, G. Pfeifer et al., "Interleukin-6-dependent survival of multiple myeloma cells involves the Stat3-mediated induction of microRNA-21 through a highly conserved enhancer," *Blood*, vol. 110, no. 4, pp. 1330–1333, 2007.

[84] F. Pichiorri, S. S. Suh, M. Ladetto et al., "MicroRNAs regulate critical genes associated with multiple myeloma pathogenesis," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 105, no. 35, pp. 12885–12890, 2008.

[85] A. M. Roccaro, A. Sacco, B. Thompson et al., "MicroRNAs 15a and 16 regulate tumor proliferation in multiple myeloma," *Blood*, vol. 113, no. 26, pp. 6669–6680, 2009.

[86] M. Lionetti, P. Musto, M. T. D. Martino et al., "Biological and clinical relevance of miRNA expression signatures in primary plasma cell leukemia," *Clinical Cancer Research*, vol. 19, no. 12, pp. 3130–3142, 2013.

[87] Y. Zhoua, L. Chena, B. Barlogiea et al., "High-risk myeloma is associated with global elevation of miRNAs and overexpression of EIF2C2/AGO2," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 107, no. 17, pp. 7904–7909, 2010.

[88] J. Chi, E. Ballabio, X. H. Chen et al., "MicroRNA expression in multiple myeloma is associated with genetic subtype, isotype and survival," *Biology Direct*, vol. 6, article 23, 2011.

[89] M. Lionetti, M. Biasiolo, L. Agnelli et al., "Identification of microRNA expression patterns and definition of a microRNA/mRNA regulatory network in distinct molecular groups of multiple myeloma," *Blood*, vol. 114, no. 25, pp. e20–e26, 2009.

[90] P. Wu, L. Agnelli, B. A. Walker et al., "Improved risk stratification in myeloma using a microRNA-based classifier," *British Journal of Haematology*, vol. 162, no. 3, pp. 348–359, 2013.

[91] N. C. Gutiérrez, M. E. Sarasquete, I. Misiewicz-Krzeminska et al., "Deregulation of microRNA expression in the different genetic subtypes of multiple myeloma and correlation with gene expression profiling," *Leukemia*, vol. 24, no. 3, pp. 629–637, 2010.

[92] F. Pichiorri, S. S. Suh, A. Rocci et al., "Downregulation of p53-inducible microRNAs 192, 194, and 215 impairs the p53/MDM2 autoregulatory loop in multiple myeloma development," *Cancer Cell*, vol. 18, no. 4, pp. 367–381, 2010.

[93] A. Rio-Machin, B. I. Ferreira, T. Henry et al., "Downregulation of specific miRNAs in hyperdiploid multiple myeloma mimics the oncogenic effect of IgH translocations occurring in the non-hyperdiploid subtype," *Leukemia*, vol. 27, no. 4, pp. 925–931, 2013.

[94] C. Y. Sun, X. M. She, Y. Qin et al., "miR-15a and miR-16 affect the angiogenesis of multiple myeloma by targeting VEGF," *Carcinogenesis*, vol. 34, no. 2, pp. 426–435, 2013.

[95] C. Fernández De Larrea, A. Navarro, R. Tejero et al., "Impact of MiRSNPs on survival and progression in patients with multiple myeloma undergoing autologous stem cell transplantation," *Clinical Cancer Research*, vol. 18, no. 13, pp. 3697–3704, 2012.

[96] J. J. Huang, J. Yu, J. Y. Li, Y. T. Liu, and R. Q. Zhong, "Circulating microRNA expression is associated with genetic subtype and survival of multiple myeloma," *Medical Oncology*, vol. 29, no. 4, pp. 2402–2408, 2012.

[97] S. Yoshizawa, J. H. Ohyashiki, M. Ohyashiki et al., "Downregulated plasma miR-92a levels have clinical impact on multiple myeloma and related disorders," *Blood Cancer Journal*, vol. 2, no. 1, article e53, 2012.

[98] S. Sevcikova, L. Kubiczkova, L. Sedlarikova, O. Slaby, and R. Hajek, "Serum miR-29a as a marker of multiple myeloma," *Leukemia and Lymphoma*, vol. 54, no. 1, pp. 189–191, 2013.

[99] C. I. Jones, M. V. Zabolotskaya, A. J. King et al., "Identification of circulating microRNAs as diagnostic biomarkers for use in multiple myeloma," *British Journal of Cancer*, vol. 107, no. 12, pp. 1987–1996, 2012.

[100] L. Kubiczkova, F. Kryukov, O. Slaby et al., "Circulating serum microRNAs as novel diagnostic and prognostic biomarkers for multiple myeloma and monoclonal gammopathy of undetermined significance," *Haematologica*, vol. 99, no. 3, pp. 511–518, 2014.

[101] R. Munker, C. G. Liu, C. Taccioli, H. Alder, and N. Heerema, "MicroRNA profiles of drug-resistant myeloma cell lines," *Acta Haematologica*, vol. 123, no. 4, pp. 201–204, 2010.

[102] X. Wang, C. Li, S. Ju, Y. Wang, H. Wang, and R. Zhong, "Myeloma cell adhesion to bone marrow stromal cells confers drug resistance by microRNA-21 up-regulation," *Leukemia and Lymphoma*, vol. 52, no. 10, pp. 1991–1998, 2011.

[103] M. A. Tessel, A. L. Benham, N. L. Krett, S. T. Rosen, and P. H. Gunaratne, "Role for microRNAs in regulating glucocorticoid response and resistance in multiple myeloma," *Hormones and Cancer*, vol. 2, no. 3, pp. 182–189, 2011.

[104] M. Y. Murray, S. A. Rushworth, L. Zaitseva, K. M. Bowles, and D. J. MacEwan, "Attenuation of dexamethasone-induced cell death in multiple myeloma is mediated by miR-125b expression," *Cell Cycle*, vol. 12, no. 13, pp. 2144–2153, 2013.

[105] M. Hao, L. Zhang, G. An et al., "Suppressing miRNA-15a/-16 expression by interleukin-6 enhances drug-resistance in myeloma cells," *Journal of Hematology and Oncology*, vol. 4, article 37, 2011.

[106] N. Amodio, M. T. di Martino, U. Foresta et al., "MiR-29b sensitizes multiple myeloma cells to bortezomib-induced apoptosis through the activation of a feedback loop with the transcription factor Sp1," *Cell Death and Disease*, vol. 3, no. 11, article e436, 2012.

[107] Z. Tian, J. J. Zhao, Y. T. Tai et al., "Investigational agent MLN9708/2238 targets tumor-suppressor miR33b in MM cells," *Blood*, vol. 120, no. 19, pp. 3958–3967, 2012.

[108] D. Ronchetti, M. Lionetti, L. Mosca et al., "An integrative genomic approach reveals coordinated expression of intronic miR-335, miR-342, and miR-561 with deregulated host genes in multiple myeloma," *BMC Medical Genomics*, vol. 1, article 37, 2008.

[109] M. Lionetti, L. Agnelli, L. Mosca et al., "Integrative high-resolution microarray analysis of human myeloma cell lines reveals deregulated miRNA expression associated with allelic imbalances and gene expression profiles," *Genes Chromosomes and Cancer*, vol. 48, no. 6, pp. 521–531, 2009.

[110] L. Chen, C. Li, R. Zhang et al., "MiR-17-92 cluster microRNAs confers tumorigenicity in multiple myeloma," *Cancer Letters*, vol. 309, no. 1, pp. 62–70, 2011.

[111] A. Lujambio, S. Ropero, E. Ballestar et al., "Genetic unmasking of an epigenetically silenced microRNA in human cancer cells," *Cancer Research*, vol. 67, no. 4, pp. 1424–1429, 2007.

[112] S. A. Melo and M. Esteller, "Dysregulation of microRNAs in cancer: playing with fire," *FEBS Letters*, vol. 585, no. 13, pp. 2087–2099, 2011.

[113] F. Sato, S. Tsuchiya, S. J. Meltzer, and K. Shimizu, "MicroRNAs and epigenetics," *FEBS Journal*, vol. 278, no. 10, pp. 1598–1609, 2011.

[114] K. I. Kozaki and J. Inazawa, "Tumor-suppressive microRNA silenced by tumor-specific DNA hypermethylation in cancer cells," *Cancer Science*, vol. 103, no. 5, pp. 837–845, 2012.

[115] C. S. Chim, K. Y. Wong, Y. Qi et al., "Epigenetic inactivation of the miR-34a in hematological malignancies," *Carcinogenesis*, vol. 31, no. 4, pp. 745–750, 2010.

[116] K. Y. Wong, R. Liang, C. C. So, D. Y. Jin, J. F. Costello, and C. S. Chim, "Epigenetic silencing of MIR203 in multiple myeloma," *British Journal of Haematology*, vol. 154, no. 5, pp. 569–578, 2011.

[117] K. Y. Wong, R. L. H. Yim, C. C. So, D. Y. Jin, R. Liang, and C. S. Chim, "Epigenetic inactivation of the MIR34B/C in multiple myeloma," *Blood*, vol. 118, no. 22, pp. 5901–5904, 2011.

[118] K. Y. Wong, R. L. Yim, Y. L. Kwong et al., "Epigenetic inactivation of the MIR129-2 in hematological malignancies," *Journal of Hematology and Oncology*, vol. 6, no. 1, article 16, 2013.

[119] M. T. di Martino, V. Campani, G. Misso et al., "In vivo activity of MiR-34a mimics delivered by stable nucleic acid lipid particles (SNALPs) against multiple myeloma," *PLoS ONE*, vol. 9, no. 2, Article ID e90005, 2014.

[120] M. T. di Martino, E. Leone, N. Amodio et al., "Synthetic miR-34a mimics as a novel therapeutic agent for multiple myeloma: in vitro and in vivo evidence," *Clinical Cancer Research*, vol. 18, no. 22, pp. 6260–6270, 2012.

[121] I. Misiewicz-Krzeminska, M. E. Sarasquete, D. Quwaider et al., "Restoration of microRNA-214 expression reduces growth of myeloma cells through positive regulation of P53 and inhibition of DNA replication," *Haematologica*, vol. 98, no. 4, pp. 640–648, 2013.

[122] D. J. Min, T. Ezponda, M. K. Kim et al., "MMSET stimulates myeloma cell growth through microRNA-mediated modulation of c-MYC," *Leukemia*, vol. 27, no. 3, pp. 686–694, 2013.

[123] M. Fabbri, R. Garzon, A. Cimmino et al., "MicroRNA-29 family reverts aberrant methylation in lung cancer by targeting DNA methyltransferases 3A and 3B," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 104, no. 40, pp. 15805–15810, 2007.

[124] N. Amodio, M. Leotta, D. Bellizzi et al., "DNA-demethylating and anti-tumor activity of synthetic miR-29b mimics in multiple myeloma," *Oncotarget*, vol. 3, no. 10, pp. 1246–1258, 2012.

[125] E. Leone, E. Morelli, M. T. di Martino et al., "Targeting miR-21 inhibits in vitro and in vivo multiple myeloma cell growth," *Clinical Cancer Research*, vol. 19, no. 8, pp. 2096–2106, 2013.

[126] Q. Xiong, Q. Zhong, J. Zhang et al., "Identification of novel miR-21 target proteins in multiple myeloma cells by quantitative proteomics," *Journal of Proteome Research*, vol. 11, no. 4, pp. 2078–2090, 2012.

[127] N. Amodio, D. Bellizzi, M. Leotta et al., "miR-29b induces SOCS-1 expression by promoter demethylation and negatively regulates migration of multiple myeloma and endothelial cells," *Cell Cycle*, vol. 12, no. 23, pp. 3650–3662, 2013.

[128] M. Kumar, Z. Lu, A. A. L. Takwi et al., "Negative regulation of the tumor suppressor p53 gene by microRNAs," *Oncogene*, vol. 30, no. 7, pp. 843–853, 2011.

[129] Y. K. Zhang, H. Wang, Y. Leng et al., "Overexpression of microRNA-29b induces apoptosis of multiple myeloma cells through down regulating Mcl-1," *Biochemical and Biophysical Research Communications*, vol. 414, no. 1, pp. 233–239, 2011.

[130] M. Rossi, M. R. Pitari, N. Amodio et al., "miR-29b negatively regulates human osteoclastic cell differentiation and function: implications for the treatment of multiple myeloma-related bone disease," *Journal of Cellular Physiology*, vol. 228, no. 7, pp. 1506–1515, 2013.

[131] A. M. Roccaro, A. Sacco, P. Maiso et al., "BM mesenchymal stromal cell-derived exosomes facilitate multiple myeloma progression," *Journal of Clinical Investigation*, vol. 123, no. 4, pp. 1542–1555, 2013.

[132] M. T. Di Martino, A. Gullà, M. E. G. Cantafio et al., "In vitro and in vivo anti-tumor activity of miR-221/222 inhibitors in multiple myeloma," *Oncotarget*, vol. 4, no. 2, pp. 242–255, 2013.

[133] K. Unno, Y. Zhou, T. Zimmerman, L. C. Platanias, and A. Wickrema, "Identification of a novel microRNA cluster miR-193b-365 in multiple myeloma," *Leukemia and Lymphoma*, vol. 50, no. 11, pp. 1865–1871, 2009.

[134] X. Gao, R. Zhang, X. Qu et al., "MiR-15a, miR-16-1 and miR-17-92 cluster expression are linked to poor prognosis in multiple myeloma," *Leukemia Research*, vol. 36, no. 12, pp. 1505–1509, 2012.

[135] S. L. Corthals, M. Jongen-Lavrencic, Y. de Knegt et al., "Micro-RNA-15a and micro-RNA-16 expression and chromosome 13 deletions in multiple myeloma," *Leukemia Research*, vol. 34, no. 5, pp. 677–681, 2010.

Hindawi Publishing Corporation
BioMed Research International
Volume 2014, Article ID 209670, 9 pages
http://dx.doi.org/10.1155/2014/209670

Research Article

Genome-Wide Screening of Cytogenetic Abnormalities in Multiple Myeloma Patients Using Array-CGH Technique: A Czech Multicenter Experience

Jan Smetana,[1,2] Jan Frohlich,[1,2] Romana Zaoralova,[2] Vladimira Vallova,[1] Henrieta Greslikova,[2] Renata Kupska,[2] Pavel Nemec,[1,2] Aneta Mikulasova,[1,2] Martina Almasi,[2,3] Ludek Pour,[4] Zdenek Adam,[4] Viera Sandecka,[4] Lenka Zahradová,[5] Roman Hajek,[2,3,5] and Petr Kuglik[1]

[1] *Department of Experimental Biology, Faculty of Science, Masaryk University Brno, Bohunice, 62500 Brno, Czech Republic*
[2] *Babak Myeloma Group, Department of Pathological Physiology, Faculty of Medicine, Masaryk University Brno, Bohunice, 62500 Brno, Czech Republic*
[3] *Department of Internal Hematooncology, University Hospital Brno, Bohunice, 62500 Brno, Czech Republic*
[4] *Department of Clinical Hematology, University Hospital Brno, Bohunice, 62500 Brno, Czech Republic*
[5] *Faculty of Medicine, University of Ostrava and University Hospital Ostrava, Poruba, 70852 Ostrava, Czech Republic*

Correspondence should be addressed to Petr Kuglik; kugl@sci.muni.cz

Received 20 February 2014; Accepted 2 May 2014; Published 2 June 2014

Academic Editor: Dong Soon Lee

Characteristic recurrent copy number aberrations (CNAs) play a key role in multiple myeloma (MM) pathogenesis and have important prognostic significance for MM patients. Array-based comparative genomic hybridization (aCGH) provides a powerful tool for genome-wide classification of CNAs and thus should be implemented into MM routine diagnostics. We demonstrate the possibility of effective utilization of oligonucleotide-based aCGH in 91 MM patients. Chromosomal aberrations associated with effect on the prognosis of MM were initially evaluated by I-FISH and were found in 93.4% (85/91). Incidence of hyperdiploidy was 49.5% (45/91); del(13)(q14) was detected in 57.1% (52/91); gain(1)(q21) occurred in 58.2% (53/91); del(17)(p13) was observed in 15.4% (14/91); and t(4;14)(p16;q32) was found in 18.6% (16/86). Genome-wide screening using Agilent 44K aCGH microarrays revealed copy number alterations in 100% (91/91). Most common deletions were found at 13q (58.9%), 1p (39.6%), and 8p (31.1%), whereas gain of whole 1q was the most often duplicated region (50.6%). Furthermore, frequent homozygous deletions of genes playing important role in myeloma biology such as TRAF3, BIRC1/BIRC2, RB1, or CDKN2C were observed. Taken together, we demonstrated the utilization of aCGH technique in clinical diagnostics as powerful tool for identification of unbalanced genomic abnormalities with prognostic significance for MM patients.

1. Introduction

Multiple myeloma (MM) is a tumor of postgerminal center isotype switched plasma cells (PCs), which are poorly proliferative but accumulate in the bone marrow leading to anemia, hypercalcemia, and lytic bone disease. Evaluation of genetic lesions associated with prognosis of MM patients is one of the most important diagnostic tools in the field [1]. Detection of chromosomal aberrations by means of standard karyotyping is limited (about 30% of cases) due to resolution and low proliferation activity of PCs [2]. This limitation can be overcome by newer techniques, such as fluorescent *in situ* hybridization (FISH) with detection rate of chromosomal aberrations (CHAs) reaching over 90% of

all cases [3]. However, this technique detects only a limited number of specific target sequences and thus provides a very limited view of the genome.

Karyotyping and FISH technique have shown that there are two major genetic subtypes in MM. Hyperdiploid MM (H-MM) is characterized by gains of odd-numbered chromosomes (e.g., chromosomes 3, 5, 7, 9, 11, 15, 19, and 21) and low incidence of *IgH* translocations and it is associated with better prognosis, whereas nonhyperdiploid MM (NH-M) is connected with worse prognosis due to frequent incidence of *IgH* translocations [4]. Several studies described prognostic significance of specific recurrent chromosomal aberrations, such as del(13)(q14)/loss of chromosome 13, del(17)(p13), gain(1)(q21), and *IgH* translocations for MM patients [5, 6]. However, current understanding of MM pathogenesis together with development of modern genome-wide screening techniques proves that common prognostic FISH panels are insufficient for description of genome heterogeneity of malignant PCs [7].

Complete analysis of the MM tumor genome by microarray techniques revealed novel recurrent copy number aberrations, such as deletions in 1p, 6q, 8p, 12q, and 16q, which are now considered additional prognostic factors to high-risk features. In addition, deletions of genes involved in regulation of the NF-κB pathway (*CYLD*, *TRAF3*, *BIRC2*, *and BIRC3*), cell cycle (*CDKN2C*, *CDKN2A*, *and CDKN2B*), or induction of apoptosis (*WWOX and FAF1*) were furthermore described by genome-wide approaches and add important information about genetic changes in MM pathogenesis [8, 9]. Recently, next-generation sequencing (NGS) techniques discovered mutations in several key genes associated with cancerogenesis, such as *K-Ras*, *N-Ras*, or sarcoma viral oncogene homolog B1 (*BRAF*) and postulated a new theory of clonal evolution of MM disease [10].

In our previous studies we showed that incidence of specific cytogenetic abnormalities, such as gain(1)(q21), del(17)(p13), and t(4;14)(p16;q32), detected by FISH in MM patients is connected with shorter overall survival for both newly diagnosed and relapsed MM patients [11, 12]. In this study, we analyzed the genomic profiles of 91 MM samples using oligonucleotide-based aCGH technique. We focused on detailed characterization of CNAs with known prognostic importance as well as CNAs connected with *IgH* translocations and incidence of homozygous deletions in our cohort of patients in terms of better understanding and subclassification of genetic heterogeneity of MM. In addition, we performed validation analysis of the FISH panel routinely used in MM diagnostic [hyperdiploidy, del(13)(q14), del(17)(p13), and gain(1)(q21)] with genome-wide approach to verify the possibility of replacing the standard I-FISH technique due to scalability of genomic profiling.

2. Material and Methods

2.1. Patients' Characteristics. The bone marrow aspirates from 91 (46 newly diagnosed and 45 relapsed) MM patients were obtained from MM patients between 2007 and 2010 from various centers in Slovakia and the Czech Republic. Patients' clinical features are summarized in Table 1. All patients were included into this study only after they signed the informed consent form approved by the ethical committee of the hospital. The enriched samples of PCs were obtained by either anti-CD138+ immunomagnetic beads (AutoMACS Pro, Miltenyi Biotec GmbH, Bergisch Gladbach, Germany) or by fluorescent-activated cells sorting (FACS Aria, BD Biosciences, San Jose, CA, USA). Detailed protocol of sorting algorithm used in our center was described elsewhere [13]. Briefly, cutoff level of 5% for CD138+ PCs infiltration in the bone marrow was established, and sorting technique (<5% FACS, >5% MACS, resp.) was used according to the manufacturer's instructions.

2.2. Microarray Analysis. Genomic DNA (gDNA) for aCGH experiments was extracted using commercially available kit (Puregene Core Kit A, Qiagen) according to manufacturer's protocol. Quality control of gDNA, digestion, labeling, and hybridization steps were performed as previously described [14]. Briefly, 1.0 μg of tumor and reference DNA were independently digested with Alu1 and Rsa1 (Promega, Madison, WI, USA) for 2 hours at 37C. Agilent Euro Female/Male was used as the normal reference in the hybridization experiments. Fluorescent labeling was made by BioPrime Total for Agilent Labeling Module (Invitrogen, Carlsbad, CA, USA) with specific fluorescent dyes Alexa3 for reference and Alexa5 for tumor DNA. Labeled reactions were cleaned up and hybridized at 65C for 24 hours. Human Genome 4×44k CGH Microarrays were scanned by Agilent Surescan C scanner with 5 μm resolution; features were extracted with Feature Extraction software and log 2 ratio data were imported and analyzed by Agilent Genomic Workbench 7.0.1.4 (Agilent Technologies, Santa Clara, CA, USA). Aberration calling was made by ADM-2 algorithm [15]. Positive aberration calls were defined by ≥3 consecutive probes and overreaching 0.2-fold change of $\log_2$ space. We used recommended default threshold 6 for ADM-2 algorithm with accuracy of aberration call confirmed on the basis of known FISH aberrations. The regions with detected CNAs were manually examined due to exclusion of copy number variant (CNV) regions and only copy number changes in exons and microRNA regions were included into further analyses. To identify and eliminate common CNVs from the study, we used default Database of Genomic Variants (http://www.openhelix.com/) for hg18. Physiological loci of the *IgH*, *IgL-k*, and *IgL-l* were also excluded from the analyses. Microarray data are available in the ArrayExpress database (http://www.ebi.ac.uk/arrayexpress/) under accession number E-MTAB-1792.

2.3. I-FISH Analysis. The cohort of 91 MM patients was examined for incidence of *IgH* translocations; furthermore, the occurrence of del(13)(q14), del(17)(p13), gain(1)(q21), and hyperdiploidy was compared with aCGH analysis in order to verify the results of whole-genome screening. The detection of PCs in the bone marrow samples was performed by

Table 1: Patients' baseline characteristics.

Sex	
Males	41
Females	50
Age median	
(at the time of therapy, years); range	69 (38–87)
Follow-up median	
(from therapy, months); range	37.8 (1.7–269.8)
Durie-Salmon stage (from therapy)	
I	9 (9.9%)
II	11 (12.1%)
III	71 (78.0%)
Stages A-B (from therapy)	
A	69 (75.8%)
B	22 (24.2%)
ISS stage (from therapy)	
1	22 (24.2%)
2	25 (27.5%)
3	44 (48.4%)
Ig isotype	
IgG	58 (63.7%)
IgA	15 (16.5%)
IgD	4 (4.4%)
IgM	1 (1.1%)
LC only	13 (14.3%)
Light chains	
Kappa	53 (58.2%)
Lambda	38 (41.8%)
Number of previous treatment lines	
None (first line treatment)	46 (50.6%)
Two	20 (22.0%)
More (>2)	25 (27.4%)
Biochemical parameter (median; min–max)	
Haemoglobin (g/L)	103.50 (66–144)
Thrombocytes (count ×109)	197.50 (27–416)
Calcium (mmol/L)	2.32 (1.47–3.64)
Albumin (g/L)	38.95 (21.1–54.1)
Creatinine (umol/L)	113.00 (54–1136)
β2-Microglobulin (mg/L)	5.18 (1.8–42.16)
Lactate dehydrogenase (ukat/L)	3.80 (1.52–22.92)
C-reactive protein (mg/L)	4.20 (0–174)
Plasma cell infiltration of bone marrow (%)	39.4 (0.80–94.60)

immunofluorescent labeling of cytoplasmic light chain (cIg-FISH), as previously reported [16], or we used CD138+ PCs obtained by cell sorting techniques. The following FISH panel of commercial DNA probes was used for analysis: LSI IGH/FGFR3 dual color probe, LSI 13q14 (RB1) spectrum green probe, LSI p53 (17p13.1) spectrum orange probe, and LSI D5S23/D5S721, CEP 9, and CEP 15 multicolor probe panel (Abbott Laboratories, Abbott Park, IL, USA). Hyperdiploidy was defined as gain of at least two of three evaluated chromosomes in a single cell. Gain(1)(q21) was assessed by homemade probe using fluorescent labeled bacterial artificial chromosome (BAC) (clone RP11-205M9); protocols for BAC isolation and labeling were followed from online resources of University of Bari, Italy (http://www.uniba.it/). Slide preparation and FISH analyses were performed according to manufacturer's protocols. We used cutoff values recommended by the European Myeloma Network [17], 20% cutoff for deletions and numerical aberrations and 10% cutoff for translocations and IgH rearrangements. At least, 100 cells were scored in each sample. Digital image analysis was assessed by fluorescent microscope Olympus BX-61 equipped with a CCD Camera Vosskuhler 1300D and Lucia KARYO/FISH/CGH imaging system (Laboratory Imaging s. r. o., Prague, Czech Republic).

3. Results

3.1. Summary of Chromosomal Aberrations in 91 MM Patients Detected by I-FISH Technique. The evaluation of unbalanced chromosomal abnormalities (hyperdiploidy, deletion of *RB1*, deletion of *TP53*, and gain/amplification 1q21) and *IgH* translocation t(4;14)(p16;q32) by FISH was performed in 91 MM patients. In our cohort, chromosomal abnormalities were detected in 93.4% (85/91) of cases. Hyperdiploidy was found in 49.5% (45/91); del(13)(q14) was detected in 57.1% (52/91); del(17)(p13) was observed in 15.4% (14/91) and gain(1)(q21) occurred in 58.2% (53/91) of samples. The t(4;14)(p16;q32) was found in 18.6% (16/86); 5 cases were discarded because of low amount of evaluated cells. Incidence of translocation t(4;14)(p16;q32) was associated with nonhyperdiploid ($P = 0.005$) cases as well as gain(1)(q21) and deletion of *RB1* ($P = 0.025$; $P = 0.052$, resp.) and corresponded with simultaneous incidence of del(13)(q14) and gain(1)(q21) ($P = 0.0047$).

3.2. Results from Whole-Genome Screening of a Cohort of 91 MM Patients Using Agilent 4×44k Microarrays. The DNA samples from 91 MM patients were analyzed by high-density oligonucleotide aCGH technique. Genome-wide screening using microarrays showed large genomic heterogeneity in MM cases and revealed copy number alterations in 100% (91/91) of samples. Graphical overview of incidence of genomic CNAs is shown in Figure 1. Overall, we found 1557 CNAs (778 gains and 779 areas of loss of genetic material); median was 16 CNAs per patient (range 1–52). The average size of aberration was 26.2 Mbp; 13% (204/1557) of all aberrations were smaller than 1 Mbp. Detailed description of CNAs found in our cohort is available in Supplementary Table 1 (see Supplementary Material available online at http://dx.doi.org/10.1155/2014/209670).

Our results confirm that there are two distinct whole-genome profiles reflecting major genetic subtypes in MM. Incidence of the extra copies of odd-numbered chromosomes is a common feature of hyperdiploid subgroup, which was found in 50.5% (46/91) of samples, whereas 49.5% (45/91)

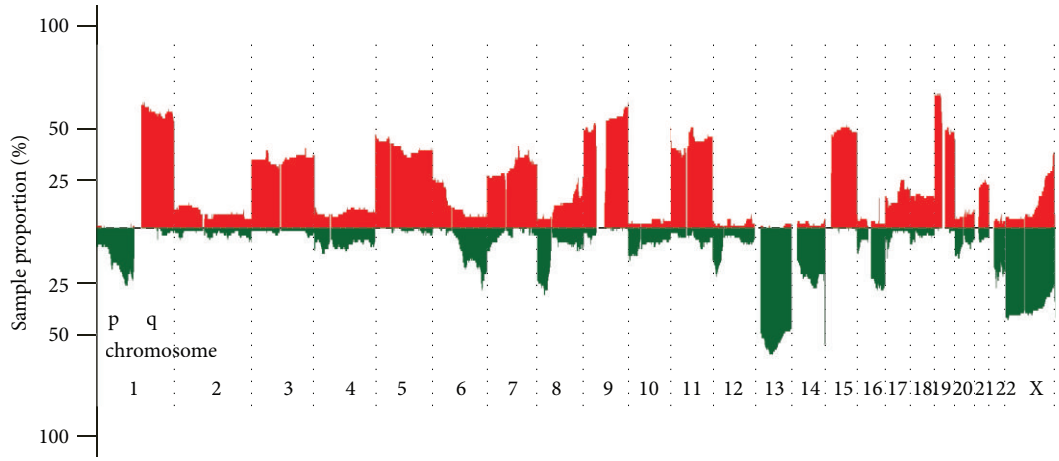


Figure 1: Graphical summary of copy number abnormalities in cohort of 91 multiple myeloma patients. Green color represents areas of loss; red corresponds with areas of gain of genetic material.

of cases were nonhyperdiploid. Most often duplicated chromosomes were chromosomes 9 and 15 (both 41.8%; 38/91), followed by chromosomes 9 (40.7%; 37/91), 19 (36.3%; 33/91), 5 (33.0%; 30/91), 11 (31.9%; 29/91), 3 (25.3%; 23/91), 7 (22.0%; 20/91), and 21 (18.7%; 17/91).

3.2.1. CNAs with Prognostic Significance in MM Diagnosis Detected by aCGH

Deletions in 1p. In our study, we found deletion of 1p in 46.2% of samples (42/91). The whole 1p arm was deleted in 16.7% (7/42) of cases. Furthermore, we identified three frequently deleted areas of 1p.

Most common deleted locus was located in 1p22.1, where we found 480 Kbp minimal deleted region (MDR), which occurred in 32.9% of cases (30/91) and included 5 genes (*HSP90B3P*, *TGFER3*, *BRDT*, *EPHAX4*, *and BTBD8*). The second frequently deleted region was found in 1p32.3 band, where we observed deletion in Fas +associated factor 1 (*FAF1*) and *CDKN2C* (p18) loci in 19.8% of cases (18/91). The third 350 Kbp MDR was detected in 1p12 in 9.9% (9/91), including loci with *MAN1A2*, *FAM46C*, and *GDAP2*.

Gain 1q. Regions of gain of genetic material in chromosome 1q were found in 71.4% (65/91) of cases; gain of whole 1q arm was detected in 50.6% (46/91) of patients. In 5.5% (5/91) of samples, we defined 10.9 Mbp minimal region of gain (MRG) in 1q21.2–1q23 including *CKS1B* and *ANP32E*, two genes associated with poor prognosis in MM.

Chromosome 17. The deletion in 17p13 locus was found in 14.3% (13/91). We found MDR of 133.5 Kbp covering 4 genes: *ATP1B2*, *TP53*, *WRAP5*, and *EFNB3*. Moreover, we found partial gain in 17q affecting area between 17q21.33 and 17qter in 12.1% (11/91), and in 5 cases (5.5%) we observed incidence of trisomy 17.

3.2.2. CNAs Associated with Chromosomes Involved in IgH Rearrangements

Chromosome 4. The loss of genetic material was frequently observed in 4p. The most common MDR was detected in 4p16.3 area in 6.6% (6/91), affecting loci of *FGFR3* and *WHSC1*. Furthermore, we found breakpoint in 4p16.3 locus in 3 cases, resulting probably from unbalanced translocation t(4;14)(p16;q32). In 7.7% of cases (7/91), we defined 7.1 Mbp MDR in 4p15.2, comprising twelve genes (*LGI2*, *SEPSECS*, *PI4K2B*, *ZCCHC4*, *ANAPC4*, *SLC34A2*, *KIAA0746*, *LOC389203*, *RBPJ*, *CCKAR*, *TBC1D19*, *and STIM2)*.

Chromosome 8. Most common aberration in chromosome 8 was loss of whole 8p, which was found in 23.1% (21/91) of cases. Aberrationsin 8p24.2 affecting *MYC* oncogene were found in 31.9% (29/91) of samples, including both gains and deletions (22%, 20/91; 9.9%, 9/91, resp.). In 7.7% (7/91), we observed breakpoint in the *MYC* locus, resulting most probably from unbalanced t(8;14) translocation.

Chromosome 11. We found an extra copy of chromosome 11 in 32.9% of cases (30/91), exclusively in the H-MM group ($P < 0.001$). We identified breakpoint in the *CCND1* locus in 4 cases suggesting incidence of t(11;14). Most often deleted region on chromosome 11 was 11q22 area. We found 4.3 Mbp MDR consisting of 22 genes in 9.9% (9/91) of cases, including loci of two genes with known function in apoptosis, and connected with the NF-κB pathway, *BIRC2* and *BIRC3*, and matrix metallopeptidase cluster.

Chromosome 14. The most common aberration was monosomy 14, which was observed in 17.6% (16/91) solely in nonhyperdiploid cases ($P < 0.001$). Common MDR was observed in 14q23 region between *AKAP5* and *ADAM21* genes in 8.8% (8/91) of cases. Another MDR was located in 14q32.22 and

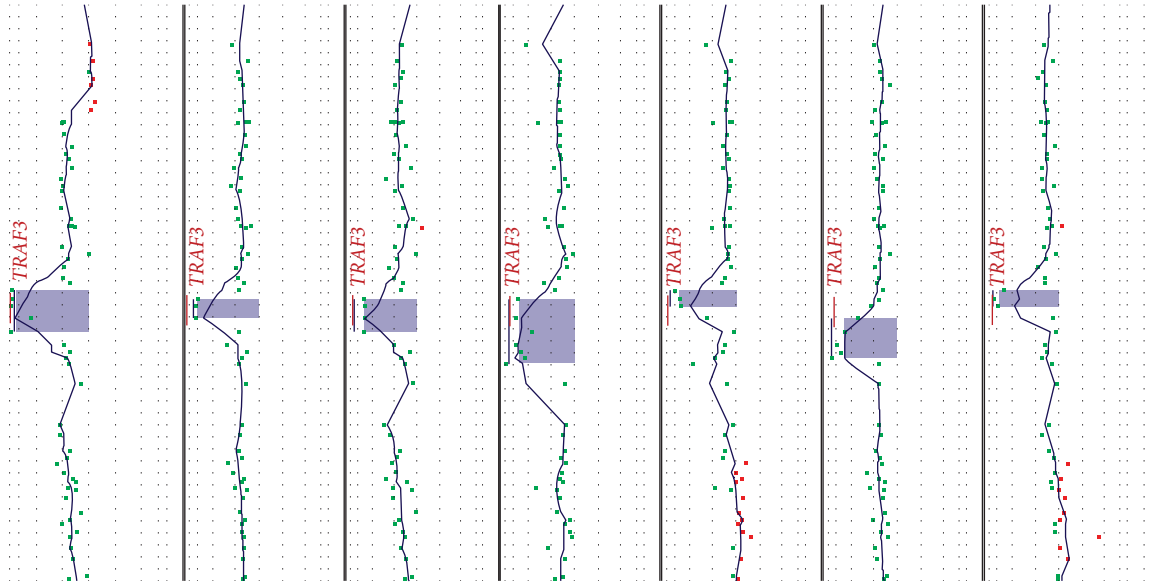


Figure 2: Schematic visualization of homozygous deletions in 14q32.33 region with highlighted *TRAF3* as main target of deletion in this area.

included 3 genes, *TRAF3*, *AMN*, and *CDC42BPB*. This region was homozygously deleted in incoherent manner (Figure 2) and occurred in 7.7% (7/91) of cases, with size of deletion varying from 48.7 Kbp to 261 Kbp.

Chromosome 16. The loss of 16q was the most frequent CNA in our cohort of patients (25.3%, 23/91). In one case with positive t(14;16)(q32;q23), we found interstitial deletion in fragile site FRA16D including *WWOX*. In the short arm of chromosome 16, we found 680 Kbp MDR in 16p13.3 area between *CLUAP1 and NAT15* in 7.7% (7/91).

3.2.3. Other Regions of Recurrent CNAs

Chromosome 6. Whereas gains were typical genetic abnormalities for 6p, interstitial deletions were commonly observed in 6q. The most frequently deleted area was 6q25. We defined MDR of size 2.1 Mbp, which was observed in 15.4% (14/91) and was comprised of *OPRM1*, *IPCEF1*, *CNKSR3*, *RBM16*, *TIAM2*, *TFB1M*, *CLDN20*, and *NOX3*. Another region with frequent deletion was 6q16.3, which was found in 11% (10/91) where we defined 3.4 Mbp MDR between *COQR* and *GRIK2*. Partial deletion of 6q (>75%) was also observed in 11.0% (10/91) of cases. In 6p, the most common CNA was gain in 6pter-6p22.3, observed in 8.7% (9/91).

Chromosome 12. The most common CNA in chromosome 12 was 69.2 Kbp deletion in 12p13. In 12.3% (13/91) of cases, we found small MDR in 12p13.1 locus including cyclin-dependent kinase inhibitor 1B (*CDKN1B*) and an endothelial cell early response protein gene *APOLD1*. In 5 cases, we observed deletion of whole 12p and 3 cases were missing the whole chromosome 12.

Chromosome 13. The loss of genetic material in chromosome 13 was the most common chromosomal aberration observed in our cohort of patients. The monosomy 13 was found in 50.6% (46/91) of cases. In 8.8% (8/91) of cases, we observed 11.2 Mbp MDR between *LRCH1* and *DIAHP3* spanning from 13q14.2 to 13q21.2 and containing genes with important roles in cancerogenesis, *RB1*, *DLEU7*, and miRNA genes *miR-15a/miR-16-1*.

Chromosome 20. The most common aberration observed in chromosome 20 was deletion of the short arm. We found loss affecting approximately 2/3 in 20p in 8.8% (8/91) of cases spanning from 20pter to 20p11.23. Gains or losses of whole chromosome 20 were observed in 7.7% (7/91) of samples (4 cases with loss and 3 cases with gain of extra copy of chromosome 20).

Chromosome 22. The most frequent CNA in chromosome 22 was loss of the whole chromosome, which was found in 15.4% (14/91) of cases. In addition, 6.6% (6/91) of cases had 33.6 Mbp deletion affecting approximately 1/3 of the 22q arm between centromere and 22q12.2 band.

3.2.4. Regions with Homozygous Deletions Detected by aCGH.

The homozygous deletions (HZDs) play an important role in cancer biology and are considered important genetic aberrations as they are able to fully inactivate genes contained within them. In our cohort of patients, HZDs were found in 30.8% (28/91) of cases. Median size was 193 Kbs (range 0.039–1.4 Mbp), and its incidence was noted more often

in nonhyperdiploid cases ($P = 0.02$). Incidence of most common HZDs is summarized in Table 2. The most frequently affected region was 14q32.32 with HZD varying from 48.7 Kbp to 261 Kbp observed in 7.7% (7/91) of cases spanning loci of genes (*RCOR1*, *TRAF3*, *AMN*, *and CDC42BPB*) in an incoherent manner. The second most common locus with HZD was 1p32.3 carrying *FAF1* and *CDKN2C*, which was deleted in 5.5% (5/91) of cases. The HZDs in chromosome 13 were also frequently observed. The HZDs were observed in 6.6% (6/91) of cases, but 13q14.2 locus was hit only in 3 cases (3.3%), varying from 52 Kbp to 206 Kbp comprising *RB1, LPAR6*, and *RCBTB2*. Notably, all single case HZDs affected tumor suppressor genes, such as *BRCA2* (13q12.3), *INTS6* (13q14.3), or *SPRY2* (13q31.1). Further loci with occurrence of HZDs were found in a single case in 1p32.3 (*PTPN14*; *ESRRG*), 3q26.3 (*PIK3CA*), 4q22 (*SMARCAD1*), 5q15 (*MCTP1*), 6q22.1 (*KPNA5*), 11q23.2 (*FAM55B*, *CADM1*), 12p31.2 (*CDKN1B*), 16q23 (*WWOX*, *MAF*), and Xq23 (*LHFPL1*, *AMOT*).

3.3. Concordance of Unbalanced Chromosomal Aberrations Detected by FISH and aCGH. In order to evaluate the possibility of replacing FISH analyses by aCGH in clinical diagnostics, the occurrence of characteristic recurrent unbalanced chromosomal aberrations was tested. The McNemar test was used for comparison of detection of hyperdiploidy, del(13)(q14), del (17p13), and gain(1)(q21) detected by I-FISH with the incidence of CNAs in those loci from our aCGH aberration list in 91 MM samples.

In our dataset, a total of 36.3% (36/91) of cases were discordant; however, we did not find statistically significant difference between results from both techniques for single aberrations. Detailed overview is shown in Table 3. While in detection of structural aberrations, concordance was over 90%, the most common discrepancy was observed in detection of the ploidy status (14.3%; 13/91). From H-MM cohort evaluated by FISH, 13.3% (6/45) of cases were classified as nonhyperdiploid by aCGH. Similarly, 15.2% (7/46) of NH-MM samples analyzed by FISH were classified as hyperdiploid by aCGH because of the incidence of extra copies of chromosomes undetectable by FISH multicolor panel. Discordant findings were also found in detection of structural aberrations by both techniques. Of the 91 patients with both aCGH and FISH results, aCGH detected 11.0% (10/91) of CNAs that were not detected by FISH [6 cases of del(13)(q14), 2 cases of del(17)(p13), and 2 cases of gain(1)(q21)]. On the contrary, I-FISH detected 11% (10/91) of abnormalities not identified by aCGH [2 cases of del(13)(q14), 3 cases of del(17)(p13), and 5 cases of gain(1)(q21), resp.].

4. Discussion

Detection of chromosomal abnormalities is one of the most important independent prognostic markers in MM pathogenesis and prognosis for patients. Similarly to many other types of hematologic malignancies, MM is characterized by numerous structural and numerical genetic lesions involving many oncogenes, tumor suppressor genes or genes involved in signaling pathways important for cell cycle, apoptosis, and so forth. [2]. While karyotyping techniques are able to detect chromosomal abnormalities roughly in 30% of cells because of the low proliferative activity, the introduction of new cytogenetic techniques, such as I-FISH or aCGH, allows us to detect genetic lesions in more than 90% of malignant PCs [18]. Expanded panel of FISH markers includes del(13)(q14)/monosomy 13, t(11;14)(q13;q32), t(14;16)(q32;p23), and hyperdiploidy. Even though I-FISH is nowadays considered as a gold standard for cytogenetic investigations in MM, it may be insufficient for description of given genetic heterogeneity. Moreover, several studies proved virtually 100% occurrence of CNAs in MM when techniques of whole-genome screening were used in MM diagnosis [19]. Based on the above-mentioned studies, we utilized the global assessment of genomic abnormalities via a high-resolution Agilent 4x44k aCGH platform and in combination with FISH in 91 MM patients. To the best of our knowledge, this is the first study of such scale in central Europe in MM patients.

4.1. FISH Assessment of Cytogenetic Aberrations Wit Prognostic Significance. Rearrangements of the *IgH* locus play important role in MM pathogenesis. Several studies showed negative prognostic impact of t(4;14)(p16;q32) in newly diagnosed or relapsed patients. In our cohort, we found t(4;14)(p16;q32) using FISH in 17.4% (15/86) of cases in agreement with previous reports [20]. The del(17)(p13) including tumor suppressor gene *TP53* is considered an important negative prognostic factor in MM pathogenesis. In our cohort, we found del(17)(p13) in 15.4% (14/91) of cases in concordance with previous observations [21]. Gain(1)(q21) and subsequently overexpression of *CKS1B* are nowadays considered as an independent prognostic factor in MM diagnosis. In our previous studies, we showed that incidence of this genetic lesion is associated with poor prognosis when detected by I-FISH in both newly and relapsed MM patients. In this cohort, we found gain(1)(q21) in 53% of cases, which is in agreement with previously published results [22].

4.2. Whole-Genome Screening by Oligo-Based Microarrays and aCGH Technique. The whole-genome screening using aCGH identified CNAs >100 Kbp in 100% of cases. Most common CNAs were found in 1p, 1q, 6p, 8p, 13q, 14q, 16q, and 22q along with gain of extra copies of odd-numbered chromosomes. Hyperdiploidy was found in nearly half of the cases (47.3%; 43/91). Within the hyperdiploid cohort of patients, we observed incidence of trisomy 11 as well as gain(1q) and del(13q) associated with worse prognosis; however, the association did not meet statistical significance ($P = 0.112$), as previously described [23].

In addition to current high-risk panel genetic abnormalities, several other CNAs associated with adverse prognosis were recently identified by genome-wide techniques. In chromosome 1, deletion in 1p32 affecting loci of *CDKN2C* and *FAF1* is connected with shorter OS. In our cohort, we defined MDR in 1p32.3 locus with incidence of this focal deletion in 19.8% (18/91), which is in good agreement with previous observations [24]. Another frequent deletion in 1p was found in 1p12, including loci of *MAN1A2*, *GDAP2*, and

Table 2: Incidence of most common homozygous deletion in a cohort of 91 multiple myeloma patients detected by array-CGH technique.

Chromosome location	Size (Mb)	Prevalence (%)	Genes
14q32.32	0.063–0.261	7.7	*RCOR1, TRAF3, AMN, CDC42BPB*
1p32.3	0.068–0.387	5.5	*FAF1, CDKN2C*
11q22.1–11q22.3	3.6–4.7	2.8	*BIRC3, BIRC2, MMP cluster*
13q14.2	0.053–0.206	2.8	*RB1, P2RY5, RCBTB2*
16q12.1-16q12.2	1.40–1.42	1.9	*CYLD, SALL1*

Table 3: Comparison of array-CGH and FISH results in evaluation of cytogenetic aberrations with known effect on prognosis in multiple myeloma patients.

		I-FISH		*P* value	Concordance
		del(13)(q14)			
		Positive	Negative		
aCGH	Positive	50	6	$P = 0.289$	**91.2**%
	Negative	2	33		
		del(17)(p13)			
		Positive	Negative		
	Positive	11	2	$P = 1.000$	**94.5**%
	Negative	3	75		
		gain(1)(q21)			
		Positive	Negative		
	Positive	48	2	$P = 0.450$	**92.3**%
	Negative	5	36		
		Hyperdiploidy			
		Positive	Negative		
	Positive	39	7	$P = 1.000$	**85.4**%
	Negative	6	37		

FAM46C. Recently, incidence of mutations and deletion of *FAM46C* were described and associated with impaired OS in MM patients [25]. In 1q, we observed common region of gain of genetic sequences in 1q21.2 region with two genes associated with negative impact on prognosis, *CKS1B*, and *ANP32E* [26]. In agreement with previous studies, our results also showed that in most cases, the whole 1q arm is affected [27]. Genetic lesions involving deletions of *TP53* in 17p13.1 were observed in 13.2% (12/91). Even though there is agreement about loss/mutation of *TP53* having negative impact on MM prognosis, MDR in 17p13.1 area in our dataset also included spermine N1-acetyltransferase *SAT2*, which has been reported to be significantly underexpressed in del(17p); it interacts with p65 subunit of the NF-κB pathway and thus is another possible candidate gene in this area [28].

4.3. Incidence and Impact of Homozygous Deletions. Homozygous deletions play important role in cancer biology and are considered to be important genetic events. By the definition, this event is able to fully inactivate genes contained within them. The most frequent region associated with incidence of HZD in our dataset was 14q32 (7.7%; 7/91). The incidence of del(14q) is commonly observed in hematological malignancies. In MM, several studies recently showed that TNF receptor-associated factor 3 (*TRAF3*) is an important target of deletion in this locus. *TRAF3* is associated with negative induction of noncanonical NF-κB pathway, enhances *BIRC2*/*BIRC3* mediated proteasome degradation of NF-κB inducing kinase (NIK), and thus increases autonomy of tumor PCs from the bone marrow microenvironment [29]. Similarly to others, *TRAF3* was the target of HZDs in our MDR in 14q32.33; its incidence was comparable with other MM studies as well as other B-lymphomas [30, 31]. Another region affected with HZD was 1q32.3 carrying loci of *FAF1* and *CDKN2C*, which occurred in 5.5% (5/91) of cases. Incidence of HZD in this region is associated with adverse prognosis in MM patients and is also common in mantle cell lymphoma patients [32]. Finally, HZD regions observed in <5% of cases have relevance in MM biology due to involvement in important signaling pathways, such as NF-κB (*CYLD*, *BIRC2*, *and BIRC3*), regulation of cell cycle (*CDKN1B*, *RB1*), or connection with apoptosis (*WWOX*, *FAF1*) [33, 34].

4.4. Comparison of FISH Evaluation and aCGH Results in Cohort of 91 MM Patients. Molecular cytogenetic analysis using I-FISH technique is still considered to be a golden standard for cytogenetic evaluation in MM diagnosis. However, genomic profiling using aCGH provides information beyond the commonly detected unbalanced genetic lesions that are observed by FISH. In our cohort, chromosomal aberrations were detected in 93.4% (85/91) of cases using I-FISH, while aCGH screening was able to detect CNAs in 100% (91/91) of cases. The concordance for loss of *RB1 (13q14)*, *TP53 (17p13)*, gain(1)(q21), and hyperdiploidy was 91.2%, 94.5%, 92.3%, and 85.4%, respectively; median of concordance for all aberrations was 91.8%. To our knowledge, no similar study was done in MM, but there are data from different hematological malignancies. Comparative studies in chronic myeloid leukemia (CLL) between FISH and aCGH showed high degree of concordance with our results, with the concordance between FISH and aCGH reaching up to 93% and 95.5%, respectively [35, 36]. Proportion of structural abnormalities missed by FISH and aCGH was 18.7% (17/91) and 17.6% (16/91), respectively, which is a little higher than in previous studies; however, we used a larger cohort of patients and highly purified CD138+ sorted cells as starting material instead of bone marrow samples, which could affect specificity of our analysis over previously published data showing discrepancy from 9 to 12% [37, 38]. In addition, several other studies in MM and CLL also showed that aCGH

is less effective when incidence of CNAs is presented to be <30% of the cells [30, 35, 39]. In our dataset, 37.5% (6/16) of cases with missed CNAs by aCGH fell within this condition (2x loss of *RB1*, 2x gain *CKS1B*, and 3x loss of *TP53*). These cases are hard to evaluate by default setting of the analytic software; however, novel computing algorithms developed for detection of mosaic samples are able to overcome this issue [40]. Primary reason for discrepancy in detection of hyperdiploidy was caused by higher false positivity of FISH evaluations, when FISH signals were scored as trisomies when only a part of the chromosome arm was duplicated but not the whole chromosome.

5. Conclusions

The results of our study showed that our complex approach comprising cell sorting, I-FISH evaluation of balanced chromosomal changes (*IgH* rearrangement and translocations associated with adverse prognosis for MM patients), and genome-wide profiling gives us a robust diagnostic tool suitable for precise evaluation of the high-risk genetic lesions. The utilization of whole-genome CGH microarrays is able to substitute routine FISH evaluations in detection of unbalanced genetic lesions with prognostic impact in MM and bring additional information about changes in genome of malignant plasma cells even though the detection of clonal aberrations in MM samples could be challenging. Altogether, combination of aCGH and I-FISH technique gives us new opportunities for description of genetic heterogeneity in MM and thus identification of novel cytogenetic features capable of discerning prognosis in MM. However, further studies focusing on genetic background of MM are needed for better understanding and characterization of role of genetic changes in MM pathology.

Conflict of Interests

All authors have no conflict of interests (including any financial relationship with companies/products) regarding the publication of this paper.

Acknowledgments

The authors would like to thank all participating members of the Czech Myeloma Group, especially all patients and their caregivers for making this work possible. The research leading to these results has received funding from the European Union Seventh Framework Programme (FP7/2007–2013) under Grant Agreement no. 278570 and OP VK CZ.1.07/2.3.00/20.0183 Project. This study was supported by research Grant MSM0021622434 from the Ministry of Education, Youth and Sports, Czech Republic, and by Grant nos. NT11154, NT13190, NT12130, and NT13492 from the Internal Grant Agency of the Ministry of Health of the Czech Republic. This research was supported by Ministry of Health, Czech Republic, for conceptual development of research organization (FNBr 65269705). The authors would also like to thank John B. Smith for proofreading the paper.

References

[1] M. Dimopoulos, R. Kyle, J.-P. Fermand et al., "Consensus recommendations for standard investigative workup: report of the International Myeloma Workshop Consensus Panel 3," *Blood*, vol. 117, no. 18, pp. 4701–4705, 2011.

[2] R. Fonseca, B. Barlogie, R. Bataille et al., "Genetics and cytogenetics of multiple myeloma: a workshop report," *Cancer Research*, vol. 64, no. 4, pp. 1546–1558, 2004.

[3] R. Fonseca, D. Harrington, M. M. Oken et al., "Biological and prognostic significance of interphase fluorescence in situ hybridization detection of chromosome 13 abnormalities (Δ13) in multiple myeloma: an Eastern Cooperative Oncology Group study," *Cancer Research*, vol. 62, no. 3, pp. 715–720, 2002.

[4] N. V. Smadja, C. Bastard, C. Brigaudeau, D. Leroux, and C. Fruchart, "Hypodiploidy is a major prognostic factor in multiple myeloma," *Blood*, vol. 98, no. 7, pp. 2229–2238, 2001.

[5] J. Shaughnessy, "Amplification and overexpression of CKS1B at chromosome band 1q21 is associated with reduced levels of p27 Kip1 and an aggressive clinical course in multiple myeloma," *Hematology*, vol. 10, no. 1, pp. 117–126, 2005.

[6] R. Fonseca, S. A. Van Wier, W. J. Chng et al., "Prognostic value of chromosome 1q21 gain by fluorescent *in situ* hybridization and increase CKS1B expression in myeloma," *Leukemia*, vol. 20, no. 11, pp. 2034–2040, 2006.

[7] N. C. Gutiérrez, J. L. García, J. M. Hernández et al., "Prognostic and biologic significance of chromosomal imbalances assessed by comparative genomic hybridization in multiple myeloma," *Blood*, vol. 104, no. 9, pp. 2661–2666, 2004.

[8] B. A. Walker and G. J. Morgan, "Use of single nucleotide polymorphism—based mapping arrays to detect copy number changes and loss of heterozygosity in multiple myeloma," *Clinical Lymphoma and Myeloma*, vol. 7, no. 3, pp. 186–191, 2006.

[9] C. Largo, S. Alvarez, B. Saez et al., "Identification of overexpressed genes in frequently gained/amplified chromosome regions in multiple myeloma," *Haematologica*, vol. 91, no. 2, pp. 184–191, 2006.

[10] M. A. Chapman, M. S. Lawrence, J. J. Keats et al., "Initial genome sequencing and analysis of multiple myeloma," *Nature*, vol. 471, no. 7339, pp. 467–472, 2011.

[11] P. Nemec, Z. Zemanova, P. Kuglik et al., "Complex karyotype and translocation t(4;14) define patients with high-risk newly diagnosed multiple myeloma: results of CMG2002 trial," *Leukemia and Lymphoma*, vol. 53, no. 5, pp. 920–927, 2012.

[12] H. Greslikova, R. Zaoralova, H. Filkova et al., "Negative prognostic significance of two or more cytogenetic abnormalities in multiple myeloma patients treated with autologous stem cell transplantation," *Neoplasma*, vol. 57, no. 2, pp. 111–117, 2010.

[13] J. Cumova, L. Kovarova, A. Potacova et al., "Optimization of immunomagnetic selection of myeloma cells from bone marrow using magnetic activated cell sorting," *International Journal of Hematology*, vol. 92, no. 2, pp. 314–319, 2010.

[14] J. Smetana, J. Fröhlich, V. Vranová, A. Mikulášová, P. Kuglík, and R. Hájek, "Oligonucleotide-based array CGH as a diagnostic tool in multiple myeloma patients," *Klinicka Onkologie*, vol. 24, pp. S43–S48, 2011.

[15] D. Lipson, Y. Aumann, A. Ben-Dor, N. Linial, and Z. Yakhini, "Efficient calculation of interval scores for DNA copy number data analysis," *Journal of Computational Biology*, vol. 13, no. 2, pp. 215–228, 2006.

[16] G. J. Ahmann, S. M. Jalal, A. L. Juneau et al., "A novel three-color, clone-specific fluorescence in situ hybridization procedure for monoclonal gammopathies," *Cancer Genetics and Cytogenetics*, vol. 101, no. 1, pp. 7–11, 1998.

[17] F. M. Ross, H. Avet-Loiseau, G. Ameye et al., "Report from the European myeloma network on interphase FISH in multiple myeloma and related disorders," *Haematologica*, vol. 97, no. 8, pp. 1272–1277, 2012.

[18] S. Kumar, R. Fonseca, R. P. Ketterling et al., "Trisomies in multiple myeloma: impact on survival in patients with high-risk cytogenetics," *Blood*, vol. 119, no. 9, pp. 2100–2105, 2012.

[19] R. Fonseca, P. L. Bergsagel, J. Drach et al., "International Myeloma Working Group molecular classification of multiple myeloma: spotlight review," *Leukemia*, vol. 23, no. 12, pp. 2210–2221, 2009.

[20] N. C. Gutiérrez, M. V. Castellanos, M. L. Martín et al., "Prognostic and biological implications of genetic abnormalities in multiple myeloma undergoing autologous stem cell transplantation: t(4;14) is the most relevant adverse prognostic factor, whereas RB deletion as a unique abnormality is not associated with adverse prognosis," *Leukemia*, vol. 21, no. 1, pp. 143–150, 2007.

[21] L. Lodé, M. Eveillard, V. Trichet et al., "Mutations in TP53 are exclusively associated with del(17p) in multiple myeloma," *Haematologica*, vol. 95, no. 11, pp. 1973–1976, 2010.

[22] J. Smetana, K. Berankova, R. Zaoralova et al., "Gain(1)(q21) is an unfavorable genetic prognostic factor for patients with relapsed multiple myeloma treated with thalidomide but not for those treated with bortezomib," *Clinical Lymphoma, Myeloma and Leukemia*, vol. 13, no. 2, pp. 123–130, 2013.

[23] D. R. Carrasco, G. Tonon, Y. Huang et al., "High-resolution genomic profiles define distinct clinico-pathogenetic subgroups of multiple myeloma patients," *Cancer Cell*, vol. 9, no. 4, pp. 313–325, 2006.

[24] H. Avet-Loiseau, C. Li, F. Magrangeas et al., "Prognostic significance of copy-number alterations in multiple myeloma," *Journal of Clinical Oncology*, vol. 27, no. 27, pp. 4585–4590, 2009.

[25] K. D. Boyd, F. M. Ross, B. A. Walker et al., "Mapping of chromosome 1p deletions in myeloma identifies FAM46C at 1p12 and CDKN2C at 1p32.3 as being genes in regions associated with adverse survival," *Clinical Cancer Research*, vol. 17, no. 24, pp. 7776–7784, 2011.

[26] B. A. Walker, P. E. Leone, L. Chiecchio et al., "A compendium of myeloma-associated chromosomal copy number abnormalities and their prognostic value," *Blood*, vol. 116, no. 15, pp. e56–e65, 2010.

[27] H. Chang, X. Qi, A. Jiang, W. Xu, T. Young, and D. Reece, "1p21 deletions are strongly associated with 1q21 gains and are an independent adverse prognostic factor for the outcome of high-dose chemotherapy in patients with multiple myeloma," *Bone Marrow Transplantation*, vol. 45, no. 1, pp. 117–121, 2010.

[28] K. D. Boyd, F. M. Ross, W. J. Tapper et al., "The clinical impact and molecular biology of del(17p) in multiple myeloma treated with conventional or thalidomide-based therapy," *Genes Chromosomes and Cancer*, vol. 50, no. 10, pp. 765–774, 2011.

[29] Y. N. Demchenko, O. K. Glebov, A. Zingone, J. J. Keats, P. Leif Bergsagel, and W. Michael Kuehl, "Classical and/or alternative NF-κB pathway activation in multiple myeloma," *Blood*, vol. 115, no. 17, pp. 3541–3552, 2010.

[30] J. J. Keats, R. Fonseca, M. Chesi et al., "Promiscuous mutations activate the noncanonical NF-κB pathway in multiple myeloma," *Cancer Cell*, vol. 12, no. 2, pp. 131–144, 2007.

[31] E. Braggio, J. J. Keats, X. Leleu et al., "Identification of copy number abnormalities and inactivating mutations in two negative regulators of nuclear factor-κB signaling pathways in Waldenström's macroglobulinemia," *Cancer Research*, vol. 69, no. 8, pp. 3579–3588, 2009.

[32] N. J. Dickens, B. A. Walker, P. E. Leone et al., "Homozygous deletion mapping in myeloma samples identifies genes and an expression signature relevant to pathogenesis and outcome," *Clinical Cancer Research*, vol. 16, no. 6, pp. 1856–1864, 2010.

[33] S. Gardam, V. M. Turner, H. Anderton et al., "Deletion of cIAP1 and cIAP2 in murine B lymphocytes constitutively activates cell survival pathways and inactivates the germinal center response," *Blood*, vol. 117, no. 15, pp. 4041–4051, 2011.

[34] R. Di Fiore, A. D'Anneo, G. Tesoriere, and R. Vento, "RB1 in cancer: different mechanisms of RB1 inactivation and alterations of pRb pathway in tumorigenesis," *Journal of Cellular Physiology*, vol. 228, no. 8, pp. 1676–1687, 2013.

[35] S. R. Gunn, M. S. Mohammed, M. E. Gorre et al., "Whole-genome scanning by array comparative genomic hybridization as a clinical tool for risk assessment in chronic lymphocytic leukemia," *Journal of Molecular Diagnostics*, vol. 10, no. 5, pp. 442–451, 2008.

[36] A. Tyybäkinoja, J. Vilpo, and S. Knuutila, "High-resolution oligonucleotide array-CGH pinpoints genes involved in cryptic losses in chronic lymphocytic leukemia," *Cytogenetic and Genome Research*, vol. 118, no. 1, pp. 8–12, 2007.

[37] A. Patel, S.-H. Kang, P. A. Lennon et al., "Validation of a targeted DNA microarray for the clinical evaluation of recurrent abnormalities in chronic lymphocytic leukemia," *American Journal of Hematology*, vol. 83, no. 7, pp. 540–546, 2008.

[38] D. P. O'Malley, C. Giudice, A. S. Chang et al., "Comparison of array comparative genomic hybridization (aCGH) to FISH and cytogenetics in prognostic evaluation of chronic lymphocytic leukemia," *International Journal of Laboratory Hematology*, vol. 33, no. 3, pp. 238–244, 2011.

[39] C. Largo, B. Saéz, S. Alvarez et al., "Multiple myeloma primary cells show a highly rearranged unbalanced genome with amplifications and homozygous deletions irrespective of the presence of immunoglobulin-related chromosome translocations," *Haematologica*, vol. 92, no. 6, pp. 795–802, 2007.

[40] R. Valli, E. Maserati, C. Marletta, B. Pressato, F. Lo Curto, and F. Pasquali, "Evaluating chromosomal mosaicism by array comparative genomic hybridization in hematological malignancies: the proposal of a formula," *Cancer Genetics*, vol. 204, no. 4, pp. 216–218, 2011.

Hindawi Publishing Corporation
BioMed Research International
Volume 2014, Article ID 647238, 7 pages
http://dx.doi.org/10.1155/2014/647238

Research Article

Clinical Comparisons of Two Free Light Chain Assays to Immunofixation Electrophoresis for Detecting Monoclonal Gammopathy

Han-Sung Kim,[1] Hyun Soo Kim,[1] Kyu-Sung Shin,[1] Wonkeun Song,[1] Hyo Jung Kim,[2] Hyeong Su Kim,[2] and Min-Jeong Park[1]

[1] *Department of Laboratory Medicine, Hallym University College of Medicine, Seoul 150-950, Republic of Korea*
[2] *Department of Internal Medicine, Hallym University College of Medicine, Seoul 150-950, Republic of Korea*

Correspondence should be addressed to Min-Jeong Park; mjpark@hallym.or.kr

Received 27 February 2014; Revised 21 April 2014; Accepted 13 May 2014; Published 27 May 2014

Academic Editor: Dong Soon Lee

Free light chains (FLCs) are useful biomarkers for the diagnosis and monitoring of various plasma cell dyscrasias. One hundred fifty-seven samples from 120 patients for screening or monitoring of monoclonal gammopathy (MG) were included. The new N Latex FLC assays (Siemens Healthcare Diagnostics GmbH, Germany) were compared with the Freelite FLC assays (The Binding Site Ltd., UK) and the results were analyzed with those of immunofixation electrophoresis (IFE). The Freelite FLC assay showed significantly wider assay ranges than the N Latex FLC assay. The correlation coefficients of the two FLC kappa (κ) assays, lambda (λ) assays, and the κ/λ ratio were 0.9792, 0.8264, and 0.9064, respectively. The concordance rate was 84.7% for the FLC κ assays, 79.6% for FLC λ, and 89.2% for the κ/λ ratio. The clinical sensitivity and specificity of the κ/λ ratios were 72.2% and 93.6% for the Freelite assay and 64.6% and 100% for the N Latex FLC assay. Two FLC assays showed good correlations and concordance. However, the clinical sensitivity of the κ/λ ratio was higher in the Freelite FLC assays; clinical specificity was higher in the N Latex FLC assay. Both FLC assays seem to have limited clinical utility in detecting MG in certain clinical settings.

1. Introduction

Monoclonal gammopathy (MG) pertains to a disease cluster involving plasma cells: it is generally detected in the serum and urine and is characterized by a clonal synthesis of monoclonal proteins. To diagnose MG, test methods for detecting monoclonal proteins, such as electrophoresis, have been employed for decades. It has been recently shown that MG induces excessive production of immunoglobulin free light chains, which remain in the blood without being bound to heavy chains [1, 2]. A test method for measuring kappa (κ) and lambda (λ) free light chains (FLCs) and calculations for the kappa-to-lambda ratio have recently been introduced [3]. The test method, known as the Freelite assay (The Binding Site Group Ltd., Birmingham, UK), has been extensively used worldwide. FLCs are one of the response criteria used in the diagnosis and treatment of the myeloma and related disorders, as described by the International Myeloma Working Group guidelines [4]. However, despite the usefulness of this method, several analytical problems have continuously been faced, including lot-to-lot variability of reagents, antigen excess, unrecognizable epitopes, and excessive polymerization [5–8]. The Freelite assay has the limitations of poor postdilution linearity and relative imprecision, as well as increased likelihood of showing false negative results due to antigen excess in patients with extremely high FLC concentration [3, 7]. To overcome these problems, a new N Latex assay (Siemens Healthcare Diagnostics GmbH, Marburg, Germany) using monoclonal antibodies has been developed and recently made available [9].

This study aimed to analyze the performance of the newly developed N Latex FLC assay compared with the Freelite FLC assay in patients with various diseases and evaluate the clinical usefulness of the two FLC assays compared to

the standard diagnostic test of immunofixation electrophoresis (IFE).

2. Materials and Methods

2.1. Patient Samples. During a 4-month period in 2012, from April to July, 157 pairs of samples of serum and urine were collected from 120 patients who had registered for laboratory screening or monitoring of MG. The study population consisted of 63 patients with MG (MG group) and 57 patients without MG (non-MG group). The clinical diagnoses of the patients in each group were determined by their physicians. The specimens in the MG group included those from newly diagnosed patients and patients undergoing treatment. The patients in the MG group had one or more monoclonal proteins in their serum or urine specimens detected on the IFE. The results of FLC assays were evaluated in relation to serum and urine IFE. This study was approved by the Institutional Review Board of Kangnam Sacred Heart Hospital in Republic of Korea.

2.2. FLC Assays. Two FLC assays for FLC κ and λ in serum were used: N Latex FLC assays (Siemens Healthcare Diagnostics GmbH, Marburg, Germany) using monoclonal Ab-based method and Freelite assays (The Binding Site Ltd., Birmingham, UK) using polyclonal Ab-based method. Both assays were performed on Behring Nephelometer II (Siemens Healthcare Diagnostics GmbH, Marburg, Germany). The N Latex assay was subjected to a prereaction step for 2 minutes prior to the assay, which allowed using a step with a higher dilution factor in the presence of a large amount of antigens, thereby reducing the false negatives caused by the antigen excess. In this study, both assays were performed by the next higher dilution factor, in addition to the automatically determined dilution factor, to preclude the possibility of false negatives caused by antigen excess.

The reference ranges of both assays were provided by the manufacturers and were as follows: N Latex FLC κ, 6.7–22.4 mg/L; Freelite κ, 3.3–19.4 mg/L; N Latex FLC λ, 8.3–27 mg/L; Freelite λ, 5.7–26.3 mg/L; N Latex FLC κ/λ ratio, 0.31–1.56; Freelite κ/λ ratio, 0.23–1.65.

2.3. Immunofixation Electrophoresis. The detection limit of IFE in serum is around 15 mg/dL. A total of 157 pairs of serum and urine samples were analyzed on IFE using an agarose gel (Helena Laboratories, Beaumont, USA). Any M-band detected in serum and/or urine IFE was considered positive for MG. The gels were evaluated by two blinded independent readers.

2.4. Comparison of Methods. We compared the principles behind the N Latex FLC assays and the Freelite assays. Correlation analysis was performed using the results for the κ, λ, and κ/λ ratios of the N Latex FLC and the Freelite assays. A qualitative comparison was performed by determining the concordance rate wherein both assays generated the same results (abnormal low, normal, and abnormal high). In addition, we investigated the cases showing discordant results between the two FLC assays.

Table 1: Study group of 120 patients.

Group	Diagnosis	Number of patients
Monoclonal gammopathy (MG)	Multiple myeloma	35
	Light chain myeloma	10
	MGUS	9
	Non-Hodgkin's lymphoma	4
	Amyloidosis	2
	Plasmacytoma	2
	Waldenström's macroglobulinemia	1
	Total	**63**
No monoclonal gammopathy (non-MG)	Chronic kidney disease	10
	Bone fracture	8
	Pneumonia	7
	Cancer	4
	Neuropathy	3
	Cerebral hemorrhage	3
	Anemia of chronic disease	2
	Iron deficiency anemia	2
	Autoimmune diseases	2
	Parkinson's diseases	2
	Miscellaneous	13
	Total	**57**

MGUS: monoclonal gammopathy of unknown significance.

2.5. Clinical Sensitivity and Specificity. The clinical sensitivity (true positive), specificity (true negative), and percent agreement of the κ/λ ratios of the N Latex FLC and Freelite FLC assays were calculated based on the clinical diagnosis and the results of the IFE analysis.

2.6. Statistical Analyses. Statistical analyses were performed using the STATA software (STATA SE v12.0, Stata Corp LP, Lakeway, USA). Spearman's rank correlation coefficients and Cohen's kappa for concordance analysis were performed. Normalized median differences pertain to the median value of all differences between the sample outcomes of the two methods as calculated in terms of percentage by $[(y-x)/(y+x)]/2$ [9].

3. Results

3.1. Clinical Characteristics of Study Populations. The study populations of 120 patients were shown in Table 1. The male to female ratio was 1.3 : 1 in the MG group and 1 : 1 in the non-MG group. No significant differences in age distribution were

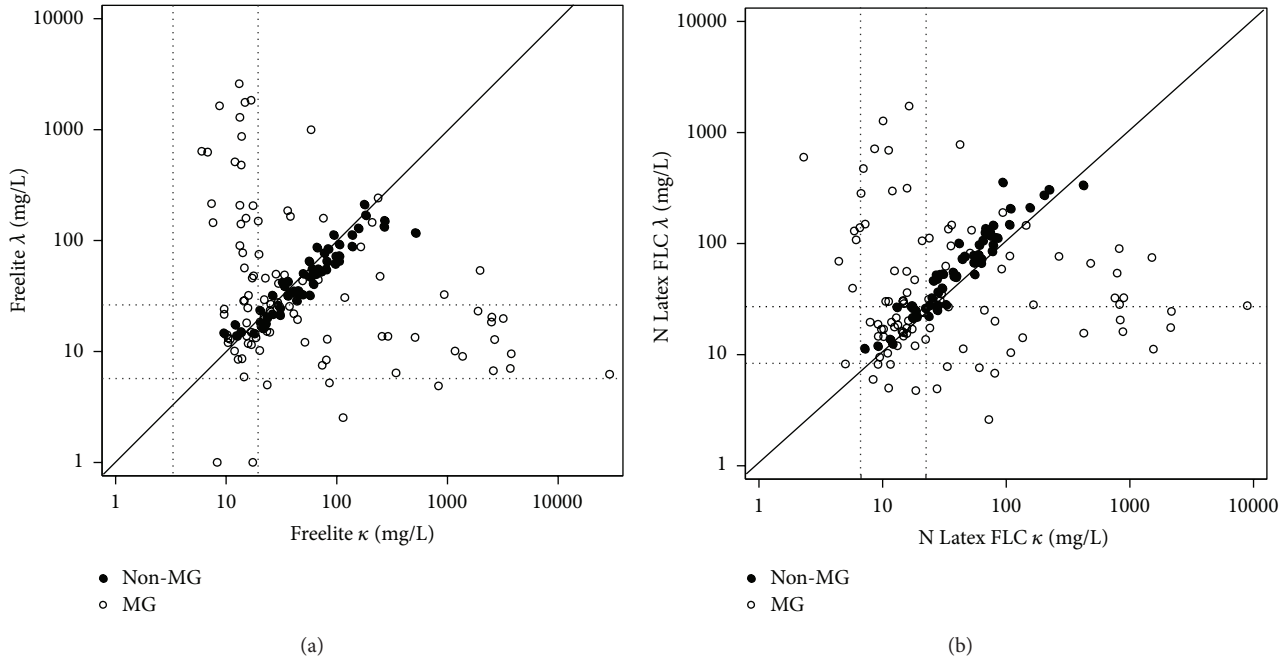


Figure 1: Free light chain κ versus λ for Freelite assay (a) and N Latex FLC assay (b) in 157 samples. Dotted lines indicate the reference ranges for the specific assays. The solid line indicates the $y = x$ axes.

observed; the mean ± SD was 66.5 ± 9.8 in the MG group versus 69.3 ± 14.4 in the non-MG group ($P > 0.05$).

3.2. Comparisons of FLC Assays

3.2.1. Quantitative Analysis. The results of the kappa and lambda FLC analyses, as measured by the two assays, for all the specimens, were plotted. The MG group showed a tendency towards the kappa or lambda axis depending upon the monoclonal type, whereas the non-MG group showed dense distribution around the line of identity in both assays. The Freelite assays revealed wider distributions of both κ and λ than the Latex assay (Figure 1).

The kappa and lambda FLC measurements provided kappa values distributed in the ranges of 6.0–29,200.0 mg/L for the Freelite assay and 2.3–8,920.0 mg/L for the N Latex assay, respectively, indicating that the Freelite assay has at least a 3-fold broader range than the Latex assay, with an excellent Pearson's coefficient of 0.9792 and with a normalized difference of −4.6%. In the case of lambda, the ranges measured using the Freelite assay and the N Latex assay were 1.0–2,590.0 mg/L and 2.6–599.0 mg/L, respectively, demonstrating that the Freelite has at least a 4-fold broader range compared to the Latex, with a Pearson's coefficient of 0.8264, which denotes a relatively low correlation compared to kappa. In contrast to κ, the normalized difference of λ was 5.3%, indicating 5.3% lower median value in the Freelite assay. The maximum value of the κ/λ ratio of the Freelite assay was 4,687.0, which was at least 10 times higher than that of the Latex assay, 323.2. The normalized difference of κ/λ ratio was −10.1%, indicating 10% higher median value in the Freelite assay. Correlation coefficients between the κ/λ ratios were remarkably low in the non-MG group, 0.5168, compared to the MG group, 0.9065 (Table 2, Figure 2).

3.2.2. Qualitative Analysis (Concordance Analysis). Based on the reference ranges provided by the manufacturers of both FLC assays, FLC results of the study population were classified into 3 groups, namely, abnormal high, normal, and abnormal low, and concordance rates were analyzed. The concordance rate between two measurements with respect to κ, λ, and κ/λ ratio was 84.7%, 79.6%, and 89.2%, respectively, for all the patients, whereas these were 81%, 76%, and 87%, respectively, in the MG group, and 91.2%, 86%, and 93%, respectively, in the non-MG group. The non-MG group exhibited a higher concordance rate for all the three items than the MG group. Cohen's kappa values of κ, λ, and κ/λ ratio were 0.70, 0.66, and 0.82, respectively. The value of κ/λ ratio, greater than 0.8, indicates a good concordance (Figure 3).

A total of 17 cases, 13 in the MG group and 4 in the non-MG group, showed discordance for the κ/λ ratio, as calculated from the two assays. Of the 13 cases in the MG group, the IFE results were in agreement with those of the Freelite assay results in 8 cases and with the Latex results in 5 cases. The 4 discordant cases in the non-MG group pertained to specimens from patients with chronic kidney disease (CKD), chronic obstructive pulmonary disease, iron deficiency anemia, and systemic lupus erythematosus (SLE), and all of them showed abnormal κ/λ ratios with the Freelite assay, suggesting false positive results compared to IFE. Taken together, the 17 discordant cases consisted of 8 (47.1%) Freelite and 9 (52.9%) N Latex results in agreement with the IFE results. Moreover, in the lambda FLC assay, 10 cases that

Table 2: Method comparison between the N Latex FLC assays and the Freelite assays.

Group	N	N Latex FLC, mg/L	Freelite FLC, mg/L	Pearson's correlation (r)	Passing-Bablok slope (95% CI)	Normalized difference (%)
All						
FLC κ	157	2.3–8920.0	6.0–29200.0	0.9792	0.76 (0.71–0.80)	−4.6
FLC λ	157	2.6–599.0	1.0–2590.0	0.8264	1.18 (0.99–1.35)	5.3
κ/λ ratio	157	0.004–323.2	0.008–4687.0	0.9064	0.45 (0.37–0.51)	−10.1
Monoclonal gammopathy						
FLC κ	100	2.3–8920.0	6.0–29200.0	0.9793	0.67 (0.61–0.75)	−5.2
FLC λ	100	2.6–599.0	1.0–2590.0	0.8388	0.78 (0.69–0.92)	0.43
κ/λ ratio	100	0.004–323.2	0.008–4687.0	0.9065	0.43 (0.35–0.53)	−8.0
No monoclonal gammopathy						
FLC κ	57	7.2–222.0	9.5–269.0	0.9867	0.78 (0.73–0.82)	−4.0
FLC λ	57	11.4–358.0	13.9–213.0	0.9286	1.72 (1.53–1.94)	8.15
κ/λ ratio	57	0.261–1.243	0.646–4.347	0.5168	0.47 (0.28–0.71)	−11.2

Table 3: Clinical sensitivity and specificity between FLC assay and immunofixation electrophoresis in 157 sera.

N Latex FLC assay	IFE			Freelite assay	IFE		
	Positive	Negative	Total		Positive	Negative	Total
Abnormal	51	0	51	Abnormal	57	5	62
Normal	28	78	106	Normal	22	73	95
Total	**79**	**78**	**157**	Total	**79**	**78**	**157**

N Latex FLC assay: agreement, 82.2%; sensitivity, 64.6%; specificity, 100%. Freelite assay: agreement, 82.8%; sensitivity, 72.2%; specificity, 93.6%.
IFE: immunofixation electrophoresis.

showed normal results in the Freelite assay revealed increased values in the N Latex assay. Eight of these 10 cases were present in high concentrations of kappa-type M-proteins.

3.2.3. Clinical Sensitivity and Specificity of the κ/λ Ratio of FLC Assays. The clinical sensitivity (true positive) and specificity (true negative) of the κ/λ ratio of N Latex FLC and Freelite FLC assays were calculated using the outcomes of the IFE analysis. The clinical sensitivity, specificity, and percent agreement were 72.2%, 93.6%, and 82.8%, respectively, for the Freelite assay, and 64.6%, 100%, and 82.2%, respectively, for the N Latex assay, indicating that the Freelite assay has higher clinical sensitivity, whereas the Latex assay has higher specificity, with the percent agreement being almost comparable (Table 3).

In the IFE-positive specimens, 20 cases with a normal FLC ratio in both assays were investigated. Six cases showed extremely low concentrations for the M-band on IFE, and 4 cases manifested intact immunoglobulin multiple myeloma (IIMM) with bound heavy and light chains. Among 5 cases of the IgM-type MG, the FLC ratios were in normal ranges in 4 cases. In addition, 3 cases of lambda-type MG, concomitant with diseases such as CKD or SLE, showed either normal or even increased ratios. Lastly, in the case of the biclonal-type MG that involves both kappa and lambda, the κ/λ ratio shifted to the M-type in higher concentration.

4. Discussion

We compared two FLC assays that have been utilized in diagnosing and monitoring plasma cell dyscrasia. The study populations consisted entirely of patients with or without monoclonal gammopathy and no normal healthy individuals were included. This is particularly important to calculate the specificity of the laboratory tests in clinical situations and our results could give more helpful information in the interpretations of FLC assays in patients with various diseases.

The results of the Freelite assay were distributed over 3–10 times wider than those of the Latex assay. This finding may

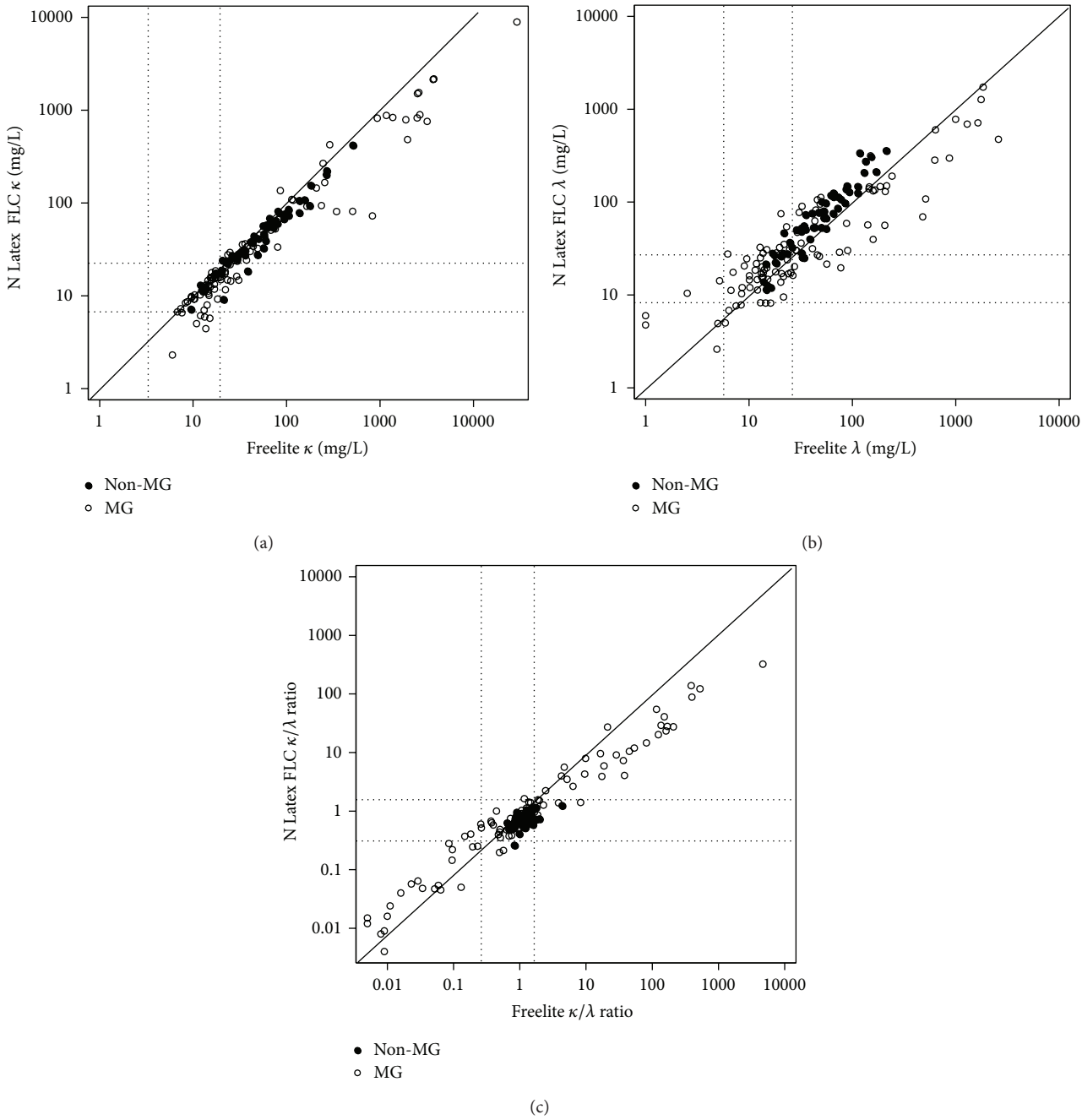


Figure 2: Method comparisons between Freelite assays and N Latex FLC assays for free light chain κ (a), λ (b), and κ/λ ratio (c) for 157 samples. Dotted lines indicate the reference ranges for the specific assays. The solid line indicates the $y = x$ axes. MG: monoclonal gammopathy.

be explained by the differences in the specificities and affinity owing to the different antibody specificity for monoclonal and polyclonal reagents [10]. Because of these considerable differences in the range of the results, these FLC assays could not be switched with each other during monitoring patients [11]. We have designed the method of this study to perform the next dilution step in addition to the autodilution steps to avoid the antigen excess problems. As a result, we could not experience any problems related to antigen excess in all study cases and the data could be analyzed entirely on the aspects of clinical utility.

In this study, the kappa FLC showed the best correlation between two assays for all specimens, followed by the κ/λ ratio and the lambda. On comparing the FLC levels according to the group, the kappa FLC showed high correlation in both groups, with the correlation coefficient being greater than 0.95, and the lambda FLC of the MG group also showed the correlation coefficient, 0.8. The κ/λ ratio of the non-MG group showed the lowest correlation, $r = 0.52$, and this is in line with the international guidelines, according to which changes in the ratio in the normal ranges should not be considered as clinically significant [3, 4]. In a previous

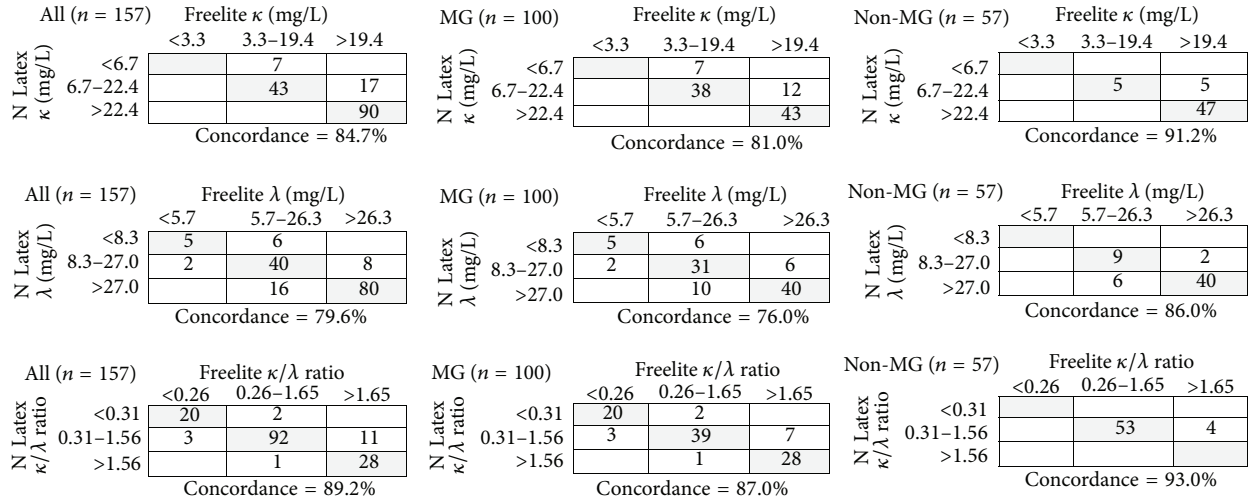


Figure 3: Concordance analysis for free light chain κ, λ, and κ/λ ratio of the Freelite assays and the N Latex FLC assays. MG: monoclonal gammopathy.

study that compared the two assays, correlation coefficients were around 0.9 for kappa and 0.7 for lambda and κ/λ ratio, indicating that the current study had relatively better outcomes [9]. This can be explained by the fact that the present study had a higher proportion of MG group specimen than the previous study.

In the concordance analysis, the overall concordance rate of the κ, λ, and κ/λ ratio between the two assays was approximately 70–80%, which was lower than one previous study, approximately 90% [9]. This result is also believed to be associated with the proportion of the groups. The non-MG group showed higher concordance rates in all the κ, λ, and κ/λ ratios compared to those of the MG group. Four cases in the non-MG group showed a discordant κ/λ ratio and were shown to be false positives in the Freelite assay. This would be relevant to broader ranges of the Freelite assay outcomes compared to those of the N Latex assay. Among the 10 discordant cases for the lambda assay in the MG group, 8 cases had very high kappa concentration in the N Latex assay. These lambda results presented false positives, indicating the same patterns reported in a previous study [6].

Comparisons of the κ/λ ratio and IFE results showed that the Freelite assay had higher clinical sensitivity than the N Latex assay, that is, 72.2% versus 64.6%, and the N Latex assay showed 100% specificity, compared to 93.6% for the Freelite assay. These results were similar with the results of a previous study [11]. The agreement rate with the IFE results was comparable in both assays, approximately 82%. The cases in disagreement with the IFE results were found in those of very low concentration of the M-proteins, IIMM, MG with CKD or polyclonal gammopathy, biclonal MG, and most cases of the IgM-type MG. Some of these findings, CKD or polyclonal gammopathy and IgM-type MG, were reported in previous studies [11, 12]. This study has extended our knowledge about the clinical settings which limit the usefulness of the FLC assays in detecting MG. Therefore, it is essential to perform the IFE along with FLC assay for detecting M-protein in such cases as very low levels of the M-protein, CKD, polyclonal gammopathy, biclonal MG, and IgM-type MG.

In terms of the limitations of this study, it may be pointed out that the MG diseases with low incidence rates such as nonsecretory myeloma, amyloidosis, and solitary plasmacytoma could not be sufficiently included on account of the relatively small sample size. By conducting large-scale studies, clinical implications of the FLC measurement for the disease group with low incidence rate will have to be further clarified.

In conclusion, the N Latex assay and the Freelite assay showed good correlations and concordance rates. When the κ/λ ratios of FLC assays and the IFE were compared, the Freelite assay showed higher clinical sensitivity and the N Latex assay showed higher specificity. Because of the obvious differences in the dynamic range between the assays, the same kind of assay should be employed for monitoring MG patients during follow-up period. Lastly, both FLC assays seem to have limited clinical utility in detecting MG in certain clinical settings.

Conflict of Interests

No potential conflict of interests relevant to this paper was reported.

References

[1] M. S. Davids, M. R. Murali, and D. J. Kuter, "Serum free light chain analysis," *The American Journal of Hematology*, vol. 85, no. 10, pp. 787–790, 2010.

[2] S. Jung, M. Kim, J. Lim et al., "Serum free light chains for diagnosis and follow-up of multiple myeloma," *Korean Journal of Laboratory Medicine*, vol. 28, no. 3, pp. 169–173, 2008.

[3] P.-Y. Briand, O. Decaux, H. Caillon, B. Grosbois, A. Le Treut, and L. Guenet, "Analytical performance of the serum free light

chain assay," *Clinical Chemistry and Laboratory Medicine*, vol. 48, no. 1, pp. 73–79, 2010.

[4] A. Dispenzieri, R. Kyle, G. Merlini et al., "International myeloma working group guidelines for serum-free light chain analysis in multiple myeloma and related disorders," *Leukemia*, vol. 23, no. 2, pp. 215–224, 2009.

[5] J. Tate, S. Bazeley, S. Sykes, and P. Mollee, "Quantitative serum free light chain assay—analytical issues," *Clinical Biochemist Reviews*, vol. 30, no. 3, pp. 131–140, 2009.

[6] J. F. M. Jacobs, R. M. J. Hoedemakers, E. Teunissen, R. G. van der Molen, and H. te Velthuis, "Effect of sample dilution on two free light chain nephelometric assays," *Clinica Chimica Acta*, vol. 413, no. 19-20, pp. 1708–1709, 2012.

[7] M. Bosmann, J. Kößler, H. Stolz, U. Walter, S. Knop, and U. Steigerwald, "Detection of serum free light chains: the problem with antigen excess," *Clinical Chemistry and Laboratory Medicine*, vol. 48, no. 10, pp. 1419–1422, 2010.

[8] M. Vercammen, P. Meirlaen, L. Broodtaerts, I. V. Broek, and X. Bossuyt, "Effect of sample dilution on serum free light chain concentration by immunonephelometric assay," *Clinica Chimica Acta*, vol. 412, no. 19-20, pp. 1798–1804, 2011.

[9] H. te Velthuis, I. Knop, P. Stam et al., "N Latex FLC—new monoclonal high-performance assays for the determination of free light chain kappa and lambda," *Clinical Chemistry and Laboratory Medicine*, vol. 49, no. 8, pp. 1323–1332, 2011.

[10] R. M. J. Hoedemakers, J. F. M. Pruijt, S. Hol et al., "Clinical comparison of new monoclonal antibody-based nephelometric assays for free light chain kappa and lambda to polyclonal antibody-based assays and immunofixation electrophoresis," *Clinical Chemistry and Laboratory Medicine*, vol. 50, no. 3, pp. 489–495, 2012.

[11] M. Drayson and H. Carr-Smith, "Clinical comparison of new monoclonal antibody-based nephelometric assays for free light chain κ and λ to polyclonal antibody-based assays and immunofixation electrophoresis," *Clinical Chemistry and Laboratory Medicine*, vol. 50, no. 3, pp. 587–588, 2012.

[12] T. D. Jaskowski, C. M. Litwin, and H. R. Hill, "Detection of κ and λ light chain monoclonal proteins in human serum: automated immunoassay versus immunofixation electrophoresis," *Clinical and Vaccine Immunology*, vol. 13, no. 2, pp. 277–280, 2006.

Hindawi Publishing Corporation
BioMed Research International
Volume 2014, Article ID 145619, 8 pages
http://dx.doi.org/10.1155/2014/145619

Research Article

Elevated Red Blood Cell Distribution Width as a Simple Prognostic Factor in Patients with Symptomatic Multiple Myeloma

Hyewon Lee,[1] Sun-Young Kong,[2] Ji Yeon Sohn,[2] Hyoeun Shim,[2] Hye Sun Youn,[1] Sangeun Lee,[1] Hyun Ju Kim,[1] and Hyeon-Seok Eom[1]

[1] *Hematologic-Oncology Clinic, Center for Specific Organs Cancer, Research Institute and Hospital, National Cancer Center, 323 Ilsan-ro, Ilsandong-gu, Goyang, Gyeonggi-do 410-769, Republic of Korea*
[2] *Department of Laboratory Medicine, Center for Diagnostic Oncology, Research Institute and Hospital, National Cancer Center, 323 Ilsan-ro, Ilsandong-gu, Goyang, Gyeonggi-do 410-769, Republic of Korea*

Correspondence should be addressed to Hyeon-Seok Eom; hseom@ncc.re.kr

Received 24 March 2014; Revised 25 April 2014; Accepted 8 May 2014; Published 21 May 2014

Academic Editor: Dong Soon Lee

Red blood cell distribution width (RDW) is a parameter reported in complete blood cell count tests, and has been reported as an inflammatory biomarker. Multiple myeloma (MM) is known to be associated with inflammatory microenvironments. However, the importance of RDW has been seldom studied in MM. For this study, 146 symptomatic myeloma patients with available RDW at diagnosis were retrospectively reviewed, and their characteristics were compared between two groups, those with high (>14.5%) and normal (≤14.5%) RDW. RDW was correlated to hemoglobin, MM stage, β2-microglobulin, M-protein, bone marrow plasma cells, and cellularity ($P < 0.001$). During induction, overall response rates of the two groups were similar ($P = 0.195$); however, complete response rate was higher in the normal-RDW group than it was in the high-RDW group ($P = 0.005$). With a median follow-up of 47 months, the normal-RDW group showed better progression-free survival (PFS) (24.2 versus 17.0 months, $P = 0.029$) compared to the high-RDW group. Overall survival was not different according to the RDW level ($P = 0.236$). In multivariate analysis, elevated RDW at diagnosis was a poor prognostic factor for PFS (HR 3.21, 95% CI 1.24–8.32) after adjustment with other myeloma-related prognostic factors. RDW would be a simple and immediately available biomarker of symptomatic MM, reflecting the systemic inflammation.

1. Introduction

Red blood cell distribution width (RDW) is one of the parameters routinely reported in the complete blood cell count test, and it reflects the size variability of mature erythrocytes in peripheral blood and ineffective erythropoiesis of bone marrow [1]. It has been used in routine practice for several decades to make a differential diagnosis for various cases of anemia, such as an iron deficiency anemia [2, 3]. Recently, RDW has been reported as an inflammatory biomarker in various conditions such as cardiovascular diseases [4, 5], acute and chronic kidney diseases [6, 7], chronic pulmonary diseases [8], and critically ill patients [9–12]. In these conditions, elevated RDW level could predict severe morbidity and mortality. Furthermore, RDW could reflect subclinical inflammation and it is associated with poor functional status dependence in the elderly [13].

Multiple myeloma is the hematologic malignancy originating from plasma cells; it is characterized by increased monoclonal protein (M-protein) and specific organ injuries resulting in hypercalcemia, anemia, renal insufficiency, and osteolytic bone lesions. The median age at diagnosis of multiple myeloma is greater than 65 years, and its incidence rapidly increases by age. The prognostic factors associated with multiple myeloma mainly reflect plasma cell burden or intrinsic characteristics of the myeloma clones. The International Staging System (ISS) and cytogenetic risk groups are well known as important prognostic models [14–16].

Inflammatory parameters such as C-reactive protein (CRP) and interleukin-6 (IL-6) at diagnosis have been also reported as prognostic in patients with multiple myeloma [17, 18]. Multiple myeloma is one of the malignancies that are associated with inflammatory microenvironments [19, 20]. Novel therapies targeting inflammatory cytokines and tumor microenvironment have been investigated in patients with multiple myeloma [21, 22]. Acute kidney injury induced by the free light chains in multiple myeloma is also associated with a cascade of inflammatory responses [23].

With regard to these characteristics of multiple myeloma, we hypothesized that RDW has a prognostic value in patients with multiple myeloma. We expected that RDW would reflect not only the tumor burden but also the global condition of the patients, including comorbidities such as age, risk of cardiovascular complications, and severity of renal impairment. Use of RDW in patients with multiple myeloma has seldom been studied; therefore, we performed a retrospective review to investigate the prognostic value of baseline RDW level at diagnosis in patients with symptomatic multiple myeloma.

2. Materials and Methods

2.1. Patients. This analysis included patients with multiple myeloma who were diagnosed and treated at the National Cancer Center, Goyang, Korea, between 2005 and 2012. Patients who were older than 20 years with previously untreated symptomatic multiple myeloma, who had been administered at least one dose of systemic chemotherapy and who had complete blood cell test results available and a reported RDW level before treatment, were enrolled. Medical records and laboratory results were retrospectively reviewed.

The diagnosis of symptomatic multiple myeloma was made when the patient had (a) 10% or more clonal plasma cells on bone marrow examination or a biopsy-proven plasmacytoma, (b) serum and/or urinary monoclonal protein (except in nonsecretory patients), and (c) evidence of end-organ damage that is related to multiple myeloma [24]. Stage was classified by the ISS [14], and a response assessment was performed based on the criteria from the International Myeloma Working Group [24]. Patients with hypodiploidy or −13 by conventional chromosome analysis were regarded as high risk. Cytogenetic abnormalities detected by fluorescent *in situ* hybridization (FISH) such as t(4;14), t(14;16), or del(17p) were also designated as high risk [24]. Data, including patients' demographics, known prognostic factors for multiple myeloma, treatments, and clinical outcomes, were collected with RDW level at the time of the first systemic chemotherapy. This study was approved by the institutional review board of the National Cancer Center, Korea, and conducted according to the Declaration of Helsinki.

2.2. Measurement of RDW. Baseline RDW level at diagnosis was defined as the value that was obtained on the nearest day within 2 weeks before the front-line treatment. RDW was measured using XE-2100 (Sysmex, Kobe, Japan). RDW is reported as a coefficient of variation (percentage) of red blood cell volume. The reference range for RDW in our institution is 11.5% to 14.5%. We defined that the RDW level was "high" when it was >14.5%.

2.3. Statistical Analysis. Based on pretreatment RDW levels, patients were divided into the high-RDW group (>14.5%) and normal-RDW group (≤14.5%). Between the two groups, patients' characteristics and survival outcomes were compared. Continuous and categorical parameters were analyzed using independent sample t-tests and x^2 tests, respectively. For survival analysis, the Kaplan-Meier method with a log-rank test was used. Progression-free survival (PFS) was measured from the date of the first treatment to the earliest date that the progression of multiple myeloma or death was documented. Initially planned induction therapy, high dose chemotherapy with autologous stem cell transplantation, and maintenance therapy were regarded as the front-line therapy. Stem cell transplantation was not censored in this survival analysis. Overall survival (OS) was defined as the duration from the first treatment to all-cause death. The prognostic value of pretreatment RDW level was validated using the Cox proportional hazards model. The significant variables with $P < 0.05$ defined in univariate survival analyses (by log-rank test) and previously well-known prognostic factors in patients with multiple myeloma such as age, performance status, stage at diagnosis, cytogenetic risk group, type of induction therapy, and stem cell transplantation were included for the multivariate analysis to validate the prognostic value of RDW. Differences were considered statistically significant when two-sided P values were <0.05.

3. Results

3.1. Patient Characteristics. A total of 146 patients were eligible for this analysis. The median age was 61 (32–83) years, and 91 (62.3%) were male. The mean baseline RDW level was 14.6%, and it ranged from 11.9% to 22.0%. Among these, 55 (27.7%) patients presented an RDW higher than the upper limit of normal range (>14.5%). The mean RDW values of normal-RDW group and high-RDW group were 13.3% (range, 11.9–14.5%) and 16.8% (range, 14.6–22.0%), respectively. Characteristics of the patients stratified according to the pretreatment RDW level are presented in Table 1. High-RDW group included more elderly patients compared to normal-RDW group, although it was not statistically significant ($P = 0.061$). The distribution of comorbidities such as diabetes mellitus, hypertension, cardiovascular diseases, malignancies other than multiple myeloma, chronic liver disease, and chronic pulmonary diseases was not different between the two groups.

Baseline RDW level correlated to hemoglobin (negative correlation, $\rho = -0.593$, $P < 0.001$), albumin level (negative correlation, $\rho = -0.386$, $P < 0.001$), serum creatinine level ($\rho = 0.208$, $P = 0.016$), β2-microglobulin ($\rho = 0.443$, $P < 0.001$), M-protein level ($\rho = 0.289$, $P = 0.002$), bone marrow plasma cell burden ($\rho = 0.370$, $P < 0.001$), and bone marrow cellularity ($\rho = 0.262$, $P = 0.002$). Patients with ISS-I disease presented with lower RDW (mean ± SD, 13.75% ± 1.69) compared to ISS-II (mean ± SD, 15.05% ± 2.19, $P < 0.001$) and ISS-III (mean ± SD, 15.61% ± 2.11, $P < 0.001$)

Table 1: Clinical characteristics of multiple myeloma patients.

	Total (n = 146)	Normal-RDW (n = 91)	High-RDW (n = 55)	P
RDW level, mean (range)	14.6 (11.9–22.0)	13.3 (11.9–14.5)	16.8 (14.6–22.0)	<0.001
Age, mean (range)	61 (32–83)	60 (32–83)	63 (41–80)	0.061
Sex, male/female	91/55	59/32	32/23	0.482
ECOG (≥2)	26/144 (18.1%)	14/80 (17.5%)	42/12 (22.2%)	0.373
Comorbidity				
Diabetes mellitus	13 (8.9%)	7 (8.0%)	6 (10.0%)	0.771
Hypertension	39 (26.7%)	24 (27.6%)	15 (25.0%)	0.850
Cardiovascular diseases	6 (4.1%)	4 (4.6%)	2 (3.3%)	1.000
Malignancies	12 (8.2%)	9 (10.3%)	3 (5.0%)	0.361
Chronic liver diseases	5 (3.4%)	3 (3.4%)	2 (3.3%)	1.000
Chronic lung diseases	6 (4.1%)	2 (2.3%)	4 (6.8%)	0.226
Hemoglobin, g/dL	10.7 (5.3–16.4)	11.4 (6.2–16.4)	9.5 (5.3–14.4)	<0.001
Platelet, $\times 10^9$/L	218 (37–691)	224 (68–555)	210 (37–691)	0.410
Creatinine, mg/dL	1.6 (1.0–9.0)	1.4 (1.0–7.0)	1.8 (1.0–9.0)	0.083
Calcium, mg/dL	9.0 (6.8–13.7)	9.2 (6.8–13.7)	8.8 (7.2–11.1)	0.004
Albumin, g/dL	3.8 (2.3–4.9)	3.9 (2.5–4.9)	3.5 (2.3–4.7)	<0.001
LDH, IU/L	199 (54–1832)	203 (54–1832)	192 (77–587)	0.762
B2MG, mg/dL	5.0 (1.2–41.9)	3.8 (1.2–18.7)	7.2 (1.6–41.9)	<0.001
CRP, mg/dL	1.20 (0.01–8.65)	0.99 (0.01–5.45)	1.53 (0.01–8.65)	0.204
ISS				
I	60 (41.7%)	50 (55.6%)	10 (18.5%)	
II	49 (34.0%)	26 (28.9%)	23 (42.6%)	<0.001
III	35 (24.3%)	14 (15.6%)	21 (38.9%)	
Unknown	2	1	1	
M-protein, g/dL	2.47 (0.01–9.31)	2.06 (0.08–6.80)	3.12 (0.01–9.31)	0.006
Light chain disease	31 (21.2%)	20 (22.0%)	11 (20%)	1.000
Nonsecretory type	5 (3.4%)	5 (5.5%)	0	0.157
Plasmacytoma	51 (34.9%)	40 (44.0%)	11 (20.0%)	0.004
Cytogenetic risk (high)	21/108 (19.4%)	12/69 (17.4%)	9/39 (23.1%)	0.613
Front-line treatment				
Radiation only	5	2	3	
Thalidomide-based	63	44	19	
Bortezomib-based	14	9	5	0.606
Bortezomib + thalidomide	9	4	5	
Lenalidomide-based	4	1	3	
Others	51	31	20	
ASCT	43 (29.5%)	31 (34.1%)	12 (21.8%)	0.136

RDW: red blood cell distribution width; ECOG: Eastern Cooperative Oncology Group; LDH: lactate dehydrogenase; B2MG: β2-microglobulin; ISS: International Staging System; ASCT: autologous stem cell transplantation.

patients (Figure 1). Extramedullary plasmacytoma was more frequent in the normal-RDW group compared to high-RDW group (44.0% versus 20.0%, $P = 0.004$).

Cytogenetic data based on conventional chromosome analysis and FISH were available for 108 (74.0%) patients. Twenty-one (19.4%) of them were stratified as high risk. The proportion of high-risk patients in the normal-RDW and high-RDW groups was not statistically different (17.4% versus 23.1%, $P = 0.613$).

The front-line treatment for symptomatic myeloma is shown in Table 1. Five (3.4%) patients received radiation therapy without any systemic chemotherapy. Ninety patients (61.6%) were administered with novel agents such as thalidomide, lenalidomide, and bortezomib as an induction regimen. Others (34.9%) received high-dose steroids alone or conventional chemotherapy, such as doxorubicin or vincristine. Among the evaluable patients, the overall response rates (ORR) were not different between the normal-RDW and high-RDW groups (82.9% versus 73.1%, $P = 0.195$). However, the complete response (CR) rate was significantly higher in the normal-RDW group compared to the high-RDW group (36.6% versus 13.5%, $P = 0.005$). After induction, autologous stem cell transplantation was performed in 43 (29.5%) patients. Among them, 31 (34.1%) were in the normal-RDW

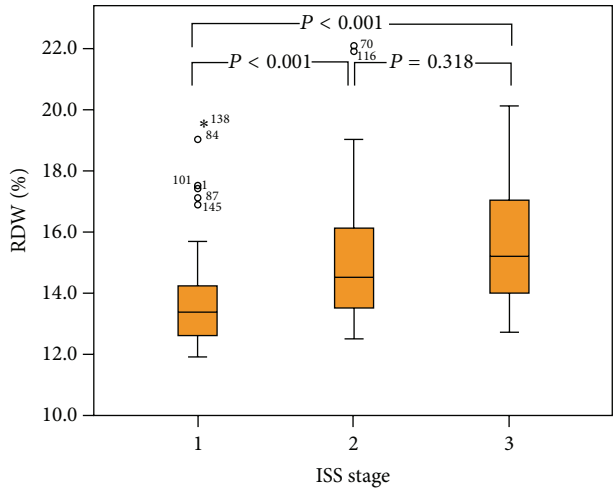


FIGURE 1: Red blood cell distribution width (RDW) level at diagnosis of multiple myeloma according to the International Staging System (ISS).

group and 12 (21.8%) were in the high-RDW group (P = 0.136).

3.2. Association between RDW Level and Clinical Outcomes. With a median follow-up of 47 (3–104) months, patients with normal-RDW showed better progression-free survival compared to high-RDW patients (median PFS, 24.2 versus 17.0 months, P = 0.029). Overall survival showed a similar tendency between the two groups; however, the difference was not statistically significant (median OS, 63.6 versus 50.6 months, P = 0.236) (Figure 2).

Univariate analyses were performed to investigate the prognostic factors affecting disease progression and death (Table 2). Baseline RDW level (HR 1.69, 95% CI 1.05–2.75, P = 0.031), performance status (HR 1.89, 95% CI 1.05–3.41, P = 0.034), hemoglobin level (HR 0.88, 95% CI 0.79–0.99), albumin level (HR 0.56, 95% CI 0.36–0.86, P = 0.008), lactate dehydrogenase (LDH) level (HR 1.84, 95% CI 1.00–3.38, P = 0.050), and β2-microglobulin level (HR 1.08, 95% CI 1.03–1.14, P = 0.002) were potential risk factors for poor progression-free survival. RDW was not prognostic for overall survival (P = 0.238). Other potential prognostic factors for overall survival in this analysis are shown in Table 2.

To exclude the effect of anemia on RDW level, we performed a subgroup analysis according to the hemoglobin level. Patients with hemoglobin >10.0 g/L and RDW >14.5% showed worse outcomes (P = 0.024 for PFS, P = 0.121 for OS) compared to patients with hemoglobin >10.0 g/L and RDW ≤14.5%. These trends were not observed in patients with hemoglobin ≤10.0 g/L (P = 0.394 for PFS, P = 0.652 for OS).

We also performed a subgroup analysis with 53 cases who were transplant-eligible and who received thalidomide-based induction to validate the prognostic value of baseline RDW level in a homogeneous population. In this subgroup analysis, normal-RDW patients were associated with prolonged PFS compared to high-RDW patients (median PFS, 34.7 versus 10.2 months, P = 0.003); however, they did not show significantly better overall survival (60.5 versus 25.0 months, P = 0.266) (Figure 3).

RDW at diagnosis in patients with symptomatic multiple myeloma was found to be an independent predictor for disease progression or death by multivariable analysis (Table 3). Patients who had RDW >14.5% at diagnosis were associated with higher risk of disease progression or death with a hazard ratio (HR) of 3.04 (95% CI 1.16–8.01, P = 0.024) compared to patients with normal RDW at diagnosis. The other factors that revealed independent predictors of progression-free survival in this analysis set were cytogenetic risk group (high risk, HR 3.78, 95% CI 1.50–9.56, P = 0.005) and type of induction regimen (novel agents, HR 0.37, 95% CI 0.16–0.86, P = 0.020).

In multivariate analysis for overall survival, RDW at diagnosis was not an independent prognostic factor (HR 0.90, 95% CI 0.36–2.26) after adjustment with age, performance status, cytogenetic risk group, ISS, LDH, hemoglobin, albumin, β2-microglobulin, type of treatment, and autologous stem cell transplantation. As a result, cytogenetic risk group (high risk, HR 4.24, 95% CI 1.12–16.09), β2-microglobulin (HR 1.14, 95% CI 1.04–1.26), type of induction regimen (novel agents, HR 0.21, 95% CI 0.07–0.60), and autologous stem cell transplantation (performed, HR 0.05, 95% CI 0.01–0.52) were significantly associated with overall survival.

4. Discussion

The present study showed that RDW level at diagnosis was associated with poor prognosis in patients with symptomatic multiple myeloma. As far as we are aware, this study is the first report to evaluate the prognostic value of RDW in patients with multiple myeloma. We showed that the patients whose RDW level was high at diagnosis experienced shorter progression-free survival compared to patients with relatively low RDW. Progression-free survival is an important surrogate marker of long-term survival in patients with multiple myeloma. Although it was not statistically significant in the presented data, overall survival in the high-RDW group seemed to be shorter compared to the normal-RDW group. Analysis for overall survival is complicated because there may be more confounding factors influencing on clinical outcomes during the long follow-up duration.

In patients with multiple myeloma, RDW level might be influenced by anemia. Anemia is one of the major symptoms of multiple myeloma together with hypercalcemia, renal insufficiency, and osteolytic bone lesions, also called CRAB signs. However, we showed that RDW was well correlated not only to the hemoglobin level (negative correlation) but also to other parameters for high tumor burden such as azotemia, M-protein, bone marrow plasma cell percentages, and ISS stages. Furthermore, anemia of multiple myeloma does not simply reflect a decrease in red cell counts, but it is also associated with impaired iron release from reticuloendothelial macrophages, which can be observed in anemia of inflammatory conditions [25]. This suggests that RDW

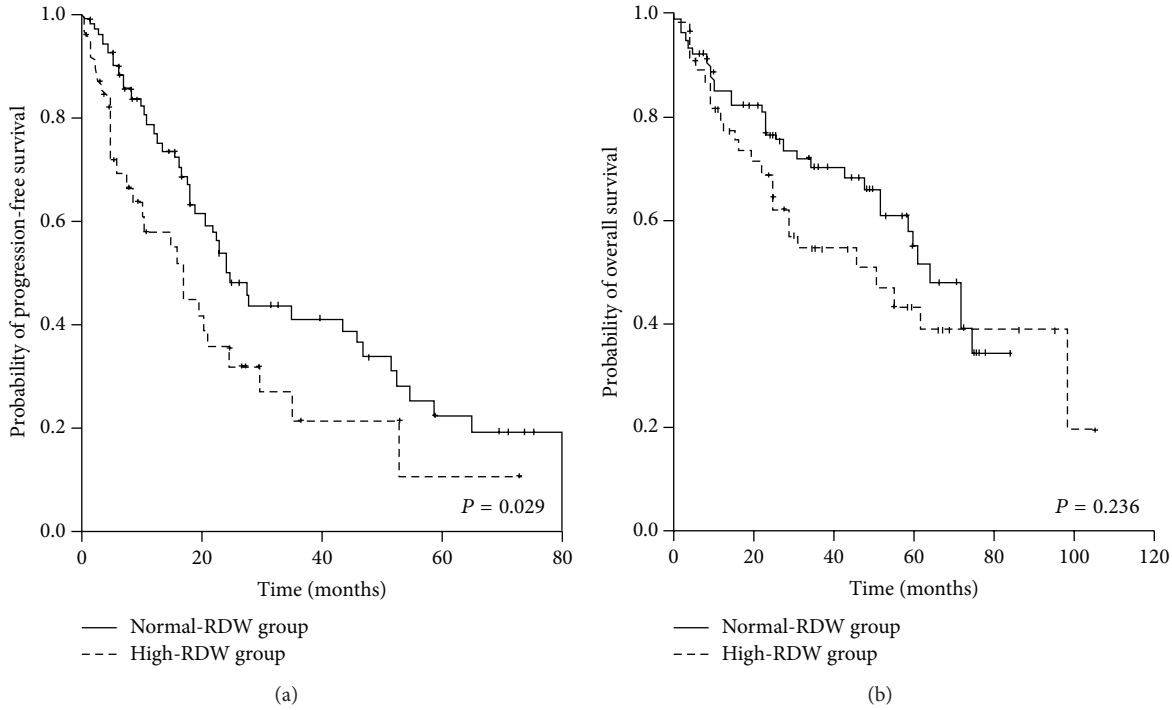


Figure 2: Survival curves according to red blood cell distribution width level at diagnosis in patients with symptomatic multiple myeloma.

Table 2: Univariate analyses for progression-free survival and overall survival.

	PFS			OS		
	HR	95% CI	*P*	HR	95% CI	*P*
RDW (%)	**1.69**	**1.05–2.75**	**0.031**	—	—	**0.238**
Age (year)	—	—	0.173	1.04	1.02–1.07	0.001
Sex (male)	—	—	0.591	—	—	0.835
ECOG (≥2)	1.89	1.05–3.41	0.034	1.82	1.01–3.28	0.048
Hemoglobin (g/dL)	0.88	0.79–0.99	0.028	0.86	0.76–0.99	0.029
Platelet ($\times 10^9$/L)	—	—	0.633	0.99	0.99-1.00	0.001
Creatinine (mg/dL)	—	—	0.539	—	—	0.127
Calcium (mg/dL)	—	—	0.435	—	—	0.443
Albumin (g/dL)	0.56	0.36–0.86	0.008	0.48	0.31–0.74	0.001
LDH (IU/L)	1.84	1.00–3.38	0.050	—	—	0.195
B2MG (mg/dL)	1.08	1.03–1.14	0.002	1.07	1.03–1.10	<0.001
M-protein (g/dL)	—	—	0.475	—	—	0.802
Light chain disease	—	—	0.722	—	—	0.282
Nonsecretory type	—	—	0.504	—	—	0.247
Plasmacytoma	—	—	0.163	—	—	0.410
Cytogenetic risk (high)	—	—	0.134	—	—	0.083
Induction with novel agents*	—	—	0.542	—	—	0.711
ASCT	—	—	0.143	0.2	0.09–0.47	<0.001

*Induction with bortezomib, thalidomide, or renalidomide.
HR: hazard ratio; CI: confidence interval; ECOG: Eastern Cooperative Oncology Group; B2MG: β2-microglobulin; LDH: lactate dehydrogenase; ASCT: autologous stem cell transplantation; RDW: red blood cell distribution width.

can reflect the overall inflammatory condition of multiple myeloma, partly influenced by combined anemia.

In line with this, there is an interesting report suggesting that hematological and inflammatory parameters, including RDW, can discriminate patients with cancer from patients without cancer in involuntary weight loss [26].

It is not surprising that RDW is prognostic in patients with multiple myeloma when we consider that it can reflect tumor burden and inflammatory conditions. We found that RDW at diagnosis was an independent prognostic factor for disease progression or death, even after the adjustment with other myeloma-associated parameters. An assessment

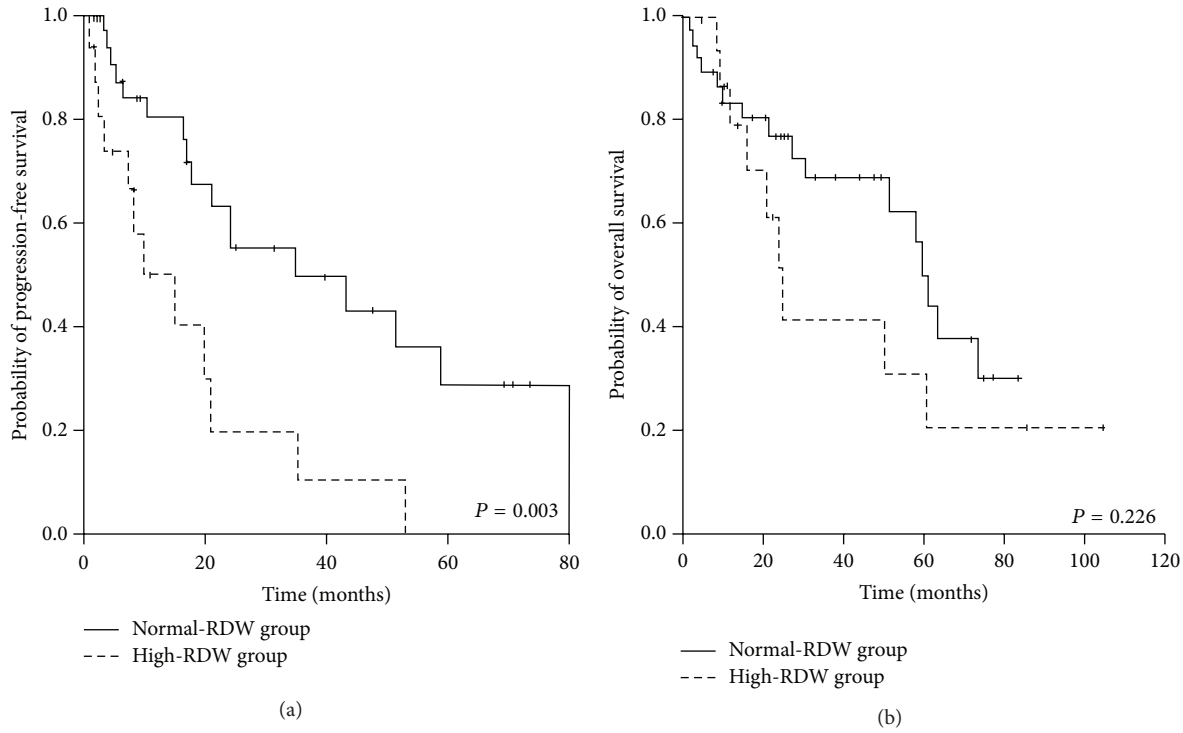


Figure 3: Survival curves according to red blood cell distribution width level at diagnosis in patients with symptomatic multiple myeloma treated with thalidomide-based induction.

Table 3: Multivariate analysis for progression-free survival.

	HR	95% CI	*P*
Age at diagnosis (year)	0.99	0.93–1.05	0.691
ECOG (≥2)	1.48	0.63–3.51	0.373
Cytogenetic risk (high)	**4.12**	**1.63–10.41**	**0.003**
B2MG (mg/L)	1.09	0.99–1.20	0.071
Albumin (<3.5 g/dL)	0.82	0.31–2.17	0.690
LDH (>normal)	1.35	0.56–3.26	0.499
Hemoglobin (>10 g/dL)	0.67	0.28–1.61	0.365
Calcium (>normal)	2.20	0.54–9.03	0.272
Induction with novel agents*	**0.34**	**0.14–0.81**	**0.014**
ASCT	0.96	0.28–3.25	0.945
High-RDW (>14.5%)	**3.21**	**1.24–8.32**	**0.016**

*Induction with bortezomib, thalidomide, or lenalidomide.
HR: hazard ratio; CI: confidence interval; ECOG: Eastern Cooperative Oncology Group; B2MG: β2-microglobulin; LDH: lactate dehydrogenase; ASCT: autologous stem cell transplantation; RDW: red blood cell distribution width.

of RDW level to predict clinical outcomes in patients with symptomatic myeloma has advantages. It can be acquired immediately when the patient is suspected of multiple myeloma to assess the patient's general condition objectively in the context of various comorbidities such as age, acute kidney injury, cardiovascular diseases, infectious condition, and malnutrition. RDW is significantly associated with increased risk in patients with heart failure [27, 28], kidney injury [6], and venous thromboembolism [29, 30], which are often encountered in myeloma patients.

Despite the increasing evidence for RDW as a prognostic factor in patients with inflammatory conditions, there are few reports addressing it in the area of oncology. Recently, there have been a few articles about the significance of RDW as a cancer biomarker. Although the prognostic value of RDW level on specific cancer types has not been studied well, there

have been some reports on breast cancer and lung cancer. Seretis et al. showed that RDW was significantly higher in patients with invasive breast cancer compared to the patient with fibroadenomas. Elevated RDW showed remarkable correlation with the size of primary tumor, the number of axillary lymph nodes, and HER2 overexpression [31]. Warwick et al. showed that preoperative RDW in patients undergoing pulmonary resections for non-small-cell lung cancer could predict mortality and long-term survival [32]. In addition, Koma et al. showed that high RDW level was associated with poor survival in patients with lung cancer [33].

As shown in our data, it has been known that RDW increases with age [34]. Increased age can be a confounding factor which could mislead to conclude that RDW is prognostic. In our data, age itself was not associated with poor prognosis of myeloma, and RDW was a significant predictive biomarker for disease progression or death even after adjustment with other confounding factors including age in multivariate analysis. With regard to both patient's age and different antimyeloma treatment according to the age at diagnosis, which determines transplant-eligible or not, we also have described the results of subgroup analysis in transplant-eligible patients who received thalidomide-based induction in the paper.

There are several limitations in this analysis. First, there may be potential bias and inaccuracy in data collection, as in most retrospective analyses. Second, patient characteristics such as treatment regimens were heterogeneous. Third, we could not find the significant correlation between RDW and CRP, an important and commonly used inflammatory marker, in our dataset. Unfortunately, there were too many missing data because CRP level was not routinely checked at diagnosis. To validate the correlation between RDW and CRP, further prospective study is warranted. Also, we only focused on RDW level at diagnosis and did not evaluate the value of dynamic change in RDW level during the disease courses. A single measurement of RDW could not account for possible variation over time and could not predict overall survival, which may be influenced by various confounding factors. Finally, the value of RDW in prediction of poor prognosis may be slightly different according to the population, because presented RDW data were collected at a single center.

Despite the limitations, this is the first documentation on the prognostic value of RDW in patients with multiple myeloma with long-term follow-up. Further prospective analysis with mechanism studies is necessary to use it widely as a practical biomarker of multiple myeloma.

5. Conclusion

Elevated RDW at diagnosis in patients with symptomatic multiple myeloma was associated with advanced disease status and poor prognosis. It would be a novel and immediately available biomarker of the activity of multiple myeloma. Although we do not know the precise mechanism, it may reflect both the inflammatory status of myeloma itself and the patient's general condition. This easy and cost-effective biomarker may be useful particularly in practice.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

Acknowledgment

This study was supported by the Grants of the National Cancer Center, Goyang, Korea, (NCC 1410200 and NCC 1110190-2).

References

[1] D. L. Simel, E. R. DeLong, J. R. Feussner, J. B. Weinberg, and J. Crawford, "Erythrocyte anisocytosis. Visual inspection of blood films vs automated analysis of red blood cell distribution width," *Archives of Internal Medicine*, vol. 148, no. 4, pp. 822–824, 1988.

[2] D. van Zeben, R. Bieger, R. K. A. van Wermeskerken, A. Castel, and J. Hermans, "Evaluation of microcytosis using serum ferritin and red blood cell distribution width," *European Journal of Haematology*, vol. 44, no. 2, pp. 106–109, 1990.

[3] K. Bhambhani and R. Aronow, "Lead poisoning and thalassemia trait or iron deficiency. The value of the red blood cell distribution width," *American Journal of Diseases of Children*, vol. 144, no. 11, pp. 1231–1233, 1990.

[4] M. Tonelli, F. Sacks, M. Arnold, L. Moye, B. Davis, and M. Pfeffer, "Relation between red blood cell distribution width and cardiovascular event rate in people with coronary disease," *Circulation*, vol. 117, no. 2, pp. 163–168, 2008.

[5] Y. Arbel, D. Weitzman, R. Raz et al., "Red blood cell distribution width and the risk of cardiovascular morbidity and all-cause mortality. A population-based study," *Thrombosis and Haemostasis*, vol. 111, pp. 300–307, 2014.

[6] H. J. Oh, J. T. Park, J. K. Kim et al., "Red blood cell distribution width is an independent predictor of mortality in acute kidney injury patients treated with continuous renal replacement therapy," *Nephrology Dialysis Transplantation*, vol. 27, pp. 589–594, 2012.

[7] Y. Solak, M. I. Yilmaz, M. Saglam et al., "Red cell distribution width is independently related to endothelial dysfunction in patients with chronic kidney disease," *The American Journal of the Medical Sciences*, vol. 347, pp. 118–124, 2014.

[8] S. Balta, M. Aydogan, S. Demirkol, M. Cakar, E. O. Akgul, and H. Sarlak, "Red cell distribution width: a novel and simple predictor of mortality in chronic obstructive pulmonary disease," *Journal of Chronic Obstructive Pulmonary Disease*, 2014.

[9] F. Wang, W. Pan, S. Pan, J. Ge, S. Wang, and M. Chen, "Red cell distribution width as a novel predictor of mortality in ICU patients," *Annals of Medicine*, vol. 43, no. 1, pp. 40–46, 2011.

[10] S. W. Purtle, T. Moromizato, C. K. McKane, F. K. Gibbons, and K. B. Christopher, "The association of red cell distribution width at hospital discharge and out-of-hospital mortality following critical illness*," *Critical Care Medicine*, vol. 42, pp. 918–929, 2014.

[11] H. S. Bazick, D. Chang, K. Mahadevappa, F. K. Gibbons, and K. B. Christopher, "Red cell distribution width and all-cause mortality in critically ill patients," *Critical Care Medicine*, vol. 39, no. 8, pp. 1913–1921, 2011.

[12] N. S. Ku, H. W. Kim, H. J. Oh et al., "Red blood cell distribution width is an independent predictor of mortality in patients with

gram-negative bacteremia," *Shock*, vol. 38, no. 2, pp. 123–127, 2012.

[13] D. de Gonzalo-Calvo, B. de Luxán-Delgado, S. Rodríguez-González et al., "Interleukin 6, soluble tumor necrosis factor receptor I and red blood cell distribution width as biological markers of functional dependence in an elderly population: a translational approach," *Cytokine*, vol. 58, no. 2, pp. 193–198, 2012.

[14] P. R. Greipp, J. S. Miguel, B. G. M. Dune et al., "International staging system for multiple myeloma," *Journal of Clinical Oncology*, vol. 23, no. 15, pp. 3412–3420, 2005.

[15] G. W. Dewald, T. Therneau, D. Larson et al., "Relationship of patient survival and chromosome anomalies detected in metaphase and/or interphase cells at diagnosis of myeloma," *Blood*, vol. 106, no. 10, pp. 3553–3558, 2005.

[16] A. K. Stewart and R. Fonseca, "Prognostic and therapeutic significance of myeloma genetics and gene expression profiling," *Journal of Clinical Oncology*, vol. 23, no. 26, pp. 6339–6344, 2005.

[17] R. Bataille, M. Boccadoro, B. Klein, B. Durie, and A. Pileri, "C-reactive protein and β-2 microglobulin produce a simple and powerful myeloma staging system," *Blood*, vol. 80, no. 3, pp. 733–737, 1992.

[18] A. Tienhaara, K. Pulkki, K. Mattila, K. Irjala, and T.-T. Pelliniemi, "Serum immunoreactive interleukin-6 and C-reactive protein levels in patients with multiple myeloma at diagnosis," *British Journal of Haematology*, vol. 86, no. 2, pp. 391–393, 1994.

[19] A. Mantovani and C. Garlanda, "Inflammation and multiple myeloma: the Toll connection," *Leukemia*, vol. 20, no. 6, pp. 937–938, 2006.

[20] R. Bataille and J. L. Harousseau, "Multiple myeloma," *The New England Journal of Medicine*, vol. 336, pp. 1657–1664, 1997.

[21] N. Nishimoto, "Interleukin-6 as a therapeutic target in candidate inflammatory diseases," *Clinical Pharmacology and Therapeutics*, vol. 87, no. 4, pp. 483–487, 2010.

[22] A. Mahindra, J. Laubach, N. Raje, N. Munshi, P. G. Richardson, and K. Anderson, "Latest advances and current challenges in the treatment of multiple myeloma," *Nature Reviews Clinical Oncology*, vol. 9, no. 3, pp. 135–143, 2012.

[23] C. A. Hutchison, V. Batuman, J. Behrens et al., "The pathogenesis and diagnosis of acute kidney injury in multiple myeloma," *Nature Reviews Nephrology*, vol. 8, no. 1, pp. 43–51, 2012.

[24] R. A. Kyle and S. V. Rajkumar, "Criteria for diagnosis, staging, risk stratification and response assessment of multiple myeloma," *Leukemia*, vol. 23, no. 1, pp. 3–9, 2009.

[25] S. Sharma, E. Nemeth, Y.-H. Chen et al., "Involvement of hepcidin in the anemia of multiple myeloma," *Clinical Cancer Research*, vol. 14, no. 11, pp. 3262–3267, 2008.

[26] C. Baicus, S. Caraiola, M. Rimbas, R. Patrascu, and A. Baicus, "Utility of routine hematological and inflammation parameters for the diagnosis of cancer in involuntary weight loss," *Journal of Investigative Medicine*, vol. 59, no. 6, pp. 951–955, 2011.

[27] J. Nunez, E. Nunez, D. Rizopoulos et al., "Red blood cell distribution width is longitudinally associated with mortality and anemia in heart failure patients," *Circulation Journal*, vol. 78, no. 2, pp. 410–418, 2014.

[28] C. A. Cauthen, W. Tong, A. Jain, and W. H. W. Tang, "Progressive rise in red cell distribution width is associated with disease progression in ambulatory patients with chronic heart failure," *Journal of Cardiac Failure*, vol. 18, no. 2, pp. 146–152, 2012.

[29] S. M. Rezende, W. M. Lijfering, F. R. Rosendaal, and S. C. Cannegieter, "Hematologic variables and venous thrombosis: red cell distribution width and blood monocyte count are associated with an increased risk," *Haematologica*, vol. 99, pp. 194–200, 2014.

[30] B. Zoller, O. Melander, P. Svensson, and G. Engstrom, "Red cell distribution width and risk for venous thromboembolism: a population-based cohort study," *Thrombosis Research*, vol. 133, no. 3, pp. 334–339, 2014.

[31] C. Seretis, F. Seretis, E. Lagoudianakis, G. Gemenetzis, and N. S. Salemis, "Is red cell distribution width a novel biomarker of breast cancer activity? Data from a pilot study," *Journal of Clinical Medicine Research*, vol. 5, no. 2, pp. 121–126, 2013.

[32] R. Warwick, N. Mediratta, M. Shackcloth, M. Shaw, J. McShane, and M. Poullis, "Preoperative red cell distribution width in patients undergoing pulmonary resections for non-small-cell lung cancer," *European Journal Cardio-Thoracic Surgery*, vol. 45, no. 1, pp. 108–113, 2014.

[33] Y. Koma, A. Onishi, H. Matsuoka et al., "Increased red blood cell distribution width associates with cancer stage and prognosis in patients with lung cancer," *PLoS ONE*, vol. 8, no. 11, Article ID e80240, 2013.

[34] R. F. Brightwell, G. P. M. Crawford, J. B. Cale, P. J. Pedler, and A. H. Bittles, "Ageing and the haematological profiles of an Australian community," *Annals of Human Biology*, vol. 25, no. 1, pp. 1–10, 1998.

Hindawi Publishing Corporation
BioMed Research International
Volume 2014, Article ID 804182, 7 pages
http://dx.doi.org/10.1155/2014/804182

Research Article

Serum Parathyroid Hormone Is a New Potential Risk Factor in Multiple Myeloma

Min-Gu Kang,[1] Eun-Jeong Won,[1] Hyun-Woo Choi,[1] Hye-Ran Kim,[2] Hyun-Jung Choi,[1] Hye-Ri Park,[3] Jong-Hee Shin,[1] Soon-Pal Suh,[1] Dong-Wook Ryang,[1] and Myung-Geun Shin[1,3]

[1] *Department of Laboratory Medicine, Chonnam National University Medical School and Chonnam National University Hwasun Hospital, 160 Ilsim-ri, Hwasun-eup, Hwasun-gun, Jeollanam-do 519-809, Republic of Korea*
[2] *Laboratory of Metabolism, National Cancer Institute, National Institutes of Health, Bethesda, MD, USA*
[3] *Brain Korea 21 Plus Project, Chonnam National University Medical School, Gwangju, Republic of Korea*

Correspondence should be addressed to Myung-Geun Shin; mgshin@chonnam.ac.kr

Received 28 February 2014; Revised 18 April 2014; Accepted 2 May 2014; Published 19 May 2014

Academic Editor: Dong Soon Lee

We hypothesized that serum PTH might be associated with various clinicopathological parameters in multiple myeloma (MM). So we investigated the implications of serum PTH in MM patients and the relationship with other risk factors of MM. A total of 115 patients who were newly diagnosed with MM were enrolled. Serum PTH level was 24.7 ± 34.9 (ranged 0.0–284.1) pg/mL. Serum levels of IgG, IgM, FLC-lambda, albumin, and LDH were in positive correlation with serum PTH. Compared to non-high PTH (<68.3 pg/mL) group, the hazard ratio (HR) for overall survival was higher for group with high PTH level (≥68.3 pg/mL) (HR, 1.710). Furthermore, the patient group with high PTH level showed inferior progression-free survival than non-high PTH group ($P = 0.056$). Interestingly, subgroup analysis showed that serum PTH level at diagnosis was associated with risk factors and clinical outcome in MM patients, especially in complete remission group, transplantation cases, ISS stage II cases, and cases without chromosome abnormality. In conclusion, this study showed that blood PTH level in MM at diagnosis was associated with risk factors and clinical outcome in MM patients.

1. Introduction

Parathyroid hormone (PTH) is synthesized and secreted by the chief cells of the parathyroid gland. PTH has a positive impact on hematopoietic stem cells by indirectly decreasing hematopoietic cell apoptosis and is currently being investigated as a potential therapeutic remedy to stimulate hematopoiesis and enhance bone marrow engraftment [1–4].

According to the previous study [5], it was hypothesized that elevated PTH may mediate the induction of multiple myeloma (MM) through the downstream biologic effects of interleukin 6 (IL-6). Meanwhile, PTH stimulates stromal-osteoblastic cells to secrete IL-6 [6]. Because this cytokine plays a key role in the development of plasma cell dyscrasias [7], high PTH levels may facilitate the emergence and growth of a plasma cell clone [8].

However, there were a limited number of studies regarding the relationship between serum PTH level and its clinicopathological implications in MM until now. Additionally, pathobiology of MM suggests that serum PTH level might be associated with clinical consequences of MM patients. So we addressed the possible relationship between serum PTH level and pathophysiology of MM patients with other various clinical parameters. This study presents data which showed the prognostic implications of serum PTH in MM patients and their relationship with other risk factors of MM.

2. Materials and Methods

A total of 115 patients who were newly diagnosed with MM were enrolled between 2006 and 2012. MM was diagnosed based on the clinical, laboratory, and radiologic findings.

The details of diagnostic criteria were as follows: monoclonal protein in serum or urine, bone marrow clonal plasma cells or plasmacytoma, and the evidence of related organ or tissue impairment (hypercalcemia, renal insufficiency, anemia, and bone lesions).

Serum PTH level was measured at the time of the diagnosis by automated 2-site chemiluminescent microparticle immunoassay of AxSYM system (Abbott Diagnostics, IL, USA) according to the manufacturer's instructions.

Chromosome analysis was performed on G-banded preparations from 48 hour bone marrow cell cultures without adding mitogens. The chromosome aberrations were described according to the International System for Cytogenetic Nomenclature 2005 and 2009. In additional to conventional cytogenetic analysis, fluorescent in situ hybridization (FISH) was applied in appropriate bone marrow specimens using 13q14.3, 13q24, and 17p13.1 (p53) deletion probes and 14q32 (IGH) break apart probe (Vysis, Des Plaines, IL, USA) according to the manufacturer's instructions.

In addition to serum PTH, other clinical parameters were reviewed for age, sex, plasma cell percentage in bone marrow, serum monoclonal protein, immunoglobulin (Ig) level, free light chain (FLC)—kappa and lambda, FLC ratio, calcium, creatinine, hemoglobin (Hb), albumin, beta-2 microglobulin, lactate dehydrogenase (LDH), international staging system (ISS) stage, international myeloma working group (IMWG) response, chromosome abnormality, bone lesion, treatment outcome, and so forth. The diagnostic criteria of MM, ISS stage, and the international myeloma working group (IMWG) response were based on the precedent review [9].

According to the serum PTH level at the time of MM diagnosis (cut-off; 68.3 pg/mL, reference range; 15–68.3 pg/mL), the study population was divided into non-high PTH group and high PTH group. Then, previously reviewed parameters of MM patients were compared between the two groups. In addition, the correlations of serum PTH level with other laboratory parameters of MM patients were examined.

The collected data were analyzed by PASW version 18.0 (SPSS Inc., Chicago, IL, USA). In detail, Spearman's correlation analysis was performed to evaluate the association between various laboratory parameters with serum PTH level. Pearson's chi-square test or Fisher's exact test was performed to calculate the significance of association between PTH group and other parameters of discrete categorical variables including gender and IMWG response. Kruskall Wallis test or Mann Whitney U test was used to compare serum PTH level according to categorical classification such as IMWG response or monoclonal protein subtype. Additionally, Mann Whitney U test was performed to compare continuous variables such as age and laboratory data between non-high PTH and high PTH group.

Time dependent Cox regression analysis was used to dissect the individual impacts of prognostic factors of overall survival (OS) of MM patients. Kaplan-Meier estimation was used to plot survival curves, and log-rank tests were used to calculate the difference of OS and progression-free survival (PFS) between high PTH group and non-high PTH group. All tests were two-tailed and a P value of less than 0.05 was considered statistically significant.

3. Results and Discussion

Serum PTH level of 115 myeloma patients was 24.7 ± 34.9 (ranged 0.0–284.1) pg/mL. The reference range of serum PTH was 15–68.3 pg/mL in our laboratory. Of the various laboratory data of MM patients, higher levels of bone marrow plasma cell percentage, monoclonal protein concentration, creatinine, beta-2 microglobulin, and lactate dehydrogenase were seen in high PTH group rather than non-high PTH group (Table 1). And calcium level was significantly different ($P = 0.016$, Figure 2(a)) by the comparison of laboratory data (continuous variables) between non-high PTH group and high PTH group (cut-off PTH level = 68.3 pg/mL, Mann Whitney U test).

Previously, Arnulf et al. [8] reported that the prevalence of monoclonal gammopathy was high in patients with primary hyperparathyroidism compared to general population and that high PTH levels might facilitate the emergence of a plasma cell clone. This is consistent with above findings of higher levels of plasma cell percentage and monoclonal protein concentration in high PTH group.

On the other hand, male patient case, case with age of above 50 years, IgG kappa monoclonal protein type, bone lesion case, ISS stage III, and progressive disease case (by IMWG response criteria) occupied the main portion in both non-high PTH group and high PTH group (Table 1). Of the various clinical parameters, gender factor (male or female) revealed significant difference between non-high PTH group and high PTH group ($P = 0.017$) (Table 1).

The serum levels of IgG, IgM, FLC-lambda, albumin, and LDH were in positive correlation with serum PTH. However, age, plasma cell percentage, monoclonal protein, IgA, FLC-kappa, FLC ratio, calcium, creatinine, Hb, and beta-2 microglobulin showed negative correlation with PTH (detailed data not shown). Among those above, IgM (rho = 0.190, $P = 0.045$) and calcium (rho = −0.220, $P = 0.043$) revealed statistically significant correlation with serum PTH (Figure 1). PTH may mediate the induction of multiple myeloma. Also, PTH can interact with other various chemokines, ligands, or hematopoietic niche. This process might influence the correlation. However, in terms of pathogenesis, the direct effect or meaning of correlation of PTH with IgM or calcium needs to be determined through further study.

In addition, serum PTH level in MM patients was not significantly different according to IMWG response ($P = 0.450$), ISS stage ($P = 0.414$), monoclonal protein subtype ($P = 0.572$), FISH result ($P = 0.105$), and chromosome analysis result ($P = 0.353$). Meanwhile, there was no significant difference of PTH according to gender ($P = 0.250$), age (above 50 years or below 50 years, $P = 0.423$), existence or nonexistence of clinical events including death ($P = 0.571$), disease progression ($P = 0.322$), bone lesion ($P = 0.207$), transplantation ($P = 0.233$), and chromosome abnormality ($P = 0.124$).

Table 1: Baseline characteristics in MM patients with non-high and high PTH level.

	Non-high PTH group (N = 108, 93.9%)	High PTH group (N = 7, 6.1%)	P
Gender*			0.017
Male	57 (52.8)	7 (100.0)	
Female	51 (47.2)	0 (0.0)	
Age*			0.351
>50 years	96 (88.9)	7 (100.0)	
≤50 years	12 (11.1)	0 (0.0)	
Mean survival (months)	51	29	0.789
Laboratory data†			
PTH (pg/mL)‡	12.5 (0.0–64.9)	91.1 (75.8–284.1)	0.000
Bone marrow-plasma cell (%)	32 (5–90)	44 (24–97)	0.184
Monoclonal protein (g/dL)	4.1 (0.0–8.7)	5.1 (1.9–6.2)	0.539
IgG (mg/dL)	2400 (151–80000)	772 (147–8430)	0.819
IgA (mg/dL)	48.0 (7.0–8630.0)	48.0 (10.0–8750.0)	0.593
IgM (mg/dL)	17.9 (5.3–153.0)	18.0 (17.0–226.0)	0.761
FLC-kappa (mg/L)	32.9 (0.3–62500.0)	27.0 (1.0–67.0)	0.298
FLC-lambda (mg/L)	20.0 (1.0–33900.0)	29.2 (2.8–867.0)	0.734
FLC ratio	2.2 (0.0–13242.4)	0.6 (0.0–16.3)	0.555
Ca (mg/dL)	8.7 (4.0–16.0)	7.9 (7.2–8.6)	0.016
Cr (mg/dL)	1.1 (0.6–12.0)	2.4 (0.7–9.4)	0.062
Hb (g/dL)	9.4 (5.0–15.0)	10.1 (5.7–13.2)	0.734
Albumin (g/dL)	3.1 (1.1–5.1)	3.5 (2.4–4.0)	0.721
β2-MG (μg/L)	3981.5 (620.4–80000)	7522.0 (2078.0–33035.5)	0.342
LD (IU/L)	356.0 (85.0–1113.0)	364.0 (239.0–714.0)	0.595
Monoclonal protein type*			0.932
IgGκ	36 (33.3)	2 (28.6)	
IgGλ	27 (25.0)	1 (14.3)	
IgAκ	20 (18.5)	2 (28.6)	
IgAλ	11 (10.2)	1 (14.3)	
Free κ	4 (3.7)	0 (0.0)	
Free λ	9 (8.3)	1 (14.3)	
Not available	1 (0.9)	0 (0.0)	
Bone lesion case*	76 (70.4)	5 (71.4)	0.953
Transplantation case*	26 (24.1)	2 (28.6)	0.788
Chromosome abnormality case*	17 (15.7)	1 (14.3)	0.918
ISS*§			0.685
Stage I	25 (23.1)	1 (14.3)	
Stage II	39 (36.1)	2 (28.6)	
Stage III	44 (40.7)	4 (57.1)	
IMWG response*			0.710
CR	23 (21.3)	2 (28.6)	
VGPR	8 (7.4)	1 (14.3)	
PR	17 (15.7)	0 (0.0)	
Progressive disease	47 (43.5)	3 (42.9)	
Stable disease	6 (5.6)	1 (14.3)	
Not available	7 (6.5)	0 (0.0)	

*Number of patients (%). †Median (range).

‡The reference range of PTH was 15–68.3 pg/mL. We have classified patient cohorts into non-high PTH group and high PTH group based on this criteria.

§International staging system: I, β2-MG <3500 μg/L and albumin ≥3.5 g/dL; II, not fitting stage I or II; stage III, β2-MG ≥5500 μg/L.

PTH: parathyroid hormone; FLC: serum free light chain; Ca: calcium; Cr: creatinine; Hb: hemoglobin; β2-MG: beta-2 microglobulin; LD: lactate dehydrogenase; κ: kappa; λ: lambda; ISS: international staging system; IMWG: international myeloma working group; CR: complete response; VGPR: very good partial response; PR: partial response.

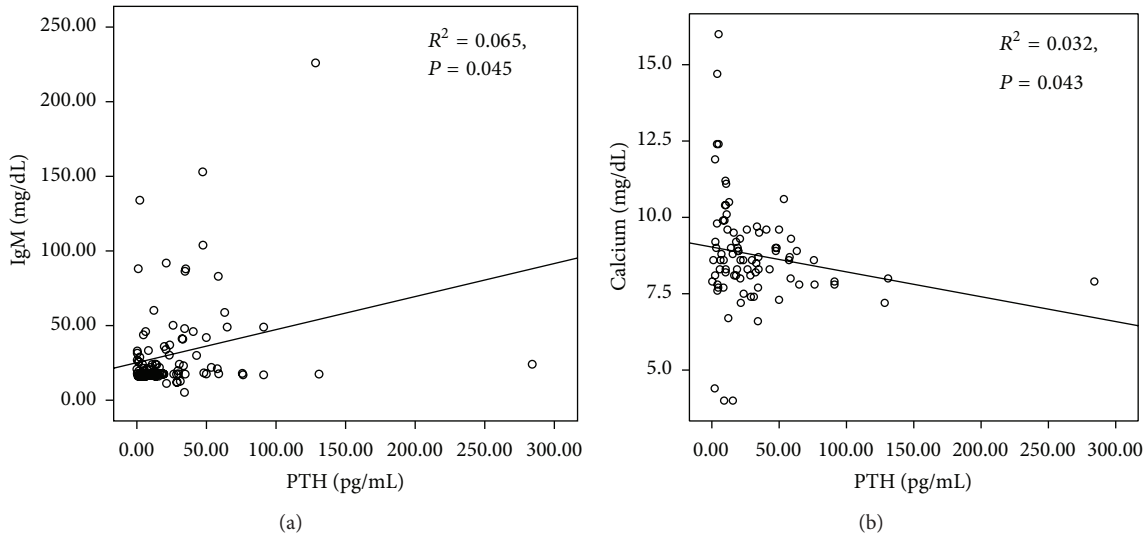


Figure 1: The correlations of PTH values with IgM (a) and calcium (b). Out of many other results of laboratory test performed for multiple myeloma patients, IgM (rho = 0.190, P = 0.045) and calcium (rho = −0.220, P = 0.043) showed meaningful correlation with serum PTH.

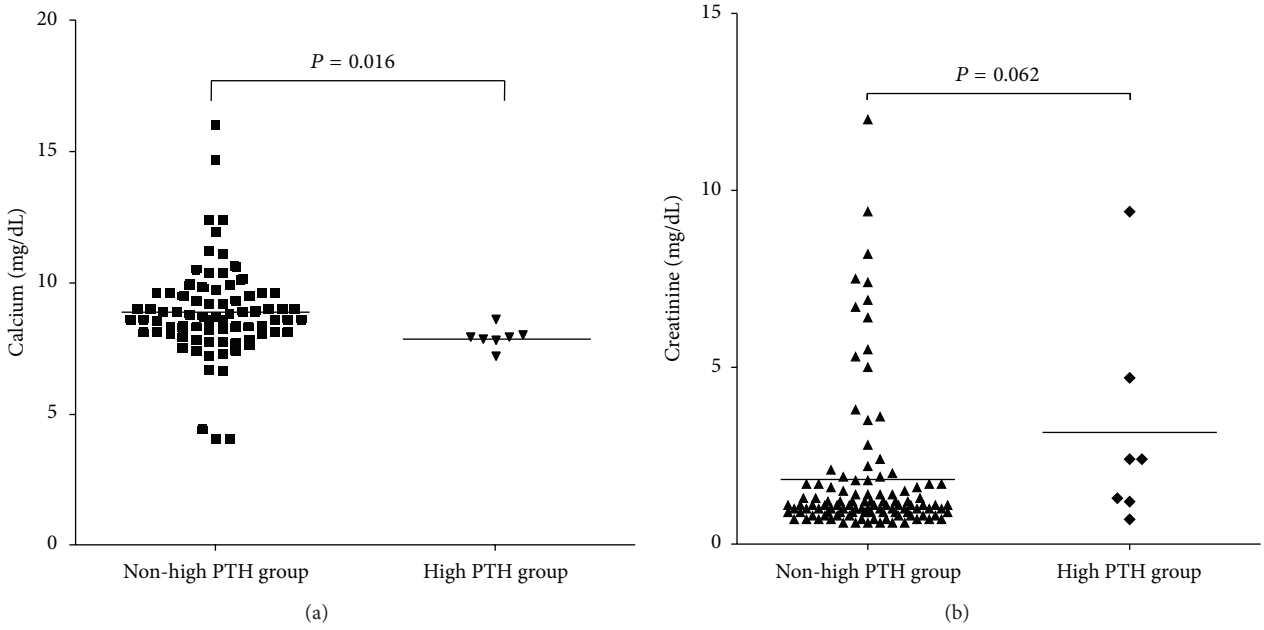


Figure 2: The comparison of calcium (a) and creatinine (b) level between non-high PTH group and high PTH group. Among the various clinical parameters of multiple myeloma patients, calcium (P = 0.016) and creatinine (P = 0.062) revealed moderate difference depending on the PTH level.

Then again, seven patients out of total 115 multiple myeloma patients did not receive treatment. Among 108 patients who were treated, 76 patients (70.4%) underwent a single set of treatment, while 32 patients (29.6%) were treated by multiple sets serially. In detail, CTD (cyclophosphamide, thalidomide, and dexamethasone) was the basic treatment modality in 48 patients, VCD (vincristine, cyclophosphamide, and dexamethasone) in 22 patients, MP (melphalan and prednisone) in 14 patients, and dexamethasone in 13 patients. And the therapeutic modalities were not significantly different between non-high PTH group and high PTH group.

According to Pirih et al. [1], PTH acts on bone marrow stromal cells to stimulate IL-6 production. Then, IL-6 synergizes with fms-like tyrosine kinase 3 ligand (Flt-3L), and it increases hematopoietic cell numbers. Namely, PTH acts with Flt-3L to maintain hematopoietic cells by limiting apoptosis. Also, Shiozawa et al. [10] showed that increasing the number of hematopoietic stem cell niche with PTH promoted metastasis, which means that hematopoietic stem

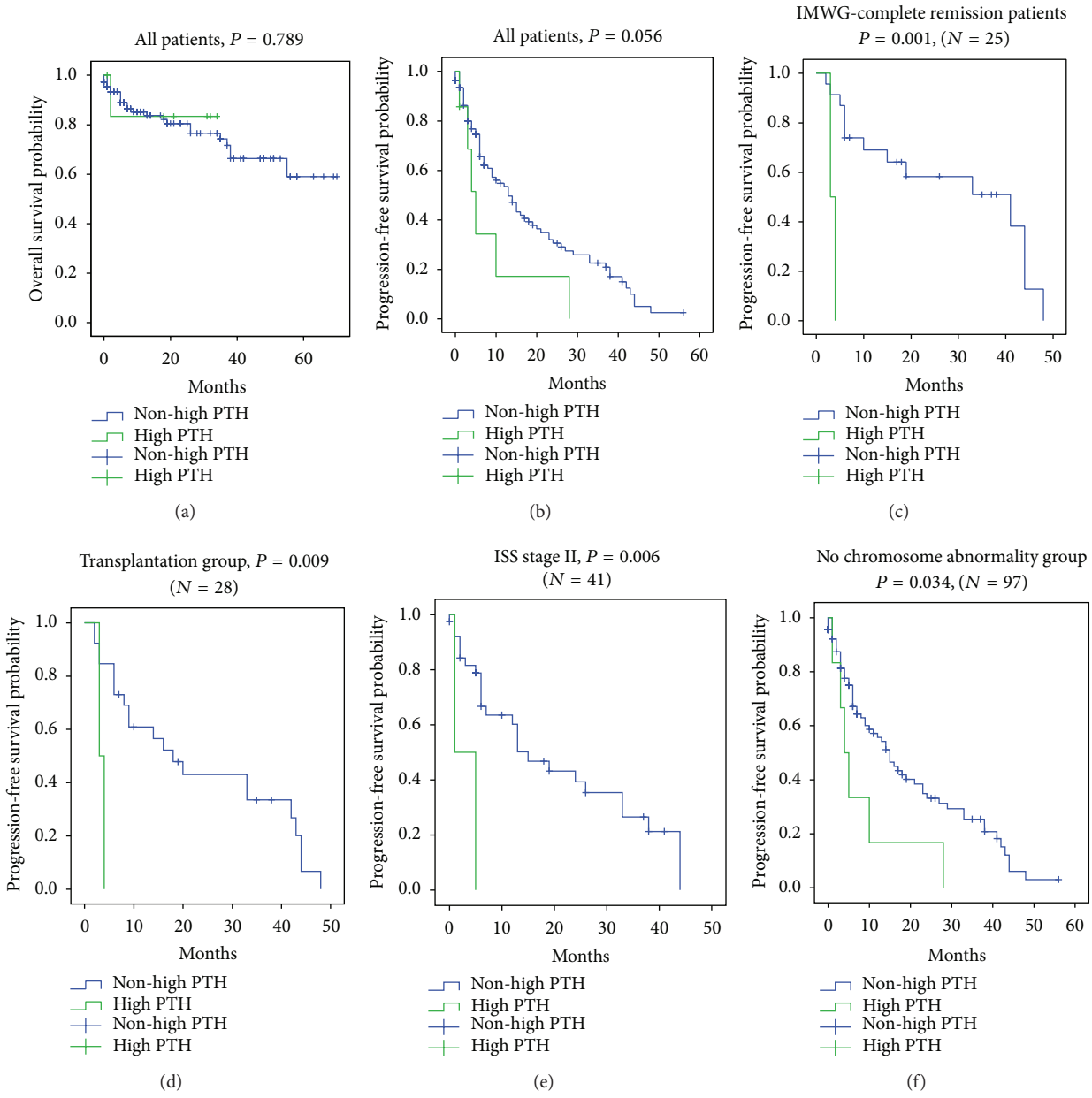


Figure 3: Impact of PTH on clinical outcome. Kaplan-Meier curves of overall survival (OS) and progression-free survival (PFS) stratified by PTH level for all patients (a-b), PFS of patients on complete remission state at the end-point of present study (c), PFS of patients with ISS stage II (d), PFS of patients with no chromosome abnormality (e), and PFS of patients who have undergone stem cell transplantation (f).

cell niche served as a specific site where disseminated cells from original cancer gain footholds in the bone marrow.

Furthermore, the role of the bone marrow microenvironment in MM has been extensively studied in many models. The bone marrow provides signals that influence the behavior of MM cells (e.g., tumor cell growth, survival, and migration). And the cellular elements of the bone marrow (e.g., mesenchymal stem cells, osteoclasts, osteoblasts, and vascular endothelial cells) interact with MM cells directly or indirectly through secretion of stimulatory cytokines and chemokines such as IL-6 that induces survival, growth advantage, and drug resistance of MM [11, 12].

As the precedent study and reports mentioned above, the present study shows interesting results as well. Compared to non-high PTH (<68.3 pg/mL) group, the hazard ratio (HR) for overall survival was higher for the group with high PTH level (≥68.3 pg/mL) (HR, 1.710; $P = 0.766$; 95% confidence interval, 0.050~58.212) (Table 2). With regard to the prognostic implication of serum PTH value, the high PTH group (≥68.3 pg/mL) showed moderate, more, inferior PFS than non-high PTH group (median, 5 months versus 13 months, $P = 0.056$; Figure 3(b)).

By contrast, Pennisi et al. [13] reported that *in vivo* PTH treatment indirectly attenuated MM progression by

Table 2: Time-dependent Cox regression analysis for the overall survival in MM patients.

Factors	Hazard ratio	95% CI	P
PTH			
≥68.3	1.710	0.050~58.212	0.766*
<68.3	1.0 (reference)		
Age	1.004	0.952~1.058	0.894
Sex			
Male versus Female	1.294	0.422~3.968	0.652
ISS stage			
I versus II	0.138	0.015~1.250	0.078
I versus III	0.171	0.017~1.683	0.130
Albumin	0.136	0.038~0.491	0.002†
β2-MG	1.0	1.000~1.000	0.963
Calcium	1.271	0.965~1.675	0.088
Creatinine	1.126	0.735~1.725	0.586
Hemoglobin	1.097	0.824~1.462	0.526
FLC ratio	1.0	1.000~1.000	0.030†

*All parameters considered; the overall P value of PTH was 0.557.
†Statistical significance.
PTH: parathyroid hormone; FLC: serum free light chain.

stimulating osteoblastogenesis and increasing osteoblast production of antimyeloma factors and by minimizing oxidative stress and inflammatory conditions in myelomatous bone. On the other hand, PTH receptors were not expressed by myeloma cells and PTH had no effect on myeloma cell growth *in vitro*. Therefore, it seems that the clinical and prognostic implication of serum PTH level in MM patients still remains a controversial topic.

Nevertheless, present study was not based on experimental findings but on the clinical and practical data of MM patients. In addition, following results of the present study are consistent with the report of Pirih et al. [1].

Although no OS differences were found between high PTH group and non-high PTH group (Figure 3(a)), subgroup analysis revealed that the patients with high serum PTH level significantly had an inferior PFS than those with non-high serum PTH level. In detail, the subgroup analysis of PFS (high PTH group versus non-high PTH group) were as follows: patients ($N = 25$) who reached the complete remission (CR) state defined by IMWG response criteria at the end of the follow-up period (median, 3 months versus 41 months, $P = 0.001$; Figure 3(c)), patients ($N = 28$) who have undergone transplantation (median, 3 months versus 18 months, $P = 0.009$; Figure 3(d)), patients ($N = 41$) who belonged to ISS stage II (median, 1 month versus 15 months, $P = 0.006$; Figure 3(e)), and patients ($N = 97$) with no chromosome abnormalities (median, 4 months versus 15 months, $P = 0.034$, Figure 3(f)).

4. Conclusion

Serum PTH level at diagnosis was associated with risk factors and clinical outcome in MM patients, especially in CR group (IMWG response), transplantation case, ISS stage II, and case without having chromosome abnormality.

Conflict of Interests

The authors have no conflict of interests to declare.

Authors' Contribution

Min-Gu Kang and Eun-Jeong Won contributed equally to this work.

Acknowledgments

This study was supported by the National Research Foundation of Korea (NRF) (grant no. 2011-0015304), the NRF Basic Science Research Program (Grant 2010–0024326), the Leading Foreign Research Institute Recruitment Program (no. 2011-0030034) through the NRF funded by the Ministry of Education, Science and Technology (MEST), and a Grant from the National R&D Program for Cancer Control, Ministry of Health & Welfare, Republic of Korea (no. 2013-1320070).

References

[1] F. Q. Pirih, M. N. Michalski, S. W. Cho et al., "Parathyroid hormone mediates hematopoietic cell expansion through interleukin-6," *PLoS ONE*, vol. 5, no. 10, Article ID e13657, 2010.

[2] G. B. Adams, R. P. Martin, I. R. Alley et al., "Therapeutic targeting of a stem cell niche," *Nature Biotechnology*, vol. 25, no. 2, pp. 238–243, 2007.

[3] J. Zhang, C. Niu, L. Ye et al., "Osteoblastic cells regulate the haematopoietic stem cell niche," *Nature*, vol. 425, no. 6960, pp. 836–841, 2003.

[4] K. K. Ballen, E. J. Shpall, D. Avigan et al., "Phase I trial of parathyroid hormone to facilitate stem cell mobilization," *Biology of Blood and Marrow Transplantation*, vol. 13, no. 7, pp. 838–843, 2007.

[5] E. P. Pest, G. McQuaker, J. A. Hunter, D. Moffat, and A. J. Stanley, "Primary hyperparathyroidsm, amyloid and multiple myeloma: an unusual association," *Scottish Medical Journal*, vol. 50, no. 1, pp. 32–34, 2005.

[6] E. M. Greenfield, M. C. Horowitz, and S. A. Lavish, "Stimulation by parathyroid hormone of interleukin-6 and leukemia inhibitory factor expression in osteoblasts is an immediate-early gene response induced by cAMP signal transduction," *Journal of Biological Chemistry*, vol. 271, no. 18, pp. 10984–10989, 1996.

[7] S. P. Treon and K. C. Anderson, "Interleukin-6 in multiple myeloma and related plasma cell dyscrasias," *Current Opinion in Hematology*, vol. 5, no. 1, pp. 40–48, 1998.

[8] B. Arnulf, D. Bengoufa, E. Sarfati et al., "Prevalence of monoclonal gammopathy in patients with primary hyperparathyroidism: a prospective study," *Archives of Internal Medicine*, vol. 162, no. 4, pp. 464–467, 2002.

[9] R. A. Kyle and S. V. Rajkumar, "Criteria for diagnosis, staging, risk stratification and response assessment of multiple myeloma," *Leukemia*, vol. 23, no. 1, pp. 3–9, 2009.

[10] Y. Shiozawa, E. A. Pedersen, A. M. Havens et al., "Human prostate cancer metastases target the hematopoietic stem cell

niche to establish footholds in mouse bone marrow," *The Journal of Clinical Investigation*, vol. 121, no. 4, pp. 1298–1312, 2011.

[11] A. Palumbo and K. Anderson, "Multiple myeloma," *The New England Journal of Medicine*, vol. 364, no. 11, pp. 1046–1060, 2011.

[12] I. M. Ghobrial, "Myeloma as a model for the process of metastasis: implications for therapy," *Blood*, vol. 120, no. 1, pp. 20–30, 2012.

[13] A. Pennisi, W. Ling, X. Li et al., "Consequences of daily administered parathyroid hormone on myeloma growth, bone disease, and molecular profiling of whole myelomatous bone," *PLoS ONE*, vol. 5, no. 12, Article ID e15233, 2010.

Hindawi Publishing Corporation
BioMed Research International
Volume 2014, Article ID 456037, 14 pages
http://dx.doi.org/10.1155/2014/456037

Review Article

Salvage Therapy of Multiple Myeloma: The New Generation Drugs

Alessandra Romano,[1,2,3] Concetta Conticello,[3] Maide Cavalli,[3] Calogero Vetro,[1,3] Cosimo Di Raimondo,[3] Valentina Di Martina,[1,3] Elena Schinocca,[1,3] Alessia La Fauci,[1,3] Nunziatina Laura Parrinello,[1,3] Annalisa Chiarenza,[3] and Francesco Di Raimondo[1,3]

[1] *Hematology Section, Department of Clinical and Molecular Biomedicine, University of Catania, Via Citelli 6, 95124 Catania, Italy*
[2] *Fondazione Veronesi, Via Lancellotti 18, 00186 Roma, Italy*
[3] *UOC di Ematologia con Trapianto di Midollo Osseo, Ospedale Ferrarotto, Via Citelli 6, 95124 Catania, Italy*

Correspondence should be addressed to Francesco Di Raimondo; diraimon@unict.it

Received 20 February 2014; Accepted 2 May 2014; Published 19 May 2014

Academic Editor: Dong Soon Lee

During the past decade, overall results of treatment of multiple myeloma (MM) have been improved and survival curves are now significantly better with respect to those obtained with historical treatment. These improvements are linked to a deeper knowledge of the biology of disease and to the introduction in clinical practice of drugs with different mechanism of action such as proteasome inhibitors and immunomodulatory drugs (IMiDs). However, MM remains in most cases an incurable disease. For patients who relapse after treatment with novel agents, the prognosis is dismal and new drugs and therapeutic strategies are required for continued disease control. In this review, we summarize new insights in salvage therapy for relapsed/refractory MM as emerging from recent clinical trials exploring the activity of bendamustine, new generation proteasome inhibitors, novel IMiDs, monoclonal antibodies, and drugs interfering with growth pathways.

1. Introduction

During the past decade, overall results of treatment of multiple myeloma (MM) have been improved and survival curves are now significantly better with respect to those obtained with historical treatment. These improvements are linked to a deeper knowledge of the biology of disease and to the introduction in clinical practice of drugs with different mechanism of action such as proteasome inhibitors (bortezomib, carfilzomib) and immunomodulatory drugs (IMiDs; thalidomide, lenalidomide, and pomalidomide) [1].

However, MM remains in most cases an incurable disease, and new drugs and therapeutic strategies are required for continued disease control. In this perspective, several new drugs are currently undergoing evaluation, and many appear very promising on the basis of reported initial results [2, 3].

The natural history of MM includes recurrence of active disease defined as relapse when salvage treatment is needed after an off-therapy period, or refractory disease if nonresponsive while on salvage therapy, or progressing within 60 days of last therapy (see the following part, [4]).

Definition of Progressive Disease in accord with MM Uniform Reporting of Clinical Trials, Report of the 2008 International Myeloma Workshop Consensus:

Definitions of Relapsed/Refractory MM

Refractory myeloma. It is a disease that is nonresponsive while being on salvage therapy or progresses within 60 days of last therapy.

Primary refractory myeloma. It is a disease that is nonresponsive in patients who have never achieved a minor response with any therapy.

Relapsed myeloma. After a period of being off therapy, it requires the initiation of salvage therapy.

Relapsed-and-refractory myeloma. It is nonresponsive while being on salvage therapy (achieved minor response (MR) or better at some point in their disease course).

Most of the studies reporting results of the new drugs are difficult to interpreter because they are small single arm studies, they deal with very heterogeneous groups of patients, and quite often relapsed and refractory patients are lumped together. In addition, there is a lack of information about the natural history of MM in the relapsed setting, after exposure to novel agents.

A recent study described the poor outcome of patients who are refractory to current treatments and provided context for interpreting trials of new drugs [5]. This study included 286 patients with relapsed MM, who were refractory to bortezomib and were relapsed following an IMiD. Median age at diagnosis was 58 years, and time from diagnosis to salvage treatment (T0) was 3.3 years. The first regimen contained bortezomib in 26% patients and an IMiD in 33% of patients. A minor or better response was achieved after at least one therapy after T0 in 44% of patients, including partial response (PR) in 32%. The median overall survival (OS) and event-free survival (EFS) from T0 were 9 and 5 months, respectively.

Thanks to new information on the biology of MM plasma cells and deeper knowledge of the metabolic pathways that the neoplastic cells use for their growth, new drugs have been developed. However, the new therapeutic strategies for relapsed and refractory patients arise not only from drugs with a new mechanism of action, targeting the deregulated pathways in MM, but also from analogs of agents already approved for treatment of MM such as new chemotherapy drugs, second and third generation of proteasome inhibitors, and new IMiDs.

In addition, it is not clear if the novel drugs should be used in monotherapy or in combination. Looking at the emerging tumor biology, combination therapy has the potential benefit of suppressing and eliminating more subclones at the same time, but patients with refractory disease may be more debilitated and unable to tolerate aggressive combination treatments. Among patients with early relapse, the use of a 3-drug regimen (bortezomib, thalidomide, and dexamethasone) improved overall response rate (ORR), depth of response, and progression-free survival with a trend toward improved overall survival [6]. Ongoing phase 3 studies will provide further evidence to address if 3-drug based regimens are better than 2 drugs, using other new agents such as carfilzomib (carfilzomib/lenalidomide/dexamethasone versus lenalidomide/dexamethasone), panobinostat (panobinostat/bortezomib/dexamethasone versus bortezomib/dexam ethasone), elotuzumab (elotuzumab/lenalidomide/dexamethasone versus lenalidomide/dexamethasone), and pomalidomide (pomalidomide/bortezomib/dexamethasone versus bortezomib/dexamethasone).

2. Novel Alkylators

Alkylators, such as melphalan and cyclophosphamide, are the backbone for the combination with the new drugs, although eventually patients develop resistance against them and the search for new alkylating agents is fully justified. In this scenario, MM researcher has shown an increased interest for bendamustine, an older alkylating drug developed behind the Iron Curtain but only recently used in Western countries.

Bendamustine is a nitrogen mustard with both purine analogue and alkylating cytotoxic effects for its unique chemical structure: a 2-chloroethylamine alkylating group (like melphalan and cyclophosphamide), a benzimidazole ring, and a butyric acid side chain (like in chlorambucil) [25]. Therefore, bendamustine can activate apoptosis and inhibit mitotic checkpoints instead of inducing necrosis alone, as all other alkylators do.

Bendamustine is effective as a single agent and in several combinations with novel agents (bortezomib, thalidomide, and lenalidomide) for the treatment of relapsed/refractory MM (Table 1).

In a phase I study involving 31 patients with progressive disease after autologous stem cell transplantation (ASCT), bendamustine was tested starting from 60 mg/m^2 on days 1 and 2 of each 28-day cycle. Dose-limiting toxicity of febrile neutropenia developed in one patient after bendamustine 100 mg/m^2 with an overall response rate (ORR) of 55%. Median duration of response was 8 months and median progression-free survival (PFS) for the whole study population was 26 weeks and for the patients receiving 90 or 100 mg/m^2 it was 36 weeks [26].

A phase I-II study including 40 MM patients previously exposed to bortezomib or alkylators tested the association of escalating doses of bendamustine 50, 70, or 90 mg/m^2 (days 1 and 4) plus bortezomib 1.0 mg/m^2 (days 1, 4, 8, and 11) for up to eight 28-day cycles [7]. The bendamustine MTD was 90 mg/m^2. The most common grade 3/4 adverse events were leucopenia (58%), neutropenia (50%), lymphopenia (45%), and thrombocytopenia (30%). ORR was 48% (one CR, two VGPR, nine PR, and seven MR) for all 40 enrolled patients, 52% (16/31) at the MTD [7].

After these encouraging results, phase II studies tested the efficacy of bendamustine in combination with bortezomib and steroids.

In 79 patients with relapsed/refractory MM, bendamustine was given at 70 mg/m^2 on days 1 and 4, bortezomib 1.3 mg/m^2 on days 1, 4, 8, and 11, and dexamethasone 20 mg on days 1, 4, 8, and 11, every 28 days up to eight cycles (BBD). With this regimen, grades 3 and 4 anemia and leukopenia were seen in 18.7% of patients, while grade 4 thrombocytopenia was observed in 6%. Grades 3 and 4 infections were observed in 20% of patients, with two deaths (3%) because of infection. In addition, a doubling of the incidence of self-assessed grade 2 neurotoxicity from baseline to cycle 8 was documented and grade 3-4 neuropathy was observed in 7% of patients at the last treatment cycle. BBD induced a very fast response with an ORR of 75.9% (including 15% CR), and PFS was 9.7 months. Preexposure to lenalidomide was correlated with a lower response rate and shorter time to progression (TTP). Of interest, incidence of response, its duration, and OS were not different between patients defined as low risk or high risk according to cytogenetics [8].

Table 1: Regimens containing bendamustine and novel agents.

Combination with novel agents: bortezomib					ORR	PFS	Reference
phase I-II	Bendamustine	90 mg	i.v.	Day 1 and day 4 every 28 days for up 8 cycles	52%	not reached	Berenson et al., 2013 [7]
	Bortezomib	1.0 mg/m^2		days 1, 4, 8, and 11 every 28 days for up 8 cycles			
BVD phase II	Bendamustine	70 mg	i.v.	Day 1 and day 4 every 28 days for up 8 cycles	76%	9.7 months	Ludwig et al., 2014 [8]
	Bortezomib	1.3 mg/m^2	i.v.	Days 1, 4, 8, and 11 every 28 days for up 8 cycles			
	Dexamethasone	20 mg	i.v.	Days 1, 4, 8, and 11 every 28 days for up 8 cycles			
BVD phase II	Bendamustine	70 mg	i.v.	Day 1 and day 8 every 28 days for up 6 cycles	71.5%	16.5 months	Offidani et al., 2013 [9]
	Bortezomib	1.3 mg/m^2	i.v.	Days 1, 4, 8, and11 every 28 days for 2 cycles and then days 1, 8, 15, and 22 for 4 cycles			
	Dexamethasone	20 mg	i.v./OS	Days 1-2, 4-5, 8-9, and 11-12 every 28 days for 2 cycles and then on days 1, 8, 15, and 22 for 4 cycles			
BPV phase II	Bendamustine	60 mg	i.v.	Day 1 and day 2 every 28 days for up 7 cycles	69%	11 months	Ponish, 2012
	Bortezomib	1.3 mg/m^2	i.v.	Days 1, 4, 8, and 11 every 28 days for up 7 cycles			
	Prednisone	100 mg	i.v.	Days 1, 2, 4, 8, and 11 every 28 days for up 7 cycles			
Combination with novel agents: IMiDs							
BTP phase I	Bendamustine	60 mg	i.v.	Day 1 and day 2 every 28 days up to maximum response or MTD	80%	11 months	Pönisch et al., 2008 [10]
	Thalidomide	50 or 100 or 200 mg	per OS	Days 1–21 every 28 days up to maximum response or MTD			
	Prednisone	100 mg	i.v./per OS	Days 1, 2, 4, 8, and 11 every 28 days up to maximum response or MTD			
BTD phase I-II	Bendamustine	60 mg	i.v.	Day 1 and day 8 every 28 days up to progression	46%	19 months	Yong et al., 2013 [11]
	Thalidomide	100 mg	per OS	Days 1–21 every 28 days up to progression			
	Dexamethasone	20 mg	i.v./per OS	days 1, 8, 15, and 22 every 28 days up to progression			
BLD	Bendamustine	75 mg	i.v.	Day 1 and day 2 every 28 days up to progression	76%	6.1 months	Lentzsch et al., 2012 [12]
	Lenalidomide	10 mg	per OS	Days 1–21 every 28 days up to progression			
	Dexamethasone	40 mg	i.v./per OS	Days 1, 8, 15, and 22 every 28 days up to progression			
BLP	Bendamustine	75 mg	i.v.	Day 1 and day 2 every 28 days up to progression	76%	48% at 18 months	Ponish, 2013
	Lenalidomide	25 mg	per OS	Days 1–21 every 28 days up to progression			
	Prednisone	100 mg	per OS	Days 1–4 every 28 days up to progression			

Similar results were obtained from the Italian group evaluating bendamustine plus bortezomib and dexamethasone (BVD) every 28 days for the first 6 cycles and then every 56 days for 6 further cycles. Seventy-five patients with relapsed/refractory MM, treated with ≤4 prior therapies and not refractory to bortezomib, were treated. Grade 3-4 hematologic toxicities (thrombocytopenia and neutropenia), neuropathy (8%), and gastrointestinal and cardiovascular events were the more frequent side effects. The ORR was 71.5% (including 16% CR). TTP was 16.5 months at a median follow-up of 12 months and 1-year overall survival was 78% [9].

In a multicentric study involving 78 relapsed-refractory MM patients BVP consisted of bendamustine 60 mg/m^2 on days 1 and 2, bortezomib 1.3 mg/m^2 on days 1, 4, 8, and 11, and prednisone 100 mg on days 1, 2, 4, 8, and 11. ORR was 69% with PFS of 11 months, superior to not-heavily pretreated patients [27]. BVP was effective also in the cohort of 36 patients with light chain-induced renal failure (creatinine clearance <60 mL/min), achieving ORR 67% [28].

The optimal dose of bendamustine associated with thalidomide is 60 mg, as shown by two independent phase 1-2 studies [10, 11]. In phase 1 study bendamustine 60 mg plus prednisone 100 mg was tested in association with increasing doses of thalidomide (50, 100, and 200 mg), without achieving the thalidomide MTD in 28 relapsed/refractory patients. Twenty-four patients responded after at least two cycles (4 CR, 6 VGPR, and 14 PR), with ORR 80% and PFS of 11 months. Only mild/moderate nonhematological side effects were observed and no patient developed dose-limiting hematotoxicity [10].

Two recent phase 1-2 studies identified MTD of bendamustine as 75 mg/m^2 when associated with lenalidomide. In the American study, involving 29 patients relapsed after a median number of 3 previous treatments, bendamustine 75 mg/m^2 (days 1 and 2) plus lenalidomide 10 mg (days 1–21), and weekly dexamethasone 40 mg resulted in ORR 76% and PFS of 6.1 months. Hematological toxicity, including 3-4 grade neutropenia, thrombocytopenia, and anemia, was responsible for discontinuation of treatment in one-third of patients. Nonhematological side effects included fatigue, diarrhea, hypocalcemia, hyperglycemia, and nausea [12].

In the German study, enrolling 21 patients in five cohorts treated with bendamustine (60 up to 75 mg/m^2), lenalidomide (10 up to 25 mg), and prednisone 100 mg, the MTD was not reached. Authors suggested the best schedule as bendamustine 75 mg/m^2 on days 1-2, lenalidomide 25 mg on days 1–21 every 28 days, and prednisone 100 mg on days 1–4, to achieve ORR 76% and PFS of 48% at 18 months [29].

Thus, bendamustine is effective in monotherapy and in combination with novel agents at reduced doses to limit toxicity but longer follow-up is needed to overcome the lack of information on overall survival.

3. Novel Proteasome Inhibitors

Bortezomib is a dipeptide boronic analog that reversibly inhibits the chymotryptic activity of the 20S subunit of the proteasome [30]. Resistance to bortezomib has been documented both in vitro and in vivo, due to upregulation of the proteasome subunits, mainly for increased levels of the β5-subunit. However, there is no clear quantitative correlation between level of resistance and the extent of β5-subunit expression. Moreover, although specific variants of the proteasome genes which encode the β subunits of the 20S proteasome (PSMB5) have been previously identified in preclinical models of bortezomib resistance, these variants were not detected in patient tumor samples collected after clinical relapse from bortezomib, which suggests that alternative mechanisms may underlie bortezomib lack of sensitivity [31].

Table 2: The most active proteasome inhibitors currently tested in relapsed/refractory myeloma patients.

Drug	Bond to proteasome	Route of administration
Carfilzomib	Reversible	i.v.
Marizomib (NPI-0052)	Irreversible	i.v.; oral
Ixazomib (MLN9708/MLN2238)	Reversible	i.v.; oral
Oprozomib (ONX0912)	Irreversible	oral
Delanzomib (CEP-18770)	Reversible	i.v.; oral

To overcome resistance to bortezomib, second and third generations of proteasome inhibitors have been developed, characterized by an irreversible bond to β5-subunit (such as carfilzomib) or binding to different subunits (such as marizomib) or the possibility of oral administration (such as ixazomib, Table 2 and Figure 1).

Carfilzomib gained FDA approval in 2012 for MM patients who have received at least two prior therapies, including bortezomib and an IMiD, and have demonstrated disease progression on or within 60 days of completion of the last therapy, as consequence of promising findings of four phase 2 clinical trials (PX-171-003-A0, PX-171-003-A1, PX-171-004, and PX-171-005).

In PX-171-003-A0, 20 mg/m^2 carfilzomib was evaluated in 46 refractory patients, with a median of 3 cycles administered [32]. Infusion reactions were observed including fever, chills, myalgia, facial swelling or flushing, vomiting, weakness, hypotension, chest tightness, or shortness of breath. Thus, prophylactic premedication with very low-dose dexamethasone (4 mg) prior to carfilzomib in cycles 1 and 2 is recommended to decrease the incidence and severity of infusion reactions.

In PX-171-003-A1, carfilzomib was tested at 20 or 27 mg/m^2 as single agent using dexamethasone as premedication in 257 patients with relapsed/refractory MM, who responded to at least one therapy. Median number of previous treatment was five, including IMiD and bortezomib and 73% of bortezomib-refractory patients [33]. ORR was 36%, with 5% VGPR (Table 3). Cytogenetic profile was available for 229 patients. ORR was comparable between patients with standard and high-risk profile, the subgroups (25.8% versus 24.6%), while time-to-event endpoints showed a trend of shorter duration in high-risk patients, including median duration of response (5.6 months versus 8.3 months) and

Table 3: New generation drugs in monotherapy or combined to novel agents.

Drug	Association to novel agents	Phase	Dosage	*N*	ORR	Reference
Carfilzomib	Monotherapy	2	20–27 mg/m^2	257	36	[13]
Carfilzomib	In combination with lenalidomide and dexamethasone	1/2	27 mg/m^2	51	78	[14]
Carfilzomib	In combination with pomalidomide	1/2	27 mg/m^2	82	70	[15]
Ixazomib	Monotherapy	1/2	2.97 mg/m^2	32	26	[16]
Pomalidomide	Monotherapy	1/2	4 mg	38	42	[17]
Pomalidomide	In combination with high dose dexamethasone	3	4 mg	455	31	[18]
Pomalidomide	In combination with cyclophosphamide and prednisone	1/2	2.5 mg	69	50	[19]
Perifosine	In combination with bortezomib	1/2	50 mg	84	41	[20]
Perifosine	In combination with lenalidomide	1	50–100 mg	32	73	[21]
Vorinostat	In combination with bortezomib	3	400 mg	637	56.2	[22]
Panobinostat	In combination with bortezomib	1b	20 mg	47	52.9	[23]
Panobinostat	In combination with bortezomib and dexamethasone	2	20 mg	55	34.5	[24]

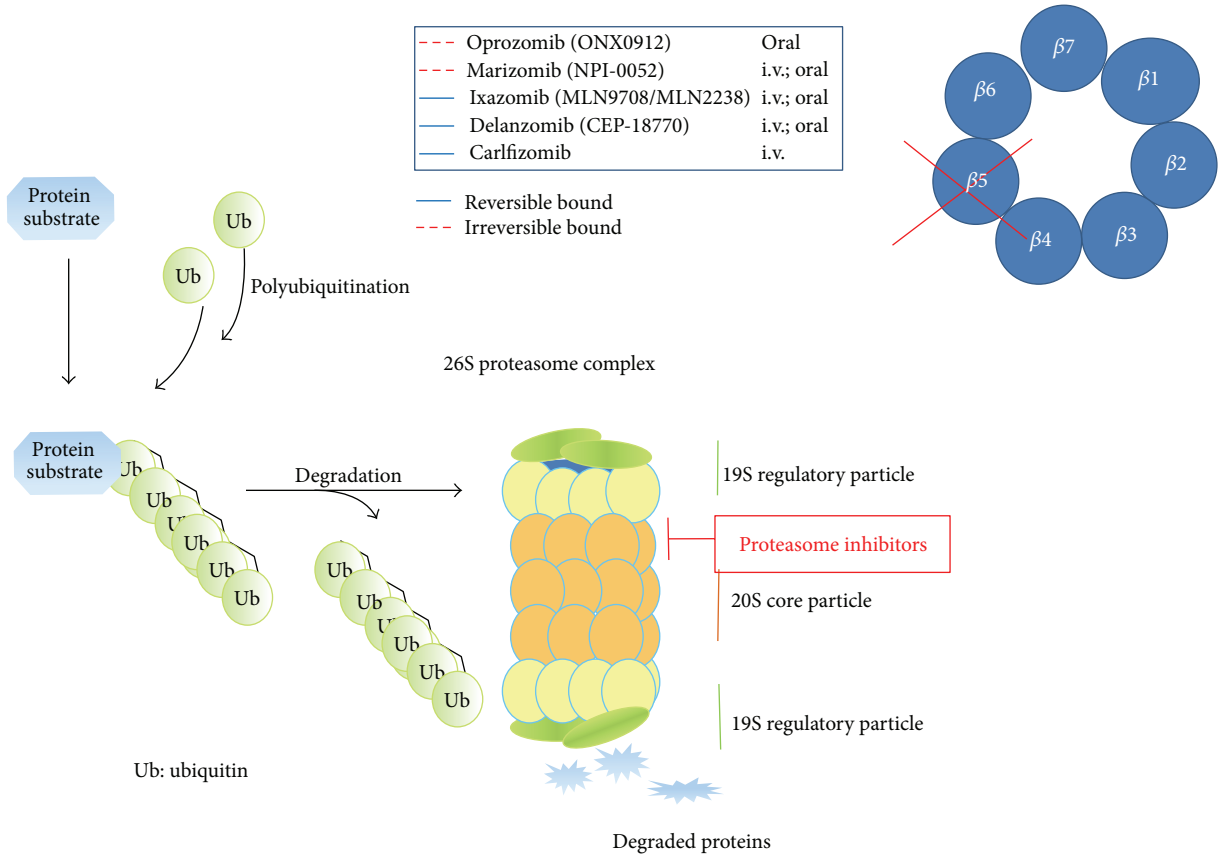


Figure 1

overall survival (9.3 versus 19.0 months). Thus, carfilzomib seems to partially overcome the impact of high-risk cytogenetics on heavily pretreated patients [13].

In the subsequent study PX-171-004, 164 relapsed/refractory patients, previously treated with more than one but less than three therapies and bortezomib naïve, received carfilzomib as single agent (20 or 27 mg/m^2) [34].

In PX-171-005, 50 relapsed/refractory MM patients with renal insufficiency received carfilzomib in monotherapy. The dose was safely escalated to the target dose (from 15 to 20 to 27 mg/m^2) used in patients with normal renal function. Carfilzomib did not appear to be associated with clinically relevant nephrotoxicity, and most patients who experienced irreversible worsening of renal function had clear evidence of progressive MM [35].

Then, a cross-trial analysis examined the safety profile of single-agent carfilzomib in 526 patients with relapsed and/or refractory MM in the above-mentioned four phase II trials upon which US approval was based [36]. Overall, the most common adverse events of any grade were fatigue, anemia,

and nausea. Aggregated cardiac-failure events (including congestive heart failure, pulmonary edema, and decreased ejection fraction) were reported in 7.2%, regardless of causality. The mortality rate was the same (7%) in patients who had baseline cardiac risk factors as it was for patients without these risk factors. Carfilzomib is not actually contraindicated in cardiopathic patients but anecdotic reports suggest a warning in elderly patients. Serial echocardiograms are being conducted in a number of ongoing carfilzomib studies, including a substudy in the randomized phase III trial ENDEAVOR (ClinicalTrials.gov identifier: NCT01568866), which is comparing carfilzomib and dexamethasone versus bortezomib and dexamethasone. Dyspnea, a common complication from the disease itself and from other MM treatments, was reported in 42.2% of patients; however, most incidences were grade 1 or 2 and transient and resolved without dose reduction or discontinuation. There are concerns that dyspnea may develop due to fluid overload, recommended up to 250 mL saline to prevent tumour-lysis syndrome rather than drug toxicity. Hydration has been previously recommended with carfilzomib treatment due to concerns of acute deterioration of renal function [36].

Higher doses of carfilzomib (up to 56 mg/m^2) can be safely infused over 30 minutes and are currently under investigation (PX-171-007, presented in abstract form at ASH 2011). In the ongoing trial ENDEAVOR mentioned above, 56 mg/m^2 carfilzomib is given in combination with dexamethasone in relapsed patients. As shown by three phase-2 studies, carfilzomib activity in monotherapy is inversely correlated with the numbers of previous treatment.

Then, carfilzomib has been tested in association with IMiDs, lenalidomide, and pomalidomide.

The combination of carfilzomib with lenalidomide and low dose dexamethasone (Rd) has shown promising results and appears to be well tolerated in 51 patients affected by relapsed MM after 1–3 prior treatments (75% previously treated with bortezomib) [14]. ORR was 78%, including 41% ≥ VGPR (Table 3). Adverse events were mainly hematological with grade 3-4 anemia, thrombocytopenia, and neutropenia. Some patients (7.5%) experienced grade 3 or 4 fatigue. Shortness of breath as well as hypertension and rare cases of significant cardiac dysfunction were reported. Due to this promising findings, the combination is being further explored in patients with relapsed MM in the randomized phase III trial ASPIRE (ClinicalTrials.gov identifier: NCT01080391), but no data are currently available.

The combination of carfilzomib with pomalidomide and low dose of dexamethasone (Car-Pom-d) was investigated in a multicenter phase I/II trial involving 82 heavily pretreated, lenalidomide-refractory MM patients [15]. The MTD was carfilzomib 27 mg/m^2 on days 1-2, 8-9, and 15-16, pomalidomide 4 mg on days 1–21, and dexamethasone 40 mg on days 1, 8, 15, and 22. Seventy-nine patients with a median of five previous treatments were evaluable for Car-Pom-d clinical activity. The regimen was tolerated well with no unexpected toxicity. Toxicities were generally reversible and manageable with G3-4 neutropenia in one-third of patients and limited G3-4 nonhematological toxicities. Nonhematological adverse events were mainly grade fatigue and dyspnea. However, a case of fatal pneumonia and a case of fatal pulmonary embolism related to treatment occurred. The ORR was 70% (with 27% VGPR, Table 3) with a median duration of response of 17.7 months. Additional 13% of patients experienced a minimal response for a total clinical benefit rate of 83% and a PFS of 9.7 months. The combination was independent from FISH/cytogenetic risk status evaluated according to mSMART profile [37]. Dr. Shah (ASH 2013, abstract number 690) gave an update of the phase I/II dose expansion trial at last ASH annual conference.

Among the new generation of proteasome inhibitors with promising activity in relapsed/refractory, certainly Ixazomib (MLN9708) deserves a mention since it is an oral, reversible, specific 20S proteasome inhibitor, currently tested in clinical trials, with promising activity and a favorable profile of tolerability.

A phase I study evaluated single-agent ixazomib given to 32 patients on days 1, 8, and 15 of a 28-day cycle, for up to 12 cycles. After MTD was assessed at 2.97 mg/m^2, ixazomib was tested in an expansion cohort of further 31 previously treated patients (median 4 lines). In this last cohort, ORR was 26%, including 25% PR (Table 3, [16]).

Oprozomib (ONX 0912) is a new orally bioavailable drug structurally analogue of carfilzomib. Like the latter, oprozomib is a potent, selective, irreversible proteasome inhibitor. In patients with advanced refractory solid tumors, MTD is 150 mg daily [38].

Several other proteasome inhibitors are currently in phase I or in a preclinical stage (Table 1, Figure 1). Future studies will be able to identify those that could represent real promises in the treatment of MM.

4. NOVEL IMiDs

Thalidomide is a racemic glutamic acid analogue, consisting of S− and R+ enantiomers that interconvert spontaneously under physiological conditions thanks to hydrolysis occurring in the liver. The S− form potently inhibits release of tumor necrosis factor (TNF alpha, which regulates apoptosis) from peripheral mononuclear blood cells and is responsible of immunological effects [39].

Thalidomide and its analogs lenalidomide and pomalidomide are effective agents in MM, known for their antiangiogenic and immunomodulatory properties thus to target at the same time neoplastic plasma cells and MM microenvironment. Recently, their molecular target has been identified in the protein complex of cereblon ubiquitin ligase that binds transcription factors of Ikaros family (IKZF-1, IKZF-3) [40–42].

Although lenalidomide arises from thalidomide, the mechanism of action can be different since thalidomide can revert lenalidomide resistance due to aberrancies in Wnt/β-catenin pathway. Under this perspective, in a phase I/II trial the combination of thalidomide and lenalidomide with dexamethasone was investigated in relapsed/refractory MM patients. After the phase I established the MTD in 25 mg lenalidomide/100 mg thalidomide/40 mg dexamethasone, 64 patients with a median of four prior lines of therapy were

enrolled in the phase II of the study. Preliminary results were presented at 2013 ASH meeting by Dr. Shah.

Another strategy to increase the therapeutic effect of lenalidomide is based on its immunological properties, since lenalidomide seems to expand and activate natural killer (NK) cells. On the other hand, myeloma cells upregulate class I antigen of the major histocompatibility complex (MHC) that are ligands for inhibitory killer immunoglobulin-like receptors (KIR) and in this way are able to avoid NK cell killing [43]. IPH2101 is a monoclonal antibody against common inhibitory KIR that increases NK cell activity through inhibition of KIR-ligand interaction. After a phase I showing that IPH2101 is safe [44], preliminary results indicated that this drug can be safely and efficiently combined with lenalidomide, as suggested by Dr. Cohen at 2013 ASH meeting.

However, the most exciting new IMiD in the setting of relapsed/refractory patients is certainly pomalidomide. After the pioneer studies of Mayo Clinic [45], Richardson et al. have recently published a study dedicated to find the MTD, safety, and efficacy of pomalidomide, in 38 patients previously exposed to both bortezomib and lenalidomide (double refractory). With an MTD of 4 mg per day on days 1 to 21 of each 28-day cycle, with or without dexamethasone (40 mg/week), ORR was 42%, including 21% ≥ PR or better and 3% CR. Toxicity was predictable and manageable, with less than 5% of peripheral neuropathy and venous thromboembolism [17].

Preliminary data of the phase 2 study MM-002 have been presented by Dr. Jagannath at 2013 ASH meeting: 221 double-refractory MM patients were randomly assigned to receive either pomalidomide alone at 4 mg days 1–21 or in combination with low-dose dexamethasone (40 mg/week, 20 mg for patients over 75 years, pom + loDex). The ORR in the pom + loDex arm was 43%, with 37% achieving at least a PR. Median PFS and OS were 4.6 months and 16.5 months, respectively, in the pom + loDex arm versus 2.6 mos and 13.6 mos in the pom alone arm. Adverse events led to at least one dose reduction in 26% of patients, with neutropenia being the most common grade 3/4 adverse event. In the pom + loDex arm, grade 1-2 neuropathy occurred in 7% of patients.

Recently, San Miguel et al. reported the final results of a phase 3 trial (MM-003) comparing pomalidomide plus low-dose dexamethasone versus high-dose dexamethasone alone (hiDex) in 455 patients with relapsed or refractory multiple myeloma previously treated with lenalidomide and bortezomib (Table 3). In double refractory patients, PFS was 3.2 versus 1.7 months for pom + loDex versus hiDex and OS was not reached versus 7.4 months, respectively. The rate of grade 3-4 anemia, thrombocytopenia, infections, and VTE was similar in the two arms but neutropenia was more frequent in the pom + loDex arm as well as febrile neutropenia [18].

A final analysis, presented at the 2013 ASH meeting by Dr. San Miguel, confirmed with a longer follow-up that pom + loDex is superior to hi-Dex in terms of response rate and survival, despite 56% of patients on hiDex arm crossed to the pom + loDex arm. In MM-003 45% of patients were aged >65 years: the combination of pom + loDex significantly improved ORR also in elderly patients. Duration of response was significantly longer for pom + loDex versus hiDex in patients aged >65 years and >70 years. The pom + loDex safety profile was consistent by age and study discontinuation due to adverse events was 6% for patients aged <65 years and 13% for patients aged >65 years.

The superiority in ORR and PFS of the pom + loDex combination over hiDex was maintained in patients with moderate renal impairment, with a baseline creatinine clearance <60 mL/min, accordingly to Dr. Dimoupolos' communication at the last ASH meeting 2013 (ASH 2013, abstract number 2939).

On the basis of previous reports indicating that the addition of clarithromycin to lenalidomide and dexamethasone could be associated with improved outcome [46], pomalidomide was tested in association with clarithromycin and low dose dexamethasone (ClaPD). Dr. Boyer presented preliminary data from 114 patients (relapsed or progressed after at least three prior therapies) at ASH 2013. ORR was 70% (with 6% CR and 17% VGPR). In most patients, PFS was sustained for >8 months that is more than double the PFS reported in patients treated with pom + loDex.

Pomalidomide was tested in combination with cyclophosphamide and prednisone in relapsed/refractory patients in 69 patients enrolled in a multicenter phase 1/2 trial. MTD was 2.5 mg/day pomalidomide, cyclophosphamide at 50 mg every other day, and prednisone at 50 mg every other day, for 6 28-day cycles, followed by pomalidomide-prednisone maintenance therapy. Thromboprophylaxis was recommended. In 55 patients treated at MTD, the ORR was 50% including 23% ≥ VGPR, median PFS was 10.4 months, and 1-year overall survival was 69%. At the MTD, grade 3 to 4 toxicities included anemia, thrombocytopenia, neutropenia with grade 3–5 infections, and thromboembolism. Treatment was discontinued for toxicity in 9% of patients [19].

However, the outcome after achieving novel agents' refractoriness is poor. At Mayo, 74 patients from among 183 patients who had relapsed after pomalidomide phase 2 trials were retrospectively evaluated. The most commonly used regimen was bortezomib based (36%), followed by autologous stem cell transplantation (13%), alkylator-steroid combination (11%), VDT-PACE (12%), and lenalidomide based (11%). The highest rate of objective response of PR or better (80%) was seen in patients treated with ASCT. Lenalidomide was active in a proportion of patients relapsing on pomalidomide, suggesting that a trial of lenalidomide in these patients could be justified [47]. In other cases patients should be addressed to clinical trials involving new drugs described below.

5. Drugs Interfering with Growth Pathways

5.1. AKT Inhibitors. After the demonstration that the PI3K/AKT pathway is constitutively active in MM, providing signals that induce proliferation, angiogenesis, and development of drug resistance, several preclinical studies have shown that PI3K/AKT inhibition was able to induce tumor inhibition and regression in cell-line and animal models [48].

Perifosine is an oral AKT inhibitor able to induce cytotoxicity of plasma cells even in the presence of bone marrow stromal cells (BMSC) that confer cell adhesion-mediated drug resistance (CAM-DR). In this perspective, perifosine has shown an in vitro synergism with bortezomib, dexamethasone, and doxorubicin and it has been evaluated in a phase I/II clinical study in combination with bortezomib and dexamethasone in 84 heavily pretreated MM patients, including patients refractory to bortezomib (73%) or to bortezomib and dexamethasone (51%). The selected dose of perifosine was 50 mg/day plus bortezomib 1.3 mg/m^2 and low-dose dexamethasone (20 mg) was added if progression occurred on perifosine plus bortezomib alone (Table 3). In 73 evaluable patients, the ORR was 41% (65% in bortezomib-relapsed and 32% in bortezomib-refractory patients). Median PFS was 6.4 months, and median OS was 25 months. Therapy was generally well tolerated and toxicities, including gastrointestinal adverse effects and fatigue, were manageable with supportive care and dose reductions. Grade ≥ 3 toxicities included thrombocytopenia (23%), neutropenia (15%), anemia (14%), and pneumonia (12%) [20].

Perifosine has been also evaluated in a phase I trial in combination with lenalidomide in relapsed and relapsed/refractory MM. Thirty-two patients received escalating doses of perifosine 50–100 mg daily and lenalidomide 15–25 mg once daily on days 1–21 of each 28-day cycle, plus dexamethasone 20–40 mg weekly. MTD was not reached and the ORR was 73% (including 13% nCR and 10% VGPR). Median PFS was 10.8 months and median OS was 30.6 months. The most common grade 1-2 adverse events were fatigue, diarrhea and grade 3-4 neutropenia, hypophosphatemia, thrombocytopenia, and leucopenia. This study also suggests that the clinical efficacy of perifosine-lenalidomide-dexamethasone is positively associated with phospho-Akt since PFS was longer in patients with high immunostaining of phospho-Akt than those with low staining [21].

Afuresertib (previously GSK2110183) is a potent, orally available, ATP competitive inhibitor of all three isoforms of AKT. A phase I trial has shown that afuresertib is well tolerated with clinical activity as single agent in heavily pretreated MM patients. After the demonstration that adding afuresertib to bortezomib promotes cell death and inhibits phosphorylation of downstream proteins in preclinical models, a clinical trial had been designed to evaluate MTD, safety, tolerability, and response rate of afuresertib, bortezomib, and dexamethasone combination. Dr. Spencer presented promising preliminary data at last ASH 2013 that will be updated soon.

5.2. Histone Deacetylase (HDAC) Inhibitors. Since histone deacetylases (HDACs) regulate cell differentiation and survival, their inhibition results in caspase-dependent and caspase-independent apoptosis. In preclinical studies, HDAC inhibitors affected the adhesion-mediated drug resistance and inhibited tumor growth in xenograft animal models of human MM. Although HDAC inhibitors as single agents have modest activity in MM [49], their combination with other antimyeloma drugs is promising, in particular with bortezomib because proteasome inhibition cooperates with HDAC inhibition of aggresome formation, leading to significant impairment of protein turnover [50]. Moreover, bortezomib transcriptional activity is favored by chromatin remodeling, that represents the molecular basis of the increasing interest in combining bortezomib with inhibitors of histone deacetylase or histone acetyltransferases [30, 51].

Given this rationale, two global multicenter clinical trials (VANTAGE 088 and 095) assessed efficacy and safety of treatment with vorinostat plus bortezomib in patients with relapsed or refractory MM.

In the VANTAGE 088, 637 patients, with a median of two previous treatments, were randomized to receive bortezomib at 1.3 mg/m^2, on days 1, 4, 8, and 11 together with oral vorinostat 400 mg (317 patients) or placebo (320 patients) given once daily on days 1–14 of each 21-day treatment cycle.

ORR was better in the vorinostat group than the placebo group (56.2% versus 40.6%, $P < 0.0001$) with 7.9% versus 5.3% of CR. Median PFS was 7.63 months in the vorinostat group and 6.83 months in the placebo group. Serious adverse events were equally distributed, and an equal percentage of patients discontinued treatment because of drug-related adverse events. However, by considering all grades, some side effects were more pronounced in the vorinostat group such as thrombocytopenia, diarrhea, nausea, and fatigue [22].

The synergistic activity of bortezomib with another pan-deacetylase inhibitor, panobinostat, was also investigated. In a phase Ib dose-escalation study, panobinostat was given orally thrice weekly every week in combination with bortezomib (21-day cycles) in 47 relapsed/refractory patients. After MTD was determined, additional 15 patients received treatment with a 1-week holiday of panobinostat, and dexamethasone was added in cycle 2. The MTD for panobinostat was 20 mg and ORR was 52.9% in the escalation phase and 73.3% in the subsequent phase. More grade 3 or 4 adverse events were in escalation phase than in the expansion phase, including thrombocytopenia, neutropenia, and asthenia [23].

This study provided the basis for a phase II clinical trial program called PANORAMA 2 (panobinostat or placebo with bortezomib and dexamethasone in patients with relapsed multiple myeloma) in patients who had a progression of disease on or within 60 days of the last bortezomib-containing regimen. In the first part of the study, patients received 8 three-week cycles of oral panobinostat (20 mg) 3 times per week on weeks 1 and 2, bortezomib in the classic schedule on weeks 1 and 2, and oral dexamethasone (20 mg) 4 times per week on weeks 1 and 2. Responsive patients were enrolled in the second part of the study, which consisted of 6-week cycles of panobinostat 3 times per week on weeks 1, 2, 4, and 5; bortezomib once a week on weeks 1, 2, 4, and 5; and dexamethasone the same day and the day after bortezomib until disease progression. Fifty-five patients were included in the study and 17 completed treatment phase 1 and entered treatment phase 2. The ORR was 34.5% in this population of bortezomib-refractory patients. One patient (1.8%) achieved a near-complete response, and 18 patients (32.7%) achieved a PR. Additional 18.2% achieved an MR with a total clinical benefit rate of 52.7%. Median duration of response was 6.0 months and median PFS was 5.4 months. OS was not reached after a median follow-up

of 8.3 months. The most common grade 3/4 adverse was thrombocytopenia (63.6%), managed with dose reduction or platelet transfusions but none of the patients discontinued treatment because of thrombocytopenia. Other common AEs were diarrhea, fatigue, anemia, neutropenia, and pneumonia [24].

Based on this demonstration of synergism between panobinostat and bortezomib, a recent study has evaluated the safety and efficacy of the combination of panobinostat with carfilzomib in relapsed and refractory MM patients. Preliminary data from 44 patients were presented at ASH 2013. Eighty percent of them had received both an IMiD and a proteasome inhibitor and 14% were considered refractory to both. Four dose levels were evaluated. Average starting dose was 20/45 mg/m^2 for carfilzomib and 30 mg for panobinostat. Maximum tolerated dose was not achieved with carfilzomib while panobinostat frequently required both dose reductions (62%) and discontinuations (21%). ORR was 64% (with 31% ≥ VGPR). Previous refractoriness to proteasome inhibitors and IMiDs did not affect ORR in total patient population. Median PFS was 6.8 months in the total population and 4.8 months in patients refractory to bortezomib. The most frequent grade 3 or 4 treatment-related adverse events were thrombocytopenia and neutropenia. Nonhematological side effects included grade 3 fatigue, diarrhea, dyspnea, and hypertension. There was also one death due to congestive heart failure with hemolytic-uremic syndrome (ASH 2013, abstract number 1937).

Although HDAC inhibitors are synergistic with proteasome inhibitors, the efficacy of the combination is lower than expected on the basis of preclinical studies. The reduced activity could be explained in part by the side effects due to a nonselective HDAC inhibition that is responsible for hyperacetylation of numerous protein networks in cells. Although the mechanism of synergism between HDAC inhibitors and bortezomib is not fully understood, the most important HDAC involved in the aggresomal formation is the HDAC6 and it should be considered the new target for inhibition. In addition, its selective inhibition could not only enhance potency, but also reduce the toxicities related to off-target effects of pan-HDAC inhibitors. One of the most promising selective HDAC inhibitors is ACY-1215, that is approximately 11-fold selective for HDAC6 over HDAC3.

Low doses of ACY-1215 combined with bortezomib induce apoptosis in MM cells and a significant delay of tumor growth and a significant prolongation of overall survival in 2 different xenograft SCID mouse models [52]. Based on these results, a study has been conceived in which ACY-1215 was tested alone (part 1, phase 1a) or in combination with bortezomib (part 2, phase 1b) in MM patients relapsed or refractory after at least two lines of treatment. In the phase 1a, 15 patients were treated at doses up to 360 mg orally on days 1–5, 8–12 schedule of 21-day cycle. No MTD was identified. Adverse events reported were elevated creatinine, fatigue, hypercalcemia, and upper respiratory infection (not attributed to ACY-1215). In the phase 1b, 22 patients received ACY-1215 on days 1–5, 8–12 with i.v. bortezomib on days 1, 4, 8, and 11 with dexamethasone per OS 20 mg on days 1, 2, 4, 5 8, 9, 11, and 12. Grade 3 or 4 gastrointestinal adverse effects were rare and hematologic adverse events were manageable with grade 3-4 thrombocytopenia observed in 19% of patients. In these heavily pretreated patients, the ORR rate was 25% with a clinical benefit rate (≥SD) of 60%. Preclinical and ongoing clinical trials are exploring the activity of ACY-1215 with carfilzomib and IMiDs, as anticipated by Dr. Vogl at the last ASH meeting.

A less mature trial is exploring the combination of escalating doses of ACY-1215 together with standard dose of lenalidomide and dexamethasone, accordingly to a communication at ASH 2013. Dr. Vorhees said that ACY-1215 was well tolerated at doses up to 160 mg on days 1–5, 8–12, and 15–19 and no DLT has been observed so far. ORR was 81%, including 1 CR and 3 VGPR. Most common adverse events, mainly grades 1 and 2, were fatigue (50%), upper respiratory tract infections (38.9%), and neutropenia (27.8%, ASH 2013, abstract number 3190).

5.3. Signal Transduction Inhibitors. A new identified target for treatment of cancer is the kinesin spindle protein (KSP), a microtubule motor protein critical to the function of proliferating cells. Filanesib (ARRAY-520-212) is a KSP inhibitor that induces aberrant mitotic arrest and rapid cell death. It has a preferentially activity on MCL-1 dependent cells including MM and it is not expected to be cross-resistant with other drugs. In a phase II study presented at last ASH by Dr. Shah, filanesib was tested either alone (at the dose of 1.5 mg/m^2 for 2 days every 2 weeks) or in combination with dexamethasone (40 mg weekly). Thirty-two patients with six median previous treatments entered the phase I (filanesib alone) and 55 patients with eight median previous treatments were enrolled in the phase II (filanesib and dexamethasone). The ORR was only 16% and the duration of response was 8 months in the single agent arm and 5 months for the combination arm. Therefore, this study confirmed the lower response rate in respect to the expectations induced by the preclinical studies [53]. However, this study explored the importance of α 1-acid glycoprotein (AAG) plasma levels in predicting the response to filanesib. AAG is an acute-phase serum protein that increases during inflammation. It binds to ARRY-520 and is responsible for increased IC_{50} for ARRY-520 in vitro. By dividing patients according to their basal AAG plasma level, the study demonstrated that all responding patients belonged to the low-level group while none of the patients with high level of AAG responded to filanesib. AAG levels correlated also with duration of response and overall survival indicating that AAG is an important selection marker for filanesib. Phase 1 studies of combination of filanesib with bortezomib or carfilzomib or lenalidomide are ongoing and preliminary results are encouraging.

6. Monoclonal Antibodies

Forty-six antigens potentially targeted by antibodies have been described in MM. Therefore a long list of monoclonal antibodies (MoAbs) is being tested either in preclinical or

in clinical studies. Three main mechanisms of action are recognized for MoAbs:

(1) direct killing of the antibody;

(2) antibody-dependent cellular cytotoxicity (ADCC), in which the binding of a MoAb to a specific target on tumor cells is responsible for a contact between tumor cells and effector cells;

(3) complement dependent cytotoxicity (CDC), in which recruitment of C1q by IgG bound to the tumor cell surface triggers a proteolytic cascade to disrupt the target cell membrane.

The advantage of MoAbs treatment relies on their relative mild toxicity that allows their combination with chemotherapy or other biological agents to be used at lower doses thus reducing the toxicity of antimyeloma treatment.

6.1. Elotuzumab, Anti CS1. The cell surface glycoprotein CS1 is constantly expressed at high levels on $CD138^+$ purified plasma cells obtained from MM patients and at low level in activated B, NK, $CD8^+$ T cells, and mature dendritic cells but not in normal tissues or stem cells.

Elotuzumab is a humanized anti-CS1 MoAb that exerts its antimyeloma mainly through ADCC mediated by NK cells but no CDC [54].

A phase 1 trial, that explored escalated doses of elotuzumab in patients with advanced relapsed/refractory MM, showed that adverse events (cough, headache, back pain, fever, and chills) were generally mild to moderate in severity but the antimyeloma efficacy was modest (26.5% only had stable disease) [55]. However, several preclinical studies have demonstrated that elotuzumab inhibits MM cell adhesion to the stroma, thus reducing drug resistance [54], and that there is a synergism between elotuzumab and other antimyeloma drugs, in particular bortezomib and lenalidomide [56]. Based on this, elotuzumab was administered together with bortezomib in a phase I/II trial in relapsed/refractory MM. An objective response was observed in 48% of evaluable patients with a median time to progression of 9.4 month. The most frequent grades 3 to 4 adverse events were lymphopenia and fatigue. Two elotuzumab-related serious adverse events (chest pain and gastroenteritis) occurred in one patient [57].

The combination of elotuzumab with lenalidomide seems to be more effective. A phase I/II study of combination of elotuzumab, lenalidomide, and dexamethasone has shown encouraging response rates in relapse/refractory MM setting [58], as recently updated at 2013 ASCO meeting [59]. In the phase I of this study, 25 patients were treated with elotuzumab 5, 10, or 20 mg/kg in 28-day cycles using standard 3 + 3 dose-escalation design. In the phase II study, 73 patients were treated with lenalidomide 25 mg/day on days 1–21 and dexamethasone 40 mg/weekly and, according to the number of previous treatment, were stratified to receive elotuzumab at the dose of 10 or 20 mg/kg i.v. on days 1,8, 15, and 22 in cycles 1 and 2 and on days 1 and 15 in cycles ≥ 3 (28 day cycles). In the phase II cohort the ORR was 84%, with higher rate observed with elotuzumab 10 mg/kg versus 20 mg/kg (92% versus 76%). CR/stringent CR was recorded in 14% of patients receiving elotuzumab 10 mg/kg versus 11% in those receiving 20 mg/kg. VGPR was obtained in 50% of patients treated with the lower dose versus 38% of patients treated with higher dose of elotuzumab. In addition, median PFS was longer in elotuzumab 10 mg/kg arm: 33.0 months versus 18.6 months. Elotuzumab was well tolerated in combination with lenalidomide/dexamethasone such that 52% of patients received therapy for ≥18 months. Most common grade 3/4 adverse events included anemia, thrombocytopenia, lymphopenia, and neutropenia without significant differences between the two arms and occurred during first 18 months of therapy.

These findings prompted 2 phase III trials of elotuzumab 10 mg/kg with lenalidomide/dexamethasone, which are currently ongoing for both relapsed/refractory MM (ELOQUENT-2) and previously untreated MM patients (ELOQUENT-1).

6.2. Daratumumab, Anti-CD38. Daratumumab is a human CD38 MoAb with broad-spectrum killing activity. Daratumumab has multiple mechanisms of action, including apoptosis and modulation of CD38 enzymatic activity, CDC, ADCC, and antibody-dependent cellular phagocytosis. In preclinical studies, daratumumab was able to kill myeloma cells and to enhance the activity of other MM treatments. Ongoing clinical trials are investigating the safety of daratumumab in combination with bortezomib or lenalidomide and dexamethasone in patients with relapsed or refractory MM.

In a study presented at ASH 2012 but not yet published, the drug was tested in 32 patients relapsed or refractory to at least two previous regimens. Doses ranging from 0.005 to 24 mg/kg were given weekly for 8 weeks for the first 16 patients and then biweekly for 16 weeks. In 26% of patients, an infusion-related reaction was observed during first full-dose infusion but without apparent relationship between dose and infusion-related reactions. Six patients across different doses experienced grade 3-4 adverse events that were related to treatment such as anemia, thrombocytopenia, bronchospasm, transaminases increase, and cytokine release syndrome. However, MTD was not reached. Daratumumab showed dose-dependent efficacy. Eight of 12 patients receiving ≥4 mg/kg daratumumab had at least a minimal response (ASH 2012, abstract number 73).

Dr. Arkenau presented at last ASH preliminary data on 11 patients indicating that the combination of daratumumab in a dose escalation design, given twice a month together with standard dose lenalidomide and dexamethasone, is safe and effective in relapsed or refractory MM patients. In this study, daratumumab at doses up to 16 mg/kg has been well tolerated (the MTD has not yet been reached) and in combination with lenalidomide and dexamethasone induced in all patients a reduction of M-component that was significant in 8 up to 11 patients (3 CR, 2 VGPR, 3 PR). The most frequent adverse events were neutropenia, gastrointestinal symptoms, bone pain, and muscle spasms. The daratumumab pharmacokinetics profile was not affected by lenalidomide and dexamethasone.

6.3. Tabalumab, Anti-BAFF. Tabalumab is a MoAb directed against membrane-bound and soluble B-cell activating factor (BAFF), a survival factor for MM. Preclinical studies have indicated an antimyeloma activity together with inhibition of osteoclastogenesis. Preliminary data have been presented at ASH 2012 and not yet published. In a phase I study, 20 relapsed/refractory MM patients were treated with a dose escalation of tabalumab (1, 10, 30, 100, or 300 mg on day 1, cycles 1–3, 5, and 7) together with bortezomib at standard biweekly dose (1.3 mg/m^2). In the expansion phase, 28 patients received tabalumab at 100 mg. Grade 3/4 toxicities included peripheral neuropathy, pneumonia, diarrhea, gastrointestinal hemorrhage, musculoskeletal pain, thrombocytopenia, neutropenia, and anemia. The ORR was 45% including 2 CR. Response was associated with lower baseline serum BAFF or IL-6 levels. Median duration of response was 7.3 months and median TTP was 4.9 months (ASH 2012, abstract number 447). A multicentric randomized phase 2/3 clinical trial is ongoing to evaluate efficacy and tolerability of dosage 100 versus 300 mg on day 1 associated with bortezomib 1.3 mg/m^2 on days 1, 4, 8, and 11 and dexamethasone 40 mg on days 1, 2, 4, 5, 8, 9, 11, and 12.

6.4. Indatuximab, Anti-CD138. Indatuximab ravtansine is an antibody-drug conjugate designed to deliver the maytansinoid cytotoxic agent, DM4, specifically to CD138+ expressing tumour cells. Indeed, CD138 is highly expressed in MM and more specific to identify neoplastic plasma cells than CD38. After binding to CD138, indatuximab ravtansine is internalized and processed in the lysosome to release lipophilic DM4 metabolites that inhibit tubulin polymerization and thus to induce cell cycle arrest and apoptosis.

Preliminary clinical data have been presented at the last ASH meeting. After a phase I dose escalation study (80, 100, and 120 mg/m^2) to determine DLT and MTD, the drug has been evaluated in a phase II in a cohort of 37 patients. Indatuximab ravtansine was administered in a 28-day cycle on days 1, 8, and 15 together with lenalidomide (25 mg/day for 21 days) and low dexamethasone (40 mg/day, days 1, 8, 15, and 22). MTD was defined as 100 mg/m^2 with anemia and mucositis reported as dose-limiting toxicities. Among the 15 evaluated patients, the ORR was 73%, including 2 CR and 4 VGPR. This ORR was maintained in lenalidomide refractory patients and was even higher (89%) in 8/9 patients treated at MTD (ASH 2013, abstract number 758).

7. Conclusions

Many new encouraging studies support an optimistic view of the future, although many dark zones remain in the pathway to cure MM. The landscape of treatment of MM is changing thanks to the new developments in understanding the biology of disease and the utilization of the new drugs although sometimes it is hard to figure out exactly the results of the most published studies in the salvage setting since enrolled cohorts are often heterogeneous and include patients with different prognosis. The new therapeutic scenario is dominated by novel therapies where drugs are able to target specific mechanisms of neoplastic cell growth. However, myeloma is quite a heterogeneous disease and neoplastic plasma cells can use several metabolic pathways in order to take a growth advantage. In addition, several studies have shown that different neoplastic clones may emerge in different phases of disease and it is possible that each clone has a different profile of drug sensitivity. It is therefore possible that each of the drugs is effective only in a subgroup of patients and within this group only during a specific phase of disease. To find the specific field of activity of each new drug will be a challenge for future studies.

Another critical point is the awareness that the new drugs often act with mechanism of action that are different from chemotherapeutic drugs, but clinicians are still accustomed to use them as chemotherapy. In many studies the object is to find the MTD while it is becoming clear that for many biological agents not always "the more is better." One example is elotuzumab: a lower dose yielded better results. New methods for measuring biological drug efficacy should be developed in the future.

In the era of the new drugs, however, it should be underlined that the "old" chemotherapy drugs still maintain a significant efficacy against myeloma and that in many cases chemotherapeutic drugs represent the backbone to which the new drugs should be added. A fine tuning of this combination is another skill that clinicians should acquire in the future.

Dealing with relapsing patients, quality of life should be one of the most important goals to be achieved. However, this kind of evaluation is often lacking in many studies and the tools for measuring it are not widely known.

In other cancers, the identification and validation of biomarkers have leaded to improvement of outcome while in MM a precise definition of high risk is still lacking. Novel biomarkers predictive of outcome include cereblon and Ikaros for IMiDs and AAG for Akt inhibitors. However, a better definition of the prognostic profile of the patients could help in interpreting the trials and could be useful to clinicians to identify treatment more appropriate for high-risk patients.

Conflict of Interests

Francesco Di Raimondo has received honoraria from Janssen-Cilag and Celgene. All other authors declare that there is no conflict of interests regarding the publication of this paper.

Acknowledgment

This work has been supported by Fondazione Veronesi that granted Alessandra Romano in the 2013 Annual Young Investigator Program.

References

[1] S. K. Kumar, S. V. Rajkumar, A. Dispenzieri et al., "Improved survival in multiple myeloma and the impact of novel therapies," *Blood*, vol. 111, no. 5, pp. 2516–2520, 2008.

[2] M. Q. Lacy, S. R. Hayman, M. A. Gertz et al., "Pomalidomide (CC4047) plus low-dose dexamethasone as therapy for relapsed

multiple myeloma," *Journal of Clinical Oncology*, vol. 27, no. 30, pp. 5008–5014, 2009.

[3] A. Badros, A. M. Burger, S. Philip et al., "Phase I study of vorinostat in combination with bortezomib for relapsed and refractory multiple myeloma," *Clinical Cancer Research*, vol. 15, no. 16, pp. 5250–5257, 2009.

[4] S. V. Rajkumar, J. Harousseau, B. Durie et al., "Consensus recommendations for the uniform reporting of clinical trials: report of the international myeloma workshop consensus panel 1," *Blood*, vol. 117, no. 18, pp. 4691–4695, 2011.

[5] S. K. Kumar, J. H. Lee, J. J. Lahuerta et al., "Risk of progression and survival in multiple myeloma relapsing after therapy with IMiDs and bortezomib: a multicenter international myeloma working group study," *Leukemia*, vol. 26, no. 1, pp. 149–157, 2012.

[6] L. Garderet, S. Iacobelli, P. Moreau et al., "Superiority of the triple combination of bortezomib-thalidomide-dexamethasone over the dual combination of thalidomide-dexamethasone in patients with multiple myeloma progressing or relapsing after autologous transplantation: the MMVAR/IFM 2005-04 randomized phase III trial from the chronic leukemia working party of the European group for blood and marrow transplantation," *Journal of Clinical Oncology*, vol. 30, no. 20, pp. 2475–2482, 2012.

[7] J. R. Berenson, O. Yellin, A. Bessudo et al., "Phase I/II trial assessing bendamustine plus bortezomib combination therapy for the treatment of patients with relapsed or refractory multiple myeloma," *The British Journal of Haematology*, vol. 160, no. 3, pp. 321–330, 2013.

[8] H. Ludwig, H. Kasparu, C. Leitgeb et al., "Bendamustine-bortezomib-dexamethasone is an active and well-tolerated regimen in patients with relapsed or refractory multiple myeloma," *Blood*, vol. 123, no. 7, pp. 985–991, 2014.

[9] M. Offidani, L. Corvatta, L. Maracci et al., "Efficacy and tolerability of bendamustine, bortezomib and dexamethasone in patients with relapsed-refractory multiple myeloma: a phase II study," *Blood Cancer Journal*, vol. 3, article e162, 2013.

[10] W. Pönisch, M. Rozanski, H. Goldschmidt et al., "Combined bendamustine, prednisolone and thalidomide for refractory or relapsed multiple myeloma after autologous stem-cell transplantation or conventional chemotherapy: results of a phase I clinical trial," *The British Journal of Haematology*, vol. 143, no. 2, pp. 191–200, 2008.

[11] K. L. Yong, C. D. Williams, F. E. Davies et al., "Identifying an optimally effective but tolerable dose of bendamustine in combination with thalidomide and dexamethasone in patients with relapsed or refractory multiple myeloma," *Blood*, vol. 122, no. 21, article 286, 2013.

[12] S. Lentzsch, O. 'Sullivan A, R. C. Kennedy et al., "Combination of bendamustine, lenalidomide, and dexamethasone (BLD) in patients with relapsed or refractory multiple myeloma is feasible and highly effective: results of phase 1/2 open-label, dose escalation study," *Blood*, vol. 119, no. 20, pp. 4608–4613, 2012.

[13] A. J. Jakubowiak, D. S. Siegel, T. Martin et al., "Treatment outcomes in patients with relapsed and refractory multiple myeloma and high-risk cytogenetics receiving single-agent carfilzomib in the PX-171-003-A1 study," *Leukemia*, vol. 27, no. 12, pp. 2351–2356, 2013.

[14] M. Wang, T. Martin, W. Bensinger et al., "Phase 2 dose-expansion study (PX-171-006) of carfilzomib, lenalidomide, and low-dose dexamethasone in relapsed or progressive multiple myeloma," *Blood*, vol. 122, no. 18, pp. 3122–3128, 2013.

[15] E. A. Stadtmauer, R. Abonour, A. D. Cohen et al., "Phase I/II dose expansion of a multi-center trial of carfilzomib and pomalidomide with dexamethasone (Car-Pom-d) in patients with relapsed/refractory multiple myeloma," *Blood*, vol. 122, no. 21, article 690, 2013.

[16] S. Kumar, W. Bensinger, T. M. Zimmerman et al., "Weekly MLN9708, an investigational oral proteasome inhibitor (PI), in relapsed/refractory multiple myeloma (MM): results from a phase I study after full enrollment," *ASCO Meeting Abstracts*, vol. 31, no. 15, supplement, article 8514, 2013.

[17] P. G. Richardson, D. Siegel, R. Baz et al., "Phase 1 study of pomalidomide MTD, safety, and efficacy in patients with refractory multiple myeloma who have received lenalidomide and bortezomib," *Blood*, vol. 121, no. 11, pp. 1961–1967, 2013.

[18] J. S. Miguel, K. Weisel, P. Moreau et al., "Pomalidomide plus low-dose dexamethasone versus high-dose dexamethasone alone for patients with relapsed and refractory multiple myeloma (MM-003): a randomised, open-label, phase 3 trial," *The Lancet Oncology*, vol. 14, no. 11, pp. 1055–1066, 2013.

[19] A. Larocca, V. Montefusco, S. Bringhen et al., "Pomalidomide, cyclophosphamide, and prednisone for relapsed/refractory multiple myeloma: a multicenter phase 1/2 open-label study," *Blood*, vol. 122, no. 16, pp. 2799–2806, 2013.

[20] P. G. Richardson, J. Wolf, A. Jakubowiak et al., "Perifosine plus bortezomib and dexamethasone in patients with relapsed/refractory multiple myeloma previously treated with bortezomib: results of a multicenter phase I/II trial," *Journal of Clinical Oncology*, vol. 29, no. 32, pp. 4243–4249, 2011.

[21] A. J. Jakubowiak, P. G. Richardson, T. Zimmerman et al., "Perifosine plus lenalidomide and dexamethasone in relapsed and relapsed/refractory multiple myeloma: a phase I multiple myeloma research consortium study," *The British Journal of Haematology*, vol. 158, no. 4, pp. 472–480, 2012.

[22] M. Dimopoulos, D. S. Siegel, S. Lonial et al., "Vorinostat or placebo in combination with bortezomib in patients with multiple myeloma (VANTAGE 088): a multicentre, randomised, double-blind study," *The Lancet Oncology*, vol. 14, no. 11, pp. 1129–1140, 2013.

[23] J. F. San-Miguel, P. G. Richardson, A. Gunther et al., "Phase Ib study of panobinostat and bortezomib in relapsed or relapsed and refractory multiple myeloma," *Journal of Clinical Oncology*, vol. 31, no. 29, pp. 3696–3703, 2013.

[24] P. G. Richardson, R. L. Schlossman, M. Alsina et al., "PANORAMA 2: panobinostat in combination with bortezomib and dexamethasone in patients with relapsed and bortezomib-refractory myeloma," *Blood*, vol. 122, no. 14, pp. 2331–2337, 2013.

[25] L. M. Leoni, B. Bailey, J. Reifert et al., "Bendamustine (Treanda) displays a distinct pattern of cytotoxicity and unique mechanistic features compared with other alkylating agents," *Clinical Cancer Research*, vol. 14, no. 1, pp. 309–317, 2008.

[26] S. Knop, C. Straka, M. Haen, R. Schwedes, H. Hebart, and H. Einsele, "The efficacy and toxicity of bendamustine in recurrent multiple myeloma after high-dose chemotherapy," *Haematologica*, vol. 90, no. 9, pp. 1287–1288, 2005.

[27] W. Ponisch, M. Bourgeois, B. Moll et al., "Combined bendamustine, prednisone and bortezomib (BPV) in patients with relapsed or refractory multiple myeloma," *Journal of Cancer Research and Clinical Oncology*, vol. 139, no. 3, pp. 499–508, 2013.

[28] W. Ponisch, B. Moll, M. Bourgeois et al., "Bendamustine and prednisone in combination with bortezomib (BPV) in the

treatment of patients with relapsed or refractory multiple myeloma and light chain-induced renal failure," *Journal of Cancer Research and Clinical Oncology*, vol. 139, no. 11, pp. 1937–1946, 2013.

[29] W. Ponisch, S. Heyn, J. Beck et al., "Lenalidomide, bendamustine and prednisolone exhibits a favourable safety and efficacy profile in relapsed or refractory multiple myeloma: final results of a phase 1 clinical trial OSHO—#077," *The British Journal of Haematology*, vol. 162, no. 2, pp. 202–209, 2013.

[30] A. Romano, C. Conticello, and F. Di Raimondo, "Bortezomib for the treatment of previously untreated multiple myeloma," *Immunotherapy*, vol. 5, no. 4, pp. 327–352, 2013.

[31] D. I. Lichter, H. Danaee, M. D. Pickard et al., "Sequence analysis of beta-subunit genes of the 20S proteasome in patients with relapsed multiple myeloma treated with bortezomib or dexamethasone," *Blood*, vol. 120, no. 23, pp. 4513–4516, 2012.

[32] S. Jagannath, R. Vij, A. K. Stewart et al., "An open-label single-arm pilot phase II study (PX-171-003-A0) of low-dose, single-agent carfilzomib in patients with relapsed and refractory multiple myeloma," *Clinical Lymphoma Myeloma and Leukemia*, vol. 12, no. 5, pp. 310–318, 2012.

[33] D. S. Siegel, T. Martin, M. Wang et al., "A phase 2 study of single-agent carfilzomib (PX-171-003-A1) in patients with relapsed and refractory multiple myeloma," *Blood*, vol. 120, no. 14, pp. 2817–2825, 2012.

[34] R. Vij, M. Wang, J. L. Kaufman et al., "An open-label, single-arm, phase 2 (PX-171-004) study of single-agent carfilzomib in bortezomib-naive patients with relapsed and/or refractory multiple myeloma," *Blood*, vol. 119, no. 24, pp. 5661–5670, 2012.

[35] A. Z. Badros, R. Vij, T. Martin et al., "Carfilzomib in multiple myeloma patients with renal impairment: pharmacokinetics and safety," *Leukemia*, vol. 27, no. 8, pp. 1707–1714, 2013.

[36] D. Siegel, T. Martin, A. Nooka et al., "Integrated safety profile of single-agent carfilzomib: experience from 526 patients enrolled in 4 phase II clinical studies," *Haematologica*, vol. 98, no. 11, pp. 1753–1761, 2013.

[37] J. R. Mikhael, D. Dingli, V. Roy et al., "Management of newly diagnosed symptomatic multiple myeloma: updated Mayo stratification of myeloma and risk-adapted therapy (mSMART) consensus guidelines 2013," *Mayo Clinic Proceedings*, vol. 88, no. 4, pp. 360–376, 2013.

[38] K. P. Papadopoulos, D. S. Mendelson, A. W. Tolcher et al., "A phase I, open-label, dose-escalation study of the novel oral proteasome inhibitor (PI) ONX, 0912 in patients with advanced refractory or recurrent solid tumors," *ASCO Meeting Abstracts*, vol. 29, no. 15, supplement, article 3075, 2011.

[39] T. D. Stephens, C. J. W. Bunde, and B. J. Fillmore, "Mechanism of action in thalidomide teratogenesis," *Biochemical Pharmacology*, vol. 59, no. 12, pp. 1489–1499, 2000.

[40] G. Lu, R. E. Middleton, H. Sun et al., "The myeloma drug lenalidomide promotes the cereblon-dependent destruction of Ikaros proteins," *Science*, vol. 343, no. 6168, pp. 305–309, 2014.

[41] A. K. Gandhi, J. Kang, C. G. Havens et al., "Immunomodulatory agents lenalidomide and pomalidomide co-stimulate T cells by inducing degradation of T cell repressors Ikaros and Aiolos via modulation of the E3 ubiquitin ligase complex CRL4," *The British Journal of Haematology*, vol. 164, no. 6, pp. 811–821, 2014.

[42] J. Kronke, N. D. Udeshi, A. Narla et al., "Lenalidomide causes selective degradation of IKZF1 and IKZF3 in multiple myeloma cells," *Science*, vol. 343, no. 6168, pp. 301–305, 2014.

[43] F. Romagné, P. André, P. Spee et al., "Preclinical characterization of 1-7F9, a novel human anti-KIR receptor therapeutic antibody that augments natural killer-mediated killing of tumor cells," *Blood*, vol. 114, no. 13, pp. 2667–2677, 2009.

[44] D. M. Benson Jr., C. C. Hofmeister, S. Padmanabhan et al., "A phase 1 trial of the anti-KIR antibody IPH2101 in patients with relapsed/refractory multiple myeloma," *Blood*, vol. 120, no. 22, pp. 4324–4333, 2012.

[45] M. Q. Lacy, J. B. Allred, M. A. Gertz et al., "Pomalidomide plus low-dose dexamethasone in myeloma refractory to both bortezomib and lenalidomide: comparison of 2 dosing strategies in dual-refractory disease," *Blood*, vol. 118, no. 11, pp. 2970–2975, 2011.

[46] F. Gay, S. V. Rajkumar, M. Coleman et al., "Clarithromycin (Biaxin)-lenalidomide-low-dose dexamethasone (BiRd) versus lenalidomide-low-dose dexamethasone (Rd) for newly diagnosed myeloma," *The American Journal of Hematology*, vol. 85, no. 9, pp. 664–669, 2010.

[47] S. Sinha, M. Lacy, J. Mikhael et al., "Response to salvage therapies and outcome in patients with multiple myeloma relapsing after pomalidomide therapy," *Leukemia*, vol. 26, no. 4, pp. 839–841, 2012.

[48] R. D. Harvey and S. Lonial, "PI3 kinase/AKT pathway as a therapeutic target in multiple myeloma," *Future Oncology*, vol. 3, no. 6, pp. 639–647, 2007.

[49] J. L. Wolf, D. Siegel, H. Goldschmidt et al., "Phase II trial of the pan-deacetylase inhibitor panobinostat as a single agent in advanced relapsed/refractory multiple myeloma," *Leukemia & Lymphoma*, vol. 53, no. 9, pp. 1820–1823, 2012.

[50] L. Catley, E. Weisberg, T. Kiziltepe et al., "Aggresome induction by proteasome inhibitor bortezomib and α-tubulin hyperacetylation by tubulin deacetylase (TDAC) inhibitor LBH589 are synergistic in myeloma cells," *Blood*, vol. 108, no. 10, pp. 3441–3449, 2006.

[51] T. Hideshima, P. G. Richardson, and K. C. Anderson, "Mechanism of action of proteasome inhibitors and deacetylase inhibitors and the biological basis of synergy in multiple myeloma," *Molecular Cancer Therapeutics*, vol. 10, no. 11, pp. 2034–2042, 2011.

[52] L. Santo, T. Hideshima, A. L. Kung et al., "Preclinical activity, pharmacodynamic, and pharmacokinetic properties of a selective HDAC6 inhibitor, ACY-1215, in combination with bortezomib in multiple myeloma," *Blood*, vol. 119, no. 11, pp. 2579–2589, 2012.

[53] B. J. Tunquist, R. D. Woessner, and D. H. Walker, "Mcl-1 stability determines mitotic cell fate of human multiple myeloma tumor cells treated with the kinesin spindle protein inhibitor ARRY-520," *Molecular Cancer Therapeutics*, vol. 9, no. 7, pp. 2046–2056, 2010.

[54] Y. Tai, M. Dillon, W. Song et al., "Anti-CSl humanized monoclonal antibody HuLuc63 inhibits myeloma cell adhesion and induces antibody-dependent cellular cytotoxicity in the bone marrow milieu," *Blood*, vol. 112, no. 4, pp. 1329–1337, 2008.

[55] J. A. Zonder, A. F. Mohrbacher, S. Singhal et al., "A phase 1, multicenter, open-label, dose escalation study of elotuzumab in patients with advanced multiple myeloma," *Blood*, vol. 120, no. 3, pp. 552–559, 2012.

[56] F. van Rhee, S. M. Szmania, M. Dillon et al., "Combinatorial efficacy of anti-CS1 monoclonal antibody elotuzumab (HuLuc63) and bortezomib against multiple myeloma," *Molecular Cancer Therapeutics*, vol. 8, no. 9, pp. 2616–2624, 2009.

[57] A. J. Jakubowiak, D. M. Benson, W. Bensinger et al., "Phase I trial of anti-CS1 monoclonal antibody elotuzumab in combination with bortezomib in the treatment of relapsed/refractory

multiple myeloma," *Journal of Clinical Oncology*, vol. 30, no. 16, pp. 1960–1965, 2012.

[58] S. Lonial, R. Vij, J. L. Harousseau et al., "Elotuzumab in combination with lenalidomide and low-dose dexamethasone in relapsed or refractory multiple myeloma," *Journal of Clinical Oncology*, vol. 30, no. 16, pp. 1953–1959, 2012.

[59] S. Lonial, S. Jagannath, P. Moreau et al., "Phase (Ph) I/II study of elotuzumab (Elo) plus lenalidomide/dexamethasone (Len/dex) in relapsed/refractory multiple myeloma (RR MM): updated Ph II results and Ph I/II long-term safety," *ASCO Meeting Abstracts*, vol. 31, no. 15, supplement, article 8542, 2013.

Hindawi Publishing Corporation
BioMed Research International
Volume 2014, Article ID 363540, 7 pages
http://dx.doi.org/10.1155/2014/363540

Research Article

MYD88 L265P Mutations Are Correlated with 6q Deletion in Korean Patients with Waldenström Macroglobulinemia

Jung-Ah Kim,[1] Kyongok Im,[2] Si Nae Park,[2] Jiseok Kwon,[2] Qute Choi,[1] Sang Mee Hwang,[1,3] Naohiro Sekiguchi,[4] Sung-Soo Yoon,[5] Dong Soon Lee,[1,2] and Seon Young Kim[1]

[1] *Department of Laboratory Medicine, Seoul National University College of Medicine, 101 Daehangno, Jongno-gu, Seoul 110-744, Republic of Korea*
[2] *Cancer Research Institute, Seoul National University College of Medicine, Seoul, Republic of Korea*
[3] *Department of Laboratory Medicine, Seoul National University Bundang Hospital, Seongnam, Republic of Korea*
[4] *Hematology Division, National Hospital Organization Disaster Medical Center, Tokyo, Japan*
[5] *Department of Internal Medicine, Seoul National University College of Medicine, Seoul, Republic of Korea*

Correspondence should be addressed to Seon Young Kim; ksuny55@gmail.com

Received 21 February 2014; Revised 14 April 2014; Accepted 14 April 2014; Published 7 May 2014

Academic Editor: Wee-Joo Chng

Waldenström macroglobulinemia (WM) is a malignant lymphoplasma-proliferative disorder with IgM monoclonal gammopathy. A recent whole-genome study identified MYD88 L265P as the key mutation in WM. We investigated *MYD88* mutations in conjunction with cytogenetic study in 22 consecutive Korean WM patients. Conventional G-banding and interphase fluorescence *in situ* hybridization (FISH) were performed at regions including 6q21 using bone marrow (BM) aspirates. Sixteen patients were subjected to Sanger sequencing-based *MYD88* mutation study. Five patients (28%) showed cytogenetic aberrations in G-banding. The incidence of 6q21 deletion was 17% by conventional G-banding and 37% by FISH. Ten patients (45%) showed cytogenetic aberrations using FISH: 6q deletion in eight (37%) and *IGH* rearrangement in four (18%). Two patients had both the 6q deletion and *IGH* rearrangement, and two had only the *IGH* rearrangement. Eleven patients (69%) presented with the MYD88 L265P mutation. *MYD88* mutations were significantly associated with the presence of 6q deletions ($P = 0.037$). Six patients with the 6q deletion for whom sequencing was possible were found to harbor *MYD88* mutations. The MYD88 L265P mutation was also associated with increased lymphocyte burden in BM biopsy. This is the first report of high frequency MYD88 L265P mutations in Korean WM patients.

1. Introduction

Waldenström macroglobulinemia (WM) is a malignant lymphoplasma-proliferative disorder characterized by bone marrow infiltration with lymphoplasmacytic lymphoma (LPL) and the secretion of monoclonal immunoglobulin M (IgM) [1–3]. Different stage B-lineage cells infiltrate into the bone marrow (BM), including small lymphocytes, lymphoplasmacytoid cells, and plasma cells [4]. Consensus recommendations from the Second International Workshop on WM diagnostic criteria require LPL with BM involvement and any concentration of IgM monoclonal gammopathy. The incidence rates for WM were 3.4/1,000,000 person-years among men and 1.7/1,000,000 person-years among women from 1988 to 1994 in the United States [5]. Meanwhile, the incidence of LPL in Korean individuals was reported to be low (0.8%) [6], although official data from the Korean national cancer registry are not available [7].

The genetic changes associated with WM are not fully elucidated. Familial clustering in WM suggests the occurrence of inherited genetic susceptibility, similar to chronic lymphocytic leukemia and multiple myeloma [2, 7]. Recently, a whole-exome sequencing study revealed a high frequency of the L265P mutation in the myeloid differentiation primary response gene 88 (*MYD88*) in WM [8]. In that study, 91% of LPL patients exhibited the MYD88 L265P mutation in their tumor cells. Depending on the sensitivity of detection method used, the reported incidence of MYD88 L265P ranges

from 70% to 100% [8–14]. The *MYD88* gene, located on 3p22, encodes a cytosolic adapter protein that plays a central role in the innate and adaptive immune responses [15].

In the present study, we analyzed the MYD88 L265P mutation status of Korean WM patients using Sanger sequencing. We also investigated cytogenetic aberrations using conventional G-banding and fluorescence *in situ* hybridization (FISH) and analyzed their correlation with MYD88 L265P mutation status.

2. Materials and Methods

2.1. Patients. A series of 22 newly diagnosed WM patients treated at Seoul National University Hospital between December 2001 and November 2012 were included for this study. At first, 23 patients who were diagnosed as WM after BM study were selected retrospectively from the hospital information system. Among them, 22 patients whose BM samples were available for cytogenetic and molecular studies were finally included. WM was diagnosed according to the consensus recommendations from the Second International Workshop on WM [2] and the World Health Organization (WHO, 2008) classification criteria on LPL [3] using BM aspiration and biopsy specimens obtained at the time of diagnosis [3]. Each patient had IgM paraproteinemia and lymphoplasmacytic infiltration of the BM.

Mononuclear cells from the initial BM aspirates of all patients were fixed in Carnoy's solution and stored at −70°C for further cytogenetic analysis. The following laboratory and clinical information was obtained for each patient: date of diagnosis and start of therapy, age, sex, ethnicity, hemoglobin level, platelet count, and levels and type of paraprotein. Furthermore, we recorded the percentage of BM lymphocytes and plasma cell infiltration, performed conventional cytogenetic analyses of BM cells by G-banding, and assessed the presence of hepatosplenomegaly, lymphadenopathies, and the number of osteolytic lesions. All BM samples were collected with informed consent, and the study was reviewed and approved by the Institutional Review Board of Seoul National University College of Medicine.

2.2. Conventional Karyotyping by G-Banding. Conventional cytogenetic data were available for 18 of the 22 patients. Cytogenetic studies using standard G-banding techniques on heparinized BM samples were performed as part of the diagnostic workup. At least 20 metaphases were analyzed whenever possible. Clonal abnormalities were defined as two or more cells with the same chromosomal gain or structural rearrangement or at least three cells with the same chromosome deletion. Karyotypes were recorded according to the International System for Human Cytogenetic Nomenclature (ISCN) 2008 [16].

2.3. BM Histological Examination. Hematopathologists reviewed Wright-stained BM smears and hematoxylin and eosin- (H&E-) stained sections of BM trephine biopsies for the percentages and patterns of BM infiltration by lymphocytes, lymphoplasmacytic cells, and plasma cells. Immunohistochemical (IHC) staining was performed using CD3, CD20, CD79a, CD138, immunoglobulin κ, and immunoglobulin λ antibodies (all from Dako, Glostrup, Denmark).

2.4. Fluorescence In Situ Hybridization. Common chromosomal abnormalities were investigated using commercial FISH probes. We used two probes to detect 6q deletions: A20/PRDM1/SHGC-79576 DNA-FISH probe (Cancer Genetics Italia S.R.I., Milano, Italy) and 6q21/MYC (8q24) dual color (Kreatech Diagnostics, Amsterdam, Netherlands). Other FISH probes, including LSI 13 *RB1* (13q14) Spectrum Orange probe, LSI *CDKN2A* (9p21) Spectrum Orange/CEP 9 Spectrum Green probe, TP53/CEP 17 FISH probe mit, LSI 1p36/1q25 probe, and LSI dual-color break-apart probe for *IGH* translocations (all from Abbott Molecular/Vysis, Des Plaines, IL), were used to detect chromosomal abnormalities that are commonly detected in multiple myeloma. Interphase FISH was performed on stored patient BM aspirate specimens. Slides were stained with FISH probes and counterstained with DAPI, and fluorescence signals were then analyzed by fluorescent microscopy (Zeiss, Göttingen, Germany). The results of FISH were recorded according to the ISCN 2008 [17]. The normal cut-off values for the deletion, amplification, or translocation of chromosomal regions were based on the mean (± three standard deviations), and the binomial distribution function [17] of 20 negative controls was analyzed. The cut-off values were 3% for 6q21, *CDKN2A*, and *TP53* deletions, 4.0% for *RB1* deletion, 1% for 1q amplification, and 2% for *IGH* translocation.

2.5. DNA Extraction and Detection of MYD88 L265P Using Sanger Sequencing. Genomic DNA was extracted from frozen BM mononuclear cells from two patients and from the unstained BM slides of 14 patients. DNA was extracted using the MagNA Pure LC DNA Isolation Kit (Roche Applied Science, Indianapolis, IN, USA) according to the manufacturer's instructions. The quality of DNA was analyzed by assessing the 260/280 absorbance ratio using an ND-1000 Spectrophotometer (NanoDrop Technologies, Wilmington, DE, USA).

Two primers (forward, 5′-CTG GCA AGA GAA TGA GGG AAT GT-3′; reverse, 5′-AGG AGG CAG GGC AGA AGT A-3′) were used to amplify a 489-base pair fragment covering the MYD88 L265P site. PCR was performed using 25 ng to 100 ng genomic DNA in 100 μL of PCR solution (10 μL of 10× MG Taq-HF buffer, 0.2 μM of each primer, 10 μL of 2 mM MG dNTPs mixture, 1 μL of MG Taq-HF polymerase (Macrogen Inc., Seoul, Korea), and distilled water). PCR was performed using an initial denaturation step of 5 min at 94°C, followed by 35 cycles of 94°C for 30 s, 62°C for 30 s, and 72°C for 60 s, with a final extension of 7 min at 72°C. The PCR products were purified and sequenced using a BigDye Terminator v3.1 cycle sequencing kit (Applied Biosystems, Foster City, CA) and an ABI 3730 XL automatic sequencer (Applied Biosystems) using the same primers described above.

2.6. Statistical Analysis. Fisher's exact test and χ^2 test were used to compare categorical variables, and the Mann-Whitney U test was used for continuous variables. Estimates of overall survival (OS) were made using the Kaplan-Meier method, and differences among survival curves were

Table 1: Clinical and laboratory characteristics of 22 patients with Waldenström macroglobulinemia.

Characteristic	Number of patients/total	(%)
Age > 65 years	11/22	50
Male sex	17/22	77
Performance status*		
0 to 2	20/22	91
3 or 4	2/22	9
B symptoms	12/22	55
Hyperviscosity	8/22	36
Cryoglobulinemia	3/10	30
Hepatomegaly	7/22	32
Splenomegaly	10/22	45
Lymphadenopathy	16/22	73
Peripheral neuropathy	2/22	9
Multiple osteolytic lesions	2/22	9
Hemoglobin ≤ 11.5 g/dL	20/22	91
Platelet < 100×10^9/L	6/22	27
Monoclonal protein type		
IgMκ	18/22	82
IgMλ	4/22	18

Ig: immunoglobulin.
*Performance status is according to Eastern Cooperative Oncology Group (ECOG) score as follows: 0: without symptoms; 1: mild symptoms not requiring treatment; 2: symptoms requiring some treatment; 3: disabling symptoms but allowing ambulation for >50% of the day; 4: ambulation < 50% of the day.

analyzed using the log-rank test. Cox proportional hazards regression analysis was used to develop a multivariate model of prognostic factors by considering the factors that were associated with survival. Statistical analyses were performed using SPSS version 17.0 (SPSS Inc., Chicago, IL, USA). P values < 0.05 were considered statistically significant.

3. Results

3.1. Clinical and Laboratory Characteristics of Patients. The baseline characteristics of the patients are summarized in Table 1. All patients were Korean, with a median age of 63.5 years (range 40–88 years). There were 17 male (77%) and five female (23%) patients. IgM monoclonal protein was observed in all patients at a median level of 2.52 g/dL (range 0.32–9.74 g/dL). Splenomegaly and lymphadenopathy were observed in 10 (45%) and 16 patients (73%), respectively.

3.2. BM Histology. Morphologic examination of BM biopsies and IHC staining revealed diffuse infiltration of CD20+ small lymphoid cells in 11 patients (50%), whereas others presented with patch or nodular infiltration of lymphoid cells. In differential counts of BM aspirations, the median percentage of small and plasmacytoid lymphocytes was 42% (range 3–93%). Four patients presented with <20% of total lymphocytes (14%, 13%, 3%, and 6%). The increase in number of plasma cells was less significant, with a median of 3% (range 0–33%). Three patients presented with >10% of total plasma cells in BM aspirates (13%, 14%, and 33%). Finally, one patient presented with more plasma cells (33%) than total lymphocytes (6%).

Table 2: Summary of interphase FISH results.

FISH	Number of patients/total	(%)
6q21 deletion	8/22	37
IGH (14q32) translocation	4/22	18
TP53 (17p13) deletion	1/22	5
1q25 amplification	1/22	5
CDKN2A (9p21) deletion	0/22	0
RB1 (13q14) deletion	0/22	0

FISH: fluorescence *in situ* hybridization.

3.3. Results of a Conventional Cytogenetic Study. Conventional cytogenetic analysis using G-banding was performed in 18 patients. Five patients (28%) exhibited structural abnormalities: three presented with 6q deletions 46,XY,14pstk+[18]/46,XY,idem,i(6)(p10)[2],46,XY,1qh+,del(6)(q23)[2]/46,XY,1qh+[22], and 45,X,−Y,1qh+,del(6)(q21),inc[3]/45,idem,add(3)(q26.2),der(3)add(3)(p?23)add(3)(q26.2)[13]/46,XY,1qh+[5] as abnormal karyotypes, which were also detected using FISH. Two patients identified as having the *IGH* rearrangement by FISH presented with abnormal karyotypes in G-banding 46,XY,t(1;14)(p?11;q32)[5]/46,XY[4] and 46,XX[14]46,XX,−1,der(1)add(1)(q42)dup(1)(q21q32)?dup(1)(q32q12),+3,add (14)(q32)[1]/46,XX[19].

3.4. Prevalence of 6q Deletion and Other Cytogenetic Abnormalities by FISH. Among the 22 patients, ten (45%) exhibited cytogenetic aberrations (Table 2). Of these, eight patients (37%) presented with 6q21 deletion (Figure 1). The t(14q32)/*IGH* rearrangement was observed in four patients (18%), amongst whom two patients had both 6q deletion and *IGH* rearrangement, and two patients had only the *IGH* rearrangement. *TP53* deletion was observed in one patient who also had a 6q deletion, and 1q amplification was detected in one patient with an *IGH* rearrangement. The *CDKN2A* (9p21) and *RB1* (13q14) FISH probes did not reveal any abnormalities. Among the ten patients with FISH abnormalities, eight had G-banding data available. Of the six patients with 6q deletions revealed using FISH, three (50%) presented normal karyotypes, whereas other three exhibited concurrent abnormalities by G-banding analysis. Two patients with both the 6q deletion and *IGH* rearrangement by FISH did not exhibit abnormalities at the 14q21 locus by G-banding, and two patients with only the *IGH* rearrangement by FISH presented with concordant abnormalities by G-banding of t(1;14)(p?11;q32) and add(14)(q32), respectively.

3.5. MYD88 L265P Mutation and Its Correlation with WM Disease Characteristics. Among the 16 patients for whom the sequencing for *MYD88* gene was possible, 11 (69%) carried the L265P mutation (Figure 2). When *MYD88* mutation-positive and mutation-negative patients were compared,

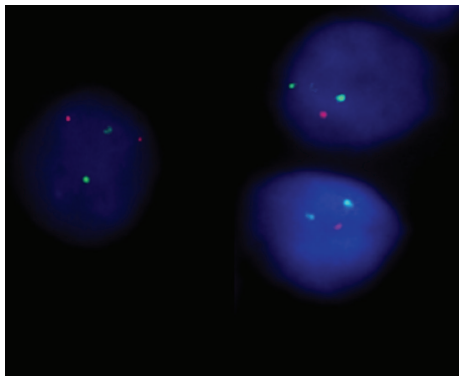


Figure 1: Interphase fluorescence *in situ* hybridization (FISH) analysis of bone marrow using a probe targeting 6q21/8q24. Abnormal cells exhibit one orange (6q21) and two green (8q24) signals, indicating the presence of a 6q deletion.

there were no significant differences in the clinical characteristics and IgM monoclonal protein levels between the two groups (Table 3). In addition, there were no significant differences in lymphocyte counts in peripheral blood and BM aspirates. However, when the lymphocyte burden in BM biopsies was compared, most patients with the MYD88 L265P mutation presented with ≥80% of BM cellularity with diffuse infiltration; in contrast, no mutation-negative patients had such a high concentration of lymphocytes in their BM biopsy ($P = 0.017$).

The *MYD88* mutation was found in all six patients with 6q deletion in whom sequencing was possible ($P = 0.037$). There was no significant difference in the presence of the *IGH* rearrangement between mutation-positive and mutation-negative patients ($P = 0.350$). However, neither of the two patients with *IGH* rearrangement without 6q deletion carried a *MYD88* mutation. When the prognosis was compared according to the presence of the MYD88 L265P mutation and 6q deletion, there was no significant difference between patients with and without *MYD88* mutations or 6q deletions (Figure 3).

When we retrospectively reviewed five *MYD88*-negative cases, one patient presented with 5.7% lymphocytes and 32.9% plasma cells in BM aspirates with the *IGH* rearrangement and 1q amplification, suggesting that a diagnosis of IgM plasma cell myeloma might be considered. Additional patients presented with 3.0% lymphocytes and 3.0% plasma cells with no FISH abnormalities, multiple large CD20 lymphoid aggregates, and low levels of monoclonal protein (0.86 g/dL), which might be more consistent with the involvement of BM in diffuse large B cell lymphoma after the review of lymph node biopsies.

4. Discussion

This is the first report describing the MYD88 L265P mutation status in Korean WM patients. MYD88 L265P mutation has been commonly reported in recent studies of WM, with frequencies of 70–100%, depending on the method and tissues used for genetic analyses. The MYD88 L265P mutation can

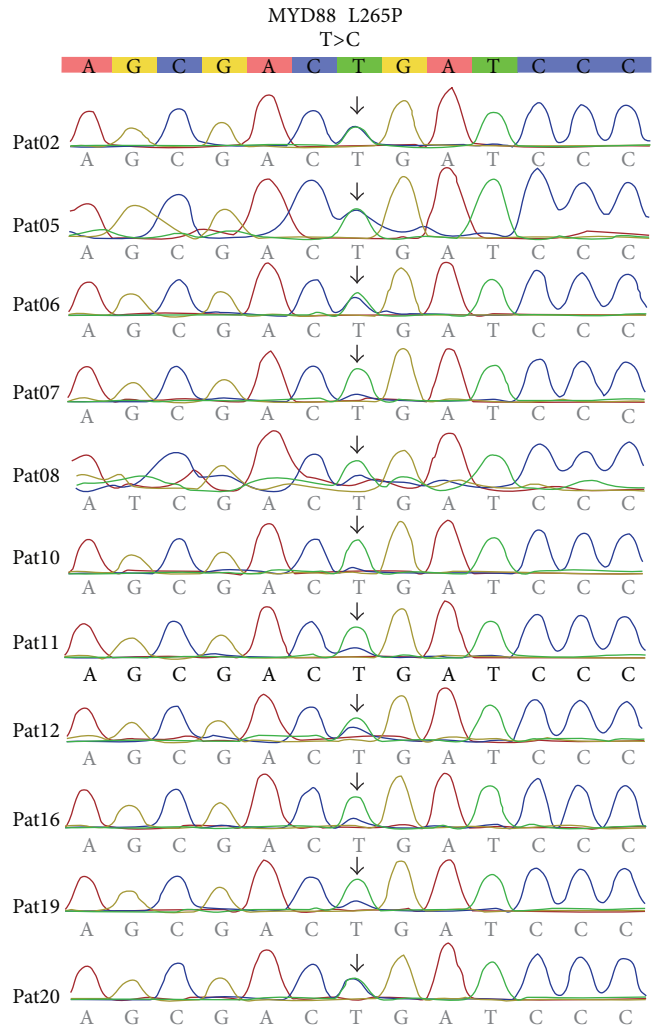


Figure 2: Somatic mutations of MYD88 L265P found in 11 Waldenström macroglobulinemia patients by Sanger sequencing method.

also be found in patients with IgM monoclonal gammopathy of unknown significance (IgM MGUS) at a lower frequency than WM, ranging from 10 to 87% [10, 18, 19]. Because the frequency of MYD88 L265P mutation is much lower in other related chronic B cell lymphoproliferative disorders such as splenic marginal zone B cell lymphoma, multiple myeloma, and chronic lymphocytic leukemia (<10%), the presence of this mutation could be a very useful diagnostic marker to distinguish WM from other B cell-related disorders and might represent a potential therapeutic target for WM [18, 19].

In this study, the frequency of the MYD88 L265P mutation was 69% in Korean patients with WM assessed by PCR and Sanger sequencing in unsorted BM cells, which is comparable with a previous study performed in Caucasian patients [9]. Therefore, we confirmed that MYD88 L265P is a major mutation that is also found in most Korean WM patients. When we compared patients with and without the MYD88 L265P mutation, mutation-positive patients tended to exhibit a higher lymphocyte burden on BM biopsy. All the patients enrolled in our study satisfied the WHO criteria, although

Table 3: Patient characteristics and interphase FISH results according to the presence of MYD88 L265P mutation.

Characteristics	MYD88 L265P (+)	MYD88 L265P (−)	P^*
Age ≥ 65 years	6/11 (55)	2/5 (40)	0.590
Male sex	9/11 (82)	3/5 (60)	0.350
B symptoms	7/11 (64)	3/5 (60)	0.889
Hyperviscosity	5/11 (46)	1/5 (20)	0.330
Splenomegaly	5/11 (46)	2/5 (40)	0.839
Lymphadenopathy	7/11 (64)	5/5 (100)	0.120
Multiple osteolytic lesions	1/11 (9)	1/5 (20)	0.541
Hemoglobin ≤ 11.5 g/dL	10/11 (91)	5/5 (100)	0.486
Platelet < 100×10^9/L	2/11 (18)	2/5 (40)	0.350
Death	2/11 (18)	2/5 (40)	0.350
IgMκ type monoclonal protein	10/11 (91)	3/5 (60)	0.142
Monoclonal protein (g/dL)	3.56 (0.50–5.43)	2.94 (0.55–9.74)	0.777
PB lymphocytes ($\times 10^6$/L)	2233 (807–6100)	1415 (1067–21775)	0.955
BM lymphocytes (%)	45.0 (14.4–93.2)	20.7 (3.0–84.7)	0.234
BM plasma cells (%)	3.7 (0–14.0)	3.2 (0–32.9)	0.691
BM cellularity (%)	85 (25–95)	60 (25–85)	0.093
High lymphocyte burden in BM biopsy[†]	7/11 (64)	0/5 (0)	0.017
6q21 deletion	6/11 (55)	0/5 (0)	0.037
IGH (14q32) translocation	2/11 (18)	2/5 (40)	0.350
P53 (17p13) deletion	1/11 (9)	0/5 (0)	0.486
1q25 amplification	0/11 (0)	1/5 (20)	0.126
Abnormal karyotype	3/7 (43)	2/5 (40)	0.921

BM: bone marrow; FISH: fluorescence *in situ* hybridization; Ig: immunoglobulin; PB: peripheral blood.
[*] P values were calculated using χ^2 test for categorical variables and Mann-Whitney U test for continuous variables between patients with or without the MYD88 L265P mutation.
[†] BM cellularity ≥ 80% with diffuse lymphocytes infiltration in BM biopsy.

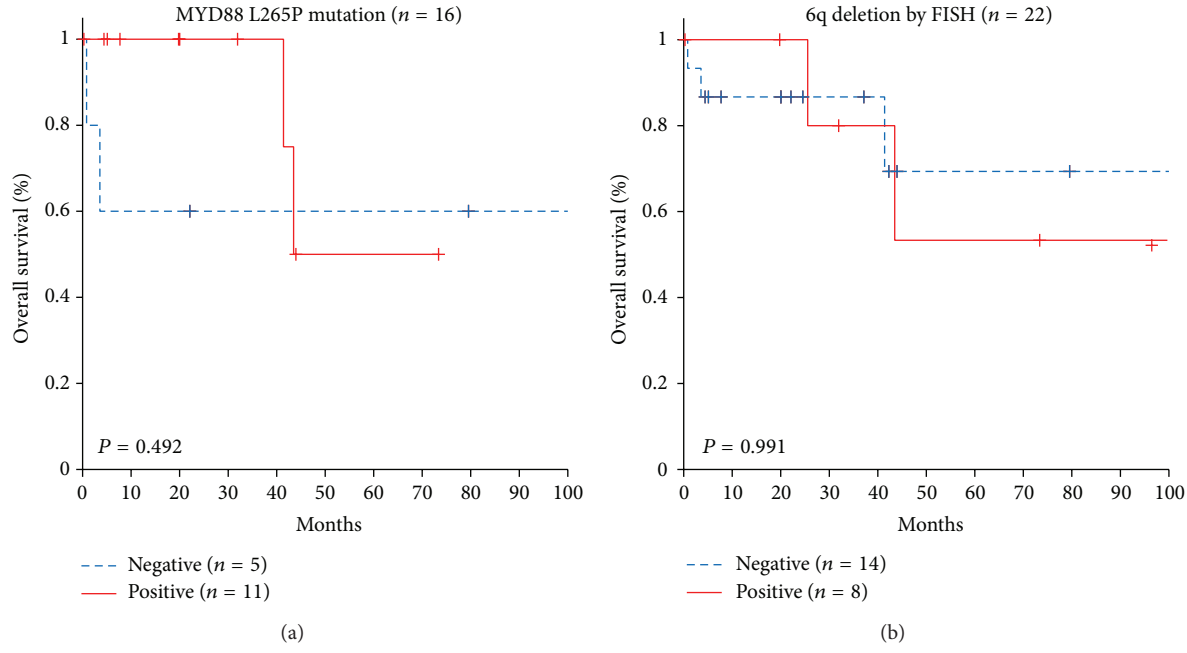


Figure 3: Overall survival of Waldenström macroglobulinemia patients according to the presence of (a) MYD88 L265P mutations and (b) 6q deletion by fluorescence *in situ* hybridization (FISH).

some mutation-negative patients were borderline between WM and other B-lymphoproliferative disorders or between IgM multiple myeloma and diffuse large B cell lymphoma. Although diagnoses are made by fulfilling the diagnostic criteria for WM, the possibility of overlapping diseases could be inferred in patients without the MYD88 L265P mutation. Clinically, the presence of MYD88 L265P mutation or 6q deletion discriminates WM from IgM MGUS [20]. Future investigation of the MYD88 L265P mutation in overlapping diseases would highlight the role of MYD88 L265P in the pathogenesis of WM and as a potential diagnostic marker.

We also investigated the correlation of cytogenetic aberrations with the MYD88 L265P mutation. The 6q deletion was the most frequent abnormality and was found in 37% of Korean patients. Previous studies performed in Western patients with WM reported the frequency of 6q deletions to be 32–54%, identified using FISH [21–24]. Previously, we reported a lower frequency of 6q deletions (10%) in Korean patients compared with Caucasians [24]. We propose that the previous low frequency of 6q deletion might be due to the small number of patients. The second most frequent abnormality in this study was the *IGH* rearrangement, which was identified in 18% of patients. Half of the patients with the *IGH* rearrangement presented with both 6q deletion and *IGH* rearrangement, whereas the other half presented with only the *IGH* rearrangement.

Interestingly, the 6q deletion was significantly associated with the presence of the MYD88 L265P mutation. All patients with 6q deletions for whom sequencing was possible harbored MYD88 L265P mutations. In contrast, patients with only the *IGH* rearrangement did not present with the MYD88 L265P mutation. The deletion of 6q is the most frequent chromosomal abnormality in WM. Candidate tumor suppressor genes identified in this region are *B lymphocyte-induced maturation protein 1* (*BLIMP1*) and *tumor necrosis factor α-induced protein 3* (*TNFAIP3* or *A20*) [25, 26]. *BLIMP1* is a transcriptional repressor that plays a pivotal role in the differentiation of B cells into plasma cells [27–29]. Therefore, deleting *BLIMP1* would block the differentiation of B cells into plasma cells. *TNFAIP3* participates in terminating NF-κB signaling, and its loss of function by deletion might enhance inflammatory, autoimmune, and malignant human diseases, including WM [30, 31]. Because *MYD88* is a key player in the activation of the canonical NF-κB pathway, which is downstream of Toll-like receptor and interleukin-1 receptor signaling [32, 33], we hypothesize that the concurrent presence of the MYD88 L265P mutation and deletion of *BLIMP1* or *TNFAIP3* enhance inflammatory reactions that contribute to the pathogenesis of WM.

In previous studies, Jimenez et al. [10] reported that there were no significant differences in 6q deletion between MYD88 L265P mutated and nonmutated groups in IgM-MGUS and WM, although higher mutation rates (84%) were found in patients with 6q deletions. Poulain et al. also reported that the 6q deletion was not significantly associated with MYD88 L265P mutation status [11]. In addition, previous studies revealed some small differences in the clinical and laboratory characteristics of carriers and noncarriers of the mutation, such as lower levels of lymphocytosis and a slightly higher IgM monoclonal component in patients with the mutation [10, 11]. These studies suggest that there are no specific clinical characteristics associated with MYD88 L265P mutation status. Consistent with these observations, we observed no specific differences in most clinical and laboratory parameters between carriers and noncarriers of the MYD88 L265P mutation. We also observed no difference in overall survival, consistent with a previous study [10]. However, we did observe a significantly higher lymphocyte burden in BM biopsy in mutated patients, regardless of peripheral blood lymphocytosis or BM lymphocyte counts. This finding is consistent with the hypothesis that more typical WM disease features occur in patients with the MYD88 L265P mutation.

The limitations of this study are the small number of patients and the use of less sensitive Sanger sequencing to detect the MYD88 L265P mutation. Because WM is a very rare disease, an additional multicenter study should be performed to allow a more comprehensive genetic analysis of Korean WM patients. In addition, studies using different molecular methods and more sophisticated approaches for assessing mutations in minor populations of malignant cells should be performed.

In conclusion, we observed a high incidence of MYD88 L265P mutation and 6q deletion in Korean WM patients. We also found a novel association between MYD88 L265P mutation and 6q deletion, though the small number of patients should be taken into consideration for interpretation of the findings of our study with caution. We suggest assessing MYD88 L265P mutation status and performing cytogenetic studies to characterize 6q deletions in WM could help with the diagnosis of WM. As such, refining the current diagnostic classification system might be attempted based on these novel findings.

Conflict of Interests

The authors declare that they have no conflict of interests regarding the publication of this paper.

Acknowledgments

This study was supported by (1) a Grant (10172KFDA993) from Korea Food & Drug Administration and (2) a Grant of the Korean Health Technology R&D Project, Ministry of Health & Welfare, Republic of Korea (A120216).

References

[1] A. Vijay and M. A. Gertz, "Waldenström macroglobulinemia," *Blood*, vol. 109, no. 12, pp. 5096–5103, 2007.

[2] R. G. Owen, S. P. Treon, A. Al-Katib et al., "Clinicopathological definition of Waldenstrom's macroglobulinemia: consensus panel recommendations from the Second International Workshop on Waldenstrom's Macroglobulinemia," *Seminars in Oncology*, vol. 30, no. 2, pp. 110–115, 2003.

[3] S. H. Swerdlow, E. Campo, N. L. Harris et al., Eds., *World Health Organization Classification of Tumours of Haematopoietic and Lymphoid Tissues*, IARC Press, 4th edition, 2008.

[4] S. P. Treon, P. Morel, V. Leblond, and J.-P. Fermand, "Report of the Third International Workshop on Waldenstrom's macroglobulinemia," *Clinical Lymphoma*, vol. 5, no. 4, pp. 215–216, 2005.

[5] F. D. Groves, L. B. Travis, S. S. Devesa, L. A. Ries, and J. F. Fraumeni Jr., "Waldenstrom's macroglobulinemia: incidence patterns in the United States, 1988–1994," *Cancer*, vol. 82, no. 6, pp. 1078–1081, 1998.

[6] Y. H. Ko, C. W. Kim, C. S. Park et al., "REAL classification of malignant lymphomas in the Republic of Korea," *Cancer*, vol. 83, no. 4, pp. 806–812, 1998.

[7] S. P. Treon, Z. R. Hunter, A. Aggarwal et al., "Characterization of familial Waldenstrom's macroglobulinemia," *Annals of Oncology*, vol. 17, no. 3, pp. 488–494, 2006.

[8] S. P. Treon, L. Xu, G. Yang et al., "MYD88 L265P somatic mutation in Waldenstrom's macroglobulinemia," *The New England Journal of Medicine*, vol. 367, no. 9, pp. 826–833, 2012.

[9] N. Gachard, M. Parrens, I. Soubeyran et al., "IGHV gene features and MYD88 L265P mutation separate the three marginal zone lymphoma entities and Waldenstrom macroglobulinemia/lymphoplasmacytic lymphomas," *Leukemia*, vol. 27, no. 1, pp. 183–189, 2013.

[10] C. Jimenez, E. Sebastian, M. C. Chillon et al., "MYD88 L265P is a marker highly characteristic of, but not restricted to, Waldenstrom's macroglobulinemia," *Leukemia*, vol. 27, no. 8, pp. 1722–1728, 2013.

[11] S. Poulain, C. Roumier, A. Decambron et al., "MYD88 L265P mutation in Waldenstrom macroglobulinemia," *Blood*, vol. 121, no. 22, pp. 4504–4511, 2013.

[12] S. P. Treon and Z. R. Hunter, "A new era for Waldenstrom macroglobulinemia: MYD88 L265P," *Blood*, vol. 121, no. 22, pp. 4434–4436, 2013.

[13] M. Varettoni, L. Arcaini, S. Zibellini et al., "Prevalence and clinical significance of the MYD88 (L265P) somatic mutation in Waldenstrom's macroglobulinemia and related lymphoid neoplasms," *Blood*, vol. 121, no. 13, pp. 2522–2528, 2013.

[14] L. Xu, Z. R. Hunter, G. Yang et al., "MYD88 L265P in Waldenstrom macroglobulinemia, immunoglobulin M monoclonal gammopathy, and other B-cell lymphoproliferative disorders using conventional and quantitative allele-specific polymerase chain reaction," *Blood*, vol. 121, no. 11, pp. 2051–2058, 2013.

[15] G. Yang, Y. Zhou, X. Liu et al., "A mutation in MYD88 (L265P) supports the survival of lymphoplasmacytic cells by activation of Bruton tyrosine kinase in Waldenstrom macroglobulinemia," *Blood*, vol. 122, no. 7, pp. 1222–1232, 2013.

[16] M. L. Slovak and L. J. Campbell, "International System of Human Cytogenetic Nomenclature," ISCN, S Karger AG, Basel, Switzerland, 2005.

[17] M. L. Slovak and L. J. Campbell, "International System of Human Cytogenetic Nomenclature," ISCN, S Karger AG, Basel, Switzerland, 2009.

[18] L. Xu, Z. R. Hunter, G. Yang et al., "MYD88 L265P in Waldenstrom macroglobulinemia, immunoglobulin M monoclonal gammopathy, and other B-cell lymphoproliferative disorders using conventional and quantitative allele-specific polymerase chain reaction," *Blood*, vol. 121, no. 11, pp. 2051–2058, 2013.

[19] M. Varettoni, L. Arcaini, S. Zibellini et al., "Prevalence and clinical significance of the MYD88 (L265P) somatic mutation in Waldenstrom's macroglobulinemia and related lymphoid neoplasms," *Blood.*, vol. 121, no. 13, pp. 2522–2528, 2013.

[20] R. F. J. Schop, S. A. Van Wier, R. Xu et al., "6q deletion discriminates Waldenström macroglobulinemia from IgM monoclonal gammopathy of undetermined significance," *Cancer Genetics and Cytogenetics*, vol. 169, no. 2, pp. 150–153, 2006.

[21] C. Terré, F. Nguyen-Khac, C. Barin et al., "Trisomy 4, a new chromosomal abnormality in Waldenström's macroglobulinemia: a study of 39 cases," *Leukemia*, vol. 20, no. 9, pp. 1634–1636, 2006.

[22] R. F. J. Schop, W. Michael Kuehl, S. A. Van Wier et al., "Waldenström macroglobulinemia neoplastic cells lack immunoglobulin heavy chain locus translocations but have frequent 6q deletions," *Blood*, vol. 100, no. 8, pp. 2996–3001, 2002.

[23] H. Chang, X. Qi, W. Xu, J. C. Reader, and Y. Ning, "Analysis of 6q deletion in Waldenstrom macroglobulinemia," *European Journal of Haematology*, vol. 79, no. 3, pp. 244–247, 2007.

[24] S.-M. Bang, J.-W. Seo, K. U. Park et al., "Molecular cytogenetic analysis of Korean patients with Waldenström macroglobulinemia," *Cancer Genetics and Cytogenetics*, vol. 197, no. 2, pp. 117–121, 2010.

[25] N. C. Gutiérrez, E. M. Ocio, J. de las Rivas et al., "Gene expression profiling of B lymphocytes and plasma cells from Waldenström's macroglobulinemia: comparison with expression patterns of the same cell counterparts from chronic lymphocytic leukemia, multiple myeloma and normal individuals," *Leukemia*, vol. 21, no. 3, pp. 541–549, 2007.

[26] S. Poulain, E. Braggio, C. Roumier et al., "High-throughput genomic analysis in Waldenström's macroglobulinemia," *Clinical Lymphoma, Myeloma and Leukemia*, vol. 11, no. 1, pp. 106–108, 2011.

[27] C. Alexander Turner Jr., D. H. Mack, and M. M. Davis, "Blimp-1, a novel zinc finger-containing protein that can drive the maturation of B lymphocytes into immunoglobulin-secreting cells," *Cell*, vol. 77, no. 2, pp. 297–306, 1994.

[28] K. L. Calame, K.-I. Lin, and C. Tunyaplin, "Regulatory mechanisms that determine the development and function of plasma cells," *Annual Review of Immunology*, vol. 21, pp. 205–230, 2003.

[29] A. L. Shaffer, M. Shapiro-Shelef, N. N. Iwakoshi et al., "XBP1, downstream of Blimp-1, expands the secretory apparatus and other organelles, and increases protein synthesis in plasma cell differentiation," *Immunity*, vol. 21, no. 1, pp. 81–93, 2004.

[30] S. G. Hymowitz and I. E. Wertz, "A20: from ubiquitin editing to tumour suppression," *Nature Reviews Cancer*, vol. 10, no. 5, pp. 332–340, 2010.

[31] A. Ma and B. A. Malynn, "A20: linking a complex regulator of ubiquitylation to immunity and human disease," *Nature Reviews Immunology*, vol. 12, no. 11, pp. 774–785, 2012.

[32] M. Muzio, J. Ni, P. Feng, and V. M. Dixit, "IRAK (Pelle) family member IRAK-2 and MyD88 as proximal mediators of IL-1 signaling," *Science*, vol. 278, no. 5343, pp. 1612–1615, 1997.

[33] H. Wesche, W. J. Henzel, W. Shillinglaw, S. Li, and Z. Cao, "MyD88: an adapter that recruits IRAK to the IL-1 receptor complex," *Immunity*, vol. 7, no. 6, pp. 837–847, 1997.

Hindawi Publishing Corporation
BioMed Research International
Volume 2014, Article ID 982504, 8 pages
http://dx.doi.org/10.1155/2014/982504

Research Article

Bone Marrow Plasma Cell Assessment before Peripheral Blood Stem Cell Mobilization in Patients with Multiple Myeloma Undergoing Autologous Stem Cell Transplantation

Sung-Eun Lee,[1] Jae-Ho Yoon,[1] Seung-Hwan Shin,[1] Ki-Seong Eom,[1] Yoo-Jin Kim,[1] Hee-Je Kim,[1] Seok Lee,[1] Seok-Goo Cho,[1] Jong Wook Lee,[1] Woo-Sung Min,[1] Chong-Won Park,[1] Myungshin Kim,[2] and Chang-Ki Min[1]

[1] *Hematology, Department of Internal Medicine, Seoul St. Mary's Hospital, College of Medicine, The Catholic University of Korea, #222 Banpo-daero, Seocho-Gu, Seoul 137-701, Republic of Korea*
[2] *Department of Laboratory Medicine, Seoul St. Mary's Hospital, College of Medicine, The Catholic University of Korea, Seoul 137-701, Republic of Korea*

Correspondence should be addressed to Chang-Ki Min; ckmin@catholic.ac.kr

Received 20 February 2014; Accepted 18 April 2014; Published 6 May 2014

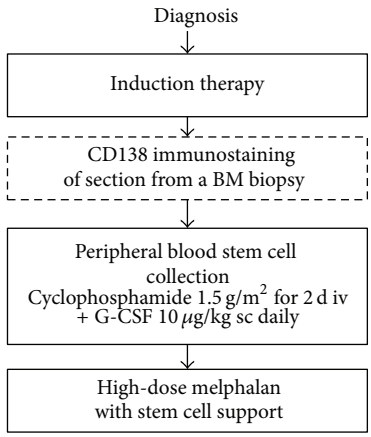
Academic Editor: Dong Soon Lee

The current definition of complete response (CR) in multiple myeloma (MM) includes negative serum and urine immunofixation (IFE) tests and <5% bone marrow plasma cells (BMPCs). However, many studies of the prognostic impact of pretransplant response have not included BMPCs. We evaluated the prognostic impact of BMPC assessment before peripheral blood stem cell (PBSC) mobilization on subsequent transplant outcomes. BMPCs were assessed by CD138, kappa, and lambda immunostaining in 106 patients. After a median followup of 24.5 months, patients with <5% BMPCs had a significantly better progression-free survival (PFS) compared to those with ≥5% BMPCs ($P = 0.005$). Patients with <5% BMPCs + serologic CR showed superior PFS compared to those with <5% BMPCs + serologic non-CR ($P = 0.050$) or ≥5% BMPCs + serologic non-CR ($P = 0.001$). Interestingly, the prognostic impact of BMPCs was more apparent for patients who did not achieve a serologic CR ($P = 0.042$) compared to those with a serologic CR ($P = 0.647$). We concluded that IFE negativity and <5% BMPCs before PBSC mobilization were important factors to predict PFS in patients with MM undergoing ASCT. Particularly, a significant impact of <5% BMPCs was observed in patients who did not achieve IFE negativity.

1. Introduction

The current definition of complete remission (CR) in multiple myeloma (MM) includes negative serum and urine immunofixation (IFE) tests and <5% bone marrow plasma cells (BMPCs) [1]. Additionally, the International Myeloma Working Group (IMWG) has proposed a stringent CR category, requiring a normal serum free light chain (FLC) ratio and the absence of clonal cells in bone marrow [2]. However, there have been several studies aimed at evaluating the role of CR in MM without including BMPCs in the definition of CR [3–5]. Moreover, uncertainty has been introduced by the arbitrary 5% limit in BMPCs in patients with negative IFE.

With the introduction of novel agents for the treatment of MM, the CR rate has increased dramatically and the prognostic impact of CR achievement before autologous stem cell transplantation (ASCT) needs to be evaluated. Kim et al. analyzed 197 MM patients treated with induction chemotherapy followed by a single ASCT and found that CR status before ASCT is an important prognostic factor for better survival outcome. In their study, approach of the CR status did not require a bone marrow examination [5].



Abbreviations:
BM: bone marrow
iv: intravenously
sc: subcutaneously

Figure 1: Study scheme.

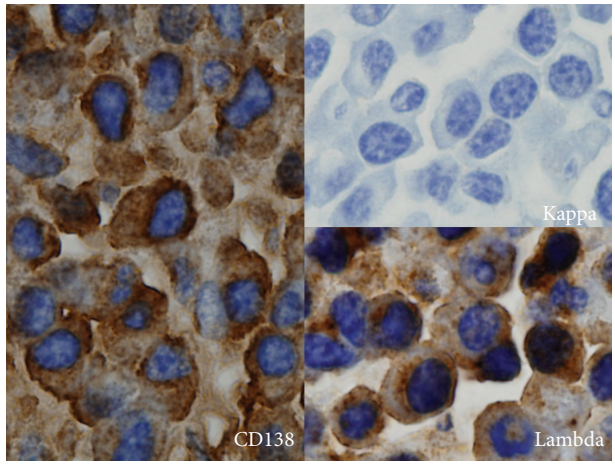


Figure 2: Representative CD138 immunostaining. Plasma cells which express CD138 reveal lambda light chain restriction. CD138, lambda, and kappa chain immunostaining were performed to confirm monoclonal plasma cell on the BM biopsy samples.

However, whether a more precise assessment of CR including bone marrow examination in addition to serologic testing is necessary has not yet been evaluated.

The goal of this study was to determine the prognostic impact of BMPCs as well as serum and urine IFE before peripheral blood stem cell (PBSC) mobilization on the outcomes following ASCT. For this approach, known prognostic factors for survival in MM were also analyzed.

2. Materials and Methods

2.1. Patient Selection. This study included 106 newly diagnosed MM patients with available bone marrow aspirates and adequate cellularity who underwent ASCT at our institution between June 2009 and September 2012. To assess the prognostic impact of BMPCs as well as serologic response before PBSC mobilization on outcomes following ASCT, patients with MM who had measurable monoclonal (M) protein levels at diagnosis were included in this study and were divided according to achievement of <5% BMPCs and/or negative protein electrophoresis (PEP)/immunofixation (IFE) results in serum and urine. The Institutional Review Board of the Catholic University of Korea approved the research protocol for data analysis and this study was conducted in accordance with the Declaration of Helsinki.

2.2. Pretransplant Bone Marrow Examination. BM aspiration and biopsy were performed before PBSC mobilization (Figure 1). Plasma cell counts were assessed after CD138 immunostaining by two examiners blinded to clinical outcomes. Lambda and kappa immunostaining were also performed to confirm monoclonal plasma cell (Figure 2). In all cases of disagreement between examiners, a common reading was organized to achieve a consensus on the count. Differential counts were recorded after a 500-cell count, and marrow cellularity was determined by both BM aspiration and biopsy section.

2.3. Treatment Regimens and Transplant Procedures. Induction chemotherapy was consisted of bortezomib ± dexamethasone ($N = 51$), bortezomib + thalidomide + dexamethasone ($N = 12$), thalidomide + dexamethasone ($N = 31$), or high-dose dexamethasone-based regimen ($N = 12$). Transplant timing was dependent on achieving more than a partial response (PR), but some patients without progressive MM resistant to different novel agents (bortezomib and thalidomide) underwent ASCT. General ASCT procedures were performed as described in previous reports [6]. Briefly, all patients were mobilized with cyclophosphamide (3 g/m^2 total) over 2 days followed by G-CSF (lenograstim, JW pharmaceutical, Seoul, Korea) at 10 μg/kg/day, subcutaneously once a day. Conditioning consisted of melphalan (100 mg/m^2) for 2 days. G-CSF (5 μg/kg/day) was administered subcutaneously to all patients from one day after transplantation until the absolute neutrophil count (ANC) was $>3.0 \times 10^9$/L. All patients received prophylactic antibiotics and an antifungal agent (fluconazole) starting 4 days before transplantation until the ANC reached 1.5×10^9/L.

2.4. Definitions and Evaluation of Response. Stage was classified by the Durie-Salmon staging system and treatment response was assessed according to the criteria from the International Myeloma Working Group [2]. Serologic CR was defined as a lack of detectable M protein in serum and urine by PEP and IFE. Patients with a deletion of chromosome 13 or hypodiploidy determined by conventional cytogenetic study or t(4;14), t(14;16), and del(17p) established by fluorescent *in situ* hybridization (FISH) of BM samples at diagnosis were stratified as high risk [2]. Overall survival (OS) from transplantation was defined as the time from ASCT to death from any cause, and surviving patients were censored at the last followup. Progression-free survival (PFS) was measured as the time from ASCT to disease progression or death (regardless of cause), whichever came first.

2.5. Statistical Analysis. Our main objectives were to evaluate the prognostic impact of BMPCs before PBSC mobilization on the probability of PFS and OS. The probabilities of PFS and OS after ASCT were plotted using the Kaplan-Meier method. Potential prognostic factors for PFS and OS were assessed using a two-tailed log-rank test including age, sex, stage at diagnosis, cytogenetic risk group, serum M protein type, induction therapy, maintenance therapy after ASCT, duration from diagnosis to ASCT, myeloma bone disease apparent on plain radiographs at diagnosis, hemoglobin level, serum LDH, creatinine, calcium, β2-microglobulin at diagnosis, and percentage of BMPCs at diagnosis. Covariates having a P value of less than 0.1 in the univariate analyses were added to Cox proportional hazards regression models, in which all P values were two-sided and statistical significance was set at $P < 0.05$. Associations between categorical variables were assessed using either χ^2 or Fisher's exact test. The Mann-Whitney U test was used to compare continuous variables.

3. Results

3.1. Patient Characteristics. A total of 106 MM patients were included in this study, of whom 59 (56%) were male and 47 (44%) were female. The median age was 56 years (range 33–65 years) and the median disease duration before ASCT was 6.7 months (range 2.9–15.6 months). Stage IIA, IIIA, and IIIB diseases at diagnosis comprised 6%, 77%, and 17% of subjects, respectively, and all patients had measurable disease at diagnosis. Among 106 patients, 39 patients (37%) had a negative IFE test in serum and urine before PBSC mobilization, while 38 (36%) and 25 (23%) of patients achieved a very good partial response (VGPR) and PR, respectively. Four patients with MM resistant to different novel agents were in the stable disease (SD). Table 1 lists the demographic information for all patients and subgroups according to BMPCs before PBSC mobilization. BM examination performed a median of 2.0 months before ASCT (range 0.4–3.7 months). Seventy-three patients (69%) had BMPCs < 5% and 33 patients (31%) showed BMPCs ≥ 5%. The characteristics between the two groups differed in administered type of induction therapy and serologic response before PBSC mobilization (Table 1).

3.2. Relation between Serologic CR and BMPCs before ASCT. Figure 3 shows the relation between serologic CR and BMPCs before PBSC mobilization. The median percentage of BMPCs was 1% (range 0–34%) and 4% (range 0–92%) for patients in serologic CR and non-CR, respectively ($P < 0.001$). Among 39 patients with serologic CR, 35 (90%) had <5% BMPCs, whereas four (10%) had ≥5% BMPCs. The serologic non-CR consisted of <5% BMPCs ($N = 38$, 57%) and ≥5% BMPCs ($N = 29$, 43%; $P < 0.001$). In addition, among 62 patients who had normal serum FLC ratio, 14 (23%) and 48 (77%) patients had ≥5% BMPCs and <5% BMPCs, respectively. For the 44 patients with abnormal serum FLC ratio, 19 (43%) and 25 (57%) patients had ≥5% BMPCs and <5% BMPCs, respectively.

3.3. Transplant Outcomes and Prognostic Factors. The median followup for survivors was 24.5 months (range 4.7–45.9 months). All patients achieved engraftment and no patients died of complications before engraftment. The 2-year PFS of patients with <5% BMPCs and ≥5% BMPCs was 55.8% and 26.9% (median PFS 27.4 versus 12.0 months; $P = 0.005$), respectively (Figure 4). The 2-year OS of patients with ≥5% BMPCs and <5% BMPCs were 89.2% and 84.0% (median OS was not reached; $P = 0.622$), respectively.

To assess prognostic factors affecting PFS and OS, univariate and multivariate analyses were performed. High risk cytogenetics ($P = 0.011$), non-CR serologic response before PBSC mobilization ($P = 0.005$), and ≥5% BMPCs before PBSC mobilization ($P = 0.006$) were potential risk factors for a lower PFS (Table 2). Considering the relationships between serologic response and BMPC percentage, patients

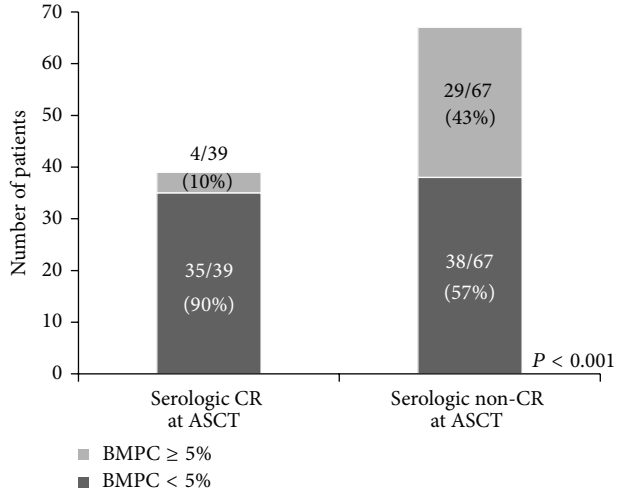


Figure 3: Relation between serologic CR and BMPCs before PBSC mobilization.

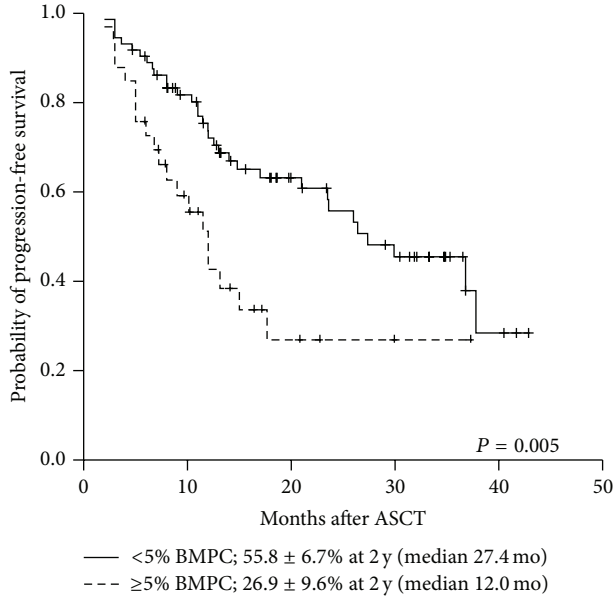


Figure 4: Progression-free survival according to premobilization bone marrow plasma cells.

Table 1: Characteristics of patients and treatment.

	All patients ($N = 106$) (%)	BMPCs before PBSC mobilization		P value
		<5% ($N = 73$)	≥5% ($N = 33$)	
Baseline characteristics				
Age, years, median (range)	56 (33–65)	56 (34–65)	56 (33–65)	ns
Patient gender (M/F)	59 (56)/47 (44)	39 (53)/34 (47)	20 (61)/13 (39)	ns
Serum M protein				ns
Light chain	30 (28)	20 (28)	10 (30)	
Other than light chain	76 (72)	53 (72)	23 (70)	
Stage at diagnosis				ns
IIA	6 (6)	6 (8)	0 (0)	
IIIA/B	100 (94)	67 (92)	33 (100)	
Cytogenetics*				ns
Standard risk	88 (83)	63 (86)	25 (76)	
High risk	18 (17)	10 (14)	8 (24)	
BMPCs at diagnosis, %, median (range)	39 (3–90)	38 (3–88)	42.3 (8–90)	ns
Myeloma bone disease on plain radiographs, yes/no	81 (76)/25 (24)	58 (80)/15 (20)	23 (70)/10 (30)	ns
Serum calcium at diagnosis, mg/dL, median (range)	8.9 (6.8–16.2)	8.9 (6.8–15.7)	9.0 (7.4–16.2)	ns
Serum creatinine at diagnosis, mg/dL, median (range)	0.9 (0.4–8.3)	0.9 (0.4–8.3)	0.9 (0.5–5.6)	ns
β2-microglobulin at diagnosis, mg/mL, median (range)	3.5 (0.2–41.4)	3.5 (0.2–41.4)	3.8 (1.6–36.0)	ns
Hemoglobin at diagnosis, g/dL, median (range)	9.8 (6.0–17.2)	9.8 (6.0–17.2)	9.5 (6.5–14.8)	ns
Serum lactate dehydrogenase at diagnosis, U/L, median (range)	374 (81–1054)	389 (165–1054)	361 (81–889)	ns
Treatment and outcomes				
Induction therapy				0.039
Bortezomib ± dexamethasone	51 (48)	36 (49)	15 (46)	
Bortezomib + thalidomide + dexamethasone	12 (11.5)	8 (11)	4 (12)	
Thalidomide + dexamethasone	31 (29)	17 (23)	14 (42)	
High-dose dexa-based regimen	12 (11.5)	12 (17)	0 (0)	
Duration from diagnosis to ASCT, months, median (range)	6.7 (2.9–15.6)	6.9 (2.9–15.6)	6.1 (3.9–15.3)	ns
Maintenance therapy after ASCT				0.742
Yes	73 (69)	51 (70)	22 (67)	
Thalidomide	50 (47)	35 (48)	15 (46)	
Bortezomib or Lenalidomide	8 (8)	3 (4)	5 (15)	
Prednisolone	15 (14)	13 (18)	2 (6)	
No	33 (31)	22 (30)	11 (33)	
Serologic response before PBSC mobilization				<0.001
CR	39 (37)	35 (48)	4 (12)	
VGPR	38 (36)	25 (34)	13 (39.5)	
PR	25 (23)	12 (17)	13 (39.5)	
SD	4 (4)	1 (1)	3 (9)	
Serologic response after PBSC mobilization				<0.001
CR	47 (44)	41 (56)	6 (18)	
VGPR	36 (34)	22 (30)	14 (42)	
PR	20 (19)	10 (14)	10 (30)	
SD	3 (3)	0 (0)	3 (9)	

ASCT: autologous stem cell transplantation; BMPCs: bone marrow plasma cells; CR: complete response; ns: not significant; PBSC: peripheral blood stem cell; PR: partial response; SD: stable disease; VGPR: very good partial response.

*High risk cytogenetics is defined as hypodiploidy or deletion 13 on conventional cytogenetics or presence of t(4; 14), t(14; 16), and del(17p) on fluorescent *in situ* hybridization and/or conventional cytogenetics. All other cytogenetic abnormalities were considered standard risk.

Table 2: Univariate analysis of potential factors affecting survival outcomes.

Potential factor	Progression-free survival		Overall survival	
	RR (95% CI)	*P* value	RR (95% CI)	*P* value
Age at diagnosis (<60 years versus ≥60 years)	0.86 (0.46–1.58)	0.624	0.55 (0.12–2.51)	0.439
Sex (F versus M)	1.09 (0.63–1.90)	0.750	4.41 (0.96–10.13)	0.056
Immunoglobulin type (others versus Light chain only)	1.01 (0.56–1.82)	0.965	1.15 (0.35–3.83)	0.816
Durie-Salmon stage at diagnosis (II versus III)	1.08 (0.57–2.06)	0.821	1.19 (0.32–4.43)	0.791
Cytogenetics (standard versus High risk)	2.22 (1.20–4.10)	0.011	5.35 (1.71–16.71)	0.004
BMPCs at diagnosis (%) (continuous)	1.00 (0.99–1.01)	0.549	0.99 (0.96–1.01)	0.254
Serum calcium at diagnosis (<10 mg/dL versus ≥10 mg/dL)	1.59 (0.85–2.97)	0.151	3.42 (1.09–10.78)	0.036
Serum creatinine at diagnosis (<2 mg/dL versus ≥2 mg/dL)	0.81 (0.38–1.72)	0.583	1.73 (0.47–6.40)	0.413
β2-microglobulin at diagnosis (<5.5 mg/dL versus ≥5.5 mg/dL)	1.56 (0.89–2.71)	0.119	1.36 (0.43–4.30)	0.601
Hemoglobin at diagnosis (≥10 g/dL versus <10 g/dL)	1.20 (0.69–2.08)	0.524	1.17 (0.37–3.70)	0.786
Serum lactate dehydrogenase at diagnosis (<450 U/L versus ≥450 U/L)	1.47 (0.85–2.56)	0.169	1.58 (0.50–5.00)	0.433
Induction therapy (novel agents* versus nonnovel)	0.97 (0.45–2.06)	0.927	0.50 (0.06–3.86)	0.504
Duration from diagnosis to ASCT (<6 mo versus ≥6 mo)	0.79 (0.45–1.39)	0.412	0.65 (0.21–2.05)	0.460
Maintenance therapy after ASCT (no versus yes)	1.02 (0.54–1.93)	0.945	0.79 (0.21–2.97)	0.723
Serologic response before PBSC mobilization (CR versus non-CR)	2.41 (1.30–4.45)	0.005	2.27 (0.61–8.44)	0.222
Serologic response after PBSC mobilization (CR versus non-CR)†	2.77 (1.53–5.04)	0.001	1.96 (0.59–6.55)	0.274
BMPCs before PBSC mobilization (<5% versus ≥5%)	2.23 (1.26–3.95)	0.006	1.35 (0.40–4.53)	0.623
Response before PBSC mobilization‡ (<5% + CR versus <5% + non-CR versus ≥5% + CR versus ≥5% + non-CR)	1.98 (0.97–4.02) 1.44 (0.32–6.41) 3.83 (1.81–8.11)	0.005	3.45 (0.69–17.18) 4.94 (0.44–54.99) 2.58 (0.43–15.61)	0.443

ASCT: autologous stem cell transplantation; BMPCs: bone marrow plasma cells; CI: confidential interval; CR: complete response; non-CR: noncomplete response; RR: relative risk.

*Novel agents for induction therapies included proteasome inhibitors and immunomodulatory drug.

†Serologic responses before and after PBSC mobilization were correlated. Therefore, serologic response after PBSC mobilization was not entered into the multivariate model.

‡BMPCs + serologic CR.

were reclassified as four groups (<5% BMPCs + serologic CR versus <5% BMPCs + serologic non-CR versus ≥5% BMPCs + serologic CR versus ≥5% BMPCs + serologic non-CR) and entered into the multivariate models. Among the factors that affected PFS determined by univariate analyses, multivariate analyses revealed that high risk cytogenetics (RR of 2.05, $P = 0.024$) was a predictive factor for a lower PFS. Based on the association of BMPCs with serologic response, <5% BMPCs + serologic non-CR (RR of 2.02, $P = 0.050$) and ≥5% BMPCs + serologic non-CR (RR of 3.60, $P = 0.001$) were significantly associated with inferior PFS, compared to <5% BMPCs + serologic CR (Table 3). In addition of the factors affecting OS in the univariate analyses, high risk cytogenetics (RR of 3.92, $P = 0.024$) was an independent factor for OS.

3.4. Separate Analysis according to Serologic Subgroups. To minutely evaluate the importance of premobilization BMPCs in assessing the prognosis after ASCT, we performed a separate analysis in two subgroups according to serologic response before PBSC mobilization (serologic CR group versus serologic non-CR group). In the serologic CR subgroup, the 2-year PFS rates were 75.4% and 37.5% (median PFS 36.8 versus 17.7 months; $P = 0.647$) for patients with <5% BMPCs and ≥5% BMPCs, respectively (Figure 5(a)). On the other hand, in the serologic non-CR subgroup, the 2-year PFS rates were 32.2% and 25.3% (median PFS 21.0 versus 12.5 months; $P = 0.042$) for patients with <5% BMPCs and ≥5% BMPCs, respectively (Figure 5(b)).

4. Discussion

Over the past 10–15 years, novel agents including immunomodulating drugs and proteasome inhibitors have been increasingly incorporated during ASCT for MM. The existing criteria for the assessment of disease response have not proven entirely satisfactory for the analysis of disease outcome before and after ASCT. CR has hitherto been defined as absence of detectable paraprotein in serum and urine according to IFE, maintained for a minimum of 6 weeks, together with <5% BMPCs on the basis of a normal number of plasma cells in the bone marrow (i.e., <4–5%) [1, 7]. However, the arbitrary 5% limit in BMPCs in patients with negative IFE results can lead to uncertainty. Moreover, the impact of BMPCs on patients with positive IFE results has remained unclear in the context of ASCT for MM. This study demonstrated that in addition to PEP and IFE in serum and urine, a pretransplant conventional BM study with CD138 immunostaining constitutes a predictor for disease progression in patients with MM undergoing ASCT. The prognostic impact of BMPCs was more apparent for patients

Table 3: Multivariate analysis of independent factors affecting survival outcomes.

Variable	E/N^*	RR (95% CI)	P value
Progression-free survival			
Cytogenetics			
Standard	39/88	1	
High risk	14/18	2.05 (1.10–3.84)	0.024
Response before PBSC mobilization†			
<5% + CR	13/35	1	
<5% + non-CR	20/38	2.02 (1.00–4.11)	0.050
≥5% + CR	2/4	1.52 (0.34–6.78)	0.583
≥5% + non-CR	18/29	3.60 (1.69–7.63)	0.001
Overall survival			
Sex			
Male	10/59	1	
Female	2/47	0.29 (0.06–1.33)	0.109
Cytogenetics			
Standard	6/88	1	
High risk	6/18	3.92 (1.20–12.81)	0.024
Ca at diagnosis			
<10 mg/dL	7/87	1	
≥10 mg/dL	5/19	2.14 (0.65–7.06)	0.214

CR: complete response; non-CR: noncomplete response; RR: relative risk.
* E/N: number of events/number of evaluable patients.
† BMPCs + serologic CR.

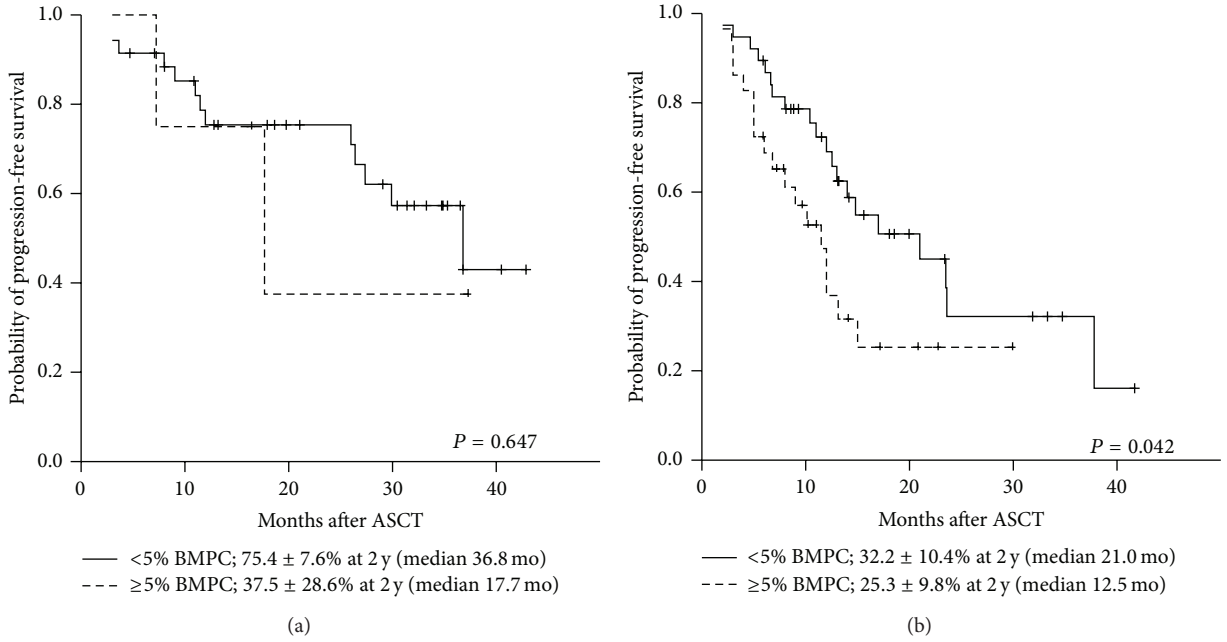


Figure 5: Progression-free survival according to premobilization bone marrow plasma cells in serologic subgroups. (a) Progression-free survival in the serologic CR group and (b) Progression-free survival in the serologic non-CR group.

who did not achieve a serologic CR compared to those with a serologic CR.

In this study, a serologic CR was defined as negative IFE for both serum and urine after induction therapy without considering BMPCs. Patients who had no detectable paraprotein on PEP without a negative IFE result (IFE either positive or not performed) were no longer classified as CR. Although it would be very unusual to achieve a serologic CR in secretory myeloma patients with persisting marrow plasma cell infiltration, we were able to detect this possibility

by a BM examination in a small number of patients (n = 4, 3.8%). CD138 immunostaining was used to objectively evaluate BMPCs because normal plasma cell morphology is not specified and morphological assessment was thought to be too subjective. Additionally, lambda and kappa chain stains were performed to confirm monoclonal plasma cell. CD138/syndecan-1 is a cell membrane proteoglycan that functions as a matrix receptor and is expressed on the surface of mature epithelial cells [8] as well as normal and neoplastic plasma cells [9]. Within the spectrum of hematologic disorders, CD138 expression is highly sensitive and specific for plasmacytic differentiation and represents an excellent marker for routine evaluation of tissue samples for plasma cell disorders. CD138 is particularly helpful for plasma cell quantification in bone marrow biopsy specimens [10]. CD138 expression, however, has been regarded as heterogeneous and nonspecific for tumor type. Therefore, caution is required in the interpretation of $CD138^+$ neoplasms for which a hematolymphoid derivation has not been established, particularly because some nonhematopoietic neoplasms might exhibit plasmacytoid features.

The use of autologous PBSCs for transplantation is associated with the risk of contamination of the graft with tumor cells; whether this impacts response rates, PFS, and OS is still debatable [11]. In this study, we could not determine whether high contamination of grafts with malignant cells [12] or *in vivo* tumor mass prior to ASCT [13] contributed to an unfavorable disease course after transplantation. In particular, the association of the percentage of pretransplant BMPCs with posttransplant PFS in patients having M protein after induction therapy has been unclear. Here, the percentage of BMPCs determined before PBSC mobilization for ASCT by means of CD138 immunostaining was associated with PFS, especially in patients who did not achieve serologic CR (Figure 5(b)). In our study, 38 of 106 patients (35.9%) had a serologic non-CR with a fraction of plasma cells <5% in BM and their survival was better than 29 patients (27.4%) with a serologic non-CR + ≥5% BMPCs. This result reflects the fact that the counting method of BMPCs using immunochemical staining of CD138, kappa, and lambda chain might detect the monoclonal plasma cells producing M protein more sensitively than usual stain such as Wright-Giemsa stain. The possible explanation was that measurement of the M-component reflects the secreted product. Our data suggested that BMPCs should be measured in patients who did not achieve a negative IFE result before PBSC collection. Additional treatment would then be required for those patients with high BMPCs. *In vivo* tumor cell purging prior to mobilization chemotherapy might be one strategy to improve the time to progression of high risk patients. Several studies have been performed to detect minimal residual disease to identify and predict patients at risk for future relapse [14, 15].

A meta-analysis found a strong association between maximal response to induction therapy and long-term survival [16]. To our knowledge, sensitivity to induction therapy measured by BMPCs at the time of ASCT has not been appropriately evaluated. Serial BM examinations are helpful, although the patchy nature of marrow involvement in myeloma makes it difficult to accurately interpret small changes in the percentage of plasma cells present. In this study, the prognostic impact of BMPC determination after induction therapy was more apparent for patients who did not achieve serologic CR compared to those with serologic CR, suggesting that the evaluation of BMPCs after induction therapy is required for all patients to predict disease progression after ASCT as well as to confirm the achievement of CR.

5. Conclusions

IFE negativity and <5% BMPCs prior to PBSC mobilization are important factors to predict PFS in patients with MM undergoing ASCT. A premobilization conventional BM study with CD138 immunostaining may constitute a useful predictor for disease progression in patients who did not achieve IFE negativity.

Conflict of Interests

The authors declare that they have no conflict of interests.

Acknowledgments

The authors acknowledge all members at the Catholic Blood and Marrow Transplantation Center, particularly the house staff, for their excellent care of the patients. This study was supported by the Korea Healthcare Technology R&D Project, Ministry of Health & Welfare, Republic of Korea (Grant no. A120175).

References

[1] J. Blade, D. Samson, D. Reece et al., "Criteria for evaluating disease response and progression in patients with multiple myeloma treated by high-dose therapy and haemopoietic stem cell transplantation. Myeloma Subcommittee of the EBMT. European Group for Blood and Marrow Transplant," *British Journal of Haematology*, vol. 102, no. 5, pp. 1115–1123, 1998.

[2] R. A. Kyle and S. V. Rajkumar, "Criteria for diagnosis, staging, risk stratification and response assessment of multiple myeloma," *Leukemia*, vol. 23, no. 1, pp. 3–9, 2009.

[3] S. V. Rajkumar, R. Fonseca, A. Dispenzieri et al., "Effect of complete response on outcome following autologous stem cell transplantation for myeloma," *Bone Marrow Transplantation*, vol. 26, no. 9, pp. 979–983, 2000.

[4] S. V. Rajkumar, R. Fonseca, M. Q. Lacy et al., "Beta2-microglobulin and bone marrow plasma cell involvement predict complete responders among patients undergoing blood cell transplantation for myeloma," *Bone Marrow Transplantation*, vol. 23, no. 12, pp. 1261–1266, 1999.

[5] J. S. Kim, K. Kim, J. Cheong et al., "Complete remission status before autologous stem cell transplantation is an important prognostic factor in patients with multiple myeloma undergoing upfront single autologous transplantation," *Biology of Blood and Marrow Transplantation*, vol. 15, no. 4, pp. 463–470, 2009.

[6] S. Lee, S. Yahng, B. Cho et al., "Lymphocyte subset analysis for the assessment of treatment-related complications after autologous stem cell transplantation in multiple myeloma," *Cytotherapy*, vol. 14, no. 4, pp. 505–512, 2012.

[7] B. G. M. Durie, J.-L. Harousseau, J. S. Miguel et al., "International uniform response criteria for multiple myeloma," *Leukemia*, vol. 20, no. 9, pp. 1467–1473, 2006.

[8] K. Elenius and L. Jalkanen, "Function of the syndecans—a family of cell surface proteoglycans," *Journal of Cell Science*, vol. 107, no. 11, pp. 2975–2982, 1994.

[9] R. C. Ridley, H. Xiao, H. Hata, J. Woodliff, J. Epstein, and R. D. Sanderson, "Expression of syndecan regulates human myeloma plasma cell adhesion to type I collagen," *Blood*, vol. 81, no. 3, pp. 767–774, 1993.

[10] F. P. O'Connell, J. L. Pinkus, and G. S. Pinkus, "CD138 (syndecan-1), a plasma cell marker immunohistochemical profile in hematopoietic and nonhematopoietic neoplasms," *American Journal of Clinical Pathology*, vol. 121, no. 2, pp. 254–263, 2004.

[11] J. F. DiPersio, A. D. Ho, J. Hanrahan, F. J. Hsu, and S. Fruehauf, "Relevance and clinical implications of tumor cell mobilization in the autologous transplant setting," *Biology of Blood and Marrow Transplantation*, vol. 17, no. 7, pp. 943–955, 2011.

[12] H. G. Kopp, S. Yildirim, K. C. Weisel, L. Kanz, and W. Vogel, "Contamination of autologous peripheral blood progenitor cell grafts predicts overall survival after high-dose chemotherapy in multiple myeloma," *Journal of Cancer Research and Clinical Oncology*, vol. 135, no. 4, pp. 637–642, 2009.

[13] A. K. Stewart, R. Vescio, G. Schiller et al., "Purging of autologous peripheral-blood stem cells using CD34 selection does not improve overall or progression-free survival after high-dose chemotherapy for multiple myeloma: results of a multicenter randomized controlled trial," *Journal of Clinical Oncology*, vol. 19, no. 17, pp. 3771–3779, 2001.

[14] M. Putkonen, V. Kairisto, V. Juvonen et al., "Depth of response assessed by quantitative ASO-PCR predicts the outcome after stem cell transplantation in multiple myeloma," *European Journal of Haematology*, vol. 85, no. 5, pp. 416–423, 2010.

[15] B. Paiva, M. Vidriales, J. Cerveró et al., "Multiparameter flow cytometric remission is the most relevant prognostic factor for multiple myeloma patients who undergo autologous stem cell transplantation," *Blood*, vol. 112, no. 10, pp. 4017–4023, 2008.

[16] H. J. K. van de Velde, X. Liu, G. Chen, A. Cakana, W. Deraedt, and M. Bayssas, "Complete response correlates with long-term survival and progression-free survival in high-dose therapy in multiple myeloma," *Haematologica*, vol. 92, no. 10, pp. 1399–1406, 2007.

Hindawi Publishing Corporation
BioMed Research International
Volume 2014, Article ID 413149, 5 pages
http://dx.doi.org/10.1155/2014/413149

Research Article

The Impact of Hyperglycemia on Risk of Severe Infections during Early Period of Induction Therapy in Patients with Newly Diagnosed Multiple Myeloma

Sung-Hoon Jung,[1] Hee-Chang Jang,[2] Seung-Shin Lee,[1] Jae-Sook Ahn,[1] Deok-Hwan Yang,[1] Yeo-Kyeoung Kim,[1] Hyeoung-Joon Kim,[1] and Je-Jung Lee[1]

[1] *Department of Hematology-Oncology, Chonnam National University Hwasun Hospital, 322 Seoyang-ro, Hwasun, Jeollanam-do 519-763, Republic of Korea*
[2] *Department of Infectious Disease, Chonnam National University Hwasun Hospital, 322 Seoyang-ro, Hwasun, Jeollanam-do 519-763, Republic of Korea*

Correspondence should be addressed to Je-Jung Lee; drjejung@chonnam.ac.kr

Received 27 February 2014; Revised 8 April 2014; Accepted 8 April 2014; Published 16 April 2014

Academic Editor: Wee-Joo Chng

The association between hyperglycemia and infections during induction chemotherapy has been reported in a number of hematologic disorders. This retrospective study evaluated the incidence of hyperglycemia during induction therapy in 155 patients with newly diagnosed multiple myeloma (MM) and its effect on serious infections during the first 60 days of induction. A total of 20 (12.9%) patients developed overt hyperglycemia (≥200 mg/dL) during induction therapy. Serious infections occurred in 28 (18.1%) of 155 patients and infection-related mortality within 2 months after treatment was 0.6% (1 patient). In a univariate analysis, overt hyperglycemia, poor performance status (≥2), International Staging System III, lymphopenia (<500/μL), and elevated serum creatinine (≥2 mg/dL) were found to be associated with serious infections. In multivariate analysis, only overt hyperglycemia (HR 7.846, 95% CI 2.512–24.503, $P < 0.001$) and poor performance status (HR 5.801, 95% CI 1.974–17.050, $P = 0.001$) remained significant. In conclusion, this study demonstrated an association between hyperglycemia and serious infections during induction therapy in patients with MM.

1. Introduction

Infection is a major cause of morbidity and mortality in patients with multiple myeloma (MM). The increased susceptibility to infection results from the interplay between antineoplastic therapies and age- and disease-related complication [1]. In a retrospective study evaluating the incidence of infection throughout the disease course in patients with MM, nearly half of the patients experienced at least one clinically significant infection in 2 months of initial chemotherapy [2]. Furthermore, one study reported that up to 10% of newly diagnosed MM patients died of infectious cause within 60 days of their diagnosis [3]. These early infections are serious problem in management of MM, but there were few studies to examine the risk factor for early infections in patients with MM.

Corticosteroid is a major treatment agent as a single agent or in combination with other agents in patients with MM. Treatment with steroid could cause hyperglycemia regardless of presence of diabetes mellitus, because it increases peripheral insulin resistance and glucose production and suppresses insulin production [4–6]. Steroid induced hyperglycemia has been reported as risk factor for poor clinical outcomes in patients with hematologic malignancies. A retrospective study reported that patients with acute leukemia having blood glucose levels above 200 mg/dL during chemotherapy including oral dexamethasone have shorter complete remission duration and poor survival outcomes and are more likely to develop infection over the next 10 years than control patients [7]. Other reports also reported that hyperglycemia is associated with increased mortality in patients with acute

myeloid leukemia, and increased risk of severe sepsis in hyperglycemic group seems to be partly responsible for the increased mortality [8]. These data suggested that steroid induced hyperglycemia may be an important risk factor for the development of severe infection in patients with MM.

In this study, we evaluated the incidence of hyperglycemia and its association with development of severe infection during early period of initial chemotherapy in patients with MM. We did not include patients with diabetes mellitus to focus on steroid induced hyperglycemia and infection risk in this study.

2. Methods

2.1. Patients. We retrospectively analyzed the records of 362 patients with newly diagnosed MM between November 2002 and February 2013 at Chonnam National University Hwasun Hospital. We excluded 82 patients who did not have available clinical and laboratory data at diagnosis and follow-up. Twenty-four patients were excluded if they had an active infection during the 7 days prior to initiation of chemotherapy. We also excluded 80 patients who received prophylactic antibiotics during first-line chemotherapy and 21 patients who had diabetes mellitus at diagnosis. Of the patients with MM, 155 were included in this study.

2.2. Measurements and Definitions. Fingerstick glucose levels were monitored at least two times a day during hospitalization for initial diagnosis: fasting glucose and 2-hour postprandial glucose. If patients showed high glucose levels during steroid containing induction therapy, fingerstick glucose levels were monitored more often (up to seven times per day). Blood glucose levels were taken at a consistent time points. Glucose levels were measured by ACCU-CHEK Inform II (Roche, Mannheim, Germany). Plasma blood glucose level was also checked in patients treated with further chemotherapy at outpatient clinic. Patients were stratified into three groups by World Health Organization criteria [9] and glucose levels obtained in any time points were used to classify patients into three groups. Mild hyperglycemia was defined as blood glucose 140–200 mg/dL on ≥2 days; overt hyperglycemia was defined as blood glucose ≥200 mg/dL on ≥1 day; all other patients were considered to have euglycemia.

The infection was defined as clinically documented (CDI) when there were clinical signs and symptoms of infection but no pathogen was isolated. If a pathogen was isolated from a blood sample or culture of any site, it was defined as microbiologically documented (MDI). The National Cancer Institute Common Terminology Criteria (NCI-CTC) for Adverse Events (version 4.0) were used to grade infectious complication. Grades 3-4 infections were classified as severe infections. A grade 3 infection was defined as a severe infection, systemic infection requiring intravenous antibiotics, antifungal, or antiviral intervention. A grade 4 infection was defined as life-threatening consequences. When severe infection developed within 60 days of induction chemotherapy, we defined it as early severe infection.

2.3. Statistical Analyses. The univariate analysis of factors associated with severe infection was performed using the χ^2 test. Among the factors, those with $P < 0.05$ were selected and included in the multivariate logistic regression analysis. $P < 0.05$ was considered significant for all analyses. All statistical computations were performed using SPSS software package version 18.0 (SPSS Inc., Chicago, IL, USA)

3. Results

3.1. Patient Population. The median age was 61 years (range, 38–81) and 34.2% of patients were ≥65 years. Of all patients, 78 (50.3%) were male. A total of 82 patients (52.9%) had MM of the IgG type and 24.5% had light-chain disease. Performance status of 0, 1, and ≥2 was found in 24, 98, and 33 patients, respectively. With regard to the International Staging System, 49 patients were stage I, 48 were stage II, and 58 were stage III. Overweight (body mass index (BMI): 25–29.9 kg/m^2) and obese (BMI ≥ 30 kg/m^2) patients were 24.5% and 4.5%, respectively. All patients received steroid containing regimen as first-line chemotherapy. Twenty-one patients (13.5%) received prednisone, and 134 patients (86.5%) received dexamethasone. A total of 131 patients (84.5%) received a thalidomide-based regimen, such as cyclophosphamide, thalidomide, and dexamethasone (CTD), melphalan, prednisolone, and thalidomide (MPT), and thalidomide and dexamethasone (TD). Ten patients (6.5%) were treated with high-dose dexamethasone only. Five patients (3.2%) received a bortezomib-based regimen. One patient (0.6%) received a combination of lenalidomide and low-dose dexamethasone. Eight patients (5.2%) were treated with other conventional chemotherapies including vincristine, adriamycin, and dexamethasone (VAD), melphalan and prednisolone (MP), and cyclophosphamide with prednisolone (CP) or dexamethasone (CD).

3.2. Incidence of Severe Infections. Severe infections occurred in 28 (18.1%) of 155 patients, defined as CDI in 25 (16.1%) and as MDI in 3 (1.9%). Among the severe infections, the most common was pneumonia, which occurred in 20 patients (71.4%). Median time to early severe infection was 20 days (range, 4–57). Three different organisms were found in patients who experienced bacteremia or fungemia: *Streptococcus pneumoniae* (1), *Enterobacter aerogenes* (1), and *Candida parapsilosis* (1). Most of the infections were cured with broad-spectrum antibiotics or antifungal agent therapy, but one patient died due to severe infection within 2 months after treatment. There were no patients who inserted central venous catheter before development of early severe infections.

3.3. Glycemic Status and Infection. A total of 20 patients (12.9%) developed overt hyperglycemia during the first 60 days of first-line chemotherapy. Forty-four patients (28.4%) were with mild hyperglycemia and 91 patients (58.7%) were with euglycemia. Severe infection was more common in overt hyperglycemic group rather than mild hyperglycemic and

euglycemic group (Table 3). There was no significant association between steroid formulation and overt hyperglycemia (9.4% in prednisone versus 13.4% in dexamethasone, $P = 1.000$).

The univariate analysis revealed that five factors were significantly associated with early severe infection (Table 1): blood sugar (≥200 mg/dL), Eastern Cooperative Oncology Group (ECOG) performance status ≥2, International Staging System III, lymphopenia <500/μL, and serum creatinine ≥2 mg/dL. In multivariate analysis, only overt hyperglycemia (HR 7.846, 95% CI 2.512–24.503, $P < 0.001$) and poor performance status (HR 5.801, 95% CI 1.974–17.050, $P = 0.001$) remained significant (Table 2). Additionally, there was no statistical difference in the development of severe infections according to the formulation of steroid (5.0% in prednisone versus 20.1% in dexamethasone, $P = 0.126$).

Table 1: Univariate analysis of risk factor for early severe infections during induction therapy ($n = 155$).

Variable	Infection rates 18.1% (28/155)	P value	OR (95% CI)
Gender			
Male	17.9% (14/78)	1.000	1.016 (0.448–2.303)
Female	18.2% (14/77)		
Age			
<65 years	21.6% (22/102)	0.129	0.464 (0.176–1.227)
≥65 years	11.3% (6/53)		
ECOG performance status			
<2	12.3% (15/122)	0.001	4.637 (1.918–11.211)
≥2	39.4% (13/33)		
Immunophenotype			
Others	19.7% (23/117)	0.470	0.619 (0.218–1.761)
Light chain	13.2% (5/38)		
Body mass index			
<25 kg/m^2	20.9% (23/110)	0.174	0.473 (0.168–1.334)
≥25 kg/m^2	11.1% (5/45)		
ISS			
I-II	10.3% (10/97)	0.002	3.915 (1.658–9.242)
III	31.0% (18/58)		
Serum creatinine, mg/dL			
<2.0	14.0% (17/121)	0.022	2.926 (1.210–7.073)
≥2.0	32.4% (11/34)		
Blood sugar during induction therapy			
<200 mg/dL	12.6% (17/135)	<0.001	8.484 (3.068–23.460)
≥200 mg/dL	55.0% (11/20)		
Early response (after 2 cycles)			
≥PR	15.0% (15/100)	0.196	1.754 (0.765–4.021)
<PR	23.6% (13/55)		
Neutropenia during induction therapy			
≥1,000/μL	17.6% (22/125)	0.793	1.170 (0.428–3.201)
<1,000/μL	20.0% (6/30)		
Lymphopenia during induction therapy			
≥500/μL	12.9% (14/108)	0.022	2.848 (1.229–6.600)
<500/μL	29.8% (14/47)		
Treatment with novel agents			
Yes	18.9% (26/137)	0.531	1.874 (0.405–8.660)
No	11.1% (2/18)		

ECOG: Eastern Cooperative Oncology Group; ISS: International Staging System; PR: partial response.

4. Discussion

Introduction of stem cell transplantation and novel antimyeloma agents, including thalidomide, bortezomib, and lenalidomide, has improved the outcome of patients with MM [10, 11]. These advances have transformed myeloma into a chronic condition. However, infections still remain the significant cause of morbidity and mortality in patients with MM [3]. Infections may occur at the rate of 1.46–4.68 infections per patient-year over the course of MM [2, 12]. Especially, the incidence of infections is 2-3 times higher during the first two months of initial chemotherapy. These early infections may be fatal and were reported as a leading cause of early death during the induction therapy. In addition, early infections frequently lead to substantial delays and dose reduction in subsequent chemotherapy with increased risk of treatment failure [2]. There were well-known various factors that contribute to the increasing risk of infection including immunoparesis, the placement of vascular catheters, type of therapy applied, extent of prior therapy, and presence of comorbidities and organ dysfunction [1]. The use of steroid also affects the development of infection. Dexamethasone-based regimens decrease in cell-mediated immunity and could increase the risk of infection by encapsulated bacterial organisms, viruses, or fungi [13]. Hyperglycemia induced by steroid could play a role of increased susceptibility of infection [14]. However, for hyperglycemia as well as other risk factors for infection, it is not known how much they are contributing to the early occurrence of the infection.

In this retrospective study, we demonstrated that MM patients in overt hyperglycemic group have significantly higher rates of severe infection rather than mild hyperglycemic and euglycemic group during 2 months of initial chemotherapy. This result is consistent with a previous study of patients with adult or childhood acute lymphoblastic leukemia [7, 15]. Although the use of systemic steroid may increase the risk of infection through effects on innate and acquired immunity, some studies support that acute hyperglycemia itself affects all major components of innate immunity and impairs the ability of the host to combat infection [16]. Acute hyperglycemia reduced neutrophil activity such as chemotaxis, formation of reactive oxygen species, and phagocytosis of bacteria, despite the accelerated migration of leukocytes into peripheral tissue [17–20]. Whether there is a

Table 2: Multivariate analysis of risk factors for early severe infections during induction therapy ($n = 155$).

	P value	HR (95% CI)
Blood sugar ≥200 mg/dL	<0.001	7.846 (2.512–24.503)
ECOG performance status ≥2	0.001	5.801 (1.974–17.050)
ISS III	0.089	2.654 (0.862–8.171)
Lymphopenia <500/μL	0.981	1.014 (0.334–3.077)
Serum creatinine ≥2 mg/dL	0.444	1.560 (0.500–4.870)

ECOG: Eastern Cooperative Oncology Group; ISS: International Staging System.

Table 3: Rates of severe infections between blood sugar groups during induction therapy ($n = 155$).

Blood sugar groups	Infection rates	P value	OR (95% CI)
<140 mg/dL	10.9% (10/91)		1 (reference)
140–200 mg/dL	15.9% (7/44)	0.422	1.532 (0.541–4.341)
≥200 mg/dL	55.0% (11/20)	<0.001	9.900 (3.299–29.709)

threshold glucose level for impaired immunity is unclear. In vitro study suggested that a threshold glucose concentration of 250 mg/dL results in impaired phagocytic function and bactericidal activity of polymorphonuclear leukocytes from nondiabetic patients [21].

While most of risk factors for infection such as immunoparesis, comorbidities, and performance status are irreversible, hyperglycemia induced by steroid could be reversible. A study showed that tight glucose control reduced infection risk in hyperglycemic neurosurgical patients [22]. Strict blood glucose control with intensive insulin therapy also has been shown to reduce morbidity and mortality among critically ill patients in a surgical intensive care unit [23]. However, it is still unclear whether strict glycemic control improves outcome in patients with MM. Some trial did not show significant difference in mortality in intensive glycemic control group [24, 25].

There were some differences in our study compared to previous studies. Patients with overt hyperglycemia during chemotherapy were smaller than previously reported (19.9% versus 58%). This may be due to the lower percentage of obese patients (3.9%) in our study. In addition, glucose levels were not checked in a standardized fashion and there was no standard for glucose control because of retrospective nature of this study. Therefore, the number of patients with overt hyperglycemia may be underestimated. In addition, we did not evaluate incidence of infections by dose of steroid, because the number of patients was relatively small and they received various induction regimens.

In conclusion, overt hyperglycemia during early period of initial chemotherapy was associated with increased risk of severe infection in patients with MM. Further prospective studies are required to evaluate the clinical outcomes in patients with MM.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

References

[1] M. Nucci and E. Anaissie, "Infections in patients with multiple myeloma in the era of high-dose therapy and novel agents," *Clinical Infectious Diseases*, vol. 49, no. 8, pp. 1211–1225, 2009.

[2] R. T. Perri, R. P. Hebbel, and M. M. Oken, "Influence of treatment and response status on infection risk in multiple myeloma," *The American Journal of Medicine*, vol. 71, no. 6, pp. 935–940, 1981.

[3] B. M. Augustson, G. Begum, J. A. Dunn et al., "Early mortality after diagnosis of multiple myeloma: analysis of patients entered onto the United Kingdom Medical Research Council trials between 1980 and 2002—Medical Research Council Adult Leukaemia Working Party," *Journal of Clinical Oncology*, vol. 23, no. 36, pp. 9219–9226, 2005.

[4] F. Ranta, D. Avram, S. Berchtold et al., "Dexamethasone induces cell death in Insulin-secreting cells, an effect reversed by exendin-4," *Diabetes*, vol. 55, no. 5, pp. 1380–1390, 2006.

[5] R. A. Rizza, L. J. Mandarino, and J. E. Gerich, "Cortisol-induced Insulin resistance in man: impaired suppression of Glucose production and stimulation of Glucose utilization due to a postreceptor defect of Insulin action," *The Journal of Clinical Endocrinology and Metabolism*, vol. 54, no. 1, pp. 131–138, 1982.

[6] L. Coderre, G. A. Vallega, P. F. Pilch, and S. R. Chipkin, "In vivo effects of dexamethasone and sucrose on Glucose transport (GLUT- 4) protein tissue distribution," *American Journal of Physiology—Endocrinology and Metabolism*, vol. 271, no. 4, pp. E643–E648, 1996.

[7] M. A. Weiser, M. E. Cabanillas, M. Konopleva et al., "Relation between the duration of remission and hyperglycemia during induction chemotherapy for acute lymphocytic leukemia with a hyperfractionated cyclophosphamide, vincristine, doxorubicin, and dexamethasone/methotrexate-cytarabine regimen," *Cancer*, vol. 100, no. 6, pp. 1179–1185, 2004.

[8] N. A. Ali, J. M. O'Brien Jr., W. Blum et al., "Hyperglycemia in patients with acute myeloid leukemia is associated with increased hospital mortality," *Cancer*, vol. 110, no. 1, pp. 96–102, 2007.

[9] M. M. Gabir, R. L. Hanson, D. Dabelea et al., "The 1997 American Diabetes Association and 1999 World Health Organization criteria for hyperglycemia in the diagnosis and prediction of diabetes," *Diabetes Care*, vol. 23, no. 8, pp. 1108–1112, 2000.

[10] S. K. Kumar, S. V. Rajkumar, A. Dispenzieri et al., "Improved survival in multiple myeloma and the impact of novel therapies," *Blood*, vol. 111, no. 5, pp. 2516–2520, 2008.

[11] H. Brenner, A. Gondos, and D. Pulte, "Recent major improvement in long-term survival of younger patients with multiple myeloma," *Blood*, vol. 111, no. 5, pp. 2521–2526, 2008.

[12] D. H. Vesole, M. M. Oken, C. Heckler et al., "Oral antibiotic prophylaxis of early infection in multiple myeloma: a URCC/ECOG randomized phase III study," *Leukemia*, vol. 26, pp. 2517–2520, 2012.

[13] N. C. Klein, C. H. Go, and B. A. Cunha, "Infections associated with steroid use," *Infectious Disease Clinics of North America*, vol. 15, no. 2, pp. 423–432, 2001.

[14] R. Khardori, A. Adamski, and N. Khardori, "Infection, immunity, and hormones/endocrine interactions," *Infectious Disease Clinics of North America*, vol. 21, no. 3, pp. 601–615, 2007.

[15] J. M. Dare, J. P. Moppett, J. P. Shield et al., "The impact of hyperglycemia on risk of infection and early death during induction therapy for acute lymphoblastic leukemia (ALL)," *Pediatric Blood & Cancer*, vol. 60, pp. E157–E159, 2013.

[16] M. Turina, D. E. Fry, and H. C. Polk Jr., "Acute hyperglycemia and the innate immune system: clinical, cellular, and molecular aspects," *Critical Care Medicine*, vol. 33, no. 7, pp. 1624–1633, 2005.

[17] B. Wierusz-Wysocka, H. Wysocki, A. Wykretowicz, and R. Klimas, "The influence of increasing Glucose concentrations on selected functions of polymorphonuclear neutrophils," *Acta Diabetologica Latina*, vol. 25, no. 4, pp. 283–288, 1988.

[18] J. M. Alexiewicz, D. Kumar, M. Smogorzewski, M. Klin, and S. G. Massry, "Polymorphonuclear leukocytes in non-Insulin-dependent diabetes mellitus: abnormalities in metabolism and function," *Annals of Internal Medicine*, vol. 123, no. 12, pp. 919–924, 1995.

[19] S. Dhindsa, D. Tripathy, P. Mohanty et al., "Differential effects of Glucose and Alcohol on reactive Oxygen species generation and intranuclear nuclear factor-κB in mononuclear cells," *Metabolism: Clinical and Experimental*, vol. 53, no. 3, pp. 330–334, 2004.

[20] A. Perner, S. E. Nielsen, and J. Rask-Madsen, "High Glucose impairs superoxide production from isolated blood neutrophils," *Intensive Care Medicine*, vol. 29, no. 4, pp. 642–645, 2003.

[21] J. D. Bagdade, R. K. Root, and R. J. Bulger, "Impaired leukocyte function in patients with poorly controlled diabetes," *Diabetes*, vol. 23, no. 1, pp. 9–15, 1974.

[22] Y. C. Ooi, T. F. Dagi, M. Maltenfort et al., "Tight glycemic control reduces infection and improves neurological outcome in critically ill neurosurgical and neurological patients," *Neurosurgery*, vol. 71, pp. 692–702, 2012.

[23] G. van den Berghe, P. Wouters, F. Weekers et al., "Intensive Insulin therapy in critically ill patients," *The New England Journal of Medicine*, vol. 345, no. 19, pp. 1359–1367, 2001.

[24] G. van den Berghe, A. Wilmer, G. Hermans et al., "Intensive Insulin therapy in the medical ICU," *The New England Journal of Medicine*, vol. 354, no. 5, pp. 449–461, 2006.

[25] F. M. Brunkhorst, C. Engel, F. Bloos et al., "Intensive Insulin therapy and pentastarch resuscitation in severe sepsis," *The New England Journal of Medicine*, vol. 358, no. 2, pp. 125–139, 2008.

Hindawi Publishing Corporation
BioMed Research International
Volume 2014, Article ID 394792, 7 pages
http://dx.doi.org/10.1155/2014/394792

Review Article

Autologous Stem Cell Transplantation in Elderly Patients with Multiple Myeloma: Past, Present, and Future

Shuji Ozaki[1] and Kazuyuki Shimizu[2]

[1] *Department of Hematology, Tokushima Prefectural Central Hospital, 1-10-3 Kuramoto, Tokushima 770-8539, Japan*
[2] *Department of Hematology, Tokai Central Hospital, 4-6-2 Sohara-higashijima, Kakamigahara 504-8601, Japan*

Correspondence should be addressed to Shuji Ozaki; ozaki@tph.gr.jp

Received 2 December 2013; Accepted 14 January 2014; Published 20 February 2014

Academic Editor: Dong Soon Lee

High-dose melphalan (200 mg/m^2) as conditioning regimen followed by autologous stem cell transplantation (ASCT) rescue has been established as a standard treatment for patients with multiple myeloma (MM) younger than 65 years of age. However, the role of ASCT in elderly patients older than 65 years remains controversial in the era of novel agents such as thalidomide, bortezomib, and lenalidomide. The efficacy and feasibility of ASCT have been shown in elderly patients by reducing the dose of melphalan to 100–140 mg/m^2. Although the clinical benefit of reduced-intensity ASCT in elderly patients has not been clearly established in comparison with that of novel agent-based induction therapy, recent studies have demonstrated that sequential strategies of novel agent-based induction therapy and reduced-intensity ASCT followed by consolidation/maintenance with novel agents translate into better outcome in the management of elderly patients. Thus, ASCT could also be a mainstay in the initial treatment of elderly MM patients, and its indication should be evaluated based on performance status and the presence of complications and/or comorbidities of each elderly patient with MM.

1. Introduction

Multiple myeloma (MM) is a plasma cell malignancy characterized by the production of monoclonal immunoglobulin and the related organ damages such as hypercalcemia, renal insufficiency, anemia, and lytic bone lesions (CRAB) [1, 2]. Most patients are elderly, aged 65 years or older, and the incidence of MM is increasing according to the aging of general population and an associated increase in life expectancy.

In the 1960s, melphalan + prednisone (MP) therapy was introduced for the treatment of MM, which extended the median survival from approximately 1.5 years to 2 years [3]. Since the late 1990s, high-dose melphalan therapy (200 mg/m^2) followed by autologous stem cell transplantation (ASCT) has been applied after induction therapy with vincristine + adriamycin + dexamethasone (VAD) in patients younger than 65 years of age, which resulted in the further improvement of survival to 5 years [4, 5]. Consequently, induction therapy + ASCT has been regarded as a standard therapy for younger patients with good health condition, and MP therapy was regarded as a standard of care for elderly patients of 65 years of age or older.

In the early phase of the 21st century, novel agents such as thalidomide, bortezomib, and lenalidomide have entered into clinical practice and become key drugs in the treatment of MM. Bortezomib-based regimens are now used as induction therapy before ASCT in transplant-eligible patients [6–9], and MP + thalidomide [10], MP + bortezomib [11], and lenalidomide + dexamethasone [12] are the widely used regimens for transplant-ineligible patients. Several clinical studies have shown an improvement of overall response rate and progression-free survival (PFS) in both transplant-eligible and transplant-ineligible patients by incorporating novel agents into antimyeloma therapy [13, 14]. However, the significant extension in overall survival (OS) has only been observed in younger patients under 60 years of age by population-based analyses (Table 1) [15–17]. Thus, the prognosis of elderly patients remains poor [18], and more effective strategies are needed to improve the outcome of those patients.

Table 1: Five-year estimates of relative survival in patients with multiple myeloma according to different age groups.

Authors (year)	Age group	Periods		P value
		1990–1992	2002–2004	
Brenner et al., (2008) [15]	50–59	38.8%	48.2%	0.001
	60–69	30.6%	36.3%	0.09
	70–79	27.1%	28.7%	0.21
		1998–2002	2003–2007	
Pulte et al., (2011) [16]	50–54	49.3%	58.3%	<0.05
	55–59	41.7%	52.5%	<0.05
	60–64	35.7%	44.4%	0.01
	65–74	32.1%	37.4%	<0.01
	≥75	19.4%	22.7%	0.06
		1988–1996	2006–2009	
Pozzi et al., (2013) [17]	<65	58.1%	74.2%	<0.001
	65–74	49.9%	72.9%	0.008
	≥75	29.2%	31.4%	0.567

In the following section, we review the clinical trials of ASCT and discuss its role in the recent treatment strategies for elderly patients with MM.

2. Efficacy and Feasibility of ASCT in Elderly Patients

Aging is likely to be associated with reduction in organ functions and drug metabolisms. Accordingly, elderly patients aged 65 years or older are usually considered as ineligible for high-dose melphalan therapy (200 mg/m^2) followed by ASCT. Thus, clinical trials of ASCT have been mostly undertaken in patients younger than 65 years of age, and reports of ASCT performed in elderly patients are hardly available.

The toxicity of each high-dose or intermediate-dose melphalan was evaluated in elderly patients by Badros et al. [19]. Four transplant-related deaths occurred among 25 (16%) patients aged 70 years or older with the melphalan dose of 200 mg/m^2, but after reducing the dose to 140 mg/m^2 transplant-related mortality was significantly reduced to 1 out of 45 (2%) patients while maintaining the efficacy. Palumbo et al. conducted a trial of 2-3 courses of ASCT with 100 mg/m^2 of melphalan in 71 patients aged 55–75 years (median, 64 years old) without comorbidities [20]. When compared with the matched-pair patients treated with MP therapy, the efficacy of ASCT was significantly superior to the MP therapy in terms of complete response (CR) rate, event-free survival (EFS), and OS, without any transplant-related death.

Thereafter, elderly patients, if in a fit medical condition, have been considered to be eligible for ASCT irrespective of the chronological age in most institutions [21, 22]. Several single institution studies have reported the experience of ASCT in elderly patients as well as in younger patients (Table 2) [23–30]. As conditioning regimen before ASCT, reduced dose of melphalan to 100–140 mg/m^2 has been used in the elderly patients. Notably, there was no significant difference in transplant-related mortality between elderly patients and younger patients. Recently, the transplant-related mortality has decreased to 3-4% probably due to the improvement of supportive therapies [31]. In terms of the efficacy, CR rate of the younger patients and that of elderly patients were 24–48% and 12–44%, respectively, and most studies have shown no significant difference in CR rates between the two age groups, younger and elderly. The median PFS for the younger patients and the elderly patients was 17–45 months and 17–29 months, respectively. Likewise, the median OS for the younger patients and the elderly patients ranged from 36 to 73 months and from 39 to 57 months, respectively, and there was no significant difference between the two age groups.

Taken together, reduced-intensity ASCT is considered to be a safe and effective therapeutic modality even in patients aged 65–75 years in a good performance status without comorbidities.

3. Efficacy of ASCT in Comparison with That of Chemotherapy

To compare the efficacy of ASCT with reduced-dose melphalan with that of conventional chemotherapy, several randomized controlled trials have been performed in elderly patients aged 65 years or older (Table 3).

Palumbo et al. conducted a randomized trial enrolling 194 patients to assess the efficacy of VAD induction therapy + tandem ASCT (100 mg/m^2 of melphalan) versus MP therapy in patients aged 50–70 years [32]. Maintenance therapy with interferon + dexamethasone was provided for the responding patients in both treatment arms. In older patients aged 65 to 70 years, the median EFS was significantly extended in the ASCT group compared with the MP group (28.0 versus 16.4 months, resp., $P = 0.023$). The median OS was also significantly extended in the ASCT group compared with the MP group (58.0 versus 37.2 months, resp., $P = 0.04$). These results suggest that intermediate-dose melphalan would be an effective treatment approach in elderly patients aged 65 to 70 years.

Table 2: Comparison of clinical outcomes of ASCT according to different age groups.

Authors (year)	Median age (range)	Number of patients	Conditioning regimen	TRM	CR	Median PFS	Median OS
Siegel et al., (1999) [23]	52 (37–64)	49	MEL 200	2%	43%	34 mo	58 mo
	67 (65–76)	49		8%	20%*	18 mo	40 mo
Sirohi et al., (2000) [24]	55 (31–64)	17	MEL 200	12%	47%	23 mo	36 mo
	67 (65–74)	17		18%	35%	24 mo	43 mo
Reece et al., (2003) [25]	52 (30–59)	382	MEL ± TBI and others	6%	34%	27 mo	39 mo
	63 (60–73)	110		5%	33%	24 mo	39 mo
Jantunen et al., (2006) [26]	57 (39–64)	79	MEL 200	1%	36%	21 mo	66 mo
	68 (65–73)	22		0%	44%	23 mo	57 mo
Gertz et al., (2007) [27]	≤65	541	MEL 200	3%	30%	17 mo	44 mo
	>65	137	MEL 140–200	3%	40%	17 mo	44 mo
Kumar et al., (2008) [28]	56 (37–65)	60	MEL 200	0%	28%	18 mo	53 mo
	72 (70–76)	33	MEL 140–200	3%	42%	29 mo	NR
El Cheikh et al., (2011) [29]	62 (60–65)	104	MEL 140–200	4%	48%	45 mo	57% at 5 yr
	69 (65–77)	82	MEL 100–200	4%	41%	27 mo**	54% at 5 yr
Muta et al., (2013) [30]	60 (51–64)	63	MEL 180–200	3%	24%	21 mo	73 mo
	67 (65–76)	25	MEL 100–200	4%	12%	17 mo	41 mo

TRM: treatment-related mortality; CR: complete response; PFS: progression-free survival; OS: overall survival; MEL: melphalan; TBI: total body irradiation; mo: months; yr: years; NR: not reached.
* $P = 0.02$, ** $P < 0.0001$.

Table 3: Results of the clinical trials of ASCT in elderly patients aged 65 years or older.

Authors (year)	Regimen	Number of patients	CR/nCR	≥PR	Median PFS/EFS	Median OS	TRM
Palumbo et al., (2004) [32]	MP	36	8%	50%	16.4 mo	37.2 mo	3%
	DAV + MEL 100	44	25%*1	68%	28.0 mo*2	58.0 mo*3	7%
Facon et al., (2007) [33]	MP	196	2%	35%	17.8 mo	33.2 mo	2%
	MPT	125	13%**1	76%**2	27.5 mo**2	51.6 mo**3	0%
	VAD + MEL 100	126	18%**2	65%**2	19.4 mo	38.3 mo	5%
Gay et al., (2013) [36]	PAD + MEL 100 + LP-L	102	53%	95%	48 mo	63% at 5 yr	8%

CR: complete response; nCR: near complete response; PR: partial response; PFS: progression-free survival; EFS: event-free survival; OS: overall survival; TRM: treatment-related mortality; MP: melphalan + prednisone; DAV: dexamethasone + doxorubicin + vincristine; MEL: melphalan; MPT: melphalan + prednisone + thalidomide; VAD: vincristine + adriamycin + dexamethasone; PAD: bortezomib + pegylated liposomal doxorubicin + dexamethasone; LP: lenalidomide + prednisone; L: lenalidomide; mo: months, yr: years.
*1 $P = 0.05$, *2 $P = 0.023$, *3 $P = 0.04$, **1 $P = 0.0008$, **2 $P < 0.0001$, **3 $P = 0.0006$ (in comparison with MP).

Facon et al. conducted a randomized trial comparing MP therapy ($n = 196$), MP + thalidomide (MPT) therapy ($n = 125$), and VAD induction therapy + tandem ASCT (100 mg/m^2 of melphalan, $n = 126$) in patients aged 65 to 75 years [33]. The median PFS was 17.8 months with MP, 27.5 months with MPT, and 19.4 months with ASCT, respectively, and there was no significant difference in PFS between the MP group and the ASCT group ($P = 0.25$). The median OS was 33.2 months with MP, 51.6 months with MPT, and 38.3 months with ASCT, and no difference was seen between the ASCT group and the MP group ($P = 0.32$). On the other hand, significantly longer PFS and OS were observed in the MPT group compared with the ASCT group ($P = 0.0002$ and $P = 0.027$, resp.). When compared with the results of Palumbo et al., the median OS with MP was similar, but the median OS with ASCT was shorter in the report of Facon et al. This was probably because maintenance therapy was not intended in the study of Facon et al.

Barlogie et al. reported that the median OS had reached to 60 months with the total therapy composed of induction therapy, tandem ASCT (140–200 mg/m^2 of melphalan), and interferon + dexamethasone maintenance therapy in 136 patients aged 65 years or older [34]. Their results showed a marked improvement of OS in elderly patients, suggesting the importance of continuous treatment after ASCT.

4. New Treatment Strategy Incorporating Novel Agents and ASCT in the Elderly

Several recent clinical trials have been designed to evaluate the efficacy and feasibility of sequential treatment strategies, for example, induction with novel agents, consolidation with ASCT, and maintenance with novel agents. Palumbo et al. and Gay et al. conducted a phase II trial of induction therapy with 4 cycles of bortezomib + pegylated liposomal doxorubicin + dexamethasone followed by tandem ASCT (100 mg/m^2 of melphalan), consolidation therapy with 4 cycles of lenalidomide + prednisone, and maintenance therapy with lenalidomide alone in patients aged 65–75 years (Table 3) [35, 36]. The median PFS was 48 months, and the 5-year estimate of survival was 63% among the total of 102 patients. In particular, the 5-year estimate of survival of the 54 patients who obtained a CR was excellent reaching to 83%. In terms of the safety profiles, three and five patients died during induction therapy and during ASCT, respectively. The treatment-related mortality was significantly higher in the older patients (5 of 26 (19%)) compared with that of the younger patients (3 of 76 (4%)) aged less than 70 years ($P = 0.024$). From these results, it would be concluded that a sequential approach including reduced-intensity ASCT may benefit patients younger than 70 years of age with good performance status and without comorbidities.

Straka et al. performed a randomized controlled trial of tandem ASCT (140 mg/m^2 of melphalan) + lenalidomide maintenance therapy comparing with continued lenalidomide + dexamethasone therapy after induction therapy of 3 cycles of lenalidomide + dexamethasone and peripheral blood stem cell collection in patients aged 60–75 years [37]. Preliminary results have demonstrated a successful collection of peripheral blood stem cells in 97% of patients given induction therapy with lenalidomide + dexamethasone. By the long-term followup the importance of ASCT for elderly patients in the era of novel agents would be clarified.

5. Current Status of the Treatment in Elderly Patients

Thus, treatment strategy employing ASCT in patients aged 65–70 years has not been well established, and the indication of ASCT varies in each institution.

Kumar et al. have reported an improvement in OS of MM patients diagnosed during 2001–2006 period and ascribed it to the beneficial effects of novel agents [38]. More recently, they have updated the outcome of 1038 patients diagnosed and treated in Mayo Clinic between 2001 and 2010 [39]. Notably, when comparing the survival data between patients diagnosed during the period of 2001–2005 and that of 2006–2010, an improvement in survival was only observed in the older patients aged ≥65 years but not in the younger patients aged <65 years. A total of 393 patients (37%) received ASCT as initial treatment. When comparing patients receiving ASCT with those who did not receive ASCT, OS was similar when the analysis was confined to the younger age group, while it was significantly prolonged in the older age group ($P < 0.01$). Therefore, it is considered that the survival has markedly improved and has almost reached to its maximum in the younger patients; however, the survival of the elderly patients has just started to increase with the use of novel agents and an increasing application of ASCT to elderly patients.

Similarly, to assess the current treatment status, the Japanese Society of Myeloma has surveyed the outcome of patients aged 65–70 years including those enrolled in clinical trials as well as those in routine practice who had received an initial treatment between the years of 2004 and 2009 in collaboration with Dr. Palumbo and his colleagues of the Turin University of the European Myeloma Network [40]. The total number of the patients was 318 (268 from the Japanese Society of Myeloma and 50 from the Turin University) composed of 167 male and 151 female patients. M protein type was IgG in 169, IgA in 80, Bence-Jones protein (BJP) in 56, IgD in 7, and other types in 6. International staging system (ISS) stage was I in 86, II in 107, III in 102, and unknown in 23, respectively. As initial treatment, 192 patients were treated with conventional chemotherapy alone such as MP and VAD therapy, 88 with conventional chemotherapy + novel drugs such as bortezomib + dexamethasone and MP + bortezomib therapy, 21 with conventional chemotherapy (VAD) + ASCT, and 17 with novel drugs (bortezomib + dexamethasone) + ASCT, respectively. As a total, thirty-eight of the 318 patients (12%) aged 65–70 years were successfully treated with ASCT without any transplantation-related mortality.

The median PFS according to the different treatment modalities was 19.1 months for conventional chemotherapy group, 24.5 months for conventional chemotherapy + novel drug group, 26.8 months for conventional chemotherapy + ASCT group, and 35.2 months for novel drug + ASCT group, respectively, and PFS was significantly longer in the novel drug group ($P < 0.01$) as well as the novel drug + ASCT group ($P < 0.04$) in comparison with that of the conventional chemotherapy group (Figure 1), respectively. As for OS, the median OS of the conventional chemotherapy group was 46.0 months with other groups being not reached. Five-year estimates of survival were 40%, 62%, 63%, and 87%, respectively, and were significantly extended in the novel drug group ($P < 0.001$), the conventional chemotherapy + ASCT group ($P < 0.02$), and in the novel drug + ASCT group ($P < 0.02$) compared with the conventional chemotherapy group (Figure 1).

From this survey, it appeared that ASCT had been performed by the physician's discretion based on patient's condition irrespective of the age of 65 years or older. Although the patient characteristics of each treatment group vary, the clinical benefit represented by PFS and OS of the conventional chemotherapy + ASCT group was almost equal to that of the novel drug group. Furthermore, the induction therapy with novel drugs followed by ASCT consolidation seemed to have contributed to the significantly improved outcome. Therefore, ASCT can be an option even in the treatment of fit elderly patients [18].

6. Conclusion

ASCT can be applied to a selected patient of 65 years of age or older when performance status is fit and no

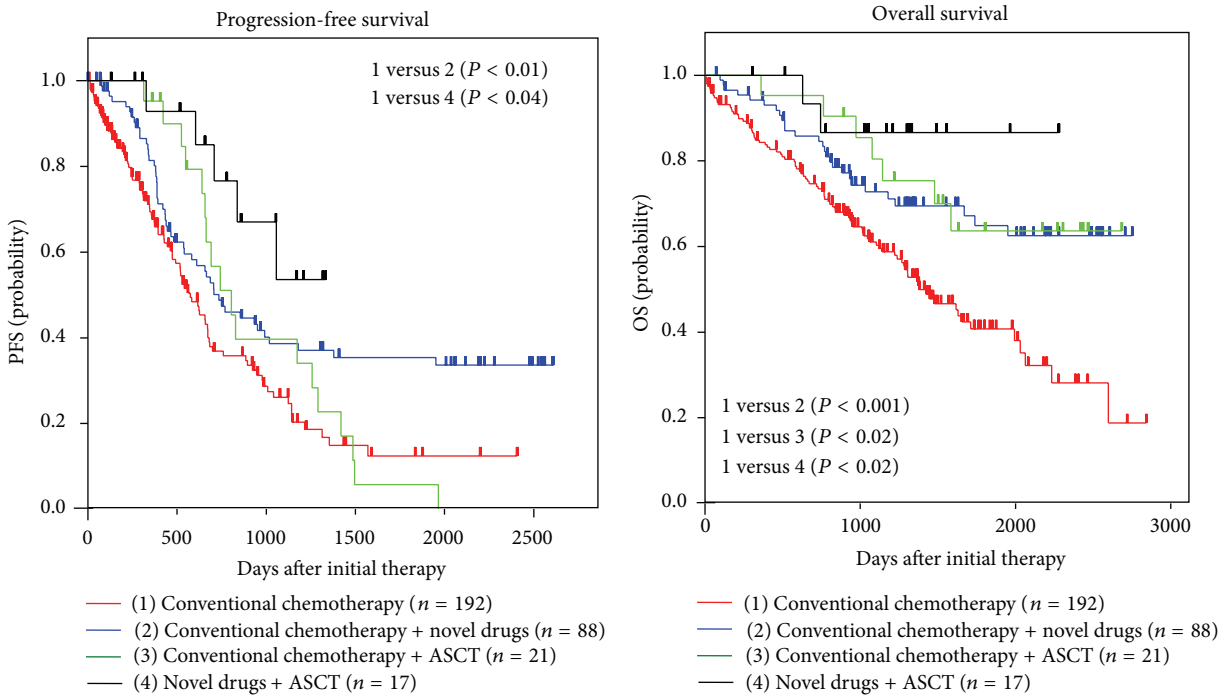


FIGURE 1: Retrospective analysis of outcome of newly diagnosed patients aged 65–70 years. Progression-free and overall survival according to the treatment groups such as conventional chemotherapy, conventional chemotherapy + novel drugs, conventional chemotherapy + ASCT, and novel drugs + ASCT is shown. Reproduced from [40] with permission from Karger.

comorbidities/complications are present. Recent studies have demonstrated that ASCT with intermediate-dose melphalan (100–140 mg/m^2) is a safe and effective treatment modality in patients younger than 70 years of age. Although the benefit of reduced-intensity ASCT had not been clearly demonstrated in the past decade, it can be a viable option if incorporated into sequential treatment strategies along with novel agents. Therefore, the indication of ASCT should be seriously considered in each elderly patient based on the performance status and the presence of complications and/or comorbidities but not on chronological age alone. The sequential approach including ASCT can be challenging but would be feasible approach to further improve the outcome of elderly patients with MM.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

Acknowledgments

The authors thank the contribution of the Japanese Society of Myeloma for their kind cooperation. This work was supported in part by a grant from International Myeloma Foundation (IMF) Japan.

References

[1] R. A. Kyle, J. A. Child, K. Anderson et al., "Criteria for the classification of monoclonal gammopathies, multiple myeloma and related disorders: a report of the International Myeloma Working Group," *British Journal of Haematology*, vol. 121, no. 5, pp. 749–757, 2003.

[2] A. Palumbo and K. Anderson, "Multiple myeloma," *The New England Journal of Medicine*, vol. 364, no. 11, pp. 1046–1060, 2011.

[3] R. Alexanian, A. Haut, A. U. Khan et al., "Treatment for multiple myeloma. Combination chemotherapy with different melphalan dose regimens," *Journal of the American Medical Association*, vol. 208, no. 9, pp. 1680–1685, 1969.

[4] M. Attal, J.-L. Harousseau, A.-M. Stoppa et al., "A prospective, randomized trial of autologous bone marrow transplantation and chemotherapy in multiple myeloma. Intergroupe Francais du Myelome," *The New England Journal of Medicine*, vol. 335, no. 2, pp. 91–97, 1996.

[5] J. A. Child, G. J. Morgan, F. E. Davies et al., "High-dose chemotherapy with hematopoietic stem-cell rescue for multiple myeloma," *The New England Journal of Medicine*, vol. 348, no. 19, pp. 1875–1883, 2003.

[6] J.-L. Harousseau, M. Attal, H. Avet-Loiseau et al., "Bortezomib plus dexamethasone is superior to vincristine plus doxorubicin plus dexamethasone as induction treatment prior to autologous stem-cell transplantation in newly diagnosed multiple myeloma: results of the IFM 2005-01 phase III trial," *Journal of Clinical Oncology*, vol. 28, no. 30, pp. 4621–4629, 2010.

[7] M. Cavo, P. Tacchetti, F. Patriarca et al., "Bortezomib with thalidomide plus dexamethasone compared with thalidomide plus dexamethasone as induction therapy before, and consolidation therapy after, double autologous stem-cell transplantation in newly diagnosed multiple myeloma: a randomised phase 3 study," *The Lancet*, vol. 376, no. 9758, pp. 2075–2085, 2010.

[8] P. Sonneveld, I. G. Schmidt-Wolf, B. van der Holt et al., "Bortezomib induction and maintenance treatment in patients with newly diagnosed multiple myeloma: results of the randomized phase III HOVON-65/ GMMG-HD4 trial," *Journal of Clinical Oncology* , vol. 30, no. 24, pp. 2946–2955, 2012.

[9] P. Sonneveld, H. Goldschmidt, L. Rosinol et al., "Bortezomib-based versus nonbortezomib-based induction treatment before autologous stem-cell transplantation in patients with previously untreated multiple myeloma: a meta-analysis of phase III randomized, controlled trials," *Journal of Clinical Oncology*, vol. 31, no. 26, pp. 3279–3287, 2013.

[10] A. Palumbo, S. Bringhen, T. Caravita et al., "Oral melphalan and prednisone chemotherapy plus thalidomide compared with melphalan and prednisone alone in elderly patients with multiple myeloma: randomised controlled trial," *The Lancet*, vol. 367, no. 9513, pp. 825–831, 2006.

[11] J. F. San Miguel, R. Schlag, N. K. Khuageva et al., "Bortezomib plus melphalan and prednisone for initial treatment of multiple myeloma," *The New England Journal of Medicine*, vol. 359, no. 9, pp. 906–917, 2008.

[12] S. V. Rajkumar, S. Jacobus, N. S. Callander et al., "Lenalidomide plus high-dose dexamethasone versus lenalidomide plus low-dose dexamethasone as initial therapy for newly diagnosed multiple myeloma: an open-label randomised controlled trial," *The Lancet Oncology*, vol. 11, no. 1, pp. 29–37, 2010.

[13] J. Martinez-Lopez, J. Blade, M.-V. Mateos et al., "Long-term prognostic significance of response in multiple myeloma after stem cell transplantation," *Blood*, vol. 118, no. 3, pp. 529–534, 2011.

[14] F. Gay, A. Larocca, P. Wijermans et al., "Complete response correlates with long-term progression-free and overall survival in elderly myeloma treated with novel agents: analysis of 1175 patients," *Blood*, vol. 117, no. 11, pp. 3025–3031, 2011.

[15] H. Brenner, A. Gondos, and D. Pulte, "Recent major improvement in long-term survival of younger patients with multiple myeloma," *Blood*, vol. 111, no. 5, pp. 2521–2526, 2008.

[16] D. Pulte, A. Gondos, and H. Brenner, "Improvement in survival of older adults with multiple myeloma: results of an updated period analysis of SEER data," *Oncologist*, vol. 16, no. 11, pp. 1600–1603, 2011.

[17] S. Pozzi, L. Marcheselli, A. Bari et al., "Survival of multiple myeloma patients in the era of novel therapies confirms the improvement in patients younger than 75 years: a population-based analysis," *British Journal of Haematology*, vol. 163, no. 1, pp. 40–46, 2013.

[18] A. Palumbo, S. Bringhen, H. Ludwig et al., "Personalized therapy in multiple myeloma according to patient age and vulnerability: a report of the European Myeloma Network (EMN)," *Blood*, vol. 118, no. 17, pp. 4519–4529, 2011.

[19] A. Badros, B. Barlogie, E. Siegel et al., "Autologous stem cell transplantation in elderly multiple myeloma patients over the age of 70 years," *British Journal of Haematology*, vol. 114, no. 3, pp. 600–607, 2001.

[20] A. Palumbo, S. Triolo, C. Argentino et al., "Dose-intensive melphalan with stem cell support (MEL 100) is superior to standard treatment in elderly myeloma patients," *Blood*, vol. 94, no. 4, pp. 1248–1253, 1999.

[21] H. D. Klepin and D. D. Hurd, "Autologous transplantation in elderly patients with multiple myeloma: are we asking the right questions?" *Bone Marrow Transplantation*, vol. 38, no. 9, pp. 585–592, 2006.

[22] J. Mehta, M. Cavo, and S. Singhal, "How I treat elderly patients with myeloma," *Blood*, vol. 116, no. 13, pp. 2215–2223, 2010.

[23] D. S. Siegel, K. R. Desikan, J. Mehta et al., "Age is not a prognostic variable with autotransplants for multiple myeloma," *Blood*, vol. 93, no. 1, pp. 51–54, 1999.

[24] B. Sirohi, R. Powles, J. Treleaven et al., "The role of autologous transplantation in patients with multiple myeloma aged 65 years and over," *Bone Marrow Transplantation*, vol. 25, no. 5, pp. 533–539, 2000.

[25] D. E. Reece, C. Bredeson, W. S. Pérez et al., "Autologous stem cell transplantation in multiple myeloma patients < 60 vs ≥ 60 years of age," *Bone Marrow Transplantation*, vol. 32, no. 12, pp. 1135–1143, 2003.

[26] E. Jantunen, T. Kuittinen, K. Penttilä, P. Lehtonen, E. Mahlamäki, and T. Nousiainen, "High-dose melphalan (200 mg/m^2) supported by autologous stem cell transplantation is safe and effective in elderly ($\geq$ 65 years) myeloma patients: comparison with younger patients treated on the same protocol," *Bone Marrow Transplantation*, vol. 37, no. 10, pp. 917–922, 2006.

[27] M. A. Gertz, M. Q. Lacy, A. Dispenzieri et al., "Impact of age and serum creatinine value on outcome after autologous blood stem cell transplantation for patients with multiple myeloma," *Bone Marrow Transplantation*, vol. 39, no. 10, pp. 605–611, 2007.

[28] S. K. Kumar, D. Dingli, M. Q. Lacy et al., "Autologous stem cell transplantation in patients of 70 years and older with multiple myeloma: results from a matched pair analysis," *The American Journal of Hematology*, vol. 83, no. 8, pp. 614–617, 2008.

[29] J. El Cheikh, E. Kfoury, B. Calmels et al., "Age at transplantation and outcome after autologous stem cell transplantation in elderly patients with multiple myeloma," *Hematology/Oncology and Stem Cell Therapy*, vol. 4, no. 1, pp. 30–36, 2011.

[30] T. Muta, T. Miyamoto, T. Fujisaki et al., "Evaluation of the feasibility and efficacy of autologous stem cell transplantation in elderly patients with multiple myeloma," *Internal Medicine*, vol. 52, no. 1, pp. 63–70, 2013.

[31] Q. Bashir, N. Shah, S. Parmar et al., "Feasibility of autologous hematopoietic stem cell transplant in patients aged $\geq$ 70 years with multiple myeloma," *Leukemia and Lymphoma*, vol. 53, no. 1, pp. 118–122, 2012.

[32] A. Palumbo, S. Bringhen, M. T. Petrucci et al., "Intermediate-dose melphalan improves survival of myeloma patients aged 50 to 70: results of a randomized controlled trial," *Blood*, vol. 104, no. 10, pp. 3052–3057, 2004.

[33] T. Facon, J. Y. Mary, C. Hulin et al., "Melphalan and prednisone plus thalidomide versus melphalan and prednisone alone or reduced-intensity autologous stem cell transplantation in elderly patients with multiple myeloma (IFM 99-06): a randomised trial," *The Lancet*, vol. 370, no. 9594, pp. 1209–1218, 2007.

[34] B. Barlogie, G. Tricot, E. Anaissie et al., "Thalidomide and hematopoietic-cell transplantation for multiple myeloma," *The New England Journal of Medicine*, vol. 354, no. 10, pp. 1021–1030, 2006.

[35] A. Palumbo, F. Gay, P. Falco et al., "Bortezomib as induction before autologous transplantation, followed by lenalidomide

as consolidation-maintenance in untreated multiple myeloma patients," *Journal of Clinical Oncology*, vol. 28, no. 5, pp. 800–807, 2010.

[36] F. Gay, V. Magarotto, C. Crippa et al., "Bortezomib induction, reduced-intensity transplantation, and lenalidomide consolidation-maintenance for myeloma: updated results," *Blood*, vol. 122, no. 8, pp. 1376–1383, 2013.

[37] C. Straka, K. Schäfer-Eckart, F. Bassermann et al., "Prospective randomized trial of Len/Dex induction followed by tandem MEL 140 with autologous blood stem cell transplantation and Len maintenance versus continued therapy with Len/Dex in myeloma patients age 60–75 years: protocol-defined safety analysis after 100 patients," *Blood*, vol. 120, no. 21, 2012.

[38] S. K. Kumar, S. V. Rajkumar, A. Dispenzieri et al., "Improved survival in multiple myeloma and the impact of novel therapies," *Blood*, vol. 111, no. 5, pp. 2516–2520, 2008.

[39] S. K. Kumar, A. Dispenzieri, M. Q. Lacy et al., "Continued improvement in survival in multiple myeloma: changes in early mortality and outcomes in older patients," *Leukemia*, 2013.

[40] S. Ozaki, T. Harada, T. Saitoh et al., "Survivalof multiple myeloma patients aged 65–70 years in the era of novel agents and autologous stem cell transplantation: a multicenter retrospective collaborative study of the Japanese Society of Myeloma and the European Myeloma Network," *Acta Haematologica*. In press.