The prognostic impact of multiparameter flow cytometry immunophenotyping and cytogenetic aberrancies in patients with multiple myeloma

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Objectives: The aim of this study was to evaluate the prognostic impact of immunophenotyping in patients with multiple myeloma (MM), as well as other markers of disease, such as serum hyaluronan and cytogenetic aberrancies.

Methods: We have prospectively analyzed the prognostic impact of antigenic markers, assessed by multiparametric flow cytometry (MFC), in a series of newly diagnosed MM patients (n = 79).

Results and discussion: Our results show that the expression of CD44, CD45, and CD28 and the absence of CD117 were associated with a significantly shorter progression free-survival (PFS). Clinical characteristics were collected; Cytogenetic aberrancies were assessed in 40 patients. Multivariate survival analyses identified that the CD117−, CD28+, CD45+, and the percentage of bone marrow plasma cells by MFC are survival predictor, along with the International Staging System stage. Interestingly, the CD117− patients were associated with chromosomal aberrancies, including del (17p), +1q21, and IgH translocations.

Conclusion: The incorporation of multiparameter flow cytometry immunophenotyping into the routine diagnostic evaluation of MM patients can help to identify patients at a high risk of progression.

Keywords: Multiple myeloma, Immunophenotyping, Flow cytometry, Cytogenetics, Hyaluronan

Introduction

Multiple myeloma (MM) is one of the most prevalent hematologic malignancy and remains incurable for most patients, with survival durations ranging from a few months to longer than 10 years.1,2 Multiparameter flow cytometry (MFC) immunophenotyping has been considered an indispensable tool for the diagnosis, classification, prognosis, and monitoring of hematologic malignancy. In MM, the use of multiparametric flow cytometry in many clinical diagnostic laboratories is currently restricted to clinical research studies and the differential diagnosis of unusual cases.3–5 However, the generation and identification of markers that allow the unequivocal identification of plasma cells (PCs) among other hematopoietic cells (such as CD138), and the identification of aberrant PC phenotypes that enable us to discriminate between normal and neoplastic PCs.6,7 It was determined that flow cytometric phenotyping could be used for the routine diagnosis of MM.8 According to European Myeloma Network recommendation for the diagnosis of MM by flow cytometry antigens, such as CD138, CD38, CD33, CD19, CD56, CD45, CD117, CD20, and CD28 must be used.7–9 In addition, antigens, such as CD45, CD56, CD117, and CD28, have been identified as prognostic markers for MM.7–9 The prognostic impact of PC immunophenotype has been broadly investigated by several researchers. Some studies suggest that markers associated with a more immature PC phenotype, such as CD20, CD45, correlate with a poor outcome.10 Also, a higher expression of CD44 and downregulation of CD56 have been associated with an extramedullary spreading of malignant PC,11,12 and the expression of CD28 has been related to disease activity.13 More recently, CD19 expression on MM PCs has been studied and shown to be an adverse prognostic marker, while CD117 was found to be associated with a favorable outcome.14 However, frequent discrepancies have been reported.
As a consequence, the clinical and prognostic value of immunophenotyping in MM remains questionable. We have prospectively analyzed the prognostic impact of a relatively high number of antigenic markers in a series of 79 newly diagnosed patients with MM uniformly treated with bortezomib and dexamethasone. Our results show that four individual markers (CD44, CD45, CD28, and CD117) may afford prognostic information.

Materials and methods

Patients’ samples and clinical details

This study was approved by institutional review board of Shanghai Jiao Tong University (Shanghai, China) and written informed consent was obtained from each subject. Seventy-nine consecutive patients with newly diagnosed MM (n = 79) were enrolled between November 2012 and December 2014 (Table 1). All patients received a MM diagnosis based on the criteria recently defined by the International Myeloma Working Group,15 and were staged according to the criteria of International Staging System (ISS).16 For the patients enrolled, they were all uniformly treated with bortezomib and dexamethasone. The median follow-up was 14 months (range 1–25 months). Bone marrow (BM) examinations were performed as routine diagnostic procedure or for evaluation purposes. Parameters collected after enrollment were age at diagnosis, gender, ISS stage, immunoglobulin (Ig) class, blood hemoglobin, serum albumin, serum calcium, creatinine, serum-beta-2-MG, and serum C-reactive protein. Fluorescence in situ hybridization (FISH) analyses were performed in 40 out of 79 patients for changes on 13q14, 1q21, 14q32, and 17p13 locus. Table 1 presents patient characteristics.

Flow cytometry analysis

Immunophenotyping was performed on fresh BM. Cells were stained with a four-color labeling system using mouse anti-human monoclonal antibodies conjugated to respective fluorochromes. Cell surface antigens were assessed by direct immunofluorescence using fluorescein isothiocyanate (FITC), phycoerythrin (PE), phycoerythrin-cyanide-5 (PE-Cy7), and allophycocyanin (APC) conjugated to monoclonal antibodies (Becton Dickinson, San Diego, CA, USA and BD Pharmingen San Diego, CA, USA). Heparinized BM cells (10^6/tube) were incubated with the following combinations of monoclonal antibodies (FITC/PE/PE-Cy7/APC): CD45/CD38/CD3/CD138, CD19/CD56/CD138/CD13, CD44/CD28/CD38/CD138, and CD20/CD117/CD38/CD138 for 15 minutes. Then, erythrocytes were lysed in 1 ml FACSTM Lysing Solution (Becton Dickinson). An aliquot just labeled with CD138-APC was used as a negative control. Data were acquired on a FACS Calibur flow cytometer (Becton Dickinson) and the FlowJo 7.6.1 software (TreeStar, Ashland, OR, USA) was used for analysis. PCs were identified on the basis of a strong CD38 expression (CD38++), expression of CD138 (CD138+), and low or negative CD45 expression, as well as on their typical light scatter properties. All investigated antigen expressions were performed on CD38++/CD138+ cells (cells from R3 as shown in Fig. 1).

Table 1: Clinical and biological characteristics of patients with multiple myeloma

| Characteristic                  | n = 79 |
|--------------------------------|--------|
| Sex                            |        |
| Male, n (%)                    | 46 (58) |
| Female, n (%)                  | 33 (42) |
| Median age, years (range)      | 64 (38–84) |
| Ig subtype, n (%)              |        |
| IgG                            | 39 (50) |
| IgA                            | 21 (27) |
| Light chain                    |        |
| Kappa                          | 6 (7)   |
| Lambda                         | 7 (9)   |
| Non-secretory                  | 6 (7)   |
| ISS, n (%)                     |        |
| I                              | 32 (40) |
| II                             | 18 (23) |
| III                            | 29 (37) |
| Median beta-2-microglobulin, mg/l (range) | 4.9 (1.2–16.3) |
| Median hemoglobin, g/dl (range) | 10.3 (2.6–16.3) |
| Median creatinine, μmol/l (range) | 89 (53–311) |
| Median calcium, mg/dl (range)   | 9.24 (7.6–15.04) |
| Median C-reactive protein, mg/dl (range) | 2.00 (0.03–4.57) |
| Median albumin, g/dl (range)    | 3.6 (2.3–5.1) |
| Median hyaluronan, μg/l (range) | 214.5 (50.4–912.8) |

Enzyme-linked immunosorbant assay

Hyaluronan protein levels were determined in BM samples from 79 patients using a sandwich-type enzyme-linked immunosorbant assay (ELISA) according to the manufacturer’s protocol (R&D Systems, Inc., Minneapolis, MN, USA). Absorbance was read at 450 nm.

BM PC sorting

Bone marrow mononuclear cells (BMMC) were obtained from BM of patients with newly diagnosed MM by density gradient centrifugation. CD138+ cells were positively selected using the MACS separation kit (Miltenyi Biotech, Auburn, CA, USA). Purity was assessed by flow cytometry and PC purity always exceeded 90%. The enriched bone marrow plasma cells (BMPCs) were prepared for the FISH analysis.
FISH Analysis

FISH analyses were performed on the above BMPCs samples of 40 patients according to the manufacturer’s instructions. Five probes were used for FISH analyses: P53 deletion probe (17p13), 1q21 amplification (+1q21) probe, retinoblastoma 1 (RB1) deletion probe (13q14), IgH double color separation probe (14q32), and D13S319 deletion probe (13q14). (Beijing GP Medical Technologies, Inc., Beijing, P.R. China). FISH patterns were considered positive or negative as shown in Fig. 2.

Statistical analysis

Estimation of progression free-survival (PFS) was performed using the method of Kaplan and Meier. The log-rank test was used for comparisons of PFS curves. In addition, Cox proportional hazard regression analysis was used to evaluate the prognostic

Figure 1 Flow cytometric detection of neoplastic plasma cells. All investigated antigen expressions were performed on CD38+/CD138+ cells (cells from R3).

Figure 2 Detection of P53 deletion (green), 1q21 amplification (red), RB1 deletion (green), IgH double color separation, and D13s319 deletion (red) by FISH in patients with MM (n = 40). The PCs are highlighted by fluorescent staining of 2-(4-amidinophenyl)-6-indolecarbamidine dihydrochloride (DAPI) (blue).
impact of the different factors in univariate models as well as in a multivariate model, together with prognostically relevant clinical factors. The Chi-square test was used to estimate differences between groups. P-values < 0.05 were considered statistically significant.

Results

The prognostic impact of BMPC counts on PFS

The median percentage of PC counts in BMPC measured by conventional morphology (CM) (27.5%; range: 1–95%) were significantly higher (P < 0.001) than that obtained by MFC (1.61%; range: 0.03–48%). Despite these differences, the PC counts with the two techniques were significantly correlated (R² = 0.58, P < 0.001, Fig. 3A). PC counts assessed by CM and MFC distinguish between groups of patients with different prognosis, with optimal cut-off values of 10 and 30% BMPC for MFC and CM, respectively. Patients with less than 10% BMPC had significantly longer PFS (median of 9 vs. 5 months; P = 0.006; Fig. 3C) than patients with or more than 30% BMPC.

Frequency of antigen expression and the impact on patients’ survival

Table 2 summarizes the frequencies and patterns of antigen expression found in the 79 patients with MM. The results show that the antigenic profile of myelomatous PC is highly diverse (Tables 2 and 3). Myelomatous PC from a minority of patients retained surface antigens typically associated with early B-cell maturation stages. CD19 expression was found in 2.5% of cases (data not shown); while CD20 and CD45 were detected in 6% (data not shown) and 23% (Table 2) of the cases, respectively. Bright expression of CD56, a marker involved in the anchorage of PC to stromal structures, was present in 84% of patients with MM. Positivity for CD117, absent in normal PC, was detected in 22% of all myeloma patients. Other antigens such as CD28 and CD33, whose expression is typically associated with non-B-cell hematopoietic lineages (activated T-lymphocytes and myeloid cells, respectively), were observed in 19 and 33% of the patients, respectively.
Table 2 Prognostic impact of the patterns of antigen expression on PFS for patients with multiple myeloma

| Patterns of antigen expression | Patients | PFS (months) |
|-------------------------------|----------|-------------|
|                              | n | % | median | 95% CI | P-value |
| CD28+                         | 15 | 19 | 7 | 4–10 | 0.005 |
| CD28−                         | 64 | 81 | 9 | 7–11 |       |
| CD117+                        | 17 | 22 | 14 | 10–18 | 0.001 |
| CD117−                        | 62 | 78 | 8 | 7–10 |       |
| CD56+                        | 66 | 84 | 8 | 6–10 | 0.35  |
| CD56−                         | 13 | 16 | 8 | 6–10 |       |
| CD45+                          | 18 | 23 | 6 | 5–7 | 0.03  |
| CD45−                          | 61 | 77 | 9 | 8–10 |       |
| CD33+                       | 26 | 33 | 6 | 4–8 | 0.54  |
| CD33−                         | 53 | 67 | 9 | 8–10 |       |
| CD44+                        | 51 | 65 | 7 | 5–9 | 0.003 |
| CD44−                         | 28 | 35 | 12 | 8–16 |       |
| CD28−CD117+                  | 12 | 15 | 4 | 2–6 | <0.001|
| CD28−CD117−                  | 14 | 18 | 16 | 13–19 |       |
| CD28−CD117+ or CD28−CD117− | 53 | 67 | 8 | 6–10 |       |

Table 2 and Fig. 4 show the impact of individual antigens on PFS for patients with MM. The patients with CD45+ expression (18 of 79 cases; 23%) had a clearly worse disease outcome as compared with CD45− cases (PFS, 6 vs. 9 months; P = 0.03; Table 2 and Fig. 4C). Over expression of CD56 (n = 66; 84%) did not significantly influence PFS (median, 8 vs. 8 months; P = 0.35) (Table 2 and Fig. 4D). Expression of CD117 (17 of 79 cases; 22%) conferred a favorable clinical outcome as defined by longer PFS (median, 14 vs. 8 months; P = 0.001; Table 2 and Fig. 4B). In contrast, expression of CD28 (n = 15; 19%) was associated with an adverse prognosis, leading to a shorter PFS (median, 7 vs. 9 months; P = 0.005; Table 2 and Fig. 4A). CD33+ positive patients (n = 26; 33%) also displayed shorter PFS, but without statistical significance (6 vs. 9 months; P = 0.54; Table 2 and Fig. 2E). On analyzing the prognostic impact of different antigen combinations, we found that based on the simultaneous assessment of CD28 and CD117 expression three different risk group categories could be identified: good prognosis, CD28− CD117+ patients, with a median PFS of 16 months; poor prognosis, CD28+ CD117− patients, with a median PFS of 4 months; and an intermediate prognosis subgroup, CD28− CD117−/CD28+ CD117+ patients, with median PFS of 8 months (Table 2 and Fig. 4F).

**Correlation between the antigen expression and clinical characteristics**

A significant relation was observed between CD28 expression, combined with CD117 and serum-beta-2-microglobulin (beta2-MG). Patients were also divided into three groups: CD28− CD117+ patients, CD28+ CD117− patients, and CD28− CD117−/CD28+ CD117+ patients. CD28+ CD117− patients vs. CD28− CD117+ patients (8.25 vs. 3.37 mg/l; P = 0.001), CD28− CD117−/CD28+ CD117+ patients vs. CD28− CD117+ patients (6.21 vs. 3.37 mg/l; P = 0.012) (Fig. 5). There was no significant difference of other clinical characteristics such as hemoglobin, serum calcemia, and creatinine between the three groups (Fig. 5).

**Serum hyaluronan and CD44 expression in MM patients**

Interestingly, a significant relation was observed between CD28 expression, combined with CD117 and serum hyaluronan. Patients were also divided into three groups: CD28− CD117+ patients, CD28− CD117− patients, and CD28− CD117−/CD28+ CD117+ patients. CD28− CD117+ patients vs. CD28− CD117− patients (6.99, 57 vs. 135.86 μg/l; P < 0.001), CD28− CD117−/CD28+ CD117+ patients vs. CD28− CD117+ patients (222.45 vs. 135.86 μg/l; P = 0.026) (Fig. 6A).

The expression of CD44 (n = 51; 65%) was associated with an adverse prognosis, leading to a shorter PFS (median, 7 vs. 12 months; P = 0.003; Fig. 6B).

**Relationship between the antigen expression and cytogenetic aberrancies**

Regarding the correlation of cytogenetics with immunophenotypic profiles, it should be noted that lack of CD117 expression was associated with high-risk cytogenetics, including 1q21 amplification, del (17p), and IgH translocation (Table 4).
Figure 4 Prognostic impact of individual phenotypic markers on survival of patients with MM ($n = 79$). (A, CD28; B, CD117; C, CD45; D, CD56; E, CD33; F, CD28 and CD117).
Survival analysis
In the study, PFS was calculated from the date of the diagnosis to the date of progression. Univariate and multivariate analyses of prognostic factors for PFS were performed in the whole series of patients \((n = 79)\). Patients were excluded if there was any missing data in the parameters collection. Analyses of prognosis factors for survival were performed using the usual clinical (% of BMPC by MFC, % of BMPC by MC, ISS, age), biological (beta-2-MG, hemoglobin, calcium, creatinine, and hyaluronan), immunophenotypic (CD117, CD33, CD28, CD56, CD45, and CD44), cytogenetic (+1q21, chromosome 13q, 17p deletion, and 14q32 rearrangement) characteristics at diagnosis. In the whole series, variables showing an independent adverse impact on PFS were more than 10% BM PC by flow cytometry \((P = 0.012)\), ISS stage \((P < 0.001)\), CD45 \((P = 0.009)\), CD28 \((P = 0.005)\), and CD117 \((P = 0.003)\) Table 3.

Discussion
In this decade, flow cytometry-based immunophenotyping has been routinely applied in many laboratories for the diagnosis, classification, monitoring of patients and identification of prognostic markers in different hematologic diseases. However, frequent discrepancies have been reported in MM, which may

Figure 5 The clinical characteristics (A, beta-2-MG; B, calcium; C, creatinine; D, hemoglobin) in three different groups (CD28⁺CD117⁻, CD28⁻CD117⁺, and CD28⁻CD117⁻/CD28⁺CD117⁺).

Figure 6 (A) The serum hyaluronan expression in MM patients in three different groups (CD28⁺CD117⁻, CD28⁻CD117⁺, CD28⁻CD117⁻/CD28⁺CD117⁺) (B) Prognostic impact of one of the hyaluronan receptors-CD44 on survival of patients with MM \((n = 79)\).
selected the BMPC counts obtained by MFC as an independent prognostic factor for overall survival ($P = 0.011$), supporting the incorporation of MFC into the routine diagnostic evaluation of MM patients and validating the clinical utility of BMPCs counting by MFC approaches. The fewer PCs detected by MFC could be explained by the existence of small PC clusters in the BM, and by the different quality of BM samples used for the two techniques, whereby there was greater peripheral blood contamination in the MFC samples. In this sense, it was found that a significantly lower percentage of PCs detected by MFC than by CM performed on first-pull BM aspirates, but similar values when the CM was performed on cytocentrifuged samples. Also, samples evaluated by CM contain cells associated with lipid-enriched spicules, whereas MFC is performed on the BM fluid, which is depleted in the lipid-adhesive PC. In fact, morphological quantification of BMPC may focus on those microaggregates where PCs are abundant, rather than from a randomly chosen field.

In this study, we have prospectively analyzed the prognostic impact of six relevant antigenic markers, assessed by MFC, in a series of 79 newly diagnosed patients with MM. Our results show that four individual markers (CD44, CD43, CD28, and CD117) may afford prognostic information. Moreover, the combinations of CD28 and CD117 allow patient stratification into three risk categories: poor risk CD28$^+$ CD117$^-$ patients (15%), intermediate CD28$^-$ CD117$^+$ and CD28$^+$ CD117$^+$ patients (56%), and good risk CD28$^-$ CD117$^+$ myeloma patients (19%). A recent Italian group published the results of an extensive study on 511 elderly patients (>65 years) with newly diagnosed MM enrolled in the GIMEMA-MM-03-05 trial with two treatment regimens. Their findings indicated that CD19$^+$/CD117$^+$ BMPC immunophenotype had an adverse impact on overall survival, and treatment with thalidomide might overcome the adverse impact. In our study, the CD19 expression was found in 2.5% of cases (only two patients). Therefore, the prognostic impact of CD19 could not be analyzed.

CD117 (C-kit) is an essential hematopoietic growth factor receptor with tyrosine-kinase activity. It is absent in normal PC but can be detected in 22% of patients with MM. Mateo et al. found that three individual markers, CD19, CD28, and CD117, were prognostically relevant. They showed that CD117 expression is associated with a significantly longer PFS, and that together with CD28 it probably represents the prognostically most important combination of phenotypic markers in MM. In our study, we also confirmed it. Patients lacking CD117 expression showed more cytogenetic aberrancies, such as +1q21, del (17p), and IgH translocation.

Table 3 Results of univariate and multivariate analyses of impact of clinical factors on survival in MM patients

| Variable | RR (95% CI) | $P$-value |
|----------|------------|-----------|
| Univariate analyses of expression correlation and clinical variables with survival |
| CD33     | 0.86 (0.52–1.43) | 0.57      |
| CD117    | 2.77 (1.45–5.39) | 0.002     |
| CD56     | 1.31 (0.72–2.39) | 0.384     |
| CD28     | 0.46 (0.25–0.83) | 0.009     |
| CD45     | 0.57 (0.33–0.98) | 0.044     |
| CD44     | 0.49 (0.29–0.82) | 0.006     |
| Hycloran | 0.54 (0.32–0.93) | 0.027     |
| Beta-2-MG| 0.30 (0.17–0.52) | <0.001    |
| Creatinine | 0.47 (0.22–0.98) | 0.04      |
| Calcium  | 0.95 (0.52–1.74) | 0.87      |
| Hemoglobin | 1.0 (0.62–1.59) | 0.98      |
| ISS      | 2.66 (1.87–3.77) | <0.001    |
| % of BMPC by MFC | 0.52 (0.30–0.90) | 0.018     |
| % of BMPC by MC  | 0.51 (0.31–0.86) | 0.011     |
| Age >65  | 0.52 (0.32–0.83) | 0.007     |
| High-risk cytogenetic | 0.55 (0.34–0.89) | 0.015     |
| Multivariate analyses, results from the Cox stepwise regression analyses |
| CD117    | 2.83 (1.42–5.63) | 0.003     |
| CD28     | 0.39 (0.20–0.76) | 0.005     |
| CD45     | 0.47 (0.27–0.83) | 0.009     |
| ISS      | 2.23 (1.52–3.29) | <0.001    |
| % of BMPC by MFC | 0.46 (0.25–0.84) | 0.012     |

95% CI: 95% confidence interval; RR: risk ratio. High-risk cytogenetic by FISH: del (17p), +1q21, IgH translocations. Cox proportional hazard regression analysis was used to assess variables in univariate and multivariate analyses. Values of $P < 0.05$ were considered statistically significant.

be due to an inappropriate study design and technical pitfalls.

Quantification of BM PCs in MM patients by CM is a mandatory test for the diagnosis and response assessment. However, the degree of BMPC infiltration by CM may vary significantly not only among but also within patients. The merit of MFC immunophenotyping to assess the prognosis is still considered unproven. In this study, we compare the BM PC counts obtained by CM and MFC. We also explore the potential prognostic impact of both techniques in 79 newly diagnosed MM patients. Although multiparameter flow cytometry generally yields lower PC counts (median percentage of 1.61 vs. 27.5%, respectively; $P < 0.001$), there is a significant positive correlation between the two techniques ($R^2 = 0.58$, $P < 0.001$). Our results show that multivariate analysis

Table 4 FISH results of 40 patients with multiple myeloma

| Chromosomal aberrancy (n = 40) | CD117$^+$ (n = 27) | CD117$^-$ (n = 13) | $P$-value |
|--------------------------------|--------------------|--------------------|-----------|
| PS(17p13)                      | 19                 | 3                  | 0.013     |
| IgH (14q32)                    | 17                 | 3                  | 0.043     |
| D13S319(13q14.3)               | 22                 | 7                  | 0.146     |
| 1q21(1q21)                     | 19                 | 4                  | 0.042     |
| RB1(13q14)                     | 19                 | 9                  | 1.000     |

Frequencies were compared using the Chi-square test. Bold font emphasizes statistically significant results.
CD28 antigen is expressed on T lymphocytes and it is an important antigen for T cell activation.21 Interestingly, recent reports suggest that CD28 could be involved in dendritic cell–PC interactions within the BM stroma, which could play an important role in myeloma cell survival.22,23 This is supported by the fact that the addition of anti-CD28 monoclonal antibody to myeloma cell cultures induces apoptosis.24 Several studies had already suggested that the expression of CD28 is associated with an increased myelomatous PC proliferation, tumoral expansion, and treatment failure.9,25 In our study, the relation between CD28 expression and cytogenetic aberrancies was not confirmed, as FISH analyses were performed in only 40 out of 79 patients and the difference was not significant.

CD44 is a single-pass, glycosylated class-I transmembrane protein involved in multiple cellular functions, including interaction with the matrix microenvironment and intracellular signaling.26,27 The extracellular portion of CD44 primarily binds to the glycosaminoglycan hyaluronan (HA), thereby contributing to cell adhesion, migration, angiogenesis, inflammation, wound healing and downstream signaling that promotes cell growth and survival.27 An extracellular domain of CD44 can be cleaved and found as a soluble entity in the serum, where it has been identified as a biomarker correlating with poor outcomes in solid tumors such as the colon, as well as hematologic malignancies such as non-Hodgkin lymphoma, and possibly myeloma.28–30 Finally, serum HA itself may be a predictive marker of a poor prognosis for MM.31 We found that the expression of CD44 was associated with an adverse prognosis, leading to a shorter PFS. Moreover, a significant relation was observed between CD28 expression, combined with CD117 and serum hyaluronan.

So far, no defined conclusions can be drawn regarding CD45 expression in MM patients. Most reports agree that CD45- phenotype represents the malignant PC population in myeloma.10 CD56 expression strongly correlates with CD45- PCs. Similarly, PC late marker CD138 is also highly expressed on CD45- cells rather than on CD45+ cells.32 Conversely, it was observed that the proliferating PC compartment, which is theoretically involved in disease progression, is included within the CD45+ bright PC fraction.33 Of note, we found that CD45 expression is associated with a significantly shorter PFS.

A total of 79 consecutive patients were enrolled in this prospective study starting on November 2012. However, FISH was introduced into our department on September 2013, and only 40 cytogenetic aberrancies could be assessed. Cytogenetics was also an important prognostic factor of MM. In order to analyze the relations between cytogenetics and immunophenotype, the 40 patients were divided into two groups: CD117+ and CD117− patients. It was shown that the CD117− patients were associated with chromosomal aberrancies, including del (17p), +1q21, and IgH translocations. These findings suggest that antigenic expression of PCs and particularly the CD117− immunophenotype may contribute to identify patients with myeloma with high risk of progression and short survival.

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