Multiple Myeloma • Research Paper

Patients with CD45 negative multiple myeloma receiving high-dose therapy have a shorter survival than those with CD45 positive multiple myeloma

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A B S T R A C T

Background and Objectives. CD45 is a critical regulator of signaling threshold in immune cells. There are clinical and animal studies suggesting that the CD45-negative phenotype is the phenotype of progressive multiple myeloma (MM). The aims of this study were to confirm this hypothesis and to test the prognostic value of CD45 expression in newly diagnosed MM patients.

Design and Methods. In a retrospective study of 95 newly diagnosed MM patients treated with high dose therapy we used 4-color flow cytometry to determine CD45 expression and correlated the immunophenotipic data with clinical data.

Results. Thirty of 95 patients (31.5%) lacked CD45 expression at diagnosis. The CD45 phenotype significantly affected the overall survival (OS) of the patients, like the most common presenting prognostic parameters analyzed including β-2-microglobulin, age and 14q32 translocations. CD45 negative MM patients had a significantly worse OS than did CD45 positive cases of MM: 28.7% cumulative survival at 4 years, median 42 months vs not reached; $p = 0.004$. Furthermore, CD45 remained the only parameter adversely affecting OS in multivariate analysis.

Interpretation and Conclusions. The CD45 negative phenotype could reflect the phenotype of progressive disease in relation to the intrinsic malignancy of the MM clone. Indeed, CD45 negative myeloma cells appear to have a greater capacity to circulate, disseminate and clone as well as being less sensitive to apoptosis.

Key words: multiple myeloma, CD45 prognostic factor, high-dose therapy.

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CD45, the first receptor-like protein tyrosine phosphatase expressed on all nucleated hematopoietic cells, is a critical regulator of signaling thresholds in immune cells.[1] Perturbation of its function may contribute to autoimmunity, immunodeficiency and malignancy. The expression of CD45 declines *in vivo* during normal plasma cell (PC) differentiation. Indeed, CD45 is brightly expressed in normal immature proliferative PC but weakly expressed in mature resting PC of the bone marrow.[2-3] In all cases of MM, a small population of myeloma cells, which always corresponds to the most proliferative myeloma cells, presents bright expression of CD45.[4] In the rest of myeloma cells, CD45 expression declines but remains detectable (CD45 positive MM) or becomes undetectable (CD45 negative MM). It has already been shown that CD45 negative MM correspond to those with myeloma cells circulating in the peripheral blood and/or to cases of advanced disease.[5-10] These data suggest that the CD45 negative phenotype could be the phenotype of progressive MM and be of negative prognostic value when present at diagnosis. Of note, it has been shown in the 5T2 murine model that both CD45 negative and CD45 positive cell subsets are able to circulate and migrate to the bone marrow but that CD45 positive MM cells have a higher bone marrow homing.[11,12]

In order to confirm our hypothesis that it is the CD45 negative rather than the positive phenotype that is the phenotype of progressive disease, we evaluated CD45 expression using flow cytometry on bone marrow PC in newly diagnosed MM patients receiving high-dose therapy (HDT) and tested the prognostic value of this expression.

Table 1. Patients' characteristics.

	All *n = 95*	*CD45 positive* *n = 65*	*CD45 negative* *n = 30*
Median age	59 (33-70)	59 (33-70)	59 (37-70)
Sex (M/F)	49/46	32/33	17/13
Stage DS II/III	23/72	16/49	7/23
Isotype G/A/BJ	65/30/10	39/20/6	16/10/4
Median β2 microglobulin (mg/L)	3.6 (1.3-20)	3.5 (1.3-17.6)	3.8 (2.1-20)
Median Calcium (mM/L)	2.3 (2.1-3.9)	2.3 (2.1-3.1)	2.3 (2.1-3.9)
Median creatinine (mM/L)	86 (50-580)	86 (50-494)	88 (58-580)
Median C-reactive protein (mg/L)	4 (0.6-119)	4.2 (0.6-112)	3.8 (2.5-119)
Median Hemoglobin (g/dL)	10.8 (4.9-14.8)	10.8 (4.9-14.8)	10.8 (5.5-13.2)
Median albumin (g/L)	42 (23.7-55)	42.5 (23.7-55)	41.1 (32-54)
C13As	45	27	18
Yes	42	30	12
No	8	8	0
Missing			
Q14q32 rearrangements			
germline	24	14	10
t(11;14)	20	15	5
t(4;14)	15	8	7
t(14;16)	1	0	1
other	28	21	7
no	7	7	0
Conditioning regimens			
High dose melphalan	76	52	24
High dose melphalan + total body irradiation	19	13	6
Disease status at transplantation			
Complete response	20	14	6
Partial response	47	32	15
Stable disease	21	14	7
Refractory disease	7	5	2

Design and Methods

In this single center retrospective trial, we studied 95 patients with newly diagnosed MM treated with HDT as part of front-line treatment. These patients were selected on the basis of the availability of CD45 immunophenotype and complete clinical data (Table 1). CD45 expression was analyzed in a four-color assay as follows: for the four color immunofluorescence staining, 5×10^5 bone marrow mononuclear cells were incubated for 20 minutes at room temperature with anti-CD45 FITC, anti-CD138 PE-Cy5 (Beckman Coulter, Miami, FL, USA), APC anti-CD38 (Becton Dickinson, San José, CA, USA) and different PE-conjugated monoclonal antibodies of interest. Cells were fixed in 1 % formaldehyde and analyzed on a FACS Calibur flow cytometer with Cell Quest Sofware (Becton Dickinson). Data acquisition was always performed in two steps : firstly, 15,000 total cells were collected; secondly, at least 1,000 PC were acquired with an activated live gate on SSC versus CD38 (strongly positive) dot plot and PC were identified using the sequential gating strategy shown in Figure 1. CD45 mean fluorescence intensities (MFI) of both lymphocytes and PC were measured. In order to compare CD45 expression on tumor cells from different patients, we calculated CD45 corrected MFI (cMFI) of PC in arbitrary units to refer to the lymphocytes as an internal standard (Figure 2). We used the following formula : CD45 corrected MFI of PC = CD45 MFI of PC/CD45 MFI of lymphocytes×100, with an arbitrary value of 100 for the lymphocytes. In all MM patients, 2 subpopulations of myeloma cells were always observed : a CD45 bright one (20% of cells, median value) and a low to negative one (80% of cells, median value) (Figure 1B). Patients were considered to have CD45 positive MM when all the tumor cells expressed CD45, that is, when there was both a CD45 bright compartment and a CD45 low (but positive i.e., CD45 cMFI > 2.5) compartment. In contrast, patients were considered to have CD45 negative MM when the majority of tumor cells (i.e., 80% median value; range: 51-99%) lacked CD45 expression (cMFI < 2.5), although these patients did have the smaller CD45 bright compartment. This cut-off value for positivity (CD45 cMFI of 2.5 units) was arbitrarily defined: it corresponds to 2 standard deviations below the mean of CD45 cMFI values of

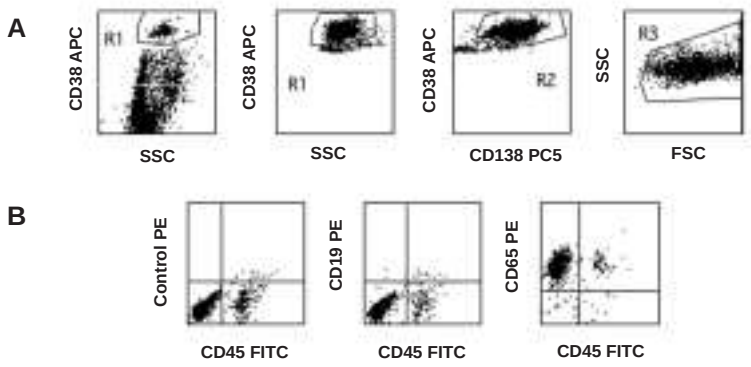

Figure 1. Sequential gating strategy used to identify PC and their phenotype. (A) An initial region (R1) was set around cells expressing a high level of CD38 with their typical side scatter (log SSC) and then a second region R2 was set on the CD38 versus CD138 to exclude contaminating events. A third region R3 was set on the light scatter of the cells satisfying R1 and R2 to exclude debris or apoptotic PCs (characterized by a lower expression of CD138 and a different light scatter distribution). (B) Dot plots display representative malignant phenotype of gated PC.

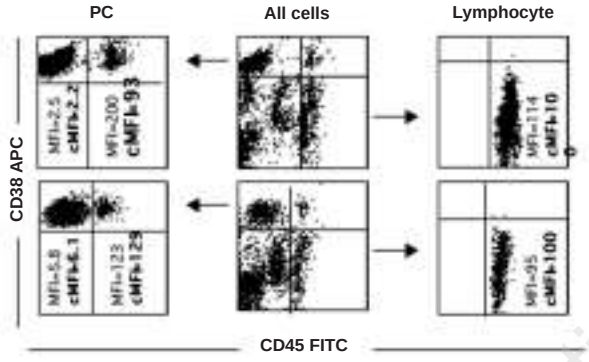

Figure 2. Mononuclear cells were stained with control PE, CD38, CD138, CD45; lymphocytes and PC CD45 MFI were determined and CD45 cMFI was calculated. Examples of positive and negative cases are shown in the bottom row and in the top row, respectively.

normal PC from 11 normal bone marrow samples (*N. Robillard et al., unpublished data*). In some of these patients, the CD45 ratio (CD45 MFI of PC divided by control MFI of PC) was measured to confirm that a CD45 cMFI value < 2.5 corresponds to true CD45 negativity (ratio ≤ 1).

Samples were also analyzed by interphase fluorescent *in situ* hybridization (FISH) with probes specific for the following chromosomal changes: chromosome 13q abnormalities, illegitimate rearrangements of the IgH gene (t(14q32)), translocations: t(4;14)(p16;q32), t(11;14)(q13;q32), and t(14;16)(q32;q23), as previously described.[13]

The presenting parameters listed in Table 1 were examined for their prognostic value on overall survival (OS) and event-free survival (EFS). OS and EFS were calculated from the time of diagnosis according to the method of Kaplan and Meier. All the parameters with significance in the univariate analysis were then included in a multivariate analysis using a Cox model.

Results

The median EFS time of the whole group was 20 months, and the actuarial OS was 60.6% at 6 years. At diagnosis, 30 patients out of 95 (31.5%) lacked CD45 on a majority of myeloma cells (group A: CD45 negative MM) and 65 retained CD45 positivity (group B: CD45 positive MM). Both groups were identical regarding presenting characteristic features, disease status at the time of HDT, and conditioning regimens used before autologous stem cell transplantation (Table 1). There was only a trend for a higher number of t(4;14) or t(14;16) in the CD45 negative group (7/30 vs 8/65 in the CD45 positive group, $p = 0.092$), and a higher number of C13As in the CD45 negative group (27/57 vs 18/30 in the CD45 positive group), although this difference did not reach statistical significance, $p = 0.03$). Response after HDT was identical in both groups and the median EFS was not statistically different in the two groups: 24.5 months in CD45 positive patients as compared with 18.3 months in patients lacking CD45 ($p = 0.29$, log-rank test). Nevertheless, patients lacking CD45 expression at diagnosis had a significantly worse OS than those retaining CD45 expression (28.7% cumulative survival at 4 years, median 42 months vs not reached, Kaplan-Meier survival analysis; $p = 0.004$, log-rank test, Figure 3). This indicates that, despite identical additional treatments at the time of relapse (thalidomide, combination chemotherapy or subsequent transplantation), the sur-

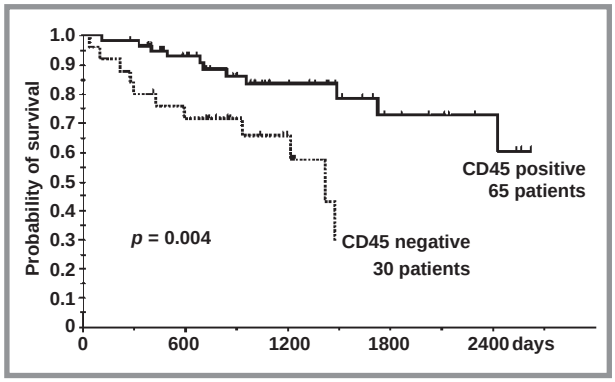

Figure 3. Survival according to CD45 phenotype.

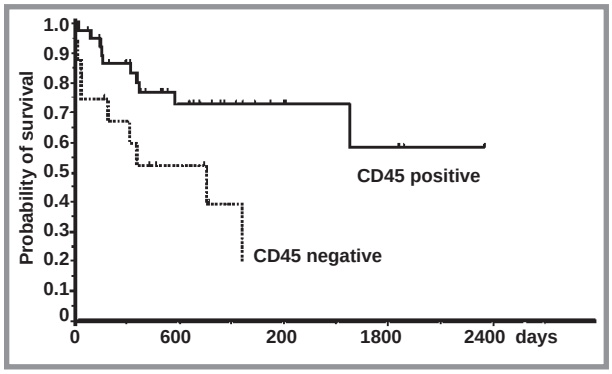

Figure 4. Survival after relapse.

vival after relapse was shorter in the CD45 negative population, and that more often in this latter group patients did not respond to further therapy (Figure 4).

In the univariate prognostic analysis, 4 parameters significantly influenced OS : CD45 phenotype, β2 microglobulin cocentration (< or > 3 mg/L), age (< or > 59 years), and chromosomal translocations. Chromosome 13q deletions did not statistically influence OS ($p = 0.12$). In the multivariate analysis, CD45 phenotype was the single parameter influencing OS.

Discussion

The CD45 negative phenotype is the phenotype of progressive disease in MM, as it is in the 5T2 mouse model.[14] Conversely, in the same murine model, Asosingh *et al*. reported that CD45 positive MM cells were associated with a longer survival.[15] We found that 20% to 30% of patients with *de novo* MM had the CD45 negative phenotype at diagnosis. Our study confirms the prognostic significance of CD45 phenotype by showing the poor outcome of patients with CD45 negative MM and conversely the better survival and outcome of those with CD45 positive MM. These results fit perfectly with the 5T2 murine model. Indeed, the major result of the current study is that the CD45 phenotype is the only prognostic parameter for OS that remains significant in the multivariate analysis. Of note, although CD45 negative MM did not differ greatly from CD45 positive MM in terms of major biochemical features, they tended to be more frequently associated with the most aggressive 14q32 translocations, such as t(4;14) and t(14;16), at presentation. This finding should be confirmed in a larger series of patients. The complete remission rate was the same in the 2 groups of patients. Thus, the poor outcome of the CD45 negative patients is probably related to other important intrinsic factors influencing malignancy such as ploidy, kinetics or other oncogenic events, especially at the time of relapse. Although the mechanisms favoring a greater malignancy of CD45 negative myeloma cells are under investigation, some points deserve comment. In a SCID-human model of MM, CD45 negative myeloma cells were more clonogenic than the others cells.[16] Furthermore CD45 negative myeloma cells were also less sensitive to apoptosis.[17] Taken together, these data already favor the concept that CD45 negative myeloma cells have a greater capacity to circulate, disseminate and clone, and thus a greater malignancy. Further prospective studies are necessary to confirm this concept, our prognostic observations and to investigate the mechanisms of disappearance of CD45 during the progression of multiple myeloma.

PM and RB both wrote the article. NR and DP performed the phenotype analyses. HA-L performed the cytogenetic analyses. PM, NM, NM are the physicians who treated the patients. JLH is the head of the Department of Clinical Hematology of the University Hospital of Nantes. RB is the head of the Central Laboratory of Hematology of the University Hospital of Nantes.

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