Leukemia Research 44 (2016) 32–39

Contents lists available at ScienceDirect

Leukemia Research

journal homepage: www.elsevier.com/locate/leukres

Research paper

The prognostic significance of CD45 expression by clonal bone marrow plasma cells in patients with newly diagnosed multiple myeloma

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ARTICLE INFO

Article history:
Received 19 October 2015
Received in revised form 2 March 2016
Accepted 8 March 2016
Available online 10 March 2016

Keywords:
CD45
Multiple myeloma
Survival

ABSTRACT

Evaluation of clonal plasma cells (PCs) in the bone marrow (BM) of multiple myeloma (MM) patients reveals two distinct clonal PC populations based on the presence or absence of CD45 expression. We explored the prognostic significance of CD45 expression by clonal PCs in the BM of MM patients in the era of novel agent therapy. All 156 MM patients seen at the Mayo Clinic, Rochester from 2009 to 2011 who had their BM evaluated by multiparametric flow cytometry were included. Patients whose BM had ≥20% of the clonal PCs expressing CD45 were classified as CD45 positive (+) and the rest as CD45 negative (−). Of these patients, the median overall survival (OS) for patients in the CD45 (+) group (n = 43, 28%) was 38 months versus not reached for the CD45 (−) group (n = 113, 72%) (P = 0.009). In a multivariable analysis, CD45 (+) status was an independent predictor of inferior OS among newly diagnosed patients with MM. CD45 expression may be a surrogate for a more aggressive phenotype of MM and warrants further investigation.

1. Introduction

Multiple myeloma (MM) is characterized by the clonal proliferation of plasma cells (PCs) in the bone marrow (BM) leading to end organ damage such as anemia, bone destruction, hypercalcemia or renal insufficiency [1]. Multiparameter flow cytometry has been utilized routinely in clinical practice to characterize the PCs in the BM as normal or malignant/clonal based on their immunophenotypic signatures [2]. CD45 is a receptor-like protein tyrosine phosphatase expressed on the surface of all nucleated hematopoietic cells and is a critical regulator of antigen mediated signaling and activation in B- and T lymphocytes [3,4]. However, its role in the biology of MM remains controversial. During normal PC development and differentiation, there is a progressive in vivo decline in CD45 expression; i.e. CD45 is brightly expressed in normal immature PCs but weakly expressed in mature PCs of the BM [5]. Consequently, evaluation of clonal PCs in the BM of MM patients reveals two distinct PC populations: CD45 negative (−) and CD45 positive (+) PCs [6,7].

A number of in vitro studies using normal and clonal PCs indicate that bright CD45 expression is seen on the most actively proliferating PCs [8]. Yet, prior clinical studies have made the counterintuitive observation that an increased proportion of CD45 (+) clonal PCs in MM is associated with a better prognosis [9–11]. Many of these prior studies utilized three or four color flow cytometry technologies and were conducted in an era devoid of novel agent induction therapy. The advent of six-color multiparameter flow cytometry in clinical practice has allowed for greater sensitivity in detecting the CD45 expressing clonal PC component. Thus, in the modern era of novel agent induction therapies, we explored the prognostic significance of CD45 expression by clonal PCs in newly diagnosed MM patients using six-color multiparameter flow cytometry.

2. Methods

We retrospectively evaluated all newly diagnosed MM patients seen at the Mayo Clinic, Rochester between October 2009 and November 2011 who had their BM samples evaluated by multipa-

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http://dx.doi.org/10.1016/j.leukres.2016.03.003
0145-2126/

Table 1
Demographic and clinical characteristics of the MM patients.

Variables	Newly diagnosed MM		
	All patients (N = 156)	CD45 + cohort (N = 43)	CD45 − cohort (N = 113)
Age[a]	67 (39–95)	66 (48–95)	68 (39–90)
Male (No, %)	94 (60%)	29 (67%)	65 (58%)
PCLI%[a]	0.8 (0–8.6)	1 (0–8.6)	0.8 (0–6)
Bone marrow PC%[a]	45 (5–100)	50 (5–95)	45 (5–100)
LDH[a]	159 (3–878)	160 (75–878)	159 (3–656)
Beta−2-microglobulin[a]	5.0 (1.7–27.2)	5.0 (2.4–27.2)	3.8 (1.4–19.3)
Creatinine[a]	1 (0.4–7.6)	1.1 (0.6–4.9)	1.0 (0.4–7.6)
Albumin[a]	3.5 (2.3–4.5)	3.5 (2.4–4.5)	3.5 (2.3–4.3)
High risk FISH (152 pts only) (No, %)	43 (28%)	7 (17%)	36 (32%)
Deletion 17p/Monosomy 17	28	4	24
t(4;14)	14	2	12
t(14;16)	7	1	6
t(14;20)	1	0	1
ISS stage at diagnosis (Available on 147 pts only) (No, %)			
	43 (29%)	12 (29%)	31 (29%)
• Stage 1	59 (40%)	13 (32%)	46(43%)
• Stage 2	45 (31%)	16 (39%)	29(28%)
• Stage 3			
Induction therapy (No, %)			
Novel agents	153 (98%)	42 (98%)	111 (98%)
	10 (6%)	1 (2%)	9 (8%)
	104 (67%)	28 (65%)	76 (67%)
• Thalidomide	56 (35%)	20 (47%)	36 (32%)
• Lenalidomide			
• Bortezomib			
Upfront post-induction ASCT (No, %)	50 (32%)	13 (30%)	37 (33%)

[a] Median(Range).

rameter flow cytometry. Approval for this study was obtained from the Mayo Clinic IRB in accordance with the federal regulations and the principles of the Declaration of Helsinki.

Immunophenotyping was performed on fresh bone marrow using six-color multiparametric flow cytometry. The fresh whole bone marrow obtained from each patient underwent lysis with ammonium chloride and then was suspended in phosphate buffered saline (PBS) with 3.0% bovine serum albumin (BSA). 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). The BM mononuclear cells were isolated by Ficoll gradient and stained with six antibodies in a single tube: kappa FITC and lambda PE (both polyclonal antibodies were obtained from Dako, Carpinteria, Ca), CD138 Percp Cy-5.5, CD19 PE Cy-7, CD38 APC, CD45 AP C-H7 (All monoclonal antibodies were obtained from BD Biosciences, San Jose, CA). The flow cytometry data was collected using the Becton Dickinson FACSCanto II instruments that analyzed 150,000 events (cells); this data was then analyzed by multi-parameter analysis using the BD FACS DIVA Software. PCs were selectively analyzed through combinatorial gating using light scatter properties and CD38, CD138, CD19, and CD45. Normal PC's were then separated from clonal PCs based on the differential expression of CD45, CD19 and polytypic immunoglobulin light chains. The clonal PCs detected were reported out as the number of clonal events/150,000 collected total events. Fig. 1 depicts a typical flow cytometry gating pattern in a patient with a kappa-restricted clonal PC population that strongly expresses CD45. The PCs were first identified based on their expression of CD138 and CD38 and then further separated from polyclonal PCs based on their lack of CD19 expression. As seen in the figure, a large portion of this selected population expresses CD45 and is kappa light chain restricted. A proportion of the patients also had their peripheral blood evaluated by six-color multiparametric flow cytometry for clonal circulating plasma cells (cPCs) as described in prior studies [12,13].

The primary end-points of the study were OS and time to next therapy (TTNT). OS was measured from the day of diagnosis to death from any cause, with censoring performed at the date of last contact. TTNT was determined from the day of diagnosis to the day of initiating the next therapy due to a documented relapse or progression of disease, with those alive and relapse free censored at the day of last follow up. Patients who had *fluorescent* in situ *hybridization* (FISH) analysis performed on their bone marrow aspirate at diagnosis were categorized as having high risk disease if they had any of the following abnormalities: t(4;14), t(14;16), t(14;20) and del17p. Host and disease variables at diagnosis that were examined for prognostic significance included: age, bone marrow plasma cell percentage, presence of high-risk FISH, ISS stage at diagnosis, plasma cell labeling index (PCLI), serum M spike, urine M spike, hemoglobin, creatinine and LDH. FISH studies and PCLI were performed as previously described [14,15]. PCLI levels of greater than 3% were considered high as they have been conventionally asso-