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Antigenic drift in relapsed extramedullary multiple myeloma: plasma cells without CD38 expression

Prashant Tembhare¹, Constance Yuan¹, Neha Korde^{2,3}, Irina Maric⁴, and Ola Landgren³

¹Flow Cytometry Unit, Laboratory of Pathology, National Cancer Institute, NIH, Bethesda, MD, USA

²Hematology Branch, National Heart, Lung, and Blood Institute, NIH, Bethesda, MD, USA

³Metabolism Branch, National Cancer Institute, NIH, Bethesda, MD, USA

⁴Hematology Section, Department of Laboratory Medicine, Clinical Center, NIH, Bethesda, MD, USA

In the past, overall survival for patients with multiple myeloma (MM) was estimated at around 3–5 years. After the introduction of high-dose melphalan chemotherapy followed by autologous stem cell rescue, and novel agents (such as bortezomib and immunomodulatory drugs [IMiDs]), the nature of treating MM has dramatically changed by nearly doubling overall survival [1]. With such an improvement in the survival of patients with MM, the question remains whether disease biology and local microenvironment interactions evolve to facilitate more highly aggressive forms of the disease, such as extramedullary relapse. Indeed, the frequency of extramedullary disease seems to be higher in the relapse setting compared to newly diagnosed patients with MM [2,3]. Although there is lack of scientific evidence, one may speculate that dynamic changes seen in the relapse setting may be supported by current novel therapeutics impacting local marrow microenvironment interactions, allowing neoplastic plasma cells to harbor and survive in a different environment. Such disease biology may not respond to conventional therapeutic strategies [4]. Also, from a diagnostic standpoint, neoplastic cells in patients with relapsed and refractory MM may or may not express conventional markers due to altered biology. These findings suggest there may be gradually increasing diagnosis- and treatment-related challenges in relapsed MM in the future.

Multiparametric flow cytometry (MFC) can distinguish normal from neoplastic plasma cells, provide prognostic information regarding progression risk in monoclonal gammopathy of undetermined significance (MGUS) [5] and smoldering multiple myeloma (SMM), and detect minimal residual disease in patients with MM post-therapy [6]. Plasma cells exhibit characteristically bright CD38 and CD138 expression, and are further defined by the expression of CD45, CD19, CD20, CD27, CD28, CD56 and CD117, amongst others [7,8],

Correspondence: Dr. Ola Landgren, National Cancer Institute, National Institutes of Health, Center for Cancer Research, Metabolism Branch, 9000 Rockville Pike, Bldg 10/Room 13N240, Bethesda, MD 20892, USA. Tel: +1-301-496-0670. Fax: +1-301-402-0172. landgreo@mail.nih.gov.

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forming the basis for the European Myeloma Network consensus methodology [8] on plasma cell analysis by flow cytometry. We describe a novel case of CD38 negative MM, its impact on the interpretation of flow cytometry analysis and its biological and clinical implications.

In brief, our patient, a 71-year-old African American male, presented with refractory MM in August 2010 after originally being diagnosed >10 years ago and having several lines of therapy, including melphalan/prednisone, autologous stem cell transplant in 2001, and lenalidomide, cyclophosphamide, bortezomib, bendamustine and pomalidomide. Recently, he displayed evidence of progressive disease during his last course of therapy, with a rising monoclonal protein concentration and development of a 3 × 4 cm soft-tissue abdominal mass.

As part of the clinical work-up, a bone marrow biopsy was performed and routinely processed for histopathological and immunohistochemical evaluation. Bone marrow aspirate and a fine needle aspirate (FNA) from the abdominal extramedullary mass were sent for flow cytometry analysis. Specimens were acquired using an eight-color multiparametric approach on a three-laser FACS Canto II (BD Biosciences, San Jose, CA) with DiVa 6.1.1 software and analyzed by FCS Express 3 software (DeNovo Software, Los Angeles, CA).

Morphological evaluation of the bone marrow biopsy revealed a hypocellular marrow with a monoclonal population of CD138 (+), CD56 (+), CD117 (+) and kappa (+) but CD38 negative plasma cells constituting 10–15% of the total cell population [Figs. 1(IA)–1(IG)]. Flow cytometric evaluation of the bone marrow aspirate showed that the plasma cells were CD138 (+), CD38 dim (+), CD19 (–), CD20 (–), CD27 dim (+), CD45 dim (+), CD56 bright (+), CD117 (+), with monoclonal intracytoplasmic kappa light chain expression [Figs. 1(IIIE)–1(IIIL)]. In the abdominal extramedullary mass, plasma cells were CD138 (+), CD38 (–), CD19 (–), CD20 (–), CD27 dim (+), CD45 (–), CD56 (+), CD117 (–) [Figs. 1(IIIA)–1(IIID)]. The CD38 (–) plasma cells were identified using CD138, CD56 and forward scatter. Corresponding cytological evaluation demonstrated atypical plasmacytoid cells. Table I illustrates the immunophenotypic differences of clonal plasma cells between bone marrow and extramedullary mass, highlighting the evolutionary immunomodulation in the neoplastic cells. To our knowledge, neoplastic plasma cells lacking CD38 expression are a novel finding. The intensity of CD38 expression by neoplastic plasma cells may often be slightly decreased as compared to normal plasma cells [7,8], but CD38 dim (+) expression is reportedly rare [9].

In the standard diagnostic setting, primary gating of plasma cells is based on CD38, CD138 and CD45, as recommended by the European Myeloma Network (EMN) consensus. Immunophenotypically, plasma cells are universally recognized by bright CD38 expression; however, in this case, extensive down-regulation of CD38 in plasma cells of the bone marrow, and lack of CD38 in plasma cells of the abdominal extramedullary mass, posed a special diagnostic challenge. Utilizing CD138, CD56 and forward scatter characteristics proved valuable in diagnosis, especially in the bone marrow, where the neoplastic plasma cells could have potentially gone undetected amidst a heterogeneous mixture of many cell types. This highlights an emerging pitfall in the EMN approach for flow cytometric isolation

of abnormal plasma cells in relapsed cases of myeloma, creating diagnostic challenges. In such situations, a multiparametric approach should be implemented.

CD38 is a transmembrane glycoprotein with a complex array of functions. It can act as a signal transducer and is involved in cellular proliferation, differentiation and lymphocyte activation [10]. It can also function as a receptor-mediated adhesion molecule via its interaction with CD31, and mediate cell adhesion to hyaluronan and other ligands [11]. Its role as an adhesion molecule suggests that CD38 may influence the interaction between plasma cells and endothelial cells and/or the bone marrow stromal microenvironment; alternatively, CD38 down-regulation may facilitate the migration of malignant plasma cells to peripheral blood or other extramedullary sites. Although CD38 is widely expressed among many hematological cell compartments, expression is highest in plasma cells, making CD38 a possible therapeutic target [12] that may potentially modify the course of this disease.

Reportedly, extramedullary plasmacytomas are immunophenotypically similar to MM, although clinical behavior is distinct [13]. Prior studies have proposed that an antigenic drift may be due to MM disease evolution, a consequence of exposure to MM treatment, or a combination of these [14]. Furthermore, aberrant changes in plasma cell antigen expression (loss of CD79a; gain of CD20, CD56 and cyclin D1) in MM correlate with a poor prognosis [14,15]. In this case, a notable antigenic drift was detected in the extramedullary mass, as compared to the bone marrow (decreased CD38, CD45 and loss of CD117) [Figs. 1(IIA)–(IID)]. This observation raises the following question: does the antigen down-regulation represent a drug-induced microenvironmental change that impacts the clonal plasma cells, or does it represent the selective survival and proliferation of an antigen negative plasma cell subpopulation that exists as a consequence of multiple therapies? Therapy-induced antigenic changes occur in hematological malignancies such as acute lymphoblastic and myeloid leukemia, making detection of minimal residual disease a challenging undertaking [16]. Certainly, therapy-induced antigenic changes could play a role here. On the other hand, CD38 expression varies significantly during B-cell maturation and differentiation to plasma cells. While it is consistently expressed among the many stages of B-cell development, the level of CD38 expression varies, and further increases in density during differentiation to plasma cells. The antigenic drift may represent the expansion of a more immature-appearing type of plasma cell (perhaps a myeloma stem-cell with features intermediate between a mature B-cell and a mature plasma cell) that may escape current therapies which currently target the most mature-appearing plasma cells exhibiting typically bright CD38 expression. The causative mechanisms behind this antigenic drift remain unclear, and are highly speculative; however, it is highly likely that a combination of MM disease evolution and the influence of local microenvironment interactions facilitated by extended MM therapy are responsible for these antigenic changes.

In summary, our novel findings of a CD38 negative extramedullary MM with a prominent antigenic drift shed important light on the application of multiparametric flow cytometry as a tool to assess extramedullary disease in MM. Given that patients with MM live longer after the introduction of novel therapies [1], it seems reasonable to believe that this phenomenon will become increasingly common. On a clinical note, since the disease biology typically is

more aggressive when there is extramedullary relapse, it is important for clinicians and flow cytometry diagnostic laboratories to be aware and suspect biological variations in this setting, so that appropriate therapy and clinical management decisions can be initiated without unnecessary delays. From a research perspective, future studies are needed to better characterize the molecular underpinnings of plasma cells in extramedullary disease relapse versus in the bone marrow, with the aim to better understand mechanisms of clonal heterogeneity and clonal evolution.

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Figure 1.

(I) (A) Paraffin sections of bone marrow biopsy showing hypocellular bone marrow with clusters of plasma cells (hematoxylin and eosin staining). Plasma cells are positive for CD138 (B), CD56 (C), CD117 (D), and show kappa light chain restriction (F). CD38 expression (E) is markedly dim to negative. Plasma cells are negative for lambda light chain (G) expression. (Original magnification $\times 200$.) (II) Multiparameter (eight-color) flow cytometric immunophenotyping of an abdominal mass fine needle aspirate (FNA) and bone marrow aspirate (FACSCanto II Flow Cytometer, BD Biosciences; FSC Express v3 analysis software, DeNovo Software). Plasma cells are highlighted in red, with background viable cells in gray, when present. Plasma cells of the FNA were CD138 (+), CD38 (-), CD19 (-), CD20 (-), CD45 (-), CD56 (+), CD27 dim (+) and CD117 (-) (A-D). Plasma cells of the bone marrow aspirate were CD138 (+), CD38 dim (+), CD19 (-), CD20 (-), CD27 dim (+), CD45 dim (+), CD56 bright (+), CD117 (+), with monoclonal intracytoplasmic kappa light chain expression (E-L). Isotype controls were utilized (I, J) for intracytoplasmic light chain staining.

Table I

Immunophenotypic variation of malignant plasma cells from extramedullary mass and bone marrow.

Immunophenotyping marker	Abdominal extramedullary mass	Bone marrow aspirate
CD38	–	Dim +
CD138	+	+
CD56	+	Bright +
CD117	–	+
CD27	Dim +	Dim +
CD45	–	Dim +
CD19	–	–
CD20	–	–

+, positive; –, negative.