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CD33 is expressed on plasma cells of a significant number of myeloma patients, and may represent a therapeutic target

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TO THE EDITOR

Multiple myeloma (MM) is a clonal malignant plasma cell disorder characterized by the accumulation of malignant cells within the bone marrow, leading to lytic bone lesions and medullary insufficiency. Despite recent advances in the management of patients with MM (high-dose chemotherapy, thalidomide and derivatives, proteasome inhibitors), the disease remains ultimately lethal within variable periods of time, ranging from a few weeks to more than 10 years. Thus, novel therapeutic approaches are highly warranted.

In the past few years, several monoclonal antibodies have been shown to present therapeutic activities in a broad range of malignancies, either for hematological malignancies or for solid tumors. One of the best examples is the rapid positioning of rituximab in the therapeutic armamentarium of CD20-positive hemopathies. Thus, the search of novel potential therapeutic targets on cell surface of malignant cells is a major objective in clinical research. For instance, we have recently shown that CD20 was expressed at the surface of malignant plasma cells of 10–15% of MM,[1] leading to an ongoing prospective therapeutic trial using rituximab.

In a systematic examination of such potential targets in MM, we conducted an expertise of the expression of CD33 on plasma cells. CD33 is usually expressed in the myeloid lineage (but not in the B-cell lineage),[2] and has been targeted by monoclonal antibodies coupled with calicheamycin (gemtuzumab ozogamicin, Mylotarg®, Wyeth, Philadelphia). This modified antibody is now used in the treatment of patients with acute myeloid leukemia (AML) who express CD33 in more than 90% of the cases, with encouraging results.[3]

We studied the expression of CD33 on bone marrow plasma cells of 65 consecutive patients with MM analyzed between December 2003 and December 2004, and on 16 human myeloma cell lines. In four patients, two samples could be analyzed, one at diagnosis and a second one at relapse. All the 69 patient bone marrow specimens were centrifuged (Ficoll-Hypaque) before labeling with the following antibodies: anti-CD45-FITC (J33), anti-CD138-PECy5 (B-B4), anti-CD38-APC (HB7), and with control-PE or anti-CD33-PE (P67.6) in a four-color assay. Labeled cells were then analyzed on a FACSCalibur flow cytometer (BD Biosciences, San Jose, CA, USA), with the CellQuest Pro software (BD Biosciences). Plasma cells were identified using a sequential gating strategy as previously described.[1] The following human myeloma cell lines have been analyzed: RPMI-8226, NCI-H929, JJN3, OPM2, U266, JIM3, KMS11, L363, MDN, NAN3, NAN4, SBN, XG1, XG2, XG6, and LP1.

CD33 was detected on plasma cells in 23 of the 65 patients with MM (35%) and in four of the 16 myeloma cell lines (25%). When detected, it was expressed on 11–100% of the malignant plasma cells (median = 54%) (Figure 1). The positivity cutoff was arbitrarily fixed at 50%. With this cutoff, 14 patients and three cell lines (RPMI-8226, NAN-4, and XG2) were positive, with a median percentage of CD33-positive plasma cells of 85% (range = 50–100%) (Table 1). Three of these 14 patients have been analyzed twice (at diagnosis and at early relapse), and similar CD33 expression was observed. CD33 expression is restricted to malignant plasma cells (not or slightly expressed on normal bone marrow plasma cells, data not shown). A similar incidence of CD33 expression has been previously reported in MM.[4,5] In CD33-positive patients, the median ratio of fluorescence intensity (RFI) was 14 (range = 6.4–29). When compared to CD33-positive AML, the median MM RFI was equivalent

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Table 1 Main characteristics of patients and cell lines with CD33 expression

	CD33 positive (%)	*Median % of CD33+ plasma cells (range)*	*t(4;14) in CD33+ cases (%)*	*t(4;14) in CD33− cases (%)*
Patients at diagnosis	14/65 (22)	85 (50–100)	5/13 (38)	6/49 (12)
Myeloma cell lines	3/16 (19)	100	0/3	5/13 (38)

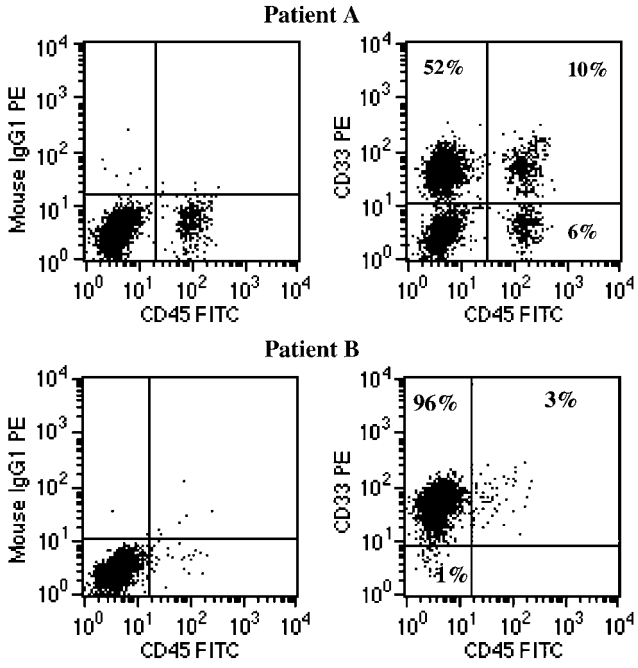

Figure 1 Flow cytometry plots (CD33 *vs* CD45), characteristic of two patients with CD33-positive myeloma. Patient A: 3.7% of the mononuclear bone marrow cells were plasma cells. Among them, 62% expressed CD33, with a similar intensity in the CD45-positive and CD45-negative plasma cell populations (mean ratio of fluorescence intensity were 13 and 12%, respectively). Patient B: 49% of the mononuclear cells were plasma cells, most of them lacking CD45 expression (mean ratio of fluorescence intensity was 16).

(data not shown). These results support the idea to use Mylotarg® in the treatment of CD33-positive MM patients. Moreover, most of the CD33-positive MM patients expressed CD33 in the large majority of plasma cells, supporting the hypothesis of a good therapeutic index. Furthermore, the CD45-positive plasma cells, which are supposed to represent the clonogenic population, expressed CD33 in a similar fashion to CD45-negative ones. Thus, the use of Mylotarg® might also target the myeloma stem cell population. CD33 is a sialic acid-dependent cell adhesion molecule, with a cytoplasmic tail bearing two tyrosine residues.[6] Phosphorylation of these residues recruits Src homology-2 domain-containing tyrosine phosphatases, leading to inhibitory intracellular signals.[7] Normally, CD33 expression is restricted to the myeloid lineage. After ligation to CD33, Mylotarg® is rapidly internalized and translocates to lysosomes, leading to the generation of radical species and ultimately to DNA cleavage.

This series of MM patients was also analyzed for the most frequent cytogenetic abnormalities (del(13), t(4;14), t(11;14)), using fluorescence *in situ* hybridization (FISH), as previously described.[8] In all, 13 of the 14 CD33-positive patients were fully characterized (no available frozen cells for the 14th patient): five presented t(4;14), five presented del(13), and three presented t(11;14). Of note, a higher incidence of t(4;14) was observed in CD33-positive patients (38 *vs* 12% in CD33-negative patients, $P = 0.06$). Thus, CD33 expression might be more frequent in the patients with the poorer prognosis, reinforcing the potential therapeutic interest of Mylotarg® in the treatment of CD33-positive MM patients. These results incite to conduct a phase II therapeutic trial testing Mylotarg® in MM.

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