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Title: Novel antigens LMA and KMA are expressed on bone marrow plasma cells from patients with AL Amyloidosis but they are not detected on normal plasma cells

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Background:

Amyloidosis (AL) is a clonal plasma cell (PC) disorder associated with secretion of immunoglobulin free light chains (FLCs); ~80% of patients produce lambda and ~20% produce kappa isotype. Fragments of the light chain variable domain play a critical role in forming amyloid fibrils that deposit in peripheral organs leading to organ dysfunction. The Mabs described here do not bind to the variable region domains or fragments of the variable and constant region domains.

We investigated 3 human monoclonal antibodies (Mabs) – 2 LambdaMabs and 1 KappaMab – that bind to cell-membrane-bound antigens called lambda myeloma antigen (LMA) and kappa myeloma antigen (KMA). LMA and KMA consist of FLCs that are not associated with heavy chain. The LambdaMabs (10B3 and 7F11) bind specifically to conformational epitopes in the lambda constant region of isotypes 1, 2 and 3 but with different affinities. KappaMab (formerly MDX-1097) binds to a conformational epitope in the kappa constant region. The conformational epitopes of LMA and KMA are presented in lipid rafts of AL and myeloma clonal PC membranes but are not found on normal PCs.

Aims:

To compare LMA or KMA expression with other PC markers in bone marrow (BM) samples from AL patients and MM patients with amyloid deposits. To determine cell lysis in PCs after incubation with the Mabs.

Methods:

Multiparametric FCM immunophenotyping was performed in BM samples with APC or PE labelled 10B3, 7F11 or KappaMab Fab'2 fragments along with CD38, CD138, BCMA, SLAM F7, CD56 and CD45 Mabs. PCs were identified by initial gating using CD38 and CD138. Ag density was calculated using QuantiBrite beads in PE. Unmanipulated BM samples from AL patients were cultured for 48 hours in the presence and absence of the antibodies and AL-PC lysis analyzed by flow cytometry

using annexin-V staining. Accordingly, CD16 expression on natural killer (NK) cells was evaluated to determine NK-cell-mediated tumor cell lysis.

Results:

A total of 31 BM samples were tested; 27 from AL patients, including newly diagnosed AL (NDAL) , treated, and relapsed, refractory AL (RRAL), and 4 from MM patients with amyloid deposits.

All samples (100%) expressed LMA (n=23) or KMA (n=8), as well as BCMA (n=30). LMA and KMA were detected on CD38 positive cells in 6 of 6 patient samples (100%) treated with daratumumab. The range of antigen densities was higher for LMA and KMA versus BCMA.

For the LambdaMabs, the 10B3 antigen density was higher than for 7F11 in 11 of 11 cases (100%). 7F11 was slightly better than 10B3 at inducing AL-PC lysis. In a limited sample set, cell lysis was higher in NDAL patients (n=6) than in RRAL patients (n=2). AL-PC lysis by all Mabs was accompanied by a decrease in the expression of CD16.

Summary/Conclusion:

LMA or KMA were detected in all samples from daratumumab-treated patients. In addition, KMA or LMA were expressed on PCs on all samples and their expression was generally higher than for BCMA. The 10B3 LambdaMab was better than the 7F11 LambdaMab at detecting LMA. KappaMab, 10B3 and 7F11 all induced PC lysis, but 7F11 appears to be better than 10B3 at inducing PC lysis. Although the increased cell lysis in NDAL vs RRAL samples observed in this sample set is too small to draw firm conclusions, these results warrant further exploration. The decrease in CD16 expression in the cell lysis assays indicates a drug-mediated effect, potentially NK cell activation.

The combination of increased and persistent antigen density following administration of standard-of-care therapies, and the specificity of these therapeutic antibodies could open new therapeutic options for AL.

LMA expression (10B3) MFI (n=4)	KMA expression (KappaMab) MFI (n=2)
1174	3547
2393	4700
196	
10170	

Table 1. LMA or KMA were detected in all samples from daratumumab-treated patients. These samples were from AL patients treated with cyclophosphamide, bortezomib, dexamethasone and daratumumab. They were gated on CD38+ cells and all samples were KMA or LMA positive. The presence of these CD38 positive cells indicated a sub-optimal response to daratumumab treatment. MFI = mean fluorescence intensity.