A Novel B Cell Antigen Designated Lambda Myeloma Antigen (LMA) Has Been Identified Using Two Fully Human Monoclonal Antibodies (Mabs) That Bind to Similar Epitopes on Plasma Cells From Patients With Plasma Cell Dyscrasias

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Introduction

Plasma cell dyscrasias (PCDs), also known as monoclonal gammopathies, are a spectrum of diseases associated with monoclonal proliferation of plasma cells (PCs) in the bone marrow and the excess production of monoclonal proteins (M proteins) consisting of immunoglobulin (Ig) and/or free light chains (FLCs).

In this study we describe two monoclonal antibodies, 10B3 and 7F11, that bind to a **cell surface lambda myeloma antigen (LMA).** We show the presence of LMA in λ human myeloma cell lines, PCD bone marrow samples, plasmacytoma tissue and normal secondary lymphoid tissues.

A phase IIb clinical trial[1] using a novel antibody called KappaMab (formerly MDX-1097) that binds to kappa myeloma antigen (KMA)[2] has recently been completed.

Methods

- Binding of the fully human monoclonal antibodies 10B3 and 7F11 was assessed on human myeloma cell lines representing lambda isotypes 1-3.
- Multiparametric flow cytometry was performed in bone marrow samples from 65 patients with various PCDs. Informed consent was obtained for testing.
- The antigen density of KMA or LMA versus BCMA was assessed.
- Tissue distribution on myeloma and normal snap frozen tissues using whole antibody 10B3-FITC was analysed.

Results

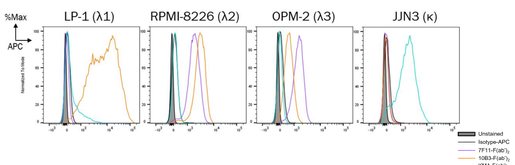

Figure 1. Binding profiles of the 7F11, 10B3 and KappaMab F(ab')$_2$ antibody fragments conjugated to APC in lambda- and kappa-positive cell lines. Antibody F(ab')$_2$ fragments were used for flow cytometry to avoid Fc binding to leukocytes. 10B3 (orange lines) bound all 3 lambda cell lines, LP-1 (λ1), RPMI-8226 (λ2) and OPM-2 (λ3). 7F11 (mauve lines) bound to RPMI-8226 (λ2) and OPM-2 (λ3). Neither 7F11 nor 10B3 bound to the kappa cell line, JJN3. KappaMab (blue lines) bound only to the JJN3 cell line. The human IgG1 isotype control (grey lines) did not bind any cell lines. Results are representative of 8 experiments.

Abbreviations: **PCDs**=Plasma cell dyscrasias, **LMA**=Lambda Myeloma Antigen, **KMA**=Kappa Myeloma Antigen, **NDMM**=newly diagnosed multiple myeloma, **RRMM**=relapsed, refractory multiple myeloma, **PCs**=plasma cells, **MGUS**=monoclonal gammopathy of unknown significance, **SMM**=smoldering multiple myeloma, **WM**=Waldenstroms macroglobulinemia, **LPD**=B cell lymphoproliferative disorder, **k**=kappa, **λ**=lambda, **BCMA**=B cell Maturation Antigen

Co-expression of KMA and BCMA was observed in 17/28 (61%) of cases of either untreated κNDMM or treated κRRMM (**Table 1**). Of the 10 untreated λNDMM cases, BCMA was expressed on 9 and LMA was expressed on 5; all 5 of these cases co-expressed BCMA and 2 co-expressed CD56. Within the 4 λRRMM cases, all expressed BCMA and 2 also expressed LMA (**Table 2**). SLAMF7 (CD319) was expressed on all patient bone marrow samples tested.

KMA and LMA expression were enriched in cases of RRMM and LMA expression was enriched in cases of AL Amyloidosis (**Tables 1-3**).

Table 1: Marker Expression for Kappa Positive Plasma Cell Dyscrasias.

Diagnosis N=43	CD269 (BCMA) n(%)	KMA n(%)	CD319 (SLAMF7) n(%)	CD56 n(%)	KMA & BCMA co-expression n(%)	KMA & CD56 co-expression n(%)
NDMM n=17	15(88)	9*(53)	17(100)	12(70)	9(53)	6(35)
RRMM n=11	8(73)	10(90)	11(100)	7*(64)	8(73)	7(64)
MGUS n=7	7(100)	4*(57)	7(100)	4**(57)	4(57)	2*(29)
SMM n=3	2(66)	1(33)	3(100)	3(100)	1(33)	1(33)
WM n=1	1(100)	0	1(100)	0	0	0
LPD n=1	1(100)	1(100)	1(100)	0	1(100)	0
Plasmacytoma n=3	3(100)	3(100)	3(100)	3(100)	3(100)	3(100)

*Includes one patient with partial expression, **includes three patients with partial expression

Table 2: Marker Expression for Lambda Positive Plasma Cell Dyscrasias.

Diagnosis N=22	CD269 (BCMA) n(%)	LMA n(%)	CD319 (SLAMF7) n(%)	CD56 n(%)	LMA & BCMA co-expression n(%)	LMA & CD56 co-expression n(%)
NDMM n=10	9(90)	5(50)	10(100)	6**(60)	5(50)	2(20)
RRMM n=4	4(100)	2*(50)	4(100)	2**(50)	2(50)	0
MGUS n=4	3(75)	1(25)	4(100)	2(50)	1(25)	0
WM n=1	1(100)	0	1(100)	0	0	0
Amyloidosis n=3	1(33)	3(100)	3(100)	3**(100)	1(33)	3**(100)

*Includes one patient with partial expression, **includes two patients with partial expression

Table 3. Antigen density of KMA (detected by KappaMab) or LMA (detected by 10B3) and BCMA was similar in the NDMM patients. In RRMM patients KMA or LMA density were both higher than BCMA density.

Diagnoses (BM samples)	KMA mean (range)	BCMA mean (range)
Untreated NDMM n=17	1655 (204-12022)	1638 (364-5370)
Treated RRMM n=11	2081 (468-7943)	1389 (350-2630)
Diagnoses (BM samples)	**LMA mean (range)**	**BCMA mean (range)**
Untreated NDMM n=10	1160 (130-3981)	1267 (395-2692)
Treated RRMM n=4	2086 (263-6664)	757 (537-1065)

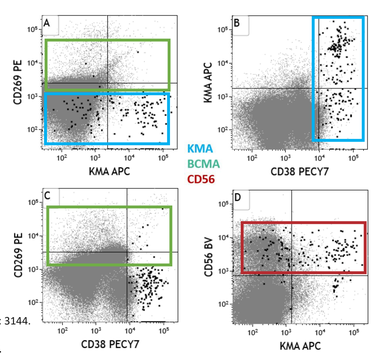

Figure 2: RRMM (KMA+ case #48) on lenalidomide treatment. There were very few plasma cells in this BM sample (<0.1%). This case showed partial expression for KMA, 60% of the plasma cells expressed bright KMA (A and B) and were CD56 positive (D) but lacked expression of CD269 (A, C).

References: [1]Kalff A et al. *Blood* (2019) 134 (Supplement 1): 3144.
[2]Asvadi P et al. *Br J Haematol.* 2015;169(3):333-343.
[3]Asvadi et al. *Haematologica* 2013;98 (Supplement 1):P756.
[4]Alameda et al. *Blood* 2021;138(17):1583-1589.

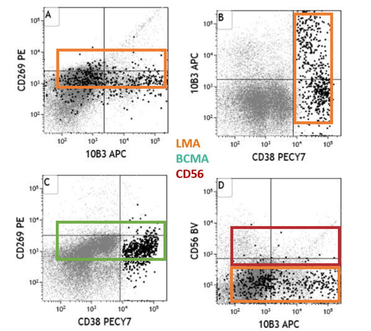

Figure 3. RRMM (LMA case #32) on lenalidomide treatment. Showed partial expression of LMA as assessed by 10B3 (A, B and D); the 10B3 positive population showed variable expression from dim to very bright. CD269 was positive (C) and CD56 was negative (D).

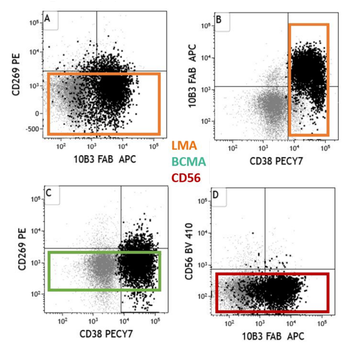

Figure 4: RRMM (LMA+ case #15). LMA (10B3) expression was positive (A, B) compared to CD269 (A and C) and CD56 (D), which were negative.

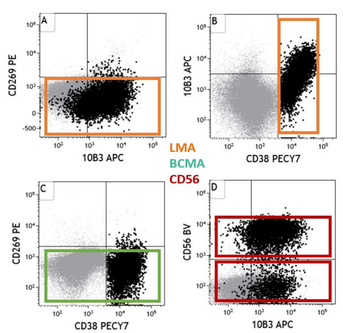

Figure 5. Amyloidosis patient (LMA+ case #36). The expression of 10B3 was variable (A and B) compared to the lack of expression for CD269 (A and C) and partial expression for CD56 (D).

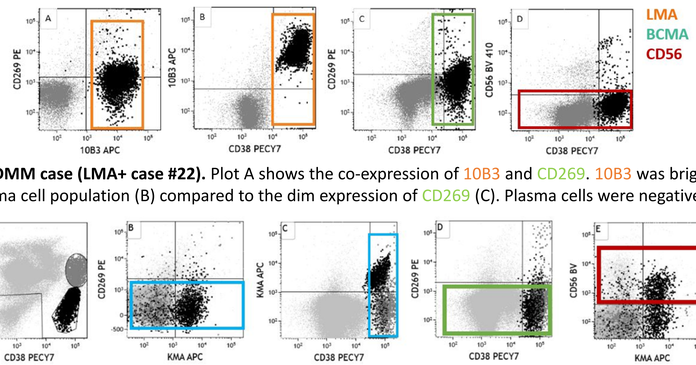

Figure 6. NDMM case (LMA+ case #22). Plot A shows the co-expression of 10B3 and CD269. 10B3 was brightly expressed on the plasma cell population (B) compared to the dim expression of CD269 (C). Plasma cells were negative for CD56 (D).

Figure 7. MGUS case (KMA+ case #36). Plot A, shows two populations of PCs, the grey population are normal PCs expressing CD45 and bright CD38, the second population (black dots) are abnormal PCs showing a lack of CD45 expression and slightly weaker CD38 expression. Abnormal PCs express KMA (B and C) have dim expression for CD269 (D) and express partial CD56 (E). Normal PCs are negative for KMA (C) and CD56 (E) but have dim expression for CD269 (B and D).

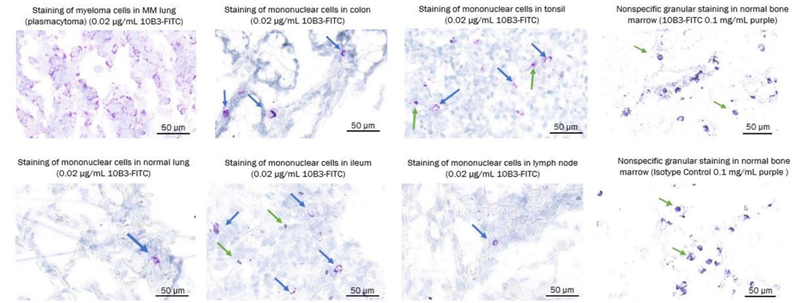

Figure 8. Immunohistochemistry staining using 10B3 was abundant in MM lung, occasional in normal secondary lymphoid tissues and nonspecific in normal bone marrow. Sections were from snap-frozen specimens and kept unfixed after sectioning during indirect chromogenic IHC assay using 10B3-fluorescein (Ventana Platform Purple®). Blue arrows: specific purple precipitates, green arrows: non-specific deep purple granular cytoplasm. Visualization was performed by light microscopy using an Axioskop (Zeiss Jena GE) up to 400x. Microphotographs were cropped from whole-section digital files acquired with an Axioscan Z1 HF (4631000285) slide scanner using a LED light source and the objective lens EC Plan Neofluar 20x/0.50 M27, and ZEN2 software blue-edition (Zeiss Microscopy, Jena, Germany).

Discussion and Conclusions

- The presence of LMA on the surface of clonal PCs in the bone marrow of PCD patients was detected using the fully human monoclonal antibodies 10B3 and 7F11 that bind to conformational epitopes in the constant region of the λ light chain isotypes λ1, λ2 and λ3 – only if it is not associated with heavy chain[3].
- KMA and LMA expression on clonal PCs did not always correlate with BCMA and/or CD56 expression.
- The antigen density of KMA and LMA is higher than that of BCMA on PCs in cases of RRMM and KMA expression is enriched suggesting a potential survival advantage and resistance to treatment.
- The presence of LMA on occasional mononuclear cells in normal secondary lymphoid tissue such as the mucosa-associated lymphoid tissue and not in normal bone marrow raises the possibility that the LMA-expressing B cell clone in PCD bone marrow has a unique origin that is peripheral to the bone marrow[4].
- There is potential to target both the λFLC and clonal PCs associated with λMM and light chain amyloidosis with the 10B3 antibody.

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