# Identification and characterization of Lambda Myeloma Antigen, LMA, as a therapeutic target in Lambda type Multiple Myeloma

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## Introduction

Multiple myeloma (MM) is an incurable malignancy of terminally differentiated plasma cells (PCs) characterized by PC accumulation in the bone marrow (BM), lytic bone disease, renal insufficiency, anemia, hypercalcemia and immunodeficiency. MM cells secrete monoclonal immunoglobulin (Ig; M protein) and/or Ig free light chain (FLC). The advent of sensitive methodologies for FLC detection has brought the significance of FLC levels in multiple conditions including MM to the fore. In MM, serum levels of FLC are used as a marker in diagnosis, response to therapy and prognosis. In the past decade, novel agents such as thalidomide, bortezomib and lenalidomide have been introduced into the clinic with significant benefit in terms of response rates and survival. However, MM remains an incurable disease and the invariable relapse of MM patients has energized the search for alternative therapeutic targets.

IST, has previously developed a chimeric monoclonal antibody (mAb), MDX-1097, against kappa myeloma antigen (KMA) which is a cell surface expressed from of kappa FLC (κFLC). A phase IIa clinical trial with MDX-1097 in κMM patients has recently concluded (Abstract No. P776).

We have identified a mAb with pan specificity against the expressed lambda (λ) LC istoypes and investigated whether a λ equivalent of KMA existed on the cell surface of λMM cells. Two mAbs, 3D12 and 4G7, both raised against λ Bence Jones Protein (BJP; FLC) were used in ELISA, surface plasmon resonance and Western blotting assays. Unlike 3D12, the 4G7 mAb demonstrated pan reactivity against all λFLC preparations under the conditions of all assays utilized. The 4G7 antibody was then used against a range of λ human multiple myeloma cell lines (λHMCLs) which encompassed the 3 dominant λFLC isotypes (isotypes 1, 2 and 3). The antibody interacted with all λHMCLs tested and its binding could only be inhibited by λFLC, and not IgG/λ, demonstrating the presence of λFLC on the cell surface of the λHMCLs. Confocal microscopy experiments further confirmed the presence of λFLC on the cell surface of the λHMCL RPMI8226 cells. The cell surface antigen thus identified was termed lambda myeloma antigen or LMA. Importantly, 4G7 also detected LMA on λMM patient derived bone marrow (BM) mononuclear cell (MNC) populations which were positive for CD38 and CD138.

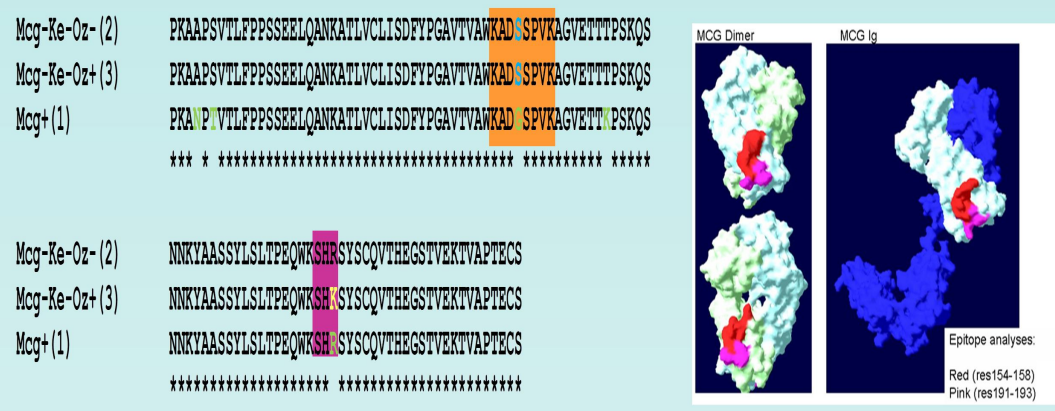
In order to provide information about the epitope of the 4G7 mAb, present on both soluble λFLC and LMA, epitope excision experiments were carried out and two non contiguous regions within the constant (C) domain of λFLC were identified as parts of the 4G7 mAb epitope. Recombinant expression of λFLC cDNA derived from the PRMI8226 and LP-1 λHMCLs on the surface of HEK-293f, and not CHO-S, cells suggested that the mature λFLC protein is retained on the cell surface most probably as a result of interaction with structural moieties within the cell membrane.

In conclusion, we have identified LMA as a cell surface antigen which can be specifically targeted by candidate mAbs with specificity similar to the 4G7 antibody. The pre-clinical development of candidate human mAbs suitable for specific targeting of MM plasma cells in λMM patients is currently under active investigation.

| Patient | Isotype | [FLC]-mg/L | %PC | LMA | Comments |
|---|---|---|---|---|---|
| 1 | NA | NA | 6 | ND | CD45+CD38+ cells detected |
| 2 | NA | 142 | 30 | + | Stained for LMA only |
| 3 | LC MM | 1372.5 | 18 | + | CD45-CD38+ cells detected |
| 4 | G | NA | 6 | ND | CD45-CD38+CD138+ cells detected |
| 5 | A | 61.6 | 13 | + | CD45-CD38+CD138+ cells detected |

## The 4G7 mAb detects LMA on primary BM cells from λMM patients

BM MNC preparations were generated by Ficoll density gradient processing of BM aspirates. Cells ($5x10^{5)}$) were stained with a combination of antibodies to determine the CD38, 138 and 45 as well as LMA (using APC labelled 4G7 and isotype control) status of the MNC population and analysed using a FACSCalibre flow cytometer. In two instances cells of the typical MM phenotype of CD38+CD45-CD138+ were detected and one of these samples was LMA positive. Two other samples were also LMA positive. NA: not available. ND: not detected. FLC: free light chain. %PC: percent BM plasma cells.




## The epitope of 4G7

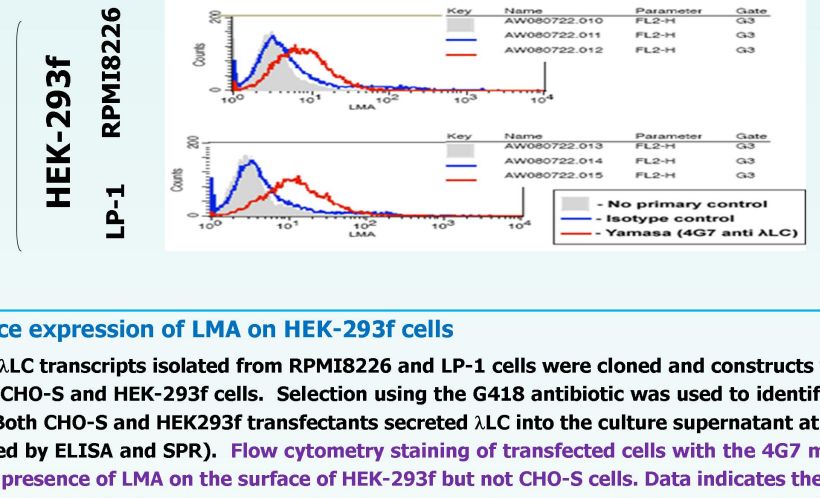
Epitope excision experiments identified two peptides as components of the 4G7 mAb epitope on λFLC. The panel on the left shows the alignment of the amino acid (aa) sequence of λFLC 1, 2 and 3 isotypes (λ LC isotypes 2 and 3 constitute 95% of the expressed λLC repertoire). Asterisks denote sequence identity. The divergent amino acids are shown in colour. The identified peptide sequences are shown in orange and cyan on the linear sequence the λLCs. The panel on the left positions the identified peptides onto the 3D structure of a λLC dimer (MCG dimer) and the MCG Ig and demonstrates that within the folded LC protein the two peptides from a contagion to create a non-idiotypic conformational epitope.

| λBJP | Biacore Response (RU) | | | |
|---|---|---|---|---|
| | 4G7 | | 3D12 | |
| Lam034 | 298 | +++ | 16 | - |
| Lam134c | 161 | ++ | 11 | - |
| Lam788a | 49 | + | 15 | - |
| Lam885 | 243 | +++ | 350 | +++ |
| Lam893c | 110 | ++ | 150 | ++ |
| MOS | -5 | - | 14 | - |
| IgGλ | 30 | -/+ | 13 | - |

| λBJP | ELISA Response | | | |
|---|---|---|---|---|
| | 4G7 | | 3D12 | |
| Lam034 | 1.416 | +++ | -0.016 | - |
| Lam134c | 1.328 | +++ | -0.024 | - |
| Lam788a | 1.399 | +++ | -0.027 | - |
| Lam885 | 1.326 | +++ | 0.890 | ++ |
| Lam893c | 1.327 | +++ | 0.509 | + |
| MOS | 1.277 | +++ | 0.001 | - |
| IgGλ | 0.532 | + | 0.117 | -/+ |
| κBJP | 0.000 | - | -0.011 | - |

## The 4G7 mAb binds all λFLCs tested in both ELISA and SPR settings

In SPR (BiaCore) experiments mAbs were immobilized on BioCore chips, preparations of λFLC were passed over the chip and the response (relative units: RU) was registered. In ELISA experiments λFLC preparations were immobilized on ELISA plates, antibody (100ng/mL) was added and binding was detected using a secondary anti-mouse antibody. In both settings, the 4G7 mAb demonstrated binding to all λFLCs tested, except to the MOS FLC in SPR experiments. The binding to IgG/λ is negligible for both 3D12 and 4G7 mAbs while no binding to κBJP was observed.

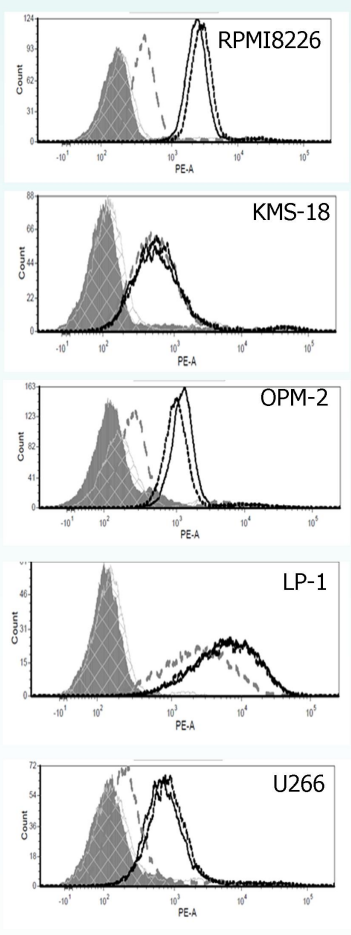


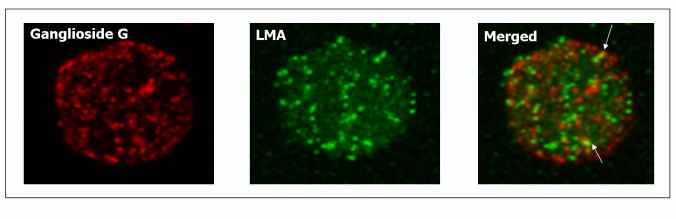

| Cell line | Isotype | FACS | |
|---|---|---|---|
| | | 3D12 | 4G7 |
| RPMI-8226 | 2 | - | + |
| KMS-18 | 1 | - | ++ |
| OPM-2 | 3 | - | + |
| LP-1 | 1 | + | ++ |
| U266 | 2 | - | + |

## The 4G7 mAb binds LMA on λHMCLs of divergent λisotype

LC cDNA was isolated from the λHMCLs (as shown in the table), sequenced and the sequence information was used to identify the isotype of the LC expressed by the cell line. The table summarizes the results of the flow cytometry staining of the cells with 3D12 and 4G7 antibodies (+ and ++ signs denote half a log and one log shift of the fluorescent peak arising from cell staining with the mAbs compared to the isotype control).

The flow histograms demonstrate the binding of 4G7 to the cell lines as marked and inhibition of this binding by λFLC only. Cells were stained with 4G7 or the isotype control antibody (+/- λFLC and IgG/λ) followed by a secondary PE labelled anti-mouse antibody. Solid grey: unstained. Hatched grey: isotype control. Outlined black: 4G7. Outlined dashed black: 4G7+IgGλ. Outlined dashed grey: 4G7+λFLC. In all instances IgG/λ does not inhibit the binding of 4G7 denoting the free light chain, and not Ig heavy chain associated, character of LMA while λFLC inhibits 4G7 binding to RPMI8226, OPM-2 and U266 cell lines. Binding of 4G7 to LP1 and KMS18 cell lines is inhibited to a lesser extent or not inhibited, respectively, due to the difference in the isotype of the LC expressed by these cell lines (isotype 1, see table) and the isotype of the FLC used in the assay (isotype 2).




## Detection of LMA on the surface of λHMCL PRMI8226 cells

Cells ($5x10^{5}$) were sequentially stained for ganglioside G [using cholera toxin subunit B (CT-B), followed by anti-CT-B Alexa Fluor 594] and LMA [using 4G7 followed by anti-mouse Ig Alexa Fluor 488] at 4 and 37°C respectively. The cell suspension was fixed and slides (poly-L-Lysine) were prepared. Images were collected on a Nikon confocal microscope (x100 magnification). Ganglioside G (a constitutive resident of the raft membrane subdomains) and LMA were detected on the surface of RPMI8226 cells. The merged image demonstrates distinct foci ganglioside G and LMA co-localization in limited instances (as shown by arrow). In the majority of instances ganglioside G and LMA were detected within distinct domains.

## Cell surface expression of LMA on HEK-293f cells

Full length λLC transcripts isolated from RPMI8226 and LP-1 cells were cloned and constructs were transfected into CHO-S and HEK-293f cells. Selection using the G418 antibiotic was used to identify transfected clones. Both CHO-S and HEK293f transfectants secreted λLC into the culture supernatant at similar levels (assessed by ELISA and SPR). Flow cytometry staining of transfected cells with the 4G7 mAb demonstrated the presence of LMA on the surface of HEK-293f but not CHO-S cells. Data indicates the sufficiency of λLC transcript in bringing about LMA cell surface expression, most probably, as a result of interaction with cell membrane structural moieties.

## Conclusions and future directions

- The presence of LMA on the cell surface of multiple λHMCLs and BM derived λMM patient PCs was demonstrated.
- A prototype mouse mAb, 4G7, with pan reactivity against λLC isotypes 1, 2 and 3 was identified.
- The epitope of the 4G7 antibody was shown to be located in the C domain of the λLC and to have a conformational character.
- Human antibodies specific for LMA are currently in pre-clinical development.

## Acknowledgements

The authors would like to thank the staff of Alfred Hospital, Melbourne for their assistance in obtaining MM patient BM samples

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ACBD Australian Centre for Blood Diseases

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