STRUCTURAL AND FUNCTIONAL VARIABILITY OF THE TUMOUR B-CELL RECEPTOR INDICATES A ROLE FOR ENVIRONMENTAL INFLUENCES ON THE BEHAVIOR OF MANTLE CELL LYMPHOMAS

D, Dutton [1], G, Chiodin[1], A, Wasik, S, Drennan[1], I, Henderson[1], G, Gaidano[3], P, Johnson[1], F, Stevenson[1], G, Packham[1], B, Sander[2], F, Forconi[1]

1 Cancer Sciences, University of Southampton, Southampton, United Kingdom,

2 Department of Laboratory Medicine, Division of Pathology, Karolinska Institutet and Karolinska University Hospital, Stockholm, Sweden

3 Division of Haematology, Department of Translational Medicine, Amedeo Avogadro University of Eastern Piedmont, Novara, Italy

INTRODUCTION

Mantle cell lymphoma (MCL) is a mature CD5$^+$ B-cell tumour defined by common phenotypic and genetic features resulting in overexpression of cyclins (most commonly cyclin D1) and subsequent alterations of cell cycle progression. MCL has a heterogeneous clinical course ranging from relatively indolent to the very aggressive. In CLL, variability of B-cell receptor (BCR) structure and function, due to antigen engagement, has provided key information on the heterogeneity of CLL behaviour and clinical course.

BCR expression is preserved in MCL, typically immunoglobulin M (IgM) and IgD. The association of immunoglobulin heavy chain variable (IGHV) status with different clinical outcomes (Navarro, 2012; Walsh, 2003) and the sensitivity to BCR-associated kinase inhibitors suggests that BCR retains functional importance in MCL.

OBJECTIVES

Our aim was to investigate the hypothesis that in MCL:

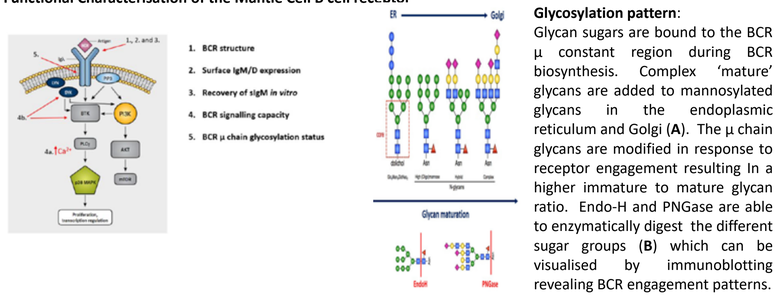
- BCR surface IgM (sIgM) levels and function are variable.
- Variability may be consequent to environmental influences acting on the BCR binding site.

METHODS

Cohort - 36 MCL patients with confirmed t(11:14) translocation.

1. **BCR structure**: IGHV-D-J transcript sequencing by polymerase chain reaction (PCR) to characterize the antigen-binding site.
2. **Surface IgM/D expression**: Immunophenotype using F(ab')2 anti-IgM/D.
3. **Recovery of sIgM *in vitro***: Surface IgM phenotyping at 0, 24, 48 and 72 hours.
4. **BCR signalling capacity**: Calcium mobilization assay and phosflow of SYK following α-IgM stimulation.
5. **BCR glycosylation status**: Immunoblotting of sIgM following EndoH or PNGase digestion to characterise the functional status of the BCR.

Functional Characterisation of the Mantle Cell B cell receptor



Glycosylation pattern:
Glycan sugars are bound to the BCR μ constant region during BCR biosynthesis. Complex 'mature' glycans are added to mannosylated glycans in the endoplasmic reticulum and Golgi (**A**). The μ chain glycans are modified in response to receptor engagement resulting In a higher immature to mature glycan ratio. Endo-H and PNGase are able to enzymatically digest the different sugar groups (**B**) which can be visualised by immunoblotting revealing BCR engagement patterns.

RESULTS (1)

In this cohort, **analysis of the IGHV and sIgM/D expression** revealed:

- An overrepresentation of MCL cases (50%) with mutated IGHV (**Figure 1**).
- Non-aggressive MCL (excluding blastoid MCL, n=30), sIgM expression was significantly higher in U-MCL (median 1219 vs 542, p=0.03) (**Figure 2**).
- The median sIgM, but not sIgD, level was significantly higher in U- and M-MCL than in CLL subgroups (p<0.01) (**Figure 3A and B**).

Signalling capacity was high but variable (range 11-100%, median 89%) and positively correlated with sIgM levels (r=0.86, p<0.01) (**Figure 5**)

Although MCL signalling was significantly higher than CLL (p<0.01) a sub-group of MCL (n=8) had low signalling capacity (CLL-like) (**Figures 6-7**).

Assessment of **MCL surface IgM recovery 'antigen-free' *in vitro***:

- 7/8 (88%) low signallers (M-IGHV (n=4), U-IGHV4-39 (n=1), 3-21 (n=2)) recovered sIgM expression during 'antigen-free' in vitro culture, in contrast to only 1/9 (11%) high signallers. (**Figure 7 & 9**).
- Basal pSYK was significantly higher in the low signalling CLL-like group (p=0.047).

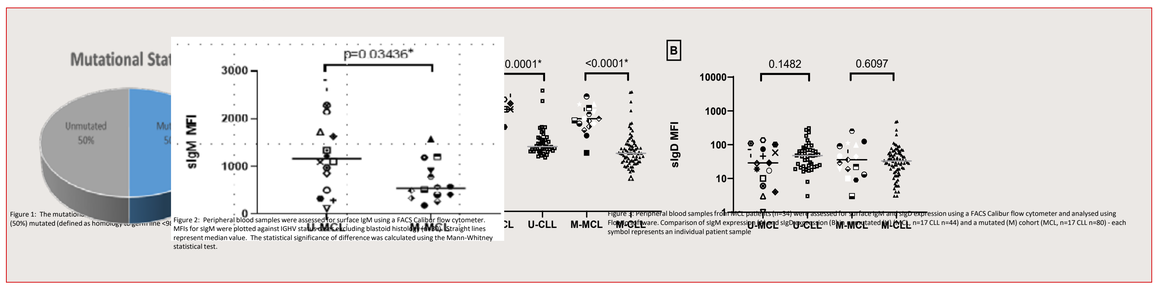
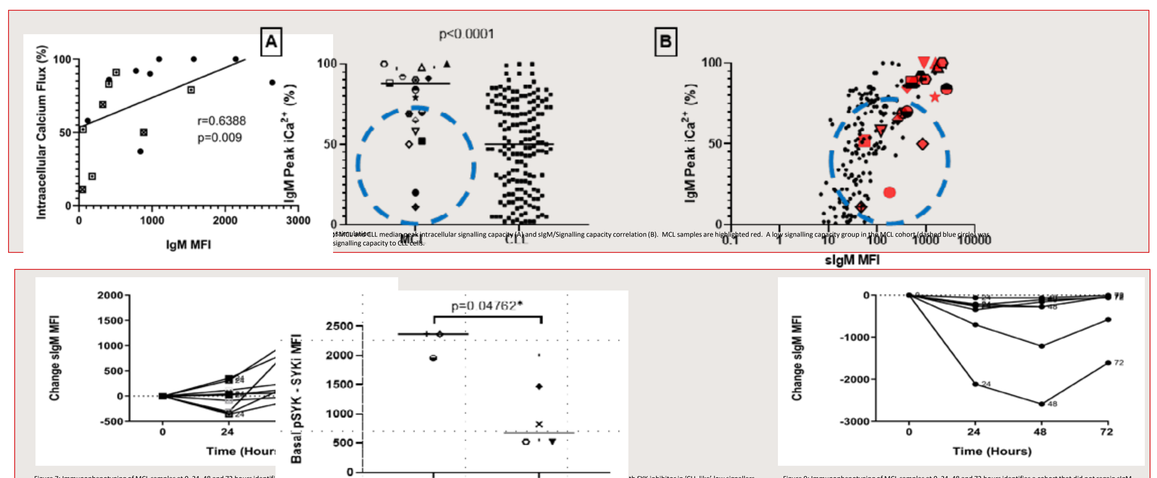


Figure 1: The mutational status of MCL cases (50%) mutated (defined as homology to germ line <98%).

Figure 2: Peripheral blood samples were assessed for surface IgM using a FACS Calibur flow cytometer. MFIs for sIgM were plotted against IGHV status excluding blastoid histology. Straight lines represent median value. The statistical significance of difference was calculated using the Mann-Whitney statistical test.

Figure 3: Peripheral blood samples from MCL patients (n=34) were assessed for surface IgM and sIgD expression using a FACS Calibur flow cytometer and analysed using FlowJo software. Comparison of sIgM expression (A) and sIgD expression (B) between mutated (M) n=17 CLL n=44) and a mutated (M) cohort (MCL, n=17 CLL n=80) - each symbol represents an individual patient sample



MCL and CLL median peak intracellular signalling capacity (A) and sIgM/Signalling capacity correlation (B). MCL samples are highlighted red. A low signalling capacity group in the MCL cohort is defined blue grey. Low signalling capacity to CLL levels.

Figure 7: Immunophenotyping of MCL samples at 0, 24, 48 and 72 hours identifi... expression. MFI at 0 hours set as 0 and MFI difference plotted at further time p... U-MCL and a dot M-MCL.

...th SYK inhibitor in 'CLL-like' low signallers

Figure 9: Immunophenotyping of MCL samples at 0, 24, 48 and 72 hours identifies a cohort that did not regain sIgM expression (<20% increase at 72 hours over baseline).

RESULTS (2)

Immunoblotting of the tumour sIgM by EndoH and PNGase digestion revealed:

- CLL-like low signalling MCLs had a mature **and immature glycan pattern** (A)
- High signalling MCL constant chains possessed mature glycan patterns (B).
- Low signallers associated with an immature glycosylation pattern, indicating prior activation (p<0.01).

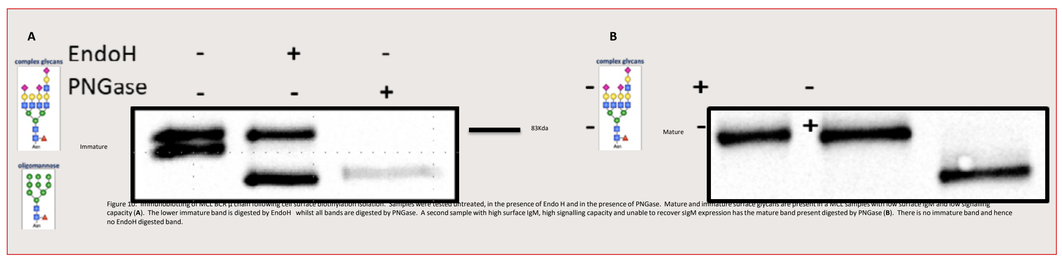


Figure 10: Immunoblotting of MCL BCR μ chain following cell surface deglycosylation reaction. Samples were tested untreated, in the presence of Endo H and in the presence of PNGase. Mature and immature surface glycans are present in 2 MCL samples with low surface IgM and low signalling capacity (**A**). The lower immature band is digested by EndoH whilst all bands are digested by PNGase. A second sample with high surface IgM, high signalling capacity and unable to recover sIgM expression has the mature band present digested by PNGase (**B**). There is no immature band and hence no EndoH digested band.

CONCLUSIONS

- In this selected cohort, MCL sIgM expression is highly variable, correlates with signalling capacity and **associates with IGHV status**.
- A subgroup possess **BCR-related features similar to CLL**.
- MCL BCRs in this subgroup recover sIgM expression *in vitro*, have high basal pSYK and have a glycosylation pattern indicative of **prior BCR engagement**.
- This process may be prominent in **indolent MCL** (M-MCL/U-IGHV3-21), where genomic aberrations are less frequent.
- The evidence in this subgroup of MCL claims analogies with CLL, in which **variable antigen-driven anergy plays a role in clinical behaviour.**

CONTACT INFORMATION

D.dutton@soton.ac.uk and F.Forconi@soton.ac.uk
Cancer Sciences Unit, MP824, Somers Building, University Hospital Southampton, Tremona Road, Southampton, SO16 6YD

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