Importance of biomarker testing in diffuse large B-cell lymphoma and mantle cell lymphoma

Includes best practice sharing by Dr Cathy Burton, consultant haematologist at Leeds Teaching Hospitals NHS Trust

A biomarker is a biological molecule found in blood, other body fluids or tissues. It can be a sign of a normal or abnormal process or of a condition or disease. A biomarker may be used to determine how well the body responds to a treatment for a disease or condition.[1]

Biomarker testing in DLBCL and MCL

- Testing for biomarkers can help clinicians deliver the right treatment to the right person at the right time.
- Biomarker testing provides new insights into the molecular and genetic basis of many types of cancer. It also provides insights into the differences in individuals with cancer at a molecular level.
- However, the challenge with biomarkers is the need for confirmation of their significance in large cohorts of people across the spectrum of disease. Due to the complexity of lymphoma, this can be extremely challenging.

Diagnostic tools for biomarker testing in DLBCL and MCL

DLBCL is the most common type of high-grade NHL. It is a rapidly growing lymphoma that arises from abnormal B cells.

MCL is a rarer subtype of B-cell NHL, which arises due to the malignant transformation of a B lymphocyte in the outer edge of a lymph node follicle (the mantle zone).

Diagnostic tools

IHC[2,3]

IHC is a technique wherein specific binding between an antibody and an antigen is used to detect and localise specific antigens in lymphoma tissues. These are most commonly detected and examined with light microscopy.

FISH[4]

FISH uses fluorescent DNA probes to target specific chromosomal locations. This results in coloured signals that can be detected using a fluorescent microscope, which indicate the presence of a biomarker.

Gene expression profiling[5,6]

Gene expression profiling measures which genes are being expressed in a cell at any given time by measuring mRNA levels. This reveals the specific pattern of genes expressed by a cell at the transcriptional level. The most powerful tool for this is microarray technology, which allows for rapid screening of thousands of genes simultaneously.

Mutational analysis[7]

Mutational analysis facilitates the identification of somatic mutations, which can be used as biomarkers for disease and prognostication. In haemato-oncology, this is often conducted as tumour genotyping.

Key biomarkers for DLBCL[8]

The most common biomarkers important in DLBCL are:

- *MYC*/BCL2
- COO

Key biomarkers for MCL[20,21]

The most common biomarkers important in MCL are:

- Cyclin D1
- Ki67
- *TP53*

How do biomarkers influence diagnosis and treatment decisions in DLBCL and MCL?

Assessment of the IPI provides clinical prognostication. The IPI includes age, tumour stage, LDH, ECOG performance status and number of extra-nodal sites. This allows segregation into risk groups but fails to accurately identify all poor-risk patients, and there is high variability of outcome within subgroups.[9]

In most cases, DLBCL can be divided into two distinct forms indicative of different stages of B-cell differentiation: germinal centre B cell (GCB) and activated B cell (ABC). IHC algorithms have been used to identify surrogate biomarkers that classify people with DLBCL into these two different categories.[10,11]

1. Germinal: **CD10, BCL6** **2.** Activated: **MUM1/IRF-4**

These IHC algorithms – also known as the Hans, Choi and Muris methods – have been variable in reproducibility.[10,12] A study comparing these methods concluded that Hans and Choi are best for predicting cell of origin and that Muris is better for predicting overall survival. IHC is used routinely, but the gold standard remains **gene expression profiling**.[12]

Gene expression profiling more reliably defines these two distinct molecular subgroups, with either a gene expression signature resembling that of GCB type or a profile with expression of genes induced during in vitro activation of peripheral blood B cells (ABC type). A third molecular subgroup called type 3 or unclassified includes cases not expressing genes characteristic of GCB or ABC subgroups.[13] Patients with a GCB profile have a significantly better overall survival than those with ABC type.[11]

People with MCL are typically divided into those with indolent MCL, who can be monitored, and those with aggressive MCL, who need prompt treatment.

Clinical and biological biomarkers are used to stratify the risk for individual MCL patients. The MIPI can be used as a scoring system for risk profiling patients based on:[22]

- Age
- ECOG performance status
- LDH
- Elevated white blood count

The MIPI score allocates patients to three risk groups: low, intermediate and high. However, due to the heterogenous nature of MCL, the MIPI falls short in fully adequately predicting prognosis. Biomarkers such as Ki67, *TP53* and other mutations can help improve the risk stratification of MCL.[21–23]

How do biomarkers affect DLBCL and MCL prognosis?

The identification of specific biomarkers using different molecular techniques can often predict the response to chemotherapy. FISH is an important biomarker tool in DLBCL; it is used to detect *MYC*, BCL2 and BCL6 gene rearrangements. Gene expression profiling has further identified biologically distinct groups beyond COO within DLBCL. Whole-exome, transcriptome and targeted sequencing has identified driver genes in DLBCL.[14]

***MYC*-R**	*MYC*-R occurs in ~10–15% of DLBCL cases. For DLBCL that carries *MYC* and BCL2 gene rearrangements (double-hit lymphoma), this subgroup has been associated with a poor prognosis after treatment with chemoimmunotherapy.[15,16]
TP53	For DLBCL with *TP53* mutations, people have generally poorer outcomes than those with wild-type *TP53*. These people have a poor overall survival and therapeutic response.[17]
MHG/ DHITsig	These similar, but not fully overlapping, groups identified by gene expression profiling have a very poor prognosis with standard chemoimmunotherapy treatment.[18,19]

Cyclin D1	IGH/cyclin D1 fusion gene associated with t(11;14)(q13;q32) is the genetic hallmark of MCL. Cyclin D1 expression is detected by IHC, and the t(11;14) is demonstrated by FISH.[21]
Ki67	Ki67 is an IHC marker that denotes tumour cell proliferation. Ki67 has been shown to have high prognostic relevance for overall survival, independently from clinical prognostic factors.[21,23]
ATM	ATM is involved in DNA damage response and is the most frequently mutated gene at baseline or diagnosis of MCL and after relapse/progression. ATM mutations have been implicated in chemotherapy resistance and poor outcomes.[23,24]
TP53	*TP53* is a tumour suppressor gene. *TP53* mutations occur in about 10–15% of MCL patients. *TP53* mutations are associated with worse outcomes, including worse overall survival and therapeutic response.[21,24]

Sequence of diagnostic tests

DLBCL: Morphology → IHC → FISH → Mutational analysis, Gene expression profiling (more commonly used in clinical trials)

MCL: Morphology → IHC → FISH → Mutational analysis

ABC, activated B cell; ATM, ataxia-telangiectasia mutation; COO, cell of origin; *DHITsig*, double-hit signature; DLBCL, diffuse large B-cell lymphoma; DNA, deoxyribonucleic acid; ECOG, Eastern Cooperative Oncology Group; FISH, fluorescence in situ hybridisation; GCB, germinal centre B cell; IGH, immunoglobulin heavy locus; IHC, immunohistochemistry; IPI, International Prognostic Index; LDH, lactate dehydrogenase; MCL, mantle cell lymphoma; MHG, molecular high grade; MIPI, Mantle Cell Lymphoma International Prognostic Index; mRNA, messenger ribonucleic acid; *MYC*-R, *MYC* rearrangement; NHL, non-Hodgkin's lymphoma; *TP53*, tumour protein 53

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