Molecular characterisation of Diffuse large B-cell lymphomas (DLBCL) diagnosed at Groote Schuur Hospital (GSH)

by

Trischke Watson

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Supervisor(s): Dr Amsha Ramburan and Dr Dharshnee Rama Chetty

DECLARATION

I, Trischke Watson, hereby declare that the work on which this dissertation/thesis is based is my original work (except where acknowledgements indicate otherwise) and that neither the whole work nor any part of it has been, is being, or is to be submitted for another degree in this or any other university.

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TABLE OF CONTENTS

LIST OF FIGURES

with no rearrangement showing two yellow or fused orange/green signals (white arrow). D: *BCL2* FISH with rearrangement showing one yellow or fused orange/green signal and one separate orange and green signal in the tumour cell nuclei (white arrow). E: *BCL6* FISH with no rearrangement showing two yellow or fused orange/green signals (white arrow). F: *BCL6* FISH with rearrangement showing one yellow or fused orange/green signal and one separate orange and green signal in the tumour cell nuclei (white arrow). G: *MYC* DFP FISH with no rearrangement showing two orange and two green signals (white arrow). H: *MYC* DFP FISH with rearrangement showing two yellow fused signals and one orange and one green signal

LIST OF TABLES

LIST OF APPENDICES

LIST OF ABBREVIATIONS

ABC	Activated B-cell
A-DH	Atypical-double hit
AID	Activation-induced cytidine deaminase
ALK	Anaplastic lymphoma kinase
A-SH	Atypical-single hit
ASHM	Aberrant somatic hypermutation
A-TH	Atypical-triple hit
BAP	Break-apart probe
BCL2	B-cell lymphoma 2
BCL6	B-cell lymphoma 6
BCL10	B-cell lymphoma 10
BCLU	B-cell lymphoma unclassifiable
BCR	B-cell receptor
BL	Burkitt's Lymphoma
BLIMP1	B lymphocyte induced maturation protein 1
CCND1	Cyclin D1
CCND3	Cyclin D3
CD10	Cluster of differentiation 10
CD19	Cluster of differentiation 19
CD20	Cluster of differentiation 20
CD21	Cluster of differentiation 21
CD22	Cluster of differentiation 22
CD30	Cluster of differentiation 30

CD38	Cluster of differentiation 38
CD40	Cluster of differentiation 40
CD70	Cluster of differentiation 70
CD79	Cluster of differentiation 79
CD83	Cluster of differentiation 83
CD138	Cluster of differentiation 138
CDKN2A	Cyclin-dependent kinase inhibitor 2A
CGH	Comparative genomic hybridisation
CHOP	Cyclophosphamide, doxorubicin, vincristine, prednisone
CNS	Central nervous system
COO	Cell of origin
CREBBP	CREBBP binding protein
CSR	Class switching recombination
DAPI	4',6-Diamidino-2-phenylindole
DEL	Double expressor lymphoma
DFP	Dual-fusion probe
DHAP	Dexamethasone, high dose cytarabine, cisplatin
DHL	Double hit lymphoma
DLBCL	Diffuse large B-cell lymphoma
DNA	Deoxyribonucleic acid
DZ	Dark zone
EBER-ISH	Epstein-Barr virus-encoded RNA *in-situ* hybridisation
EBNA-1	Epstein-Barr nuclear antigen 1
EBV	*Epstein-Barr virus*

EPOCH	Etoposide, prednisone, vincristine [Oncovin], cyclophosphamide
ESHAP	Etoposide, methylprednisolone, high dose cytarabine, cisplatin
ETV6	ETS variant transcription factor 6
EZH2	Enhancer of zest homolog 2
FFPE	Formalin-fixed, paraffin-embedded
FISH	Fluorescence *in-situ* hybridisation
FL	Follicular lymphoma
GAS5	Growth arrest specific 5
GAPDH	Glyceraldehyde-3-phosphate dehydrogenase
GCB	Germinal centre B-cell
GEP	Gene expression profiling
gp120	Glycoprotein 120
H&E	Haematoxylin & eosin
H_2O	Water
HAART	Highly active antiretroviral therapy
HCl	Hydrochloric acid
HGBCL	High-grade B-cell lymphoma
HHV8	Herpes Virus Type 8
HIV	*Human Immunodeficiency Virus*
HL	Hodgkin lymphoma
HMRN	Haematological Malignancy Research Network
HREC	Human Research Ethics Committee
IG	Immunoglobulin
IGEPAL	Octylphenoxypolyethoxyethanol

IGH	Immunoglobulin heavy chain
IGK	Immunoglobulin kappa light chain
IGL	Immunoglobulin lambda light chain
IHC	Immunohistochemistry
IL21	Interleukin 21
IMVP-16	Ifosfamide, methotrexate, and etoposide
IPI	International prognostic index
IRF4	Interferon regulatory factor 4
ISH	*In-situ* hybridisation
Ki-67	Marker of proliferation
KMT2D	Histone-lysine N-methyltransferase 2D
LBCL	Large B-cell lymphoma
LCP1	Lymphocyte Cytosolic Protein 1
LDH	Lactate dehydrogenase
LMO2	LIM-only protein 2
LMP1	Latent membrane protein 1
LMP2	Latent membrane protein 2
LRMP	Lymphoid-restricted membrane protein
LSI	Locus specific identifier
LZ	Light zone
mTORC1	Mammalian target of rapamycin gene
MAX	MYC-associated factor X
MBNL1	Muscle blind like splicing regulator 1
MCL1	Myeloid leukaemia 1

MCL	Mantle cell lymphoma
MIME	Mesna, ifosfamide, methotrexate, and etoposide
miR-127	microRNA 127
MLPA	Multiplex ligation-dependent probe amplification
MUM1	Multiple myeloma 1
MRC	Medical Research Council
MYC	Myelocytomatosis oncogene homolog
MYD88	Myeloid differentiating factor 88
MZL	Marginal zone lymphoma
NACA	Nascent-polypeptide-associated complex alpha polypeptide
NaOH	Sodium hydroxide
NAPA	N-ethylmaleimide-sensitive factor attachment protein Alpha
NF-κB	Nuclear factor kappa-beta
NGS	Next-generation sequencing
NHL	Non-Hodgkin lymphoma
NHLS	The National Health Laboratory Service
NOS	Not otherwise specified
NOTCH1	Neurogenic locus notch homolog protein 1
P17	Matrix protein
P21/CKI	Potent cyclin-dependent kinase inhibitor
PAX5	Paired box protein 5
PBL	Plasmablastic lymphoma
PIM1	Proto-oncogene serine/threonine-protein kinase 1
PMBL	Primary mediastinal large B cell lymphoma

PRDM1/BLIMP1	PR domain zinc finger protein 1/B-lymphocyte-induced maturation protein 1
PI3K	Phosphoinositide-3-kinase
RAG	Recombination-activating gene
RASGEF1A	RasGEF Domain Family Member 1A
R-CHOP	Rituximab-CHOP
RNA	Ribonucleic acid
SA	South Africa
SFRS3	Serine and arginine rich splicing factor 3
SGK1	Serum/glucocorticoid regulated kinase 1
SHM	Somatic hypermutation
SLL	Small lymphocytic leukaemia
SOCS1	Suppressor of cytokine signalling 1
SOX11	SRY-box transcription factor 11
SSC	Sodium chloride sodium citrate
TdT	Terminal deoxynucleotidyl transferase
TET2	Tet methylcytosine dioxygenase 2
THL	Triple hit lymphoma
TNF	Tumour necrosis factor
TNFAIP3	Tumour necrosis factor, alpha-induced protein 3
TNFRSF14	TNF receptor superfamily member 14
TP53	Tumour protein 53
TRADD	Tumour necrosis factor receptor type 1-associated DEATH domain protein
TRAF	Tumour necrosis factor receptor–associated factor

UCT	University of Cape Town
V(D)J	Variable diversity joining gene
VH	Variable region of heavy chain
VIG	Variable region of IG genes
WHO	World Health Organisation

ABSTRACT

Background: Diffuse large B-cell lymphoma (DLBCL) is an aggressive high-grade neoplasm characterised by a diverse spectrum of clinical, morphological, immunophenotypic, and molecular features and response to therapy and survival. It is the most common lymphoma subtype diagnosed in South Africa. While the exact pathogenesis of DLBCL is not fully understood, genetic alterations in oncogenes such as *MYC*, *BCL2* and *BCL6* as well as viral oncogenes, contribute to its development and progression. In the World Health Organisation (WHO) classification of haematolymphoid tumours, the classification of DLBCL requires testing for *MYC, BCL2* and *BCL6* genetic alterations to determine subtypes such as DLBCL with *MYC* and *BCL2* rearrangements ("double-hit") or DLBCL with rearrangements of *MYC* and *BCL2* with *BCL6* rearrangement ("triple-hit"). Cases without rearrangements, single rearrangements or copy number variation (CNV) are classified as DLBCL, Not Otherwise Specified (NOS). Double-hit (DHL) or triple-hit (THL) lymphomas typically have a worse prognosis and outcome compared to DLBCL, NOS. Fluorescence *in-situ* hybridisation (FISH) is the gold standard cytogenetic method for detecting specific genetic alterations (rearrangement, gain, amplification, and loss) in cancer cells. Our centre's current diagnostic workup of DLBCLs does not include FISH for *MYC, BCL2* and *BCL6*. This information is not only necessary for the classification of DLBCLs but is also critical for patient prognosis and treatment decisions.

Aim and Objective: This study aimed to determine the profile of *MYC, BCL2* and *BCL6* gene alterations in a cohort of DLBCL cases diagnosed at Groote Schuur Hospital (GSH) between 2015 and 2021 using FISH.

Methods: FISH for *MYC, BCL2*, and *BCL6* alterations was performed on formalin-fixed paraffin wax-embedded (FFPE) tissue according to manufacturer instructions. Each case was assessed for rearrangement and CNV. Appropriate statistical analyses were conducted to correlate the molecular alterations with histopathological data. The clinical data like prognosis and treatment outcomes for these cases were not available.

Results: The patient cohort comprised 75 cases. The age range was between 18 and 83 years with 31 (41.3%) females and 44 (58.7%) males. HIV data was known in 72 cases, with 30/72 patients (41.7%) being HIV positive and 42 (58.3%) being HIV negative. Fifty cases yielded a result with all the FISH probes tested (66.7%). The *MYC FISH* results comprised 13/50 (26.0%) cases with rearrangement, 9/50 (18.0%) cases with CNV and 28/50 (56.0%) with no aberrations. *BCL2* FISH yielded 6/50 (12.0%) cases with rearrangements, 6/50 (12.0%) with CNV and 38/50 (76.0%) with no aberrations. *BCL6* FISH yielded 9/50 (18%) cases with rearrangements, 12/50 (24%) with CNV and 29/50 (58.0%) with no

aberrations. Using this data, cases were classified resulting in 45/50 (90.0%) DLBCL, NOS cases, 1/50 (2.0%) DLBCL with *MYC* and *BCL2* rearrangement (DHL) and 1/50 (2.0%) DLBCL with rearrangement of *MYC, BCL2* and *BCL6* (THL). There were 3/50 (6.0%) EBV-positive DLBCL, NOS cases. The DHL and THL cases both were from the GCB subtype. The DLBCL, NOS cases comprised 26/45 (57.8%) GCB and 19/45 (42.2%) non-GCB subtypes. The EBV-positive DLBCL, NOS cases were all non-GCB subtypes. Of statistical significance, 13/47 (27.7%) of HIV-positive cases were of the GCB subtype (P=0.028). Also, 10/28 (35.7%) of the cases with *BCL2* aberrations were from the GCB subtype (P=0.029). When the immunohistochemistry (IHC) results were correlated with the FISH results, all cases with *BCL2* aberrations were positive for BCL2 IHC expression (P=0.038). Atypical hits were observed in 23/50 (46.0%) cases and in 33/50 (66.0%) of cases, one or more aberrations were present.

Conclusion: This was the first study at GSH to investigate the spectrum of *MYC, BCL2* and *BCL6* gene alterations in a cohort of DLBCLs. The *MYC* gene was rearranged the most (26.0%) followed by *BCL6* (18.0%) and *BCL2* (12.0%). The *BCL6* gene showed the most copy number variations (24.0%) followed by *MYC* (18%) and *BCL2* (12.0%). These findings were largely consistent with other studies. The effect HIV has on the germinal centre was noted in our study, highlighting the role HIV plays in the development of DLBCL. Although rearrangements and protein expression are frequently co-expressed, MYC, BCL2 and BCL6 IHC as a surrogate for predicting rearrangements cannot be used. Regarding the classification, 90.0% of cases were DLBCL, NOS. DHL and THL were an uncommon finding and while they account for a very low prevalence, they can only be identified by cytogenetic testing in a diagnostic setting. This highlights the invaluable need for FISH as a diagnostic tool in lymphoma classification. In 66.0% of our cases, there was at least 1 *MYC, BCL2* or *BCL6* aberration present. Only 34.0% of DLBCLs, in this cohort, were without alterations in any of the 3 genes tested. This finding highlights the genetic heterogeneity of DLBCL and reaffirms the role of these oncogenes in the pathogenesis of DLBCLs.

CHAPTER 1: Literature Review

1.1 Introduction

Globally the incidence of cancer is increasing at an alarming rate. It is the second most common cause of death and one in nine individuals are believed to develop cancer. In South Africa (SA), a low to middle-income country, the burden of cancer is felt greatly as it is the cause of death in 11.7% of people nationally. It is projected that by the year 2030, the number of cancer cases will double the current numbers. Many factors contribute to this increase including tobacco use, alcohol use, unhealthy diets, obesity, and infections (Ferlay et al., 2024; Finestone and Wishnia, 2022). One of the most significant contributing factors to cancer in SA is the *Human Immunodeficiency Virus (*HIV*).* Approximately 8,45 million people or 13.9% of the population live with HIV (STATS-SA, 2022). People living with HIV have an increased risk of developing cancer specifically lymphoma (Finestone and Wishnia, 2022).

Lymphoid neoplasms arise from normal lymphocytes which undergo malignant transformation at various stages of cell differentiation, growing and multiplying in an uncontrolled manner. Lymphomas are grouped into 2 major categories namely, Hodgkin lymphomas (HLs) and non-Hodgkin lymphomas (NHLs), by the World Health Organisation (WHO) (Morton et al., 2007; Naseem et al., 2020; Swerdlow et al., 2017). NHL comprises both B- and T-cell lymphomas but more than 80% of NHLs are mature B-cell lymphomas with diffuse large B-cell lymphoma (DLBCL) being the most common subtype observed (De Leval, 2020; Naseem et al., 2020; Wang, 2023c). DLBCL is a clinically aggressive disease and heterogeneous in its clinical, biological and its morphology presentation (Chen et al., 2019a).

1.2 Epidemiology

DLBCL accounts for 30-40% of adult NHLs globally. A small male predominance is observed. It is common among the elderly with more than 30% of patients being older than 75 years (Sehn and Salles, 2021; Thandra et al., 2021). In SA, 38.2% of all NHLs are DLBCL, making it the most common subtype (Wang, 2023c). Multiple risk factors such as genetic, clinical, occupational, environmental factors and immune suppression can contribute to the development of DLBCL (Sehn and Salles, 2021). Immune suppression can result from post-transplantation drug therapy and in SA from HIV infection. In a SA study, DLBCL was observed in 43% of NHLs due to the high prevalence of people living with immunosuppression (Pather and Patel, 2022).

1.3 Aetiology/pathophysiology

1.3.1 B-cell formation

Haematopoietic stem cells in primary lymphoid organs give rise to B- and T-cells. Specifically, B-cell development starts in the bone marrow where after successful V(D)J recombination, naive B-cells with a receptor (BCR) are expressed (Basso and Dalla-Favera, 2015). The naive B-cell stage is reached when functional immunoglobulin (IG) heavy and light chains are expressed. Negative selection occurs when B-cells are reactive to autoantigens, and through apoptosis are eliminated or driven into an anergic state. Positive selection occurs when B-cells mature with a functional BCR co-expressing IGM and IGD, through splicing the variable region of the *heavy chain* gene (*VH*). Mature naive B-cells migrate from the bone marrow and circulate in peripheral blood and secondary lymphoid organs as resting lymphocytes (Figure 1.1) (Li, 2021).

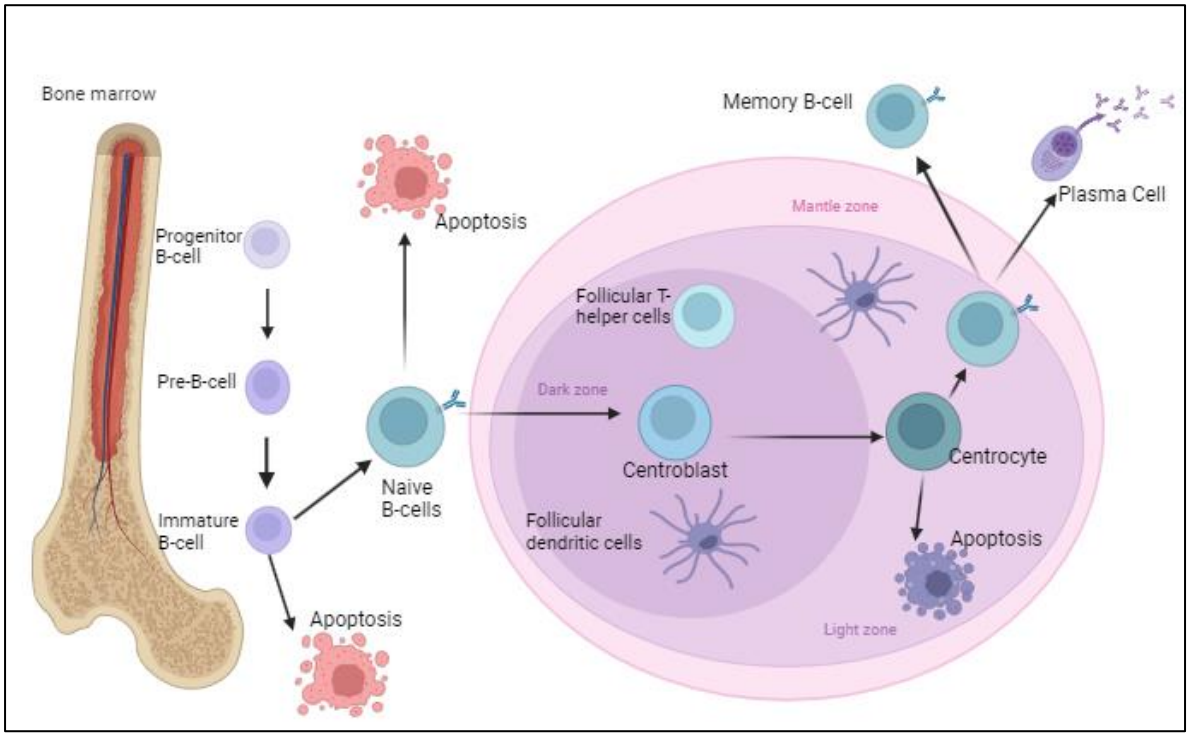

Figure 1.1: Normal B-cell development and activation (Adapted from Li, 2021)

In circulation or as resting lymphocytes, mature B-cells encounter antigens and undergo transformation and proliferation becoming either antibody-secreting plasma cells or memory B-cells. Some of the antibody-secreting plasma cells aggregate into the primary follicles together with T-helper cells where germinal centres (GC) are formed in response to the engagement of antigen-expressing

cells (Basso and Dalla-Favera, 2015; Holmes et al., 2020). Already residing B-cells are displaced into the mantle zone which surrounds the GC (Figure 1.1) (Li, 2021). The GC comprises two distinct zones, the dark zone (DZ) and the light zone (LZ). In the DZ most cells are highly proliferating centroblasts while the LZ contains centrocytes together with follicular dendritic cells, T-cells, and macrophages (Basso and Dalla-Favera, 2015).

The DZ represents the site where somatic hypermutation (SHM) and class switch recombination (CSR) occurs which results in B-cells that produce high-affinity antibodies of different isotype classes (Basso and Dalla-Favera, 2015; Holmes et al., 2020). These two processes are dependent on an enzyme which converts cytidine into uracil in the affected DNA strand called activation-induced cytidine deaminase (AID) (Figure 1.1). The change to uracil causes these sites to be targeted by error-prone DNA repair mechanisms (Li, 2021). SHM occurs when GC B-cells modify the variable region of their IG genes (*VIG*). This results in the BCR changing affinity to the antigen by introducing single-nucleotide substitutions. When centroblasts have mutations they migrate to the LZ where affinity selection occurs by the introduction of apoptosis. Another AID-dependent combination process in GC as well as outside the GC is CSR. During CSR, *Cμ* and *Cδ* genes are replaced with *Cα*, *Cε*, or *Cγ* genes CSR causes alterations in BCR signalling capacity as well as antibody function modifications. GC B-cells recirculate multiple times and undergo multiple cycles of proliferation, mutation and positive selection and eventually differentiate into high-affinity antibody-secreting plasma cells or resting memory B-cells and leave the GC. Memory B-cells can be reactivated by encountering the antigen, contributing to an improved secondary immune response (Holmes et al., 2020; Li, 2021).

1.3.2 DLBCL aetiology/pathogenesis

DLBCL arises either *de novo* or may transform from low-grade lymphoma, most commonly follicular lymphoma (FL) (Cai et al., 2015). At any stage of differentiation, B-cells can undergo a malignant transformation because of chromosomal mutations (Gouveia et al., 2012; Holmes et al., 2020; Lossos, 2005). The same genetic pathways that allow the development of high-affinity BCR of distinct isotype classes are also implicated in the malignant transformation of B-cells (Basso and Dalla-Favera, 2015).

1.3.2.1 Pathways and molecular events involved in DLBCL pathogenesis

Chromosomal rearrangements can be roughly classified into three types based on the features of the breakpoints. They can result from errors in the recombination-activating gene (RAG)-mediated V(D)J recombination process, errors in the CSR process and rearrangements occurring as by-products of aberrant SHM (ASHM) mechanism (Basso and Dalla-Favera, 2015; Singh and Briggs, 2016). Additional DNA breaks are introduced in the CSR mechanism and these breaks occur not only in the GC B-cells but in the activated B-cells (ABC) as well. The AID enzyme seems to play a role in these events and abnormal mutation occurs due to continued expressions (Chan, 2010). Notably, mutations have an impact on genes that are not typically known as GC-SHM targets. This is predicted for genes that are translocated to the IG locus, but SHM also has an impact on genes that are not translocated. Neoplastic cells go through ASHM, or cells can extensively be exposed to SHM. Neoplastic transformation can be due to rare mutations that are not typically found in normal germinal centre B-cell (GCB) cells and are chosen because they are beneficial for tumour cell growth or survival. Lastly, the DNA repair mechanisms can malfunction resulting in lymphomagenesis. The *Myelocytomatosis Oncogene Homolog* (*MYC*)*, B-cell lymphoma 2 (BCL2)* and *B-cell lymphoma 6 (BCL6) genes* are recurrent genetic targets involved in the pathophysiology of DLBCL (Lossos, 2005). Infections like *Epstein-Barr Virus (EBV)* and HIV contribute to the immunosuppression of patients which has a direct effect on the development of DLBCL (Bilajac et al., 2022).

1.3.3 Pathogenesis of DLBCL with viral oncogenes

One of the primary risk factors for the development of DLBCL is immunosuppression caused by HIV and EBV. Acquired immunodeficiency syndrome (AIDS) patients with a low CD4+ cell count seem to be at higher risk of developing DLBCL (Gessese et al., 2023).

1.3.3.1 *Human Immunodeficiency Virus (HIV)*

There is still no clarity on whether HIV itself leads to cellular transformation during lymphomagenesis. During HIV infection, the B-cell compartment frequently displays the development of a hyperactivation phenotype, which is characterised by a wide range of physiological changes. Additional HIV-induced effects on the B-cell compartment, include effects on memory B-cells and immunological fatigue, which is characterised by a decreased proliferative response. In the background of genetic abnormalities and a dysregulated immune surveillance, HIV stimulates chronic

antigen activation, cytokine dysregulation and coinfection with oncogenic viruses (De Carvalho et al., 2021; Re et al., 2019)

Oxidative stress is induced through the release of HIV viral proteins by infected cells or expressing cells. Through this action, they are known to trigger malignant transformation, infect epithelial cells and enhance tumorigenic features of transformed or malignant cells (Bilajac et al., 2022). The HIV genome includes a set of retroviral genes (*gag, pol* and *env*) involved in the lymphomagenesis of NHL. *Gag* codes for structural proteins as well as matrix proteins like matrix protein p17. The p17 protein has a complex function by viral entry through the cellular cytoplasm and regulates the early and late stages of viral replication. Variants of p17 can promote B-cell growth which leads to malignant B-cell transformation (Gloghini et al., 2013). *Pol*, on the other hand, encodes for proteins responsible for HIV replication like reverse transcriptase and protease enzymes. Lastly, *env* encodes glycoprotein, gp120 (Da Silva and De Oliveira, 2011). B-cells are activated through the glycoprotein gp120 and induce CSR with the variable heavy chain 3 (VH3). IGM is switched with IGG and IGA, due to AID upregulation. Further, gp120 interacts with C-type lectin receptors on B-cells and CD21-dependent interactions. The modulation of cytokine release, which deregulates B-cell differentiation and activation, results in DNA changes (Bilajac et al., 2022; Gloghini et al., 2013).

HIV proteins can incorporate T-cell CD40L and stimulate B-cells through CD40 receptors' interaction, leading to cytokine secretion. Virons that express CD40L-activated AID in B-cells can potentially lead to SHM and CSR with IG genes and oncogenesis in unrelated genes (Gloghini et al., 2013). These viral effects on B-cells have a large contribution to the development of DLBCL. In comparison to the uninfected population, people living with HIV have a much higher risk of developing lymphoma (Bilajac et al., 2022).

Due to errors in the CSR and SHM the tumour suppressor gene *TP53* is overexpressed in B-cell lymphomas. In 50% of HIV-related DLBCL cases the overexpression of MYC and BCL2 as well as SHM involving Proto-oncogene serine/threonine-protein kinase (PIM1), Paired box protein 5 (PAX5), and Thyroid transcription factor-1, is observed as additional pathogenic factors. A worse overall survival is observed in studies when MYC is overexpressed in HIV-positive patients. This is due to the role the *MYC* gene plays in cell proliferation, development, and apoptosis. Defects in the *MYC* gene may, due to its role in the cell cycle, cause the dysregulation of several other genes. In HIV-related DLBCL, a GCB

subtype is related to defective BCR signalling and ABC-like enrichment in MYC targets and cell cycle components. Additionally, increased cytotoxic T-cells are observed, with hyperproliferation and *MYC* rearrangements (Gessese et al., 2023).

1.3.3.2 *Epstein-Barr Virus (EBV)*

Transmission of EBV occurs from host to host *via* saliva and starts a primary infection in the oesophageal epithelium or the oropharynx. Infection occurs in two phases namely the lytic and latent phases (Shibusawa et al.,2021; Thompson, 2004). In the lytic phase, virus replication starts in the infected B-cells and lyses the infected cells. When EBV infects the B-cell and they escape cytotoxic elimination, it results in a latent infection. B-cells escape this process by downregulating antigen expression and when the infected cells eventually pass through the GC, they exit as EBV-infected memory B-cells (Shibusawa et al., 2021). The latent phases are grouped into four stages: latency 0, I, II, and III. They are grouped according to cell type, the time since infection and the local environment. The difference between the groups is the viral genes they express (Sausen et al., 2023). Latency 0 occurs in memory B-cells when no viral proteins are expressed except EBV-encoded ribonucleic acid (EBER). In latency I, the expression of Epstein-Barr nuclear antigens (EBNA) 1 is present together with EBER expression. In latency II, expression of latent membrane proteins (LMPs) 1 and 2 as well as EBNA1 is present (Sausen et al., 2023; Shibusawa et al., 2021). Finally, in latency lll infected B-cells express a full complement of viral proteins. The expression includes Epstein-Barr nuclear antigens (EBNAs 1, 2, 3A, 3B and 3C and EBNA leader protein), latent membrane proteins (LMP1, 2) and EBV-encoded RNA (EBER) (Shibusawa et al., 2021).

The association of EBV in Burkitt's lymphoma (BL) was previously seen as a casual occurrence, transforming B-cells. Over time, the true effect was noted in an array of cancers, including DLBCL (Ross et al., 2023). The pathogenesis of EBV-related DLBCL is mainly due to immunosuppression (Gessese et al., 2023). Certain malignancies occur during a specific EBV latent stage. DLBCL is associated with latency type ll and rarely type lll (Sausen et al., 2023). Oncogenic mechanisms in DLBCL are related to LMP1 expression most frequently. It plays a role in transforming B-cells and increases the development of B-cell lymphomas (Carbone et al., 2009). DLBCL with an immunoblastic morphology is usually associated with immunocompromised patients. Due to the low T-cell immunity against EBV the immunoblastic variant it is mostly affected. LMP1 and 2 increase the expression of CD23 and CD40, which is involved in cell proliferation and B-cell activation, as well as the expression of BCL2 which is an anti-apoptotic protein (Carbone et al., 2009; Gessese et al., 2023). Genetic alterations can occur

due to the *mammalian target of rapamycin* (mTORC1) gene which is stimulated by LMP2A mediators. The *mTORC1* gene is mainly responsible for controlling cell cycle proliferation and may promote monoclonal and polyclonal proliferation when prolonged antigen stimulation by HIV occurs, which is an important key factor in lymphomagenesis. EBV is seen in 30-60% of tumour cells and even higher (80-100%) in the immunoblastic variant of DLBCL (Gessese et al., 2023). The influence of LMP1 on DLBCLs is anticipated to be highest in tumours displaying a post-GC plasmacytic differentiation profile, according to the correlation between the expression of LMP-1 and BCL6, a marker for GC B-cells (Carbone et al., 2009). EBV can prompt nuclear factor kappa-light-chain-enhancer (NF-κB) responses by LMP1 imitating CD40, through the Tumour necrosis factor receptor type 1-associated DEATH domain protein (TRADD) and Tumour necrosis factor receptor-associated factor (TRAF) proteins and activates the NF-kB signalling pathway to promote cell proliferation (Marques-Piubelli et al., 2020; Shibusawa et al., 2021).

1.4 Localisation

DLBCL can present in both nodal and extra-nodal sites as single or multiple rapidly enlarging masses (Magangane et al., 2020). About 40% of patients present with extra-nodal disease, with the gastrointestinal tract being the most common extra-nodal site. Other extra-nodal sites include the bone, testes, liver, thyroid, kidneys, spleen, salivary glands, and adrenal glands. The risk of spreading to the central nervous system (CNS) is high when the kidneys and the adrenal glands are involved (Swerdlow et al., 2017).

1.5 Clinical features

DLBCL is associated with lymphomatous granulomatosis or chronic inflammation and B symptoms, experienced by 30% of patients which include the swelling of lymph nodes, fever, fatigue, weight loss and night sweats (Mamgain et al., 2022; Martelli et al., 2013; Swerdlow et al., 2017). Lactate dehydrogenase (LDH) levels in patients are elevated. Clinical features in patients with HIV are more aggressive and adverse than in those without HIV. HIV-positive patients also present with more extra-nodal disease, high-grade histology, and poorer prognoses. Due to myelosuppression, HIV-positive patients have a great risk of contracting secondary infections like tuberculosis, hepatitis B infection, herpes viral infections, sepsis, organ dysfunction and bone marrow involvement (Patel et al., 2015; Pather and Patel, 2022).

1.6 Diagnostic features

The diagnosis of DLBCL requires a combination of clinical, histological, and molecular features.

1.6.1 Microscopic features and morphological subtypes

In DLBCL, total architectural effacement is demonstrated by the diffuse proliferation of medium or large lymphoid cells with nuclei that are larger or the same size as those of normal macrophages and double in size of normal lymphocytes. Morphologically DLBCL comprises centroblastic, immunoblastic and anaplastic variants (Swerdlow et al., 2016). The centroblastic variant is the most commonly observed in about 80% of DLBCL. The centroblasts are medium to large-sized lymphoid cells that have round to oval shape nuclei and the nuclei are vesicular with fine chromatin. Two to four small nucleoli are present adjacent to the nuclear membrane. The cytoplasm is moderate, usually basophilic or amphophilic. Tumours can comprise up to 90% of centroblasts or can have a polymorphic composition with a mixture of centroblasts, centrocytes and immunoblasts. Nuclei in the tumour cells can be multilobulated, especially in extra-nodal tumours (Figure 1.2 A) (Insuasti-Beltran, 2022; Li et al., 2018,).

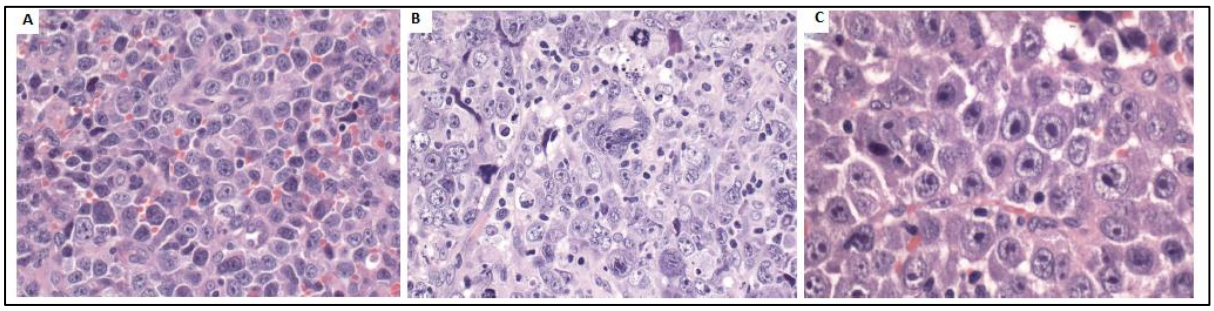

Figure 1.2: Morphological subtypes in DLBCL; A: Centroblastic variant [Haematoxylin & Eosin (H&E) stain, ×400]; B: Anaplastic variant (H&E stain, ×400); C: Immunoblastic variant (H&E stain, ×630) (Chen et al., 2019a).

The immunoblastic variant accounts for about 8-10% of cases. About 90% of the cells in this variant are immunoblasts with considerable basophilic cytoplasm. The cells have a single trapezoid-shaped centrally located nucleolus with chromatin attached to the nuclear membrane. To differentiate this variant from extramedullary involvement by a plasmablastic lymphoma (PBL), it is important to use clinical and immunophenotyping findings (Figure 1.2 B) (Chen et al., 2019a; Insuasti-Beltran, 2022; Li

et al., 2018). The anaplastic variant is the least common morphological subtype and is characterised by cells that resemble Hodgkin/Reed-Sternberg cells. The tumour cells are large with bizarre pleomorphic nuclei and moderate to abundant cytoplasm. Partial or extensive sinusoidal patterns may be seen (Figure 1.2 C) (Insuasti-Beltran, 2022; Li et al., 2018).

1.6.2 Immunohistochemical features

Immunohistochemistry (IHC) is a diagnostic technique that detects specific proteins in cells by using antigen-antibody interactions. B- or T-cells are indistinguishable on Haematoxylin & Eosin (H&E)-stained slides and IHC is critical in lymphoma diagnosis to determine cell lineage, maturation phase and degree of cell proliferation (Table 1.1) (Cho, 2022; Li et al., 2018,).

In DLBCL, at least one pan B-cell marker such as CD19, CD20, CD22, CD79a or PAX5 is expressed. CD3, a T-cell marker, in the atypical cells, is negative (King and Lam, 2020; Li et al., 2018). Markers CD10, BCL6 and Multiple Myeloma 1 (MUM1) have variable expression, depending on the tumour's cell of origin (COO) (Li et al., 2018). The CD10 marker is found normally in centrocytes and centroblasts of reactive GC and is expressed in 30-60% of DLBCL cases (Li et al., 2018). BCL6 is expressed in centrocytes and centroblasts of the GC as well as in activated B-cells. BCL6 expression is positive in 60-90% of cases. MUM1 is expressed in plasma or post-GCB cells with an expression frequency of 35-65% in DLBCL (Cho, 2022).

MYC and BCL2 expression varies depending on the cutoff value used to consider positivity. MYC is considered positive when ≥ 40% of the nuclei are expressing MYC and BCL2 is considered positive when ≥ 50% of the tumour cells are positive (King and Lam, 2020; Li et al., 2018). MYC expression has a frequency of 30-50% while BCL2 expression is observed in 20-35% of cases. The co-expression of MYC and BCL2 proteins is described as double-expressor lymphoma (DEL) (Rungwittayatiwat et al., 2021). DELs make up approximately 26% of DLBCL cases and are associated with an ABC or post-GC COO (Chen et al., 2023a; Liu and Barta, 2019,).

Variable expression of CD30 is observed in 10-20% of cases associated with the anaplastic variant and is an indicator of favourable prognosis (King and Lam, 2020; Swerdlow et al., 2017). CD5 is observed in 5-10% of the cases and is associated with post-GC B-cells. In the absence of cyclin D1 and/or SRY-

box transcription factor 11 (SOX11) expression, DLBCL with CD5 positivity can be distinguished from the pleomorphic or blastoid variant of mantel cell lymphoma. Cyclin D1 has an expression frequency of 1.5-15% of cases (Li et al., 2018; Liu and Barta, 2019).

Table 1.1: Immunohistochemical markers expressed in DLBCL (Chen et al., 2019a; Li et al., 2018; Sun et al., 2016).

Antigen/markers	Frequency in DLBCL	Phase of expression	Significance
CD20	100%	Immature B-cell, Mature B-cell, Germinal centre	Diagnosis and therapeutic target
CD19	Almost always	Immature B-cell, Mature B-cell, Germinal centre,	
CD22	Almost always	Mature B-cell, Germinal centre	
CD79a	Almost always	Mature B-cell, Germinal centre	Diagnosis
PAX5	Often	Immature B-cell, Mature B-cell, Germinal centre	
CD5	5-10%	Post-germinal centre	Prognostic
CD30	10-20%	Anaplastic cells	
CD10	30-60%	Pre-germinal centre, Germinal centre	COO determination and prognosis
BCL6	60-90%	Germinal centre and activated B-cells	
MUM1	35-65%	Activated B-cells/post-germinal centre	
Ki-67	80-90%	All phases of the cell cycle	Proliferative marker
MYC	30-50%	Pre-germinal centre and Germinal centre	Prognostic (Depend on co-expression)
BCL2	20-35%	Reactive germinal centres and neoplastic follicles	Prognostic

The Ki-67 proliferation index in DLBCL varies usually between 80-90% and occasionally exceeds 90% (King and Lam, 2020; Li et al., 2018). If the cell size, CD10 expression, and Ki-67 positivity do not differentiate BL from DLBCL, immunohistochemical staining for BCL2, BCL6 and MUM1 should be

undertaken. Cases of BL are almost always positive for BCL6, but rarely for BCL2. In contrast, all three of these markers vary greatly between DLBCL instances (King and Lam, 2020).

1.6.3 Cell of origin subtypes

The classification of COO is a prognostic tool, advised by the WHO as an important sub-classification to include in the diagnosis of DLBCL. The distinction between DLBCL subgroups based on their COO was first published in 2000 by Alizadeh et al with the use of gene expression profiling (GEP) (Alizadeh et al., 2000). GEP can determine the derivation of tumour cells grouping them into GCB, ABC, or unclassifiable subtypes (Pasqualucci, 2019; Placa et al., 2022). GEP is the gold standard, but in the clinical setting, it introduces some challenges due to its high cost and proposed technical constraints (Hans et al., 2004; Hwang et al., 2013). Due to these constraints, several practical IHC algorithms have been proposed. These algorithms include those proposed by Hans et al, Choi et al and Visco et al. The Choi algorithm uses IHC stains FOXP1, GCET1, CD10 and MUM1, the Visco-Young algorithm uses CD10, FOXP1 and BCL6 and the Hans algorithm uses CD10, BCL6 and MUM1 to distinguish between the subtypes (Choi et al., 2009; Hans et al., 2004; Song et al., 2023; Visco et al., 2012). Using IHC reduces the cost and time for the determination of the COO (Zamo et al., 2022).

The most widely used algorithm is the Hans algorithm (Figure 1.3) (Beham-Schmid, 2017; Li et al., 2018; Visco et al., 2012). In correlation to GEP, the Hans algorithm yields similar results and the three antibodies used are more easily available than those in the other algorithms (Hans et al., 2004; Placa et al., 2022). Markers are considered positive when ≥ 30% of the tumour cells are positive (Swerdlow et al., 2017). When CD10 is positive independent of the results of the other two markers, the tumour is classed as GCB (Cho, 2022). MUM-1 expression suggests non-GCB/ABC type. When BCL6 is expressed it is classified as GCB if the MUM1 expression is negative (Cho, 2022). In 50% of the DLBCL GCB subtype, co-expression of MUM1 and BCL6 occurs (Swerdlow et al., 2017). The incidences of these subtypes vary, due to multiple factors such as patient age, geographical location and methodology used. GCBs are usually in 60% of DLBCL cases and non-GCBs in 40% of cases (Liu and Barta, 2019). This classification has been shown to have prognostic significance and to correlate with significant differences in tumour molecular aetiology. Recent research has revealed that non-GCB lymphomas differ significantly in their clinical and treatment outcome and are more sensitive to medications than GCB lymphomas. Thus, COO determination may guide patient therapy options in addition to prognostication (Jaffe et al., 2017; Mamgain et al., 2022; Willenbacher et al., 2020).

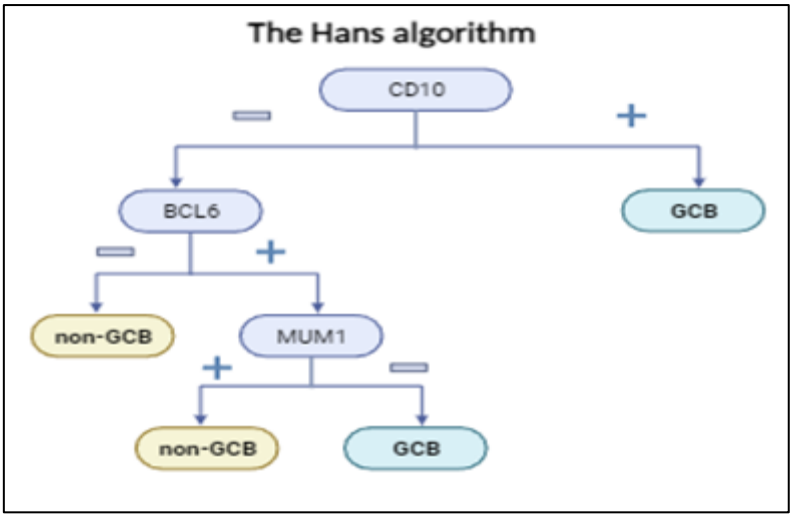

Figure 1.3: The Hans algorithm (Adapted from Hwang et al., 2013)

1.7 Classification

The classification of DLBCL has evolved over the years. The development of diagnostic tools has enabled the progression of classification systems since more are aware of the molecular makeup of tumours. The classification of DLBCL according to the 5th WHO *Classification of Tumours of Haematopoietic and Lymphoid Tissues* resulted in 17 distinct entities outside DLBCL, NOS (Table 1.2). Some entities that were provisional in the 4th edition became definite subtypes and in some, major revisions were executed (Chadburn et al., 2023).

Table 1.2: The subtypes and types of large B-cell lymphomas

<table>
<tr><th colspan="2">Diffuse large B-cell lymphoma, not otherwise specified (NOS)</th></tr>
<tr><td colspan="2">Morphological subtypes: Centroblastic, Immunoblastic, Anaplastic</td></tr>
<tr><td colspan="2">Molecular subtypes: GCB and non-GCB</td></tr>
<tr><td colspan="2">Genetic subtype: Diffuse large B-cell lymphoma with MYC and BCL6 rearrangements</td></tr>
<tr><th colspan="2">Other lymphomas of large B-cells</th></tr>
<tr><td>• ALK-positive large B-cell lymphoma
• DLBCL associated with chronic inflammation
• DLBCL/HGBCL with MYC and BCL2 rearrangements
• EBV-positive DLBCL, NOS
• Fibrin-associated large B-cell lymphoma
• Fluid overload-associated large B-cell lymphoma
• High-grade B-cell lymphoma with 11q aberration
• Intravascular large B-cell lymphoma</td><td>• Large B-cell lymphoma with Interferon regulatory factor 4 (IRF4) rearrangement
• Lymphomatoid granulomatosis
• Mediastinal grey zone lymphoma
• Plasmablastic lymphoma
• Primary cutaneous diffuse large B-cell lymphoma, leg type
• Primary large B-cell lymphoma of immune-privileged sites
• Primary mediastinal large B-cell
• T-cell/histiocyte-rich large B-cell lymphoma</td></tr>
</table>

1.8 Differential diagnosis

The differential diagnosis of DLBCL comprises a long list of different lymphomas since microscopically, large lymphoid cells may be observed in virtually any lymphoid tissue, benign or malignant, and nodal or extra-nodal (Hu et al., 2011).

1.8.1 High-grade B-cell lymphoma, NOS (HGBCL, NOS) with *MYC* and *BCL2* with or without *BCL6* rearrangements

Morphologically this classification has features intermediate between BL and DLBCL. Commonly a starry-sky pattern with high-grade morphology is present with abundant apoptosis and a high mitotic

rate (Medeiros et al., 2017). The presence of gene rearrangements must be considered to fulfil the criteria. Gene copy number variations like amplifications and gains, are not recognised in the classification (Chen et al., 2019a). These cases are described as either double-hit lymphomas (DHL) (*MYC* and *BCL2*) or triple-hit lymphomas (THL) (all 3 rearrangements) (Chadburn et al., 2023). However, the 5th edition of the WHO classification of haematopoietic tumours only considers cases with *MYC* and *BCL2* rearrangements as a distinct entity and are classified as HGBCL or DLBCL with *MYC* and *BCL2* rearrangements. These cases are usually of GCB origin and are genetically distinct (Chadburn et al., 2023). Cases however that have only *MYC* and *BCL6* rearrangements are classified as DLBCL, NOS or HGBCL, NOS according to their morphological features. Cases with *MYC* and *BCL6* rearrangements without *BCL2* usually have a more diverse genetic profile and are of non-GCB origin (Chadburn et al., 2023).

As previously termed, DHL and THL make up 4-16% of DLBCL (Liu and Barta, 2019). *MYC* can be translocated with IG or non-IG partners. Rearrangements with IG partners seem to have a worse prognosis than those with non-IG partners. Previously cases that arose from FL into DLBCL with rearrangements were excluded from this classification and only *de novo* cases could be included (Chen et al., 2019a). Patients with DHL/THL present with aggressive advanced-stage disease and do not respond to traditional Rituximab-CHOP (R-CHOP) therapy. They have high LDH levels, extra-nodal involvement, and a high International Prognostic Index (IPI) score (King and Lam, 2020; Li et al., 2018) *MYC/BCL2* DHLs are the most frequent subtype of DHL, accounting for around 70% of cases with a concurrent GCB origin, and *MYC/BCL6* DHL accounting for approximately 34% of cases with a non-GCB origin likelihood (Dinneen et al., 2020; Marino et al., 2021). THL is observed in 15% of cases and is associated with the GCB subtype (Li et al., 2018; Liu and Barta, 2019).

Single-hit lymphomas (SHL) cases are those that morphologically fall into the categories of DLBCL, NOS, or HGBCL, NOS with just a single *MYC* rearrangement. Similar to DHL and THL, SHL has been associated with a poor prognosis for survival (Ok and Medeiros, 2020). It is still unclear if cases with amplifications or copy number variants of *MYC, BCL2*, and/or *BCL6* genes have a significant impact on clinical behaviour and biological presentation. They are classified as HGBCL, NOS or DLBCL, NOS (Chadburn et al., 2023).

1.8.2 HGBCL, NOS

There is still a small subset of tumours considered to be cytologically high-grade with a blastoid morphology or intermediate features between DLBCL, NOS, and BL and lacking *MYC, BCL2* and *BCL6* rearrangement (Bouroumeau et al., 2021). In the 5th edition of the WHO, they are classified as HGBCL, NOS. HGBCL, NOS has a morphological appearance with medium-shaped cells, with a starry-sky appearance or often a blastoid/ Burkitt-like cytology. Data on HGBCL, NOS is very sparse, and the use of this classification should be used sparingly (King et al., 2023; Nasr et al., 2019). Terminal deoxynucleotidyl transferase (TdT) and Cyclin D1 IHC can be done to exclude mantle cell lymphoma (MCL) and Burkitt-like B-cell lymphoma (B-LBL), especially in cases with a blastoid morphology. HGBCL, NOS are usually associated with the GCB subtype and the expression of MYC and BCL2 is variable. DH/TH rearrangements should be excluded with FISH testing. The genetic findings of HGBCL, NOS require more studies as rearrangements of genes are observed variably (Chen et al., 2019a).

1.8.3 Follicular lymphoma

Grade 3 FL is morphologically remarkably similar to the centroblastic variant of DLBCL. IHC should be performed for follicular dendritic cells which may be useful in challenging instances to identify big, confluent follicles from DLBCL. FL can develop into DLBCL, but with genetic testing, this can be identified (Hu et al., 2011). CD21, CD25 and CD35 are used to identify follicular dendritic cells. The *BCL2* and *BCL6* rearrangement is observed in 80% and 10-15% of cases, respectively (Medeiros et al., 2017).

1.8.4 Small cell lymphoma (SLL)

SLL is a common low-grade lymphoma which is part of the DLBCL differential diagnosis. SLL contains many large cells and is CD5-positive. A process called Richter's transformation can transform SLL into DLBCL. DLBCL with Richter's transformation should only be made if large, disorganised sheets of cells are seen (Hu et al., 2011).

1.8.5 Mantle cell lymphoma (MCL)

This is a high-grade lymphoma which can be easily distinguished from DLBCL based on morphology and IHC results. When MCL has a blastoid or pleomorphic appearance, it can be morphologically like DLBCL. With CD5, SOX11 and Cyclin D1 expression, these two can be separated (Hu et al., 2011;

Swerdlow et al., 2017). FISH can be used to further distinguish MCL from DLBCL. MCL is known for t(11;14) between the *Cyclin D1* and an *IG* gene. In the absence of the rearrangements, it favours DLBCL and FISH for *MYC, BCL2* and *BCL6* can be performed to confirm (Webb et al., 2023).

1.8.6 Burkitt's lymphoma (BL)

BL cells are medium in size and pleomorphic, thus resembling DLBCL. The starry-sky appearance is present in cells with numerous mitoses. Cell markers CD10, CD20, CD22 and BCL6 are positive in BL and are BCL2 negative with the Ki-67 proliferative index almost 100%. Apoptosis and mitosis are abundant and infiltrating T-cells are less common. The *MYC gene t*(8;14) rearrangement is characteristic of BL (Hu et al., 2011, Mamgain et al., 2022; Medeiros et al., 2017; Swerdlow et al., 2017).

1.8.7 High-grade B-cell lymphoma with 11q aberration

A similar morphological pattern to BL is observed, but a more pleomorphic appearance is noted. CD10 is positive with a variable expression of BCL2. The Ki-67 proliferation index is generally high. No *MYC* rearrangements are involved but chromosomal alterations involving 11q with telomeric losses and proximal gains are observed (Chadburn et al., 2023).

1.8.8 Primary mediastinal large B-cell lymphoma (PMBL)

PMBL stems from thymus B-cells presenting as a large mediastinal mass in young females with occasional invasion of nearby structures. DLBCL with secondary mediastinal involvement should be excluded from this diagnosis. Like the centroblastic and immunoblastic subtypes of DLBCL, PMBL morphologically is heterogeneous with large cells and abundant cytoplasm with round to oval nuclei. The occasional Reed-Steenberg cells raise the suspicion of HL. PMBL expresses pan B-cell markers; in 75-82% of cases, CD30 expression is observed but not as strong as in HL. CD23 expression is seen in 70% of cases. Gains are usually observed for the 9p24 genes (Martelli et al., 2013).

1.8.9 EBV-positive DLBCL, NOS

In DLBCL, EBV positivity is seen in 5-10% of cases (Frontzek et al., 2023; Wang, 2023c). This percentage does vary from country to country. It is usually found among HIV/AIDS individuals as well as organ

transplant recipients (Wang, 2023c). EBV-positive DLBCL was historically seen as a separate entity from DLBCL, due to its heterogenous features. In the current 5th edition of the WHO classification, it is categorised as a DLBCL, NOS subgroup (Ross et al., 2023). It is recognised as DLBCL where most of the tumour cells are EBV-positive. This diagnosis is excluded when an underlying immune deficiency or dysregulation is present. Previously it was described as a disease of the elderly, but studies show that it occurs in younger patients as well. Morphologically it presents with a large B-cell that has a variable appearance (Kurz et al., 2023). It is usually polymorphous and in areas of necrosis Reed-Sternberg-like cells are present. EBER expression is positive and CD20 and CD79a are expressed (Martelli et al., 2013; Kurz et al., 2023). In up to 40% of EBV-positive DLBCL cases, CD30 is expressed. In some cases where a monomorphic sheet of cells is present, it can be indistinguishable from DLBCL, NOS. Most cases are non-GCB subtypes. This disease presents with EBV latency either I, II, or III and expresses LMP1 and dominance of EBER reactivity (Kurz et al., 2023; Ross et al., 2023). Based on factors like geographical area and the age of patients the prognosis may differ (Ross et al., 2023).

1.8.10 CD20 negative differentials

The differentiation of PBL, lymphoblastic lymphoma, *Herpes Virus Type* 8 (HHV8)-positive large B-cell lymphoma (LBCL) primary effusion lymphoma and anaplastic lymphoma kinase (ALK)-positive large B-cell lymphoma, can easily be made as CD20 is negative in the tumour cells, thus excluding them as a possible diagnosis (Martelli et al., 2013; Medeiros et al., 2017). In a few of these differential diagnoses, FISH can be employed to test for specific rearrangement associated with these lymphomas. In PBL the *MYC* gene is rearranged in 50% of cases, while in ALK-positive LBCL, *ALK* is rearranged in all cases (Medeiros et al., 2017).

1.9. Genetic profile

1.*9.1 Myelocytomatosis Oncogene Homolog (MYC)*

The *Myelocytomatosis Oncogene Homolog* (*MYC*), located on chromosome 8q24, is a proto-oncogene that encodes for the MYC protein (Almeida et al., 2022; Chisholm et al., 2015; Swerdlow et al., 2017) MYC is responsible for transcriptional expression in 15% of genes. *MYC* regulates multiple cellular events like cell cycle, cell growth, self-renewal, survival, metabolism, protein synthesis, and differentiation (Figure 1.4). Functions are carried out through direct activation or inhibiting gene transcription and transcriptional amplification (Casey & Felsher, 2018).

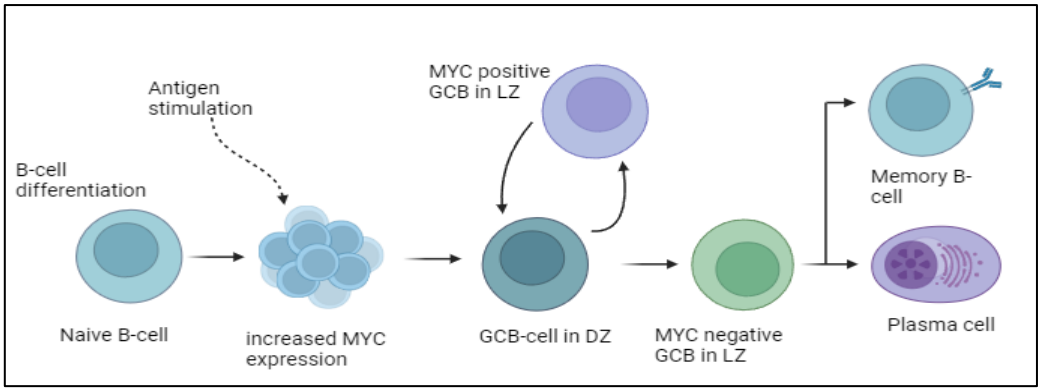

Figure 1.4: *MYC* in B-cell development and dysregulation (Adapted from Karube and Campo, 2015).

1.9.1.1 *MYC* functional regulation

The three main functional regulations of MYC can be broken up into transcription, micro-RNAs and apoptosis (Cai et al., 2015). As a transcription factor, MYC binds with MYC-associated factor X (MAX), which is dependent on the enhancer box (E-BOX) DNA sequence and the recruitment of specific co-activator complexes. Transcription is activated when MYC-MAX heterodimers bind to the E-box and *MYC* transcriptional activation starts that is mediated by binding to acetyltransferases. With the aid of an adaptor TRRAP, a transition from the G0 or G1 phase to the S phase of the cell cycle occurs. This cell phase transition activates DNA replication, protein biosynthesis and cell proliferation. Another function of MYC includes the downregulation of the cell cycle inhibitors and activation of Cyclin D1 expression and cyclin-dependent kinases (CDKs) (Cai et al., 2015).

MYC regulates micro-RNAs that function as oncogenes or tumour suppressor genes. *MYC* as an oncogene downregulates phosphatase and tensin homolog deleted on chromosome ten (PTEN), Tumour protein 53 (TP53) and E2F1 which induces the activation of the phosphoinositide-3-kinase (PI3K) pathway and inhibits apoptosis. *MYC* represses the *microRNA 127* gene with its tumour suppression function by employing histone deacetylases (HDACs). These microRNAs are vital in the regulation of neoplastic development like apoptosis, proliferation, and cell differentiation, which targets genes including *BCL2, TP53* and *enhancers of zest homolog 2* (*EZH2*). A MYC function that is related to the increased aggressiveness in lymphomas is the fact that MYC can function as an amplifier of transcribed genes (Cai et al., 2015).

MYC plays a part in the cellular apoptosis of cells, and it involves multiple pathways. Apoptosis is induced when MYC is overexpressed leading to DNA damage, resulting in the activation of *TP53* response. The MYC protein expression indirectly downregulates proteins like BCL2 which is an anti-apoptotic protein, and it upregulates BIM which is a pro-apoptotic element. These contributing apoptotic elements explain why MYC needs other mechanisms in the progression of tumours (Cai et al., 2015).

MYC also has a significant role in the formation of B-cell GCs, where it is expressed in different subsets of cells after the interaction with antigens and T-cells (Korac et al., 2017). In the GC following the upregulation of BCL6, MYC is transcriptionally inhibited and the DZ of the GC is formed. After the upregulation of NF-kB and MUM1 expression, MYC is re-expressed in some cells in the LZ. These cells will re-enter the DZ and undergo IG SHM. MYC-negative cells in the LZ exit the GC either as plasmablasts or memory B-cells. MYC is repressed in plasma cells with the introduction of B lymphocyte-induced maturation protein 1 (BLIMP1) and BCL6. Although the biological significance of MYC suppression in DZ B-cells is unknown, the initial burst of MYC expression presumably involves the induction or amplification of transcriptional programmes associated with many of MYC's known functions (Basso and Dalla-Favera, 2015). When combined with IG genes, the *MYC* gene is linked to the development of B-cell oncogenesis (Cho et al., 2021, Karube and Campo, 2015).

In up to 70% of lymphomas, the MYC oncoprotein is found to be deregulated. Genetic alterations seen in lymphomas are associated with *MYC* alterations caused by unusual expression. It can be due to activation of signal transduction pathways [e.g., Neurogenic locus notch homolog protein 1 (NOTCH) or receptor tyrosine kinases (TKs)] or direct alterations of *MYC* (Martínez-Martín et al., 2023).

1.9.1.2 Overexpression of MYC in DLBCL

In DLBCL MYC overexpression is observed in 40% of cases (Xia and Zhang, 2020). It seems that MYC upregulation overcomes BCL6 or BLIMP1 repressors and their inhibitory effect in differentiated B-cells (Ott et al., 2013). To escape apoptosis, other genetic events contribute to promoting proliferation and oncogenesis. Some of these genes include *BCL2* and *BCL6* expression and *TP53* mutations. An increase in MYC expression leads to genetic events that can lead to *MYC* gene amplification, rearrangements, point mutations, and copy number alterations (Karube and Campo, 2015; Riedell and Smith, 2018; Xia and Zhang, 2020). For *MYC* to drive tumorigenesis, it acquires additional mutations for events like

apoptosis and cell ageing. Events like apoptosis need to be inactivated for MYC to be carcinogenic (Martínez-Martín et al., 2023)

1.9.1.3 Rearrangement of *MYC* in DLBCL

MYC gene rearrangements are observed in 5-14% of DLBCL cases (Almeida et al., 2022; Ott et al., 2013). *MYC* rearrangements are linked with an increase in proliferation. In the event of gene dysregulation and mutations, it affects the level of MYC protein expression (Riedell and Smith, 2018). The most frequent rearrangement partners with *MYC* are *IG* genes. The t(8;14) rearrangement occurs when *MYC* is juxtaposed to the *IGH* gene on chromosome 14q32. Less commonly, t(2;8) and t(8;22) involve the *IGK* (2p12) or *IGL* (22q11) light chain genes (Chisholm et al., 2015). In DLBCL about 5% of cases with *MYC* rearrangements have a non-IG rearrangement partner. Non-IG partners include 1p36, 3p25, 3q27(*BCL6*), 4p13,5q13, 9p13 (*PAX5*), 12p11, and 13q31 (Karube and Campo, 2015; Riedell and Smith, 2018). Patients with non-IG rearrangements are known to have better outcomes than those with *IG-MYC* rearrangement (Ok and Medeiros, 2020).

1.9.1.4 *MYC* copy number variation (CNV) in DLBCL

In addition to *MYC* rearrangements, CNVs can occur in DLBCL cases. *MYC* gains are observed in 19-38% of cases and amplification is reported in 2-20% of cases (Riedell and Smith, 2018). Amplifications of the *MYC* gene are most frequently observed in the GCB-DLBCL subtype (Xia and Zhang, 2020).

1.9.2 *B-cell lymphoma 2 (BCL2)*

The *B-cell lymphoma 2 (BCL2)* gene, located on chromosome 18q21, encodes for an outer mitochondrial membrane protein (Almeida et al., 2022; Shivakumar and Armitage, 2006). Its primary role in B-cells is to maintain cellular viability by balancing cell survival and apoptosis inhibition (Riedell and Smith, 2018; Sesques and Johnson, 2017).

1.9.2.1 The function of BCL2 in B-cells

The BCL2 protein regulates the differentiation and development process by four conserved BCL2 homology (BH) domains which are located on the BCL2 protein, known as BH1, BH2, BH3, and BH4. They are involved in protein-to-protein interactions and apoptotic regulation. BCL2 expression is

strictly controlled in healthy cells and the protein it produces guards against apoptosis by preventing pro-apoptotic proteins like BAX and BAK from acting. BCL2 is crucial in cells that are subjected to apoptotic triggers such as DNA damage, hypoxia, or the removal of growth factors. By preventing the release of cytochrome-c from the mitochondria, BCL2 prevents the activation of the mitochondrial pathway of apoptosis under these circumstances (Singh and Briggs, 2016). In the event of dysregulation, it can lead to tumorigenesis. Both direct and indirect mechanisms can lead to dysregulation of BCL2. Direct mechanisms include SHM, translocations and amplifications. Indirect mechanisms include the loss of loss of Myeloid leukaemia 1 (MCL1) and the activation of signalling pathways. Like MYC, BCL2 by itself cannot induce full tumorigenesis. However, with MYC and BCL2 in cooperation, they can initiate carcinogenesis and maintain cell survival (Martínez-Martín et al., 2023). Chromosomal rearrangements and gene amplifications with an increase in NF-kB signalling are observed in the oncogenesis of DLBCL (Riedell and Smith, 2018).

1.9.2.2 Overexpression, amplification, and gains of *BCL2* in DLBCL

Amplification of the 18q21 locus causes overexpression of the *BCL2* gene. *BCL2* amplification and/or overexpression is found in a variety of lymphoid neoplasms and appears to be a crucial event in the development of DLBCL (Almeida et al., 2022). *BCL2* is a true oncogene, although cells overexpressing BCL2 must undergo additional genetic changes before becoming evident in lymphoma (Sesques and Johnson, 2017). BCL2 overexpression is observed in association with MYC expression and other oncogenes involved in DLBCL development (Riedell and Smith, 2018). BCL2 protein is expressed in 30-50% of DLBCLs and 75% of HGBLs but not in BL or normal GCB cells (Sesques and Johnson, 2017). The gain of the *BCL2* however is observed mostly in the non-GCB subtype and 14% of cases associated with overexpression (Tomas-Roca et al., 2021).

1.9.2.3 *BCL2* rearrangements in DLBCL

BCL2 transcription is dysregulated in the event of a t(14;18) which connects the junctional segment (JH) of the *IGH* gene and the *BCL2* gene. This results in the targeting of the *BCL2* oncogene by SHM, a process that is generally limited to the IG loci. (Rosenthal and Younes, 2017, Singh and Briggs, 2016). Implementation of the IG's normal SHM process by the *BCL2* gene places the gene under the control of AID, resulting in a high rate of mutations (Figure 1.5) (Singh and Briggs, 2016). In 20-30% of DLBCLs, *BCL2* rearrangements are mostly observed in the GCB subtype (Rosenthal and Younes, 2017; Swerdlow et al., 2017; Tomas-Roca et al., 2021).

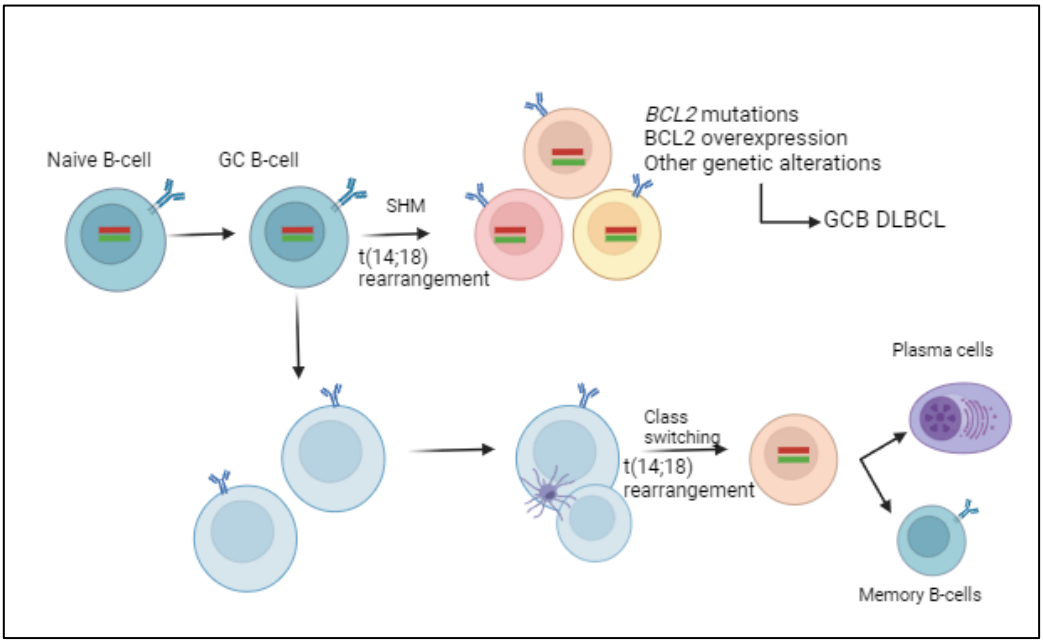

Figure 1.5: *BCL2* in normal and DLBCL B-cell formation (Adapted from Singh and Briggs, 2016)

1.9.3 *B-cell lymphoma 6 (BCL6)*

The *B-cell lymphoma 6 (BCL6)* gene is a proto-oncogene located on chromosome 3q27 (Gouveia et al., 2012). It is a transcriptional repressor, which regulates a vast transcriptional network that promotes GC development and maintenance.

1.9.3.1 Function and dysregulation

BCL6 is required for GC formation during the immune response and is involved in the silencing of over 1000 genes. These genes include target genes that control DNA damage and keep proliferation in check. BCL6 is also needed for B-cells to exit the GC and for plasma cell differentiation, making sure that GCB cells have enough time to undergo SHM with IG genes (Cardenas et al., 2017; Martínez-Martín et al., 2023). BCL6 is expressed in the interfollicular zone in a small subset of naive B-cells that have been successfully activated by antigen and T-cell interactions. Interferon-regulatory factor 8 (IRF8), IRF4/MUM1 and MEF2B25 all contribute to BCL6 production, however, the signals that activate these transcription factors and BCL6 remain unknown. After induction, BCL6 expression levels are fine-tuned through an autoregulatory process in which BCL6 binds to its promoter to negatively inhibit transcription (Basso and Dalla-Favera, 2015). The dissection of the BCL6-controlled transcriptional network has revealed a significant number of target genes involved in driving and regulating the GC

reaction. These genes define *BCL6's* role. In response to DNA damage the BCL6 protein blocks genes involved in the cell cycle differentiation, and inflammation as well as modulating apoptosis, making GC B-cells less sensitive to DNA damage (Basso and Dalla-Favera, 2015; Gouveia et al., 2012).

BCL6 promotes cell proliferation by repressing the expression of Potent cyclin-dependent kinase inhibitor (p21), which is a cell cycle inhibitor. BCL6 targets the *PR domain zinc finger protein 1 (PRDM1)* gene directly. The *PRDM1* gene expression is required for terminal differentiation to plasma cells. *PRDM1* encodes for plasma cells regulator BLIMP1. The transcriptional downregulation of *BCL6* for the promotion of its degradation is directed by multiple pathways. Following BCR, CD40 and toll-like receptor signalling, transcriptional repression of BCL6 occurs by the activation of NF-κB, mediated by IRF4 (Basso and Dalla-Favera, 2015). Antibody production changes in this stage, from membrane-bound to secreted and loses its proliferative capacity. In normal function, BCL6 represses cytokine production in macrophages and thus the lack of BCL6-mediated repression would produce excessive cytokine release, increasing inflammation. In the context of malignancies, the perturbation of DNA repair and TP53 may contribute to normal CG B-cell functions by repressing DNA damage responses and leading to mutations promoting aggressive lymphomas. (Wagner et al., 2011). Due to *BCL6*'s involvement in all these cellular processes, it is easy to recognise how deregulation can result in the oncogenesis of B-cells (Cardenas et al., 2017). BCL6 interestingly also targets MYC and BCL2 in normal conditions. It downregulates MYC and BCL2 proteins. In the event of direct or indirect BCL6 dysregulation, BCL6 cross-talks with proteins which are involved in chromatin modifications. These proteins include EZH2, CREBBP binding protein (CREBBP) and Histone-lysine N-methyltransferase 2D (KMT2D). These modifications can lead to the imbalance of BCL6. (Martínez-Martín et al., 2023)

1.9.3.2 *BCL6* in DLBCL

In the case of BCL6 dysregulation, DNA damage responses are suppressed by the inhibition of terminal differentiation. Chromosomal rearrangements target the *BCL6* locus placing the intact protein coding sequence of *BCL6* downstream of the regulatory regions provided by the partner chromosomes. *BCL6* rearrangements prevent the BCL6 protein from being downregulated in post-GCB cells (Riedell and Smith, 2018). Regions include the *IGH* locus as well as gene promoters that are involved throughout B-cell development both germinal and post germinal. The major breakpoint on the *BCL6* locus is at 3q27. A 3q27 breakpoint can result in the *Interleukin 21 (IL-21) receptor* located on chromosome 16, being placed under the BCL6 promotor. Both IL-21 and BCL6 increase in expression which may

contribute to lymphomagenesis (Sesques and Johnson, 2017). Breakpoints are located on the switch region of *IGH* which is indicative of it occurring at the late stage of B-cell development (Wagner et al., 2011). The *BCL6* rearrangement is the most frequent aberration in DLBCL (Nakamura et al., 2022). It is observed in almost 30-40% of DLBCL cases (Almeida et al., 2022; Basso and Dalla-Favera, 2015,). BCL6 protein and *BCL6* rearrangements contribute to DLBCL pathogenesis but are not linked to resistance to therapy (Sesques and Johnson, 2017). *BCL6* have many rearrangement partners which include 26 IG and non-IG partners such as *Growth Arrest Specific 5* (*GAS5), ALK, Muscleblind Like Splicing Regulator 1 (MBNL1),* Glyceraldehyde-3-phosphate dehydrogenase *(GAPDH), Nascent-polypeptide-associated complex alpha polypeptide (NACA), Lymphoid-restricted membrane protein (LRMP), Lymphocyte Cytosolic Protein 1 (LCP1), IL21, N-ethylmaleimide-sensitive factor Attachment Protein Alpha (NAPA), MYC, RasGEF Domain Family Member 1A (RASGEF1A), Serine and arginine rich splicing factor 3 (SFRS3)* (Gopalakrishnan et al., 2024; Jarosova et al., 2016; Larson et al., 2020).

1.9.4 Atypical hits in DLBCL

Apart from DHL and THL cases in DLBCL, atypical hits can be present in DLBCL. They are defined as cases that have alterations other than *MYC* rearrangements. Atypical-single hit (A-SH) refers to cases with a single alteration of *BCL2* or *BCL6* or a *MYC* copy number variation (CNV). Atypical double hits (A-DH) are cases that have 2 alterations present other than a *MYC* rearrangement. This will include *BCL2* and *BCL6* alterations occurring simultaneously or *MYC* CNV co-rearranged with *BCL2* or *BCL6* alterations. Atypical-triple hits (A-TH) are described as cases that have CNV of *MYC* together with *BCL2* and *BCL6* alterations (Herrera et al., 2018; Huang et al., 2019). Patients who have these alterations seem to have a slightly worse prognosis than those without (Huang et al., 2019).

1.9.5 Other genetic subtypes

There are different studies which classify mutational profiles with the use of next-generation sequencing techniques. The following classification schemes as being proposed: LymphGen, Harvard and the Haematological Malignancy Research Network (HMRN), UK. All these subtypes have a different impact on patient prognosis, but it is still unclear how these classifications impact the treatment plan in practice. Table 1.3 outlines the classification schemes, their subtypes and further information on DLBCL, NOS mutational subtypes (Chapuy et al., 2018; Lacey et al., 2020; Runge et al., 2021; Schmitz et al., 2018; WHO classification of tumours editorial board, 2022; Wright et al., 2020).

Table 1.3: Proposed mutational subtypes of large B-cell lymphomas

HMRN, UK (Lacey et al., 2020; Runge et al., 2021)					
Subtype	Associated abnormalities	COO association	Comparison to other classifications	Similar lymphomas	Prognosis
MYD88	*MYD88, PIM1, CD79B, ETV6 CDKN2A*	Strong association non-GCB	MCD, C5	Extra-nodal lymphomas	Poor
BCL2	*EZH2, BCL2, CREBBP, TNFRSF14;* KMT2D	Strong association GCB-type	*EZB*, C3	Transformed FL	Favourable
SGK1	*SOCS1; CD83, SGK1,*	Predominant GCB	C4	Features of PMBCL	Favourable
NOTCH2	*NOTCH2, BCL10, TNFAIP3, CCND3, SPEN* and *CD70* mutations.	Predominantly non-GCB	C1 or BN2	MZL	Poor
LymphGen (Wright et al., 2020; Schmitz et al., 2018					
MCD	*MYD88* and *CD79B* mutations	Mostly post-germinal centre/non-GC	C5/MYD88	Extra-nodal lymphomas	Poor
BN2	*BCL6 and NOTCH2* mutations	Unclassified mostly	C1/NOTCH2	MZL	Intermediate
N1	*NOTCH1* mutations	Non-GC	NOTCH1	Richter's	Poor
EZB	*EZH2* mutations and *BCL2* R	Mostly GCB	C3, BCL2	FL	Intermediate
ST2	*SGK1* and *TET2*	Mostly GCB	C4, SGK1	HL	Favourable
A53	Aneuploidy and *TP53*	GCB and non-GCB	C2	None	Intermediate
NEC	*NOTCH1, REL* amplification, *TP53*	Mostly non-GCB	N1	Richter's	Poor

Harvard (Chapuy et al., 2018)					
Subtype	Associated abnormalities	COO association	Comparison to other classifications	Similar lymphomas	Prognosis
C0	No detectable drivers	Unclassified		T-cell or histocyte-rich LBCLs	Favourable
C1	*BCL6* rearrangement, *NOTCH*2 mutations	Mostly non-GCB	*NOTCH2*/ BN2	MZL	Favourable
C2	*TP53* and *CDKN2A* alterations	GCB and non-GCB	A53	N/A	Intermediate
C3	*BCL2 R, PTEN, KMT2D, EZH2* aberrations and *CREBBP*	Mostly GCB	BCL2 and EZB	FL, DLBCL-GCB	Intermediate and poor in GCB
C4	With BCR/PI3K, JAK/STAT and BRAF pathway alterations	Mostly GCB	ST2/SGK1	FL, DLBCL-GCB	Favourable
C5	N/A	Mostly GCB	MCD/MYD88	Extra-nodal lymphomas	Intermediate in non-GC

Key: CDKN2A= Cyclin-dependent kinase inhibitor 2A; PI3K= phosphoinositide 3-kinase; *TNFAIP3=* Tumour necrosis factor, alpha-induced protein 3, CCND3= Cyclin D3, CD70= Cluster of differentiation 70; R= rearrangement; SGK1= serum/glucocorticoid regulated kinase 1; TET2= Tet methylcytosine dioxygenase 2; ETV6= ETS Variant Transcription Factor 6 , *TNFRSF14=* TNF Receptor Superfamily Member 14, SOCS1= Suppressor of cytokine signalling 1, N/A= not applicable

1.10 Treatment

DLBCL subtypes differ significantly in their clinical and treatment outcome. Treatment depends on the clinical risk factor and the stage of the disease. The disease can be classified as a limited-stage or advanced-stage disease (Willenbacher et al., 2020). The chemotherapy regimens administered in DLBCL include CHOP [cyclophosphamide, doxorubicin (adriamycin), vincristine, and prednisone]. CHOP is given to patients with early-stage (stage IA or IIA non-bulky) DLBCL with 3-4 cycles together with radiation therapy of 6 cycles (Mamgain et al., 2022). While R-CHOP is administered to patients with advanced-stage DLBCL. It is currently the most widely used treatment for DLBCL. Approximately 60-70% of patients are cured using this therapy. The addition of Rituximab is an anti-CD20 therapy

used in cases that show CD20 expression. Although outcomes look good 30-40% of patients have a relapse when treated with R-CHOP (Li et al., 2018).

In the case of HGBCL-DHL, EPOCH (etoposide, prednisone, vincristine [oncovin], cyclophosphamide, doxorubicin) together with Rituximab, is suggested for the treatment due to a high relapse rate and poor survival (Mamgain et al., 2022). When patients relapse the salvage chemotherapy regimens used include DHAP (dexamethasone, high-dose cytarabine, and cisplatin), ESHAP (etoposide, methylprednisolone, high-dose cytarabine, and cisplatin), MIME (mesna, ifosfamide, methotrexate, and etoposide) and IMVP-16 (ifosfamide, methotrexate, and etoposide) (Herbst, 2021; Li et al., 2018)

Patients with HIV usually receive highly active antiretroviral therapy (HAART) treatment before or after their lymphoma diagnosis (Magangane et al., 2020). There are multiple attempts to improve this therapy, but no improved patient outcomes have been noted (Swerdlow et al., 2017). Supportive care is included for patients with a combination of antifungals, antibiotics growth factors, and anti-retroviral therapy. Although a lot of progress has been made, the general response to therapy is lower in patients with HIV. The management of HAART is important in the management of DLBCL (Patel et al., 2015). Stem cell transplantation and high-dose salvage chemotherapy are given to patients who are HIV-negative with a poor response to CHOP and relapsed patients. Palliative radiotherapy and/or chemotherapy are given to patients with HIV with refractory disease or who have relapsed (Magangane et al., 2020).

The advancement of molecular classification and clinical trials in DLBCL has revealed multiple treatment strategies targeting different subsets of the disease like the tumour type, COO as well as genetic events and pathways. According to studies, these new treatment opportunities could in the future replace chemotherapy. These therapeutic targets include BCL6 inhibitors, BCL2/MYC inhibitors Toll-Like receptor inhibition, NF-*k*B inhibition as well as immune checkpoint inhibitors (Tomas-Roca et al., 2021).

1. 11 Prognosis

The prognosis of each case is influenced by several factors like the patient's age, the stage of the disease and the tumour's immunophenotypic and molecular profile.

1.11.1 Clinical factors and immune status

Patients with DLBCL older than 60 years have a worse overall prognosis (Chen et al., 2019a). Other factors that influence prognosis, include the immune status of a patient or infection with a virus. As discussed earlier HIV seems to play the biggest role and has a poor overall prognosis. EBV-positive DLBCL is usually seen in older patients and the prognosis varies (Sehn and Salles, 2021). In a South African study by Magangane et al., HIV-positive patients with DLBCL had a significantly poorer survival and prognosis, but this outcome seems to be influenced by the treatment of patients and whether HAART was used for each patient (Magangane et al., 2020).

1.11.2 Immunophenotype

Patients with DEL profile are more likely to have a poor prognosis and poor performance status and response to treatment with traditional R-CHOP therapy. When DELs are compared to those without such abnormalities, or with only one of the proteins being expressed, an inferior prognosis was observed (Chen et al., 2023a; King and Lam, 2020; Riedell and Smith, 2018). In approximately 30% of DLBCL NOS, patients with overexpression of BCL2 and MYC had a poorer prognosis, but overall, patients with DEL had a more favourable prognosis compared to those with DHL (Li et al., 2018). Patients with only BCL2 expression also had a poorer prognosis than those with MYC expression alone (Sehn and Salles, 2021).

1.11.3 Cell of origin

Patients with the non-GCB subtype DLBCL have a worse prognosis than those with the GCB subtype (Liu and Barta, 2019). The non-GCB subtype is associated with a high risk of relapse to the CNS (Sehn and Salles, 2021).

1.11.4 Molecular profile

In the overall setting, patients with rearrangements and genetic alterations have a worse prognosis than those who do not. The presence of a *MYC* rearrangement according to several studies compared to other *MYC* counterparts has a worse prognosis and an inferior overall survival (OS) rate when treated with R-CHOP. When *MYC* has an IG rearrangement partner a poorer prognosis and OS are noted compared to non-IG partners (Liu and Barta, 2019; Rosenwald et al., 2019). In SHL the prognosis

is still inconclusive due to variable morphology and the presence or absence of gene CNV noted in some cases (Chen et al., 2019a).

The co-occurrence of *BCL2* and *BCL6* rearrangements (HGBCL, DHL/THL) in cases when treated with R-CHOP is much worse than DEL and DLBCL, NOS. Patients experience advanced-stage disease and are usually much older. The median OS is 1.5 years and disease progression, and relapse are frequent (Chen et al., 2019a; Rosenwald et al., 2019). Compared to DLBCL, NOS the prognosis of HGBCL, NOS is worse, however when compared to DHL a better prognosis is forecasted in some studies and other studies suggest a similar outcome, especially in cases with single-hit *MYC* rearrangements. Treatment with R-CHOP is dismal with relapse and short OS with a low response rate to salvage chemotherapy. Other treatment strategies should be considered. The presence of CNV together with another gene rearrangement has shown in some studies to have a poor prognosis or a similar prognosis to cases that have a DHL. These results are still variable in some studies and need more investigation (Chen et al., 2019a; Zayac et al., 2023).

1.12 Methods used in the clinical practice to determine chromosomal alterations in DLBCL

There are several methods available to test for rearrangements and CNV in DLBCL. These include fluorescence *in-situ* hybridisation (FISH), next-generation sequencing (NGS), multiplex ligation-dependent probe amplification (MLPA) and comparative genomic hybridisation (CGH) (Larson et al., 2020).

1.12.1 Fluorescence *in-situ* hybridisation (FISH)

FISH is a method which can detect chromosomal abnormalities (rearrangement, amplification, deletion, or gain). Specifically, interphase FISH is performed on FFPE tissue mounted on a glass slide. The specimen DNA is denatured to its single-stranded form and then allowed to hybridise with the fluorescently labelled probes. These hybridised probes are then visualised with the aid of a fluorescence microscope fitted with the appropriate excitation and emission filters. Two different probes can be employed, a dual-fusion probe (DFP) and a break-apart probe (BAP) (Chu et al., 2019; Garimberti and Tosi, 2010). Each probe in a pair of DFP is labelled with a different fluorophore and binds to a different chromosomal region. DFPs are created to extend the rearrangement breakpoint regions in the two separate genes participating in a reciprocal rearrangement, as opposed to BAP, which is situated near the rearrangement breakpoint and is less sensitive (Figure 1.6).

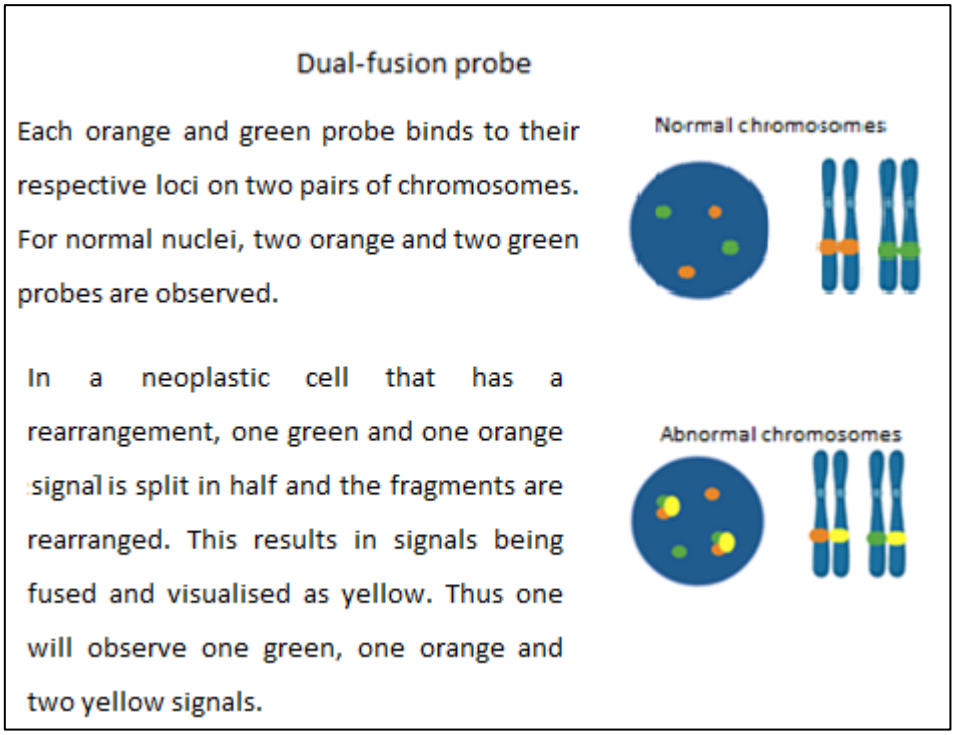

Figure 1.6: Anticipated FISH hybridisation pattern in DFPs. Normal intact signal (top) and abnormal (bottom) (Adapted from Ventura et al., 2006)

BAPs are made up of pairs of two distinct probes that are each labelled a different colour. In a normal diploid cell, two sets of orange/green fused signals will be observed since each binds to sequences around the identified breakpoint region in a locus or gene of interest. Orange and green signals will be detected in addition to the typical fused signal in a neoplastic cell where one allele has been divided by a rearrangement, hence the term "break-apart" (Figure 1.7) (Ventura et al., 2006).

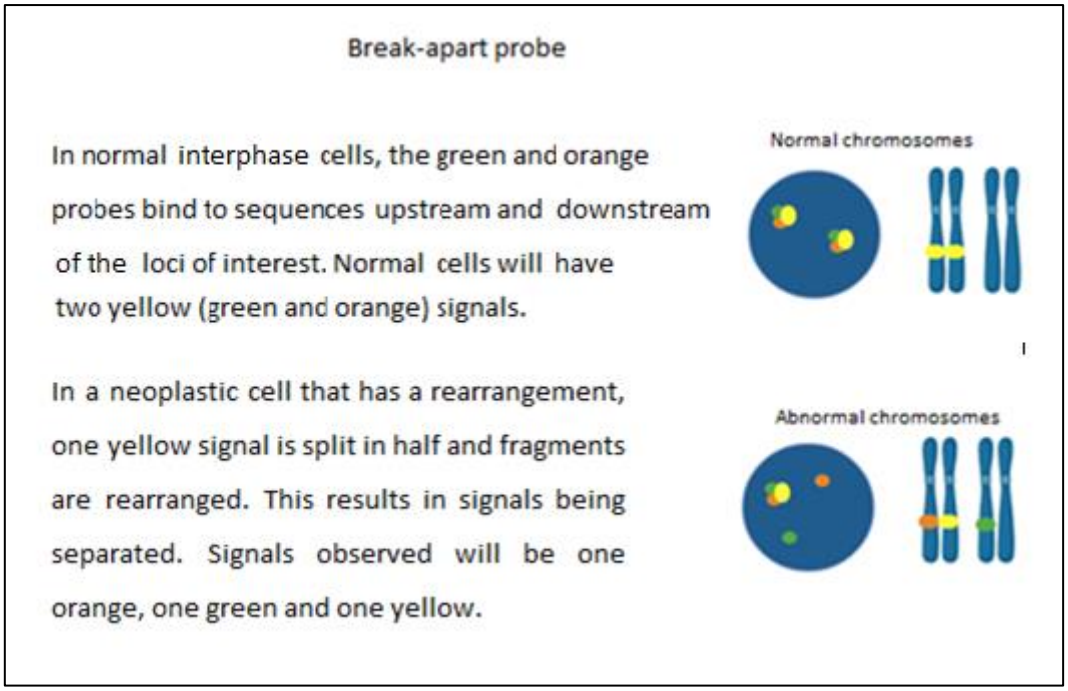

Figure 1.7: Anticipated FISH hybridisation pattern in BAP. Normal intact signal (top) and abnormal (bottom) (Adapted from Ventura et al., 2006)

FISH is the gold standard method in the clinical setting to detect genetic alterations. Interphase FISH, has great sensitivity and specificity and can be tested on formalin-fixed, paraffin-embedded (FFPE) specimens (Larson et al., 2020). The drawbacks of FISH include the need for fluorescence microscopy and skilled personnel to interpret results. Other challenges include signal fading, background autofluorescence, and the potential for issues with morphological characteristics like the presence of non-neoplastic cells, tissue architecture, necrosis, and cellularity (Garimberti and Tosi, 2010, Ventura et al., 2006). The use of *MYC, BCL2* and *BCL6* FISH is important as 10% of DLBCL, has either DH or TH. Patients with DHL or THL have a better outcome when therapy is adjusted for them according to this diagnosis rather than the standard R-CHOP treatment. Some challenges arise due to the cost of FISH in some clinical settings. This affects the decision on which cases should get tested (Pavlovsky, 2019). Not all DLBCL cases can be tested for *MYC, BCL2* and *BCL6* rearrangements so some guidelines have been proposed. An algorithmic approach is proposed that tests cases initially for the *MYC* rearrangement. If there is a *MYC* rearrangement, *BCL2* and *BCL6* reflex testing will be done (Larson et al., 2020). The Royal College of Pathologists (UK) have proposed that GCB origin cases be tested for *MYC* rearrangements and if present it is followed by *BCL2* and *BCL6* FISH. The College of American Pathologists (CAP) has a similar approach but suggests that when MYC protein is positive (70% cutoff

value), the possibility of *MYC* rearrangement is possible and these cases should be sent for *MYC* FISH followed by *BCL2* and *BCL6* FISH (Ta et al., 2022).

1.12.2 Next-generation sequencing (NGS)

NGS technology is employed to perform millions of multiple independent sequencing reactions in a short period. Results are captured as individual sequences of multiple DNA targets. Sequencing can be performed on a whole genome, exome, or a targeted gene panel. With NGS, mutations, DNA copy number variations and rearrangements can be detected (Hastings et al., 2016; Qin, 2019; Xu-Monette et al., 2020). The method comprised the fragmentation of DNA, library preparation, massively parallel sequencing, and analysis with bioinformatics for variant or mutation annotation and interpretation (Qin, 2019). There are currently several commercial and custom in-house created NGS panels for lymphomas that are used in some clinical settings. The majority of these are amplicon-based and concentrated on certain gene alterations that are often mutated in lymphomas (Mansouri et al., 2022). Multiple DNA sample sources can be used for sequencing which includes small amounts of frozen, fixed, FFPE or fine needle aspiration samples (Hastings et al., 2016). Complex genetic events such as rearrangements and gene fusions, include a wide range of genetic partners and involve highly variable breakpoints. Due to the variability of these breakpoints, large genomic regions need to be tested, leading to increased costs. Unfortunately, due to this high cost, it is not feasible to introduce this technique in the routine clinical setting. It has been suggested that smaller alternative-focused sequencing strategies should be used but, it still needs prior knowledge of which fusion should be analysed (Solomon and Arcila, 2020). Other limitations of NGS include a limited frequency of allele variant detection, test result interpretation is challenging and there is an uncertainty of the use of results in the diagnosis and classification of DLBCL (Sun and Pfeifer, 2019).

1.12.3 Comparative genomic hybridisation (CGH)

This is a molecular technique that can be used to detect copy number changes in tumour cells. It uses genome scanning methods with a high-density oligonucleotide array to compare the tumour's extracted DNA to a commercially available pooled control of normal DNA (Cai et al., 2015; Gunn et al., 2007). The principle is based on FISH. Two DNA samples, one the sample being tested and the second control sample, are labelled with fluorochromes. Two different colour fluorochromes are used for the samples, respectively. The two samples are then hybridised together, and the results are imaged with a fluorescence microscope, karyotyped, and then computerised. The fluorescence intensities are

analysed by the computer and the values are arranged in a CGH profile. The CGH profile is expressed in a curve with the ratio of fluorochrome intensities over each chromosome (Das, 2020). Almost any kind of clinical specimen can be used including blood and FFPE, but fresh or frozen tissue is preferred (Das, 2020; Weiss et al., 1999). This method is used widely as cell culturing is not needed for this method. Compared to FISH, CGH is a more advanced method as it is not limited to the resolution of the microscope (Das, 2020). CGH can be time-consuming with several limitations like the inability to detect balanced chromosomal alterations and reduced sensitivity compared to other methods like PCR. Sensitivity can be influenced by the contamination of normal cells among tumour cells (Gunn et al., 2007).

1.12.4 Multiplex ligation-dependent probe amplification (MLPA)

The MLPA method uses multiple probes to detect copy numbers of DNA sequences. These probes are designed with a 5' and a 3' half probe with a unique specific target sequence. Amplification with PCR of all the probes can occur simultaneously. The first step in MLPA is the denaturation and fragmentation of the sample DNA. This is followed by the hybridisation of the MLPA probe and the denatured DNA. A ligase enzyme is added which connects the probe and is then amplified with PCR. The two half probes can only be ligated and amplified when a perfect match without a single gap after hybridisation is present. The PCR product is separated by electrophoresis or with a high-resolution capillary electrophoresis-based single-strand conformation polymorphism system, and visualised (Fu et al., 2022).

MLPA can identify imbalances and CNVs of certain loci available in the reaction kit. Only a small amount of tumour DNA is needed to perform this analysis. This approach is sensitive, has a short processing time, and is cost-effective because it does not require a dedicated platform making it easy to perform (Hastings et al., 2016; Solomon and Arcila, 2020; Xu-Monette et al., 2020,). The downfall of MLPA is that regions tested in a sample are kit-dependent, which results in balanced rearrangements not being detected. Inaccuracy may occur due to genomic instability with the tumour having multiple genetic alterations and the possibility of normal DNA contamination (Hastings et al., 2016). The pitfall of this method is that there are a lot of pre-analytical factors that can affect results. The quality of the tissue processing, fixation conditions and the effect of decalcification solutions all have an impact on the sensitivity of the PCR (Sun and Pfeifer, 2019).

1.13 Rationale for this study

DLBCL is a high-grade, aggressive, and clinically heterogeneous tumour. Despite intensive therapy a subset of patients still have poor outcomes. The investigation of the tumour's spectrum of *MYC, BCL2* and *BCL6* alterations by FISH allows classification according to the most recent WHO guidelines. This also identifies high-risk subgroups which clinicians can treat accordingly. The current diagnostic workflow in our centre does not include FISH as part of the routine workup of lymphomas. This is the first study to investigate the genetic abnormalities of *MYC, BCL2* and *BCL6* in a cohort of DLBCLs diagnosed at Groote Schuur Hospital.

1.14 Aims and Objectives

1. To determine the profile of *MYC, BCL2* and *BCL6* genetic abnormalities in cases of DLBCL diagnosed at Groote Schuur Hospital by using fluorescence *in-situ* hybridisation.
2. To correlate the data generated with histopathological parameters and patient demographics using simple statistical tests.

CHAPTER 2: Materials and methods

2.1 Ethics approval

This study was granted scientific approval by the Department of Pathology Research Committee, Faculty of Health Sciences, University of Cape Town. Thereafter, the protocol was approved by the Human Research Ethics Committee (HREC) of the Faculty of Health Sciences, University of Cape Town (HREC Ref Number: 739/21) (Appendix 1). Ethics was renewed annually.

2.2 Funding

The project was funded by the NHLS Research Trust Grant as well as the Medical Research Council (MRC) Self-Initiated Research Grant. Funding was used to buy reagents and consumables for the project.

2.3 Sample selection

Cases diagnosed as 'DLBCL' at Groote Schuur Hospital (GSH) Division of Anatomical Pathology between 2015 and 2021 were requested from the National Health Laboratory Service (NHLS) by applying to the Academic Affairs and Research Management System (AARMS). The case data was retrieved from the NHLS Corporate Data Warehouse database. The IHC results (CD10, CD20, BCL2, BCL6, MUM1, and Ki-67) and EBER-ISH were extracted from the histopathology reports. The MYC IHC is not routinely done and results for some cases were extracted from a previous study by Cassim et al (Cassiem et al., 2020). FFPE and slides were retrieved from the archives. The slides were reviewed with the supervising pathologist and medical scientist, and the diagnosis was confirmed. Cases showing poor fixation or not enough tumour tissue were excluded. Patient demographics were recorded, in addition to all diagnostic IHC and ISH results. This information was collated into a Microsoft Excel spreadsheet and patient identifiers were removed to ensure patient confidentiality. The cases were then assigned a randomised study number. All study information and materials were securely stored for the duration of the study.

2.4 Sample size calculation

The sample size required was calculated with a sample size calculation:

$$\frac{Z^2 p(1-p)}{d^2}$$

Z^2= Standard normal variate

p= Expected proportion in population based on previous studies

d= Absolute error or precisio

2.5 Immunohistochemistry

Previous IHC tests were done in the IHC laboratory at the NHLS division of Anatomical Pathology GSH. Each test was either done on a Ventana XT or Benchmark Ultra autostainer (Roche Diagnostics, Basel,Switzerland). Each antibody and its manufacturer and cutoff values are described in Table 2.1.

Table 2.1: Immunohistochemistry marker

Antibody (clone)	Manufacturer	Cutoff value and staining
CD20cy (Clone L26)	DAKO/Agilent, Santa Clara, CA, USA	Diffusely positive membranous staining
BCL2 (Clone 124)	DAKO/Agilent, Santa Clara, CA, USA	50% cytoplasmic staining
BCL6 (PG-B6p)	DAKO/Agilent, Santa Clara, CA, USA	40% nuclear staining
CD10 (SP67)	Ventana (Roche Diagnostics, Basel, Switzerland)	40% membranous staining
MUM1 (clone MUM1p)	DAKO/Agilent, Santa Clara, CA, USA	40% nuclear staining
c-MYC (Y69)	Ventana (Roche Diagnostics, Basel, Switzerland)	40%
KI-67 (Clone MIB-1)	DAKO/Agilent, Santa Clara, CA, USA	70-90% nuclear staining
INFORM EBER probe	Ventana (Roche Diagnostics, Basel, Switzerland)	50% nuclear staining

2.6 Fluorescence *in-situ* hybridisation (FISH)

The FISH was processed by the student and the analysis was done by the principle investigator.

2.6.1 Preparation of slides

For each FISH test, an area measuring 5mmx5mm of the tumour was identified on the H&E slide. Areas showing crush artefact, haemorrhage and necrosis were avoided. Sections of 4µm were cut with a Leica rotary microtome RM2125 RTS (Leica Biosystems Inc GmbH, Wetzlar, Germany), and mounted on HISTOBOND® (Paul Marienfeld GmbH& Co, Lauda-Königshofen, Germany) slides. The slides were dried overnight in an incubator (Memmert GmbH, Schwabach, Germany) at 37°C. The next day the slides were dewaxed in 3 changes of xylene, for 5 minutes in each and subsequently with 3 changes of graded alcohol concentrations of 70%, 85% and 100% (Appendix 2) for 2 minutes in each. Thereafter the slides were treated with 0.2N hydrochloric acid (HCl) (Merck KGaA, Darmstadt, Germany) (Appendix 2) at 37°C for 20 minutes. The slides were then washed in distilled water for 5 minutes. Antigen retrieval was performed in a waterbath at 80°C using 1M sodium thiocyanate (Merck KGaA, Darmstadt, Germany) (Appendix 2) for 30 minutes. The slides were then washed with distilled water for 5 minutes. Depending on the tissue type, tissue sections were digested in a 0.05% pepsin/0.01N HCL (Merck KGaA, Darmstadt, Germany) at 37°C in a waterbath for between 16 to 34 minutes. After the desired digestion was reached the slides were washed in distilled water for 2 minutes. Using grading alcohols, the slides were dehydrated for 2 minutes in each and left to dry in a darkened cupboard. After the slides dried completely the probes (Abbott Laboratories, Illinois, USA.) *MYC* (Figure 2.1), *BCL6* (Figure 2.2), *BCL2* (Figure 2.3) or *IGH/MYC*/CEP 8 (Figure 2.4) was prepared (Appendix 2). The probe mix (5µl) was applied to the sections. The sections were coverslipped and the edges were sealed using Fixogum rubber cement (Marabu GmbH & Co. KG Bietigheim-Bissingen, Germany). The slides were put on the hybridiser (DAKO Agilent Technologies, Glostrup, Denmark) and denaturation at 73°C for 5 minutes followed by hybridisation at 37°C for 24 hours. When the hybridisation was completed, slides were washed in a post-hybridisation wash buffer (Appendix 2) at room temperature for 2 minutes and then washed with post-hybridisation wash buffer at 74°C in a waterbath for 2 minutes and 10 seconds. The slides were then allowed to dry in a dark cupboard. When completely dried, the slides were mounted with 5µl 4',6-Diamidino-2-phenylindole (DAPI) II (Abbott Laboratories, Illinois, USA). The coverslip's edges were sealed with clear nail polish and allowed to dry in a dark cupboard before analysis. The positive control for each probe was a previously tested DLBCL case while a reactive lymph node served as the negative control. Several parameters such as the appropriate pre-treatment procedure and digestion time were optimised.

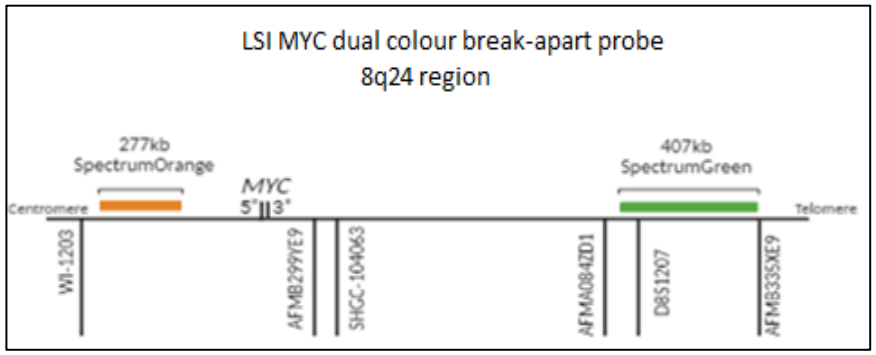

Figure 2.1: Diagrammatic representation of the *MYC* BAP map (Adapted from Abbott Laboratories, Illinois, USA)

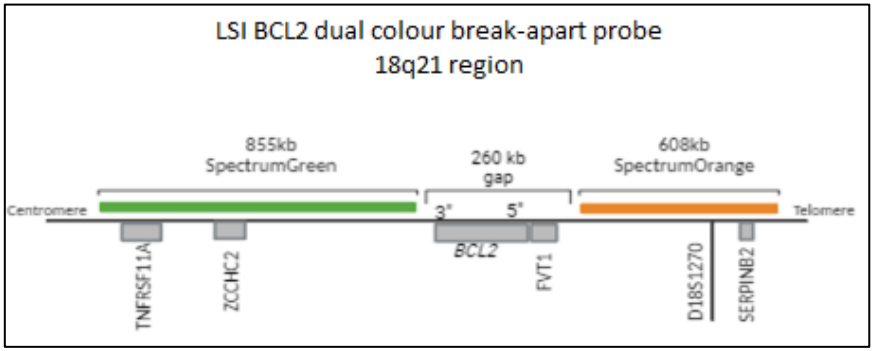

Figure 2.2: Diagrammatic representation of *BCL6* BAP map (Adapted from Abbott Laboratories, Illinois, USA)

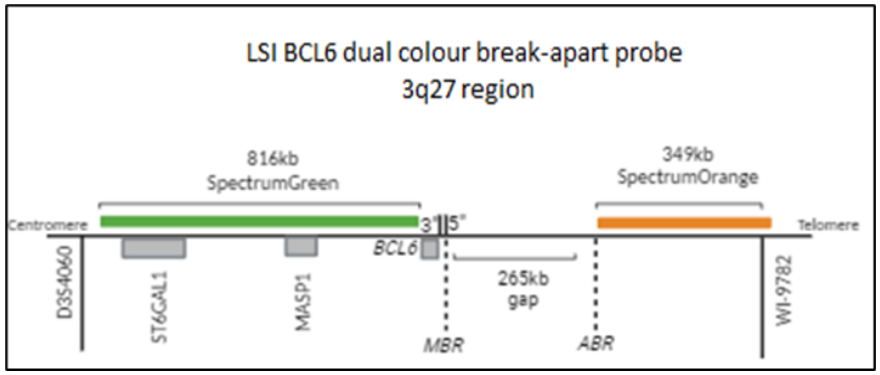

Figure 2.3: Diagrammatic representation of the *BCL2* BAP map (Adapted from Abbott Laboratories, Illinois, USA)

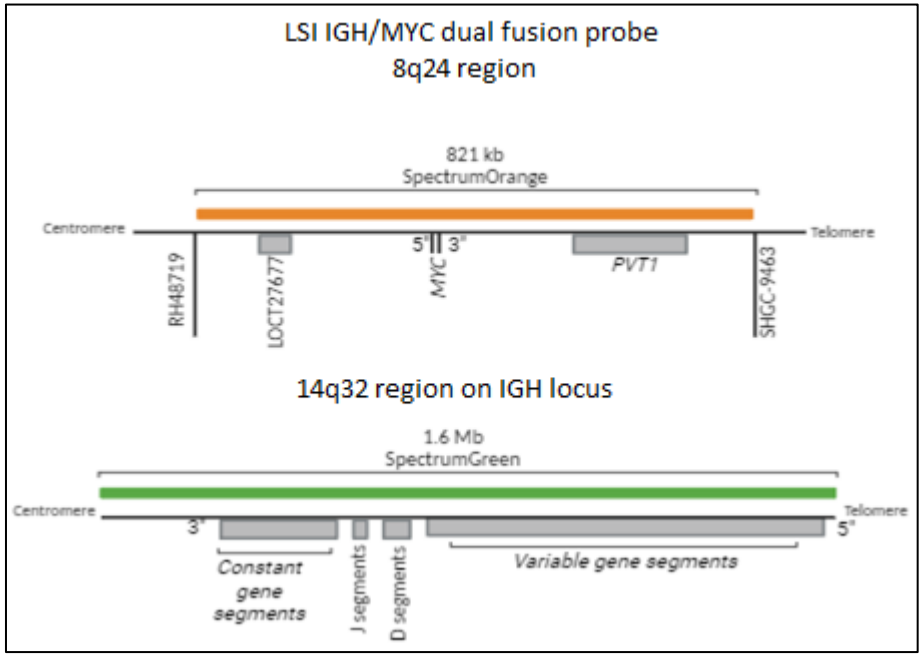

Figure 2.4: Diagrammatic representation of the *IGH/MYC*/CEP 8 FISH probe map (Adapted from Abbott Laboratories, Illinois, USA)

2.6.2 Analysis and interpretation of FISH

The slides were viewed using a Zeiss Axioskop 40FL fluorescent microscope, HBO 100 (Carl Zeiss AG, Oberkochen, Germany), which has the appropriate excitation and emission filters (Carl Zeiss AG, Oberkochen, Germany). The images were captured using AxioVision SE64 Rel.4.9.1 software (Carl Zeiss AG, Oberkochen, Germany). Single images using each filter (DAPI, spectrum orange and spectrum green) were captured and overlayed to produce the triple pass image. The signals were enumerated on a minimum of 200 non-overlapping tumour nuclei. Scoring was recorded on a FISH scoring template for each case and probe. The scoring criteria were as follows: Using the BAP, no rearrangement was observed by <5% of cells showing 1-O, 1-G and 1-O/G signal pattern while rearrangement was observed by ≥5% of cells showing 1-O, 1-G, and 1-O/G signal pattern out of 200 cells. Gain was observed by the presence of 3-4 O/G signals, while amplification was identified by >4 O/G signals. With the DFP, no rearrangement was observed by <5% of cells with 1-O, 1-G and 2-O/G signals while rearrangement was observed by ≥5% of cells with 1-O, 1-G, and 2-O/G fusion signals.

2.7 Data and Statistical Analysis

During data analysis for each FISH probe, gains and amplifications were first grouped as CNV then rearrangements and CNV were grouped as aberrations. Cases were considered as DHL when both *MYC* and *BCL2* rearrangements were present, and THL when *MYC*, *BCL2* and *BCL6* rearrangements were present. Atypical hits were considered as A-SH when cases had a single aberration of *BCL2* or *BCL6* or a *MYC* CNV. A-DH were cases with two aberrations present other than a *MYC* rearrangement. A-TH were cases that have *MYC* CNV together with *BCL2* and *BCL6* aberrations.

The demographic and histopathology data were reported as frequencies or medians. The FISH data together with histopathology data was compared using the Chi-squared test or Fisher's exact test. Depending on the sample size, the latter were used. Results were considered significant when the P value was less than 0.05. The Statistical Package for Social Sciences (SPSS) v.23.0 (SPSS, Inc., Chicago, IL) was used for the statistical analysis.

CHAPTER 3: Results

3.1 Case selection

The Corporate Data Warehouse provided data for cases diagnosed as DLBCL at GSH from 01-01-2015 to 31-12-2021. The previously stained slides from 350 cases that met the inclusion criteria were retrieved from the archives. The diagnosis of each case was reviewed and confirmed. Thereafter, the paraffin blocks were retrieved. A total of 75 cases had enough tissue for further study. The immunohistochemical and molecular data extracted from the histopathology reports and generated by this study, respectively appear in Appendix 3. The sample size was calculated using the standard error of 5% and the precision error of 5%. The expected proportion of 10% was used, which gave a sample size of 49 cases.

3.2 Patient demographic data

The cohort comprised 44/75 (58.7%) males and 31/75 (41.3%) females (Table 3.1). The age range was between 18 and 83 years while the median age was 48 years. Almost half of the cohort (38/75; 50.7%) was between 41 and 60 years (Figure 3.1). The HIV status of 72 patients was known; 30/72 (41.7%) were HIV positive and 42/72 (58.3%) were HIV negative. The biopsy site was extra-nodal in 38/75 (50.7%) cases and the lymph node in 37/75 (49.3%) of cases. Of the extra-nodal sites, the most common were the ear, nose, and throat area (11/75; 14.7%) followed by skin (8/75; 10.7%) (Table 3.1).

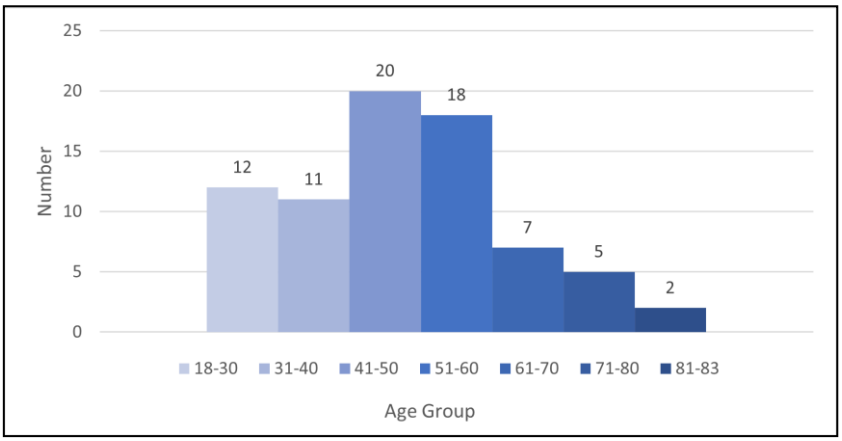

Figure 3.1: Patient age range summary

Table 3.1: Summary of patient demographic data

Patient demographic		**Total**
Sex	Male	44
	Female	31
Age (years)	Range	18-83
	Median	48
HIV status	Positive	30
	Negative	42
	Unknown	3
Site of biopsy	Nodal	37
	Extra-nodal	38

3.3 Immunophenotype, *in-situ* hybridisation (ISH) and the Hans algorithm classification

CD20 was positive in all cases (75/75; 100.0%). CD10 was positive in 36/73 (49.3%) cases and negative in 37/73 (50.7%) cases while BCL6 was positive in 55/75 (73.3%) cases and negative in 20/75 (26.7%) cases. BCL2 was positive in 46/73 (63.0%) cases and negative in 27/73 (36.9%) cases. MYC was negative in 30/37 (81.1%) cases and positive in 7/37 (18.9%) cases. Double expression (DE) of MYC and BCL2

was observed in 4/7 (57.1%) of cases. Due to the small number of these cases, the DE phenotype was not explored further. MUM1 was positive in 46/66 (69.7%) cases and negative in 20/66 (30.3%) cases. Lastly, EBER-ISH was negative in 50/54 (92.6%) cases and positive in 4/54 (7.4%) cases. COO determination using the Hans algorithm resulted in 42/75 (56.0%) cases classified as GCB subtype and 33/75 (44.0%) cases were non-GCB subtype (Table 3.2).

Table 3.2: Summary of immunohistochemistry, *in-situ* hybridisation, and cell of origin data

Immunohistochemistry	**Positivity**
CD20	75/75 (100.0%)
CD10	36/73 (49.3%)
BCL6	55/75 (73.3%)
BCL2	46/73 (63.0%)
MYC	7/37 (18.9%)
MUM1	46/66 (69.7%)
***In-situ* hybridisation**	
EBER-*ISH*	4/54 (7.4%)
Cell of origin	
Non-GCB	33/75 (44.0%)
GCB	42/75 (56.0%)

3.4 FISH verification

To verify the probes used in this study, previously tested DLBCLs with *MYC, BCL*2 and *BCL6* rearrangements served as the positive control while a reactive lymph node served as the negative control. The signal patterns observed in the controls were consistent with the manufacturer's information (Figure 3.2).

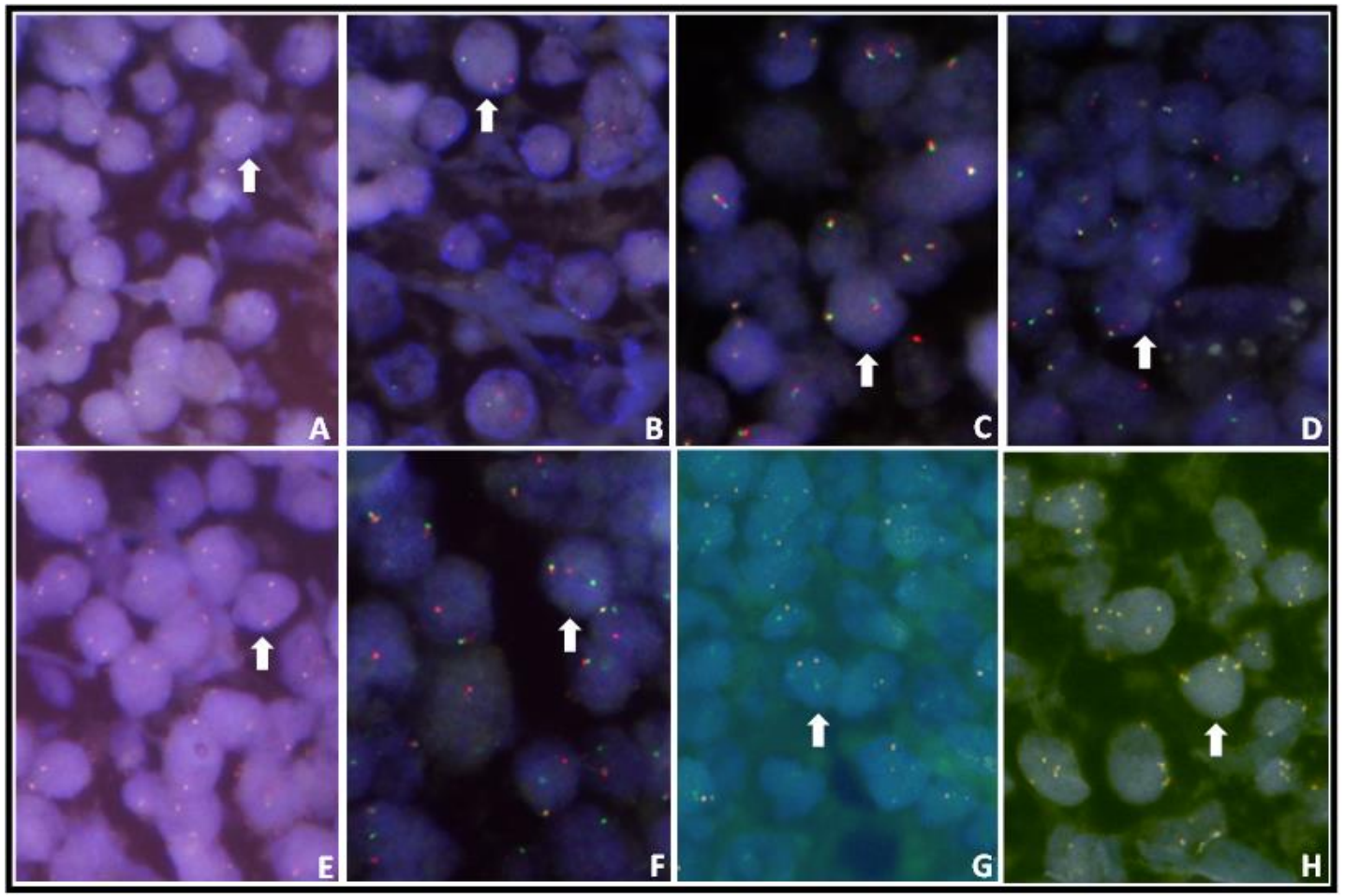

Figure 3.2: Triple pass overlayed images showing FISH results. A**:** *MYC* FISH with no rearrangement showing two yellow or fused orange/green signals (white arrow). B**:** *MYC* FISH *with* rearrangement showing one yellow or fused orange/green signal and one separate orange and green signal in the tumour cell nuclei (white arrow). C**:** *BCL2* FISH with no rearrangement showing two yellow or fused orange/green signals (white arrow). *D*: *BCL2* FISH with rearrangement showing one yellow or fused orange/green signal and one separate orange and green signal in the tumour cell nuclei (white arrow). E**:** *BCL6* FISH with no rearrangement showing two yellow or fused orange/green signals (white arrow). F: *BCL6* FISH *with* rearrange*ment* showing one yellow or fused orange/green signal and one separate orange and green signal in the tumour cell nuclei (white arrow). G: *MYC* DFP FISH with no rearrangement showing two orange and two green signals (white arrow). H: *MYC* DFP FISH with rearrangement showing two yellow fused signals and one orange and one green signal.

3.5 FISH results

Of the 75 cases tested, only 50/75 (66.7%) cases yielded a result for all 3 probes.

3.5.1. *MYC* FISH

Using the dual colour *MYC* BAP, 32/50 (64.0%) cases showed no rearrangement (Figure 3.2 A), 9/50 (18.0%) cases showed rearrangement (Figure 3.2 B), 8/50 (16.0%) cases showed gain and 1/50 (2.0%) case showed an amplification. In the cases with no rearrangement, the *MYC* DFP showed rearrangements in a further 4/32 (12.5%) cases. When the *MYC* BAP and *MYC* DFP results were combined, there were 28/50 (56.0%) cases with no rearrangement, 13/50 (26.0%) with rearrangement, 8/50 (16.0%) with gain and 1/50 (2.0%) with amplification. Nine (18.0%) cases showed CNV. Twenty-two (44.0%) cases showed aberrations and 28/50 (56.0%) cases showed no aberrations (Table 3.3). SHL cases were observed in 11/50 (22.0%) of cases.

3.5.2 *BCL2* FISH

The *BCL2* BAP yielded 38/50 (76.0%) cases with no rearrangements (Figure 3.2 C), 6/50 (12.0%) cases with rearrangement (Figure 3.2 D), 4/50 (8.0%) cases with gain and 2/50 (4.0%) cases with amplification. CNV was observed in 6/50 (12.0%) cases. No aberrations were observed in 38/50 (76.0%) cases and 12/50 (24.0%) had aberrations (Table 3.3).

3.5.3 *BCL6* FISH

BCL6 FISH yielded 29/50 (58.0%) cases with no rearrangements (Figure 3.2 E), 9/50 (18.0%) cases had rearrangements (Figure 3.2 F), 9/50 (18.0%) cases had gains and 3/50 (6.0%) cases had amplification. CNVs were observed in 12/50 (12.0%) cases. No aberrations were observed in 29/50 (58.0%) cases and 21/50 (42.0%) cases had aberrations (Table 3.3).

Table 3.3: Summary of FISH data

	***MYC* (n=50)**	***BCL2* (n=50)**	***BCL6* (n=50)**
No rearrangement	28 (56.0%)	38 (76.0%)	29 (58.0%)
Rearrangement	13 (26.0%)	6 (12.0%)	9 (18.0%)
Gain	8 (16.0%)	4 (8.0%)	9 (18.0%)
Amplified	1 (2.0%)	2 (4.0%)	3 (6.0%)
Copy number variation	9 (18.0%)	6 (12.0%)	12 (24.0%)
No aberration	28 (56.0%)	38 (76.0%)	29 (58.0%)
Aberration	22 (44.0%)	12 (24.0%)	21 (42.0%)

3.6 WHO classification 5th edition

Of the 50 cases, 45/50 (90.0%) were DLBCL, NOS, NOS (Table 3.4). There was one case (2.0%) which was DLBCL with *MYC* and *BCL2* rearrangement (DH), one case (2.0%) was DLBCL with rearrangement of *MYC, BCL2* and *BCL6* (TH) and 3 cases (6.0%) remained as EBV-positive DLBCL, NOS. The DHL and THL cases were all GCB subtypes, while the DLBCL, NOS cases comprised 26/45 (57.8%) GCB subtypes and 19/45 (42.2%) non-GCB subtypes. The EBV-positive DLBCL, NOS cases were all non-GCB subtype.

Table 3.4: Classification of cases

WHO classification	**Diagnosis**	**Number**
4th edition	DLBCL, NOS	71/75 (94.7%)
	EBV-positive DLBCL, NOS	4/75 (5.3%)
5th edition	DLBCL, NOS	45/50 (90.0%)
	DLBCL with *MYC* and *BCL2* rearrangements	1/50 (2.0%)
	DLBCL with rearrangement of *MYC, BCL2* and *BCL6*	1/50 (2.0%)
	EBV-positive DLBCL, NOS	3/50 (6.0%)

3.7 Atypical hits

A-SH was present in 9/50 (18.0%) cases, A-DH were in 9/50 (18.0%) cases, A-TH in 2/50 (4.0%) cases and no aberrations were present in 17/50 (34.0%) cases (Table 3.5).

Table 3.5: Atypical hits

Atypical hits	Total (n=50)
A-SH	9 (18.0%)
A-DH	9 (18.0%)
A-TH	2 (4.0%)
No aberrations	17 (34.0%)

3.8 Statistical correlations

3.8.1 HIV status versus cell of origin

Thirteen HIV-positive cases belonged to the GCB subtype compared to 4 cases that belonged to the non-GCB subtype (*P*= 0.028) (Table 3.6).

Table 3.6: Statistical correlation between HIV status and cell of origin subtype

Cell of origin subtype	**HIV status (n=47)**		**P Value**
	Positive	Negative	
Non-GCB	4	17	***P= 0.028***
GCB	13	13	

3.8.2 Immunohistochemistry data versus FISH data

All cases with *BCL2* rearrangement (n=5) showed BCL2 expression and while this was not significant (*P*=0.082 and *P*=0.093) when *BCL2* aberrations were considered, 10 cases showed BCL2 expression

while only 1 case was BCL2 negative (*P*=0.038) (Table 3.6). There were no significant correlations between *MYC* and *BCL6* aberrations and their IHC expression respectively (data not shown).

Table 3.7: Statistical correlations between BCL2 immunohistochemistry and *BCL2* FISH data

BCL2 FISH	**BCL2 immunohistochemistry (n=49)**		**P Value**
	Positive	Negative	
Rearrangement	5	0	*P*=0.093
Gain	4	0	
Amplifications	1	1	
No rearrangement	21	17	
Copy number variations	5	1	*P*=0.082
Rearrangement	5	0	
No rearrangement	21	17	
Aberrations	10	1	Fisher's exact test ***P=0.038***
No aberrations	21	17	

3.8.3 FISH data versus cell of origin

All cases with *BCL2* rearrangements (n=6) belonged to the GCB subtype (*P*=0.046) (Table 3.8). There were 10 cases with *BCL2* aberrations that were GCB subtypes compared to 2 non-GCB subtype cases (*P*=0.029). There were no significant correlations between *MYC* and *BCL6* aberrations and COO respectively (data not shown). There was a trend though that *MYC and BCL6* aberrations were also more commonly observed in the GCB subtype. *MYC* aberrations were observed in 15 cases with the GCB subtype compared to 7 cases that were non-GCB subtype (*P*=0.325) while for *BCL6*, 10 cases were from the GCB subtype and 11 cases from the non-GCB subtype (*P*=0.310).

Table 3.8: Statistical correlations between FISH data and COO

BCL2 FISH	**Cell of origin (n=50)**		***P* Value**
	non-GCB	GCB	
Rearrangement	0	6	*P*=0.058
Gain	2	2	
Amplifications	0	2	
No rearrangement	20	18	
Copy number variations	2	4	***P=0.046***
Rearrangement	0	6	
No rearrangement	20	18	
Aberrations	2	10	***P=0.029***
No aberrations	20	18	

CHAPTER 4: Discussion

Since the advent of next-generation sequencing technologies, the molecular profiling of tumours has advanced in great leaps. This has impacted the classification of tumours, and this is evident in the latest WHO classification of haematolymphoid tumours. Molecular profiling allows the identification of DLBCL subgroups which have poor prognoses and do not respond to standard treatment regimens. This is the first study at our centre to use the most recent revisions of the WHO classification (5th ed), to classify DLBCL cases. With the use of FISH, we identified 45 (90.0%) DLBCL, NOS cases, 1 case (2.0%) of DLBCL with rearrangements involving *MYC* and *BCL2* and 1 case (2.0%) of DLBCL with rearrangements involving *MYC, BCL2*, and *BCL6.* Three (6%) cases were EBV-positive DLBCL, NOS. The *MYC* gene was the most common genetic aberration, accounting for 44% of cases while *BCL6* aberrations and *BCL2* aberrations were found in 42% and 24% of cases, respectively. Our cohort had 41.7% HIV-positive cases, and more than half (66.0%) of the cases had an aberration present. Although not without some limitations, FISH proved to be a useful tool in the detection of molecular aberrations, especially in a diagnostic setting. Our findings highlight the importance of testing for these oncogenes, shedding light on the genetic heterogeneity of DLBCL.

4.1 Patient demographics

Our patient cohort consisted of 44 males and 31 females. This is in keeping with previous research which shows a slight male predominance in the adult population (Appendix 4) (Bellas et al., 2014; Cassidy et al., 2020; McPhail et al., 2018). Notably, the prognosis is typically poorer for men (Ting et al., 2019). With a median age of 48, the age distribution of our patient group varied from 18 to 83 years old. Although DLBCL usually affects people over the age of 30, our wide age range shows that this tumour is rather non-discriminate in the adult population. Since cases were selected randomly rather than consecutively, we acknowledge the possibility of selection bias in our findings. It is interesting to note that similar age ranges were observed by two more investigations (Appendix 4) which also reported similar age ranges of 18 to 86 years and 18 to 88 years, respectively (Bellas et al., 2014; Ting et al., 2019).

Extra-nodal involvement was present in 50.7% compared to 49.3% of nodal biopsies (49.3%). Our findings align with results from other studies with an almost equal representation of nodal and extra-

nodal tumour sites (Appendix 4) (Bellas et al., 2014; Chapman et al., 2021; Ting et al., 2019). One study showed that extra-nodal involvement was associated with a poorer overall prognosis, and certain sites pose a risk of central nervous system (CNS) involvement (Ollila and Olszewski, 2018). The ear, nose, and throat region emerged as the most biopsied extra-nodal site in our cohort (11/75; 14.7%). Ollila et al. suggest that when treated with rituximab-based chemoimmunotherapy, this specific site exhibits improved OS and a reduced risk of CNS involvement (Ollila and Olszewski, 2018).

The HIV pandemic in South Africa has led to an increase in NHLs, especially DLBCL (Morton et al., 2014) since there is a 60-to-200-fold increased risk of developing NHL among people living with HIV (Liapis et al., 2013). In our cohort, the distribution of HIV showed 41.7% of the cases being HIV-positive and 58.3% HIV-negative. HIV is known for its unique influence on the manifestation of DLBCL, by affecting cells through the disruption of CRS and SHM (Pather and Patel, 2022). According to other studies,including other South-African studies, the demographic profile of the HIV-positive population usually includes younger adults, which could explain our younger age range (Magangane et al., 2016; Pather and Patel, 2022; Vaughan et al., 2022). In our HIV-positive population, we observed a slight male predominance (10/17; 58.8%). In a Chinese study, comparing DLBCL characteristics in HIV-positive and HIV-negative patients, they found that the HIV-positive DLBCL population had different pathological features than those without (Zhou et al., 2022). DLBCL associated with immunosuppression and HIV had an immunoblastic morphology and was known to have dysregulation of the *MYC* gene. Immunoblastic features include plasmacytoid cells and cells which contain more than 90% of immunoblasts. DLBCL is an aggressive disease, the addition of immunosuppression due to HIV leads to an even worse possible prognosis and OS (Gloghini et al 2013; Zhou et al., 2022). A South African study found similar results where the HIV-positive population had a worse OS (Magangane et al., 2020). A study was done comparing the prognosis and response to treatment of HIV-positive and HIV-negative populations with DLBCL. They found that HIV-positive patients with *MYC* rearrangements had a worse outcome (Ramos et al., 2020).

4.2 Immunohistochemistry findings

With the use of previous IHC results, we recognised certain expression patterns of our cohort. Although some data was limited, DEL and COO subtypes were identified. These variables were later used for correlation with molecular results. There are currently no universally accepted IHC markers

that can serve as surrogate markers for the presence of *MYC*, *BCL2* and *BCL6* rearrangements (Cho et al, 2021; Ting et al., 2019).

4.2.1 MYC and BCL2 overexpression and DEL

MYC and BCL2 overexpression have been widely studied to be a diagnostic tool and prognostic indicator in DLBCL (Bellas et al., 2014; Khan et al., 2021; Willenbacher et al., 2020). In our cohort, MYC expression was observed in 18.9% (7/37) of cases, reflecting a limited availability of results, given that MYC IHC is not routinely done in the diagnostic workup of DLBCLs. In the literature, MYC expression ranges between 14-62% (Appendix 4) (Bellas et al., 2014; Chen et al., 2019b; Collinge et al., 2021; Huang et al., 2019, Khan et al., 2021, Naseem et al., 2020; Timlin et al., 2018; Willenbacher et al., 2020). Although our MYC expression was within the range, the expression is relatively low, given that *MYC* was our most rearranged gene. Cutoff values across studies did however vary (Bellas et al., 2014; Cassidy et al., 2020; Green et al., 2012; Kluk et al., 2012; Naseem et al., 2020). The 5th edition of WHO suggests a cutoff value of ≥40% to define MYC overexpression. One study suggested that when the expression of MYC was over 50%, the presence of an *MYC* rearrangement was evident (Kluk et al., 2012). However, Green et al, suggested an overexpression of ≥70% was suggestive of rearrangements (Green et al., 2012). Due to the lack of MYC IHC results we could not explore the relationship between MYC expression and *MYC* rearrangements. Only one out of 13 (7.7%) cases could be identified as having both a rearrangement and MYC expression. Of the other rearranged cases 3/13 (23.1%) had no MYC expression and 9/13 (69.2%) had no data available. The use of MYC expression to indicate *MYC* rearrangements seems to have different findings across studies. The prognostic significance of MYC overexpression and *MYC* rearrangements seems to be controversial. According to other studies, the presence of *MYC* rearrangements was known to have a poor prognostic impact on patients. They suggest that the early detection of these rearrangements can have a positive impact on patient treatment planning, especially in patients with SHL, DHL as well as THL (Salam et al., 2020; Wang., 2023c). Different reasons can contribute to these different findings. The sample sizes of these studies varied from as little as 81 cases (Salam et al, 2020) to as many as 2383 cases (Rosenwald et al., 2019). The studies that found a prognostic significance were the studies with smaller sample sizes (81 and 145 cases) compared to the studies that found no prognostic significance (2102 and 2383 cases) (Cho et al., 2021; Rosenwald et al., 2019; Salam et al., 2020; Wang, 2023c). Another study suggested that the reason *MYC* rearrangements were non-contributory to prognosis was that they did not consider SHL separately and focused on DHL and THL only (Cho et al., 2021). Case selection bias could also play a role in varied findings.

In our study, we observed BCL2 expression in 31/50 (63.0%) cases which align with the range reported in numerous studies of between 5-78% (Appendix 4) (Akyurek et al., 2012, Bellas et al., 2014, Huang et al., 2019, Chen et al., 2019b, Willenbacher et al., 2020, Khan et al., 2021, Naseem et al., 2020). The wide range points to several influences on IHC results. Again, across diagnostic settings and studies, different cutoff values are used ranging from 40-50% (Bellas et al., 2014; Naseem et al., 2020). Also, the use of different antibody clones and manufacturers plays a role in the sensitivity of the antibody.

Double expresser lymphomas were identified in 4/7 (57.1%) cases. While our sample size was limited, our findings slightly exceeded those reported in various studies where DEL prevalence ranged from 10-47%. (Appendix 4) (Bellas et al., 2014; Cassidy et al., 2020; Chen et al., 2019b; Herrera et al., 2018 ; Huang et al., 2019 ; Timlin et al., 2018; Willenbacher et al., 2020). One study suggested that the DEL subgroup included fast-proliferating tumours and was a possible reason for the poorer prognosis. A supporting study found that cases with DEL have a poorer prognosis than cases without a DEL profile and they usually have a non-GCB subtype (Abdelhamid et al., 2023; Hashmi et al., 2021; Wang et al., 2023a). A study by Bettelli et al showed that the presence of BCL2 expression in DEL was the contributing factor which led to a worse prognosis in patients (Bettelli et al., 2021). The recognition of DEL as an indicator of a worse prognosis underscores its potential utility in the diagnostic setting, influencing both diagnostic and therapeutic decision-making (Rho et al., 2022, Krull et al., 2020). Reflex testing in DLBCL cases is highly suggested, especially in tumours that have bulky mass DLBCL. The early detection of DEL could lead to swift treatment and prophylactic CNS treatment (Naseem et al., 2020; Wang et al., 2023b). Although DEL has prognostic implications it is not an indicator of rearrangements. Although rearranged cases often have an overexpression of MYC, BCL2 and BCL6 proteins, the reverse is not always true. Overexpression/expression can occur without rearrangements present (Blomme et al., 2024). In this study, 1 case with no BCL2 expression had *BCL2* rearrangement while 3 cases with *MYC* rearrangement showed no MYC expression while *BCL6* rearranged cases had 2 cases with no BCL6 IHC expression. There are a few possible explanations for the reason protein expression of MYC, BCL2 and BCL6 can occur in the absence of a rearrangement. DLBCL is a heterogeneous disease with multiple subtypes. Each subtype has its molecular characteristics and due to this, the protein expression might be different from subtype to subtype (Zhang et al., 2024). Another possibility is the technical aspects of IHC staining. Interpretation, detection level, different protocols across laboratories and fixation, affect the outcome of ICH results (Kim et al., 2016). Regulatory mechanisms can be affected when rearrangements occur which affects protein production. These include miRNAs which can influence protein production (Getaneh et al., 2019).

4.2.2 CD10, BCL6, MUM1 and the Hans algorithm classification

Cell of origin subtypes were almost equal with a slight presence of the GCB subtype (56.0%) as opposed to the non-GCB subtype (44.0%). In the literature, there appear to be slightly more non-GCB subgroup cases which ranged from 25.8-91% (Appendix 4), while non-GCB subtypes ranged from 6-74.2% (Bellas et al., 2014; Chapman et al., 2021; Cassidy et al., 2020; Chen et al., 2019b; Huang et al., 2019; Khan et al., 2021; McPhail et al., 2018; Mehta et al., 2020; Oliveira et al., 2017; Timlin et al., 2018; Tsai et al., 2021; Zhang et al., 2018). Five of the 13 studies had COO findings that were similar to our study (Bellas et al., 2014; Chapman et al., 2021; Mehta et al., 2020; Oliveira et al., 2017; Tsai et al., 2021). Different results in the COO subtypes can be due to factors like the IHC methodology/classifier used and patient demographics (Liu and Barta, 2019). A recent study found that the COO subtype in the same tumour can change due to disease progression. In the event of therapies being COO-targeted, testing for these subtypes should be done across all tumour sites in a patient (Sugitani et al., 2023).

In this study, we observed that the GCB subtype was more common in the HIV-positive population (13/47; 27.7%) (*P*= 0.028). While this is consistent with a study done by Vaughan et al., another study found that HIV-positive and HIV-negative populations have an equal distribution of COO subtypes (Cassim et al., 2020; Pather and Patel, 2022). Due to alterations in tumour biology observed in HIV-positive patients and poor COO detection using different IHC techniques, this may account for the disparate results across investigations. Also, HIV-encoded proteins and virions that promote lymphomagenesis may be linked to these mechanisms (Cassim et al., 2020). The proliferation index in HIV-positive DLBCL cases is found to be higher than those without which suggests a more aggressive tumour biology (Vaughan et al., 2022). However, OS in correlation with COO subtypes presented no significant difference in a previous SA study (Pather et al., 2013). Another possible explanation for the GCB subtype predominance in HIV-positive DLBCL can be attributed to immune dysregulation, oncogenic viral factors, and genetic alterations. Normal B-cell functions are dysregulated when the immune system is dysregulated resulting in the loss of CD4 cells. Neoplastic GCB cells survive due to T-cells impairing GC reactions leading to GCB DLBCL development (Bilajac et al., 2022; Gesssese et al., 2023). The GCB subtype generally has a better prognosis than the non-GCB subtype (Gessese et al, 2023)

4.3 Fluorescence *in-situ* hybridisation results

4.3.1 *MYC*

In this study, *MYC* showed the most rearrangements in 13 (26.0%) cases. While the literature points to *BCL6* as the gene most commonly rearranged, our observed frequency aligns with studies showing a range of 2.5-56% (Appendix 4) (Akyurek et al., 2012; Almeida et al., 2022; Baptista et al., 2022; Cassidy et al., 2020; Chapman et al., 2021; Cucco et al., 2020; Foot et al., 2011; Horn et al., 2013; Huang et al., 2019; Li et al., 2023; Meriranta et al., 2020; Oliveira et al., 2017; Ott, 2017; Perry et al., 2013; Rosenwald et al., 2019; Salam et al., 2020; Ting et al., 2019; Tourneret et al., 2021; Tsai et al., 2021; Tzankov et al., 2014; Vaughan et al., 2022; Willenbacher et al., 2020; Zhang et al., 2018). Five other studies studied also showed *MYC* as their most rearranged gene (Chapman et al., 2021; Foot et al., 2011; Li et al., 2023; Perry et al., 2013; Vaughan et al., 2022). One study found that *MYC* rearrangement was more frequently observed in high-grade morphology DLBCL cases (Li et al., 2023). Two of these studies suggested a correlation between HIV and a high incidence of *MYC* rearrangement (24.6% vs 10% in the HIV-negative population) (Chapman et al., 2021; Vaughan et al., 2022). The SA study showed that HIV-positive patients had a more aggressive tumour biology (Vaughan et al., 2022). It is noted that HIV has indirect as well as direct mechanisms leading to the development of DLBCL. Increased MYC expression due to *MYC* rearrangements is observed, specifically in HIV-positive GCB DLBCL cases. In our cohort 7/17 (41.2%) HIV-positive cases had a *MYC* rearrangement. Although not statistically significant this supports studies that found increased *MYC* dysregulation in the HIV-positive population (Gessese et al., 2023). A study found that HIV-positive DLBCL cases with *MYC* rearrangement were more frequently from the GCB subtype and had *BCL2* rearrangements (Pagani et al., 2024).

Twenty-two per cent (11/50) of our cases were SHL. The cases with the concurrent *BCL6* rearrangement were included as a SHL since it is also no longer a DHL according to the new WHO. The impact of SHL is not as widely studied and the frequency ranges between 8.2-40% (Herrera et al., 2018; Horn et al., 2013; Rosenwald et al., 2019; Tsai et al., 2021; Tzankov et al., 2014). A study. These studies found that SHL were no different from cases without *MYC* rearrangements (Cho et al., 2021; Rosenwald et al., 2019).

MYC CNV was present in 18.0% (9/50) of our cohort. A comprehensive look at other studies showed a range of between 0.7-13% (Cho et al., 2019; Huang et al., 2019; Salam et al., 2020; Zhang et al, 2018). Our findings are slightly higher, but no significant correlations were observed regarding *MYC* CNVs in our cohort. Although the impact of *MYC* CNVs is still controversial, studies indicated that the presence of CNVs has an overall worse impact on survival and prognosis compared to those without and should not be disregarded (Schieppati et al., 2020).

There was an absence of *MYC* aberrations in 56.0% of cases. A possibility exists that some *MYC* aberrations could have been missed due to some factors. The *MYC* probes used in the study were BAP and DFP which only cover a certain allele of the *MYC* gene. In our study, 12.5% (4/32) of cases that were negative for *MYC* BAP, had a rearrangement when tested with the DFP. BAP has a higher sensitivity than DFP and is easier to interpret (Chen et al., 2019a). However, in another study, with a similar approach to our *MYC* testing methodology, there was a 4.0% false negative rate when only the BAP was used (King et al., 2019). *MYC* BAP probes frequently miss distal telomeric *MYC* events, but rearrangement *IGH*/*MYC* DFP may produce false negative results due to cryptic insertional processes. The sensitivity of commercially available *MYC* BAP probes varies depending on the extent of breakpoint area coverage. Some probes lack the distal 5'*MYC* or 3'*MYC* gene coverage. The detection of *MYC* rearrangements is diagnostically significant and some recommended that both *MYC* BAP and *MYC* DFP be used together to maximise sensitivity (Chen et al., 2019a; Larson et al., 2020; May et al., 2010). Currently, in our setting, the use of both probes cannot be implemented, due to the high cost of probes.

While MYC expression by itself cannot be a surrogate for *MYC* rearrangement detection, a study found that CD38 bright IHC expression together with ≥55% MYC IHC expression is highly predictive of DHL and THLs (Alsuwaidan et al., 2022). Another study used IHC marker LIM-only protein 2 (LMO2) in combination with CD10 and MYC IHC as an indicator for *MYC* rearrangements. LMO2 is a protein which is responsible for the regulation of haematopoiesis. In the case of *MYC* rearrangement LMO2 expression is low and CD10 is expressed. They also found that compared to MYC IHC expression as a predictor of *MYC* rearrangement, LMO2 IHC was more reproducible, and they suggested that diagnostic centres should include LMO2 in their diagnostic workup (Papaleo et al., 2023).

Due to the significant role MYC plays in lymphomagenesis, studies have attempted to, directly and indirectly, use MYC as a druggable target. A direct strategy targeted disrupting the interaction between MAX and MYC. Most studies targeted MYC indirectly. These indirect methods include the activation of certain pathways like NF-κB and the PI3K pathway or the loss of Myeloid Leukemia 1. Currently, there is no information regarding the extent to which tumour cells depend on MYC activity and the extent MYC needs to be inhibited for it to be effective in causing cell death and inhibition of proliferation. Although progress has been made in some drug trials, the difficulty remains due to the structure of MYC. The structure of MYC is quite disordered making it difficult for target compounds to bind with high affinity or groove into the compounds. Unlike other oncoproteins, MYC lacks catalytic activity and cannot be blocked with enzyme inhibitors. Using MYC as a therapeutic target, tumourigenesis could be reversed, and it could inhibit or shrink tumour cell growth (Duffy et al., 2021; Martínez-Martín et al., 2023).

4.3.2 *BCL2*

The *BCL2* gene showed the least rearrangements (12.0%; 6/50). Our study is consistent with several other studies (Appendix 4) showing rearrangement between 4.37-74.5% (Akyurek et al., 2012; Baptista et al., 2022; Cassidy et al., 2020; Chapman et al., 2021; Cucco et al., 2020; Foot et al., 2011; Horn et al., 2013; Huang et al., 2019; Li et al., 2023; Meriranta et al., 2020; Oliveira et al., 2017; Perry et al., 2013; Rosenwald et al., 2019; Salam et al., 2020; Ting et al., 2019; Tourneret et al., 2021; Tsai et al., 2021; Tzankov et al., 2014; Vaughan et al., 2022; Willenbacher et al., 2020; Zhang et al., 2018). In our study, all cases with *BCL2* gene aberrations had a BCL2 expression (10/11; 90.9%) (*P=0.038)*. This is in keeping with another study that showed BCL2 was almost always expressed in *BCL2* rearranged cases (87.5%) (Tsai et al., 2021). The mechanism of *BCL2* aberrations is known to prevent apoptosis, increase cell survival, and prompt the cell to attain secondary chromosomal alterations (Salam et al., 2020). Due to the significant co-existence of BCL2 expression and *BCL2* aberrations, one could use BCL2 IHC and a GCB subtype, as an indicator for possible *BCL2* genetic abnormalities. In our cohort, *BCL2* aberrations were more common in the GCB subtype. All cases with *BCL2* rearrangements (n=6) belonged to the GCB subtype (*P*=0.046). Most studies show that cases with *BCL2* aberrations have a GCB subtype (Bolen et al., 2020; McPhail et al., 2018; Morton et al., 2014; Zhang et al., 2018). One study in particular found that all their cases with *BCL2* alterations were from the GCB subtype *(*Bellas et al., 2014). These cases seem to have a worse prognostic outcome (Bellas et al., 2014; Schieppati et al., 2020). A recent study explored the prognostic and survival outcomes of different genetic

subgroups in DLBCL and found *BCL2* rearrangements in both DHL and atypical-DH to have a worse outcome than cases without (Blomme et al., 2024). The influence of BCR and NF-κB signalling pathways leads to rearrangements, mutations, copy number variations and increased transcription. When MYC induces apoptosis, BCL2 blocks tumour cell death. In the event of BCL2 and MYC acting together the impact on the tumour progression is accelerated. This causes resistance to therapy (Almasmoum, 2024). According to a study looking at the mechanism of BCL2 behaviour in lymphomas, they found *BCL2* rearrangements to be secondary to other genetic events that occur. They found that *BCL2* rearrangements enable the cell to escape apoptosis and trigger the cell to develop secondary *BCL2* aberrations. This can lead to the NF-kB pathway being dysregulated leading to an increase in BCL2 protein expression in the non-GCB subtype (Salam et al., 2020). The BCL2 protein promotes resistance to therapy in cells and can enhance the growth of tumour cells (Zhang et al., 2018). DLBCL patients are known to have drug resistance. This is due to the blocking of drug-induced apoptosis, specifically due to the deregulation of BCL2. These mechanisms can be used as a druggable target in DLBCL (Roh et al., 2023). Studies doing this showed cell death in drug-resistant lymphomas can be restored. Alone or in combination with other treatments, the BCL2 inhibitors can overcome drug resistance (Maji et al., 2018).

4.3.3 *BCL6*

With a rearrangement frequency of 18.0% (9/50), *BCL6* was the second most rearranged gene in our cohort. Although it has been described to be the most frequently rearranged gene in DLBCL, our rearrangement frequency still fell in line with other studies which ranged between 3.0-33.0%. (Appendix 4) (Akyurek et al., 2012; Baptista et al., 2022; Cassidy et al., 2020; Chapman et al., 2021; Cucco et al., 2020; Foot et al., 2011; Horn et al., 2013; Huang et al., 2019; Li et al., 2023; Meriranta et al., 2020; Oliveira et al., 2017; Perry et al., 2013; Rosenwald et al., 2019; Salam et al., 2020; Ting et al., 2019; Tourneret et al., 2021; Tsai et al., 2021; Tzankov et al., 2014; Vaughan et al., 2022; Willenbacher et al., 2020; Zhang et al., 2018). *BCL6* rearrangement is usually found in the GCB subtype (Zhang et al., 2018). *BCL6* CNVs were more common in our cohort. Although this does not carry the same weight as rearrangements, this proves the dysregulation of *BCL6* in DLBCL. Little research has been done on CNVs and their role in the overall management of DLBCL. However, it has been suggested that patients whose tumours show *BCL6* CNV with a non-GCB subtype have a worse prognosis (Foot et al., 2011). Interestingly there were no significant correlations made between BCL6 expression and *BCL6* aberrations in this study. Studies have shown that when BCL6 expression was present there is a high

probability that there was a *BCL6* aberration (Zhang et al., 2018). Different factors can contribute to these BCL6 IHC results. One of these contributors can be our study population which has a slight GCB predominance. Another possibility described by Salam et al., suggested that the cutoff values for IHC differ from other institutions as well as the scoring system used (Salam et al., 2020). Some use a cutoff of 50% while others use 30% (Ting et al., 2019; Yan, et al.,2014). Due to *BCL6*'s function as a nuclear transcriptional repressor, gene aberrations involving *BCL6* are said to be the basis of tumourigenesis. Overexpression of BCL6 protein directly controls cellular processes like proliferation, differentiation, and apoptosis, enabling the development of tumours. In normal GCB cells, BCL6 inhibits MYC and BCL2 expression. A study showed that *BCL6* rearrangements have a high probability of having BCL6 protein overexpression (Zhang et al., 2018). Although not significant, our study showed that the rearrangements and protein expression levels were almost all co-expressed. Out of our 9 cases that had a *BCL6* rearrangement, 7 had a BCL6 protein expression. This points to a role *BCL6* rearrangements can have on the cellular processes of tumour cells and the progression of DLBCL.

Knowing the mechanism of BCL6 action in the development of DLBCL, as well as the structural characterisation of BCL6-corepressor complexes, makes BCL6 an excellent druggable target. With BCL6 as the target, the compounds bind to BCL6 block corepressor recruitment and kill tumour cells. BCL6 inhibitors can inhibit both non-GCB and GCB cells as both require BCL6 to survive. A recent study even found BCL6 inhibitors (WK500B) to be an oral therapeutic drug in conjunction with chemotherapy in DLBCL treatment (Cardenas et al., 2017; Xing et al., 2022).

4.3.4 Atypical hits

Atypical hits were present in almost half of our cohort (20/50; 40%). This percentage surpassed other studies examined which had atypical hits in 5.4-22.0% of cases (Blomme et al., 2024; Herrera et al., 2018; Huang et al., 2019; Tourneret et al., 2021). While our study did not establish correlations between atypical cases and other variables, the high number of these observations present may indicate that atypical hits are a prevalent characteristic of our DLBCL cohort. A study showed that A-DH and A-TH, have a similar poor OS compared to those of typical DHL and THL (Huang et al., 2019). This finding underscores the clinical significance of atypical hits, emphasising the need for a more comprehensive understanding of their impact on disease progression and patient outcomes. Studies which compared atypical hits, without any rearrangements, found that there were no adverse

prognostic implications than those with rearrangements present. They had similar outcomes to those that had no aberrations (Blomme et al., 2024; Tourneret et al., 2021). In our study, it is noted that A-SH with *BCL6* aberrations (6/9; 66.7%) were predominantly from the non-GCB subtype and A-SH with BCL2 aberrations (2/9;22.2%) were mostly GCB subtype. One of the few studies to investigate atypical hits in the context of patient outcomes in DLBCL suggests that both rearrangements and atypical hits should be investigated (Tourneret et al., 2021).

4.3.5 Overall genetic profile of DLBCL cohort

Interesting observations were made regarding our cohort's overall genetic profile. In 66.0% of our cases, a genetic aberration was present. This is more than half of our cohort. Although these cases do not fall under the subtypes of DLBCL it sheds light on the genetic heterogeneity of DLBCL and the need for further research. As seen in other studies, DLBCL comprises multiple genetic subtypes. These genetic subtypes all have different prognostic outcomes. This highlights the need for individualised diagnostic approaches in DLBCL (Crombie and Armand, 2020).

4.4 WHO classification

Most of our cohort (90.0%) were classified as DLBCL, NOS. A small proportion of cases had molecular characteristics, classifying them as DHL and THL. Only 1 (2.0%) case each was DHL and THL. In studies observed, DHL occurs in 1.3-23% and THL in 0.4-3% of DLBCL cases (Herrera et al., 2018; Horn et al., 2013; Oliveira et al., 2017; Rosenwald et al., 2019; Timlin et al., 2018; Tsai et al., 2021; Zhang et al., 2018). We had low frequencies of DHL and THL, but it still fell in line with other studies. The low number could be due to our small cohort and that DHL and THL typically occur in the elderly (Huang et al., 2018; Ting et al., 2019). Another contributing factor could be the different WHO criteria used regarding the classification of DHL and THL across studies. In the course of our study, the WHO updated the classification of DLBCL which resulted in DLBCL with *MYC* and *BCL6* rearrangements being removed from being regarded as DHL. Cases with this rearrangement profile are now classified as DLBCL, NOS. One (2.0%) case in our cohort had a *MYC* and *BCL6* rearrangement. A study by Blomme et al confirmed that cases with this profile did not have worse prognostic outcomes compared to those without this rearrangement. A possible reason for the removal of *BCL6* is the fact that *BCL6* rearrangement partners are very broad. Some of these partners have other roles than dysregulation and activation (Blomme et al., 2024). Patients with DHL and THL have an overall worse prognosis. The

worse prognosis in DHL and THL highlights the importance of the detection of molecular subgroups, as they benefit from more aggressive treatment (Almeida et al., 2022; McPhail et al., 2018; Schieppati et al., 2020). The COO subtype of DHL and THL are in most cases GCB according to studies (Cho et al, 2021; Collinge et al., 2021; Huang et al., 2018). Although our cohort only had two of these cases, and both cases had a GCB subtype. Our study did not correlate tumour morphology with genetic findings. A study showed that tumour morphology had an impact on the outcome of patients. Cases with a blastoid morphology or a B-cell lymphoma unclassifiable (BCLU) morphology are frequently the cases with *MYC, BCL2* and *BCL6* rearrangements. They suggest that in the screening for DHL and THL cases, morphology, immunophenotype and COO subtype should be used in conjunction to identify these tumours (Thirunavukkarasu et al., 2022). With the addition of this information in the pathology report, treatment strategies could be individualised.

EBV-positive DLBCL was found in 4/75 (5.3%) cases of our cohort. This is consistent with another study that found 4.7% of their pooled Western DLBCL cohort were EBV-positive (Hwang et al., 2021). Another frequent observation in EBV-positive cases is the fact that they are usually from the non-GCB subtype (Chapman et al., 2021). All EBV-positive DLBCL, NOS cases in this study were non-GCB subtypes. EBV-positivity is known to be associated with HIV-positivity (Gessese et al., 2023). In our cohort 2 of our EBV-positive cases were HIV-positive and 1 was negative. Regarding the FISH results, 2 cases showed no aberrations while 1 case showed *MYC* and *BCL6* gain. This DLBCL subtype also requires further study. The 25/75 cases that did not have FISH results were classified as DLBCL, NOS according to the 5th WHO edition.

4.5 Challenges regarding classifying DLBCL according to new WHO

Due to the necessity for molecular profiling in the recent updates of the WHO, the major challenge is the accessibility of FISH and other genetic tests (Pavlovsky, 2019). There is currently no consensus on the diagnostic criteria and cutoff values for FISH, making diagnostic and treatment strategies vary (Hwang et al., 2013; Xia and Zhang, 2020). As seen in the different studies the cutoff values for rearrangements range between 5-15% (Akyurek et al., 2012; Bellas et al., 2014; Horn et al., 2013). Due to the lack of consensus, diagnostic tools and diagnostic confidence, some cases are left unclassified (Ta et al., 2022). Different studies have different selection criteria for cases to undergo FISH testing (Mehta et al., 2020). Some suggest that all 3 probes should be tested for an initial diagnosis while

others suggest testing for *MYC* rearrangement first and if rearranged, test for *BCL2* and *BCL6* (Alsuwaidan et al., 2022; Larson et al., 2020; Rosenwald et al., 2019). Although FISH is cost-effective, compared to other techniques to detect aberrations, some institutions still struggle with the cost of cytogenetic tests. Thus, in some institutions, only selected cases are sent for genetic testing (Almeida et al., 2022). Although FISH is widely performed, there is still a lack of consensus when it comes to the cutoff values and case selection criteria for FISH. Some centres use MYC IHC expression of more than 40% for the selection for FISH testing, whereas some centres do FISH on all DLBCL cases regardless of their MYC IHC results. A United Kingdom study recently confirmed that there was a lack of consensus when it came to treatment management as well. There was a lack of studies to support treatment strategies and studies have conflicting results (El-Sharkawi et al., 2023).

4.6 Study limitations

Due to its retrospective nature, our study encountered some limitations. Room was created for selection bias, due to tissue availability, and the unavailability of patient clinical data and some demographic and IHC data. MYC IHC results were limited, which restricted us from fully understanding DELs as well as MYC IHC expression versus *MYC* aberrations. The need for improved resources such as expanded IHC panels FISH is required not only for lymphoma diagnosis but for other malignancies as well. Additionally, patient treatment as well as patient OS results, was not available and better insight into our DLBCL cohort behaviour was not possible. Another limitation of this study pertains to the FISH methodology itself. Several cases in our cohort (25/75) did not yield FISH results. Possible reasons for this could be the over or under-fixation of cells. When cells are not fixed properly cells do not digest enough or are over-digested even when the digestion times are optimised. This impacts the probe's ability to hybridise to the tissue. In most of these cases, one or both probes of a case did not hybridise and were not viewed. Formalin fixation times are not standardised and usually vary from case to case. This has an adverse impact on molecular testing (Petersen et al., 2004).

A study done investigating cryptic events found that FISH probes have some limitations. They found that in the case of HGBCL-DHL or THL, due to some genetic events cryptic *MYC* and *BCL2* rearrangements were not identified (Hilton et al., 2019). There are currently two types of *MYC* probes commercially available for the testing of aberrations. Suppliers of probes include Abbott Laboratories, DAKO and Zytovision (Tourneret et al., 2021; Willenbacher et al., 2020). These include BAP and DFP.

Both probes have their advantages and disadvantages. Rearrangements can occasionally be missed when using the *MYC* BAP (Blomme et al., 2024). Our study did use this approach and 5/32 (15.6%) of cases that had a negative result with the BAP were positive for *MYC* rearrangements using the DFP. Both probes cover the breakpoint region that is the most common in DLBCL rearrangements. The advantage of the *MYC* DFP is that it detects cryptic rearrangements, which are not detected by the BAP. It is suggested that both the BAP and DFP should be employed to detect *MYC* aberrations if available (May et al., 2010; Munoz-Marmol et al., 2013). Unfortunately, the cost of probes is still a limitation in our setting and implementing this approach would not be feasible. According to 2 studies observed the Vysis probe showed better results (May et al., 2010). In our study, we also made use of Vysis probes.

An expert or skilled person is needed to interpret FISH results reliably which limits the amount of people that can interpret FISH results (Cassidy et al., 2020). With that said, there is also a lack of consensus on the cutoff values for results which poses a limitation to the uniformity of FISH used as the gold standard to detect molecular aberrations. The variability in some of our results can be due to the change in the current edition of the WHO. Studies before 2022 used the 2008 and 2016 WHO classifications. In their studies, BCL6 rearrangements were included in their DHL classification. Thus, our comparison to these studies differs somewhat from our DHL numbers.

4.7 Future directions

The limitations in this study, shed some light on potential future directions of this study. One of these possible outcomes, is a further look into the patient treatment and outcome of our cohort, as multiple cases had a genetic aberration present. Acknowledging the latter, future studies should examine the mutational landscape of DLBCL, NOS and their subclassifications, which can be useful to patient management and prognostication. These genetic alterations can be detected with the use of NGS techniques. Genetic subtypes of DLBCL based on their shared genomic aberrations like *BCL6*, *BCL2, TP53 EZH2* and *Myeloid differentiating factor 88 (MYD88*) have given new insight into DLBCL (Chapuy et al., 2018). These proposed genetic subtypes have not been investigated in our cohort. They can provide further information, especially since our cohort differs from studies that do not have an HIV-prevalent cohort. Due to the cost implications of tests like NGS, other approaches can be attempted to screen and identify DLBCL with rearrangements (Chen et al., 2023a). The TP53 IHC expression has

been utilised in studies to provide prognostic information. They found that cases with TP53 overexpression and abnormalities had a worse OS than those without (Huang et al.,2018; Song et al., 2021).

In the public sector, the request for FISH on all DLBCL cases is not feasible. Affordable screening tools would be valuable. Other studies have proposed LMO2 IHC which should be tested in our setting (Papaleo et al., 2023). Due to the cost of FISH being a challenge in certain diagnostic settings, a multi-probe approach should be considered. With this approach, both *BCL2* and *BCL6* rearrangements could be detected simultaneously. It is performed on the same slide, reducing cost as well as turnaround time. A study tested this approach and found the method to be useful. They had no false positives or false negatives, and the interpretation of the test was manageable. They recommended using this approach as it has diagnostic advantages in the detection of aggressive DLBCL (Marino et al., 2021).

CHAPTER 5: Conclusion

The aim and objectives were met in this study, by successfully classifying 50 DLBCL cases according to the 5th edition of the WHO classification of haematolymphoid tumours with the use of FISH. To our knowledge, this was the first study at GSH to investigate the spectrum of *MYC, BCL2* and *BCL6* gene alterations in a cohort of DLBCLs. Our findings were largely consistent with other studies. The *MYC* gene was rearranged the most (26.0%) followed by *BCL6* (18.0%) and *BCL2* (12.0%). The effect HIV has on the germinal centre was noted in our study, highlighting the role HIV plays in the development of DLBCL. Although rearrangements and protein expression are frequently co-expressed, the use of MYC, BCL2 and BCL6 IHC as a surrogate for predicting rearrangements cannot be used. Regarding the classification, 90.0% of cases were DLBCL, NOS. DHL and THL were an uncommon finding and while they account for a very low prevalence, they can only be identified by cytogenetic testing in a diagnostic setting. This highlights the invaluable need for FISH as a diagnostic tool in lymphoma classification. In 66.0% of our cases, there was at least 1 *MYC, BCL2* or *BCL6* aberration present. Only 34.0% of DLBCLs, in this cohort, were without alterations in any of the 3 genes tested. This finding highlights the genetic heterogeneity of DLBCL and reaffirms the role of these oncogenes in the pathogenesis of DLBCLs. The unique heterogeneity of DLBCL encourages the use of molecular testing on DLBCL cases as a reflex test. Other studies have highlighted how these genetic alterations influence clinical outcomes, therapeutic response, and OS. With this knowledge attempts can be made to improve treatment options and create more individualised and efficient treatment plans for patients suffering from this difficult hematologic neoplasm.

REFERENCES

ABDELHAMID, T. M., GABER, A. A., ABDELFATTAH, R. M., ALGAMAL, D. A., HAMDY, O. & MOHAMED, G. 2023. The significance of concurrent MYC and BCL2 expression in Egyptian patients with diffuse large B-cell NHL. *Pathology - Research and Practice,* 253**,** 154973.

AKYUREK, N., UNER, A., BENEKLI, M. & BARISTA, I. 2012. Prognostic significance of MYC, BCL2, and BCL6 rearrangements in patients with diffuse large B-cell lymphoma treated with cyclophosphamide, doxorubicin, vincristine, and prednisone plus rituximab. *Cancer,* 118**,** 4173-83.

ALIZADEH, A. A., EISEN, M. B., DAVIS, R. E., MA, C., LOSSOS, I. S., ROSENWALD, A., BOLDRICK, J. C., SABET, H., TRAN, T., YU, X., POWELL, J. I., YANG, L., MARTI, G. E., MOORE, T., HUDSON, J., JR., LU, L., LEWIS, D. B., TIBSHIRANI, R., SHERLOCK, G., CHAN, W. C., GREINER, T. C., WEISENBURGER, D. D., ARMITAGE, J. O., WARNKE, R., LEVY, R., WILSON, W., GREVER, M. R., BYRD, J. C., BOTSTEIN, D., BROWN, P. O. & STAUDT, L. M. 2000. Distinct types of diffuse large B-cell lymphoma identified by gene expression profiling. *Nature,* 403**,** 503-11.

ALMASMOUM, H. A. 2024. Molecular complexity of diffuse large B-cell lymphoma: a molecular perspective and therapeutic implications. *Journal of Applied Genetics,* 65**,** 57-72.

ALMEIDA, R., ABRANTES, C., GIGLIANO, D., OLIVEIRA, R. C., TEIXEIRA, P., VIEGAS, M., RODRIGUES, Â. & JULIÃO, M. J. 2022. Clinical and Pathological Features of Double-Hit and Triple-Hit High-Grade B-Cell Lymphomas: A Retrospective Study from Three Portuguese Tertiary Centers. *International Journal of Hematology-Oncology and Stem Cell Research,* 16**,** 94-102.

ALSUWAIDAN, A., KODURU, P., FUDA, F., MANUEL JASO, J., CHEN, M., ROSADO, F., LUU, H. S., SWEED, N., GARCIA, R., DOUCET, M., DESAI, N. B., KUMAR, K. A., AWAN, F. T., RAMAKRISHNAN GEETHAKUMARI, P. & CHEN, W. 2022. A Combined Biomarker of Bright CD38 and MYC ≥55% Is Highly Predictive of Double-/Triple-Hit High-Grade B-Cell Lymphoma. *American Journal of Clinical Pathology,* 158**,** 338-344.

BAPTISTA, M. J., TAPIA, G., MUNOZ-MARMOL, A. M., MUNCUNILL, J., GARCIA, O., MONTOTO, S., GRIBBEN, J. G., CALAMINICI, M., MARTINEZ, A., VELOZA, L., MARTINEZ-TRILLOS, A., ALDAMIZ, T., MENARGUEZ, J., TEROL, M. J., FERRANDEZ, A., ALCOCEBA, M., BRIONES, J., GONZALEZ-BARCA, E., CLIMENT, F., MUNTANOLA, A., MORALEDA, J. M., PROVENCIO, M., ABRISQUETA, P., ABELLA, E., COLOMO, L., GARCIA-BALLESTEROS, C., GARCIA-CARO, M., SANCHO, J. M., RIBERA, J. M., MATE, J. L. & NAVARRO, J. T. 2022. Genetic and phenotypic characterisation of HIV-associated aggressive B-cell non-Hodgkin lymphomas, which do not occur specifically in this population: diagnostic and prognostic implications. *Histopathology,* 81**,** 826-840.

BASSO, K. & DALLA-FAVERA, R. 2015. Germinal centres and B cell lymphomagenesis. *Nature Reviews Immunology,* 15**,** 172-84.

BEHAM-SCHMID, C. 2017. Aggressive lymphoma 2016: revision of the WHO classification. *Memo,* 10**,** 248-254.

BELLAS, C., GARCIA, D., VICENTE, Y., KILANY, L., ABRAIRA, V., NAVARRO, B., PROVENCIO, M. & MARTIN, P. 2014. Immunohistochemical and molecular characteristics with prognostic significance in diffuse large B-cell lymphoma. *PLoS One,* 9**,** e98169.

BETTELLI, S., MARCHESELLI, R., POZZI, S., MARCHESELLI, L., PAPOTTI, R., FORTI, E., COX, M. C. C., DI NAPOLI, A., TADMOR, T., MANSUETO, G. R., MUSTO, P., FLENGHI, L., QUINTINI, M., GALIMBERTI, S., LALINGA, V., DONATI, V., MAIORANA, A., POLLIACK, A. & SACCHI, S. 2021. Cell of origin (COO), BCL2/MYC status and IPI define a group of patients with Diffuse Large B-cell Lymphoma (DLBCL) with poor prognosis in a real-world clinical setting. Leukemia Research, 104, 106552.

BILAJAC, E., MAHMUTOVIC, L., LUNDSTROM, K., GLAMOCLIJA, U., SUTKOVIC, J., SEZER, A. & HROMIC-JAHJEFENDIC, A. 2022. Viral Agents as Potential Drivers of Diffuse Large B-Cell Lymphoma Tumorigenesis. *Viruses,* 14.

BLOMME, S., DE PAEPE, P., DEVOS, H., EMMERECHTS, J., SNAUWAERT, S. & CAUWELIER, B. 2024. Alternative genetic alterations of MYC, BCL2, and/or BCL6 in high-grade B-cell lymphoma (HGBL) and diffuse large B-cell lymphoma (DLBCL): Can we identify different prognostic subgroups? *Genes Chromosomes Cancer,* 63**,** e23211.

BOLEN, C. R., KLANOVA, M., TRNENY, M., SEHN, L. H., HE, J., TONG, J., PAULSON, J. N., KIM, E., VITOLO, U., DI ROCCO, A., FINGERLE-ROWSON, G., NIELSEN, T., LENZ, G. & OESTERGAARD, M. Z. 2020. Prognostic impact of somatic mutations in diffuse large B-cell lymphoma and relationship to cell-of-origin: data from the phase III GOYA study. *Haematologica,* 105**,** 2298-2307.

BOUROUMEAU, A., BUSSOT, L., BONNEFOIX, T., FOURNIER, C., CHAPUSOT, C., CASASNOVAS, O., MARTIN, L., MCLEER, A., COL, E., DAVID-BOUDET, L., LEFEBVRE, C., ALGRIN, C., RASKOVALOVA, T., JACOB, M. C., VETTIER, C., CHEVALIER, S., CALLANAN, M. B., GRESSIN, R., EMADALI, A. & SARTELET, H. 2021. c-MYC and p53 expression highlight starry-sky pattern as a favourable prognostic feature in R-CHOP-treated diffuse large B-cell lymphoma. *The Journal of Pathology: Clinical Research,* 7**,** 604-615.

CAI, Q., MEDEIROS, L. J., XU, X. & YOUNG, K. H. 2015. MYC-driven aggressive B-cell lymphomas: biology, entity, differential diagnosis, and clinical management. *Oncotarget,* 6**,** 38591-616.

CARBONE, A., CESARMAN, E., SPINA, M., GLOGHINI, A. & SCHULZ, T. F. 2009. HIV-associated lymphomas and gamma-herpesviruses. *Blood,* 113**,** 1213-24.

CARDENAS, M. G., OSWALD, E., YU, W., XUE, F., MACKERELL, A. D., JR. & MELNICK, A. M. 2017. The Expanding Role of the BCL6 Oncoprotein as a Cancer Therapeutic Target. *Clinical Cancer Research,* 23**,** 885-893.

CASEY, S. B., V AND FELSHER, DW 2018. The MYC oncogene is a global regulator of the immune response. *The American Society of Hematology*, 131

CASSIDY, D. P., CHAPMAN, J. R., LOPEZ, R., WHITE, K., FAN, Y. S., CASAS, C., SEVERSON, E. A. & VEGA, F. 2020. Comparison Between Integrated Genomic DNA/RNA Profiling and Fluorescence In Situ Hybridization in the Detection of MYC, BCL-2, and BCL-6 Gene Rearrangements in Large B-Cell Lymphomas. *American Journal of Clinical Pathology,* 153**,** 353-359.

CASSIM, S., ANTEL, K., CHETTY, D. R., OOSTHUIZEN, J., OPIE, J., MOHAMED, Z. & VERBURGH, E. 2020. Diffuse large B-cell lymphoma in a South African cohort with a high HIV prevalence: an analysis by cell-of-origin, Epstein-Barr virus infection and survival. *Pathology,* 52**,** 453-459.

CHADBURN, A., GLOGHINI, A. & CARBONE, A. 2023. Classification of B-Cell Lymphomas and Immunodeficiency-Related Lymphoproliferations: What's New?. *Hematology,* 4**,** 26-41.

CHAN, W. J. 2010. Pathogenesis of diffuse large B cell lymphoma. *International Journal of Hematology,* 92**,** 219-30.

CHAPMAN, J. R., BOUSKA, A. C., ZHANG, W., ALDERUCCIO, J. P., LOSSOS, I. S., RIMSZA, L. M., MAGUIRE, A., YI, S., CHAN, W. C., VEGA, F. & SONG, J. Y. 2021. EBV-positive HIV-associated diffuse large B cell lymphomas are characterized by JAK/STAT (STAT3) pathway mutations and unique clinicopathologic features. *British Journal of Haematology,* 194**,** 870-878.

CHAPUY, B., STEWART, C., DUNFORD, A. J., KIM, J., KAMBUROV, A., REDD, R. A., LAWRENCE, M. S., ROEMER, M. G. M., LI, A. J., ZIEPERT, M., STAIGER, A. M., WALA, J. A., DUCAR, M. D., LESHCHINER, I., RHEINBAY, E., TAYLOR-WEINER, A., COUGHLIN, C. A., HESS, J. M., PEDAMALLU, C. S., LIVITZ, D., ROSEBROCK, D., ROSENBERG, M., TRACY, A. A., HORN, H., VAN HUMMELEN, P., FELDMAN, A. L., LINK, B. K., NOVAK, A. J., CERHAN, J. R., HABERMANN, T. M., SIEBERT, R., ROSENWALD, A., THORNER, A. R., MEYERSON, M. L., GOLUB, T. R., BEROUKHIM, R., WULF, G. G., OTT, G., RODIG, S. J., MONTI, S., NEUBERG, D. S., LOEFFLER, M., PFREUNDSCHUH, M., TRUMPER, L., GETZ, G. & SHIPP, M. A. 2018. Molecular subtypes of diffuse large B cell lymphoma are associated with distinct pathogenic mechanisms and outcomes. *Nature Medicine,* 24**,** 679-690.

CHEN, B.-J., FEND, F., CAMPO, E. & QUINTANILLA-MARTINEZ, L. 2019a. Aggressive B-cell lymphomas—from morphology to molecular pathogenesis. *Annals of Lymphoma,* 3**,** 1-1.

CHEN, H., QIN, Y., LIU, P., YANG, J., GUI, L., HE, X., ZHANG, C., ZHOU, S., ZHOU, L., YANG, S. & SHI, Y. 2023a. Genetic Profiling of Diffuse Large B-Cell Lymphoma: A Comparison Between Double-

Expressor Lymphoma and Non-Double-Expressor Lymphoma. *Molecular Diagnosis & Therapy,* 27**,** 75-86.

CHEN, J., SUN, L., DAI, Y., ZHANG, L., YANG, K., HAN, X., DING, X., GAO, H., ZHOU, X. & WANG, P. 2023b. Clinical pathology of primary central nervous system lymphoma in HIV-positive patients-a 41 Chinese patients retrospective study. *Annals of Diagnostic Pathology,* 63**,** 152108.

CHEN, Y., CHEN, H., CHEN, L., ZHENG, X., YANG, X., ZHENG, Z., ZHENG, J., YANG, T., LIU, T., YANG, Y. & HU, J. 2019b. Immunohistochemical overexpression of BCL-2 protein predicts an inferior survival in patients with primary central nervous system diffuse large B-cell lymphoma. *Medicine (Baltimore),* 98**,** e17827.

CHISHOLM, K.M., BANGS, C.D., BACCHI, C.E., MOLINA-KIRSCH, H., M., CHERRY, A., NATKUNAM, Y. 2015. Expression Profiles of MYC Protein and MYC Gene Rearrangement in Lymphomas. *American Journal of Clinical Pathology,* 39.

CHO, J. 2022. Basic immunohistochemistry for lymphoma diagnosis. *Blood Research,* 57**,** 55-61.

CHO, Y. A., HYEON, J., LEE, H., CHO, J., KIM, S. J., KIM, W. S. & KO, Y. H. 2021. MYC single-hit large B-cell lymphoma: clinicopathologic difference from MYC-negative large B-cell lymphoma and MYC double-hit/triple-hit lymphoma. *Human Pathology,* 113**,** 9-19.

CHOI, W. W., WEISENBURGER, D. D., GREINER, T. C., PIRIS, M. A., BANHAM, A. H., DELABIE, J., BRAZIEL, R. M., GENG, H., IQBAL, J., LENZ, G., VOSE, J. M., HANS, C. P., FU, K., SMITH, L. M., LI, M., LIU, Z., GASCOYNE, R. D., ROSENWALD, A., OTT, G., RIMSZA, L. M., CAMPO, E., JAFFE, E. S., JAYE, D. L., STAUDT, L. M. & CHAN, W. C. 2009. A new immunostain algorithm classifies diffuse large B-cell lymphoma into molecular subtypes with high accuracy. *Clinical Cancer Research,* 15**,** 5494-502.

CHU, Y. H., HARDIN, H., ZHANG, R., GUO, Z. & LLOYD, R. V. 2019. In situ hybridization: Introduction to techniques, applications and pitfalls in the performance and interpretation of assays. *Semin Diagnostic Pathology,* 36**,** 336-341.

COLLINGE, B., BEN-NERIAH, S., CHONG, L., BOYLE, M., JIANG, A., MIYATA-TAKATA, T., FARINHA, P., CRAIG, J. W., SLACK, G. W., ENNISHI, D., MOTTOK, A., MEISSNER, B., CHAVEZ, E. A., GERRIE, A. S., VILLA, D., FREEMAN, C., SAVAGE, K. J., SEHN, L. H., MORIN, R. D., MUNGALL, A. J., GASCOYNE, R. D., MARRA, M. A., CONNORS, J. M., STEIDL, C. & SCOTT, D. W. 2021. The impact of MYC and BCL2 structural variants in tumours of DLBCL morphology and mechanisms of false-negative MYC IHC. *Blood,* 137**,** 2196-2208.

CUCCO, F., BARRANS, S., SHA, C., CLIPSON, A., CROUCH, S., DOBSON, R., CHEN, Z., THOMPSON, J. S., CARE, M. A., CUMMIN, T., CADDY, J., LIU, H., ROBINSON, A., SCHUH, A., FITZGIBBON, J., PAINTER, D., SMITH, A., ROMAN, E., TOOZE, R., BURTON, C., DAVIES, A. J., WESTHEAD, D. R., JOHNSON, P.

W. M. & DU, M. Q. 2020. Distinct genetic changes reveal evolutionary history and heterogeneous molecular grade of DLBCL with MYC/BCL2 double-hit. *Leukemia,* 34**,** 1329-1341.

CROMBIE, J. L. & ARMAND, P. 2020. Diffuse Large B-Cell Lymphoma's New Genomics: The Bridge and the Chasm. *Journal of Clinical Oncology,* 38**,** 3565-3574.

DA SILVA, S. R. & DE OLIVEIRA, D. E. 2011. HIV, EBV and KSHV: viral cooperation in the pathogenesis of human malignancies. *Cancer Letters,* 305**,** 175-85.

DAS, R., NATARAJAN, S., AGRAWAL, S., & BHATTACHARYA, I. 2020. Towards analysing comparative genomic hybridisation method. *Journal of Advanced Scientific Research,* 11.

DE CARVALHO, P. S., LEAL, F. E. & SOARES, M. A. 2021. Clinical and Molecular Properties of Human Immunodeficiency Virus-Related Diffuse Large B-Cell Lymphoma. *Frontiers Oncology,* 11**,** 675353.

DE LEVAL, L. J., ES. 2020. Lymphoma Classification. *The Cancer Journal,* 26.

DINNEEN, K., TIMLIN, D. M., O'HARE, K., WALKER, J., CASTRICIANO, G., CONNOLLY, Y., GRANT, C., BACON, L., VANDENBERGHE, E., DUNNE, B., JEFFERS, M. & FLAVIN, R. 2020. Incidence of single hit Bcl-2 and Bcl-6 rearrangements in DLBCL: the Irish experience. *Journal of Clinical Pathology,* 73**,** 689-690.

DUFFY, M. J., O'GRADY, S., TANG, M. & CROWN, J. 2021. MYC as a target for cancer treatment. *Cancer Treatment Reviews,* 94**,** 102154.

EL-SHARKAWI, D., SUD, A., PRODGER, C., KHWAJA, J., SHOTTON, R., HANLEY, B., PEACOCK, V., PENG, Y. Y., ARASARETNAM, A., SHARMA, S., ALDRIDGE, F., SHARMA, B., WOTHERSPOON, A., CHEUNG, B., DE LORD, C., JOHNSTON, R., KASSAM, S., PETTENGEL, R., LINTON, K., GREAVES, P., COOK, L., NARESH, K. N., CWYNARSKI, K., EYRE, T. A., CHAU, I., CUNNINGHAM, D. & IYENGAR, S. 2023. A retrospective study of MYC rearranged diffuse large B-cell lymphoma in the context of the new WHO and ICC classifications. *Blood Cancer Journal,* 13**,** 54.

FERLAY, J., ERVIK, M., LAM, F., LAVERSANNE, M., COLOMBET, M., MERY, L., PIÑEROS, M., ZNAOR, A., SOERJOMATARAM, I., BRAY, F . 2024.*Global Cancer Observatory: Cancer Today. Lyon, France: International Agency for Research on Cancer*. Available from: https://gco.iarc.who.int/today, accessed [20 February 2024]

FINESTONE, E. & WISHNIA, J. 2022. Estimating the burden of cancer in South Africa. *South African Journal of Oncology,* 6.

FOOT, N. J., DUNN, R. G., GEOGHEGAN, H., WILKINS, B. S. & NEAT, M. J. 2011. Fluorescence in situ hybridisation analysis of formalin-fixed paraffin-embedded tissue sections in the diagnostic work-up of non-Burkitt high grade B-cell non-Hodgkin's lymphoma: a single centre's experience. *Journal of Clinical Pathology,* 64**,** 802-8.

FRONTZEK, F., STAIGER, A. M., WULLENKORD, R., GRAU, M., ZAPUKHLYAK, M., KURZ, K. S., HORN, H., ERDMANN, T., FEND, F., RICHTER, J., KLAPPER, W., LENZ, P., HAILFINGER, S., TASIDOU, A., TRAUTMANN, M., HARTMANN, W., ROSENWALD, A., QUINTANILLA-MARTINEZ, L., OTT, G., ANAGNOSTOPOULOS, I. & LENZ, G. 2023. Molecular profiling of EBV associated diffuse large B-cell lymphoma. *Leukemia,* 37**,** 670-679

FU, X., SHI, Y., MA, J., ZHANG, K., WANG, G., LI, G., XIAO, L. & WANG, H. 2022. Advances of multiplex ligation-dependent probe amplification technology in molecular diagnostics. *Biotechniques*.

GARIMBERTI, E. & TOSI, S. 2010. Fluorescence in situ Hybridization (FISH), Basic Principles and Methodology. *In:* BRIDGER, J. M. & VOLPI, E. V. (eds.) *Fluorescence in situ Hybridization (FISH): Protocols and Applications.* Totowa, NJ: Humana Press.

GESSESE, T., ASRIE, F. & MULATIE, Z. 2023. Human Immunodeficiency Virus Related Non-Hodgkin's Lymphoma. *Blood Lymphat Cancer,* 13**,** 13-24.

GETANEH, Z., ASRIE, F. & MELKU, M. 2019. MicroRNA profiles in B-cell non-Hodgkin lymphoma. *Ejifcc,* 30**,** 195-214

GLOGHINI, A., DOLCETTI, R. & CARBONE, A. 2013. Lymphomas occurring specifically in HIV-infected patients: from pathogenesis to pathology. *Seminars in Cancer Biology,* 23**,** 457-67.

GOPALAKRISHNAN, V., ROY, U., SRIVASTAVA, S., KARIYA, K. M., SHARMA, S., JAVEDAKAR, S. M., CHOUDHARY, B. & RAGHAVAN, S. C. 2024. Delineating the mechanism of fragility at BCL6 breakpoint region associated with translocations in diffuse large B cell lymphoma. *Cellular and Molecular Life Sciences,* 81**,** 21.

GOUVEIA, G. R., SIQUEIRA, S. A. C. & PEREIRA, J. 2012. Pathophysiology and molecular aspects of diffuse large B-cell lymphoma. *Revista Brasileira de Hematologia e Hemoterapia,* 34**,** 447-451.

GUNN, S. R., ROBETORYE, R. S. & MOHAMMED, M. S. 2007. Comparative genomic hybridization arrays in clinical pathology: progress and challenges. *Molecular Diagnosis & Therapy,* 11**,** 73-7.

GREEN, T. M., YOUNG, K. H., VISCO, C., XU-MONETTE, Z. Y., ORAZI, A., GO, R. S., NIELSEN, O., GADEBERG, O. V., MOURITS-ANDERSEN, T., FREDERIKSEN, M., PEDERSEN, L. M. & MØLLER, M. B. 2012. Immunohistochemical double-hit score is a strong predictor of outcome in patients with diffuse large B-cell lymphoma treated with rituximab plus cyclophosphamide, doxorubicin, vincristine, and prednisone. *Journal of Clinical Oncology,* 30**,** 3460-7.

HANS, C. P., WEISENBURGER, D. D., GREINER, T. C., GASCOYNE, R. D., DELABIE, J., OTT, G., MULLER-HERMELINK, H. K., CAMPO, E., BRAZIEL, R. M., JAFFE, E. S., PAN, Z., FARINHA, P., SMITH, L. M., FALINI, B., BANHAM, A. H., ROSENWALD, A., STAUDT, L. M., CONNORS, J. M., ARMITAGE, J. O.

& CHAN, W. C. 2004. Confirmation of the molecular classification of diffuse large B-cell lymphoma by immunohistochemistry using a tissue microarray. *Blood,* 103**,** 275-82.

HASHMI, A. A., IFTIKHAR, S. N., NARGUS, G., AHMED, O., ASGHAR, I. A., SHIRAZI, U. A., AFZAL, A., IRFAN, M. & ALI, J. 2021. Double-Expressor Phenotype (BCL-2/c-MYC Co-expression) of Diffuse Large B-Cell Lymphoma and Its Clinicopathological Correlation. *Cureus,* 13**,** e13155.

HASTINGS, R. J., BOWN, N., TIBILETTI, M. G., DEBIEC-RYCHTER, M., VANNI, R., ESPINET, B., VAN ROY, N., ROBERTS, P., VAN DEN BERG-DE-RUITER, E., BERNHEIM, A., SCHOUMANS, J., CHATTERS, S., ZEMANOVA, Z., STEVENS-KROEF, M., SIMONS, A., HEIM, S., SALIDO, M., YLSTRA, B., BETTS, D. R. 2016. Guidelines for cytogenetic investigations in tumours. *European Journal of Human Genetics,* 24**,** 6-13.

HERBST, M. 2021. Fact Sheet on Diffuse Large B-Cell Lymphoma, *CANSA*

HERRERA, A. F., RODIG, S. J., SONG, J. Y., KIM, Y., GRIFFIN, G. K., YANG, D., NIKOLAENKO, L., MEI, M., BEDELL, V., DAL CIN, P., PAK, C., ALYEA, E. P., BUDDE, L. E., CHEN, R., CHEN, Y. B., CHAN, W. C., CUTLER, C. S., HO, V. T., KORETH, J., KRISHNAN, A., MURATA-COLLINS, J. L., NIKIFOROW, S., PALMER, J., PIHAN, G. A., PILLAI, R., POPPLEWELL, L., ROSEN, S. T., SIDDIQI, T., SOHANI, A. R., ZAIN, J., KWAK, L. W., WEISENBURGER, D. D., WEINSTOCK, D. M., SOIFFER, R. J., ANTIN, J. H., FORMAN, S. J., NADEMANEE, A. P. & ARMAND, P. 2018. Outcomes after Allogeneic Stem Cell Transplantation in Patients with Double-Hit and Double-Expressor Lymphoma. *Biology Blood Marrow Transplant,* 24**,** 514-520.

HILTON, L. K., TANG, J., BEN-NERIAH, S., ALCAIDE, M., JIANG, A., GRANDE, B. M., RUSHTON, C. K., BOYLE, M., MEISSNER, B., SCOTT, D. W. & MORIN, R. D. 2019. The double-hit signature identifies double-hit diffuse large B-cell lymphoma with genetic events cryptic to FISH. *Blood,* 134**,** 1528-1532.

HOLMES, A. B., CORINALDESI, C., SHEN, Q., KUMAR, R., COMPAGNO, N., WANG, Z., NITZAN, M., GRUNSTEIN, E., PASQUALUCCI, L., DALLA-FAVERA, R. & BASSO, K. 2020. Single-cell analysis of germinal-center B cells informs on lymphoma cell of origin and outcome. *Journal of Experimental Medicine,* 217.

HORN, H., ZIEPERT, M., BECHER, C., BARTH, T. F., BERND, H. W., FELLER, A. C., KLAPPER, W., HUMMEL, M., STEIN, H., HANSMANN, M. L., SCHMELTER, C., MOLLER, P., COGLIATTI, S., PFREUNDSCHUH, M., SCHMITZ, N., TRUMPER, L., SIEBERT, R., LOEFFLER, M., ROSENWALD, A., OTT, G. & GERMAN HIGH-GRADE NON-HODGKIN LYMPHOMA STUDY, G. 2013. MYC status in concert with BCL2 and BCL6 expression predicts outcome in diffuse large B-cell lymphoma. *Blood,* 121**,** 2253-63.

HU, Y., YANG, K. & KRAUSE, J. R. 2011. Diffuse large B-cell lymphoma, differential diagnosis, and molecular stratification. *North American Journal of Medicine and Science,* 4.

HUANG, S., NONG, L., WANG, W., LIANG, L., ZHENG, Y., LIU, J., LI, D., LI, X., ZHANG, B. & LI, T. 2019. Prognostic impact of diffuse large B-cell lymphoma with extra copies of MYC, BCL2 and/or BCL6: comparison with double/triple hit lymphoma and double expressor lymphoma. *Diagnostic Pathology,* 14**,** 81.

HUANG, W., MEDEIROS, L. J., LIN, P., WANG, W., TANG, G., KHOURY, J., KONOPLEV, S., YIN, C. C., XU, J., OKI, Y. & LI, S. 2018. MYC/BCL2/BCL6 triple hit lymphoma: a study of 40 patients with a comparison to MYC/BCL2 and MYC/BCL6 double hit lymphomas. *Modern Pathology,* 31**,** 1470-1478.

HWANG, J., SUH, C. H., WON KIM, K., KIM, H. S., ARMAND, P., HUANG, R. Y. & GUENETTE, J. P. 2021. The Incidence of Epstein-Barr Virus-Positive Diffuse Large B-Cell Lymphoma: A Systematic Review and Meta-Analysis. *Cancers (Basel),* 13.

HWANG, H. S., YOON, D. H., SUH, C., PARK, C. S. & HUH, J. 2013. Prognostic value of immunohistochemical algorithms in gastrointestinal diffuse large B-cell lymphoma. *Blood Research,* 48**,** 266-73.

INSUASTI-BELTRAN, G. 2022. Diffuse Large B-Cell Lymphomas: From Morphology to Genomic Profiling. *Lymphoma - Recent Advances IntechOpen*.

JAFFE, E. S., BARR, P. M. & SMITH, S. M. 2017. Understanding the New WHO Classification of Lymphoid Malignancies: Why It is Important and How It Will Affect Practice. *American Society of Clinical Oncology Educational Book***,** 535-546.

JAROSOVA, M., KRIEGOVA, E., SCHNEIDEROVA, P., FILLEROVA, R., PROCHAZKA, V., MIKESOVA, M., FLODR, P., INDRAK, K. & PAPAJIK, T. 2016. A Novel Non-Immunoglobulin (non-Ig)/BCL6 Translocation in Diffuse Large B-Cell Lymphoma Involving Chromosome 10q11.21 Loci and Review on Clinical Consequences of BCL6 Rearrangements. *Pathology and Oncology Research,* 22**,** 233-43

KARUBE, K. & CAMPO, E. 2015. MYC alterations in diffuse large B-cell lymphomas. *Seminar of Haematology,* 52**,** 97-106.

KHAN, U. T., KELLY, M., DODD, J., FERGIANI, S., HAMMER, B., SMITH, J., ARUMAINATHAN, A., ATHERTON, M., CARTER, A., RACU-AMOASII, I., KALAKONDA, N., PETTITT, A. & MENON, G. 2021. Role of MYC and BCL2 expression in a cohort of 43 patients with DLBCL: a retrospective study. *Journal of Clinical Pathology,* 74**,** 816-818.

KIM, S. W., ROH, J. & PARK, C. S. 2016. Immunohistochemistry for Pathologists: Protocols, Pitfalls, and Tips. *Journal of Pathology and Translational Medicine,* 50**,** 411-418.

KING, J. F. & LAM, J. T. 2020. A Practical Approach to Diagnosis of B-Cell Lymphomas With Diffuse Large Cell Morphology. *Archives of Pathology & Laboratory Medicine,* 144**,** 160-167.

KING, R. L., HSI, E. D., CHAN, W. C., PIRIS, M. A., COOK, J. R., SCOTT, D. W. & SWERDLOW, S. H. 2023. Diagnostic approaches and future directions in Burkitt lymphoma and high-grade B-cell lymphoma. *Virchows Archive,* 482**,** 193-205.

KING, R.L., MCPHAIL, E.D., MEYER, R.G., VASMATZIS, G., PEARCE, K., SMADBECK, J.B., KETTERLING, R.P., SMOLEY, S.A. GREIPP, P.T., HOPPMAN, N.L., PETERSON, J.F. & BAUGHN, L.B. 2019. False-negative rates for MYC fluorescence in situ hybridization probes in B-cell neoplasms. *Haematologica,* 104**,** e248-e251.

KLUK, M. J., CHAPUY, B., SINHA, P., ROY, A., DAL CIN, P., NEUBERG, D. S., MONTI, S., PINKUS, G. S., SHIPP, M. A. & RODIG, S. J. 2012. Immunohistochemical detection of MYC-driven diffuse large B-cell lymphomas. *PLoS One,* 7**,** e33813.

KORAC, P., DOTLIC, S., MATULIC, M., ZAJC PETRANOVIC, M. & DOMINIS, M. 2017. Role of MYC in B Cell Lymphomagenesis. *Genes (Basel),* 8.

KRULL, J. E., WENZL, K., HARTERT, K. T., MANSKE, M. K., SARANGI, V., MAURER, M. J., LARSON, M. C., NOWAKOWSKI, G. S., ANSELL, S. M., MCPHAIL, E., HABERMANN, T. M., LINK, B. K., KING, R. L., CERHAN, J. R. & NOVAK, A. J. 2020. Somatic copy number gains in MYC, BCL2, and BCL6 identifies a subset of aggressive alternative-DH/TH DLBCL patients. *Blood Cancer Journal,* 10**,** 117.

KURZ, K. S., OTT, M., KALMBACH, S., STEINLEIN, S., KALLA, C., HORN, H., OTT, G. & STAIGER, A. M. 2023. Large B-Cell Lymphomas in the 5th Edition of the WHO-Classification of Haematolymphoid Neoplasms-Updated Classification and New Concepts. *Cancers (Basel),* 15.

LACEY, S. E., BARRANS, S. L., BEER, P. A., PAINTER, D., SMITH, A. G., ROMAN, E., COOKE, S. L., RUIZ, C., GLOVER, P., VAN HOPPE, S. J. L., WEBSTER, N., CAMPBELL, P. J., TOOZE, R. M., PATMORE, R., BURTON, C., CROUCH, S. & HODSON, D. J. 2020. Targeted sequencing in DLBCL, molecular subtypes, and outcomes: a Haematological Malignancy Research Network report. *Blood,* 135**,** 1759-1771.

LARSON, D. P., PETERSON, J. F., NOWAKOWSKI, G. S. & MCPHAIL, E. D. 2020. A practical approach to FISH testing for MYC rearrangements and brief review of MYC in aggressive B-cell lymphomas. *Journal of Hematopathology,* 13**,** 127-135.

LI, S., QIU, L., XU, J., LIN, P., OK, C. Y., TANG, G., MCDONNELL, T. J., JAMES YOU, M., KHANLARI, M., MIRANDA, R. N. & MEDEIROS, L. J. 2023. High-grade B-cell lymphoma (HGBL)-NOS is clinicopathologically and genetically more similar to DLBCL/HGBL-DH than DLBCL. *Leukaemia,* 37**,** 422-432.

LI, S., YOUNG, K. H. & MEDEIROS, L. J. 2018. Diffuse large B-cell lymphoma. *Pathology,* 50**,** 74-87.

LI, W. 2021. Pathogenesis and Pathology of Paediatric Lymphoma. *In:* GALLAMINI, A. & JUWEID, M. (eds.) *Lymphoma.* Brisbane (AU): Exon Publications

LIU, Y. & BARTA, S. K. 2019. Diffuse large B-cell lymphoma: 2019 update on diagnosis, risk stratification, and treatment. *American Journal of Haematology,* 94**,** 604-616.

LASSOS, I. S. 2005. Molecular pathogenesis of diffuse large B-cell lymphoma. *Journal of Clinical Oncology,* 23**,** 6351-7.

MAGANGANE, P. S., MOHAMED, Z. & NAIDOO, R. 2020. Diffuse large B-cell lymphoma in a high human immunodeficiency virus (HIV) prevalence, low-resource setting. *South African Journal of Oncology,* 4.

MAGANGANE, P., SOOKHAYI, R., GOVENDER, D. & NAIDOO, R. 2016. Determining protein biomarkers for DLBCL using FFPE tissues from HIV-negative and HIV positive patients. *Journal of Molecular Histology,* 47**,** 565-577.

MAJI, S., PANDA, S., SAMAL, S. K., SHRIWAS, O., RATH, R., PELLECCHIA, M., EMDAD, L., DAS, S. K., FISHER, P. B. & DASH, R. 2018. Bcl-2 Antiapoptotic Family Proteins and Chemoresistance in Cancer. *Advances in Cancer Research,* 137**,** 37-75.

MAMGAIN, G., SINGH, P. K., PATRA, P., NAITHANI, M. & NATH, U. K. 2022. Diffuse large B-cell lymphoma and new insights into its pathobiology and implication in treatment. *Journal of Family Medicine and Primary Care,* 11**,** 4151-4158.

MANSOURI, L., THORVALDSDOTTIR, B., LAIDOU, S., STAMATOPOULOS, K. & ROSENQUIST, R. 2022. Precision diagnostics in lymphomas - Recent developments and future directions. *Seminars in Cancer Biology,* 84**,** 170-183.

MARINO, F. Z., AQUINO, G., BRUNELLI, M., SCOGNAMIGLIO, G., PEDRON, S., RONCHI, A., COZZOLINO, I., SPARANO, L., BOTTI, G., PANICO, L., DE CHIARA, A., FRANCO, R. & GRUPPO EMATOPATOLOGIA, C. 2021. High performance of multiplex fluorescence in situ hybridization to simultaneous detection of BCL2 and BCL6 rearrangements: useful application in the characterization of DLBCLs. *Virchows Archiv,* 479**,** 565-573.

MARQUES-PIUBELLI, M. L., SALAS, Y. I., PACHAS, C., BECKER-HECKER, R., VEGA, F. & MIRANDA, R. N. 2020. Epstein-Barr virus-associated B-cell lymphoproliferative disorders and lymphomas: a review. *Pathology,* 52**,** 40-52.

MARTELLI, M., FERRERI, A. J., AGOSTINELLI, C., DI ROCCO, A., PFREUNDSCHUH, M. & PILERI, S. A. 2013. Diffuse large B-cell lymphoma. *Critical Reviews in Oncology/Haematology,* 87**,** 146-71.

MARTÍNEZ-MARTÍN, S., BEAULIEU, M. E. & SOUCEK, L. 2023. Targeting MYC-driven lymphoma: lessons learned and future directions. *Cancer Drug Resistance,* 6**,** 205-222.

MAY, P. C., FOOT, N., DUNN, R., GEOGHEGAN, H. & NEAT, M. J. 2010. Detection of cryptic and variant IGH-MYC rearrangements in high-grade non-Hodgkin's lymphoma by fluorescence in situ hybridization: implications for cytogenetic testing. *Cancer Genetics and Cytogenetics,* 198**,** 71-5.

MCPHAIL, E. D., MAURER, M. J., MACON, W. R., FELDMAN, A. L., KURTIN, P. J., KETTERLING, R. P., VAIDYA, R., CERHAN, J. R., ANSELL, S. M., PORRATA, L. F., NOWAKOWSKI, G. S., WITZIG, T. E. & HABERMANN, T. M. 2018. Inferior survival in high-grade B-cell lymphoma with MYC and BCL2 and/or BCL6 rearrangements is not associated with MYC/IG gene rearrangements. *Haematologica,* 103**,** 1899-1907.

MEDEIROS, L. J., MIRANDA, ROBERTO N. 2017. *Diagnostic Pathology Lymph Nodes and Extranodal Lymphomas,* Canada, Elsevier, Inc.

MEHTA, A., VERMA, A., GUPTA, G., TRIPATHI, R. & SHARMA, A. 2020. Double Hit and Double Expresser Diffuse Large B Cell Lymphoma Subtypes: Discrete Subtypes and Major Predictors of Overall Survival. *Indian Journal of Haematology Blood Transfusion,* 36**,** 627-634.

MERIRANTA, L., PASANEN, A., ALKODSI, A., HAUKKA, J., KARJALAINEN-LINDSBERG, M. L. & LEPPA, S. 2020. Molecular background delineates the outcome of double protein expressor diffuse large B-cell lymphoma. *Blood Advances,* 4**,** 3742-3753.

MORTON, L. M., KIM, C. J., WEISS, L. M., BHATIA, K., COCKBURN, M., HAWES, D., WANG, S. S., CHANG, C., ALTEKRUSE, S. F., ENGELS, E. A. & COZEN, W. 2014. Molecular characteristics of diffuse large B-cell lymphoma in human immunodeficiency virus-infected and -uninfected patients in the pre-highly active antiretroviral therapy and pre-rituximab era. *Leuk Lymphoma,* 55**,** 551-7.

MORTON, L. M., TURNER, J. J., CERHAN, J. R., LINET, M. S., TRESELER, P. A., CLARKE, C. A., JACK, A., COZEN, W., MAYNADIE, M., SPINELLI, J. J., COSTANTINI, A. S., RUDIGER, T., SCARPA, A., ZHENG, T. & WEISENBURGER, D. D. 2007. Proposed classification of lymphoid neoplasms for epidemiologic research from the Pathology Working Group of the International Lymphoma Epidemiology Consortium (InterLymph). *Blood,* 110**,** 695-708.

MUNOZ-MARMOL, A. M., SANZ, C., TAPIA, G., MARGINET, R., ARIZA, A. & MATE, J. L. 2013. MYC status determination in aggressive B-cell lymphoma: the impact of FISH probe selection. *Histopathology,* 63**,** 418-24.

NAKAMURA, Y., ITOH, Y., KAKEGAWA, E., UCHIDA, Y., ICHIMURA, T. & SASAKI, A. 2022. Biallelic BCL6 rearrangements by dual t(3;14)(q27;q32) and t(3;22)(q27;q11) translocations in diffuse large B-cell lymphoma. *Journal of Clinical and Experimental Hematopathology,* 62**,** 268-272.

NASEEM, M., ASIF, M., KHADIM, M. T., UD-DIN, H., JAMAL, S. & SHOAIB, I. 2020. The Frequency of Double Expresser in Selected Cases of High Grade Diffuse Large B-Cell Lymphomas. *Asian Pacific Journal of Cancer Prevention,* 21**,** 1103-1107.

NASR, M.R., PERRY, A. M. & SKRABEK, P. 2019. Lymph Node Pathology for Clinicians. Switzerland: *Springer*.

OK, C. Y. & MEDEIROS, L. J. 2020. High-grade B-cell lymphoma: a term re-purposed in the revised WHO classification. *Pathology,* 52**,** 68-77.

OLIVEIRA, C. C., MACIEL-GUERRA, H., KUCKO, L., HIRAMA, E. J., BRILHANTE, A. D., QUEVEDO, F. C., DA CUNHA, I. W., SOARES, F. A., NIERO-MELO, L., DOS REIS, P. P. & DOMINGUES, M. A. 2017. Double-hit lymphomas: clinical, morphological, immunohistochemical and cytogenetic study in a series of Brazilian patients with high-grade non-Hodgkin lymphoma. *Diagnostic Pathology,* 12**,** 3.

OLLILA, T. A. & OLSZEWSKI, A. J. 2018. Extranodal Diffuse Large B Cell Lymphoma: Molecular Features, Prognosis, and Risk of Central Nervous System Recurrence. *Current Treatment Options in Oncology,* 19**,** 38.

OTT, G. 2017. Aggressive B-cell lymphomas in the update of the 4th edition of the World Health Organization classification of haematopoietic and lymphatic tissues: refinements of the classification, new entities, and genetic findings. *British Journal of Haematology,* 178**,** 871-887.

OTT, G., ROSENWALD, A. & CAMPO, E. 2013. Understanding MYC-driven aggressive B-cell lymphomas: pathogenesis and classification. *Blood,* 122**,** 3884-91.

PAPALEO, N., CLIMENT, F., TAPIA, G., LUIZAGA, L., AZCARATE, J., BOSCH-SCHIPS, J., MUNOZ-MARMOL, A. M., SALIDO, M., LOME-MALDONADO, C., VAZQUEZ, I. & COLOMO, L. 2023. Round-robin testing for LMO2 and MYC as immunohistochemical markers to screen MYC rearrangements in aggressive large B-cell lymphoma. *Virchows Archiv*.

PAGANI, C., RUSCONI, C., DALLA PRIA, A., RAVANO, E., SCHOMMERS, P., BASTOS-OREIRO, M., VERGA, L., GINI, G., SPINA, M., ARCAINI, L., STEFFANONI, S., DALU, D., CRUCITTI, L., LORENZI, L., BALZARINI, P., CATTANEO, C., BONGIOVANNI, L., ROSENWALD, A., FACCHETTI, F., BOWER, M., FERRERI, A. J. M., ROSSI, G., TUCCI, A. & RE, A. 2024. MYC rearrangements in HIV-associated large B-cell lymphomas: EUROMYC, a European retrospective study. *Blood Advances,* 8**,** 968-977

PASQUALUCCI, L. 2019. Molecular pathogenesis of germinal center-derived B cell lymphomas. *Immunological Reviews,* 288**,** 240-261.

PATEL, M., PHILIP, V., OMAR, T., TURTON, D., CANDY, G., LAKHA, A. & PATHER, S. 2015. The Impact of Human Immunodeficiency Virus Infection (HIV) on Lymphoma in South Africa. *Journal of Cancer Therapy,* 06**,** 527-535.

PATHER, S., MOHAMED, Z., MCLEOD, H. & PILLAY, K. 2013. Large cell lymphoma: correlation of HIV status and prognosis with differentiation profiles assessed by immunophenotyping. *Pathology and Oncology Research,* 19**,** 695-705.

PATHER, S. & PATEL, M. 2022. HIV-associated DLBCL: Clinicopathological factors including dual-colour chromogenic in situ hybridisation to assess MYC gene copies. *Annals of Diagnostic Pathology,* 58**,** 151913.

PAVLOVSKY, A. 2019. Better understanding and new challenges in high grade lymphomas. *Annals of Lymphoma,* 3**,** 13-13.

PERRY, A. M., CROCKETT, D., DAVE, B. J., ALTHOF, P., WINKLER, L., SMITH, L. M., AOUN, P., CHAN, W. C., FU, K., GREINER, T. C., BIERMAN, P., GREGORY BOCIEK, R., VOSE, J. M., ARMITAGE, J. O. & WEISENBURGER, D. D. 2013. B-cell lymphoma, unclassifiable, with features intermediate between diffuse large B-cell lymphoma and burkitt lymphoma: study of 39 cases. *British Journal of Haematology,* 162**,** 40-9.

PETERSEN, B. L., SØRENSEN, M. C., PEDERSEN, S. & RASMUSSEN, M. 2004. Fluorescence In Situ Hybridization on Formalin-fixed and Paraffin-Embedded Tissue: Optimizing the Method. *Applied Immunohistochemistry & Molecular Morphology,* 12**,** 259-265.

PLACA, J. R., DIEPSTRA, A., LOS, T., MENDEVILLE, M., SEITZ, A., LUGTENBURG, P. J., ZIJLSTRA, J., LAM, K., DA SILVA, W. A., JR., YLSTRA, B., DE JONG, D., VAN DEN BERG, A. & NIJLAND, M. 2022. Reproducibility of Gene Expression Signatures in Diffuse Large B-Cell Lymphoma. *Cancers (Basel),* 14.

QIN, D. 2019. Next-generation sequencing and its clinical application. *Cancer Biol Med,* 16**,** 4-10.

RAMOS, J. C., SPARANO, J. A., CHADBURN, A., REID, E. G., AMBINDER, R. F., SIEGEL, E. R., MOORE, P. C., RUBINSTEIN, P. G., DURAND, C. M., CESARMAN, E., ABOULAFIA, D., BAIOCCHI, R., RATNER, L., KAPLAN, L., CAPOFERRI, A. A., LEE, J. Y., MITSUYASU, R. & NOY, A. 2020. Impact of Myc in HIV-associated non-Hodgkin lymphomas treated with EPOCH and outcomes with vorinostat (AMC-075 trial). *Blood,* 136**,** 1284-1297

RE, A., CATTANEO, C. & ROSSI, G. 2019. Hiv and Lymphoma: from Epidemiology to Clinical Management. *Mediterranean Journal of Hematology and Infectious Diseases,* 11**,** e2019004.

RIEDELL, P. A. & SMITH, S. M. 2018. Double hit and double expressors in lymphoma: Definition and treatment. *Cancer,* 124**,** 4622-4632.

ROH, J., CHO, H., PAK, H. K., LEE, Y. S., LEE, S. W., RYU, J. S., CHAE, E. J., KIM, K. W., HUH, J., CHOI, Y. S., JEONG, S. H., SUH, C., YOON, D. H. & PARK, C. S. 2022. BCL2 super-expressor diffuse large B-cell lymphoma: a distinct subgroup associated with poor prognosis. *Modern Pathology,* 35**,** 480-488.

ROH, J., PAK, H. K., JEONG, S., HWANG, S., KIM, D. E., CHOI, H. S., KIM, S. J., KIM, H., CHO, H., PARK, J. S., JEONG, S. H., CHOI, Y. S., HAN, J. H., YOON, D. H. & PARK, C. S. 2023. The comprehensive expression of BCL2 family genes determines the prognosis of diffuse large B-cell lymphoma. *Biochemical and Biophysical Research Communications,* 673**,** 36-43.

ROSENTHAL, A. & YOUNES, A. 2017. High grade B-cell lymphoma with rearrangements of MYC and BCL2 and/or BCL6: Double hit and triple hit lymphomas and double expressing lymphoma. *Blood Review,* 31**,** 37-42.

ROSENWALD, A., BENS, S., ADVANI, R., BARRANS, S., COPIE-BERGMAN, C., ELSENSOHN, M. H., NATKUNAM, Y., CALAMINICI, M., SANDER, B., BAIA, M., SMITH, A., PAINTER, D., PHAM, L., ZHAO, S., ZIEPERT, M., JORDANOVA, E. S., MOLINA, T. J., KERSTEN, M. J., KIMBY, E., KLAPPER, W., RAEMAEKERS, J., SCHMITZ, N., JARDIN, F., STEVENS, W. B. C., HOSTER, E., HAGENBEEK, A., GRIBBEN, J. G., SIEBERT, R., GASCOYNE, R. D., SCOTT, D. W., GAULARD, P., SALLES, G., BURTON, C., DE JONG, D., SEHN, L. H. & MAUCORT-BOULCH, D. 2019. Prognostic Significance of MYC Rearrangement and Translocation Partner in Diffuse Large B-Cell Lymphoma: A Study by the Lunenburg Lymphoma Biomarker Consortium. *Journal of Clinical Oncology,* 37**,** 3359-3368.

ROSS, A. M., LEAHY, C. I., NEYLON, F., STEIGEROVA, J., FLODR, P., NAVRATILOVA, M., URBANKOVA, H., VRZALIKOVA, K., MUNDO, L., LAZZI, S., LEONCINI, L., PUGH, M. & MURRAY, P. G. 2023. Epstein-Barr Virus and the Pathogenesis of Diffuse Large B-Cell Lymphoma. *Life (Basel),* 13.

RUNGE, H. F. P., LACY, S., BARRANS, S., BEER, P. A., PAINTER, D., SMITH, A., ROMAN, E., BURTON, C., CROUCH, S., TOOZE, R. & HODSON, D. J. 2021. Application of the LymphGen classification tool to 928 clinically and genetically characterised cases of diffuse large B cell lymphoma (DLBCL). *British Journal of Haematology,* 192**,** 216-220.

RUNGWITTAYATIWAT, S., BOONSAKAN, P., CHANTRATHAMMACHART, P., PUAVILAI, T., PUKIAT, S., PHUSANTI, S., BOONYAWAT, K., WACHARAPORNIN, P., ANGCHAISUKSIRI, P., UNGKANONT, A., CHUNCHARUNEE, S. & NIPARUCK, P. 2021. Treatment Outcomes and Clinical Relevance in Patients with Double Expressor DLBCL. *Mediterranean Journal of Hematology and Infectious Diseases,* 13**,** e2021063.

SALAM, D., THIT, E. E., TEOH, S. H., TAN, S. Y., PEH, S. C. & CHEAH, S. C. 2020. C-MYC, BCL2 and BCL6 Translocation in B-cell Non-Hodgkin Lymphoma Cases. *Journal of Cancer,* 11**,** 190-198.

SAUSEN, D. G., BASITH, A. & MUQEEMUDDIN, S. 2023. EBV and Lymphomagenesis. *Cancers (Basel),* 15.

SCHIEPPATI, F., BALZARINI, P., FISOGNI, S., RE, A., PAGANI, C., BIANCHETTI, N., MICHELI, L., PASSI, A., FERRARI, S., MAIFREDI, A., BOTTELLI, C., LEOPALDO, R., PELLEGRINI, V., FACCHETTI, F., ROSSI, G. & TUCCI, A. 2020. An increase in MYC copy number has a progressive negative prognostic impact in patients with diffuse large B-cell and high-grade lymphoma, who may benefit from intensified treatment regimens. *Haematologica,* 105**,** 1369-1378.

SCHMITZ, R., WRIGHT, G. W., HUANG, D. W., JOHNSON, C. A., PHELAN, J. D., WANG, J. Q., ROULLAND, S., KASBEKAR, M., YOUNG, R. M., SHAFFER, A. L., HODSON, D. J., XIAO, W., YU, X., YANG, Y., ZHAO, H., XU, W., LIU, X., ZHOU, B., DU, W., CHAN, W. C., JAFFE, E. S., GASCOYNE, R. D.,

CONNORS, J. M., CAMPO, E., LOPEZ-GUILLERMO, A., ROSENWALD, A., OTT, G., DELABIE, J., RIMSZA, L. M., TAY KUANG WEI, K., ZELENETZ, A. D., LEONARD, J. P., BARTLETT, N. L., TRAN, B., SHETTY, J., ZHAO, Y., SOPPET, D. R., PITTALUGA, S., WILSON, W. H. & STAUDT, L. M. 2018. Genetics and Pathogenesis of Diffuse Large B-Cell Lymphoma. *New England Journal of Medicine,* 378**,** 1396-1407.

SEHN, L. H. & SALLES, G. 2021. Diffuse Large B-cell lymphoma. *New England Journal of Medicine,* 384**,** 842-858.

SESQUES, P. & JOHNSON, N. A. 2017. Approach to the diagnosis and treatment of high-grade B-cell lymphomas with MYC and BCL2 and/or BCL6 rearrangements. *Blood,* 129**,** 280-288.

SHIBUSAWA, M., KIDOGUCHI, K. & TANIMOTO, T. 2021. Epstein-Barr Virus-Positive Diffuse Large B Cell Lymphoma. *In:* GALLAMINI, A. & JUWEID, M. (eds.) *Lymphoma.* Brisbane (AU): Exon Publications

SHIVAKUMAR, L. & ARMITAGE, J. O. 2006. Bcl-2 gene expression as a predictor of outcome in diffuse large B-cell lymphoma. *Clin Lymphoma Myeloma,* 6**,** 455-7.

SINGH, K. & BRIGGS, J. M. 2016. Functional Implications of the spectrum of BCL2 mutations in Lymphoma. *Mutation Research - Reviews in Mutation Research,* 769**,** 1-18.

SOLOMON, J. P. & ARCILA, M. E. 2020. Molecular Diagnostics of Non-Hodgkin Lymphoma. *Cancer Journal,* 26**,** 186-194.

SONG, J. Y., DIRNHOFER, S., PIRIS, M. A., QUINTANILLA-MARTINEZ, L., PILERI, S. & CAMPO, E. 2023. Diffuse large B-cell lymphomas, not otherwise specified, and emerging entities. *Virchows Archiv,* 482**,** 179-192.

SONG, J. Y., PERRY, A. M., HERRERA, A. F., CHEN, L., SKRABEK, P., NASR, M. R., OTTESEN, R. A., NIKOWITZ, J., BEDELL, V., MURATA-COLLINS, J., LI, Y., MCCARTHY, C., PILLAI, R., WANG, J., WU, X., ZAIN, J., POPPLEWELL, L., KWAK, L. W., NADEMANEE, A. P., NILAND, J. C., SCOTT, D. W., GONG, Q., CHAN, W. C. & WEISENBURGER, D. D. 2021. Double-hit Signature with TP53 Abnormalities Predicts Poor Survival in Patients with Germinal Center Type Diffuse Large B-cell Lymphoma Treated with R-CHOP. *Clinical Cancer Research,* 27**,** 1671-1680.

STATS-SA. 2022. Statistical release P0302 Mid-year population estimates 2022. 1-34.

SUGITANI, A., FUKUHARA, S., SHIBATA, M., ICHIHARA, R., FURUKAWA, H. & MAESHIMA, A. M. 2023. Diffuse large B-cell lymphoma with composite germinal centre and non-germinal centre types: A report of two cases. *Journal of Clinical and Experimental Hematopathology,* 63**,** 181-186.

SUN, L. & PFEIFER, J. D. 2019. Pitfalls in molecular diagnostics. *Seminars of Diagnostic Pathology,* 36**,** 342-354.

SUN, R., MEDEIROS, L. J. & YOUNG, K. H. 2016. Diagnostic and predictive biomarkers for lymphoma diagnosis and treatment in the era of precision medicine. *Modern Pathology,* 29**,** 1118-42.

SWERDLOW, S. H., CAMPO, E., PILERI, S. A., HARRIS, N. L., STEIN, H., SIEBERT, R., ADVANI, R., GHIELMINI, M., SALLES, G. A., ZELENETZ, A. D. & JAFFE, E. S. 2016. The 2016 revision of the World Health Organization classification of lymphoid neoplasms. *Blood,* 127**,** 2375-90.

SWERDLOW S.H, Campo, E., HARRIS, N.L., JAFFE, E.S., PILERI, S.A., STEIN, H. & THIELE, J. (eds). 2017. WHO Classification of Tumours of Haematopoietic and Lymphoid Tissues*(Revised 4th edition).* Lyon*: International Agency for Research on Cancer*

TA, R., YANG, D., HIRT, C., DRAGO, T. & FLAVIN, R. 2022. Molecular Diagnostic Review of Diffuse Large B-Cell Lymphoma and Its Tumor Microenvironment. *Diagnostics (Basel),* 12.

THANDRA, K. C., BARSOUK, A., SAGINALA, K., PADALA, S. A., BARSOUK, A. & RAWLA, P. 2021. Epidemiology of Non-Hodgkin's Lymphoma. *Medical Science (Basel),* 9.

THIRUNAVUKKARASU, B., BAL, A., PRAKASH, G., MALHOTRA, P., SINGH, H. & DAS, A. 2022. Screening Strategy for Detecting Double-Hit Lymphoma in a Resource-Limited Setting. *Appl Immunohistochemistry and Molecular Morphology,* 30**,** 49-55.

THOMPSON, M. A. K., R 2004. Epstein-Barr Virus and Cancer. *American Association for Cancer Research.,* 10**,** 803–821.

TIMLIN, D. M., O'HARE, K., WALKER, J., CASTRICIANO, G., CONNOLLY, Y., GRANT, C., BACON, C. L., VANDENBERGHE, E., DUNNE, B., JEFFERS, M. & FLAVIN, R. 2018. FISH studies in DLBCL: correlations with cell of origin: the Irish experience. *Journal of Clinical Pathology,* 71**,** 947-948.

TING, C. Y., CHANG, K. M., KUAN, J. W., SATHAR, J., CHEW, L. P., WONG, O. J., YUSUF, Y., WONG, L., SAMSUDIN, A. T., PANA, M., LEE, S. K., GOPAL, N. S. R., PURI, R., ONG, T. C., BAHARI, S. K., GOH, A. S. & TEOH, C. S. 2019. Clinical Significance of BCL2, C-MYC, and BCL6 Genetic Abnormalities, Epstein-Barr Virus Infection, CD5 Protein Expression, Germinal Center B Cell/Non-Germinal Center B-Cell Subtypes, Co-expression of MYC/BCL2 Proteins and Co-expression of MYC/BCL2/BCL6 Proteins in Diffuse Large B-Cell Lymphoma: A Clinical and Pathological Correlation Study of 120 Patients. *International Journal of Medical Sciences,* 16**,** 556-566.

TOMAS-ROCA, L., RODRIGUEZ, M., ALONSO-ALONSO, R., RODRIGUEZ-PINILLA, S. M. & PIRIS, M. A. 2021. Diffuse Large B-Cell Lymphoma: Recognition of Markers for Targeted Therapy. *Haematology,* 2**,** 281-304.

TOURNERET, A., ALAME, M., RIGAU, V., BAUCHET, L., FABBRO, M., DE OLIVEIRA, L., CACHEUX, V., COSTES, V. & LACHERETZ-SZABLEWSKI, V. 2021. BCL2 and BCL6 atypical/unbalanced gene rearrangements in diffuse large B-cell lymphoma are indicators of an aggressive clinical course. *Journal of Clinical Pathology,* 74**,** 650-656.

TSAI, C. C., SU, Y. C., BAMODU, O. A., CHEN, B. J., TSAI, W. C., CHENG, W. H., LEE, C. H., HSIEH, S. M., LIU, M. L., FANG, C. L., LIN, H. T., CHEN, C. L., YEH, C. T., LEE, W. H., HO, C. L., LAI, S. W., TZENG, H. E., HSIEH, Y. Y., CHANG, C. L., ZHENG, Y. M., LIU, H. W., YEN, Y., WHANG-PENG, J. & CHAO, T. Y. 2021. High-Grade B-Cell Lymphoma (HGBL) with MYC and BCL2 and/or BCL6 Rearrangements Is Predominantly BCL6-Rearranged and BCL6-Expressing in Taiwan. *Cancers (Basel),* 13.

TZANKOV, A., XU-MONETTE, Z. Y., GERHARD, M., VISCO, C., DIRNHOFER, S., GISIN, N., DYBKAER, K., ORAZI, A., BHAGAT, G., RICHARDS, K. L., HSI, E. D., CHOI, W. W., VAN KRIEKEN, J. H., PONZONI, M., FERRERI, A. J., YE, Q., WINTER, J. N., FARNEN, J. P., PIRIS, M. A., MOLLER, M. B., YOU, M. J., MCDONNELL, T., MEDEIROS, L. J. & YOUNG, K. H. 2014. Rearrangements of MYC gene facilitate risk stratification in diffuse large B-cell lymphoma patients treated with rituximab-CHOP. *Modern Pathology,* 27**,** 958-71.

VAUGHAN, J., PERNER, Y. & WIGGILL, T. 2022. Diffuse Large B-cell Lymphoma in the public sector of Johannesburg, South Africa, in the era of widescale Anti-retroviral therapy use. *Journal of Acquired Immune Deficiency Syndromes*.

VENTURA, R. A., MARTIN-SUBERO, J. I., JONES, M., MCPARLAND, J., GESK, S., MASON, D. Y. & SIEBERT, R. 2006. FISH analysis for the detection of lymphoma-associated chromosomal abnormalities in routine paraffin-embedded tissue. *Journal of Molecular Diagnostics,* 8**,** 141-51.

VISCO, C., LI, Y., XU-MONETTE, Z. Y., MIRANDA, R. N., GREEN, T. M., LI, Y., TZANKOV, A., WEN, W., LIU, W. M., KAHL, B. S., D'AMORE, E. S., MONTES-MORENO, S., DYBKAER, K., CHIU, A., TAM, W., ORAZI, A., ZU, Y., BHAGAT, G., WINTER, J. N., WANG, H. Y., O'NEILL, S., DUNPHY, C. H., HSI, E. D., ZHAO, X. F., GO, R. S., CHOI, W. W., ZHOU, F., CZADER, M., TONG, J., ZHAO, X., VAN KRIEKEN, J. H., HUANG, Q., AI, W., ETZELL, J., PONZONI, M., FERRERI, A. J., PIRIS, M. A., MOLLER, M. B., BUESO-RAMOS, C. E., MEDEIROS, L. J., WU, L. & YOUNG, K. H. 2012. Comprehensive gene expression profiling and immunohistochemical studies support application of immunophenotypic algorithm for molecular subtype classification in diffuse large B-cell lymphoma: a report from the International DLBCL Rituximab-CHOP Consortium Program Study. *Leukemia,* 26**,** 2103-13.

WAGNER, S. D., AHEARNE, M. & KO FERRIGNO, P. 2011. The role of BCL6 in lymphomas and routes to therapy. *British Journal of Haematology,* 152**,** 3-12.

WANG, X., HE, J., HE, H., SHUAI, Y., WANG, L., LI, Y., HUANG, Y., YU, K., ZHAO, M., XIE, T. & LI, D. 2023a. Myc rearrangement and concurrent high protein expression of C-Myc/Bcl2 carry an adverse prognosis in diffuse large B-cell lymphoma. *Annals of Diagnostic Pathology,* 66**,** 152165.

WANG, Y., LIU, D., ZHANG, X., ZHANG, M., LI, S., FENG, X., DONG, M., MA, S., QIAN, S., WANG, Z., ZHANG, Y., WANG, P., MEI, S. & CHEN, Q. 2023b. MYC overexpression but not MYC/BCL2 double

expression predicts survival in bulky mass diffuse large B-cell lymphoma patients. *Cancer Medicine,* 12**,** 18568-18577.

WANG, S. S. 2023c. Epidemiology and aetiology of diffuse large B-cell lymphoma. *Seminars of Hematology*

WEBB, F., MOREY, A., MAHLER-HINDER, C., GEORGOUSOPOULOU, E., KOO, R., PATI, N. & TALAULIKAR, D. 2023. Comprehensive FISH testing using FFPE tissue microarray of primary lymph node tissue identifies secondary cytogenetic abnormalities in Mantle Cell Lymphoma. *Cancer Genetics,* 274-275**,** 75-83.

WEISS, M. M., HERMSEN, M. A., MEIJER, G. A., VAN GRIEKEN, N. C., BAAK, J. P., KUIPERS, E. J. & VAN DIEST, P. J. 1999. Comparative genomic hybridisation. *Journal of Molecular Pathology,* 52**,** 243-51.

WHO CLASSIFICATION OF TUMOURS EDITORIAL BOARD. 2022. Haematolymphoid tumors [Internet; beta version ahead of print (in progress)]. *International Agency for Research on Cancer*. (WHO classification of tumors series, 5th ed.). Lyon (France). Available from: https://tumourclassification.iarc.who.int.

WIENAND, K. & CHAPUY, B. 2021. Molecular classification of aggressive lymphomas-past, present, future. *Hematology Oncology,* 39 Supplement 1**,** 24-30.

WILLENBACHER, E., WILLENBACHER, W., WEGER, R., DOMINIK, W., MANZL, C. & BRUNNER, A. 2020. Patients with double/triple copy number gains on C-MYC, BCL2, and/or BCL6 treated with standard chemotherapy have a similarly poor prognosis than those with high-grade B cell lymphoma with C-MYC and BCL2 and/or BCL6 rearrangements: a single-center experience on a consecutive cohort of large B cell lymphomas. *Annals of Hematology,* 99**,** 2125-2132.

WRIGHT, G. W., HUANG, D. W., PHELAN, J. D., COULIBALY, Z. A., ROULLAND, S., YOUNG, R. M., WANG, J. Q., SCHMITZ, R., MORIN, R. D., TANG, J., JIANG, A., BAGAEV, A., PLOTNIKOVA, O., KOTLOV, N., JOHNSON, C. A., WILSON, W. H., SCOTT, D. W. & STAUDT, L. M. 2020. A Probabilistic Classification Tool for Genetic Subtypes of Diffuse Large B Cell Lymphoma with Therapeutic Implications. *Cancer Cell,* 37**,** 551-568 e14.

XIA, Y. & ZHANG, X. 2020. The Spectrum of MYC Alterations in Diffuse Large B-Cell Lymphoma. *Acta Haematologica,* 143**,** 520-528.

XING, Y., GUO, W., WU, M., XIE, J., HUANG, D., HU, P., ZHOU, M., ZHANG, L., ZHANG, Q., WANG, P., WANG, X., WANG, G., WU, H., ZHOU, C., CHEN, Y., LIU, M., YI, Z. & SUN, Z. 2022. An orally available small molecule BCL6 inhibitor effectively suppresses diffuse large B cell lymphoma cells growth in vitro and in vivo. *Cancer Letters,* 529**,** 100-111.

XU-MONETTE, Z. Y., ZHANG, H., ZHU, F., TZANKOV, A., BHAGAT, G., VISCO, C., DYBKAER, K., CHIU, A., TAM, W., ZU, Y., HSI, E. D., YOU, H., HUH, J., PONZONI, M., FERRERI, A. J. M., MOLLER, M. B., PARSONS, B. M., VAN KRIEKEN, J. H., PIRIS, M. A., WINTER, J. N., HAGEMEISTER, F. B., SHAHBABA, B., DE DIOS, I., ZHANG, H., LI, Y., XU, B., ALBITAR, M. & YOUNG, K. H. 2020. A refined cell-of-origin classifier with targeted NGS and artificial intelligence shows robust predictive value in DLBCL. *Blood Advances,* 4**,** 3391-3404.

YAN, L. X., LIU, Y. H., LUO, D. L., ZHANG, F., CHENG, Y., LUO, X. L., XU, J., CHENG, J. & ZHUANG, H. G. 2014. MYC expression in concert with BCL2 and BCL6 expression predicts outcome in Chinese patients with diffuse large B-cell lymphoma, not otherwise specified. *PLoS One,* 9**,** e104068.

ZAMO, A., GERHARD-HARTMANN, E., OTT, G., ANAGNOSTOPOULOS, I., SCOTT, D. W., ROSENWALD, A. & RAUERT-WUNDERLICH, H. 2022. Routine application of the Lymph2Cx assay for the subclassification of aggressive B-cell lymphoma: report of a prospective real-world series. *Virchows Archiv,* 481**,** 935-943.

ZAYAC, A. S., LANDSBURG, D. J., HUGHES, M. E., BOCK, A. M., NOWAKOWSKI, G. S., AYERS, E. C., GIRTON, M., HU, M., BECKMAN, A. K., LI, S., MEDEIROS, L. J., CHANG, J. E., STEPANOVIC, A., KURT, H., SANDOVAL-SUS, J., ANSARI-LARI, M. A., KOTHARI, S. K., KRESS, A., XU, M. L., TORKA, P., SUNDARAM, S., SMITH, S. D., NARESH, K. N., KARIMI, Y. H., EPPERLA, N., BOND, D. A., FAROOQ, U., SAAD, M., EVENS, A. M., PANDYA, K., NAIK, S. G., KAMDAR, M., HAVERKOS, B., KARMALI, R., OH, T. S., VOSE, J. M., NUTSCH, H., RUBINSTEIN, P. G., CHAUDHRY, A. & OLSZEWSKI, A. J. 2023. High-grade B-cell lymphoma not otherwise specified: a multi-institutional retrospective study. *Blood Advances,* 7**,** 6381-6394.

ZHANG, C., STELLOO, E., BARRANS, S., CUCCO, F., JIANG, D., TZIONI, M. M., CHEN, Z., LI, Y., SWENNENHUIS, J. F., MAKKER, J., RASO-BARNETT, L., LIU, H., EL-DALY, H., SOILLEUX, E., SHAH, N., NAGUMANTRY, S. K., KYAW, M., PRAHLADAN, M. P., TOOZE, R., WESTHEAD, D. R., FEITSMA, H., DAVIES, A. J., BURTON, C., JOHNSON, P. W. M. & DU, M. Q. 2024. Non-IG::MYC in diffuse large B-cell lymphoma confers variable genomic configurations and MYC transactivation potential. *Leukemia,* 38**,** 621-629.

ZHANG, Y., WANG, H., REN, C., YU, H., FANG, W., ZHANG, N., GAO, S. & HOU, Q. 2018. Correlation Between C-MYC, BCL-2, and BCL-6 Protein Expression and Gene Translocation as Biomarkers in Diagnosis and Prognosis of Diffuse Large B-cell Lymphoma. *Front Pharmacology,* 9**,** 1497.

ZHOU, M., CHENG, J., ZHAO, H., YANG, M., YU, W., QIN, J., LANG, G., TAO, R., CAO, Q., HUANG, Y., ZHU, B. & XU, L. 2022. Clinical Features, Phenotypic Markers and Outcomes of Diffuse Large B-Cell Lymphoma between HIV-Infected and HIV-Uninfected Chinese Patients. *Cancers (Basel),* 14.

APPENDIX 1: Ethics approval and annual renewal

UNIVERSITY OF CAPE TOWN
Faculty of Health Sciences
Human Research Ethics Committee

Room 45, E-52- Old Main Building
Groote Schuur Hospital
Observatory 7925
Telephone [021] 406 6492
Email: hrec-enquiries@uct.ac.za
Website: www.health.uct.ac.za/fhs/research/humanethics/forms

22 November 2021

HREC REF: 739/2021

Dr A Ramburan
Division of Anatomical Pathology
Room 4.08.1 Falmouth Building-FHS
Email: amsha.ramburan@uct.ac.za
Student: trischkewatson@gmail.com

Dear Dr Ramburan

PROJECT TITLE: MOLECULAR CHARACTERISATION OF DIFFUSE LARGE B-CELL LYMPHOMAS (DLBCL) DIAGNOSED AT GROOTE SCHUUR HOSPITAL (GSH) - MSC MED CANDIDATE- MS. TRISCHKE WATSON-SUB-STUDY LINKED TO 501/2018

Thank you for submitting your study to the Faculty of Health Sciences Human Research Ethics Committee (HREC) for review.

It is a pleasure to inform you that the HREC has **formally approved** the above-mentioned study.

This approval is subject to strict adherence to the HREC recommendations regarding research involving human participants during COVID -19, dated 17 March 2020: 06 July 2020 & 01 July 2021.

Approval is granted for one year until the 30 November 2022.

Please submit a progress form, using the standardised Annual Report Form if the study continues beyond the approval period. Please submit a Standard Closure form if the study is completed within the approval period.
(Forms can be found on our website: www.health.uct.ac.za/fhs/research/humanethics/forms)

The HREC acknowledge that the student: Ms Trischke Watson also be involved in this study.

Please quote the HREC REF 739/2021 in all your correspondence.

Please note that the ongoing ethical conduct of the study remains the responsibility of the principal investigator.

Please note that for all studies approved by the HREC, the principal investigator **must** obtain appropriate institutional approval, where necessary, before the research may occur.

UNIVERSITY OF CAPE TOWN

FACULTY OF HEALTH SCIENCES
Human Research Ethics Committee

FHS016: Annual Progress Report / Renewal

HREC office use only (FWA00001637; IRB00001938)			
This serves as notification of annual approval, including any documentation described below.			
☑ Approved	Annual progress report	Approved until/next renewal date	30.11.2024
☐ Not approved	See attached comments		
Signature Chairperson of the HREC/ Designee		Date Signed	6/11/2023

Note: Please email this form and supporting documents (if applicable) in a combined pdf file to:
hrec-enquiries@uct.ac.za.
Please clarify your plan for research-related activities during COVID-19 lockdown.
Please use the latest form found on our website:
http://www.health.uct.ac.za/fhs/research/humanethics/forms

HUMAN RESEARCH ETHICS COMMITTEE
- 6 NOV 2023
HEALTH SCIENCES FACULTY
UNIVERSITY OF CAPE TOWN

Comments to PI from the HREC

Principal Investigator to complete the following:

1. Protocol information

Date (when submitting this form)	3 November 2023		
HREC REF Number	739/2021	Current Ethics Approval was granted until	30 November 2023
Protocol title	Molecular characterisation of Diffuse large B-cell lymphomas (DLBCL) diagnosed at Groote Schuur Hospital (GSH)		
Protocol number (if applicable)			
Are there any sub-studies linked to this study?	☐ Yes	X No	
If yes, could you please provide the HREC Reference number for all sub-studies? **Note:** A separate FHS016 must be submitted for each sub-study.			
Principal Investigator	Dr Amsha Ramburan		
Department / Office Internal Mail Address	Division of Anatomical Pathology Falmouth Building Room 4.08.1		

1.1 Does this protocol receive US Federal funding?	☐ Yes	X No

(Note: Please complete the Closure form (FHS010) if the study is completed within the approval period)

APPENDIX 2: Preparation of reagents

20 x SCC (3M sodium chloride; 0.3M sodium citrate) pH5.3:

66g of 20xSCC was added to 200ml sterile water (H_2O). It was well mixed, and the pH was adjusted to 5.3 with the addition of 1N HCl. The final volume was made up to 250ml by adding sterile H_2O.

Post hybridisation wash buffer (2xSCC/0.3% Octylphenoxypolyethoxyethanol (IGEPAL) CA-630):

50ml of 20XSCC (pH 5.3) was added to 420ml of sterile H_20 and mixed thoroughly. Using 1N NaOH the pH was adjusted to pH 7.25 and subsequently 1.5 ml of IGEPAL CA-630 was added. The final volume was made up to 500ml with sterile H_2O.

70% Ethanol:

350ml absolute ethanol was added to 150ml of sterile H_2O.

85% Ethanol:

425ml of absolute ethanol was added to 75ml of sterile H_2O.

0.2N HCl:

1ml of concentrated HCl (32%=10.4%) was added to 49ml of sterile H_2O.

0.01N HCl:

1.5ml of 0.2N HCl was added to 28.5ml of sterile H_2O.

10% pepsin:

500mg of pepsin was added to 5ml of sterile H_2O. Aliquots were stored at -20˚C.

Pepsin working solution:

75µl of 10% pepsin was added to 30ml of 0.1N HCl.

1M Sodium thiocyanate buffer:

81.7g of sodium thiocyanate was added to 800ml of sterile H_2O. It was mixed well and topped up to 1000ml with sterile H_2O.

Preparation of probe mix:

Number of sections	**X1**
Hybridisation buffer	3.5µl
Sterile H_2O	1.0µl
Probe	0.5µl
Total volume	5.0µl

APPENDIX 3: Anonymised raw case data

Case nr.	Age	Sex	HIV	Site	Immunohistochemistry								Previous WHO classification (3rd and 4th edition)	COO	FISH					Atypical hits	WHO classification (5th edition)
					CD20	CD10	BCL6	MUM1	BCL2	MYC	EBER-ISH	KI-67(%)			*MYC BAP*	*MYC DFP*	*MYC combined*	*BCL2*	*BCL6*		
1	72	F	N	LN	P	P	P	N	N	ND	N	40	DLBCL,NOS	GCB	NA	NA	NA	NA	NA	NA	DLBCL,NOS
2	83	F	N	Tonsil	P	N	P	P	P	N	N	85-90	DLBCL,NOS	non-GCB	NA	NA	NA	NA	NA	NA	DLBCL,NOS
3	81	F	N	Nasal	P	N	P	P	P	N	N	60	DLBCL,NOS	non-GCB	NA	NA	NA	NA	R	A-SH	DLBCL,NOS
4	48	F	N	LN	P	P	P	P	P	N	N	60	DLBCL,NOS	GCB	G	ND	G	G	NA	A-DH	DLBCL,NOS
5	56	F	N	Tonsil	P	N	N	P	N	P	N	70	DLBCL,NOS	non-GCB	NA	NA	NA	NA	R	A-SH	DLBCL,NOS
6	64	F	P	Subcutaneous tissue	P	P	P	ND	N	N	N	85-88	DLBCL,NOS	GCB	NA	NA	NA	NA	NA	NA	DLBCL,NOS
7	63	F	N	LN	P	P	P	ND	P	N	U	85-90	DLBCL,NOS	GCB	A	ND	A	A	G	A-TH	DLBCL,NOS
8	45	F	P	LN	P	P	P	P	P	N	N	75	DLBCL,NOS	GCB	G	ND	G	NA	R	A-DH	DLBCL,NOS
9	49	M	N	Skin	P	P	P	N	P	ND	N	60-70	DLBCL,NOS	GCB	NA	NA	NA	NA	NA	NA	DLBCL,NOS
10	52	M	N	Tonsil	P	N	P	P	P	N	N	80-90	DLBCL,NOS	non-GCB	NA	NA	NA	NA	R	A-SH	DLBCL,NOS
11	31	M	N	LN	P	N	N	P	P	N	N	60-70	DLBCL,NOS	non-GCB	NA	NA	NA	NA	R	A-SH	DLBCL,NOS
12	38	M	N	LN	P	N	N	P	P	N	N	75	DLBCL,NOS	non-GCB	G	ND	G	NA	NA	A-SH	DLBCL,NOS
13	52	M	N	Small bowel	P	N	P	P	P	N	N	ND	DLBCL,NOS	non-GCB	R	ND	R	G	A	SHL	DLBCL,NOS
14	30	M	P	Inguinal mass	P	P	P	P	P	N	N	95	DLBCL,NOS	GCB	R	ND	R	NA	NA	SHL	DLBCL,NOS
15	44	M	P	LN	P	P	P	ND	N	N	N	90	DLBCL,NOS	GCB	R	ND	R	NA	NA	SHL	DLBCL,NOS

Key: A= amplification; DNW= did not work; F= female; G= gain; LN= lymph node; M= male; N= negative; NA= no aberration; ND= not done; P= positive; R= rearrangement; U= unknown

Case nr.	Age	Sex	HIV	Site	Immunohistochemistry								Previous WHO classification (3rd and 4th edition)	COO	FISH					Atypical hits	WHO classification (5th edition)
					CD20	CD10	BCL6	MUM1	BCL2	MYC	EBER-ISH	KI-67(%)			*MYC BAP*	*MYC DFP*	*MYC combined*	*BCL2*	*BCL6*		
16	59	M	P	LN	P	N	N	P	P	N	P	>90	EBV-positive DLBCL,NOS	non-GCB	NA	NA	NA	NA	NA	NA	EBV-positive DLBCL,NOS
17	45	M	P	LN	P	P	P	ND	N	ND	ND	80-85	DLBCL,NOS	GCB	R	ND	R	NA	NA	SHL	DLBCL,NOS
18	45	M	N	LN	P	N	P	ND	P	ND	ND	70-80	DLBCL,NOS	non-GCB	NA	NA	NA	G	R	A-DH	DLBCL,NOS
19	36	M	P	Omentum	P	P	N	P	N	ND	N	90	DLBCL,NOS	GCB	R	ND	R	A	A	SHL	DLBCL,NOS
20	72	F	N	Ovary	P	P	P	N	P	ND	ND	95	DLBCL,NOS	GCB	NA	NA	NA	NA	NA	NA	DLBCL,NOS
21	57	M	N	LN	P	P	N	N	P	ND	ND	60-70	DLBCL,NOS	GCB	G	ND	G	R	A	A-TH	DLBCL,NOS
22	79	F	N	LN	P	N	P	P	P	ND	N	70	DLBCL,NOS	non-GCB	NA	NA	NA	NA	NA	NA	DLBCL,NOS
23	73	M	U	Skin	P	N	P	N	N	ND	N	70	DLBCL,NOS	GCB	G	ND	G	NA	G	A-DH	DLBCL,NOS
24	36	M	P	Parotid gland	P	P	P	ND	P	N	N	80	DLBCL,NOS	GCB	NA	NA	NA	G	G	A-DH	DLBCL,NOS
25	49	F	N	Tonsil	P	N	P	P	N	P	N	100	DLBCL,NOS	non-GCB	G	ND	G	NA	G	A-DH	DLBCL,NOS
26	58	M	P	Nasophary nx	P	N	P	P	P	ND	N	85	DLBCL,NOS	non-GCB	NA	NA	NA	NA	R	A-SH	DLBCL,NOS
27	66	M	N	LN	P	P	P	N	N	ND	N	80	DLBCL,NOS	GCB	NA	R	R	NA	NA	SHL	DLBCL,NOS
28	50	M	P	LN	P	P	P	P	N	ND	N	85	DLBCL,NOS	GCB	NA	R	R	NA	NA	SHL	DLBCL,NOS
29	42	M	P	Skin	P	P	P	P	N	ND	N	80	DLBCL,NOS	GCB	NA	NA	NA	NA	NA	NA	DLBCL,NOS
30	58	F	N	LN	P	P	P	P	ND	ND	N	85	DLBCL,NOS	GCB	NA	NA	NA	R	NA	A-SH	DLBCL,NOS
31	42	F	P	LN	P	P	P	P	P	ND	N	80	DLBCL,NOS	GCB	NA	NA	NA	NA	NA	NA	DLBCL,NOS
32	63	M	N	LN	P	N	N	N	N	N	N	90	DLBCL,NOS	non-GCB	NA	DNW	NA	NA	NA	NA	DLBCL,NOS
33	42	F	P	Liver	P	P	P	N	N	ND	ND	95	DLBCL,NOS	GCB	NA	NA	NA	NA	NA	NA	DLBCL,NOS

Key: A= amplification; DNW= did not work; F= female; G= gain; LN= lymph node; M= male; N= negative; NA= no aberration; ND= not done; P= positive; R= rearrangement; U= unknown

Case nr.	Age	Sex	HIV	Site	Immunohistochemistry								Previous WHO classification (3rd and 4th edition)	COO	FISH					Atypical hits	WHO classification (5th edition)
					CD20	CD10	BCL6	MUM1	BCL2	MYC	EBER-ISH	KI-67(%)			*MYC BAP*	*MYC DFP*	*MYC combined*	*BCL2*	*BCL6*		
34	27	F	U	LN	P	N	P	P	P	ND	N	85-90	DLBCL,NOS	non-GCB	NA	NA	NA	NA	NA	NA	DLBCL,NOS
35	47	M	P	Skin	P	N	N	P	N	ND	N	95	DLBCL,NOS	non-GCB	R	ND	R	NA	G	SHL	DLBCL,NOS
36	34	F	P	Axilla	P	N	P	N	N	ND	N	95	DLBCL,NOS	GCB	G	ND	G	NA	G	A-DH	DLBCL,NOS
37	23	F	N	LN	P	N	P	P	N	ND	P	90	EBV-positive DLBCL,NOS	non-GCB	NA	NA	NA	NA	NA	NA	EBV-positive DLBCL,NOS
38	62	M	N	LN	P	N	P	N	P	ND	N	70	DLBCL,NOS	non-GCB	NA	NA	NA	NA	NA	NA	DLBCL,NOS
39	59	M	N	Axilla	P	N	P	P	N	ND	N	80	DLBCL,NOS	non-GCB	NA	NA	NA	NA	NA	NA	DLBCL,NOS
40	54	M	N	LN	P	N	N	P	P	ND	N	75	DLBCL,NOS	non-GCB	NA	R	R	NA	NA	SHL	DLBCL,NOS
41	33	M	N	Mediastinum	P	P	P	P	P	ND	N	80	DLBCL,NOS	GCB	NA	ND	NA	NA	NA	NA	DLBCL,NOS
42	48	F	P	LN	P	ND	N	N	N	ND	P	90	EBV-positive DLBCL,NOS	non-GCB	G	ND	G	NR	G	A-DH	EBV-positive DLBCL,NOS
43	48	M	N	LN	P	N	P	P	P	ND	N	70	DLBCL,NOS	non-GCB	NA	NA	NA	NA	G	A-SH	DLBCL,NOS
44	57	M	N	LN	P	ND	P	P	P	ND	N	60-70	DLBCL,NOS	GCB	R	ND	R	R	NA	DHL	DLBCL with *MYC* and *BCL2* R
45	27	M	N	LN	P	P	P	ND	P	ND	N	80	DLBCL,NOS	GCB	NA	NA	NA	R	NA	A-SH	DLBCL,NOS
46	28	M	N	LN	P	N	N	P	P	ND	N	70	DLBCL,NOS	non-GCB	NA	R	R	NA	NA	SHL	DLBCL,NOS
47	55	M	N	Vertebra	P	P	P	N	P	N	ND	65	DLBCL,NOS	GCB	NA	NA	NA	R	G	A-DH	DLBCL,NOS
48	76	M	N	LN	P	P	N	P	P	ND	ND	85	DLBCL,NOS	GCB	NA	NA	NA	NA	NA	NA	DLBCL,NOS
49	56	F	P	Thigh	P	P	P	P	P	ND	N	>95	DLBCL,NOS	GCB	R	ND	R	NR	R	SHL	DLBCL,NOS
50	67	M	U	Adrenal gland	P	P	P	P	P	P	ND	>85	DLBCL,NOS	GCB	R	ND	R	R	R	THL	DLBCL with *MYC, BCL2* and *BCL6* R

Key: A= amplification; DNW= did not work; F= female; G= gain; LN= lymph node; M= male; N= negative; NA= no aberration; ND= not done; P= positive; R= rearrangement; U= unknown

Case nr.	Age	Sex	HIV	Site	Immunohistochemistry								Previous WHO classification (3rd and 4th edition)	COO	FISH					Atypical hits	WHO classification (5th edition)
					CD20	CD10	BCL6	MUM1	BCL2	MYC	EBER-ISH	KI-67(%)			*MYC BAP*	*MYC DFP*	*MYC combined*	*BCL2*	*BCL6*		
51	36	F	N	Tonsil	P	N	P	N	P	N	ND	70	DLBCL,NOS	GCB	DNW	ND	DNW	DNW	DNW	ND	DLBCL,NOS
52	47	F	N	Tonsil	P	P	P	P	P	P	ND	90	DLBCL,NOS	GCB	DNW	ND	DNW	DNW	DNW	ND	DLBCL,NOS
53	29	F	N	LN	P	N	P	N	N	N	N	ND	DLBCL,NOS	GCB	DNW	ND	DNW	DNW	DNW	ND	DLBCL,NOS
54	62	F	N	Skin	P	P	N	P	P	N	N	40	DLBCL,NOS	non-GCB	ND	ND	ND	ND	DNW	ND	DLBCL,NOS
55	38	F	P	LN	P	N	N	N	N	N	ND	80	DLBCL,NOS	non-GCB	ND	ND	ND	ND	DNW	ND	DLBCL,NOS
56	37	F	P	LN	P	P	N	P	P	N	N	50	DLBCL,NOS	non-GCB	ND	ND	ND	DNW	DNW	ND	DLBCL,NOS
57	18	F	P	Lacrimal gland	P	N	P	P	p	N	N	80	DLBCL,NOS	non-GCB	ND	ND	DNW	DNW	DNW	ND	DLBCL,NOS
58	59	F	N	Cervix	P	N	P	P	N	N	ND	90	DLBCL,NOS	non-GCB	ND	ND	DNW	DNW	DNW	ND	DLBCL,NOS
59	52	M	N	Skin	P	P	P	N	P	N		80-85	DLBCL,NOS	GCB	DNW	ND	DNW	DNW	ND	ND	DLBCL,NOS
60	47	M	N	Testis	P	P	P	N	N	P	ND	70	DLBCL,NOS	GCB	DNW	ND	DNW	DNW	ND	ND	DLBCL,NOS
61	53	M	N	Spinal epidural	P	N	N	P	P	N	N	80	DLBCL,NOS	non-GCB	DNW	ND	DNW	DNW	ND	ND	DLBCL,NOS
62	57	M	N	Testis	P	P	N	P	P	N	N	90	DLBCL,NOS	non-GCB	DNW	ND	DNW	DNW	ND	ND	DLBCL,NOS
63	24	M	P	LN	P	P	P	P	P	P	ND	70	DLBCL,NOS	GCB	DNW	ND	DNW	NR	NA	ND	DLBCL,NOS
64	52	M	P	Cecum	P	P	P	ND	N	P	N	75-80	DLBCL,NOS	GCB	ND	ND	ND	DNW	DNW	ND	DLBCL,NOS
65	49	M	P	Vertebra	P	N	P	N	P	P	N	80	DLBCL,NOS	GCB	ND	ND	ND	ND	DNW	ND	DLBCL,NOS
66	19	M	P	LN	P	ND	P	P	N	N	ND	80	DLBCL,NOS	non-GCB	DNW	ND	DNW	DNW	DNW	ND	DLBCL,NOS
67	25	M	P	Brain	P	ND	N	ND	P	N	P	80-90	EBV-positive DLBCL,NOS	non-GCB	ND	ND	ND	ND	DNW	ND	EBV-positive DLBCL,NOS
68	40	M	P	LN	P	ND	P	N	N	P	ND	95%	DLBCL,NOS	GCB	R	ND	R	DNW	DNW	ND	DLBCL,NOS

Key: A= amplification; DNW= did not work; F= female; G= gain; LN= lymph node; M= male; N= negative; NA= no aberration; ND= not done; P= positive; R= rearrangement; U= unknown

Case nr.	Age	Sex	HIV	Site	Immunohistochemistry								Previous WHO classification (3rd and 4th edition)	COO	FISH					Atypical hits	WHO classification (5th edition)
					CD20	CD10	BCL6	MUM1	BCL2	MYC	EBER-ISH	KI-67(%)			*MYC BAP*	*MYC DFP*	*MYC combined*	*BCL2*	*BCL6*		
69	40	F	P	Skin	P	ND	N	P	P	N	ND	95	DLBCL,NOS	non-GCB	ND	ND	ND	ND	DNW	ND	DLBCL,NOS
70	44	F	N	Nasal	P	N	P	P	P	P	N	90	DLBCL,NOS	GCB	NR	ND	NR	ND	DNW	ND	DLBCL,NOS
71	55	F	N	LN	P	N	N	P	P	N	ND	80	DLBCL,NOS	non-GCB	DNW	ND	DNW	DNW	DNW	ND	DLBCL,NOS
72	42	M	P	LN	P	P	P	P	N	P	N	80	DLBCL,NOS	GCB	DNW	ND	DNW	ND	R	ND	DLBCL,NOS
73	27	M	P	LN	P	ND	P	P	N	P	N	85	DLBCL,NOS	GCB	ND	ND	ND	ND	DNW	ND	DLBCL,NOS
74	29	F	P	LN	P	ND	P	N	ND	P	N	ND	DLBCL,NOS	GCB	ND	ND	ND	ND	DNW	ND	DLBCL,NOS
75	48	M	N	Oropharynx	P	ND	P	P	P	P	ND	85-90	DLBCL,NOS	GCB	ND	ND	ND	ND	DNW	ND	DLBCL,NOS

Key: A= amplification; DNW= did not work; F= female; G= gain; LN= lymph node; M= male; N= negative; NA= no aberration; ND= not done; P= positive; R= rearrangement; U= unknown

APPENDIX 4: Summary of previous studies

First author and year	Numbers	Probe information	Cutoff values	Findings	MYC Findings	BCL2 Findings	BCL6 Findings
Foot 2011	162 non-Burkitt high-grade B-cell non-Hodgkin's lymphomas (HG-BNHL).	MYC, BCL6: DAKO BAP IGH/BCL2 and IG-MYC: ABOTT DFP	NA	160/162 cases worked abnormalities in 118/160 (74%)	R: 35/160 (22%) G/CNV:31/160 (19%) A: 0/160 (0%)	R: 28/160 (18%) G: 44/160 (2%) A: 4/160 (3%)	R: 18% (29/160) G: 35/158(22%) A:0/158 (0%)
Akyurek 2012	250 DLBCL	*BAP: MYC, BCL2* and *BCL6* (Dako)	>10%	239/250 Successful	R:6/239 (2.5%)	R:15/239 (6.28%) IHC: 75/ (53%)	R:29/239 (12.13%) IHC: 77/ (53%)
Perry 2013	39 BCLU	DFP: IGH/MYC, IGH/BCL2 BAP: MYC, BCL6		BCLU median age: >69 years Abnormalities in 80% of cases DH:8/35(23%) CNV:14/35	R: 17/35 (48.6%) IGH/MYC:12/17	R:5/35(14%)	R: 1/35(3%)
Horn 2013	506 DLBCL	BAP: Vysis LSI BCL2-, BCL6 MYC (Vysis/Abbott Molecular Diagnostics)	MYC: 15% BCL2: 13% BCL6: 12%	SHL: 14/35 (40%) DH: 17/350 (4.9%) TH: 4/350 (1.1%)	R:36/407 (8.8%) IHC:90/ 283(31.8%)	R: 52/384 (13.5%) IHC: 313/393(79.6%)	R: 116/404(28.7%) IHC: 284/343 (82.8%)
Bellas 2014	100 DLBCL	BAP: Abott laboratories *MYC, BCL2* and *BCL6*	FISH: >10% IHC MYC: 40% and 50%	Males:53; Females: 47 Median age:61 Age range: 18–88 years Nodal: 50%; Extranodal: 50% GCB: 51% ; Non-GCB: 49% DEL: 21/100 (21%) = non-GC DHL: 7% and THL: 2%	Aberrations: 30/100 (30%) R: 15/100 IHC: 29/ 100 (29%) Gene and protein expression:13/100 (13%)	R: 20 /100 (20%) A:35 / 100 IHC: 62/ 100 (62%),	Aberration: 52/100 (52%) R:29/100 (29%) A: 30 /100 (30%) IHC: 67 /100 (67%)
Tzankov 2014	563 DLBCL	BAP: MYC, BCL2 and BCL6 DFP: IGH/MYC/CEP VYSIS Abott	IGH/MYC: >6.5% MYC: >4% BCL2: >3% BCL6: >1.5%	DH (8/563 (1.4% TH: 2/563 (0.4%)	R:20/432 (4.6%) G: 55/432(13%) A: 3/432 (0.7%)	R:80/440 (18%) A: 4/440 (1%) G: 54/440 (12%)	R:121/376 (32%) A: 6/376 (1.6%) G:89/376 (24%)
Morton. *2014*	177 NHL			HIV-:116; HIV+: 51 HIV+ 100% male between 28-58years. 37years median non-GCB; R: 31% HIV-: Even male and female (65years median, range 16-90); Extra nodal=61% R: 47%	GCB predominance (HIV-)	GCB predominance (HIV-)	Non-GCB predominance
Chang 2016	153 DLBCL	BAP DAKO Japan *BCL2, MYC, BCL6, IGH/MYC*		BCL2=GCB MYC=non-GCB DH:3/153 2% (2 BCL6/MYC 1 MYC/BCL2)	R:6/153 (3.9%)	R:7/153 (4.6%)	R:40/153 (26.1%)

Key: NA= Not available; BCLU= B-cell lymphoma unclassifiable R=rearrangement; A= amplification; G= gain; CNV= copy number variations; HIV-= HIV-negative; HIV+= HIV-positive; LSI= Locus specific identifier ; DLBCL= Diffuse large B-cell lymphoma ; HGBCL= high grade B-cell lymphoma ; NOS= not otherwise specified

First author and year	Numbers	Probe information	Cutoff values	Findings	MYC Findings	BCL2 Findings	BCL6 Findings
Oliveira 2017	120 BCLU, DLBCL, BL	BAP: MYC and BCL6 (Vysis Abbott) BCL2 (Dako)	5%	DH:3/120 (2.5%) DLBCL GCB: 34/70 (48.6%) Non-GCB:36/70 (51.4%)	DHL: 3/3(100%) DLBCL: R:3/72(4.2%)	DHL:2/3(66.7) DLBCL: 11/72(15.3%)	DHL:1/3(33.3%) DLBCL: 9/72(12.5%)
Timlin 2018	367 DLBCL			DH/TH: 38/367 (10%) GCB: 222/367 (60%) Non-GCB:145/367 (40%) DEL: 92/227 (41%)	R:77/367 (21%) IHC: 136/237 (57%)	NA	NA
Li 2018	212 DLBCL			DE: 42/212 (19.8%)	IHC: 76/212 (35.8%)	IHC:145/212(58.4%)	IHC: 178/212 (84.0%)
Herrera 2018	50 de novo DLBCL, 25 patients transformed indolent lymphoma 3 BCLU.	*NA*	NA	DEL: 31/78 (47.0%) Atypical hits: 17/78 (22.0%) SHL: 11/78 (14.0%) DHL: 10/78 (13.0%)	IHC:40/78 (51.3%) R: 21/78 (27.0%)	NA	NA
Chen 2019	82 PCNS DLBCL			GCB:15/82 (30.5%) Non-GCB: 49/82(59.8%) COO unclassifiable: 8/82 DEL: 44/49 (22.4%)	IHC: 16/49 (32.7%)	IHC: 40/58(69%)	IHC: 61/73 (83.6%)
Zhang 2019	67 DLBCL	MYC, BCL2 and BCL6 (Beijing Jinpujia Company)	3%	non-GCB:61.9% (26/42) GCB: 16/42 (38.1%) DH:2/42(4.76%) (1 MYC/ BCL2 and 1 MYC/BCL6)	R:10/42 (23.8%) A:2.4%	R:18/42 (42.9%) A:11/42 (26.19%)	R:6/42 (14.29%) A: 3/42 (7.14%)
Rosenwald 2019	2,383 DLBCL	*MYC, BCL2* and *BCL6* BAP (Abbott Molecular) IGH/MYC	NA	53/264 failed DH with *BCL2*: 82/264 (32.8%) DH with *BCL6*: 31/264 (11.7%) TH: 26/264 (9.8%)	R: 264 / 2383 (11%) *MYC* SHL: 72/264 (27.2%)	R:82/264 (31.0%)	R:31/264(11.7%)
Huang 2019	130 DLBCL			GCB: 50/130 (38.5%) Non-GCB: 80/130 (61.5%) DEL: 51/130 (39.2% DHL: 8/130 (6.2%) THL: 2/130 (1.5%) Atypical DH: 7/130 (5.4%) Atypical THL: 5/130 (3.8%	G: 13/130 (10.0%) A: 1/130 (0.8%) R: 13/130 (10.0%) IHC: 61/130 (46%)	G: 22/130 (16.9%) A: 4/130 (3.1%) R: 19/130 (14.6%) IHC: 98/130 (75.4%) IHC+R: 3 /130 (2.3%)	G: 18/130 (13.8%) A: 1/130 (0.8%) R: 22/130 (16.9%) IHC: 61/130 (46.9%)
Ting 2019	120 cases of de novo DLBCL			Age: 18 to 86 years Nodal: 43.3% Extra-nodal: 56.7% non-GCB: 74.2% /120 GCB: 25.8% DH:2/120 EBV positive: 8/120 (6.7%) DEL:16/120 (13.3%)	R:7/120 (5.8%)	R:7/120 (5.8%)	R: 17/120 (14.1%),

Key: NA= Not available; BCLU= B-cell lymphoma unclassifiable R=rearrangement; A= amplification; G= gain; CNV= copy number variations; HIV-= HIV-negative; HIV+= HIV-positive; LSI= Locus specific identifier ; DLBCL= Diffuse large B-cell lymphoma ; HGBCL= high grade B-cell lymphoma ; NOS= not otherwise specified

First author and year	Numbers	Probe information	Cutoff values	Findings	MYC Findings	BCL2 Findings	BCL6 Findings
Vaughan 2019	77 DLBCL	*MYC, BCL2 and BCL6* (Probe details =NA)	NA	DHL: 3/77 (3.9%)	*R*: 17/77 (22.1%).	R:0/55 (0%)	*R:* 7/48 (15.6%)
McPhail 2019	100 DLBCL or BCLU			Male: 64/100 Female: 36/100 Age: median= 61 (29-87) years. GCB: 91/100 (91%) Non-GCB: 6/100 (6%) DEL: 30 / 37 (81%)	IHC: 37/ 43 (86%)	IHC: 76 /f84 (90%)	N/A
Naseem 2020	74 high-grade DLBCL cases	*IHC: DAKO probes*	IHC cutoff: MYC=>40% BCL2= >50%	DEL: 11/74 (14.8%) TE: 2/74	IHC: 14%	IHC: 5%	
Willenbacher 2020	78 DLBCL,NOS/BCLU	*MYC, BCL2, and BCL6* BAP (ZytoLight)	CNGs were diagnosed when there were ≥ 3 fusion signals/ cell.	DLBCL, NOS DH: 6/78 (7.7%) BCLU DH: 1 (1.3%) BCLU TH: 1 (1.3%) CNGs: 8(10%)	R: 3/77 (4%) IHC: 17/84 (20.2%)	R:8/ 76 (10%) IHC: 49/84 (58.3%)	R: 10/70 (13%) IHC: 47/84 (56.0%)
Cassidy 2020	69 LBCL	BAP: *MYC a*nd *BCL-6* DFP: *IGH/MYC* and *IGH/BCL2* Vysis (Abbott Molecular)	IHC: Myc=40% BCL2= 50%	Age: average= 60 years Range= 33-92 years Male: 38/69 (55%) Female:31/69 (45%) GCB: 44/69 (64%) Non-GCB: 25/69 (36%) DEL: 30/69 (45%)	R: 4/69 (5.6%) *IGH/MYC* 6/69 (8.7%) non–IGH/MYC	R: 18/69 (26%)	R:19 /69(27.5%)
Mehta 2020	172/200 DLBCL selected	ZytoVision. BAP *BCL2, BCL6* and *MYC*	15%	DHL: 7/172 (4.1%) DEL: 20/172 (11.6%) DHL =DEL: 6/7 GCB: 85/172(49.4%) Non-GCB: 87/172 (50.6%)	NA	NA	NA
Cassim 2020	181 DLBCL NOS HIV-:131/181 (72.4%) HIV+: 50/181 (27.6%)	*NA*	NA	131 HIV- (median age 52 years) 50 HIV-positive (median age 39 years) HIV-positive: non-GCB= 24/ 50 (48%) GCB= 26/50 (52%) Nodal: 128/181 (71%) Extra-nodal: 53/181 (29%) DE: 9%	NA	NA	

Key: NA= Not available; BCLU= B-cell lymphoma unclassifiable R=rearrangement; A= amplification; G= gain; CNV= copy number variations; HIV-= HIV-negative; HIV+= HIV-positive; LSI= Locus specific identifier ; DLBCL= Diffuse large B-cell lymphoma ; HGBCL= high grade B-cell lymphoma ; NOS= not otherwise specified

First author and year	Numbers	Probe information	Cutoff values	Findings	MYC Findings	BCL2 Findings	BCL6 Findings
Salam 2020	81 B-cell NHL	BAP: *MYC, BCL2* and BCL6 (Dako)		DH: 1/57 (1.8%) TH: 3/57 (5.3%)	R:4/57 (7.0%) G: 5/57 (8.8%)	R: 3/57 (5.3%) G:13/57 (22.8%)	R: 10 /57(17.5%) G:6/57 (10.5%)
Cucco 2020	553 DLBCL cases	Dako BAP MYC Abbott MYC BAP and IGH/MYC DFP *BCL2 and BCL6:* manufacturer NA	NA	THL: 11/553 (1.9%) DHL:73/553 (13.2%) MYC/BCL2 DH: 51/553 (9.2%); MYC/BCL6 DH: 22/553 (4.0%)	R: 125/553 (22.6%)	R: 136/553 (24.6%)	R: 97/553 (17.5%)
Meriranta 2020	181 DLBCL	NA	NA	DEL: 48/147 (33%)	R:18/130 (14%)	R:24/152 (16%) A/G:36/181 (20%)	R:25/145 (17%) G: 23/181(13%)
Tsai 2021	282 patients with DLBCL	BAP: *MYC, BCL2* and BCL6	FISH: 20%	SH: 23/ 282 (8.2%) GCB: 116/282(41.1%) Non-GCB: 166/282 (58.9%) DH and TH: 24/282 (8.5%)	R: 47/ 282 (16.7%) IHC: 162/282(57.44%)	R: 8/47 (17.0%) IHC:244/282 (86.5%)	R:16/47 (34.0%) IHC: 213/282 (75.3%)
Cho 2021	2102 LBCL 480 suitable 241 selected for FISH testing	BAP: MYC BCL2.BCL6 (VYSIS) LSI IGH/ MYC/CEP 8 tricolour DFP Abbott .	R: 5% G: > 3%	DLBCL: 181/241 DHL: 18/241 (7.4%) THL: 3/241 (1.2%) HGBCL, NOS:39/241 (16.2%)	R: 55/241 (22.8%) CNV: 19/241 (7.9%) IGH/*MYC* DFP: 5/6 (83.3%)	NA	NA
Collinge 2021	802 DLBCL	BAP: *MYC* and *BCL2* IHC: MYC and BCL2	R: 5% G: 25%	HGBL-DH/TH-BCL2 = GCB predominantly *MYC* and *BCL2* R= increased protein expression *BCL2* CNVs = increased protein expression / *MYC* CNVs were not.	G: 20% (152/771) R: 13% (104 /771) GCB R= 4% (11/274) MYC IHC: 70%	R: 28% (206 /740) G: 21% (155/740) CNV= increased protein expression	N/A
Khan 2021	43 DLBCL	*NA*	NA	GCB: 24/43 (56%) Non-GCB: 9/43 (21%) Unknown COO: 10/43 (23%) DEL: 19/41 (46%)	IHC: 26/42 (62%) R: 4/20 (20%)	IHC:31/41 (78%) R: 5/20 (25%)	
Tourneret 2021	97 DLBCL	BAP *BCL6, BCL2* (DAKO) *MYC* BAP Vysis	15%	Atypical R:19/ 97 (19,6%)	R:38/95 (40%)	R:70/94 (74.5%)	R: 5/19 (26%)
Baptista 2022	75 HIV-associated aggressive B-NHL and 47 DLBCL	*MYC, BCL6 BCL2* BAP DFP *MYC; IGH/MYC*/CEP8	Not available		R: 7/47 (14.9%) IGH/MYC: 5/47	R: 2/47 (4.3%)	13/47 (27.7%)
Chapman 2022	30 HIV-DLBCL	IGH/*BCL2* and *IGH/MYC* and BAP *MYC* and *BCL6* Vysis	*IGH/BCL2* and *IGH/MYC*: ≥ 10% BAP *MYC* and *BCL6*: >15%	Median age: 46 years Site: extra-nodal (67%) Non-GCB: 13/26(50%) GCB: 13/26 (50%) EBER +: 13 (48%) EBER +=non-GCB (70%)	R: 9/16 (56%) 4/16 EBER POS	R:0/16 (0%)	R:3/16 (19%)

Key: NA= Not available; BCLU= B-cell lymphoma unclassifiable R=rearrangement; A= amplification; G= gain; CNV= copy number variations; HIV-= HIV-negative; HIV+= HIV-positive; LSI= Locus specific identifier ; DLBCL= Diffuse large B-cell lymphoma ; HGBCL= high grade B-cell lymphoma ; NOS= not otherwise specified

First author and year	Numbers	Probe information	Cutoff values	Findings	MYC Findings	BCL2 Findings	BCL6 Findings
Pather 2022	51	*MYC CISH*		HIV+ GC=48/90 (53%) Non-GCB=42/90 (47%) HIV- GCB=12/21 (57%) Non-GCB=9/21 (43%) DEL: 28/110 (26%) 25% HIV+ 25% HIV-	CISH=46 successful		
Li 2023	136 HGBL, NOS DLBCL/HGBL-224 DLBCL-217	*MYC and BCL6* BAP IGH /BCL2 DFP	NA		HGBCL, NOS: R: 59/136 (43%) DLBCL/HGBL-DH: 224/224 (100) DLBCL, NOS: 28/217 (13%)	HGBCL, NOS: BCL2-R in 12/93 (13%). DLBCL/HGBL-DH:. 224/224 (100%) DLBCL, NOS 27/120 (23%)	HGBCL, NOS: R: 8/82 (10%) DLBCL/HGBL-DH: 76/224 (34%) DLBCL, NOS: 12/75 (16%)
Key: NA= Not available; BCLU= B-cell lymphoma unclassifiable R=rearrangement; A= amplification; G= gain; CNV= copy number variations; HIV-= HIV-negative; HIV+= HIV-positive; LSI= Locus specific identifier ; DLBCL= Diffuse large B-cell lymphoma ; HGBCL= high grade B-cell lymphoma ; NOS= not otherwise specified							

APPENDIX 5: Turn it in report

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12 April 2024