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Wästerlid, Tove

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A vibrant cosmic nebula with swirling clouds of pink, purple, and blue, punctuated by numerous bright, multi-colored stars. The nebula's structure is complex, with dense regions and wispy filaments.

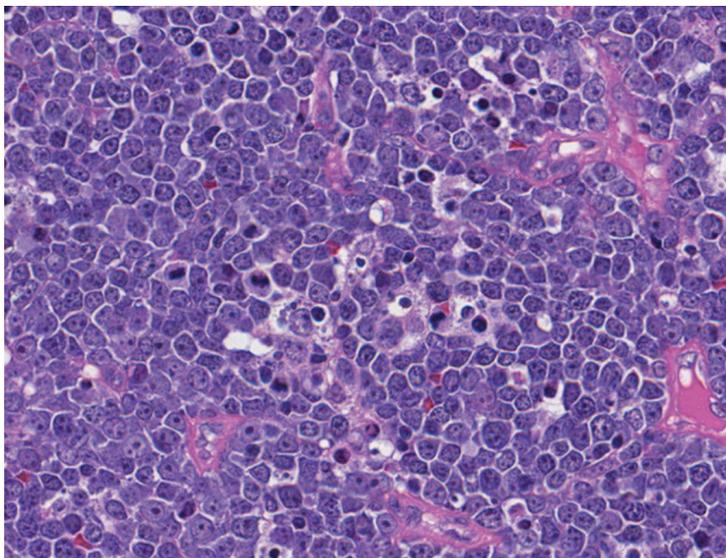
Burkitt lymphoma and diffuse large B-cell lymphoma

– therapeutic strategies and pathogenetic mechanisms

TOVE WÄSTERLID

ONCOLOGY AND PATHOLOGY | FACULTY OF MEDICINE | LUND UNIVERSITY





Burkitt lymphoma is one of the most aggressive tumours known, with a doubling time of approximately 24 hours. Owing to its rapid growth, and subsequent frequent apoptoses of tumour cells, Burkitt lymphoma is histologically characterised by a "starry sky" appearance, as pictured above. This morphological appearance is created by scattered macrophages that contain the ingested apoptotic tumour cells.



Burkitt lymphoma and diffuse large B-cell lymphoma – therapeutic strategies and pathogenetic mechanisms

A population based perspective

Tove Wästerlid, MD



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DOCTORAL DISSERTATION

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Faculty opponent

Associate professor Martine E.D. Chamuleau, VU University Medical Centre, Department
of Haematology, Amsterdam, The Netherlands

Supervisor

Associate professor Mats Jerkeman, Lund University, Department of Clinical Sciences,
Oncology and Pathology, Lund, Sweden

Co-supervisor

Professor Sara Ek, Lund University, Department of Immunotechnology, CREATE Health,
Lund, Sweden

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Title and subtitle: Burkitt lymphoma and diffuse large B-cell lymphoma – therapeutic strategies and pathogenetic mechanisms. A population based perspective.		
Abstract: <p>Burkitt lymphoma (BL) is a rare, aggressive disorder constituting ~1% of all non-Hodgkin lymphoma. Diffuse large B-cell lymphoma (DLBCL) is more common, accounting for ~30% of malignant lymphoma. Standard treatment for adult BL and for certain subgroups of patients with DLBCL remains to be defined due to paucity of randomised trials performed. The focus in this thesis lies on the effect of prognostic factors and treatment on outcome for patients with these two aggressive lymphomas, using unselected, population based patient cohorts.</p> <p>In the first and second study, prognostic factors and efficacy of treatment regimens used for adult BL patients were investigated using data from the Swedish lymphoma registry (SLR) and Danish lymphoma registry (study two). Age was determined the most important predictor of adverse prognosis, and improvement in outcome during the study period was restricted to patients aged ≤ 65. Also, the superiority of high-intensive chemotherapy regimens compared to low-intensive treatment was confirmed, whereas the role of the monoclonal antibody rituximab remained undefined.</p> <p>In the third study, the impact of dose-dense chemotherapy administration and addition of etoposide were evaluated for adult DLBCL patients, using data from a six-year period, collected from the SLR. Among all patients, there was no evidence of a difference in outcome between examined regimens. However, when restricted to patients ≤ 65, the addition of etoposide to the R-CHOP-14 regimen was associated with superior outcome.</p> <p>In study number four, the frequency and potential clinical implications of expression of the transcription factor SOX11 in adult BL was investigated. SOX11 is aberrantly expressed in various hematopoietic and solid malignancies and appears to affect clinicopathological characteristics. Fourteen of 45 examined adult BL samples expressed SOX11 and its presence did not impact overall survival, in our material. In contrast, SOX11 knockdown in a BL cell line resulted in increased cellular proliferation, suggesting a potential growth regulatory role for SOX11 in BL.</p> <p>Collectively, the studies included in this thesis provide real-world data regarding the effect on outcome of patient characteristics and treatment in adult BL and DLBCL. Although optimal treatment needs to be established in a randomised setting, this work emphasises the importance of high-intensive treatment and provides unselected, population based information on clinicopathological factors that affect outcome. Novel therapeutic strategies are warranted particularly for elderly patients, but will hopefully contribute to improve survival and decrease toxicity for all adult BL and DLBCL patients. Additionally, results presented in this thesis may possibly serve as comparative data for future population based studies.</p>		
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Burkitt lymphoma and diffuse large B-cell lymphoma – therapeutic strategies and pathogenetic mechanisms

A population based perspective

Tove Wästerlid, MD



LUND
UNIVERSITY

Lund University, Department of Clinical Sciences, Oncology
and Pathology, Lund, Sweden

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tove.wasterlid@med.lu.se

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Life, London, this moment of June

Virginia Woolf

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List of papers

This thesis is based on the following papers, which will be referred to in the text by their Roman numerals.

- I. Population based study of prognostic factors and treatment in adult Burkitt lymphoma: a Swedish Lymphoma Registry study.
Wästerlid T, Jonsson B, Hagberg H, Jerkeman M.
Leuk Lymphoma. 2011;52:2090-2096.
- II. Impact of chemotherapy regimen and rituximab in adult Burkitt lymphoma: a retrospective population based study from the Nordic Lymphoma Group.
Wästerlid T, Brown PN, Hagberg O, Hagberg H, Pedersen LM, D'Amore F, Jerkeman M.
Ann Oncol. 2013 Jul;24(7):1879-86.
- III. Impact on survival of addition of etoposide to primary chemotherapy in diffuse large B-cell lymphoma: a Swedish Lymphoma Registry study.
Wästerlid T, Hartman L, Szekely E, Jerkeman M.
Hematol Oncol. 2015 Sep 15. doi: 10.1002/hon.2256.
- IV. Frequency and Clinical Implications of SOX11 Expression in Burkitt Lymphoma.
Wästerlid T, Nordström L, Freiburghaus C, Pedersen M, Nørgaard P, Gang AO, Brown PN, Dictor M, Jerkeman M, Ek S.
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My contributions to the papers

Paper I

I was responsible for analysis of data and writing the manuscript.

Paper II

I participated in the study design and collection of data through review of medical records, and was responsible for data analysis and for writing the manuscript.

Paper III

I participated in the study design and was responsible for data analysis and for writing the manuscript, as well as managing all communication with co-authors and journal.

Paper IV

I participated in the design of the study and collection of data and was responsible for data analysis and for writing the manuscript. I was introduced to the laboratory work performed, and participated in the immunohistochemical analysis. I also managed all communication with co-authors and journal.

Selected abbreviations

ABC	Activated B-cell
ADCC	Antibody Dependent Cell-mediated Cytotoxicity
ALL	Acute Lymphoblastic Leukaemia
AID	Activation Induced Deaminase
ASCT	Autologous Stem Cell Transplantation
BCR	B-cell Receptor
BCLU	B-cell Lymphoma Unclassifiable
BFM	Berlin Frankfurt Munster
BL	Burkitt Lymphoma
CALGB	Cancer and Leukemia Group B
CDC	Complement Dependent Cytotoxicity
CHOP	Cyclophosphamide, doxorubicin, vincristine, prednisone
CHOEP	Cyclophosphamide, doxorubicin, vincristine, etoposide, prednisone
CI	Confidence Interval
CLL	Chronic Lymphocytic Leukaemia
CNS	Central Nervous System
CODOX-M/IVAC	Cyclophosphamide, cytarabine, doxorubicin, leucovorin, methotrexate, vincristine/ifosfamide, etoposide, cytarabine, IT methotrexate
CR	Complete Remission
CSR	Class Switch Recombination
DA-EPOCH	Dose Adjusted Etoposide, prednisone, vincristine, cyclophosphamide, doxorubicin
DLBCL	Diffuse Large B-cell Lymphoma
EBV	Epstein Barr Virus
EFS	Event Free Survival
FL	Follicular Lymphoma
GC	Germinal Centre

GCB	Germinal Centre B-cell
GEP	Gene Expression Profile
GMALL	German Multicenter study group for adult Acute Lymphoblastic Leukemia
HGBL	High Grade B-cell Lymphoma
HMG	High Mobility Group
HR	Hazard Ratio
Hyper-CVAD	Hyperfractionated cyclophosphamide, vincristine, doxorubicin, dexamethasone
IHC	Immunohistochemistry
IPI	International Prognostic Index
LDH	Lactate Dehydrogenase
LMB	Lymphome Malins de Burkitt
MCL	Mantle Cell Lymphoma
NHL	Non Hodgkin Lymphoma
NGS	Next Generation Sequencing
NOS	Not Otherwise Specified
OS	Overall Survival
PS	Performance Status
RCC	Regional Cancer Centre
SCR	Swedish Cancer Registry
SEER	Surveillance, Epidemiology and End Results
SLR	Swedish Lymphoma Registry
SHM	Somatic Hypermutation
TMA	Tissue Microarray
WHO	World Health Organisation

Introduction

Malignant lymphomas are clonal tumours originating from lymphoid cells that have acquired oncogenic mutations at distinct stages of differentiation. The history of lymphoma begins in 1832, when Thomas Hodgkin first described a malignant tumour in lymph nodes, which later became known as Hodgkin lymphoma [1]. The landscape of lymphoid neoplasms has since proven to be a complex and heterogeneous one, comprising more than 70 different entities in the current version of the WHO classification [2].

Rather bluntly, lymphomas can be divided into Hodgkin and Non-Hodgkin lymphoma (NHL). The latter group is stratified, according to cell of origin, into mature B-cell neoplasms, mature T- and NK-neoplasms, post-transplant lymphoproliferative disorders and histiocytic and dendritic cell neoplasms. Further subdivision is based on the widely differing clinical characteristics, morphology, phenotype and molecular profiles of the various lymphomas [2].

Due to the diversity of lymphoid neoplasms, and the ever evolving knowledge regarding their disease biology, consensus regarding the classification of lymphomas has proven enormously challenging. During the last 50 years a range of different classification schemes have been proposed, and variously utilised around the world [3-6]. In 2001, a 3rd update of the WHO classification was published, based on previous classifications, representing a world-wide agreement among more than 50 experts. So far, this classification in its 4th version, published in 2008 and recently updated 2016, is the closest to achieve a golden standard for classifying hematopoietic malignancies. However, it is a continuously evolving document containing several provisional entities [2, 7].

The multi-faceted role of the immune system is reflected in the heterogeneous clinical presentations of lymphoid malignancies, ranging from indolent entities to some of the most aggressive, fastest growing tumours known. Advances in medical research during recent decades have led to a greater understanding of the underlying biology of lymphomas. With modern treatment, the outcome of patients with lymphoid cancers have vastly improved [8].

The focus in this thesis lies on Burkitt lymphoma (BL) and diffuse large B-cell lymphoma not otherwise specified (DLBCL NOS), both aggressive mature B-cell neoplasms.

B-cell lymphomagenesis

B-cells function in the humoral immune system by secreting high-affinity antibodies as well as by recognising and presenting antigens. The aim of B-cell development is to generate B-cells with a broad repertoire of antigen recognition that produce antigen-specific immunoglobulins (Ig). To achieve this, B-cells undergo a strict selection process during the course of their maturation, as well as several processes altering the gene segments coding the heavy and light chains of antibodies [9, 10]. That mechanisms involved in the pursuit of producing high-affinity B-cells are also implicated in malignant transformation, is evident by the fact that lymphomas of B-cell origin account for approximately 95% of all lymphoid neoplasms, despite that the ratio between T- and B-cells in the human body is similar [11-13].

In the bone marrow, the development of B-cells is initiated by a process termed V(D)J recombination, in which the Ig heavy- and light-chain genes are reassembled. Only B-cells where this rearrangement results in the expression of a surface antibody functioning as an antigen receptor, the B-cell receptor (BCR), are chosen for survival. Moreover, these newly formed B-cells are required to pass the first, of several, autoreactive checkpoints before they are allowed to enter the blood stream as mature naïve B-cells [9, 10, 14].

Further risk for DNA damage and oncogenic translocations occur in the next step of B-cell differentiation, which is initiated by binding of antigen to the BCR on a circulating, naïve B-cell. At this stage, naïve B-cells enter T-cell rich areas of secondary lymphoid organs, where they form and aggregate into germinal centres (GC). The GC is the site where B-cells undergo clonal expansion, as well as the two other mechanisms involved in remodelling of Ig loci, somatic hypermutation (SHM) and class switch recombination (CSR) [15].

The GC consists of a dark and a light zone. The dark zone harbours B-cells undergoing rapid proliferation and SHM of the V-region in Ig-genes, with the aim to increase antigen affinity. In the light zone, a fraction of B-cells is subject to CSR, in which the constant region of Ig heavy chain is rearranged to create different isotypes of antibody. Also in the light zone, B-cells are selected on the basis of the affinity of their BCR, to either progress into plasma- or memory B-cells, re-enter the dark zone for further modification of Ig-genes or undergo apoptosis (Figure 1) [15, 16].

The GC reaction is initiated, and controlled, by a complex transcriptional network, which has only recently begun to be elucidated [15]. One component is activation-induced cytidine deaminase (AID), which triggers both SHM and CSR. Although well regulated, this enzyme is not entirely specific to the Ig locus, thus resulting in the risk for mutations in oncogenes and breaks in DNA leading to genomic

instability and translocations [16]. In turn, AID is upregulated via B cell lymphoma 6 (BCL6). BCL6 is thought to be a master regulator of the GC reaction by sanctioning a transcriptional network enabling alterations of Ig gene segments, impairing terminal B-cell differentiation and increasing the threshold of response to DNA damage. Also, in order for B-cells to exit the GC and differentiate, down-regulation of BCL6 is essential [15].

The GC is the site of origin for several B-cell lymphomas, evidenced by the presence of hypermutated V-regions in their Ig loci [14]. Genome sequencing has revealed that distinct lymphoma subtypes correspond to specific stages of GC development (Figure 1) [15-20]. It appears that many mature B-cell neoplasms adopt the gene expression program of their normal GC B-cell counterparts and exploit it in conjunction with genetic lesions that allow them to abrogate autoregulatory circuits of GC phases, blocking further maturation and enabling malignant development [15, 16]. The importance of retaining some normal B-cell physiology for survival of most B-cell lymphomas, is exemplified by the fact that a functional BCR is preserved in a majority of B-cell lymphomas. Thus, the, for B-cell lymphomas typical, Ig chromosomal translocations predominantly target the non-functional Ig alleles [21].

In summary, a combination of accumulated oncogenic mutations and translocations during the venturesome B-cell development ultimately enables the B-cell to evade normal regulatory apoptosis, resulting in the development of a malignant clone, and subsequent evolution of a B-cell lymphoma.

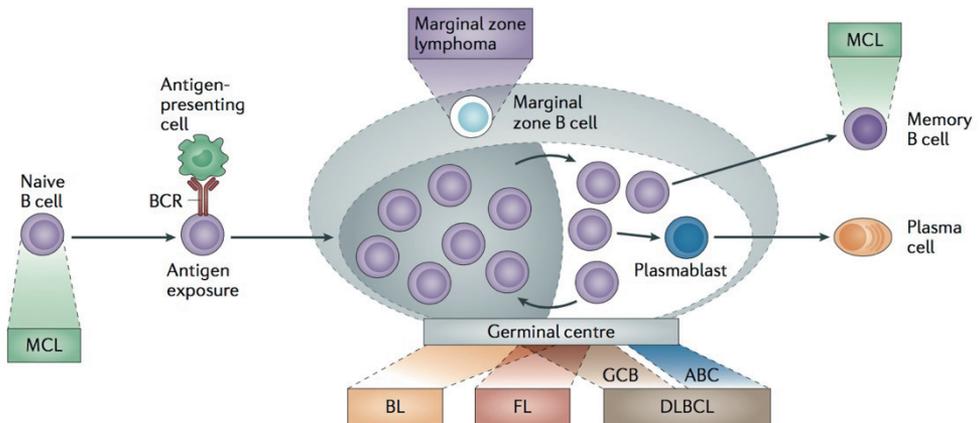


Figure 1. B-cell development and putative cell-of-origin for some B-cell malignancies. Mantle cell lymphoma (MCL) is thought to predominantly originate from naïve B-cells, although 15–40% show evidence of SHM. BL, DLBCL and Follicular lymphoma (FL) exhibit a germinal centre phenotype resembling cells from the GC light zone. However, a proportion of BL exhibit profiles more reminiscent of the dark zone and the Activated B-cell like (ABC) DLBCL share similarities with the pathway to plasma cell differentiation, the plasmablast stage. From [22]. Reprinted by permission from Macmillan Publishers Ltd: Nature Reviews Cancer © 2014.

Background

Burkitt lymphoma

In spite of the fact that BL is a rare disorder, constituting only 1-2% of NHL [8, 11, 12], this neoplasm has served a compelling role in contributing to the general knowledge of tumorigenesis. BL was first described by the Irish surgeon Denis Burkitt in Uganda in 1958 [23]. Children in Africa presenting with rapidly growing tumours of the jaw had been described previously [24, 25], but Burkitt was the first to compile a description of a number of cases. Moreover, he was involved in further characterisation and mapping of its distribution in Africa [26, 27]. In addition, Burkitt served as a link in the detection of the first tumour-associated virus when he supplied the virologists Epstein and Barr with a sample of endemic BL. From this specimen, the first virus particles of the Epstein-Barr virus (EBV) were extracted in 1964 [28]. Furthermore, BL samples were also implicated when the first oncogenic chromosomal translocation was described [29-31], and was the first lymphoma found to be associated with human immunodeficiency virus (HIV) [32]. Thus, that BL is sometimes nicknamed the “Rosetta stone” of cancer, is not surprising.

Classification and epidemiology

There are three clinical subtypes of BL: endemic (eBL), sporadic (sBL) and immunodeficiency-associated BL [33]. Although these variants share similar morphology, immunophenotype and largely conform with regard to genetic profile [34-37], they differ in incidence pattern and exhibit some unique clinical features.

Sporadic BL is a rare disorder found in areas that lack endemic malaria. It affects all ages and accounts for approximately 1% of adult lymphomas, equalling ~15 BL patients per year in Sweden [11, 12]. Due to smaller numbers of NHL in children, sBL accounts for 30% of lymphomas in this group [11, 12]. Incidence rates appear to be bimodally age-specific, with a peak incidence in children and a later peak at age >60 [38, 39]. It is more prevalent among white males [8, 11]. sBL is associated with EBV to a lesser degree, with EBV present in only 10-20% of cases [40-42].

Endemic BL is predominantly a paediatric disease with a high incidence of approximately 3-6 cases/100 000 children, accounting for 30-50% of paediatric cancer in areas with holoendemic malaria [43]. It was the variant of BL first described by Denis Burkitt and because of its relatively high occurrence is designated 'endemic'. More than 95% of eBL are associated with EBV [41, 43-45]. Clinically, it differs by frequently presenting with involvement of the jaw [46].

The immunodeficiency-subtype was first recognised in the setting of HIV, where BL accounts for 10-40% of HIV-associated lymphomas [32]. Discrepant to other HIV-associated malignancies, BL often occurs among patients with CD4 counts >200 [39, 47]. In the USA, the incidence rate per year is 22/100 000 [39]. Approximately 30-40% are associated with EBV [48, 49]. Genetically, it is more similar to eBL than sBL [35]. The risk of BL among other immunosuppressed patients is increased, but not as high as among HIV-infected individuals [50].

Aetiology

The aetiology of BL, and of NHL overall, is yet to be deciphered [51]. Regarding sBL little is known of its aetiology, and it is considered to arise 'sporadically', hence its name. Based on epidemiological observations, some causative agents have been considered for BL.

EBV

Due to the epidemiological observations that nearly all cases of eBL harbour EBV, a link between BL and EBV has been suspected, and for eBL later confirmed [44, 45]. However, due to the lack of EBV in the majority of sBL and HIV-associated BL it cannot be a requirement for pathogenesis. It is hypothesized that the mechanism by which EBV may aid malignant transformation is to induce an immortalised state of the B-cells it infects, thus enabling EBV-carrying cells to avoid apoptosis even with acquired oncogenic mutations. Also, EBV is thought to promote genomic instability and allow infected cells to avoid immune surveillance [41, 43, 48, 52, 53].

Malaria

The study of eBL in regions with holoendemic plasmodium falciparum malaria have revealed a synergistic effect of EBV and malaria in causing eBL [44, 45, 54]. The main tumorigenic mechanisms of malaria are thought to be that it increases the expression of AID in B-cells, augmenting the mutational load [55-57]. Also, malaria promotes EBV-dysregulation, which expands the number of EBV-infected cells, thus increasing the probability for survival of cells carrying DNA damage.

Moreover, malarial infection causes cell hyperplasia, may activate chronic BCR-signalling, and appears to impair cytotoxic T-cell control [48, 55].

Other

Immunosuppression is believed to contribute to tumorigenesis by inflicting increased EBV load and dysfunctional immune regulatory mechanisms [51]. Neither environmental factors nor the use of tobacco or alcohol have been associated with BL, although some claim that arboviruses and certain plant toxins contribute to the formation of eBL [58]. Epidemiological observations indicate that BL among patients aged <50 is associated with presence of eczema and is inversely correlated with allergy [59].

Clinical presentation

BL is the fastest growing tumour known, with a doubling time of approximately 25 hours [40]. Because of this, patients frequently present with rapidly disseminating extranodal disease (~40%) and with tumour bulk >10 cm. Often, clinical and laboratory evidence of tumour lysis syndrome, such as elevated serum lactate dehydrogenase (S-LDH) and uric acid levels is seen. In sBL and immunodeficiency-associated BL the abdomen is the most common site of involvement. Presenting symptoms may include nausea, vomiting, gastrointestinal bleeding as well as symptoms imitating appendicitis. Other commonly affected areas include lymphadenopathy in the head- and neck region [33, 48]. Involvement of the bone marrow and central nervous system (CNS) is seen in approximately 30% and 15% of cases, respectively [40].

Diagnosis, morphology and immunophenotype

Diagnosis of BL is based on a combination of histopathological examination of the tumour, relevant laboratory investigations and CT imaging, as well as careful clinical examination and medical history. Also, with confirmation of BL diagnosis, examination of cerebrospinal fluid and bone marrow for malignant cells, should be performed. To improve the accuracy of staging, PET-CT is recommended [48, 60].

Typical morphology for BL is a monotonous, diffuse growth pattern with intermediate-sized B-cells with abundant, basophilic cytoplasm and multiple, prominent nucleoli without cleaves or folds (Figure 2). High proliferation rates, with Ki-67 $\geq 95\%$, and frequent presence of apoptotic tumour cells ingested by macrophages create the, for BL characteristic, “starry sky” appearance [33, 40, 61]. BL immunophenotyping show cells of GC B-cell lineage with expression of CD19,

CD20, CD22, CD79a, CD10 and BCL6, without expression of TdT, CD5 and CD34 [33, 61].

The diagnosis of typical BL is well-defined. However, the distinction between borderland cases is notoriously difficult and BL diagnosis should be made by experienced haematopathologists. When examining the diagnostic reproducibility of lymphoma, the agreement of haematopathologists was lowest for BL with only approximately 50 % concordance [4].

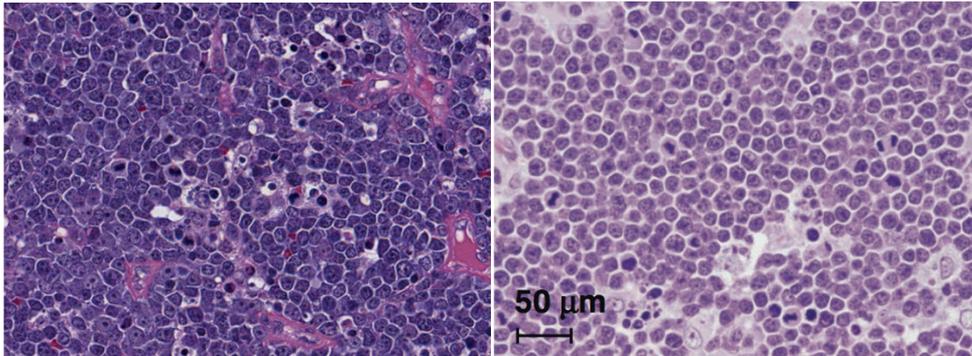


Figure 2. Two specimens depicting morphology of BL. A) Haematoxylin and eosin stain of a BL specimen, x400. Intermediate-sized lymphocytes with round, fairly monotonous nuclei and multiple indistinct nucleoli. Numerous tingible macrophages create a 'starry sky' appearance. From [62]. Reprinted by permission from Elsevier: Surgical Pathology Clinics © 2016. B) Another example of morphologic appearance of BL. From [63]. Reprinted by permission from Macmillan Publishers Ltd: Nature genetics © 2012.

Molecular background and pathogenesis of BL

Typically, the BL genome is less complex than those of other B-cell lymphomas and characterised by the lymphoma-initiating, hallmark *MYC*-rearrangement, t(8;14) [64, 65]. Since the discovery of this translocation in 1982 [29, 31, 66], much has been learnt regarding both the role of *MYC* as a proto-oncogene and of the genomic profile of BL.

The role of MYC

The t(8;14) is found in 80 % of BL cases and involves juxtaposition of the *MYC*-gene on chromosome 8 to the Ig heavy chain enhancer elements of chromosome 14, leading to a constitutive expression of *MYC* in BL. The remaining 20% of BL carry alternative variants of *MYC* translocation, with the *MYC* gene placed adjacent to the κ or λ light chain loci on chromosome 2 t(2;8) and 22 t(8;22), respectively [67]. There is an ongoing debate as to whether BL without *MYC* translocation exist, with reports of 7-10% of BL lacking this aberration [65, 68]. However, recent studies using more sophisticated techniques reveal the number of truly *MYC*-negative BL

cases to be much smaller [69-71]. Thus, some of the BL cases previously reported to lack *MYC* translocation may have been false negatives, due to technical insensitivity of FISH to detect all translocations [61]. However, these truly *MYC*-negative BL cases have been shown to exhibit a recurrent 11q aberration and may constitute a distinct BL subset [70]. One suggested target oncogene, in the gained region of 11q, is *PAFAH1B2*, which was selectively overexpressed in cell lines harbouring the 11q aberration and previously associated with *IGH* translocations and oncogene activation in chronic lymphocytic leukaemia [70]. In addition, aberrations of 11q has been found also in *MYC*-rearranged aggressive lymphomas, that exhibited a favourable outcome despite harbouring a more complex genome [72]. In recognition of these findings, a provisional entity termed “Burkitt-like lymphoma with 11q aberration” was added in the most recent WHO classification [2].

The oncogenic potential of *MYC* overexpression was first demonstrated in mice in 1985 [73]. Since then, deregulation of *MYC* has been found to be implicated in the pathogenesis of ~70% of all human tumours [74, 75]. Also, *MYC* translocations are found in several other lymphoma types, although occasionally involving juxtaposition to non-Ig genes, suggesting it may not be a primary oncogenic event, as in BL [76]. *MYC* is a global transcription factor that is estimated to govern approximately 10-15 % of genes in the genome, controlling several aspects of survival and proliferation of cells [76]. Intriguingly, the effect of *MYC* activation is diverse and dependent on the specific gene programs active in a cell, as *MYC* does not bind to promoters of silent genes [75]. In general, it exerts an oncogenic effect by driving cells through the cell cycle, promoting cell growth, adhesion and migration, as well as inducing angiogenesis and chromosomal instability, all contributing to malignant transformation (Figure 3). However, in non-malignant

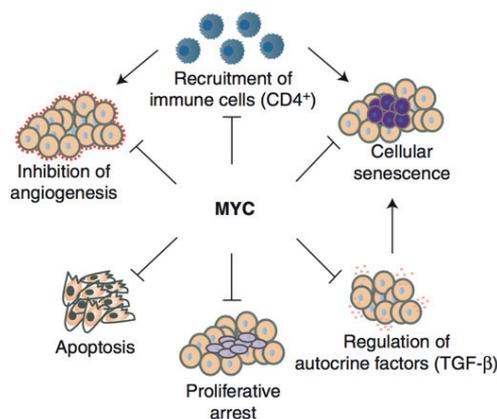


Figure 3. Selected effects of MYC. Activation of *MYC* leads to tumorigenesis through suppression of critical safeguards such as apoptosis, proliferative arrest, differentiation, inducing angiogenesis and chromosomal instability. From [74]. Reprinted by permission from Cold Spring Harbor Laboratory Press © 2014.

cells, the processes governed by MYC are tightly regulated by pro-apoptotic cascades also initiated by MYC [74-76]. Thus, *MYC* aberration alone is not sufficient to drive tumorigenesis but must be combined with synergistic aberrations, allowing the malignant clone to overcome these auto-regulatory mechanisms.

Wider molecular profile of BL and pathogenic clues

In recent years, the genetic landscape of BL has been unravelled by way of next generation sequencing (NGS) and gene expression profiling (GEP), providing new suggestions as to which the necessary additive genetic abnormalities may be [19, 20, 65, 77, 78]. GEP-studies of BL show a distinct molecular profile of BL with a relatively homogeneous transcriptome. Compared to GEP of DLBCL, BL displays a higher expression of MYC target genes and a subgroup of GC B-cell genes but lower levels of genes coding targets of the pro-survival NF- κ B-pathway and of major histocompatibility complex class I genes [65, 77]. Also, GEP of molecular BL (mBL) cases reveal a phenotype similar to that of GC dark zone cells, with the exception of expression of MYC-genes, which are normally repressed by BCL6 in dark zone cells [16].

Genome-wide sequencing of BL has revealed several novel oncogenic pathways. Of considerable interest is the finding that approximately 70% of sporadic and immunodeficiency-associated BL demonstrate either activating mutations of the transcription factor *TCF3* (11-37%) or inactivating mutations of its inhibitor *ID3* (38-68%), which are implicated in the PI3K signalling pathway [19, 20, 78, 79]. PI3K-signalling mediates survival of mature B-cells and is the pathway activated by tonic (antigen-independent) BCR-signalling [80]. Normally, PI3K-signalling does not occur in dark zone B-cells [15]. However, in BL, dysregulated activity of *TCF3* appear to induce this antigen-independent BCR-signalling [20, 63]. That it may be the establishment of this PI3K-mediated tonic BCR-survival signal that allow malignant BL cells to counteract the pro-apoptotic effect of MYC, is supported by a mouse model in which constitutive *MYC*-activation in combination with PI3K-activity generated lymphomas similar to BL [81]. Also of interest is the fact that other lymphomas such as DLBCL lack lesions in the *TCF3* and *ID3* genes [19, 20]. Likewise, the presence of *TCF3* and *ID3* appear to be restricted to the GC dark zone. Hence lending further support for a GC dark zone origin of BL, and that these lesions may be pathogenic for BL [16] (Figure 4).

Other frequently occurring somatic mutations, found in BL, include activating mutations of *CCND3* (38%), which promotes proliferation and is also a direct target of *TCF3* [20], and *GNAI3*, which may be involved in GC B-cell migration [15, 78, 79]. *TP53* is mutated in approximately 35% of cases, also potentially contributing to counterbalance the pro-apoptotic effect of MYC [19, 20, 77, 79]. The *MYC* gene itself is one of the most frequently mutated genes in BL (40-70%) [20, 78].

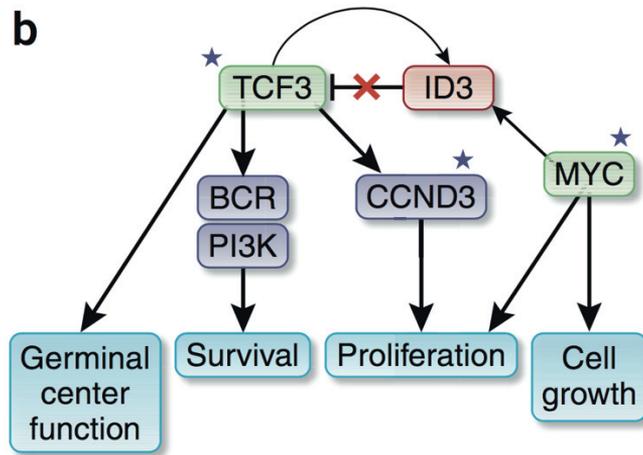


Figure 4. Selected oncogenic pathways in BL. BL frequently harbours mutations in *ID3*, *TCF3* and *CCND3* that activate the PI3K pathway. The t(8:14) translocation present in BL dysregulates *MYC*. From [63]. Reprinted by permission from Macmillan Publishers Ltd: Nature genetics © 2012.

That pathogenesis may differ between sBL and eBL is indicated by some distinct molecular characteristics. For example, the *CCND3*-mutation is much less frequent in eBL [20]. Also, the location of the break-point of the *MYC* translocation varies between these two entities. The translocation seen in eBL appears to be acquired due to aberrant SHM in early GC-phases whereas the translocation mechanism in sBL is suggested to be faulty CSR in the GC light zone, prior to re-entry into the GC dark zone [15, 34, 55].

Furthermore, a recent study revealed the presence of distinct genetic lesions in paediatric and adult BL, indicating that biologic heterogeneity may contribute to the difference in outcome between these populations [82]. In paediatric BL, 13q amplification, 7q gains and copy-neutral loss of heterozygosity of 5q was more common. Amplification of 13q may confer to lymphomagenesis by increasing expression of the *MIR17HG* cluster, the host gene for *miR17-92*, that counteracts MYC-induced apoptosis and reduces PTEN expression, thus activating the PI3K-pathway. In contrast, all adult cases harboured *ID3* mutations, compared to 42% of paediatric patients and also 18q alterations were more frequent in adult BL, possibly conferring a worse prognosis by loss of the tumour suppressor *DCC* [82, 83].

Prognostic factors and staging systems

Determining prognostic factors in BL has proven challenging due to small cohorts available, select study populations included in treatment trials and a scant number of studies performed specifically to examine prognostic determinants [84]. That

survival rates reported in clinical trials have not been matched in population based observations indicate that prognostic scores developed from clinical trials may not necessarily be applicable to the general BL population [84]. Also, improved treatment regimens may have diminished the impact of some prognostic factors of previous clinical significance.

Large, but not comprehensive, cohorts from the Surveillance, Epidemiology and End Results (SEER) database in USA show age, black ethnicity and Ann Arbor stage III-IV to be of significant prognostic value. In these studies, data regarding laboratory investigations was not available, wherefore those parameters as prognostic factors were not evaluated [8, 85]. A small study of 40 Asian patients with BL showed performance status (PS) ≥ 2 , engagement of the bone marrow and/or central nervous system (CNS) and stage IV to be indicative of prognosis [86]. In another small, early study on 42 African BL-samples, factors that reflect tumour burden affected outcome, such as stage and elevated LDH [87].

Regarding age, there is a general consensus that it is a powerful determinant of outcome in BL [8, 33, 85, 88]. However, there is some variation regarding at what age the threshold for high-risk is, with both age 40 and 60 used [85, 88, 89]. The influence of age is likely multifactorial and may reflect inferior tolerance to treatment, administration of less intensive regimens and/or differing tumour biology. Surprisingly, a recent prospective trial did not find age to be of prognostic value, attributing this to successful dose reduction for patients >55 [90]. Other parameters found to have prognostic value in clinical BL trials include advanced stage, poor PS, involvement of the bone marrow and/or CNS, presence of B-symptoms and elevated levels of LDH as well as low haemoglobin and serum albumin concentration [89, 91-93]. Furthermore, failure to achieve complete remission (CR) 4-6 weeks after treatment, is known to be a dismal prognostic marker in BL [92, 94]. More recently, molecular parameters such as a higher level of karyotypic complexity and genomic imbalances have proved to be predictive of outcome [79, 95, 96].

No staging system is specifically attuned for BL. The Murphy/St Jude system has been used for paediatric patients and the Ann Arbor system, originally developed for Hodgkin lymphoma, for adult patients. Both systems mainly depict anatomical distribution, with acknowledgment to the presence of B-symptoms [97, 98]. Because of the frequency of disseminated disease in adult BL, the use of Ann Arbor is inadequate due to limitations in fully describing the extent of extranodal engagement. In turn, the Murphy/St Jude system was developed while surgery was still a part of BL care [61]. To create a scoring system better adapted for aggressive NHL the International Prognostic Index (IPI) was developed. The IPI takes into account Ann Arbor stage III-IV, age >60 , elevated S-LDH, PS ≥ 2 and presence of >1 extranodal site [99] (Table I).

Table I. Risk stratification according to IPI

PROGNOSTIC INDEX	NUMBER OF RISK FACTORS	RISK GROUP
INTERNATIONAL PROGNOSTIC INDEX (IPI)	0-1	Low risk
	2	Low-intermediate risk
	3	High-intermediate risk
	4-5	High risk
AGE-ADJUSTED IPI (AA-IPI)	0	Low risk
	1	Low-intermediate risk
	2	High-intermediate risk
	3	High risk

Risk factors: age >60; number of extranodal sites >1; elevated lactate dehydrogenase (LDH), performance status ≥ 2 , Ann Arbor stage III or IV. Age and number of extranodal sites are not included in the age-adjusted IPI.

While well validated, expansions and adaptations of the IPI has since been created for several lymphoma subtypes, albeit not yet for BL [100, 101]. An alternative prognostic score has also been proposed for BL, with a larger emphasis on age, ethnicity and stage [84, 85]. With novel imaging techniques it is likely that staging methods will advance and incorporate PET-scans, which have shown high sensitivity for BL [102].

The possibility to stratify BL patients into reliable risk groups is of utmost importance in order to tailor treatment accordingly, and thus spare low-risk patients unnecessary toxic therapy while not withholding potentially curative treatment for high-risk patients.

Treatment of Burkitt lymphoma

Theoretical and historical background to treatment

Because of its rapidly proliferating nature, efficacious BL treatment needs to be promptly initiated, and exploit the constant re-entry of tumour cells into the cell cycle. Also, to avoid restitution and development of chemo-resistance due to the enhanced growth rate of the remaining viable malignant clone in between cycles, course intervals need to be short. Thus, the rationale for BL treatment is currently short-duration, dose-intensive chemotherapy regimens consisting of multiple agents with synergetic cytotoxic mechanisms, with drugs either fractionated or infused to maintain serum-concentrations for at least 48-72h [33, 61, 103]. The importance of adequate initial treatment is emphasised by the fact that BL appears to be a "one-shot" disease with limited treatment options in a refractory or relapsed setting [40, 104]. Also, due to the propensity for CNS relapse in BL, intensive CNS-prophylaxis should be incorporated [40].

A brief history of the development of BL treatment

Just as BL was originally discovered in Africa, it was also there that the foundations for its treatment were laid. It was quickly established that neither surgery nor radiotherapy were adequate treatment strategies for BL patients. Therefore, this patient group were subjected to a range of, then available, cytotoxic agents, in a varying systematic fashion [103, 105]. Nonetheless, BL quickly proved to be exquisitely chemo-sensitive and was one of the first malignancies where cure by chemotherapy only was achieved, by Burkitt himself in 1967 [106]. In particular, response was seen with the use of cyclophosphamide, the anti-folate methotrexate and anti-microtubule agent vincristine. The alkylating agent cyclophosphamide is the most effective single agent in BL with a cell-cycle independent effect, although it preferentially kills during cell cycle wherefore a fractionated administration schedule augments its efficacy, while decreasing its toxicity [107-109].

Additionally, in the beginning of the 1970s, these three drugs proved to be non-cross resistant and to act synergistically, thus providing the backbone for combination therapy still utilised [110, 111]. Further improvement was subsequently achieved with the realisation of the importance of prophylactic CNS treatment in BL, with incorporation of intrathecal methotrexate or cytarabine resulting in improved overall survival (OS) [112].

Despite never proven to have effect as single agents in BL, anthracyclines and steroids were commonly incorporated in BL treatment regimens when their beneficial effects were seen in other lymphomas [103, 113]. With the addition of the anthracycline doxorubicin to a combination of cyclophosphamide, vincristine and prednisone by McKelvey in 1976, the CHOP-regimen was formed, which has subsequently been of paramount importance in treatment of NHL [114]. However, the success of this regimen among other NHL was not replicated in BL patients, and outcome was also poor with regimens developed for acute lymphoblastic leukaemia (ALL) [115, 116].

Consequently, in the 1980s several novel regimens specifically targeting the high growth fraction of BL and its propensity to spread to the CNS were introduced, primarily for paediatric patients. With these intensive regimens BL was, for the first time, curable in a majority of patients with 2-year disease-free-survival of approximately 80% [117-119]. Simultaneously, attempts were made to improve outcome also for adult BL. For example, the Stanford regimen (cyclophosphamide, doxorubicin, vincristine, prednisone, methotrexate and IT methotrexate) resulted in an encouraging OS of 67% [120]. Similar results were seen with the Vanderbilt regimen (high-dose cyclophosphamide, methotrexate, bleomycin, vincristine, and doxorubicin) [121]. In contrast, results of other intensified regimens were less successful. For example, when using a combination of NHL regimens the OS-rate was 52% [122].

At this time, several regimens were successfully modified from the effective paediatric regimens, significantly improving outcome also among adult BL patients. OS-rates >70% were achieved, establishing that treatment with regimens similar to those used in children was warranted. These adapted protocols include the French Lymphome Malins de Burkitt (LMB) regimens; the German Berlin-Frankfurt-Munster (BFM) protocols; POG 8617 and CODOX-M/IVAC [94, 119, 123-129]. Furthermore, some treatment schemes were specifically developed for adult BL and/or B-ALL, based on paediatric treatment principles. Among these are Hyper-CVAD and protocols from the Cancer and Leukemia Group B (CALGB) [89, 130]. All these regimens consist of a similar therapeutic strategy with 3-8 cycles of short-duration, high-intensive chemotherapy combinations. Commonly incorporated agents include high-dose fractionated cyclophosphamide, vincristine, doxorubicin, high-dose methotrexate, etoposide, prednisone, dexamethasone as well as intrathecal methotrexate and/or cytarabine. Doses and administration schedule vary across the various versions of protocols. During the years, several dose-modifications have been explored to optimise outcome while avoiding intolerable toxicities [124, 131]. Some versions of the LMB, BFM and CALGB protocols incorporate a cytoreductive, pre-phase cycle to decrease tumour burden and thus minimise the risk for tumour lysis [123, 126, 130].

Concurrently with adaptations of paediatric regimens, immunotherapy was starting to develop and, while not yet a part of treatment, was to become an integral part in treatment of many NHL [132]. So far, the most appreciable drug has been the monoclonal CD20 antibody rituximab. Rituximab is thought to reinforce treatment both by inducing apoptotic pathways by itself, but also by sensitising tumour cells to chemotherapy agents, potentially overcoming previous drug resistance [132, 133]. As one target of rituximab is thought to be BCL2, the addition of rituximab to BL treatment was initially not as evidently beneficial as in other CD20-expressing lymphomas [134-136].

Supportive care in BL treatment

Because of the intensive nature of BL treatment, sophisticated supportive care is essential. Of importance is tumour lysis prophylaxis, consisting of rigorous hydration, allopurinol and/or rasburicase [40]. Frequent complications to treatment include severe myelosuppression, mucositis and neutropenic fever. Thus, prophylactic bacterial, viral and fungal treatment are often incorporated into treatment regimens, as well as blood product support [33]. Use of granulocyte colony-stimulating factors (G-CSF) may permit use of sufficient dose-intensity, although contradictory results of its value have been reported [89, 94, 129, 137].

Current treatment of BL

Despite the vast improvement in outcome attained after implementing a paediatric therapeutic strategy also for adult patients, optimal BL treatment is yet to be determined due to the paucity of randomised trials performed. Currently, treatment varies according to local practice, with a range of intensive regimens to choose from. Comparison of treatment protocols is aggravated by considerable disparity in published series. Firstly, treatment evaluations often comprise modest-size patient cohorts using diverse entry criteria, resulting in heterogeneous patient characteristics. There are often major differences in median age and proportion of patients with adverse prognostic characteristics such as CNS engagement. Secondly, the chemotherapy evaluated is complex, with the use of multiple agents in various doses, fluctuating number of cycles administered as well as alternating administration methods. Thirdly, differential diagnostic difficulties and frequent changes in lymphoma classification has made creation of analogous treatment cohorts challenging. Lastly, the use of incongruent methods for risk stratification complicates a direct comparison of regimens.

R-CODOX-M/IVAC

The CODOX-M/IVAC (cyclophosphamide, cytarabine, doxorubicin, leucovorin, methotrexate, vincristine/ifosfamide, etoposide, high-dose cytarabine, IT methotrexate) regimen is one of the most commonly administered. It was first reported as a paediatric regimen by Magrath *et.al.* in the 1980s. In 1996 it was shown to be equally effective for adult BL, with a cohort of 41 patients (20 adults, median age 25) achieving a 2-year event-free-survival (EFS) rate of 92% [94, 117]. Patients are stratified into low- or high risk cohorts to receive either three cycles of CODOX-M, or two cycles each of CODOX-M and IVAC, respectively (Table II). Here, low risk is defined as lack of bulky disease, completely resected abdominal disease and normal LDH. In earlier studies, toxicity was severe and many adult patients were unable to complete all therapy, mainly attributed to the high dose of methotrexate of 6,7 g/m² [91]. Thus, in a subsequent adult BL trial, a dose-modified version was evaluated, with reduced cyclophosphamide, capped vincristine at 2 mg and reduction in IT cytarabine from 70 to 50 mg, as well as of i.v. methotrexate from 6,7 to 3 g/m², achieving abated toxicity and a 2-year OS of 71% [138]. Subsequently, this dose-modified version has been the mainstay for use when treating adult patients with CODOX-M/IVAC [139]. Since the advent of rituximab, its addition to CODOX-M/IVAC has been evaluated in several retrospective and prospective studies, demonstrating that it is a tolerable combination that may improve outcome, with reported 2-4 year OS rates of 77-89% (Table III) [140-145].

BFM/GMALL/NHL regimens

The German multicenter study group for adult acute lymphoblastic leukemia (GMALL) have developed several BL-specific protocols based on the original paediatric BFM-protocols. Generally, they consist of a pre-phase treatment of cyclophosphamide and prednisone, followed by six cycles of alternating chemotherapy including ifosfamide, teniposide, vincristine, cytarabine, high-dose methotrexate, dexamethasone and doxorubicin, as well as triple IT therapy of cytarabine, methotrexate and dexamethasone (Table II). For the original NHL-83 protocol, 8-year OS-rate was 49%, which was slightly improved when methotrexate was escalated to 1,5 g/m² and ifosfamide added to the NHL-86 version [126]. However, further increase in methotrexate aggravated toxicity without improving OS, wherefore in the current version (NHL-2002), patients ≤55 receive 1,5 g/m² and those >55 receive a further reduction to 0,5g/m² [146]. For young patients, the same group investigated whether methotrexate infusion time could be shortened from 24 to 4 hours and found that 4 hour infusion, as well as dose reduction from 5 to 1g/m², was non-inferior for low-, but not high-risk patients [147]. The NHL-2002 regimen has been evaluated in several prospective trials in Germany, Spain and Italy, yielding 3-5 year OS rates of 73-80% (Table III) [90, 148, 149].

Hyper-CVAD

The Hyper-CVAD regimen was originally developed for acute lymphoblastic leukemia and BL. Patients receive 4 cycles of hyperfractionated cyclophosphamide, vincristine, doxorubicin and dexamethasone alternated with 4 cycles of methotrexate and high-dose cytarabine (Table II). In the original study from 1999, 3-year OS rate was only 49% with a prominent dichotomy in outcome between patients aged ≤60/>60 with 3-year OS differing from 77% to 17%, between these groups [89]. The results with Hyper-CVAD were much improved in a subsequent study with addition of rituximab, resulting in a 3-year OS rate of 89% [150].

CALGB and LMB regimens

Using a similar backbone as in the BFM protocols, both the LMB and CALGB-groups have developed efficacious regimens. Both utilise a pre-phase cycle of cyclophosphamide and prednisone, followed by alternating chemotherapy cycles, according to risk. In the CALGB 9251, patients received 3 cycles each of ifosfamide, methotrexate, vincristine, cytarabine, etoposide, dexamethasone and cyclophosphamide, methotrexate, vincristine, doxorubicin, dexamethasone. Also, in this protocol, patients received cranial irradiation and 12 doses of IT-therapy, resulting in major neurologic toxicity, with an OS rate of only 57% [130, 151]. Subsequently, cranial irradiation has been dropped and rituximab added (CALGB study 10 002), resulting in a 4-year OS rate of 78% [152]. In the LMB regimens patients are stratified according to completely resected disease (low-risk), presence

Table II. Content comparison of three chemotherapy regimens used to treat adult BL.

R-CODOX-M/R-IVAC	Dose	Days	BFM/NHL-2002	Dose	Days
R-CODOX-M			Prephase:		
Cyclophosphamide	800 mg/m2	1	Cyclophosphamide	200 mg/m2	1-5
	200 mg/m2	2-5	Prednisone	60 mg/m2	1-5
Doxorubicin	40 mg/m2	1	A: Cycle 1+3		
Vincristine	1.5 mg/m2	1 + 8	Rituximab	375 mg/m2	7
IT Cytarabine	70 mg	1+3	Dexamethasone	10 mg/m2	8-12
Rituximab	375 mg/m2	9	Vincristine	2mg	8
Methotrexate	≤65: 3 g/m2	10	Ifosfamide	800 mg/m2	8-12
	>65: 1 g/m2		Methotrexate*	≤55: 1.5 g/m2	8
IT Methotrexate	15 mg	15		>55: 0.5 g/m2	11-12
R-IVAC			Teniposide	100 mg/m2	11-12
Etoposide	60 mg/m2	1-5	Cytarabine	150 mg/m2x2	8
Ifosfamide (w mesna)	≤65: 1.5 g/m2	1-5	IT Cytarabine	40mg	12
	>65: 1 g/m2		IT Methotrexate	15mg	
Cytarabine	≤65: 2 g/m2	1+2	B: Cycle 2+4		
	>65: 1 g/m2		As A +		2-6
Rituximab	375 mg/m2	3 +7	Cyclophosphamide	200 mg/m2	5-6
IT Methotrexate	15 mg	5	Doxorubicin	25 mg/m2	
			C: Cycle 3+6		1
For LR: 3 cycles R-CODOX-M			Rituximab	375 mg/m2	2-6
For HR: 4 cycles, alternating R-CODOX-M and R-IVAC			Dexamethasone	10mg/m2	2
			Vindesine	3mg/m2	2
			Methotrexate	1.5g/m2	5-6
			Etoposide	250 mg/m2	6
			Cytarabine	2g/m2 x2	
R-HYPER-CVAD	Dose	Days			
Cycle 1,3,5,7:					
Cyclophosphamide	300 mg/m2x2	1-3			
Doxorubicin	50 mg/m2	4			
Vincristine	2mg	4+11			
Dexamethasone	40 mg/m2	1-4, 11-14			
Rituximab	375 mg/m2	1+11			
Cycle 2,4,6,8:					
Methotrexate	1g/m2	1			
Cytarabine	3g/m2 x2	2+3			
Rituximab	375 mg/m2	2+8			
Every cycle:					
IT Methotrexate	12 mg	2			
IT Cytarabine	100 mg	7			

Intravenous administration if not otherwise specified. IT = intrathecal, LR = low-risk, HR = high-risk.

of CNS and/or bone-marrow disease (high-risk), or all other patients not fulfilling either criteria (intermediate risk), to receive 3, 8 or 5 chemotherapy cycles, respectively. In a retrospective study this approach reached a 3-year OS rate of 74% [123], and when evaluated prospectively in 72 adult BL patients, 2-year OS rate was 70% [153]. In a more recent study, the low-risk group was omitted and patients in the intermediate and high-risk groups received the LMB 84 and 89 regimens

(including high-dose methotrexate of 4-8g/m²), respectively. Also, they were randomised to receive or not receive rituximab, resulting in 3-year OS rates of 83% and 70%, respectively [93].

DA-EPOCH-R

Using a different approach to the high-intensive scheduling of other regimens, with prolonged exposure to low concentrations of chemotherapy agents, the infusional EPOCH (etoposide, prednisone, vincristine, cyclophosphamide, doxorubicin) regimen was developed to achieve a continuous exposure of tumour cells to cytotoxic agents and to decrease toxicity [154]. Initially introduced as a regimen for relapsed DLBCL, it has subsequently been modified with addition of rituximab and individual dose adjustment according to neutrophil counts, and shown efficacy also for BL and other aggressive lymphomas. In a study consisting of 30 patients, of which 19 had sBL, outcome was encouraging with a 100% 7-year OS, although in a favourable patient cohort with a median age of only 25 and only one with CNS disease [155]. Preliminary follow-up results from the development of this study included 77 patients stratified to 3 courses without CNS-prophylaxis if classed as low-risk, or 6 cycles including IT methotrexate for high-risk patients, demonstrating a 2-year OS of 88% [156] (Table III). In contrast to the fractionated administration of cyclophosphamide utilised in other regimens, cyclophosphamide in DA-EPOCH-R is administered as a single bolus dose during 15 minutes per cycle [157].

Other high-intensive regimens

In addition to these regimens several other variations of short-cycle, high-intensive regimens have been developed. Two separate studies have tried to maximise the use of cyclophosphamide because of its efficacy as a single-agent in BL and that dose escalation of cyclophosphamide is thought to be more feasible for elderly patients compared to other agents due to its lymphoablative, rather than myeloablative effect [158]. In the regimen developed by Kujawski *et.al.* 4 g/m² of cyclophosphamide was administered per cycle, while eliminating other agents such as cytarabine, etoposide and ifosfamide, resulting in a 3-year OS rate of 72% [159]. In the BASIC regimen, doxorubicin was omitted in favour of high-dose escalation of cyclophosphamide, yielding a 3-year OS rate of 57% among elderly patients [158]. Moreover, successful adaptation of the paediatric POG8617 as well as another modified paediatric regimen have been utilised in Italy [160, 161] (Table III).

The role of rituximab in BL treatment

As mentioned, the impact of rituximab in BL treatment is not as extensively studied as in other NHLs. However, the tolerability and value of this agent in other NHL led to its common incorporation in most regimens used to treat BL. The exact mechanism by which rituximab exerts its effect is not fully understood, but it is

thought to induce cell lysis both via direct induction of apoptosis, as well as via complement-mediated or antibody-dependent cytotoxicity [162].

Although compared to an historical control group, the addition of rituximab to Hyper-CVAD appeared to significantly improve outcome [150]. In CODOX-M/IVAC, a retrospective comparison of 80 patients where 50% received rituximab, a superior outcome with a 3-year OS of 77% versus 66% among those administered versus not receiving rituximab was seen [143]. Regarding BFM/NHL-regimens, all recent prospective trials have included rituximab and an indication of superior results compared to historical data is seen, although whether the improvement in outcome is due to the addition of rituximab or other modifications to the regimen is hard to elucidate [90, 146, 148]. Recently, the results of the first prospective, randomised trial of rituximab addition to BL was published, with rituximab combined with the LMB 84 and 86 protocols. In this study, outcome was favourable among patients receiving rituximab with a 3-year OS of 83% compared to 70% for patients not administered rituximab [93]. Likewise, a meta-analysis evaluating rituximab addition to BL treatment found that immunochemotherapy was associated with improved OS [163].

Treatment of elderly BL patients

The treatment of elderly BL patients poses a particular challenge due to the extensive toxicity associated with BL protocols. Evaluation of regimens for the elderly population is complicated by the relative infrequency of this population included in clinical trials, as well as use of arbitrary thresholds for what is classified as ‘elderly’. In a retrospective review of BL treatment for patients aged >40 the conclusion was that this cohort should receive intensive treatment if in any way deemed feasible, and that further clinical trials specifically for this age group are warranted [88]. Currently, with improved supportive care, more patients aged >40 are included in clinical trials. Also, a particularly beneficial effect of for example rituximab and the use of DA-EPOCH have been suggested for this cohort [61, 150]. Moreover, use of careful dose-modifications for elderly patients may contribute to improve outcome for this population [90, 149].

Treatment of HIV-positive BL patients

Initially, HIV-positive BL patients were thought to not tolerate intensive BL regimens and were thus not included in early treatment trials. However, during the past decade several intensive regimens have been evaluated for this cohort, establishing that a similar treatment approach as to sBL is both tolerable and advantageous [164-167]. This may be attributable to the success of modern anti-retroviral therapy, which is often administered concurrently to chemotherapy.

Table III. Results from prospective trials of upfront therapy for adult BL.

REGIMEN	REFERENCE	R	N	MEDIAN AGE (RANGE)	OS %	CR %	COMMENT
CODOX-M/IVAC	Magrath <i>et.al.</i> [94] 1996	No	20	25 (18-59)	4y 74	89	RS. C:3-4
	Mead <i>et.al.</i> [91] 2002	No	52	35 (15-60)	2y 73	77	RS. C: 3-4 Omitted vincristine.
	Lacasse <i>et.al.</i> [138] 2004	No	14	47 (18-65)	2y 71	86	Dm. RS. C3-4
	Mead <i>et.al.</i> [139] 2008	No	53	37 (17-76)	2y 67	-	Dm. RS C:3-4
	Corazzelli <i>et.al.</i> [140] 2012	Yes	30	52 (25-77)	4y 82	93	Dm. C:4
	Evens <i>et.al.</i> [144] 2013	Yes	25	44 (23-70)	2y 89	92	Dm. RS. C:3-4
HYPER-CVAD	McMillan <i>et.al.</i> [145] 2015	Yes	150	38 (20-64)	2y 80	71	High-risk patients
	Thomas <i>et.al.</i> [89] 1999	No	26	58 (17-79)	3y 49	81	B-ALL patients
BFM/GMALL-B-ALL/ NHL	Thomas <i>et.al.</i> [150] 2006	Yes	31	46 (17-77)	3y 89	86	Protective environment. 14/31 B-ALL
	Hoelzer <i>et.al.</i> [126] 1996	No	24	33 (15-58)	8y 49	63	NHL-83
	Hoelzer <i>et.al.</i> [126] 1996	No	35	36 (18-65)	4y 51	74	NHL-86
	Intermesoli <i>et.al.</i> [148] 2013	Yes	105	47 (17-78)	3y 79	79	NHL-2002 48% B-ALL, 14% HIV+
	Ribera <i>et.al.</i> [90] 2013	Yes	108	44 (15-83)	4y 73	85	Dm for >55. 32% HIV+
	Hoelzer <i>et.al.</i> [149] 2014	Yes	363	42 (16-85)	5y 80	88	Dm for >55
	CALGB	Lee <i>et.al.</i> [130] 2001	No	54	44 (18-71)	5y 52	80
Rizzieri <i>et.al.</i> [151] 2004		No	52 40	44 (18-72) 50 (17-78)	3y 54 50	79 68	CALGB 9251. Use of CNS RT.
Rizzieri <i>et.al.</i> [152] 2014		Yes	105	43 (19-79)	4y 78	83	CALGB 10002. G-CSF
LMB	Divine <i>et.al.</i> [153] 2005	No	72	33 (18-76)	2y 70	72	LMB95. C: 3-8, RS

	Ribrag <i>et.al.</i> [93] 2016	Yes No	128 129	47 (18->60)	3y 83 70	-	RS. LMB84 or LMB86
DA-EPOCH	Dunleavy <i>et.al.</i> [155] 2013	Yes	19	25 (15-88)	7y 100	-	
	Dunleavy <i>et.al.</i> [156]	Yes	77	45 (19-78)	2y 88	-	RS. C:3-6 + IT. 26% HIV+
OTHER	Di Nicola <i>et.al.</i> [161] 2004	Yes	22	35.5 (18-76)	2y 77	77	Italian paediatric protocol
	Van Imhoff <i>et.al.</i> [168] 2005	No	27	36 (15-64)	5y 81	81	CT + ASCT
	Kujawski <i>et.al.</i> [159] 2007	No	11	51 (33-71)	3y 72	91	High-dose CHOP
	Todeschini <i>et.al.</i> [160] 2012	Yes	46	39 (17-77)	5y 72*	94	POG 8617 regimen. R in 50% of patients
	Kasamon <i>et.al.</i> [158] 2013	Yes	21	53 (34-75)	3y 57	76	BASIC regimen

R = rituximab, N = number of patients, OS = overall survival, CR = complete remission, y=year, Dm = dose-modified, RS = Risk stratified, C = number of treatment cycles. RT = radiotherapy. IT = intratechal treatment, CT =chemotherapy, ASCT = autologous stem cell transplantation. *EFS, OS data not available

The role of other treatment modalities in BL

Because of the rapid response to aggressive chemotherapy alone, the use of other treatment modalities in BL is limited. However, the role of autologous stem cell transplantation (ASCT) at CR has been evaluated. With a less intensive induction regimen, 3-year OS was 45% in a consecutive case series [169]. In contrast, the HOVON-group achieved 5-year OS rates of 81% following ASCT after using brief initial high-dose chemotherapy consisting of cyclophosphamide, doxorubicin, etoposide, mitoxantrone and prednisone [168]. Interestingly, no graft-versus-BL effect is seen in this disease. This may be due to the high proliferation rate of BL, thus diminishing potential positive effects of using allogeneic transplantation for BL [170]. Regarding radiotherapy, there is no established use in BL, although there are some reports of potential effect in a relapsed setting [171].

Salvage treatment in BL

There is no established salvage treatment available for BL, and prognosis is dismal at relapse or failure to respond to primary treatment [33]. Few studies have evaluated regimens for these patients, and remaining therapeutic options are few, as patients have often already been subjected to the most active agents for BL. In this setting, ASCT or allogeneic transplantation may represent an alternative. In relapsed patients with chemo-sensitive disease who underwent ASCT, a 3-year OS of 37% was reported, although only 7% for patients with chemo-refractory relapse [104].

Diffuse Large B-cell Lymphoma

DLBCL is the most common NHL-subtype among adults and accounts for 20-40% of adult lymphoma [12, 172]. Age-standardised incidence rates range from approximately 4-7/100 000, with a higher incidence among caucasians and males [12, 173]. In Sweden, incidence is 5.5/100 000/year, yielding approximately 500 cases per annum [174]. Although more common among elderly patients, with a median age of 70, DLBCL occurs among all age groups. Its aetiology is largely unknown but is thought to be associated with immunosuppression, genetic susceptibility, autoimmune disease, various infectious agents as well as other environmental factors [172, 175]. Also, some DLBCL evolve from transformed cases of less aggressive lymphoma [2]. During past decades, the heterogeneity of DLBCL has been increasingly acknowledged. Despite morphological similarity this disease is likely to consist of several biologically disparate entities [176].

Diagnosis

DLBCL diagnosis is based on histopathological report of an adequate biopsy sample as well as careful clinical examination and staging via CT, with or without PET, and bone marrow aspirate and biopsy. Spinal tap analysis is recommended for high-risk patients [177]. Typically, immunostaining show expression of CD19, CD20, CD22 and CD79a. As implied by its name, the morphological features of DLBCL include large B-cells that grow in a diffuse pattern resulting in complete effacement of normal lymph node architecture [172](Figure5). In the WHO classification, multiple morphological variants are recognised, reflecting the molecular genetic diversity of DLBCL. The heterogeneous subgroup DLBCL NOS is the most numerous [2].

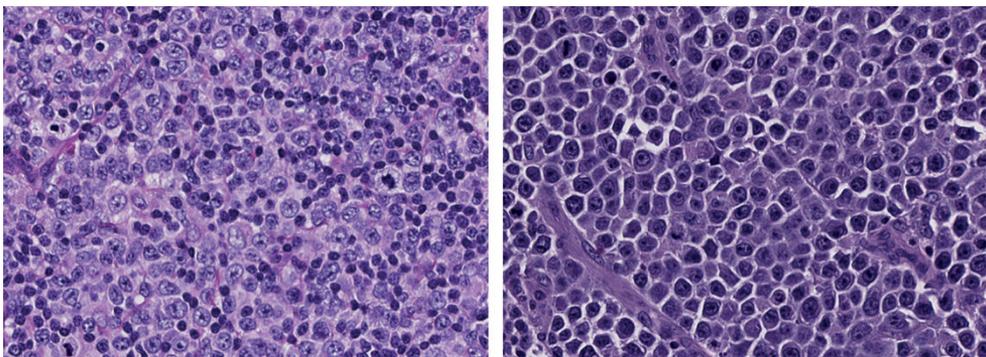


Figure 5. Morphology of DLBCL. Haematoxylin and eosin stains x400. To the left DLBCL with centroblastic morphology with multiple small nucleoli, reminiscent of cells in the GC dark zone. To the right DLBCL with immunoblastic morphology, with prominent central nucleoli or other features suggestive of plasmacytic differentiation. From [62]. Reprinted by permission from Elsevier: Surgical Pathology Clinics © 2016.

Clinical presentation and prognostic factors

DLBCL is an aggressive disorder with a rapidly fatal course without treatment. Encouragingly, it is now readily curable in the majority of patients by administration of adequate immunochemotherapy [178, 179]. However, the 20-40% of patients that still suffer from refractory or relapsed disease constitute a subgroup where improved therapy is warranted [180]. Common presenting symptoms include rapid enlargement of a lymph node, with extranodal presentation seen in 40% of patients. In 15% bone marrow engagement is found, and approximately 30% of patients present with B-symptoms [172]. Staging is performed using the Ann Arbor system, describing the anatomical extent of disease [60]. In DLBCL, approximately 25% of patients present with stage I or II, respectively, and 50% demonstrate disseminated disease (stage III-IV) [174]. Advanced stage disease is often defined as Ann Arbor stages III-IV or stages I-II with associated B-symptoms or bulky disease ($\geq 10\text{cm}$), constituting approximately 75% of patients [172, 177].

Reported prognostic factors include advanced age, number of extranodal sites, elevated levels of S-LDH, PS score, stage, bulky disease and involvement of CNS as well as presence of other comorbidity [174, 181]. For DLBCL, the IPI continues to be the most robust prognostic tool (Table 1, page 24), although it lacks the capacity to recognise a subgroup with $<50\%$ survival in the rituximab era [182, 183]. Originally, four distinct risk groups were identified with 5-year OS rates ranging from 26-73% [99]. In the rituximab era, corresponding rates for low- and high-risk, respectively, were reported to be 59-91% [182]. Thus, various IPI adaptations have been proposed, such as the R-IPI, which discriminates three, rather than four risk groups [183]. The E-IPI utilises an age cut-off of 70, identifying more distinct subgroups in patients aged 60-80 [184]. Most recently, the NCCN-IPI enhanced stratification by extending the age and S-LDH categorisation, and differentiating between specific extranodal presentations [101]. Currently, the impact of molecular profile on prognosis is increasingly acknowledged [185-189]. Also, parameters such as low absolute lymphocyte/monocyte count, elevated serum immunoglobulin free light chains as well as vitamin D deficiency may confer inferior outcome [190].

Molecular background and pathogenesis

Advances in, and increased availability of, genetic technology and profiling within recent decades have contributed to an explosive improvement in the understanding of DLBCL biology, involving development of a novel molecular taxonomy [17, 18, 186]. It is now generally appreciated that the DLBCL NOS subgroup consists of at least two molecular subtypes according to GEP classification, with differing cell of origin (COO) and clinical outcome. The germinal centre B-cell (GCB) and activated B-cell (ABC) subtypes account for approximately 85% of all DLBCL, while some

cases remain unclassifiable. In GEP studies, ~10% of cases have been recognised as primary mediastinal B-cell lymphoma (PMBL), a separate diagnostic entity in the WHO classification [176, 186, 187, 191, 192]. In accordance with their distinct molecular phenotype, each subtype exhibits some exclusive genetic lesions. However, some oncogenic pathways appear to be shared, including lesions that subvert *BCL6* regulation and immune recognition (*B2M*) as well as lesions in chromatin modifiers, affecting epigenetic regulation (*CREBBP*, *EP300*, *MLL2*) [15, 18]. The exact effects of these lesions are as yet incompletely understood. Dysregulated *BCL6* is thought to contribute to pathogenesis via several mechanisms, such as suppression of DNA damage response through p53 repression [193], augmenting the proliferative phenotype [194] and blocking terminal differentiation [195]. Overall, the genetic landscape of DLBCL is complex, with significant variation in the number of tumour-acquired lesions and ~30 clonally represented lesions per DLBCL case. In addition, most identified alterations are seen in only a fraction of cases [18]. Furthermore, there is increasing evidence that the microenvironment and its inflammatory response also affect pathogenesis in DLBCL [192].

As the name implies, GCB DLBCL is believed to derive from GC light zone B-cells and thus frequently express proteins detected in normal B-cells, such as *BCL6* and *CD10* as well as evidence of ongoing SHM [17, 18, 186]. Genetic lesions restricted to the GCB subtype include t(14;18) translocation, leading to *BCL2* overexpression in 35% of cases, conferring a pro-proliferative and anti-apoptotic effect on tumour cells [196]. Mutations of the histone methyltransferase *EZH2* is found among ~21% and regulates the GCB phenotype in concert with *BCL6*, contributing to GC proliferation and impairing terminal differentiation [197, 198]. Moreover, amplification of *miR-17-92* is seen in ~13% and its presence is mutually exclusive to deletion of *PTEN*, found in ~10% of cases [18, 199]. *MiR-17-92* acts synergistically with *MYC* and also inhibits *PTEN*, which in turn results in constitutive activation of the PI3K-pathway, inducing growth and survival [200]. Also, *C-REL* amplification and *MDM2* overexpression, affecting the p53 pathway, are restricted to the GCB subtype (Figure 5) [192]. Although some conflicting results have been reported, the GCB subtype is generally associated with favourable outcome compared to the ABC variant, and is the subtype most often seen in younger patients with DLBCL [186, 191].

The ABC subtype is thought to derive from B-cells committed to plasmablastic differentiation, just prior to GC exit. Its pathogenesis is characterised by two features: constitutive activation of the NF- κ B pathway and blockade of plasmacytic differentiation [18, 199, 201]. The NF- κ B signalling pathway mediates cell survival, proliferation and inhibits apoptosis. The aberrant activation is sustained in the ABC subtype by multiple genetic alterations. Approximately 20% harbour lesions in *CD79A* or *CD79B*, causing chronic BCR-signalling to activate the NF- κ B pathway

[202]. Alternatively, 10% carry activating mutations of *CARD11*, allowing NF-κB activation independent from upstream signalling. Moreover, ~35% exhibit *MYD88* mutation and 30% have inactivated *TNFAIP3*, both enabling constitutive NF-κB activation [18]. Other genetic lesions specific to the ABC subtype relate to the blockade of terminal differentiation, which is mediated either by *BCL6* translocation or inactivation of *PRDM1*, via for example *SPIB* mutations, seen in ~25% [15]. It has been hypothesised that the loss of several tumour suppressors in the ABC subtype blocks the effect of chemotherapy, potentially conferring the inferior outcome seen in ABC DLBCL [203]. In contrast to GCB, *BCL2* is overexpressed via gene amplification rather than translocation in the ABC subtype. Also, combined overexpression of *MYC* and *BCL2* protein (dual-expressors) is more frequent in the ABC cohort, also potentially contributing to the poorer prognosis of patients with ABC DLBCL [188]. Lastly, the ABC subtype is more common among elderly DLBCL patients [187].

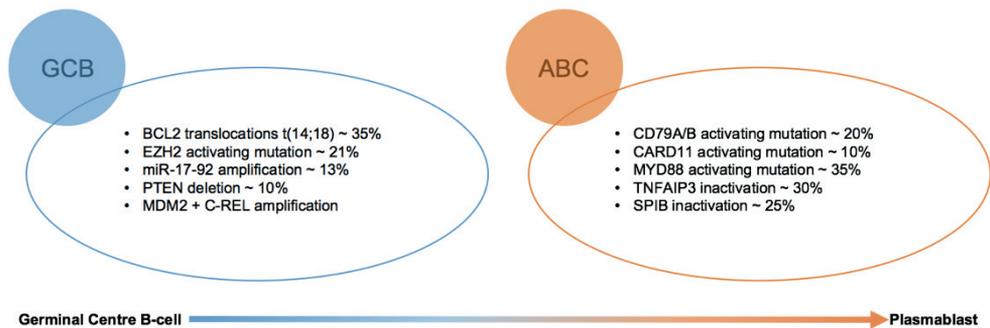


Figure 5. A selection of cell of origin-associated genetic alterations for the GCB and ABC subtypes of DLBCL.

Current upfront treatment of DLBCL

Standard treatment of DLBCL has remained similar since the 1970s, when the successful addition of doxorubicin to cyclophosphamide, vincristine and prednisone created the CHOP regimen, and became one of the first curative treatments of DLBCL [114]. Similar to BL, adequate and prompt treatment is essential to achieve long-term survival in DLBCL. In the following decades, attempts to better the outcome achieved with CHOP focused on adding various agents to the regimen. Some promising results were seen in phase 2 trials, but in a randomised study comparing these 2nd and 3rd generation regimens to standard CHOP, there was more toxicity without evidence of superiority, hence establishing CHOP as standard of care [204].

Subsequently, the German lymphoma study group (DSHNL) performed two randomised studies to evaluate increased dose-density (biweekly administration compared to every 21 days) and addition of etoposide (CHOEP) to CHOP in both younger and older (>60) patients. In the younger cohort, CHOEP but not dose-density improved outcome, whereas CHOP-14 was beneficial for patients >60 [205, 206]. A major treatment advance occurred with the addition of rituximab to CHOP (R-CHOP), with the first randomised study demonstrating improved 2-year OS rates of 70% vs 57% for R-CHOP vs CHOP alone [207]. The improved outcome achieved with R-CHOP was subsequently confirmed for all age groups in other randomised studies [208, 209], and in a large population based cohort [210]. However, in the rituximab era, the previously reported positive effect of etoposide among low-risk patients aged ≤ 60 diminished in the MInT study [179, 209]. Also, two randomised studies did not find dose-dense administration (R-CHOP-14) to be superior, thus establishing R-CHOP-21 as standard therapy [211, 212], although 6 cycles of R-CHOP-14 was also determined feasible for patients >60 in the RICOVER-60 trial [213].

Generally, choice of treatment is based on age and risk stratification according to IPI, Ann Arbor stage and presence of bulky disease [177]. Despite advances in molecular categorisation and mounting evidence that patients with different DLBCL subtypes benefit from differing treatment, patients largely continue to be treated in a uniform fashion. For example, treatment effect of agents that inhibit the NF- κ B pathway, such as lenalidomide, ibrutinib and bortezomib, appear to be restricted to the ABC subgroup [214-216]. Also, certain sites of extranodal involvement require special treatment considerations, such as CNS involvement, where CNS prophylaxis is warranted.

Currently, the only subgroup where first-line standard therapy is not entirely defined is the young high- and high-intermediate risk patients (aaIPI ≥ 2) [177]. In the rituximab era, one randomised study demonstrated superiority of increased dose-intensity in this population, comparing R-ACVBP (doxorubicin, cyclophosphamide, vindesine, bleomycin and prednisone) with R-CHOP [217]. Three-year OS for the R-ACVBP cohort was 92%, and among patients who received this regimen the inferior outcome associated with the ABC-subtype, when treated with R-CHOP, diminished [218]. However, the significant hematologic toxicity of R-ACVBP somewhat restricts its clinical use, which was also largely the case when Hyper-CVAD was evaluated in high-risk patients with DLBCL ≤ 60 [219]. The use of first-line HDT + ASCT in DLBCL has been controversial and is as yet not proven superior to chemotherapy alone [220]. Other treatment options proposed for this subgroup include etoposide-containing regimens such as R-CODOX-M/IVAC, DA-EPOCH-R and R-CHOEP-14 [221-226].

The role of etoposide

The cytotoxic agent etoposide is a cell cycle dependent topoisomerase II inhibitor that has shown effect both as a single-agent and in chemotherapy combinations [227-229]. In vitro, the exposure of tumour cells to inhibitors of topoisomerase II agents has been shown to promote the p53-p21 pathway, which increases the apoptotic stress response and allows exploitation of the rapid tumour proliferation by independently activating the check point kinase 2 that induces apoptosis [230, 231]. In addition, inhibition of topoisomerase II appears to mediate downregulation of BCL6, thus counteracting its oncogenic effects [232]. Moreover, etoposide has been suggested to penetrate into cerebral spinal fluid and may contribute to reduce the incidence of CNS relapse [233].

As mentioned, incorporation of etoposide to CHOP improved outcome among low-risk DLBCL patients ≤ 60 in the pre-rituximab era [205]. It has been suggested that mechanisms essential for rituximab mediated cellular cytotoxicity, such as presence of NK-cells, may be compromised by increased haematological toxicity caused by etoposide, thereby explaining the subsequent lack of superiority of R-CHOEP-21 to R-CHOP-21 [179, 209]. Nonetheless, addition of etoposide to R-CHOP-14 (R-CHOEP-14), has been evaluated in several prospective and retrospective analyses and deemed both efficacious and tolerable for young, high-risk patients, thus still representing a valid treatment option in this cohort [223, 224, 234, 235].

In a population based study R-CHOEP-14 was superior to R-CHOP-14 in patients with $aaPI \geq 2$, with beneficial effect primarily seen in patients with the GCB subtype [223, 236]. A randomised study comparing R-CHOEP-14 with R-MegaCHOEP + ASCT found higher event- and progression free survival in the R-CHOEP-14 arm, with an encouraging 3-year OS rate of 84.6% [224]. Almost identical results were obtained in a subsequent study from the same group, where high risk DLBCL patients aged ≤ 60 were randomised to receive CHOEP-14 with either 6 or 12 applications of rituximab, without additional effect of extra doses of rituximab [235]. Similar outcome was seen in a prospective phase II NLG study of R-CHOEP-14 with systemic CNS-prophylaxis [225]. Furthermore, in another Nordic phase II trial, high-risk patients received four cycles of R-CHOEP-14 in addition to two cycles of R-CHOP-14 and systemic CNS prophylaxis with high dose methotrexate and intrathecal liposomal cytarabine, resulting in a CR rate of 79% and 2-year OS rate of 90% [237] (Table IV).

Finally, etoposide is included in the DA-EPOCH-R regimen, which has shown promising outcome in high-risk DLBCL patients of all ages in prospective, multi-centre studies achieving 2-10-year OS rates of 64-84% (Table IV) [221, 222, 238, 239]. Currently, DA-EPOCH-R is being compared with R-CHOP in the ongoing CALGB 50303 randomised trial.

Table IV. Reported overall survival rates of etoposide-containing regimens in the rituximab era

REGIMEN	REFERENCE	YEAR	N	MEDIAN AGE (RANGE)	OS %	COMMENT
R-CHOEP-21	Pfreundschuh <i>et.al.</i> [179]	2006 + 2011	181	47 (35-54)	6y 75.1	Low-risk patients. Randomised.
R-CHOEP-14	Adde <i>et.al.</i> [234]	2006	38	58 (27-65)	2y 79	Observational
	Gang <i>et.al.</i> [223]	2011	159	49 (27-60)	4y 75	Population based
	Schmitz <i>et.al.</i> [224]	2012	136	50 (18-60)	3y 84.6	Randomised, prospective
	Holte <i>et.al.</i> [225]	2013	156	54 (18-65)	3y 81	Phase II
	Schmitz <i>et.al.</i> [235]	2015	78	47 (18-60)	2y 82	Phase II. Rituximab x12
	Leppä <i>et.al.</i> [237]	2016	140	56 (20-64)	2y 90	Phase II. CNS prophylaxis
DA-EPOCH-R	Garcia-Suarez <i>et.al.</i> [221]	2007	33	55 (21-76)	2y 75	Observational
	Wilson <i>et.al.</i> [238]	2008	72	50 (19-85)	5y 80	Phase II
	Wilson <i>et.al.</i> [222]	2012	69	58 (23-83)	5y 84	Phase II
	Purroy <i>et.al.</i> [239]	2015	81	60 (21-77)	10y 63.6	Phase II

N = number of patients, OS = overall survival, y=year

The effect of DA-EPOCH-R appears to vary according to COO, with particularly impressive OS rates of up to 94% 5-year OS in the GCB subtype compared to 58% in the ABC variant [222]. This has been attributed to the rapid proliferation of GC B-cells and their frequent overexpression of BCL6, thus potentially making GCB tumour cells more sensitive to treatment with topoisomerase II inhibitors [240]. Perhaps the restricted beneficial effect of etoposide to patients aged ≤ 60 , in the pre-rituximab era, may reflect the higher frequency of the GCB subtype among young DLBCL patients. That etoposide may be particularly efficacious in cases with BCL6 overexpression is supported by a small, long-term follow-up of DA-EPOCH-R, where 10-year OS was 100% for patients harbouring a BCL6 rearrangement [239].

The diagnostic grey zone between BL and DLBCL

During several decades, clinicians and pathologist have strived to better distinguish BL from DLBCL, and to identify the intermediate group of very aggressive large B-cell lymphoma that exhibit either atypical cytogenetics, morphology or

immunophenotype, that preclude a definitive diagnosis of DLBCL NOS or BL. In the recently updated WHO classification, the provisional subgroup previously termed “B-cell lymphoma, unclassifiable, with features intermediate between DLBCL and BL” (BCLU) was superseded by two other categories named “High-grade B-cell lymphomas (HGBL), with *MYC* and *BCL2* and/or *BCL6* rearrangements” or “HGBL, not otherwise specified (NOS)” if lacking a *MYC* and *BCL2* and/or *BCL6* rearrangement [2]. That there are several other prior nomenclatures such as ‘Burkitt-like lymphoma’ and ‘atypical BL’ illustrates the protracted diagnostic difficulty of this subgroup [5, 7] (Table V).

In favour for the existence of an as yet incompletely characterised subgroup, molecular studies have recognised that cases clinically and biologically intermediate between BL and DLBCL also represent a true intermediate grey zone of the mutational spectrum [65, 77, 241-244]. In the studies by Dave and Hummel *et.al.* 16-34% of cases defined as molecular BL (mBL) through GEP, were classified as DLBCL or BCLU according to current classification criteria, and 22% of the aggressive lymphomas studied, exhibited a molecular profile intermediate between mBL and non-mBL [65]. Moreover, another molecular BL classifier found that 28% of DLBCL cases carrying a *MYC*-rearrangement exhibited molecular features

Table V. Overview of the current WHO classification of DLBCL NOS, BL and the intermediate grey zone

NOMENCLATURE IN WHO 2016 CLASSIFICATION	CHANGE FROM THE 2008 CLASSIFICATION
DIFFUSE LARGE B-CELL LYMPHOMA NOS	<ul style="list-style-type: none"> • Distinction of GCB vs non-GCB may affect therapy • Coexpression of <i>MYC</i> and <i>BCL2</i> new prognostic marker (double-expressor)
BURKITT LYMPHOMA	<ul style="list-style-type: none"> • No diagnostic changes, but recognition of ~70% carry ID3 och TCF3 mutations
BURKITT LYMPHOMA WITH 11Q ABERRATION*	<ul style="list-style-type: none"> • New provisional entity that closely resembles BL but lacks <i>MYC</i> rearrangement • More complex karyotype compared to BL
HIGH-GRADE B-CELL LYMPHOMA, WITH <i>MYC</i> AND <i>BCL2</i> AND/OR <i>BCL6</i> TRANSLOCATIONS	<ul style="list-style-type: none"> • New category for all DHL/THL lymphomas other than follicular or lymphoblastic lymphomas • Morphological appearance should be noted in comment
HIGH-GRADE B-CELL LYMPHOMA, NOS	<ul style="list-style-type: none"> • Replaces the 2008 category of B-cell lymphoma unclassifiable, with features intermediate between DLBCL and BL (BCLU), together with the above category • Includes blastoid-appearing large B-cell lymphomas and cases lacking <i>MYC</i> and <i>BCL2</i> or <i>BCL6</i> translocations that would formerly have been called BCLU

Adapted from [2]. *Provisional category

more consistent with a BL diagnosis [245]. Also intriguing is that the *ID3* mutation, initially proposed to be restricted to mBL, has been found in 21-67% of cases designated as BCLU [241, 242], while the presence of *ID3* lesions in GCB DLBCL remained low at 3.5% [242]. The BCLU cases with *ID3* lesions also commonly carried mutations typical for DLBCL, thus not representing cases consistent with a BL diagnosis either [242]. To further increase the intricacy of BL diagnosis, clinicopathological characteristics atypical for BL, such as *BCL2* expression, have been found in cases of mBL [65, 245, 246].

The pathological features of intermediate cases, that most often differ from typical BL, include Ki-67 <90%, lower frequency of *MYC*-translocations (33-90%, and similarly to DLBCL, more commonly to a non-Ig-partner) and presence of concurrent *BCL2*- or *BCL6*-rearrangements in 47-78% [65, 247-249]. In addition, the previously termed BCLU cases, harbour an overall higher genetic complexity compared to mBL. Also, these patients present a higher median age and frequency of adverse prognostic features, in addition to an often poor response to conventional chemotherapy [243, 249, 250]. Morphological features are presented in figure 6.

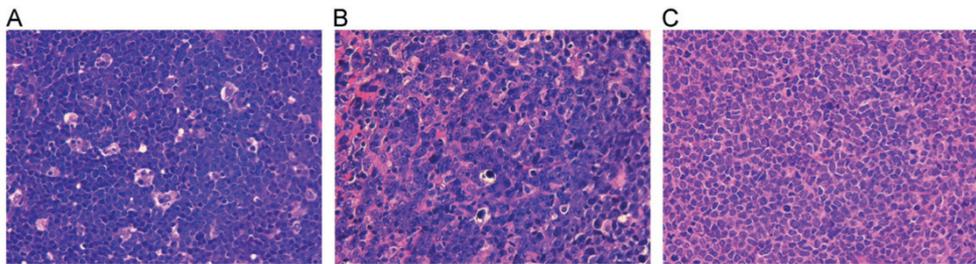


Figure 6. Morphological findings of *MYC*-rearranged lymphomas A) BL case with uniform, medium-sized lymphoma cells with small nuclei. Macrophages that have ingested apoptotic cells create a “starry-sky” pattern. B) DLBCL case with typical large lymphoma cells with abundant cytoplasm as well as large and prominent nucleoli. C) BCLU case with slightly larger lymphoma cells with more irregular nuclei, compared to BL. Magnification x400. From [251]. Reprinted by permission from Elsevier: Seminars in Hematology © 2015.

As reflected by the current classification nomenclature, an important subset of these intermediate cases are the so-called “double-hit” or “triple-hit” lymphomas (DHL/THL), that carry concurrent *MYC*- and *BCL2* and/or *BCL6*-rearrangements. Albeit a heterogeneous group, patients with DHL typically present with aggressive clinical characteristics and have inferior survival to non-DHL DLBCL and BCLU [248, 249, 252-254]. Preceding the current WHO classification, where all DHL and THL are included in the “HGBL with *MYC* and *BCL2* and/or *BCL6* rearrangements” category, the estimated frequency of DHL in DLBCL NOS was ~6%, with occurrence nearly exclusive to the GCB phenotype. In the prior BCLU entity, frequency was higher at 32-78% [253]. Data is contradictory regarding whether *MYC*-rearrangement alone (single-hit) confers inferior outcome [250, 255-258], or

if its detrimental prognostic effect is dependent on concomitant translocations [249, 252], although a recent meta-analysis found independent prognostic impact of all *MYC*-aberrations [259]. Furthermore, the importance of *MYC*-translocation partner have recently been emphasised, with inferior survival restricted to IG-*MYC* in some studies [256, 260]. Additionally, ‘double-expressor’ (DE), cases with positive staining of *MYC* and *BCL2* protein on IHC, have attracted increasing attention. DE are more common than DHL, particularly in the ABC subtype, and is not concordant with the presence of an actual rearrangement. Although associated with worse prognosis, outcome reported for DE is not as discouraging as for DHL [188, 252, 261].

Treatment of HGBL with MYC and BCL2 and/or BCL 6, and HGBL NOS

Due to the relative rarity of these cases and subsequent paucity of randomised trials specific for this subgroup, optimal standard treatment is not yet defined. However, most trials concordantly report unsatisfactory outcome with immunochemotherapy, indicating that this entity may require more intensive treatment [249, 252, 262, 263]. Two retrospective studies including DHL cases of both DLBCL and BCLU morphology found improved PFS when patients received BL regimens, such as Hyper-CVAD, CODOX-M/IVAC or DA-EPOCH-R, compared to R-CHOP [262, 263]. However, the higher median age of this intermediate group compared to BL patients may limit the potential clinical use of these intensive regimens. Thus, support has been lent for the use of the DA-EPOCH-R regimen, which has demonstrated to be tolerable also for elderly patients. In a phase II study of DA-EPOCH-R in patients with a *MYC*-rearrangement, preliminary reported survival was encouraging and the negative prognostic impact of *MYC*-translocation diminished [264]. In contrast, a single centre case series reported that the use of this regimen in 7 *MYC*-rearranged DLBCL patients did not improve on the outcome achieved with R-CHOP [265]. Furthermore, outcome at relapse is dismal, and first-line SCT has also not demonstrated a survival benefit [263]. Thus, novel treatment modalities and targeted therapy is warranted for this population.

SOX11

The transcription factor SOX11 is a member of the SOX gene family, which consists of over 20 proteins grouped together because they contain a similar DNA-binding high-mobility group (HMG) domain. This domain was originally identified in *SRY*, the sex-determining gene on the Y chromosome, hence the name SOX (SRY box containing) [266]. *SOX* genes control cell fate and differentiation, and are subdivided into eight groups (A-H) according to the degree of homology within, and outside, the HMG domain [266]. SOX11 is grouped together with SOX4 and

SOX12 in subgroup C. SOX4 is known to be an important transcription factor in both B- and T-cell lymphocytes, and thought to be crucial for B-cell development [267]. Although overlapping functions in the SOXC group have been suggested, no physiological role for SOX11 is known in haematopoiesis [267].

Instead, SOX11 is important for tissue remodelling and neuronal development in embryogenesis, during which SOX11 is transiently expressed [268]. In adults, SOX11 is absent in most differentiated tissues, although it continues to play an important role in neurogenesis, where it is believed to regulate neuronal progenitor cells [269]. Interestingly, after downregulation in normal adult tissue, SOX11 appears to be reactivated during tumorigenesis and is aberrantly expressed in several tumour types, including mantle cell lymphoma (MCL), malignant glioma, ovarian prostate, nasopharyngeal, gastric, and breast cancer [270-276]. In addition to MCL, SOX11 is present on transcriptional level in chronic lymphocytic leukaemia (CLL) [277]. Also, expression of SOX11 has been reported in 33-50% of BL, in studies aiming to determine whether expression of SOX11 was restricted to MCL [278-280].

Deregulation of SOX11 appears to be caused not by mutations in the SOX11 gene but via epigenetic modifications of its promoter, with unmethylated DNA and presence of activating histone marks associated with SOX11 overexpression. That hypermethylation, and thus silencing, of SOX11 is seen in several lymphoid neoplasms have led to speculations of a potential tumour suppressor role [281-283]. However, as SOX11 appears to be physiologically silenced in the adult hematopoietic system, DNA hypermethylation of SOX11 in lymphoid tissue may be a functionally dormant phenomenon [282].

SOX11 as a prognostic marker

The role of SOX11 has been most extensively studied in MCL, where its presence is almost universal and it functions as a diagnostic antigen [270, 280, 284]. However, whether SOX11 is a marker for adverse or favourable outcome is fraught with controversy, with conflicting results seen not only regarding MCL, but also in solid malignancies.

In MCL, there are reports that SOX11 expression confers a superior outcome, supporting the tumour suppressor hypothesis [285-288]. In contrast, lack of SOX11 has also been proposed as a marker for the non-nodal, indolent subtype of MCL and that presence of SOX11 mediates a worse outcome [289-293].

In other neoplasms, a tumour suppressor role with SOX11 expression constituting a favourable prognostic indicator, is seen in gastric, nasopharyngeal, prostate and high-grade epithelial ovarian cancer [272-275, 294]. The presence of SOX11 in breast cancer has been associated with superior outcome [276], but also adverse

prognosis in the basal-like subtype [295]. Also in CLL, the presence of SOX11 mRNA was associated with inferior prognosis [277].

Targets and proposed cellular functions of SOX11

In line with the conflicting data regarding prognostic influence of SOX11, various target genes and transcriptional programs regulated by SOX11 have been proposed, potentially facilitating both repressive, and inductive, effects on tumour growth.

In support of the tumour suppressor hypothesis, gene chip analysis *in vitro* demonstrated that SOX11 is associated with induction of cell cycle regulatory pathways such as Rb-E2F and TGF- β , decreasing tumour growth in lymphoproliferative cell lines [281]. Also, SOX11 knock-down in MCL cell lines resulted in increased proliferation and more aggressive tumours in mice [296]. GEP of MCL cell lines have revealed several potential target and co-regulated genes for SOX11, such as hypoxia-inducible factor 2 (HIF-2), which may have a tumour suppressor function [297, 298]. Additionally, one functional study found a SOX11-mediated repression of the WNT-pathway, which controls pro-proliferative genes such as *MYC*, resulting in decreased proliferation rates in MCL [299].

In contrast, a number of functional studies have outlined several oncogenic pathways of SOX11 in MCL. In a mouse model, SOX11 was demonstrated to promote tumour growth, contributing to a more aggressive disease course. The same study identified 366 genes affected by SOX11 knock-down, of which *PAX5* was one of the major targets. Silencing of SOX11 decreased *PAX5*, which in turn increased *BLIMP1*, promoting a shift toward plasmacytic differentiation [300]. In line with these results, plasma cell differentiation was significantly more frequent in SOX11-negative MCL tumour samples, indicating that SOX11 may contribute to lymphomagenesis by blocking terminal B-cell differentiation in MCL [301]. Furthermore, *in vivo* studies reveal that SOX11 increase vascular tube formation, endothelial cell proliferation, cell migration and angiogenic pathways through regulation of platelet derived growth factor A (*PDGFA*), contributing to a more aggressive MCL phenotype [302]. In addition, SOX11 was recently described to directly repress *BCL6*, preventing SOX11 expressing MCL cells to enter the GC [303].

Knowledge regarding the functional role of SOX11 in other neoplasms than MCL is so far limited. Because the HMG-domain on SOX proteins is known to increase its DNA-binding affinity and specificity by interacting with other transcription factors, it is probable that target genes may vary according to the molecular environment, due to the presence of differing tissue-specific co-transcription factors [266].

Aims of this work

The overall objective of the work presented in this thesis was to investigate how prognostic and clinicopathological factors, as well as choice of chemotherapy regimen, affect overall survival in population based data sets of adult patients with BL and DLBCL.

With a scarcity of randomised trials performed to evaluate treatment for adult BL, and for certain cohorts of DLBCL patients, the ambition was that population based data may contribute knowledge regarding what therapeutic option to choose, and validate the applicability of current treatment strategies in the general population, not fully represented in clinical trials. Also, increased insight into the influence of clinicopathological factors on prognosis may guide treatment stratification and identify patient cohorts most in need of novel treatment options, aiding design of future clinical trials. The specific aims of the studies included were:

- To examine prognostic factors for OS in a population based data set of adult BL patients, and to analyse the efficacy of chemotherapy regimens specified in the Swedish lymphoma registry (**Paper I**).
- Determine the efficacy of the chemotherapy regimens used to treat adult BL patients in Sweden and Denmark, and evaluate the impact of rituximab addition as well as whether outcome improved during the study observation period (**Paper II**).
- To compare chemotherapy regimens used to treat adult DLBCL patients in Sweden, and investigate if there is a beneficial effect of addition of etoposide and/or dose-dense chemotherapy in a population based data set (**Paper III**).
- To investigate the frequency of SOX11 positive BL cases on immunohistochemistry (IHC) staining and correlate its expression to clinical and pathological parameters (**Paper IV**).

Patients

All patients in the studies included in this thesis were identified through the Swedish Lymphoma Registry (SLR), and for papers II and IV also via the lymphoma registry of the Danish lymphoma group, within the collaborative framework of the Nordic Lymphoma Group.

The SLR was established in 2000 by the Swedish Lymphoma Group. Due to the complexity of malignant lymphoma and their characterisation, the aim was to expand the data included in the Swedish Cancer Registry (SCR), which was initiated in 1958. Cases of cancer are reported to the SCR in a double manner through both the pathologist and clinician responsible for diagnosis, but does not include detailed clinical parameters. The SLR functions as a registry for quality control in Swedish health care, and it is administered through regional cancer centres (RCC). Complete registration of all lymphoma cases in the SLR is attempted via collaboration with the SCR, which notify the appropriate RCC at registration of a lymphoma in the SCR. The RCC subsequently initiate a case file that is sent out to the health care clinic responsible for the patient. From 2008 and onwards registration has been managed by a web-based report system. Data from the SLR are presented in annual reports (www.swedishlymphoma.com). Compared to the SCR, the coverage of the SLR is ~95% of all lymphoma cases diagnosed in Sweden [304]. Initially, the registry's content was restricted to clinical characteristics, but since 2007 detailed data regarding treatment and response has been added.

The lymphoma registry of the Danish Lymphoma Group (LyFo) was initiated in 1983, with initial coverage limited to Western Denmark. In 1999 the registry was expanded to include all newly diagnosed patients with lymphoma in Denmark. It also issues annual reports (www.lymphoma.dk) and coverage is cross-referenced with the Central Danish Cancer Registry as well as the Danish Central Registry of Pathology [223].

All BL and DLBCL patients included in this thesis were diagnosed according to the pathology guidelines specified by the WHO classification at the time of diagnosis. Data regarding survival status were collected from the respective national population registries, without access to cause of death.

Relevant ethical approval was obtained from local ethics committees in Sweden and Denmark, respectively (reference numbers: 73/2008, 2014/854, H4-4-2013-115).

Paper I

The study population consisted of adult BL patients diagnosed with BL in Sweden from 1 January 2000 to 31 March 2010. A total of 156 patients were registered with a BL diagnosis in the SLR during this period.

Paper II

This study was performed as a collaborative study with the Danish Lymphoma Group and included all 258 patients diagnosed with BL in Sweden and Denmark from 1 January 2000 to 31 December 2009, registered in the respective national lymphoma registries. For Swedish patients diagnosed prior to 2007, a review of medical records was performed to collect data on treatment.

Paper III

The study population consisted of all adult patients diagnosed with DLBCL in Sweden during a six-year period from 1 January 2007 to 31 December 2012, as registered in the SLR. Patients with CNS involvement were excluded (n=173), resulting in a study population of 3443 patients.

Paper IV

The study population included 45 adult patients registered with a BL diagnosis in the Danish and Swedish Lymphoma Registries, from the Capital Region of Denmark (diagnosed 2002-2011, n=25) or Southern Sweden (diagnosed 2000-2009, n=20), with paraffin blocks available. In addition to the adult BL cohort, nine paediatric BL cases from Denmark were obtained and analysed for SOX11 expression, without clinical data available.

Methods

Statistics

Paper I–IV

In all studies, the Kaplan–Meier method was used to estimate OS rates. To compare survival curves the log-rank test was utilised. Crude and adjusted hazard ratios (HR) were calculated using Cox proportional hazards regression.

Paper I

Risk factors for OS were analysed using the Cox proportional hazards model, with hazard ratios presented as the mean values for the entire time interval. The variables used for the multivariable analysis had all shown statistical significance for predicting overall survival with p-values of 0.05 or less in the univariate analysis. Pearson χ^2 -tests were computed to evaluate interrelationships among prognostic factors. All statistics were calculated in SPSS version 19.

Paper II

For frequency tabulation of, e.g. clinicopathological features, prognostic factors and treatment regimens, the Pearson χ^2 -tests and non-parametric tests were utilised. All P-values were two sided and values were regarded statistically significant if $P < 0.05$. All statistical calculations were performed with SPSS version 20.

Paper III

In multivariable analyses the effect of chemotherapy was adjusted for WHO PS (linear), S-LDH, gender, bulky disease, stage (as a factor on four levels) and age. Age was modelled as a restricted cubic spline with five knots, to more truthfully allow the effect of increased age on survival to vary in impact among different age (Figure 7). To test the stability of results and to further reduce the risk of bias because of differences in age and prognostic factors between patients receiving versus not receiving etoposide, stratified Cox regression was performed, thus allowing for different baseline hazards across strata. The strata were defined by age in eight groups, including patients up to 65 years (analysis adjusting for S-LDH, PS, stage, gender and bulky disease) as well as age in eight groups separated for age-adjusted IPI (analysis adjusting for gender and bulky disease). Data was analysed in

STATA version 13 (for Kaplan–Meier estimation and Cox regression) and SPSS version 22 (for patient characteristics).

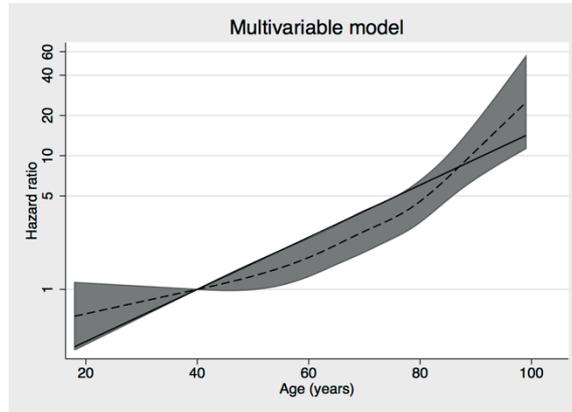


Figure 7. The effect (hazard ratio) of age in a multivariable analysis when modelled as a linear covariate (whole line) compared to when modelled as splines (dotted line).

Paper IV

Clinicopathological features, prognostic factors and treatment regimens were compared between groups with Pearson χ^2 -tests and independent samples t-test. Data was analysed in SPSS version 22.

Immunohistochemistry

Immunohistochemistry (IHC) is a widely used technique to analyse the expression of specific proteins in tumour tissue, via utilisation of antibodies directed against the protein of interest. In Paper IV, IHC was performed both on tissue samples assembled into tissue microarray (TMA) (Swedish cases), and on whole tissue sections (Danish cases).

For more than a decade, TMA technology has been a well-established and commonly used method to perform tissue-saving IHC analyses of multiple tumour markers. TMA technology has demonstrated good concordance with results from IHC performed on whole tissue sections, despite the comparatively small amount of tumour tissue in each core biopsy [305]. The TMA blocks analysed in paper IV were constructed according to the method described by Kononen *et.al.* [306] using formalin fixed paraffin embedded (FFPE) tissue sections from Swedish BL cases that were haematoxylin and eosin-stained, with representative areas subsequently selected. One mm in diameter FFPE tissue cores were then transferred in duplicate

to a recipient paraffin block. In paper IV, IHC BL TMA and whole tissue sections were stained for SOX11 using the monoclonal antibody SOX11-C1, developed in-house to improve sensitivity and specificity of SOX11-staining, as compared to previously used polyclonal antibodies with a broad batch-to-batch variation and cross-reactivity to other SOXC-group proteins [279]. The fraction of positive nuclei was scored and samples divided into groups as follows; negative (any staining in <30% of tumour cell nuclei) and positive staining (any staining in >30% of tumour cell nuclei). Standard staining for other immunohistochemical markers was performed, as described in further detail in the method section of paper IV.

In vitro model

To investigate the relation between SOX11 and growth in BL in Paper IV, an *in vitro* cell-line-based model was developed. Two different BL cell lines were used; BJAB (naturally expressing SOX11) and Raji (no SOX11 expression). *In vitro* transient knock-down of SOX11 was performed via SOX11-specific siRNA mediated gene silencing. Both nucleofection with a scrambled sequence (non-functional) and a GFP-producing plasmid were used as controls, to certify the success of the siRNA transfection.

The effect was measured in level of SOX11 protein expression at 48 hours, in a western blot analysis. As a control, the same procedure was performed on the SOX11 expressing MCL cell line Z-138. Western blot analysis is a powerful method for the immunodetection of proteins, especially if present at low levels. The principle of western blot is to separate proteins according to their molecular weight using gel electrophoresis and then transfer the proteins to a membrane to allow subsequent identification and quantification of a selected protein, via staining with antibodies specific to the target protein [307]. The protein data presented in paper IV is representative of three independent assays.

Assessment of cell proliferation of both BL cell lines as well as the MCL cell line Z-138, with and without SOX11 knock-down, was performed at 0 (reference value), 24, 48 and 72 hours. Level of cell proliferation was determined via detection of the radiolabelled agent methyl-14C-thymidine, measured by using the excitation effect of ionising radiation on the scintillation material and detecting the resultant light pulses. The uptake of methyl-14C-thymidine is cell cycle specific, with incorporation restricted to proliferating cells. Thus, the level of thymidine incorporation is proportional to the amount of cell proliferation [308]. All proliferation values were normalised towards the untreated control. More immersive

details regarding the methods used to develop the *in vitro* model are presented in paper IV.

Methodological considerations

Paper I-IV

In all papers, the primary measure of outcome was overall survival. Survival status was collected from the respective national population registries, without access to cause of death. Thus, for the elderly population in particular, some causes of death may not have been lymphoma-related, and calculated hazard ratios may consequently be exaggerated due to age-associated excess mortality. For elderly patients, calculation of relative survival might be considered. Also, as neither progression nor relapse data, or data regarding other events, was available in the registry, progression and/or event free survival could not be assessed. Finally, the observational study design of all four studies preclude complete exclusion of residual confounding.

Paper I

Treatment data was only available for 44.5% of the study population, hence questioning the feasibility of analysing treatment outcomes in this study. Typically, when developing a prognostic index, a validation cohort, separate from the derivation cohort is mandatory. This was not done for the proposed index in paper I, thus its validity remains to be determined.

Paper III

Due to large disparities between treatment groups, we were precluded to perform a case control comparison, which may have been the preferable method to analyse the impact of etoposide. A case control approach would have provided groups with comparable patient characteristics, only differing in administration of etoposide, perhaps providing optimal isolation of its effect. Instead, both a stratified comparison and treatment-intensity associated analyses were performed, thus affirming the stability of our results, although complete exclusion of bias is deterred in very heterogeneous cohorts.

Paper IV

The use of immunohistochemical analysis is associated with several well-known limitations, such as individual assessment and technical aspects affecting the interpretation of results. Additionally, for SOX11 expression on IHC, there is as yet no consensus regarding criteria for a positive result. In MCL, a good correlation of

SOX11 expression on protein and mRNA level is seen without evidence of an obvious cut-off value. Thus, a dichotomised division has been proposed where cases with weak and/or variable staining considered SOX11 positive [309]. The cut-off value used in paper IV is based on data from an MCL study in which the effect of expression of SOX11 on outcome was associated with the 30% cut-off value used by us [279, 288]. The development of monoclonal SOX11 antibodies (as used in paper IV) have considerably improved reliability for both sensitivity and specificity, compared to prior polyclonal antibodies [279, 310].

The number of variables possible to adjust for was limited due to the small study population. Consequently, some disparities in characteristics between compared groups may remain, increasing the risk of residual confounding.

Results

Impact of treatment and prognostic factors on OS in BL

Both Paper I and Paper II describe prognostic factors and impact of chemotherapy regimen on OS for adult patients with BL. In paper II, the cohort was expanded to include patients from the Danish lymphoma registry. Also, additional treatment data for Swedish patients diagnosed prior to 2007 was added through review of medical records. The study population in paper I consisted of 156 patients and in paper II of 258 patients.

Median age in both studies was 56 (range 16-93 and 15-93, respectively). Two-year OS for the whole population was 61.6% and 57.7% in paper I and II, respectively. Median follow-up time for surviving patients in paper I was 41 months and 58 months in paper II. The male to female ratio was similar in both papers, 2.6:1. Likewise, patient characteristics were comparable, presented in part for paper II in Table VI. In paper I treatment data were available for 44.5% of the population, the corresponding rate in paper II was 79.5%.

Age is the most important prognostic indicator

Advanced age predicted adverse prognosis in both paper I and II. It was the sole variable independently associated with impaired OS in both studies. In paper I, advanced age, poor PS, elevated levels of LDH were all associated with inferior survival at the univariate level. In the multivariable analysis, both age and PS >1 retained independent prognostic importance (PS>1 HR: 3.0 95% CI:1.7-5.3 $p<0.01$). Additionally, in paper II, presence of B-symptoms and bone marrow involvement also correlated with adverse prognosis at univariate level (Table VI). Neither gender, Ann Arbor stage, CNS involvement nor presence of bulky disease had significant prognostic impact in any of the studies, although there was a trend for inferior survival of female patients in paper I. Several prognostic factors were associated with each other. Advanced age correlated with poor PS, elevated LDH and a high number of extranodal sites. In turn, elevated LDH was associated with the presence of bulky disease, PS>1 and stage III-IV.

There was a striking dichotomy in outcome when stratifying the study population into age groups in both paper I and II, with an HR of 6.4 for patients aged ≥ 40 in paper I (95% CI: 2.3-17.7, $p < 0.01$) and 4.5 for patients aged ≥ 60 (95% CI: 2.6-7.8, $p < 0.01$) (Figure 8 & 9).

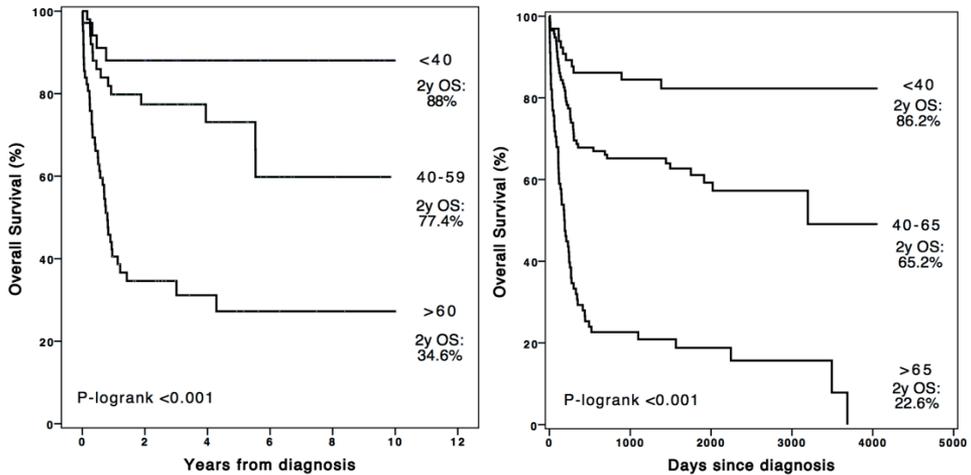


Figure 8 & 9. Overall survival for adult patients with BL grouped according to age. Figure 8 presenting data from paper I (n=156), Figure 9 presenting data from paper II (n=258).

Table VI. Estimated 2-year OS and hazard ratios for prognostic factors according to univariable and multivariable Cox regression analysis. Data presented from paper II.

VARIABLE	N (%)	2Y OS (%)	UNIVARIABLE ANALYSIS			MULTIVARIABLE ANALYSIS		
			HR	95% CI	p	HR	95% CI	p
AGE								
<40	65 (25)	86.2	-	-	-			
40-65	115 (45)	65.2	4.6*	2.5-8.6	<0.01			
>65	78 (30)	22.6	4.5*	3.1-6.4	<0.01	1.04	1.0-1.1	<0.01
PS								
0-1	163 (65)	72.6						
2-4	89 (35)	32.4	3.5	2.4-5.1	<0.01	1.9	0.9-3.9	0.09
LDH								
<ULN	52 (22)	84.6						
>ULN	188 (78)	52	3.3	1.8-6.1	<0.01	1.7	0.7-4.3	0.3
BONE MARROW								
	91 (35)	49.4	1.5	1.0-2.1	0.03	0.9	0.5-1.8	0.8
B-SYMPTOMS								
	141 (55)	50.3	1.8	1.2-2.6	<0.01	1.6	0.8-3.6	0.2

*HR for all patients ≥ 40 and >65 , respectively. In the multivariable analysis, age was analysed as a continuous variable. Year of diagnosis, CNS disease and treatment also included in multivariable analysis (Table 1, paper II).

The IPI may not be ideal for risk stratification of BL patients

Despite including both Ann Arbor stage and extent of extranodal disease, that did not impact outcome for BL patients in neither paper I nor II, the IPI identified two distinct risk groups (IPI 1-2 2-year OS: 81.3% and IPI 3-5 2-year OS: 51%) in paper I. However, when excluding stage and number of extranodal sites in a proposed modified prognostic index in paper I, three risk groups with more distinct survival rates were distinguished (2-year OS: 91.2%, 58.4% and 27.5% for point 0-1, 2 and 3, respectively).

Outcome has improved for BL patients aged ≤ 65

In paper II, the study population was divided into two groups according to year of diagnosis; 2000-2004 and 2005-2009. Two-year OS rates were 52.6% and 61.3% for patient diagnosed in the earlier and later time period, respectively. For the whole population, year of diagnosis did not have independent prognostic value. However, when stratifying the study population according to age above or under 65 years, a statistically significant secular improvement in OS was demonstrated for the younger age group (2000-2004: 2-year OS 64.1%; 2005-2009: 79.4%; HR=0.5, 95% C. I.: 0.3-0.9, $p=0.02$). Corresponding 2-year OS rates for patients aged >65 were 21.7% and 22.9%, respectively (Figure 10 & 11).

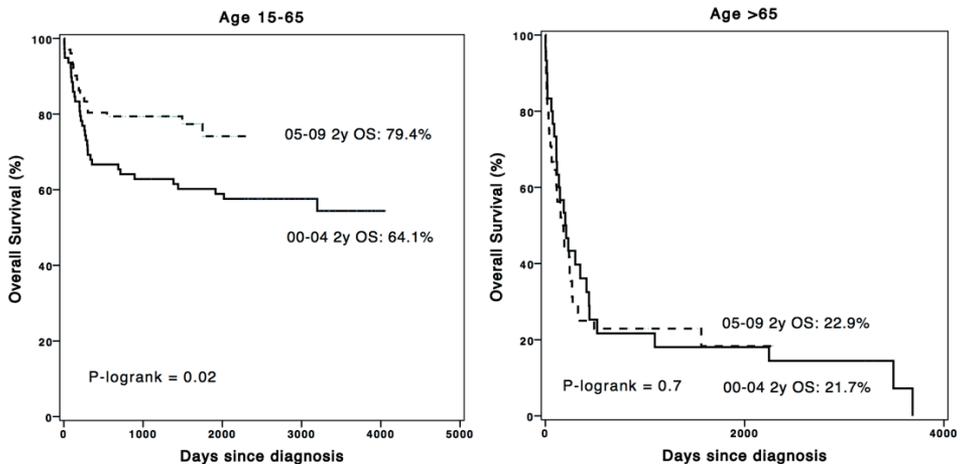


Figure 10 & 11. Overall survival according to year of diagnosis, 2000-2004 or 2005-2009 (broken line), for patients aged 15-65 (Figure 10) and >65 (Figure 11).

Intensive chemotherapy regimens are associated with superior OS

In paper II, treatment data were available for 205/258 patients. Among these, 64% were treated with a high-intensive regimen (BFM, Hyper-CVAD or CODOX-M/IVAC). The distribution, median age and 2-year OS for patients administered the various chemotherapy regimens, used in Denmark and Sweden during the study period, are presented in Table VII. In addition to presenting at a higher median age, patients who received CHOP/CHOEP or no treatment more frequently exhibited WHO PS>1 and elevated S-LDH. No difference in the presence of these factors was seen among the other regimens.

Table VII. Distribution of chemotherapy regimens, median age, 2-year OS rates and use of rituximab

	BFM	HYPER-CVAD	CODOX-M/IVAC	CHOP/CHOEP	OTHER	NO TREATMENT
N (%)	71 (34)	29 (14)	32 (16)	49 (24)	18 (9)	6 (3)
MEDIAN AGE	40	56	42	66	67.5	81
2-YEAR OS (%)	81.7	82.8	68.6	38.8	33.3	0
RITUXIMAB						
YES	43 (60.5)	28 (97)	14 (44)	15 (31)	6 (33)	0 (0)
NO	11 (15.5)	0 (0)	18 (56)	20 (41)	8 (45)	6 (100)
MISSING	17 (24)	1 (3)	0 (0)	14 (28)	4 (22)	0 (0)

Complete table including distribution of all prognostic variables are presented in Table 2, paper II.

Patients who received the more low-intensive CHOP/CHOEP regimen (2-year OS 38.8%) demonstrated significantly inferior outcome compared to patients administered the high-intensive regimens (BFM, Hyper-CVAD or CODOX-M/IVAC, combined 2-year OS 78.7%), irrespective of age differences and use of rituximab (HR = 2.0 95% CI 1.0-4.1, $p=0.04$) (Figure 12).

High-intensive regimens demonstrated equal efficacy

OS-rates were similar for patients who received high-intensive regimens (Figure 12), and there was no evidence of a survival benefit for patients administered any of the high-intensive regimens, also when considering distributional differences in age and use of rituximab (Table VIII).

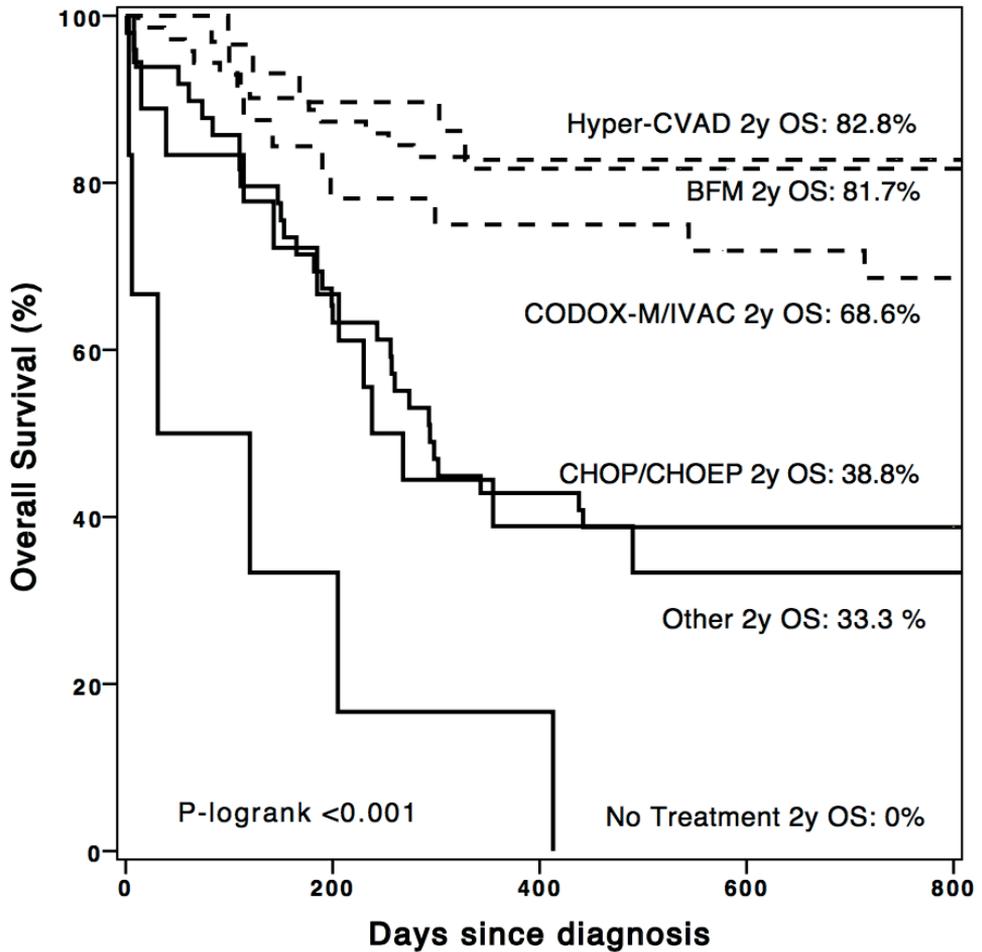


Figure 12. Overall survival according to chemotherapy regimen. Data presented from Paper II. High-intensive regimens (broken lines) significantly superior to CHOP/CHOEP.

Table VIII. Multivariable analysis of overall survival restricted to intensive chemotherapy regimens

	HR	95% CI	P
AGE*	1.04	1.02-1.1	<0.01
RITUXIMAB	0.98	0.6-1.7	0.98
BFM	-	-	-
HYPER-CVAD	0.67	0.2-2.1	0.49
CODOX/M-IVAC	2.1	0.9-5.1	0.24

*Continuous variable. The BFM regimen was used as the reference category. Data presented from paper II.

The effect of addition of rituximab to BL treatment remains elusive

Of the 205 patients with treatment data available in paper II, information regarding use of rituximab was known for 163. Of these, 111 patients (68%) received rituximab and demonstrated a 2-year OS of 70.3%. The corresponding rate for patients treated without rituximab (n=52) was 55.8%. Rituximab was mainly used from 2005 and onwards, and all patients treated with Hyper-CVAD concurrently received rituximab. In univariable analysis, patients receiving rituximab demonstrated superior outcome (HR=0.57, 95% CI:0.34-0.94, $p=0.03$). However, this favourable effect diminished when adjusting for age and chemotherapy regimen (Table VIII). Numerically, there appeared to be a discrepancy of rituximab impact in combination with different regimens, but there was no evidence for improved outcome with rituximab when examining regimens individually (Figure 13-15).

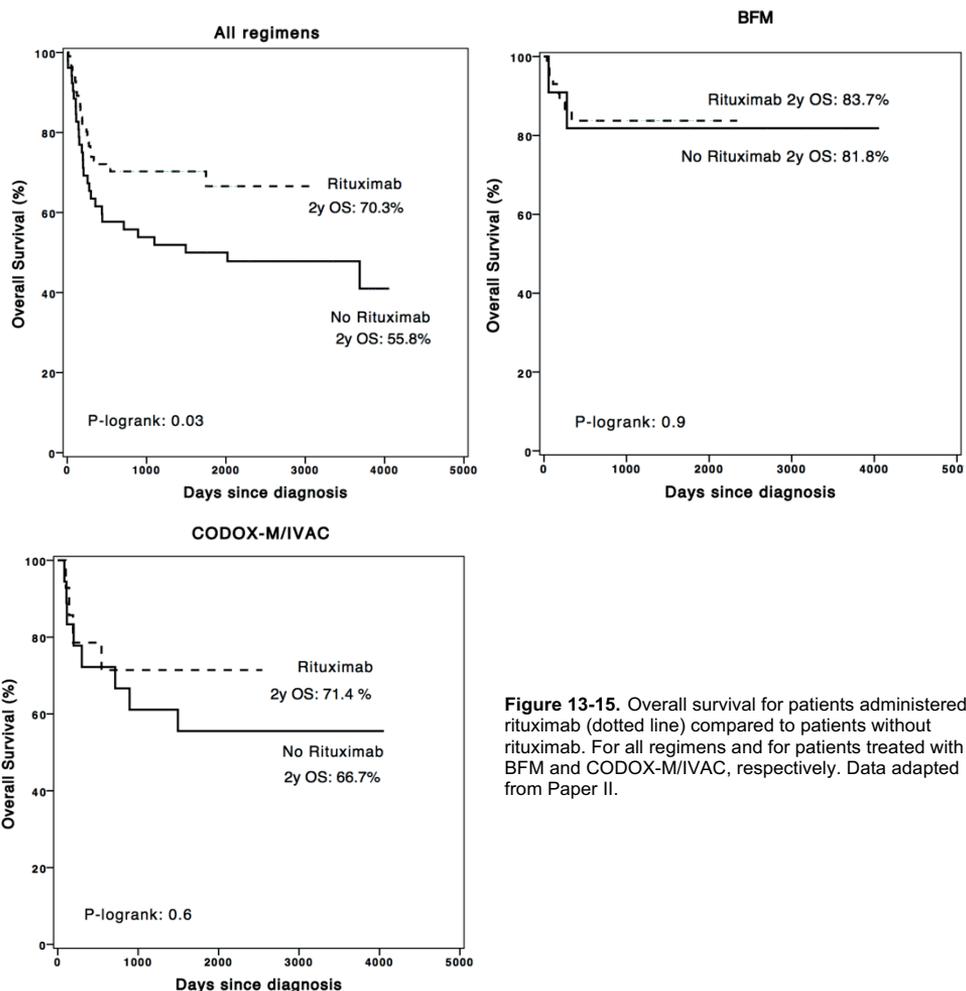


Figure 13-15. Overall survival for patients administered rituximab (dotted line) compared to patients without rituximab. For all regimens and for patients treated with BFM and CODOX-M/IVAC, respectively. Data adapted from Paper II.

Frequency and clinical implications of SOX11 in BL

In paper IV, the aim was to investigate the frequency of SOX11 positive BL cases in a joint cohort from Sweden and Denmark, and correlate its expression to clinical and pathological parameters. Also, we examined the relation between SOX11 and growth in BL cell lines.

The study population was collected from Denmark and southern Sweden and consisted of 45 adult BL patients. Median age was 49 (range 18-86) and median follow-up time for surviving patients was 74 months.

SOX11 was expressed in a minority of adult BL patients

Fourteen patients (31%) in the adult population expressed nuclear staining of SOX11 (Figure 16). Median age in the SOX11 positive group was 53 years, compared to 44 in the SOX11 negative cohort, but the difference was not significant ($p=0.7$). SOX11 expressing BL patients more often presented with elevated LDH, but did not differ with regard to presence of other prognostic factors. The extent of SOX11 positive and negative BL patients who received high-intensive regimens was similar. However, three patients in the SOX11 positive subgroup received no treatment compared to one patient in the SOX11 negative cohort. The BL cases without treatment were subsequently excluded from remaining analyses to minimise treatment bias. There were no differences in immunohistochemical expression of CD10, BCL6, BCL2 or p53 expression between the two groups.

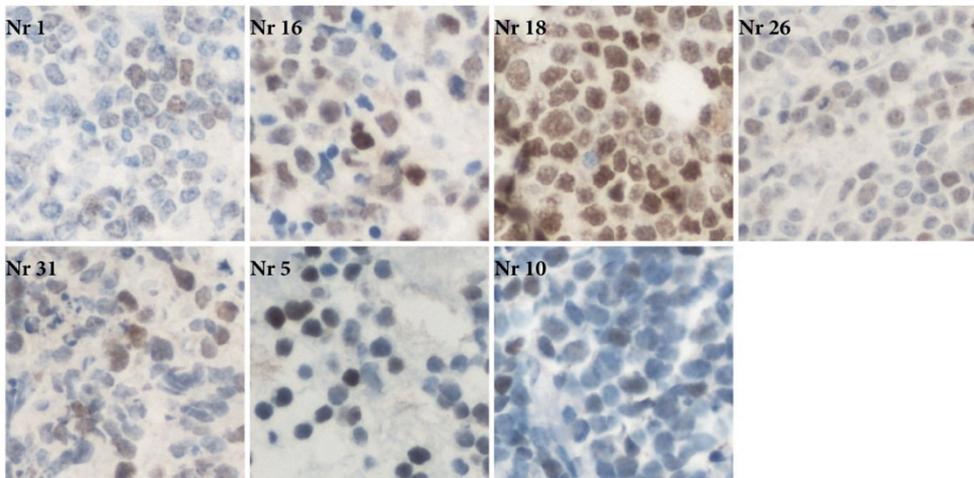


Figure 16. SOX11 staining among adult Burkitt Lymphoma. Among the positive cases (31% of total cases) the intensity and fraction of SOX11 varied (see positive cases 1, 16, 18, 26, 31 and negative cases 5 and 10).

SOX11 expression in this BL cohort did not impact prognosis

When excluding patients who were not administered treatment, OS rates for the SOX11 positive and negative cohorts were 73 % and 86 %, respectively (Figure 17). There was no evidence for a difference in outcome between SOX11 positive and negative BL patients, when adjusting for age and use of low-intensive treatment regimens (CHOP or other) (HR: 1.9 95 % CI: 0.4-8.7, $p=0.4$).

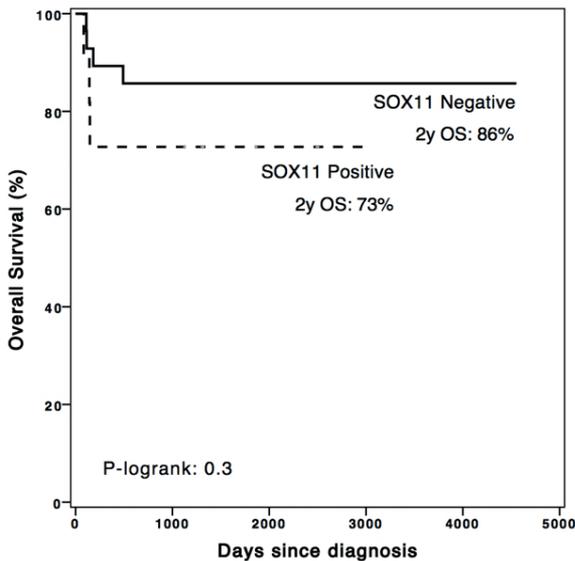


Figure 17. Overall survival of patients with either SOX11 positive or negative disease

SOX11 expression may be more frequent in paediatric BL

In nine paediatric BL patients, where tissue material but no clinical data were available, 5/9 (56%) expressed nuclear staining of SOX11.

SOX11 knock-down in a BL cell line (BJAB) results in increased cellular proliferation

In an *in vitro* model of BL using the SOX11 expressing BJAB cell line, siRNA mediated gene silencing resulted in a significant decrease of SOX11 protein after 48 hours (Figure 18A) and a 25% increase in proliferation at 48 hours (Figure 18B). The Raji cell line does not express SOX11 and no change in proliferation was consequently detected.

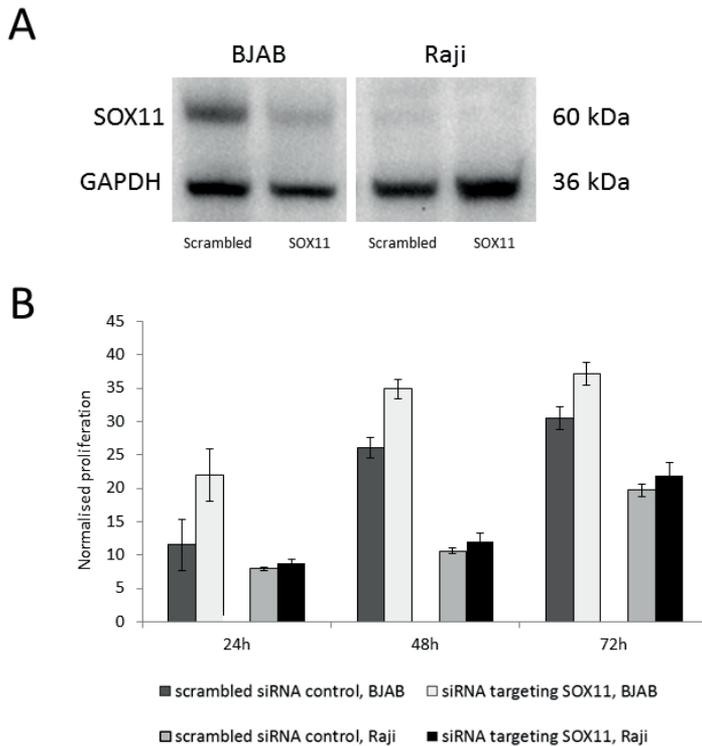


Figure 18. Effect of the siRNA induced knock-down of the SOX11 gene in BJAB on A) protein level at 48 hours and B) proliferation at 0, 24, 48 and 72 hours. The Raji cells line does not express SOX11 and proliferation is not affected by siRNA. The protein data is representative of four independent assays. In B) all values are normalised towards the untreated control and the error bars show \pm standard deviation.

Impact of addition of etoposide to chemotherapy in DLBCL

Paper III compared outcome, described as overall survival, for adult DLBCL patients treated with various chemotherapy regimens, with special emphasis on the addition of etoposide and dose-dense administration. The study population consisted of 3443 patients - all adult patients diagnosed with DLBCL NOS without CNS involvement in Sweden 2007-2012. Median follow-up time for surviving patients was 47.4 months. Median age was 70 (range 18-105), and there was a slight male predominance of 55%. Data on treatment were available for 2838 patients (82%).

Age and distribution of other prognostic factors differed between patients administered various regimens

R-CHOP-14 was the most commonly administered regimen (42%). Apart from a higher median age, patient characteristics were favourable among patients administered R-CHOP-21, with a lower proportion of patients with elevated S-LDH, Ann Arbor stage III-IV and presence of bulky disease in the R-CHOP-21 group compared to the other regimens. As expected, the most intensive regimen, R-CHOEP-14, was more frequently administered to younger, poor-prognosis patients and median age was highest among patients who received no treatment or other regimens.

Table IX. Patient characteristics and overall survival stratified according to treatment.

	R-CHOP-21	R-CHOP-14	R-CHOEP-14	OTHER	NO TREATMENT
N (%)	910 (32)	1196 (42)	158 (6)	373 (13)	201 (7)
MEDIAN AGE (RANGE)	76 (26-99)	64 (18-90)	50 (18-78)	80 (18-96)	83 (44-105)
FIVE-YEAR OS (%)	56	70	84	34	3.7
S-LDH:					
<ULN	494 (56)	360 (30)	23 (15)	149 (42)	47 (32)
>ULN	390 (44)	821 (70)	134 (85)	206 (58)	103 (69)
WHO PS:					
0-1	751 (83)	993 (83)	130 (82)	237 (65)	50 (28)
2-4	154 (17)	197 (17)	28 (18)	128 (35)	131 (72)
STAGE:					
I-II	509 (58)	452 (38)	42 (27)	172 (51)	52 (35)
III-IV	373 (42)	734 (62)	116 (73)	166 (49)	97 (65)

Missing data not reported in this version, complete table presented in paper III.

No beneficial effect of dose-dense administration – equal efficacy of R-CHOP-14 and R-CHOP-21 among all age groups

Patients administered R-CHOEP-14 had a superior 5-year OS rate of 84% compared to 70% for R-CHOP-14 and 56% for R-CHOP-21 (Figure 19). In a univariable Cox regression analysis, there was strong evidence of lower HR rates for both R-CHOEP-14 and R-CHOP-14 compared to R-CHOP-21. However, when adjusting for distributional difference in prognostic factors, there was no remaining evidence of an overall difference between the studied chemotherapy regimens (Table X).

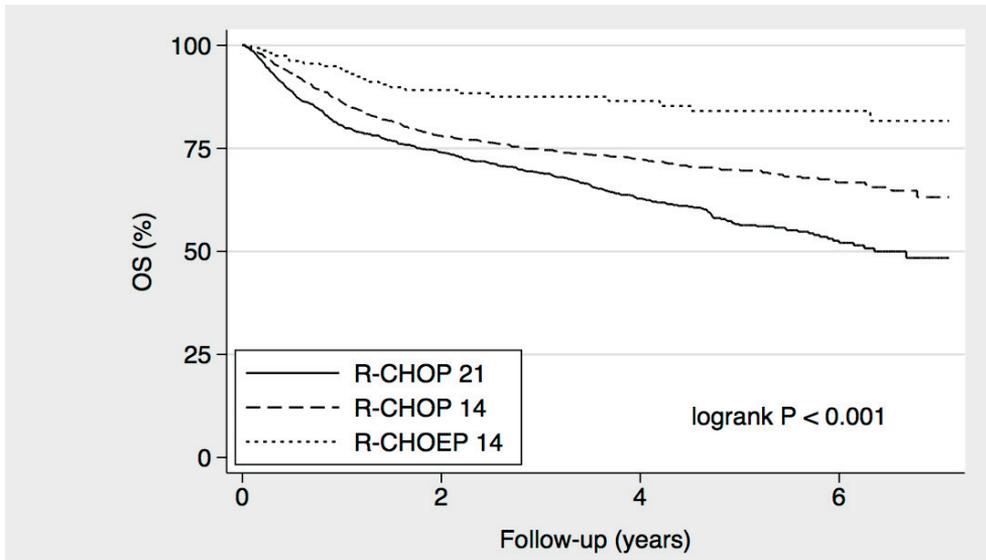


Figure 19. Overall survival curves according to chemotherapy regimen. Data presented from paper III.

R-CHOEP-14 is associated with improved OS among patients aged ≤ 65

In a subgroup analysis restricted to patients eligible to receive etoposide in terms of toxicity tolerance, 1304 patients aged 65 and under were included. Of these, 201 patients received R-CHOP-21, 657 R-CHOP-14 and 155 R-CHOEP-14. Also in this cohort, patients administered R-CHOP-21 demonstrated favourable prognostic features apart from a higher median age. Complete patient characteristics are presented in Table 1, paper III. Five-year OS rates were 85%, 78% and 84% for the patients who received R-CHOP-21, R-CHOP-14 and R-CHOEP-14 respectively. When adjusting for prognostic factors, R-CHOEP-14 was associated with a lower HR compared to R-CHOP-21 (HR: 0.49 95% CI: 0.3-0.9 $p=0.028$, Table X) and also in a direct comparison with R-CHOP-14 (HR: 0.64 95% CI: 0.4-1.0 $p=0.06$).

Table X. Univariable and multivariable analysis of overall survival according to chemotherapy regimen

		UNIVARIABLE ANALYSIS			MULTIVARIABLE ANALYSIS		
		HR	95% CI	P	HR	95% CI	P
ALL PATIENTS	R-CHOP-21	1		<0.001	1		0.15
	R-CHOP-14	0.69	0.6-0.8	<0.001	0.91	0.8-1.1	0.3
	R-CHOEP-14	0.32	0.2-0.5	<0.001	0.63	0.4-1.0	0.06
PATIENTS ≤ 65	R-CHOP-21	1		0.02	1		0.06
	R-CHOP-14	1.6	1.1-2.5	0.02	0.76	0.5-1.2	0.3
	R-CHOEP-14	1.1	0.7-2.0	0.6	0.49	0.3-0.9	0.028

Multivariable analysis adjusted for WHO performance status (linear), S-LDH, gender, bulky disease, stage (as a factor on 4 levels) and age. Age included as a spline with five knots. R-CHOP-21 was used as the reference category.

Discussion and future perspectives

Population based data

All four studies included in this thesis are based on population based data, identified via national registries. Although utilisation of population based data entails several unique advantages, it is also associated with some important limitations.

One of the major assets of population based data is the inclusion of patient groups that are frequently under-represented in clinical trials and case series from large referral centres. Strict selection criteria are often applied in clinical trials. Accordingly, patients of advanced age, with poor performance status and/or comorbidities are generally not represented in clinical trials [88]. Thus, this precludes assessment of optimal treatment and prognostic factors for these patient groups. While case series often describe consecutive patients treated at a certain centre, they frequently depict the experience from large academic referral hospitals for rare diagnoses, such as BL. Hence, albeit not employing exclusion criteria, these series may suffer from referral-bias created by the selection of patients that tend to be referred to academic centres. Again, potentially resulting in an under-representation of old and frail patients.

Consequently, population based data may function as a valuable complement to clinical trials and case series. The non-selection of patients in population based studies probably enable the most accurate and complete disease description. Also, by including the entire spectrum of patients with a certain disease, identification of true risk groups is facilitated. Another benefit of population based data is its surveillance potential, allowing evaluation of patterns and trend over a certain time period. Additionally, for rare entities such as BL, the relatively large number of patients available when using population based data is advantageous.

Concurrently, the inclusion of all patients will also incur some of the disadvantages associated with the analysis of population based data. The non-selectivity of patients may introduce a large number of confounding factors due to the potentially very heterogeneous population created. Thus, elucidation of causal relations between associations may be difficult.

Furthermore, potential incomplete register recordings constitute a limitation. For example, for some individual cases certain parameters may not have been documented and some variables may not have been registered at all, resulting in missing data. For example, in the studies presented in this thesis, missing treatment data was a recurrent issue. In both paper II and paper III ~20% of patients did not have information regarding treatment available. In both studies, the cohort with missing treatment data presented with a higher median age and inferior outcome. Hence, this indicates that information on treatment may be missing due to these patients, to a larger extent, having received no treatment. Moreover, registration of individual prognostic parameters was missing, although to a lesser degree. However, due to the small number and random distribution of these individual missing variables, they are unlikely to have introduced bias in the presented series.

Additionally, evaluation of further variables would have been valuable when assessing the aims of this thesis. For example, data regarding relapse, salvage therapies, toxicity and health-related quality of life would obviously have been of interest when comparing efficacy of chemotherapy regimens. However, excessive toxicity of a certain regimen would likely have translated into an effect on overall survival, which ultimately is the primary end-point of interest. Also, pathological parameters such as data regarding IHC and FISH would have been of interest.

With regard to the two lymphoma subtypes studied in this thesis, another important aspect to consider are the changes in diagnostic classification during the study observation period. Because of the large number of cases included, with widespread geographical distribution and sometimes lack of remaining tissue material, central pathology review was not feasible to perform. Indubitably, this increases the likelihood that the cohorts studied in this thesis include cases that would not be classified as BL or DLBCL NOS, according to current diagnostic criteria. For these diagnostic entities, this predicament is additionally augmented by the mounting evidence for the existence of an as yet incompletely characterised intermediate diagnostic grey zone, as described in the background. However, the fact that the population studied in this thesis may be heterogeneous also represents a unique possibility to more truly depict the real-world clinical scenario, where tricky intermediate cases cannot simply be excluded from clinical trials but must be allocated appropriate treatment. Thus, the series presented here may reflect a unique collective outcome of both typical, as well as the less typical, cases. This information is increasingly relevant as many grey zone cases have an aggressive clinical presentation and likely benefit from similar high-intensive regimens, as used for BL treatment [254, 262, 263].

Prognostic factors in BL

The extremely rapid proliferation of BL accentuates the importance of prompt initiation of adequate treatment [33]. It is largely a “one-shot” disease with high curability with first-line treatment but dismal prognosis at relapse due to limited availability of effective salvage regimens [104, 149, 150]. Thus, expeditious diagnosis and reliable risk stratification of patients is crucial to enable swift administration of optimal initial treatment to improve patients’ chances for survival.

Thence, it was of interest to try and establish variables of prognostic significance from a data set that represents the full spectrum of BL patients.

The influence of age is likely multifactorial

In paper I and paper II we demonstrate the vast impact of age on BL prognosis, both by confirming its strong independent effect on overall survival and also by demonstrating the isolated lack of improvement in outcome for patients aged >65 during the study period. The considerably inferior OS rates reported for older patients clearly substantiates the need for novel treatment strategies for this cohort. These results are concordant with data reported from both clinical trials and population based studies [85, 88, 89, 173].

Presumably, the influence of age is multifactorial. Firstly, optimal treatment for elderly patients is less well defined as patients aged over 65 constitute a minority in clinical trials performed [311], although this number is increasing due to advances in supportive care [88]. Also, excessive caution toward the toxicity associated with high-intensive regimens may affect treatment choice and withhold potentially curative treatment for elderly patients, as evidenced in previous studies and in both paper I and II where elderly patients were consistently over-represented in the cohorts who received no, or low-intensive treatment [40, 88, 173].

However, concurrently, a second explanation for inferior outcome in this cohort may be toxicity itself. Several clinical trials report worse toxicity rates and more frequent non-completion of treatment among elderly patients in clinical trials [89, 130, 139]. A suggestive explanation is the known association of age with serious comorbidity in lymphomas [312, 313]. Encouragingly, dose-modifications of CODOX-M/IVAC [138-140] as well as use of low-concentration exposure to chemotherapy agents, such as in DA-EPOCH-R show encouraging feasibility also for elderly patients [156]. Furthermore, Ribera *et.al.* reported that adverse prognosis associated with age diminished in the Burkimab trial, and attributed this to the dose-reduction of methotrexate in the NHL-2002 regimen for patients aged >55 [90].

However, this result was not replicated in a subsequent study by GMALL, using the same protocol [149].

Thirdly, the inferior outcome seen among elderly BL patients may represent a biological difference in BL disease, although reports on this issue somewhat differ. Several studies have been unable to detect differences in presence and frequency of chromosomal abnormalities [65, 77, 95, 96, 243, 246, 314] or miRNA levels [34] between typical paediatric and adult BL cases. In contrast, one earlier study found a higher number of genomic imbalances in adult BL [315]. More recently, Havelange *et al.* demonstrated an age-related heterogeneity in presence of genomic anomalies between adult and paediatric BL cases [82]. Additionally, population based epidemiological studies have revealed an age-dependent bimodal incidence of BL, indicating a potential heterogeneity in both aetiology and biology of BL among different age groups [38, 316-318].

Lastly, the inferior prognosis of elderly patients may be due to a higher frequency of misclassified cases among older patients. Cases intermediate between BL and DLBCL exhibit both a higher median age and level of karyotypic complexity. This affects disease biology and response to treatment, and the adverse outcome of grey zone cases has been confirmed in multiple studies [242, 243, 248, 253, 319]. Thus, if a larger proportion of elderly patients represent intermediate cases, this would contribute to the poor outcome seen in this fraction of patients.

That improvement in survival was restricted to patients aged ≤ 65 is in accordance with other epidemiological studies [8, 85, 173, 320]. In paper II, median age was higher in the later time period among patients treated with low-intensive regimens, indicating that advances in supportive care may have enabled administration of more toxic regimens also to older patients, although not yet of benefit for the most elderly. It is reasonable to assume that the fact that only a small minority of patients aged >65 received high-intensive regimens largely explain the dismal outcome and lack of improvement over time, demonstrated for this cohort.

Other prognostic factors and future prognostic scoring systems

In addition to age we establish that PS score and level of LDH influence outcome to a certain extent. PS score >1 had independent prognostic value in paper I, which diminished when also adjusting for CNS involvement and treatment in paper II. That these variables failed to sustain independent prognostic significance in multivariable analysis is likely explained by the extensive interrelationships present among the studied prognostic factors. This is not surprising, as several parameters measure similar disease characteristics. For example, total tumour burden is reflected in several variables, such as elevated LDH, presence of B-symptoms and bulky

disease, as well as in the dissemination of the disease, depicted via stage and extent of extranodal disease.

Prognostic factors reported from clinical BL trials are heterogeneous, possibly explained by statistical noise and selection criteria of included patients. However, apart from age, elevated LDH, failure to achieve CR, PS score >1, stage, bone marrow involvement as well as anaemia, low albumin, high creatinine and presence of circulating blasts have been reported to be associated with inferior outcome [61, 89, 91-93, 120, 126, 139, 148, 153].

Few studies have specifically examined prognostic factors for BL. Two SEER based studies found older age, black ethnicity and Ann Arbor stage III-IV to predict adverse outcome, but laboratory investigations and PS score were not examined [8, 85]. The impact of ethnicity in these series was attributed to potential unequal access to care [8]. Another prognostic study established factors associated with tumour burden to confer worse outcome, such as elevated LDH and uric acid as well as stage [87]. A small Asian study with unadjusted data demonstrated prognostic influence of PS >2, stage IV as well as bone marrow and CNS involvement [86].

In contrast, neither Ann Arbor stage nor extent of extranodal disease affected outcome in our BL study population. This conforms with the rapid and disseminated growth of BL, which the Ann Arbor staging system is not constructed to accurately portray [40]. Accordingly, in both paper I and II a majority of patients presented with stage IV disease. Both Ann Arbor stage and extent of extranodal disease are incorporated in the IPI. Thus, risk stratification of BL patients according to the IPI may not be ideal.

For several other NHL subtypes, the IPI has been modified to better suit specific entities, and been re-validated in the rituximab era [101, 183, 321]. As this has not been performed for BL, optimal risk stratification for BL is lacking. In Paper I, we proposed a modified IPI excluding stage and number of extranodal sites. Although this approach would need to be validated in a larger, independent cohort it may be a feasible strategy. However, with rapid advances in molecular profiling and improved staging opportunities via PET, it is likely that enhanced staging as well as augmented use of biological markers will be of relevance in future prognostic scoring systems [102, 190].

The prognostic consequence of genomic complexity is increasingly acknowledged. Multiple studies demonstrate that concurrent chromosomal aberrations in addition to t(8;14) affect prognosis [79, 95, 96, 322-324], although which chromosomal gains and/or losses that are of most prognostic value remains to be elucidated. Intriguingly, BL cases that harbour chromosome 11q aberrations, accounting for the novel provisional WHO classification category, typically exhibit a higher overall chromosomal complexity, but in spite of this demonstrate excellent survival rates

similar to typical BL cases with a less complex genome [70, 72]. For other lymphomas, such as DLBCL, there is an ongoing debate as to which variables that will affect prognosis and/or treatment choice in the era of biologic agents [190]. Likewise, in an updated prognostic index for follicular lymphoma, integration of gene mutations improved prognostication [325].

Moreover, the parameters that affect outcome will likely evolve in relation to advances in treatment and risk stratification. For example, an interesting result from our studies is the lack of prognostic relevance of CNS involvement. CNS disease was frequently associated with inferior outcome in early BL treatment trials [118, 120]. Since then, the prognostic relevance of CNS disease has lessened [89, 91, 93, 149]. However, its significance varies between clinical trials, even with application of similar protocols, and some trials still report adverse outcome of patients with CNS engagement [90, 92, 139, 150]. This heterogeneity may reflect differences in the studied populations, with differing median age and inhomogeneous inclusion of HIV-positive patients. However, that we and others demonstrate abated consequences of CNS involvement may indicate that aggressive CNS prophylaxis with i.v. and intrathecal cytarabine and methotrexate successfully eradicates CNS disease.

In the era of targeted therapy, it is probable that development of biomarkers indicative of which patients that may benefit from certain treatment, as well as improved risk- and response assessment will be pivotal.

Frequency and clinical implications of SOX11 in BL

As presented in Paper IV, SOX11 expression was expressed in ~1/3 of adult BL patients and was not associated with outcome in our cohort. Our reported prevalence rate is in accordance with prior rates demonstrated in smaller subsets of BL evaluated for SOX11 expression [278, 280].

As discussed in the background section, the prognostic bearing of SOX11 is heterogeneous and results of its impact on survival differ even among the same malignancy. For example, its role as a diagnostic antigen for mantle cell lymphoma (MCL) is established [270, 280] whereas its prognostic influence is under continued debate [286-290, 293]. Similarly, it is associated with both superior [272, 276], and inferior outcome in solid malignancies [295].

That SOX11 did not impact outcome for adult BL, in our material, may be multifactorial. Firstly, the studied cohort was small, calling for some caution when interpreting our results. Furthermore, there was a numerical discrepancy in patients who received no treatment between the SOX11 negative and positive cases in our

cohorts. To diminish this treatment bias, these cases were excluded from survival analysis, hence further decreasing the number of cases analysed.

However, assuming that our results are robust, the lack of prognostic impact of SOX11 may be explained by the diversified functions of the SOX proteins in varying molecular environments [266]. That the major regulation of the *SOX11* gene appears to occur via epigenetic modifications and that SOX11 genes are promiscuous in their DNA-binding capacity, accentuates the role of specific, contiguous events for its effector function and capacity to affect several pathways [281-283, 298]. However, that SOX11 appears to be physiologically silenced via DNA hypermethylation in the adult hematopoietic system and is not expressed in normal adult tissue [270], suggests some neoplastic influence to its aberrant expression in ~30% of adult BL cases. This is also supported by the detection of increased proliferation in BL cell lines on SOX11 knockdown, demonstrated in our study. Similar effect of SOX11 on growth in cell lines has been observed in other malignancies [281].

It should be noted, that experiments on cell lines may differ from real life specimens and thus these results warrant further validation. For example, it has been proposed that the divergent results regarding the prognostic influence of SOX11 in MCL is due to differences in clinical characteristics of studied samples [286, 289, 290, 299] and varying use of polyclonal and monoclonal antibodies [279, 310]. In addition, it has been speculated that results may differ according to whether experiments were performed in experimental systems *in vivo* or *in vitro*. In several *in vitro* and *in vivo* studies, a putative tumour suppressor role of SOX11 has been suggested [272, 281, 283, 296, 298, 299], whereas the opposite has also been reported [300-302]. Thus, it may be speculated that the non-physiologically high levels of SOX11 expression in experimental and cell line systems, may act in an anti-proliferative fashion not seen at physiological levels in mature cells [291]. In contrast, several studies indicate no difference in SOX11 impact dependent on how strong the immunohistochemical staining is, but rather that SOX11 should be dichotomously scored as negative or positive (any staining, even weak) [309, 310].

In concordance with the proposed heterogeneous function of SOX11, is the lack of association with SOX11 and BCL6 expression in our study on adult BL. Recently, it has been reported that SOX11 defines the two different subtypes of MCL via transcriptional repression of BCL6 [303]. The same study stimulated SOX11 transduction in BL cell lines and found that this decreased levels of BCL6 expression. However, in our study, no correlation between SOX11 and BCL6 expression was noted. Thus, this function, as well as the plasma cell differentiation conferred via SOX11 mediated regulation of *PAX5* that has been observed in MCL [300, 301], may not occur in BL due to the different molecular environment.

Another interesting result from our study on SOX11 expression in BL is that 5/9 of the paediatric BL cases analysed, expressed SOX11. Albeit no clinical data were available for this cohort, and that the number of examined cases was small, this may concur with a hypothesis that SOX11 may foremost be present in typical BL cases, with a less complex karyotype [243, 253, 278]. It is likely that a proportion of the adult BL cases included in our study represent intermediate, grey zone cases and perhaps the proportion of SOX11 positive cases would have been higher in a cohort restricted to typical BL cases.

SOX11 as a therapeutic target

In MCL, where various signalling pathways have been proposed, SOX11 could potentially function as a therapeutic target. For example, targeting the increased angiogenesis induced by SOX11 mediated *PDGFA* regulation could be viable in SOX11 positive MCL [302, 326]. Also, the use of epigenetic modulators to treat lymphomas is increasingly recognised [327], and regulation of SOX11 expression by way of histone modification has been demonstrated [272]. Thus, epigenetic modification may constitute a potential method to target SOX11. Whether similar therapeutic targeting may be viable for SOX11 expressing BL cases remains to be elucidated.

Current optimal treatment of BL

Due to the rarity of adult BL, standard treatment remains to be defined owing to the paucity of randomised trials. Similar to treatment of paediatric BL, as covered in the background, there are several high-intensive, multiagent regimens with extensive CNS prophylaxis, that demonstrate excellent efficacy in adult BL [93, 144, 149, 150, 152]. However, the associated toxicity and need for sophisticated supportive care, largely preclude their administration to elderly and/or frail patients, as well as to patients with endemic BL, predominant in countries where access to advanced supportive care may be inadequate [105]. Additionally, the real-world highest OS rate of 82.8% reported for the regimens studied in this thesis, will ideally be improved upon in the future.

In paper I and paper II we confirm the dismal outcome of BL patients who receive the more low-intensive regimens (CHOP/CHOEP), as well as the 100% mortality rate without treatment. No difference in outcome was observed in a comparison of the three high-intensive regimens used to treat adult BL in Sweden and Denmark, when adjusting for age and use of rituximab, wherefore no particular high-intensive regimen can be specifically recommended based on our results. Our reported OS rates are in accordance with prospective clinical trials of these regimens, where

toxicity rates for these regimens are also comparable [139, 149, 150] (Table III, p. 32). In our series, the 2-year OS rate for patients who received CODOX-M/IVAC was numerically lower compared to BFM and Hyper-CVAD. A conceivable explanation for this discrepancy are some potentially misdiagnosed cases in that cohort, as four patients who were treated with CODOX-M/IVAC relapsed and died more than one year after diagnosis, a relapse pattern not typical for BL [33, 61].

Regarding their composition and projected dose-intensity of included agents, these three high-intensive regimens are comparable. Hyper-CVAD does not include etoposide, but instead applies a higher doxorubicin dose (Table II, p. 29). The number of cycles varies from 3-8, dependent on regimen and risk stratification, but overall projected doses of cyclophosphamide/ifosfamide, vincristine, methotrexate and cytarabine are fairly similar. The complex administration schedules and varying number of cycles administered, aggravates a comparison of their efficacy according to their respective composition.

Currently, the first randomised study ever performed for adult BL is enrolling patients and will compare R-CODOX-M/IVAC with DA-EPOCH-R. The importance of cyclophosphamide has often been emphasised in BL treatment, and although its effect is cell-cycle independent, fractionated administration has been recommended to augment its effect and decrease toxicity [107, 108]. Interestingly, cyclophosphamide is administered as a single bolus-dose for 15 minutes in the DA-EPOCH-R regimen. Despite this, the infusional approach with prolonged exposure to the other cytotoxic agents at lower concentrations, has demonstrated favourable results [155, 156]. Perhaps the lack of fractionated cyclophosphamide in this regimen is compensated by the fact that the total dose of cyclophosphamide with a full course of six cycles is 7447 mg/m² [157], as compared to 3000 and 3200 mg/m² for the BFM and CODOX-M/IVAC, respectively [139, 149]. Thence, this regimen may be a toxicity-decreasing, feasible alternative not only for the elderly and/or frail but for all BL patients, wherefore the results from this randomised trial will be eagerly anticipated.

Albeit rituximab addition was associated with improved outcome at univariable level in paper II, its independent effect diminished in combination with treatment and age. Also, we noted a numerical disparity of its additive effect in combination with various regimens. Consequently, the role of rituximab in BL treatment remained unanswered. The exact mechanisms via which rituximab exerts its effect are not entirely elucidated. As one proposed target has been BCL2, the rationale for rituximab in BL treatment has not been as strong as for other NHL. However, recently the first randomised trial evaluating rituximab for adult BL was published, and demonstrated improved outcome in combination with the LMB regimen [93]. Favourable outcome was also reported in a prospective trial of the BFM regimen (NHL-2002) in combination with rituximab [149]. Similarly, improved outcome

with rituximab compared to historical controls have been observed for Hyper-CVAD and CODOX-M/IVAC [140, 143, 144, 150]. Thus, rituximab is now commonly incorporated in treatment of adult BL, and its addition to BL treatment in Sweden and Denmark, mainly from 2005 and onwards, may have contributed to the improved OS of patients ≤ 65 in the later time period, demonstrated in paper II.

To summarise, current treatment of adult BL calls for the use of high-intensive regimens composed of alkylating, anti-folate, anti-microtubule agents, anthracyclines, topoisomerase inhibitors and steroids, in combination with CNS directed therapy. The results presented in this thesis further demonstrate the dismal outcome with low-intensive treatment. Thus, although toxicity is a legitimate concern, it seems reasonable to modify therapy only when absolutely necessary in individual patients. However, to avoid potentially life-threatening toxicity, novel approaches are definitely warranted, and under rapid evolution, as discussed below.

The role of etoposide in DLBCL treatment

In paper III, no difference in efficacy was observed between R-CHOP-14, R-CHOP-21 and R-CHOEP-14 in the study population as a whole, in accordance with randomised trials that did not detect any advantage of dose-dense administration [211, 212]. Nonetheless, we also demonstrate that addition of etoposide to R-CHOP-14 conferred superior survival when restricting analysis to the cohort eligible to receive etoposide in terms of toxicity (age ≤ 65).

The addition of etoposide to the CHOP-regimen was pioneered more than 25 years ago and has since been a treatment option for high-risk patients with DLBCL [227, 228, 328]. Based on several rituximab-era studies that determined R-CHOEP-14 to be an effective and feasible regimen for young, high-risk DLBCL patients [179, 223-225, 234, 235, 237] (Table IV, p. 40), this is the subgroup for which current treatment guidelines in Sweden recommend administration of R-CHOEP-14. Therefore, the large differences in patient characteristics between treatment groups in our population based material is not surprising. Both in the whole population and in the cohort aged ≤ 65 , patients who received R-CHOEP-14 were significantly younger but more often presented with elevated LDH, bulky disease and stage III-IV. Because of this, the improved outcome of etoposide addition became evident only when adjusting for these differences in patient characteristics. Due to the wide distributional differences in age and other parameters, the number of matching controls available for the various regimens was too small to perform a comparison based on matched controls. Instead, to minimise the risk of bias due to confounding, we tested the stability of our results in a stratified analysis with patients grouped according to age and IPI-score, with consistent results. Thus, our results, in

combination with several prospective and retrospective studies (Table IV) indicate that, among patients aged 65 and under, R-CHOEP-14 constitutes a valid treatment option.

Another etoposide-containing regimen that has demonstrated promising outcomes for DLBCL is the DA-EPOCH-R regimen. As evident by the dosage comparison in table XI, dose intensity is comparable between these regimens. As presented in Table IV (p. 40), reported OS rates are also congruent. An often emphasised advantage of DA-EPOCH-R is its decreased toxicity and tolerability among all ages. This may be due to the dose-adjusted administration schedule that allows for individual dose adaption [157, 239]. Drug clearance appears to be inversely correlated with age, thus elderly exhibit higher serum concentrations at standard doses, and hence may benefit most from individual dose-adjustment to decrease risk for excessive toxicity [157]. Albeit often reported to be well tolerated, clinical studies of CHOEP regimens uniformly report higher rates of myelosuppression ranging from 39-79% with grade 3-4 myelosuppression [205, 223, 224, 234], as compared to ~35% without etoposide [205], and ~50% per cycle with DA-EPOCH-R [221, 222, 239]. Also, both R-CHOEP-14 and DA-EPOCH-R have been associated with a higher risk for secondary myelodysplastic syndrome and acute myeloid leukaemia [221, 224, 237].

Table XI. Comparison of total administration dose of full course R-CHOEP-14 and DA-EPOCH-R in mg/m²

	R-CHOEP-14	DA-EPOCH-R
CYCLOPHOSPHAMIDE	4500	7447
DOXORUBICINE	300	397
VINCRIStINE	12	9.6
ETOPOSIDE	1800	1986
PREDNISONE	3000	3600
RITUXIMAB	2250	2250

R-CHOEP-14 dose intensity based on 6 cycles, according to administration schedule reported in [224]. DA-EPOCH-R dose intensity based on 6 courses, with maximal dose escalation, as in [157].

In our material, we did not have access to data regarding cell of origin or expression of BCL6. Both the GCB subtype [222, 236] and BCL6 expression [239] have been reported to confer superior outcome of etoposide-containing regimens. Thence, this data would have been of interest to further delineate which patients that are likely to benefit most from the increased toxicity associated with addition of etoposide.

Although overexpression of BCL6 may occur among all DLBCL, the targets of its transcriptional repression may differ in the GC compared to post-GC [194, 195]. For GCB DLBCL, it has been hypothesised that tumour survival and growth is sustained predominantly via decreased DNA damage response and high tumour proliferation, in part mediated via the repression of p53 by BCL6 [193], as compared to via high anti-apoptotic protein levels in the non-GCB subtype [180,

186, 203]. Thus, the reported topoisomerase II-induced downregulation of BCL6 [232], as well as promotion of the p53-p21 pathway and check-point activation generated by prolonged exposure to topoisomerase II inhibitors, demonstrated *in vitro* [230], may be especially beneficial in the GCB subtype [157, 222]. Also, the rapid proliferation of GC B-cells may confer increased sensitivity toward the cell-cycle dependent effect of etoposide, and perhaps contributes to explain its activity in BL. The augmented effect of etoposide in GC B-cells may also account for that the pre-rituximab era profitability of CHOEP was restricted to young patients [205, 206], where the GCB subtype is more frequent [186, 191]. However, this age disparity can also be due to poor tolerability of CHOEP among elderly patients [206].

Thus far, both prospective and retrospective studies of DLBCL have indicated a particularly promising efficacy of DA-EPOCH-R in the GCB-subtype, with 5-year EFS up to 100% [222, 236], supporting these theoretical assumptions. Hopefully, the question of whether the effect of etoposide may be COO-dependent will be settled with the use of gene microarrays to evaluate differing efficacy according to COO, in the ongoing CALGB 50303 randomised trial that compares R-CHOP with DA-EPOCH-R for untreated DLBCL. Also, the advent of novel targeted therapies will presumably further augment the value of information on tumour specific properties and COO.

Targeted therapy – the future?

Concurrent with the substantial advances in knowledge, and mapping, of the genetic landscape of BL and DLBCL, strategies to improve outcome for patients with these diseases have largely shifted. Prior attempts to improve outcome through addition of various chemotherapeutic agents have generally been abandoned, in favour for the development of a vast number of tumour specific therapeutic targets.

This relatively new, rapidly developing field will likely harbour more effective, less-toxic treatment choices for the future. However, the endeavour to evaluate all available specific agents in various potential combinations with chemotherapy as well as other novel agents, poses an immense challenge, and affirms the importance of development of reliable biomarkers to enable optimal treatment stratification.

Novel CD20 antibodies

The success of rituximab has yielded the development of several successors. Rituximab is a type I antibody, thought to favour complement- (CDC) and antibody-dependent cellular cytotoxicity (ADCC) but induce weaker direct cell death. Thus, type II antibodies such as obinutuzumab (GA101) have been developed to augment

ADCC and direct apoptosis, on expense of minimal CDC activity. In BL cell lines, obinutuzumab achieved a higher rate of apoptosis than rituximab [329], as well as in other NHLs [330]. In phase III trials of CLL and FL, superior outcome with obinutuzumab compared to rituximab have been demonstrated [331]. However, preliminary reports from the phase III GOYA trial, that compares obinutuzumab addition to CHOP (G-CHOP) with R-CHOP as first-line treatment for DLBCL, failed to meet its primary endpoint of improved PFS with G-CHOP. Another novel CD20 antibody is ofatumumab, a type I antibody designed to increase CDC, compared to rituximab. Despite promising *in vitro* effect this compound has so far shown limited clinical efficacy for DLBCL and BL [332]. For example, in the ORCHARRD study, where ofatumumab was compared to rituximab in combination with salvage chemotherapy for refractory/relapsed DLBCL, no difference in efficacy was found [333]. In addition, there are multiple other type I, II and bi-specific CD20 antibodies (Table XII) evaluated in phase I/II trials for various NHLs, but cogent data regarding their effect is still lacking [334].

One of the proposed mechanisms for the diminished effect of etoposide in combination with rituximab is that the ADCC effect of rituximab may be decreased by the added toxicity [209]. Perhaps the enhanced ADCC effect observed in novel CD20 antibodies will counteract this effect and so increase the expediency of CD20 antibodies in combination with CHOEP. Other evaluated strategies for further exploitation of the anti-CD20 effect in DLBCL, is use of dose-dense administration, longer exposure and maintenance rituximab. However, these have largely failed to affect outcome, although longer exposure may be efficacious for male patients with DLBCL [335, 336].

Potential therapeutic targets for BL

As one of the hallmarks of BL, MYC constitutes an obvious treatment target. However, transcription factors are notoriously difficult to target and MYC has long been denominated an “undruggable target”. Also, the central role of MYC by governing ~15% of the genome have raised concern for severe toxicities if constrained [337, 338]. Consequently, other strategies have been explored. For example, successful abrogation of MYC gene expression has been achieved by interfering with MYC transcription via use of the small-molecule BET bromodomain inhibitor JQ1 [339]. In BL and other NHL murine models, this agent has decreased tumour volume as well as augmented response to rituximab and other agents [340-342]. Another approach to target MYC is inhibition of Aurora kinase A and B. Aurora kinases are overexpressed in MYC-driven malignancies and several *in vitro* studies have observed enhanced apoptosis with these agents [343, 344]. A phase II study of alisertib, a small-molecule inhibitor of aurora kinase A, demonstrated an overall response rate (ORR) of 27% in aggressive refractory/relapsed NHL, including both cases of DLBCL and BL [345].

The revelation of distinct molecular abnormalities in BL have not yet translated into therapeutic targeting of, for example, ID3 or TCF3. However, their implication in the PI3K signalling pathway suggest that PI3K inhibition may be a feasible addition to BL treatment. So far, a plethora of various PI3K-inhibitors have been developed, of which idelalisib is most evaluated, and approved for relapsed or resistant CLL and FL [346]. Other agents include TGR1202, copanlisib and duvelisib, which are currently assessed in several phase I/II trials to determine efficacy and evaluate the prospect of an improved safety profile, compared to idelalisib [346, 347]. Furthermore, the PI3K pathway may be affected via mTOR-inhibition. For example, temsirolimus has demonstrated effect in BL cell lines in combination with epigenetic modulation [348]. Similarly, dual inhibition of PI3K and histone deacetylases affected growth and migration in BL cells [349]. Also, the tonic BCR signalling in BL has been studied to identify novel effectors, identifying proteins that constitute potential future drug targets [350]. Additionally, antiviral therapy in EBV positive BL may be viable, in combination with agents that induce the expression of targetable EBV related kinases, such as cyclophosphamide [351, 352].

Other conceivable targets in BL include CCND3, that may be targeted by way of cyclin-dependent kinase inhibition, currently under development [20, 55, 337]. Finally, the presence of *GNA13* aberrations in BL may be exploited to decrease growth and block dissemination of BL tumour cells [353].

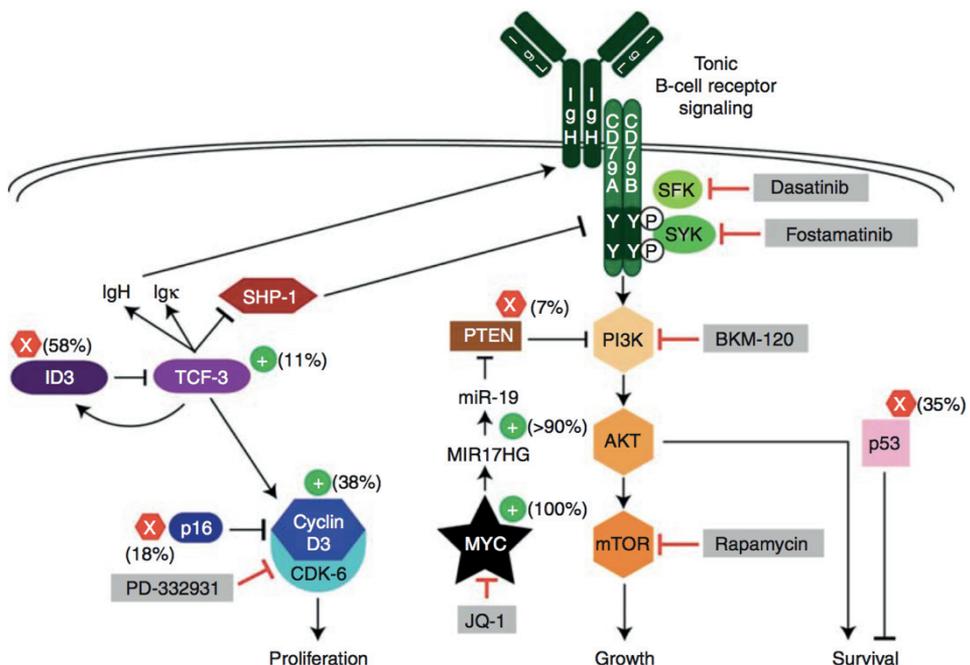


Figure 20. Selected oncogenic pathways in BL. Potential drugs to block these deregulated pathways outlined in grey boxes. From [20]. Reprinted by permission from Macmillan Publishers Ltd: Nature © 2012.

Potential therapeutic targets common for all DLBCL

In line with increased understanding of the genomic heterogeneity according to COO in DLBCL, distinct targets in the various subtypes is plausible. However, some potential targets are shared. These include agents affecting expression of BCL6 and BCL2, that can be overexpressed in all subtypes. For BCL6 there is a scarcity of reported inhibitors, although there are reports of small molecule repressors with *in vivo* and *in vitro* effect in DLBCL [354, 355]. BCL2 targeting has reached further and encouraging effects of BH3 mimetics such as venetoclax (ABT-199), that has largely superseded the more toxic navitoclax, have been reported, both as a single agent and in combination with other novel targets and chemotherapeutic agents [356-358]. Currently, venetoclax is approved for treatment of relapsed or refractory CLL. For NHLs, venetoclax in combination with immunochemotherapy is currently evaluated in various phase I/II trials [356, 359].

Despite that non-GCB DLBCL express PTEN, and hence lack that depletory mechanism to activate the PI3K/Akt/mTOR pathway, constitutive phosphorylation of Akt is present also in the non-GCB subtype, suggesting that there is another mechanism for this activation in non-GCB DLBCL [360]. In accordance, influence on this pathway has been achieved via mTOR inhibition in all DLBCL subtypes. Everolimus demonstrated single-agent effect for relapsed aggressive NHL [361]. Subsequently, a phase I study of everolimus in combination with R-CHOP-21 as first-line treatment has shown promising results with a 96% CR rate and 100% 2-year EFS rate, with similar results among both GCB and non-GCB DLBCL [362, 363]. In contrast, adjuvant everolimus in the phase III PILLAR-2 trial failed to improve DFS [364].

Furthermore, for DLBCL that overexpress CD30, brentuximab vedotin may be a valid addition to treatment [365]. Likewise, the feasibility of the abundance of emerging check-point inhibitors, engineered to restore anti-tumour effects of T-cells, should presumably continue to be evaluated among all subtypes [366]. However, effect appears to be dependent on expression of PD-L1/2 and recently expression of PD-L1 was found to be associated with the non-GCB subtype and EBV positivity, suggesting that immunotherapy targeting this ligand may be beneficial particularly in this subgroup [367]. Other check-point inhibitors include the genetically modified autologous CAR T-cells CTL019, and the CD3/CD19 bispecific antibody blinatumomab. CTL019 has demonstrated encouraging ORR in a phase II study of relapsed NHL, although associated with recurring cytokine release syndrome [368]. Likewise, feasibility and an ORR of 43% has been reported for blinatumomab [369, 370].

Finally, mutations in histone-modifying enzymes occur in ~50 % of all DLBCL cases, and although these mutations are not associated with outcome [18, 371], several epigenetic modifiers have been developed. Examples include vorinostat,

CUDC-907 and panbinostat, that have all demonstrated preliminary activity in lymphomas and could presumably be efficacious among all DLBCL [327, 372, 373].

Potential therapeutic targets for GCB DLBCL

The constitutive activation of the PI3K-pathway caused either by the GCB specific *miR-17-92* amplification or deletion of *PTEN* imply that similar mechanisms to inhibit PI3K-signalling as discussed above for BL may be efficacious also in GCB DLBCL. Similarly, the SYK inhibitor fostamatinib may be most effective in lymphomas with tonic BCR activating, with responses restricted to the GCB subtypes in a phase II trial of refractory and/or relapsed DLBCL [374].

Another anomaly restricted to the GCB subtype is *EZH2*, for which several inhibitors has been developed [197]. For instance, a favourable safety profile and several responses were observed with the *EZH2* inhibitor tazemetostat in a phase I study. Surprisingly, effects were seen also among cases with wild-type *EZH2*, indicating that this strategy could be feasible for all DLBCL [375]. Similarly to BL, 20-30% of GCB DLBCL carry *GNA13* aberrations, thus constituting a potential target also for this entity [353].

Potential therapeutic targets for non-GCB DLBCL

The activation of the NF- κ B pathway in non-GCB DLBCL is a frequent target. Down-regulation of this pathway has been achieved via various targets in chronic BCR signalling. For example, inhibition of Bruton's tyrosine kinase (BTK) using ibrutinib has demonstrated superior effect for non-GCB DLBCL [216]. Currently, addition of ibrutinib to R-CHOP is compared to R-CHOP alone in non-GCB DLBCL in the PHOENIX trial. Likewise, the immunomodulatory lenalidomide has been shown to reduce the inferior outcome associated with non-GCB and is included in several ongoing phase III trials, such as the ROBUST trial where its addition is compared to R-CHOP alone [215].

Additionally, the blunt inhibition of NF- κ B by the proteasome inhibitor bortezomib has demonstrated a distinct effect for the non-GCB subtype [214], although the ongoing randomised REMoDL-B trial includes all subtypes, with continued inclusion of GCB DLBCL recommended also after an interim analysis [376]. In contrast, preliminary data from the Pyramid trial that compares R-CHOP with and without addition of bortezomib in non-GCB did not detect a significant efficacy advantage of bortezomib addition to R-CHOP [377]. Finally, the PKC β -pathway is an integral part of BCR-signalling in the non-GCB subtype, and also activates the NF- κ B pathway. Therefore, the PKC β inhibitor enzastaurin was hypothesised to be efficacious in non-GCB DLBCL, with promising results in the first phase I/II trial [378]. However, in the phase III PRELUDE trial with DLBCL patients at high-risk of relapse failed to demonstrate a beneficial adjuvant effect of this agent [379].

Table XII. Selected potential novel therapeutic targets for BL, DLBCL and HGBL

TARGET	AVAILABLE AGENTS	POTENTIALLY EFFECTIVE FOR
MYC BET INHIBITORS AURORA A/B KINASE INHIBITORS	JQ1 Alisertib	BL, HGBL with MYC and BCL2 and/or BCL6 translocations
PI3K	Idelalisib TGR1202 Copanlisib Duvelisib Dactolisib Buparlisib (BKM-120)	BL, GCB DLBCL
NOVEL CD20 ANTIBODIES	Obinutuzumab Ofatumumab Veltuzumab Ocrelizumab Tositumumab Ublituximab	BL, DLBCL, HGBL
BCL2	Navitoclax Venetoclax	BCL2 expressing DLBCL & HGBL
BCL6	HSP90	BL, BCL6 expressing DLBCL & HGBL
MTOR	Sirolimus Temsirolimus Everolimus	GCB & non-GCB DLBCL
NF-κB BTK INHIBITORS	Ibrutinib Acalabrutinib	Non-GCB DLBCL
PROTEASOME INHIBITORS MICROENVIRONMENT	Bortezomib Lenalidomide	
SYK	Fostamatinib	BL? GCB DLBCL
CDK-INHIBITORS	Palbociclib Flavopiridol Abemaciclib	BL?
Gα13	-	BL, GCB DLBCL
EPIGENETIC MODIFIERS	Vorinostat Azacitidine CUDC-907 Panobinostat	BL, DLBCL
CD30	Brentuximab vedotin	CD30 expressing DLBCL
CHECK-POINT INHIBITORS	Pembrolizumab Nivolumab Durvalumab CTL019 Blinatumomab	DLBCL, BL?
EZH2	Tazemetostat	GCB DLBCL. Non-GCB DLBCL?
PKCβ	Enzastaurin	Non-GCB DLBCL

Conclusions

Paper I

Population based data on adult BL patients indicated that:

- Age and poor performance status (>1) independently predict adverse overall survival.
- High-intensive chemotherapy regimens are associated with superior overall survival.

Paper II

In this bi-national, population based cohort of adult BL:

- Outcome with high-intensive chemotherapy regimens was superior to low-intensive treatment.
- A favourable effect of rituximab addition diminished when adjusting for chemotherapy regimen and prognostic factors.
- Improved outcome during the study period was restricted to patients aged ≤ 65 .

Paper III

Real-world data regarding treatment of adult DLBCL patients demonstrated:

- No overall difference in efficacy between R-CHOP-14, R-CHOP-21 and R-CHOEP-14.
- R-CHOEP-14 is associated with superior overall survival in patients aged ≤ 65 .

Paper IV

- SOX11 is expressed in a minority of adult BL patients and did not impact outcome in our cohort.
- In a BL cell line, decreased levels of SOX11 result in increased proliferation, which may suggest a growth regulatory role for SOX11 in BL.

Concluding remarks

Ideally, improved outcome in BL and DLBCL will be achieved. Plausibly, personalised treatment that maximises the benefit of every treatment agent, for every individual lymphoma patient, will contribute to this progress. Increased insight into the influence of clinicopathological factors on prognosis may identify patient populations with particular benefit of certain therapeutic strategies. Thus, the population based data presented in this thesis may hopefully contribute to improve outcome for adult BL and DLBCL patients, by identifying poor prognostic subgroups and confirming the importance of high-intensive induction therapy.

While chemotherapy combinations will likely remain the backbone of treatment for some time, it is probable that the emergence of novel targets will substantially impact future care of patients with lymphoma. In order to enable a feasible evaluation of the abundance of novel agents, rational selection and application to patients with a theoretical advantage will be pivotal. Consequently, development of reliable biomarkers will indubitably be of great importance.

Also, the rarity of adult BL, with the first randomised clinical trial ever performed just underway, and the relative scarcity of certain DLBCL subgroups, augments the challenge of implementing and determining efficacy of novel therapeutic strategies. Extensive molecular profiling in clinical trials, and experiences of performance of agents among other aggressive lymphomas, with similar genetic alterations, may have to be utilised to try and predict what agents may be of additive effect for individual patients. To reliably evaluate novel agents, mitigation of the potential effect of agents due to administration among patients without the targeted molecular alteration, will have to be avoided.

Thus far, experience of treatment with novel agents is often restricted to phase I/II studies that often consist of heterogeneous cohorts with refractory and/or relapsed lymphomas of various morphology, somewhat constraining evaluation of side-effects and efficacy. Prior treatment may engender unique changes to the characteristics of the tumour cell and its microenvironment, as well as the patients' immunological responses, affecting both treatment efficacy and distorting the development of certain toxicities. Also, potential interactions of targeting several signalling pathways remain to be delineated. Therefore, encouraging results of phase I/II studies may not infallibly be replicated in the first-line setting.

One of the major potential advantages of targeted therapy is the possibility to decrease toxicity, with a prospect that they may constitute feasible treatment options for patients where frailty and/or intolerance to toxicity is a limitation. However, while several novel agents demonstrate a favourable toxicity profile [361, 375], it is not uniformly so. For example, three phase III studies of idelalisib in combination with chemotherapy for treatment of indolent NHLs were suspended due to excessive adverse events without improved outcome. Thence, certain innovative treatment strategies may also be withheld the populations too frail to receive high-intensive chemotherapy. In addition, excessive toxicity observed emphasises the current lack of comprehensive knowledge regarding possible interactions between novel agents and traditional therapeutic strategies. With respect for the possibility of unexpected side-effects, novel combinations should likely be approached with some caution, and enhances the importance of thorough evaluation.

Similarly, experience in other lymphoma further highlight the fact that there is yet a lot to learn about molecular profiles and their effect on cell function and that prediction of response is not always intuitive. An example is the lack of expansive response to BCL2 inhibitors in FL, as BCL2 is overexpressed in the majority of FL [380].

As mentioned, another vital means to improve outcome for adult BL and DLBCL patients is development of more accurate, easily applicable molecular biomarkers for finer classification within both typical BL and DLBCL, as well as improved identification and management of the intermediate, aggressive HGBL.

With molecularly guided, individualised and stricter therapeutic stratification, sufficiently numerous study populations will be scarce, even among the more populous DLBCL entity. Thence, international collaborations will be necessary to perform conclusive studies and it is likely that non-prospective studies will continue to play a role. Population based data may aid evaluation of novel agents in rare diseases. Thus, the population based data presented in this thesis may potentially serve as a future source for comparison of prior real-world treatment data.

Populärvetenskaplig sammanfattning

Lymfom är en grupp tumörsjukdomar som uppstår i en särskild typ av vita blodkroppar (lymfocyter), som utgör en viktig del av kroppens immunförsvar. Lymfom är en stor och heterogen sjukdomsgrupp. För närvarande finns fler än 70 olika lymfomtyper klassificerade, med unika egenskaper beroende på ursprungscell och uppkomstmekanism. Lymfom kan vara alltifrån mycket aggressiva tillstånd med ett snabbt sjukdomsförlopp till stillsamma kroniska sjukdomar.

Burkitt lymfom (BL) och diffust storcelligt B-cellslymfom (DLBCL) är båda två mycket aggressiva sjukdomar som utgår från olika utvecklingsstadier av en sorts lymfocyter som benämns B-celler. BL är en mycket ovanlig sjukdom som drabbar cirka 15 personer per år i Sverige. Det är en av de mest aggressiva tumörarterna som finns. Tillväxttakten är mycket snabb, med en dubbling av tumörstorleken varje dygn. Till följd av detta är sjukdomsförloppet hastigt, och utan behandling dör alla patienter inom några månader. DLBCL är en av de vanligaste lymfomtyperna bland vuxna och drabbar 500 personer per år i Sverige. Typiska symptom för både BL och DLBCL är plötslig lymfkörteltillväxt, viktnedgång, feber och svettningar.

Med hjälp av intensiv behandling med cellhämmande läkemedel (cytostatika) och antikroppsbehandling, riktad direkt mot tumörcellerna, är båda dessa tillstånd botbara. På grund av att BL är en så ovanlig sjukdom har jämförande studier för att avgöra vilken behandlingskombination som är mest effektiv ej kunnat genomföras. För DLBCL råder fortfarande viss ovisshet kring vilken som är den bästa behandlingen för särskilda patientkategorier. Med tanke på att cirka 20-40% av patienter med BL respektive DLBCL inte botas från sin sjukdom finns ett behov av förbättrad behandling.

Den här avhandlingen baseras på studier av patienter med BL och DLBCL insamlade från Svenska lymfomregistret, samt i studie två och fyra också från Danska lymfomregistret. Populationsbaserade studier såsom de här är ett värdefullt komplement till andra studietyper, då de inkluderar alla patientkategorier och skapar större studiepopulationer vid ovanliga sjukdomar, såsom BL. Syftet har varit att utvärdera de cytostatikabehandlingar som använts för att behandla BL och DLBCL i Sverige och Danmark (för BL), samt att identifiera faktorer som påverkar prognosen. Målet har varit att öka kunskapen kring optimal behandling och att identifiera patientgrupper i behov av specifik och/eller förbättrad behandling.

I studie ett och två analyserades data på alla vuxna patienter som diagnostiserats med BL i Sverige och Danmark (studie två) under en 10-årsperiod, totalt 258 patienter. Vi undersökte prognostiska faktorer och letade efter eventuella skillnader i effekt mellan de behandlingsalternativ som använts. Den viktigaste patientberoende parametern för överlevnad var ålder. Vi fann en påtagligt sämre överlevnad bland patienter äldre än 65 år, oavsett behandling. Vi visade också att de högintensiva behandlingarna botade patienter i större utsträckning än de lågintensiva. Den patientgrupp som framför allt fick mindre intensiv behandling var de äldre, vilket sannolikt beror på att denna patientgrupp ofta inte anses tåla biverkningarna av högintensiv behandling. Vidare sågs förbättrad överlevnad under studieperioden endast bland patienter som var 65 år eller yngre. En slutsats som kan dras från de här studierna är att intensiv behandling bör erbjudas alla patienter som bedöms tåla den, oavsett ålder, och att det behövs nya behandlingsalternativ för äldre och sköra patienter.

I studie tre ingick vuxna patienter med DLBCL. Vi undersökte skillnader i effekt av att ge cytotatikabehandling varannan jämfört med var tredje vecka, och huruvida tillägg av cytotatikasorten etoposid, till standardbehandlingen R-CHOP, förbättrade överlevnaden. I hela gruppen sågs ingen skillnad i utfall beroende på behandlingsintervall eller tillägg av etoposid. Bland patienter som var 65 år eller yngre förbättrades dock överlevnaden vid tillägg av etoposid, vilket antyder att denna cytotatikaregim är ett möjligt behandlingsalternativ för DLBCL patienter som är 65 år eller yngre.

I den sista studien undersöktes förekomsten och potentiell klinisk inverkan av uttryck av transkriptionsfaktorn (protein som styr genuttryck) SOX11 i 45 stycken tumörprover från vuxna patienter med BL. SOX11 finns inte i friska celler, men har hittats i flera olika cancersorter, där dess förekomst har visat sig påverka prognos. Uttryck av SOX11 fanns i 14/45 av de undersökta proverna, men påverkade inte överlevnad i vårt material av vuxna patienter med BL. I ett experiment utfört på BL-celler sågs dock ökad tillväxt då uttrycket av SOX11 nedreglerades, vilket skulle kunna tyda på att SOX11 har en roll i styrningen av tillväxt i BL.

Sammanfattningsvis har arbetena i denna avhandling bidragit med populationsbaserad information avseende prognostiska faktorer för BL och effekt av olika behandlingsalternativ för både BL och DLBCL. Optimal behandling behöver fastställas i kontrollerade, randomiserade studier men i avsaknad av sådana kan registerbaserad forskning bidra med värdefull information. För närvarande sker stora framsteg inom målinriktad behandling, med flera nya läkemedel under utveckling. Individualiserad behandling med tumorspecifika läkemedel skulle kunna bidra till minskad biverkningsmängd, och således förbättra överlevnaden särskilt för patienter med för dåligt allmäntillstånd för att erhålla den idag mest effektiva, högintensiva behandlingen.

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