Molecular definition of Burkitt Lymphoma
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Chapter 1 | General introduction
GENERAL INTRODUCTION

Lymphomas are malignancies of the lymphoid tissue. They are divided in Hodgkin’s Lymphoma (HL) and Non-Hodgkin’s Lymphoma (NHL). Whereas HL is a relatively homogenous disease, NHL encompasses around 60 different entities, each with its own clinical presentation, morphology, treatment and prognosis. Burkitt lymphoma (BL) is the most aggressive form of NHL with a doubling time of about 24 hours. While it is relatively rare in adults, it is the most common type of NHL in children. Diffuse large B-cell lymphoma (DLBCL) is a closely related type of NHL. It is the most common type of NHL seen in adult patients and the second most common in children. While BL is considered a homogeneous disease, DLBCL shows clear clinical, morphological and biologic heterogeneity. A subset of DLBCL cases shares features with BL. The choice between a diagnosis of BL or DLBCL for such cases is hard to make, while it has important clinical consequences concerning treatment and prognosis. In this thesis we defined a molecular profile for BL and then tried to answer the question whether these “gray-zone” cases should be considered as BL, DLBCL or deserve their own diagnostic entity. In this chapter a general introduction on BL and the diagnostic dilemma with these “gray-zone” cases is given.

The discovery of Burkitt lymphoma

BL was first described in 1958 by the British surgeon Denis P. Burkitt as a sarcoma presenting either in the jaw or the abdomen of African children. In 1960 O’Conor and Davies described this lesion as a type of lymphoma instead of sarcoma. The first ideas on the pathogenesis of the disease came from an epidemiological study. During a 100 day safari in 1961, Burkitt observed a relationship between the incidence of BL and certain geographic and climatic factors, which later appeared to match the distribution of malaria falciparum. Three years later Epstein and colleagues discovered the Epstein-Barr virus (EBV) in a large number of endemic BL cases and suggested a causative role for the virus in the pathogenesis of this lymphoma. However, the common presence of EBV infection ruled against its role as a single cause for the lymphoma. Neither could the co-occurrence with malaria, which provided either a hyperplastic or immunodeficient environment, fully explain its pathogenesis.

New input in the pathogenesis of the disease came from a genetic point of view. In 1972 Manolov and Manolova described a recurring genetic abnormality in BL cell lines involving an extra band on the telomeric end of the long arm of chromosome 14. Although several groups found that the telomeric region of the long arm of chromosome 8q translocated towards specific bands on chromosome 2, 14 or 22 in both endemic and sporadic BL cases during the following years, it was not before 1981 that the oncogene MYC was discovered to be the target of the recurrent translocations. In the following years it became clear that MYC overexpression played an pivotal role in BL, but that additional oncogenic
events were needed for the development of BL, as MYC overexpression alone is not sufficient for tumorgenesis.\textsuperscript{16,17}

**The clinical variants of Burkitt lymphoma have their own epidemiology and clinical presentation**

Three distinct clinical variants of BL are observed: the endemic, the sporadic and the immunodeficiency-related variant (Table 1).\textsuperscript{18} The endemic variant, occurring in equatorial Africa and Papua-New Guinea, represents the most common form of pediatric lymphoma in these regions. It normally presents with a large extranodal mass either in the jaw or the abdomen in children around the age of 5, preferably males. It is rarely seen in adults. Incidence rates in children are around 1:10,000\textsuperscript{19} with a male:female ratio of about 2.5:1.\textsuperscript{20,21} There is a high association with EBV with over 95\% of the cases being positive for EBV.\textsuperscript{22} Epidemiological studies have also described a relationship with malaria infection\textsuperscript{4,6} and exposure to a certain type of domestic bush, *Euphorbia Tirucalli*.\textsuperscript{23}

The sporadic variant occurs throughout the world, mainly in children and young adults. Incidence rates are much lower (2.5:1,000,000), but with a similar male:female ratio of 2.5:1. It usually presents with an abdominal mass, most often originating from the ileocecal region. Other common locations are the ovaries, kidneys or breasts. Lymph node involvement is more often seen in adults. Bone marrow involvement and/or leukemia are only seen in cases with extensive disease. In contrast with endemic BL, EBV is only seen in a minority of sporadic cases (15-20\%).\textsuperscript{24}

The immunodeficiency-related variant is most commonly associated with the Human Immunodeficiency Virus (HIV) and often represents one of the first AIDS defining symptoms. BL is only rarely seen in the context of other immunodeficiency states (e.g. as post-transplant lymphoproliferative disease, PTLD). Incidence rates of the immunodeficiency-related variant vary between 2:1,000 in children with AIDS\textsuperscript{25} and 1:1,000 in adult AIDS patients,\textsuperscript{26} with a male:female ratio of 2:1. In contrast with the other two variants, nodal presentation as well as bone marrow involvement is relatively common. EBV is identified in 25-40\% of HIV associated BL cases.

**WHO criteria for morphology and immunophenotype of Burkitt lymphoma**

Although it has carried a number of different names throughout the years and different lymphoma classifications (Table 2), BL has always been considered a separate and unique entity. According to the morphological criteria of the 2001 World Health Organization (WHO) Classification of Tumours of Haematopoietic and Lymphoid Tissues,\textsuperscript{18} classic BL cases (most endemic and the majority of the sporadic cases) show a monotonous, cohesive growth pattern. The tumor cells are medium-sized (the nuclei are about the same size as the tingible body macrophages) and have a strong basophilic cytoplasm. The nuclei are round and contain multiple, paracentrally located nucleoli. The proliferation rate is very high;
Table 1. Characterization of the three clinical variants of Burkitt lymphoma.

<table>
<thead>
<tr>
<th>Criteria</th>
<th>endemic BL</th>
<th>sporadic BL</th>
<th>immunodeficiency / AIDS related BL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age and gender</td>
<td>Mostly young boys</td>
<td>Bimodal age distribution, with a peak at 5-10, mostly boys and rise after the age of 60, both male and female</td>
<td>Adolescents / adults</td>
</tr>
<tr>
<td>Kinetics</td>
<td>Rapidly growing, bulky</td>
<td>Rapidly growing, bulky</td>
<td>Rapidly growing, bulky</td>
</tr>
<tr>
<td>Primary presentation</td>
<td>Jaw &gt;&gt; ileocecal / mesenteric, ovary, kidney, breasts</td>
<td>Ileocecal / mesenteric, ovary, kidney, breasts; in adults more nodal</td>
<td>Mostly nodal, CNS</td>
</tr>
<tr>
<td>Primary leukemia</td>
<td>Not</td>
<td>Primary type of ALL-L3 (FAB classification)</td>
<td>Primary type of ALL-L3 (FAB classification)</td>
</tr>
<tr>
<td>Secondary involvement</td>
<td>CNS</td>
<td>CNS, may become leukemic</td>
<td>CNS, may become leukemic, extensive dissemination</td>
</tr>
<tr>
<td>Histology, low power</td>
<td>Cohesive, starry sky, apoptosis may be abundant, few residual lymphocytes</td>
<td>Cohesive, starry sky, apoptosis may be abundant, few residual lymphocytes</td>
<td>Cohesive, starry sky, apoptosis may be abundant, few residual lymphocytes</td>
</tr>
<tr>
<td>Histology, high power</td>
<td>Medium sized, round nuclei, equal or smaller than of macrophages (in atypical BL some variation is size and contour), granular chromatin, multiple medium sized nuclei, or (atypical) less and more larger nuclei; basophilic cytoplasm, often containing many lipid droplets</td>
<td>Medium sized, round nuclei, equal or smaller than of macrophages (in atypical BL some variation is size and contour), granular chromatin, multiple medium sized nuclei, or (atypical) less and more larger nuclei; basophilic cytoplasm, often containing many lipid droplets</td>
<td>Medium sized, round nuclei, equal or smaller than of macrophages (in atypical BL some variation is size and contour), granular chromatin, multiple medium sized nuclei, or (atypical) less and more larger nuclei; basophilic cytoplasm, often containing many lipid droplets; may be more abundant (plasmacytic differentiation)</td>
</tr>
<tr>
<td>Immunophenotype</td>
<td>sIgM+, IgD-, k+/l+, CD20+, CD10+, bcl6+, bcl2-, TdT+, Ki-67/MIB-1 &gt;95%, other features less well known</td>
<td>sIgM+, IgD-, k+/l+, CD20+, CD10+, bcl6+, bcl2-, TdT+, MUM1- or weak, CD38+, CD77+, CD138-, TCL1+, CD44-, Ki-67/MIB-1 &gt;95%; rare cases may be CD5+</td>
<td>SlgM+ and often also slgM+, IgD-, k+/l+, CD20+, CD10+, bcl6+, bcl2-, TdT-, MUM1- or weak, CD38+, CD77+, CD138-, TCL1+, CD44-, Ki-67/MIB-1 &gt;95%</td>
</tr>
<tr>
<td>Genetic</td>
<td>t(8;14)(q24;q32), (q24;q11) or t(2;8)(p11;q24) in 90-100%</td>
<td>t(8;14)(q24;q32) in 80-85%, t(8;22)(q24;q11) in 10-15%, or t(2;8)(p11;q24) in 5%</td>
<td>t(8;14)(q24;q32) in 80-85%, t(8;22)(q24;q11) in 10-15%, or t(2;8)(p11;q24) in 5%</td>
</tr>
<tr>
<td>MYC and IGH breakpoints</td>
<td>8q24: breakpoints more often far upstream of MYC; IGH breakpoints more often at VDJ region (mediated by somatic mutations)</td>
<td>8q24: breakpoints more often directly upstream of MYC or in intron 1; IGH breakpoints more often at switch sites (variable, also downstream of Sµ) mediated by class switch recombination</td>
<td>8q24: breakpoints more often directly upstream of MYC or in intron 1; IGH breakpoints more often at switch sites (variable, also downstream of Sµ) mediated by class switch recombination</td>
</tr>
</tbody>
</table>
therefore many mitotic figures can be observed. The rate of apoptosis is also very high, resulting in a so-called “starry-sky”-like appearance caused by the multiple macrophages that have phagocytized the apoptotic debris.

Two morphologic variants are described in the WHO classification. First, the atypical BL variant (aBL; sometimes also called Burkitt-like lymphoma - BLL) shows greater pleomorphism in nuclear size and shape compared with classic BL. In addition, nucleoli are less numerous and more prominent. For the diagnosis a growth fraction of or close to 100% (as determined by the Ki-67/MIB-1 labeling index) and a proven or strongly suspected MYC translocation are required. Secondly, the plasmacytoid variant is often seen in immunodeficiency related cases of BL, with eccentric cytoplasm and often a single, centrally located nucleolus. A certain degree of pleomorphism may also be observed, just as in the atypical variant.

All BL cells should express surface IgM, pan-B cell antigens, including CD19, CD20, CD22 and CD79 and the germinal center markers CD10 and bcl6. The cells should be negative for CD5, CD23, CD138, bcl2 and terminal deoxynucleotidyl transferase (TdT). The very high proliferation index is represented by a Ki-67/MIB-1 staining of close to 100% of cells.

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**Table 1 continued**

<table>
<thead>
<tr>
<th>Criteria</th>
<th>endemic BL</th>
<th>sporadic BL</th>
<th>immunodeficiency / AIDS related BL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Somatic hypermutations</td>
<td>High, not ongoing, possibly antigen selection</td>
<td>Intermediate, not ongoing, no evidence of antigen selection</td>
<td>High, not ongoing, possibly antigen selection</td>
</tr>
<tr>
<td>Other genetic abnormalities</td>
<td>In 40% no other abnormalities. Limited data available</td>
<td>In 40% no other abnormalities. Gains: 1q, 7, and 12; losses: 6q, 17p, 13q32-q34</td>
<td>Not different from sBL</td>
</tr>
<tr>
<td>Epstein Barr virus</td>
<td>&gt;95%; latency type I (EBNA1, EBER)</td>
<td>15-20%; latency type I (EBNA1, EBER)</td>
<td>25-40%; latency type I (EBNA1, EBER); associated with relatively high counts of CD4+ cells</td>
</tr>
</tbody>
</table>

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**Table 2. Burkitt lymphoma throughout the different lymphoma classifications.**

<table>
<thead>
<tr>
<th>Classification</th>
<th>Introduced in</th>
<th>Name</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rappaport</td>
<td>1966</td>
<td>Undifferentiated lymphoma, Burkitt type</td>
</tr>
<tr>
<td>Lukes-Collins</td>
<td>1974</td>
<td>Small non-cleaved follicular centre cell lymphoma</td>
</tr>
<tr>
<td>Kiel</td>
<td>1974</td>
<td>Burkitt lymphoma</td>
</tr>
<tr>
<td>Working Formulation</td>
<td>1982</td>
<td>Small non-cleaved lymphoma, Burkitt type</td>
</tr>
<tr>
<td>Updated Kiel</td>
<td>1988</td>
<td>Burkitt lymphoma</td>
</tr>
<tr>
<td>REAL</td>
<td>1994</td>
<td>Burkitt lymphoma</td>
</tr>
<tr>
<td>WHO</td>
<td>2001</td>
<td>Burkitt lymphoma</td>
</tr>
</tbody>
</table>
A MYC translocation is the genetic hallmark of Burkitt lymphoma

The genetic hallmark of BL is translocation of the MYC gene (located at cytoband 8q24) towards one of the immunoglobulin (IG) loci. Such a translocation is the prerequisite for the diagnosis of BL and therefore should be present in all cases of BL. The typical t(8;14) (q24;q32) translocation juxtaposes the MYC gene to the immunoglobulin heavy chain locus, which occurs in approximately 80% of BL patients, whereas the variant t(2;8)(p11;q24) and t(8;22)(q24;q11) translocations involve the kappa and lambda immunoglobulin light chain loci in about 5% and 15% of BL, respectively. All three translocations result in constitutive over-expression of MYC.

These translocations are regarded as aberrant products of the immunoglobulin diversification process. In B-cells V(D)J recombination, somatic hypermutation (SHM) and class switch recombination (CSR) allow the production of antibodies with an almost unlimited diversity (Figure 1). As these mechanisms involve double-strand DNA breaks, mistakes can easily lead to translocation events, especially when another double-strand break is nearby. Based on the location of the breakpoints in the IgH locus at either the IgH switch regions or within or downstream of rearranged V genes, aberrant CSR and SHM are regarded as the main causes of an IG-MYC breakpoint in BL. Recently, activation-induced cytidine deaminase (AID) was described to have an important role in both CSR and SHM and the translocation process. It is also thought that AID mistargets MYC in a process of parallel CSR and in this way facilitates the translocation event.

![Figure 1. Antibody diversification through V(D)J recombination, class switch recombination (CSR) and somatic hypermutation (SHM).](image)

V(D)J recombination (left) involves the variable regions and occurs in two steps. In the first rearrangement, a D gene segment is rearranged to a J segment, forming a DJ joint. In the second rearrangement, a V segment is rearranged to a DJH joint, forming a VDJ joint. Which VDJ segments are joined determines the specificity of the antibody. In the human IgH locus on chromosome 14, there are about 50 functional V, 27 D and 6 J gene segments. In class switch recombination (middle), the expressed heavy chain constant region (C) gene is replaced by a downstream C gene. The recombination process involves deletion of the DNA between repetitive DNA regions (switch regions, sμ, sγ and sα) upstream of the recombining C genes. The specificity of the antibody remains unaltered, but the effector functions of the antigen receptor are changed. Somatic hypermutation (right) introduces point mutations and also some deletions and duplications specifically into the V region genes and flanking sequences, further improving the specificity of the antibody. The mutations are indicated by ·.
In addition to the *IG-MYC* breakpoint other genetic abnormalities have also been described in BL. Mutation and/or deletion of *TP53* is commonly described in both BL patients and cell lines. The most commonly reported numerical abnormalities are (partial) gain of the q-arm of chromosome 1 and gain of the whole chromosome 7. Although a pathogenetic role in addition to *MYC* overexpression is suggested, the molecular targets in these regions still have to be identified.

**MYC overexpression has a strong oncogenic potential**

The biological hallmark of BL is a deregulated overexpression of *MYC*. This overexpression is mainly caused by the translocation event, juxtaposing *MYC* to one of the *IG* enhancers. In addition, negative regulatory sequences within *MYC* are often deleted or mutated, further enhancing *MYC* transcription and stability, and thus activity. The myc protein is a transcriptional regulator, which functions through heterodimerization with max. Myc regulates up to 15-20% of all genes in the human genome and over-expression of *MYC* has many biological consequences, most of which underlie its oncogenic potential (Figure 2). The most important consequence of *MYC* overexpression is increased proliferation, as myc enhances cell cycle progression by induction of CDK2 and CDK4 through CDC25A; induction of Cyclin D and E; and down-regulation of p21 and p27. To sustain this high proliferation rate, myc can regulate a number of metabolic pathways, of which induction of LDH-A is the best known target. Myc can also up-regulate genes involved in nucleotide and protein synthesis and iron metabolism. As tumor cells tend to persist in cell-cycle it is no surprise that myc inhibits genes that result in cell differentiation, as this requires the cell to exit the cell-cycle. In addition to the increased proliferation, overexpression of *MYC* induces genomic instability, which may result in additional oncogenic hits. This genomic instability is further enhanced by the immortalization of cells via hTERT, a direct target of myc, which permits the indefinite maintenance of telomeres. Finally, myc downregulates several adhesion molecules (e.g. LFA-1), extra-cellular matrix proteins and HLA which might enable BL to escape immune surveillance.

In contrast to these oncogenic effects of *MYC* overexpression, myc also induces apoptosis. The p53-dependant apoptotic pathway is triggered by myc targets CDC25A and p14, whereas a p53-independent pathway is induced by LDH-A. It seems inevitable that additional molecular or genetic events occur to counteract these pathways, otherwise BL could not survive. As said, mutations and/or deletion of p53 are frequent in BL leading to such a result. But many other genes in the p53 pathway (e.g. via homozygous deletion of *p14ARF/p16INK*) could result in a similar defect in the apoptosis pathway and in consequence contribute to the pathogenesis of BL.
General introduction

Chapter 1

BL requires a specific treatment

Historically, treatment of BL consisted of monotherapy with cyclophosphamide or prolonged chemotherapeutic regimens with an induction, consolidation and maintenance phase, adapted from the earlier ALL-regimens. However such therapies resulted in relatively low cure rates (CR rates less than 50% and only 10-20% of long-term survival). With the introduction of short duration, intensive multi-agent chemotherapy in the early 1980s, cure rates improved considerably. Such intensive chemotherapeutic regimens are thought to be most appropriate for BL, as they maintain effective serum drug concentrations for at least 48-72 hours. Since the proliferation rate is extremely high in BL, with doubling times around 24-48 hours, almost all tumor cells will have passed through the cell cycle in that period of time and consequently be affected by the cytostatic drugs. Furthermore, the risk of tumor repopulation between cycles and development of drug resistance, due to the very short doubling time, is kept as low as possible by keeping the intervals between chemotherapy courses as short as possible and using combinations of non-cross-resistant agents.

Figure 2. The oncogenic potential of MYC overexpression.
MYC overexpression has a great oncogenic potential via a number of mechanisms.
Current pediatric regimens according to the Société Française d’Oncologie Pédiatrique (SFOP), Berlin-Frankfurt-Münster (BFM) and Pediatric Oncology Group (POG) protocols yield 2 year survival rates between 60-80% in high risk and even up to 100% in low-risk pediatric BL patients (reviewed by C Patte).\textsuperscript{66} The application of these regimens in adult patients resulted in somewhat lower, but still very good survival.\textsuperscript{64} As the survival percentages are now that high, challenges lie within the field of new therapies that lower the significant toxicity of these aggressive treatment regimens without lowering the treatment results.

### A subset of DLBCL shows features of BL

DLBCL is a type of NHL closely related to BL, and the most common type of NHL seen in adult patients (30-40% of all NHL) as well as the second most common type in children (around 25% of all NHL). It shows clear clinical, morphological and biologic heterogeneity (many reviews).\textsuperscript{67-70} Efforts to subdivide DLBCL in different subgroups have had considerable success. Since the discovery by Alizadeh et al in 2000 that DLBCL can be categorized in clinically relevant subtypes based on gene expression,\textsuperscript{71} subsequent researchers have discovered a number of molecular subtypes (of which the germinal center B-cell like (GCB) and the activated B-cell like (ABC) subtypes gained most support), each with its own prognosis.\textsuperscript{72,73}

Within the wide spectrum of DLBCL, a subset exists with characteristics of BL (Figure 3). These so called “gray-zone” cases have always been a problem in lymphoma classifications, and it could not be decided whether they should have their own entity or should be considered a variant of either BL or DLBCL. In the Working Formulation\textsuperscript{74} and REAL classification\textsuperscript{75} these cases had their own (provisional) entity (small non-cleaved, non-Burkitt lymphoma and Burkitt-like lymphoma, respectively). However, these entities had a very poor reproducibility.\textsuperscript{76} Therefore, it was decided in the 2001 WHO classification to consider these cases as either DLBCL or a variant of BL (atypical BL). Atypical BL cases showed greater pleomorphism in nuclear size and shape, with fewer and less prominent nucleoli than BL, but required a growth fraction of nearly 100% and a proven or strong presumptive evidence of a MYC translocation.\textsuperscript{18} All other cases were considered to be DLBCL, creating a somewhat artificial and debatable split. Although these criteria seem to be straightforward, the distinction was still hard to make in daily practice. This is problematic as the distinction between BL and DLBCL has more than semantic consequences. BL patients show only limited response to standard DLBCL therapy, i.e. R-CHOP, whereas DLBCL patients respond relatively successfully to this far less toxic regimen and therefore should not be routinely exposed to standard intensive BL therapy.

Another group of BL-mimickers are progressed indolent lymphomas (e.g. follicular lymphoma (FL) and mantle cell lymphoma (MCL) cases) that have gained a MYC translocation during their progression.\textsuperscript{77,78} These cases also show a Burkitt-like morphology and a high growth fraction, mimicking BL. However, in addition to the breakpoint in or around MYC, these cases usually harbor a translocation involving BCL2 or CCND1, which is considered
to be the primary oncogenic event. These “double-hit” lymphomas show a very aggressive clinical course and often do not respond to therapy at all, resulting in a very poor outcome.  

Figure 3. Morphology and IHC of two “gray-zone” cases. 
The upper case involves a 32 year old male patient, with a tumor in the ileocecal region. In the upper left figure a low power magnification (10x) of an H&E staining can be appreciated. It shows a cohesive growth pattern with a “starry-sky” appearance, which suggest the diagnosis of BL. Upon immunohistochemical analysis, the tumor also has a BL immunophenotype (CD10+, bcl2-, bcl6+) and harbors a MYC translocation, again suggesting the diagnosis of BL. However at a high power magnification (100x, oil immersion, upper right), the relatively large and heterogeneous nuclei contradict the diagnosis of BL and favor a diagnosis of DLBCL. 
The lower case involves a 36 year old female patient, with an abdominal tumor. At low power magnification (10x, lower left) a BL morphology can be appreciated with a cohesive growth pattern and a clear “starry-sky” appearance. The inlay in the lower left figure also shows the high percentage of Ki-67 positive cells. However, at a high power magnification (100x, oil immersion, lower right), the nuclei appear atypical. In addition the results of immunohistochemistry (CD10+, bcl2+, bcl6+) and FISH (both a MYC and a BCL2 translocation were identified) suggest an other diagnosis than BL. Up to recently such cases were diagnosed as DLBCL rather than BL. In the novel WHO classification published in 2008, such cases are designated as B-cell lymphoma unclassifiable, with intermediate features between DLBCL and BL (“double-hit lymphoma”); see chapter 6.
SCOPE OF THIS THESIS

In this thesis we set out to determine a molecular profile for BL. In addition we searched for an answer whether the “gray-zone” cases with characteristics of both BL and DLBCL should be considered as BL, DLBCL or deserve their own diagnostic entity.

In chapter 2 we describe the epidemiology and clinical presentation of BL in the Netherlands. We show a bimodal age-distribution with a peak at the pediatric age and a steady increase after the age of 50. We also show a strong male preponderance, which is especially present in pediatric patients. A number of clinical differences is observed between pediatric and adult patients, suggesting that we might be looking at different diseases in children and adults.

In chapter 3 we describe the definition of BL based on currently used techniques (morphology, immunohistochemistry (IHC) and fluorescent insitu hybridization (FISH)). We show that classic BL and DLBCL cases can easily be recognized, but that problems arise in the gray-zone in between and that additional techniques are needed to decide whether these “gray-zone” cases should be considered as BL or DLBCL.

In chapter 4 we used gene expression profiling from RNA gene expression arrays, to distinguish BL from DLBCL. We show that BL has a characteristic gene expression profile, which is identical to the profile of atypical BL. We also show that with this robust, high throughput technique a distinction between BL and DLBCL can be made in the majority of cases, but that a small subset of cases remains, for which it is hard to make a clear-cut decision. These discrepant BL cases represent the molecular gray-zone.

In chapter 5 we compared the genetic profile of classic and atypical BL cases with the profile of these discrepant BL cases. We show that there are no major genetic differences between classic and atypical BL cases, but the discrepant cases differ greatly in their genetic make-up. We also demonstrate the effect of different genetic aberrations on the gene expression profile of BL.

In chapter 6 we used karyotyping data from all BL patients described in literature and determined a (cyto)genetic profile for BL. We show that BL typically harbors an IG-MYC translocation, relatively few additional numerical aberrations and no additional translocation of either BCL2, BCL6 or CCND1. We then compared the profile of this core subset of BL with the profile of other B-NHL (mainly DLBCL) harboring a MYC translocation and groups of “gray-zone” cases, which all appeared to be more genetically complex. Based on these data we suggest that the “gray-zone” cases should not be considered as BL.

In chapter 7 we present data on the clinico-pathological heterogeneity in a high-risk cohort of DLBCL patients, treated with a high-dose intensive chemotherapeutic regimen and autologous stem cell transplantation. We show that the distinction between GCB and ABC subtypes remains of prognostic value in this group of DLBCL.
In chapter 8 the different chapters are summarized. The distinction between BL and DL-BCL is discussed in more detail and put in perspective of similar research projects performed in the same era. At the end a number of future perspectives are presented.

REFERENCES


