Integrative genomic and clinical analysis of follicular lymphoma

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Contents

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Abbrevitations

A	adenine
ABC	activated B-cell
AIDS	acquired immune deficiency syndrome
arrayCGH	array comparative genomic hybridization
ASCT	autologous stem cell transplantation
aUPD	acquired uniparental disomy
BAC	Bacterial artificial clone
BCR	B-cell receptor
BL	Burkitt's lymphoma
BM	bone marrow
BTK	Bruton tyrosine kinase
C	cytosine
CD	cluster of differentiation
cDNA	complementary DNA
CGH	comparative genomic hybridization
CHOP	combination of cyclophosphamid, doxorubicin, vincristin, prednisolon
chr	chromosome
CLL	chronic lymphatic leukemia
CNA	copy number alteration
CNP	copy number polymorphism
CR	complete remission
CSR	class-switch recombination
CT	computer tomography
DBS	double strand break
DC	dendritic cell
DLBCL	diffuse large B-cell lymphoma
DNA	deoxyribonucleic acid
EBV	Epstein-Barr virus
FDC	follicular dendritic cell
FGF	fibroblast growth factor
FISH	fluorescence in situ hybridization
FL	follicular lymphoma
FL1-2	follicular lymphoma grade 1-2
FL3a	follicular lymphoma grade 3a
FL3b	follicular lymphoma grade 3b
FLIPI	follicular lymphoma international prognostic index
FTH	follicular helper T-cell
G	guanine
GCB	germinal center B-cell
GEF	guanine-nucleotide exchange factor
Gy	gray
H.pylori	*Helicobacter pylori*
HAART	highly active anti-retroviral therapy

HD	high-dose chemotherapy
HIV	human immunodeficiency virus
HL	Hodgkin's lymphoma
HTLV-1	human T-cell leukemia/lymphoma virus type 1
Ig	immunoglobulin
IgH	immunoglobulin heavy chain
IGH	immunoglobulin heavy chain gene cluster
IgL	immunoglobulin light chain
IGV	immunoglobulin variable regions
IgVH	variable region of immunoglobulin heavy chain
IHC	immunohistochemistry
IL	interleukin
INF	interferon
ITAM	immunoreceptor tyrosine-based activation motif
LDH	lactate dehydrogenase
LLMPP	leukemia and lymphoma molecular profiling project
LOH	loss of heterozygozity
LPS	lipopolysaccharide
MALT	mucosa associated tissue
MAPK	mitogen-activated protein kinase
MHC	major histocompatibility complex
NCI	National Cancer Institute
NFκB	nuclear factor kappa B transcription factor
NHL	non-Hodgkin's lymphoma
NK-cell	natural killer cell
NO	nitrogen monoxide
NHL	non-Hodgkin's lymphoma
OS	overall survival
PAC	P1-derived artificial clone
PAMP	pathogen-associated molecular pattern
PCF	piecewise constant fitting
PCR	polymerase chain reaction
PET	positron emission tomography
PFS	progression free survival
PI 3-kinas	phosphinositol 3-kinase
PKC	protein kinase C
PLC-γ	phospholipase C
PR	partial remission
PRR	pattern recognition receptor
R	rituximab
RIC-allo	reduced intensity conditioning allogenic stem cell transplantation
RNA	ribonucleic acid
ROS	reactive oxygen species
RS-cell	Reed-Sternberg cell
SEER	Surveillance, Epidemiology and End Results

SHM	somatic hypermutation
SNP	single nucleotide polymorphism
T	thymine
TCR	T-cell receptor
TGFβ	transforming growth factor beta
Th-cell	T-helper cell
TLR	Toll-like receptor
TNFα	tumor necrosis factor alfa
Treg	T-regulatory cell
UV	ultra violet
VEGF	vascular endothelial growth factor
WHO	World Health Organization

List of included papers

Paper 1:

Non-Hodgkin's lymphoma with t(14;18): clonal evolution patterns and cytogenetic-pathologic-clinical correlations

Hege Vangstein Aamot, Emina Emilia Torlakovic, Marianne Brodtkorb Eide, Harald Holte, Sverre Heim

J Cancer Res Clin Oncol (2007) 133:455-470

Paper 2:

High-dose chemotherapy with autologous stem cell support for patients with histologically transformed B-cell non-Hodgkin's lymphomas

Marianne Brodtkorb Eide, Grete Fossum Lauritzen, Gunnar Kvalheim, Arne Kolstad, Unn Merete Fagerli, Martin Mainsenhölder, Bjørn Østenstad, Øystein Fluge, Jan Delabie, Harald Aarset, Knut Liestøl and Harald Holte

Br J Haematol (2011) 152(5):600-10

Paper 3:

Genomic alterations reveal potential for higher grade transformation in follicular lymphoma and confirm parallel clonal evolution of tumor cell clones

Marianne Brodtkorb Eide, Knut Liestøl, Ole Christian Lingjærde, Marit E. Hystad, Stine H. Kresse, Leonardo Meza-Zepeda, Ola Myklebost, Gunhild Trøen, Hege Vangstein Aamot, Harald Holte, Erlend Bremertun Smeland and Jan Delabie

Blood (2010) 116:1489-1497

Paper 4:

Whole-genome integrative analysis reveals expression signatures predicting transformation in follicular lymphoma

Marianne Brodtkorb, Ole Christian Lingjærde, Kanutte Huse, Gunhild Trøen, Marit Hystad, June Myklebust, Harald Holte, Jan Delabie and Erlend Bremertun Smeland

Blood (2013) blood-2013-07-512392; published ahead of print Dec 19, 2013, doi:10.1182/blood-2013-07-512392

Aims of the study

The overall aim of the work described in this thesis was to obtain deeper insight into the molecular mechanisms underlying disease progression in follicular lymphoma (FL), to identify markers for prediction of transformation and survival in FL, and to assess the effect of existing treatments and generate hypotheses for new therapeutic targets. More specifically:

- In Paper 1, we aimed to characterize the frequency and pattern of secondary chromosomal aberrations in t(14;18)(q32;q21)-carrying non-Hodgkin's lymphoma and relate this to morphological subtypes and clinical outcome.

- In Paper 2, we aimed to assess the effect of high-dose chemotherapy with autologous stem cell transplantation (HD-ASCT) in patients with transformation of indolent B-cell lymphoma, and a prospective national multi-center study was performed for this purpose. In this paper, we further sought to assess the effect of in vitro purging of stem cell grafts on patient survival following HD-ASCT with ASCT.

- In Paper 3, we aimed to further investigate the role of genomic aberrations in FL and to shed light on clonal evolution in disease relapse and progression. For this purpose, a genome-wide investigation of DNA copy number alterations in serial biopsies of FL was performed, and the relation to clinical parameters was investigated.

- In Paper 4, we aimed to identify gene signatures and signaling pathways of significance for the progression and transformation of FL. For this purpose, a whole-genome integrative analysis of DNA copy number and gene expression data from serial FL biopsies in patients with and without transformation was performed.

General introduction

Cancer is a major health problem worldwide and increasing incidence rates has been the trend in developed countries for decades. According to WHO and the GLOBOCAN project (http://globocan.iarc.fr/) the incidence of cancer was 12.6 million and the mortality exceeded 7.6 million in 2008. Cancer has for long been considered a health problem related to lifestyle and mainly affecting the elderly population. As the treatments for several cancer types have improved, the number of people who have recovered from or live with cancer increases and late effects of cancer treatment affect life quality and employability of a considerable number of people. According to the Norwegian Cancer Registry there were 28 271 new cancer cases in Norway in 2010 and the incidence rate has increased steadily since the registration started in 1951. Then again, the incidence rates of colon cancer, lung cancer, bladder cancer and breast cancer show trends for decreasing rates during the last five year period, possibly reflecting an effect of preventing measures. In the less developed parts of the world, the incidence of cancer increase as communicable diseases are better controlled. Although the incidence is lower, the relative mortality and morbidity of cancer is much higher in less developed countries due to delay (or lack of) diagnosis and limited availability of treatment and palliative care.

The four main categories of cancer diseases are the carcinomas arising from cells covering bodily surfaces, the sarcomas arising from connective tissue, muscle and bone, the tumors of the central nervous system and the hematological malignancies. The latter comprise a heterogeneous group of leukemias, myelomas and lymphomas. The lymphomas are divided into Hodgkin's and the non-Hodgkin's lymphomas which are further subdivided into several specific disease entities. The main focus of this thesis is follicular lymphomas, one of the most common types of non-Hodgkin's lymphoma.

Common characteristics of cancers

Cancer results from a multi-step process in which various genetic mutations accumulate over time and confer growth advantages to a cell. The development of a tumor has many parallels to the Darwinian evolution of species; randomly acquired mutations endow cells with new properties, and natural selection favors the expansion of cell clones that achieve growth advantages within the surrounding microenvironment. The incidence of cancer increases with age, consistent with a multi-step accumulation of mutations over many years underlying malignant transformation. The rare cases of childhood cancer are more often associated with germline mutations, consistent with the need for fewer somatic mutational steps and shorter time to malignant transformation.

Cancer cells are endowed with a set of fitness traits that constitutes "The hallmarks of Cancer", as reviewed by D. Hanahan and R.A. Weinberg in 2000 and 2011 and listed in Figure 1

(Hanahan & Weinberg, 2000;Hanahan & Weinberg, 2011). The exact molecular mechanisms underpinning each of the hallmark properties vary between cancer types and even between individual cancers of the same type. Each property may be acquired at different time points of cancer evolution (Vogelstein *et al*, 2013). The molecular pathways leading to cancer are heterogeneous; single oncogenes may influence several signaling pathways that in turn enable several of the hallmarks.

Common characteristics of cancer

1) Self-sufficiency in growth signals

2) Insensitivity to anti-growth signals

3) Evasion apoptosis

4) Limitless replicative potential

5) Sustained angiogenesis

6) Tissue invasion and metastasis

7) Deregulating cellular energetic

8) Avoiding immune destruction

9) Tumor-promoting inflammation

10) Genetic instability

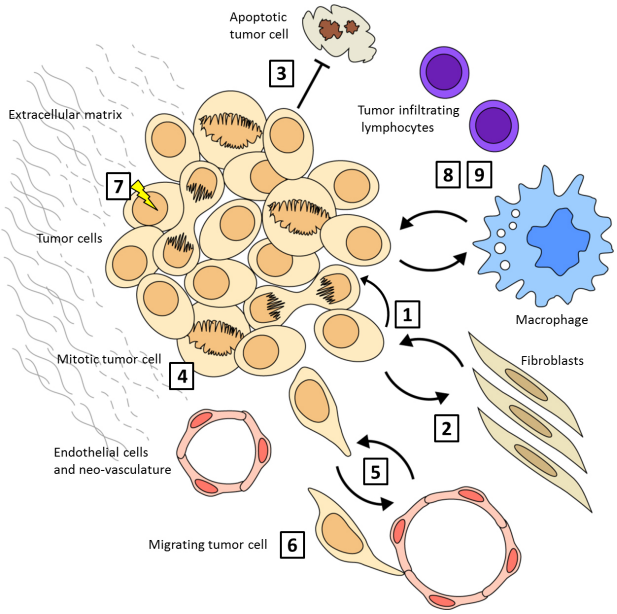

Figure 1. Common characteristics of cancer. **1)** Cancer cells may attain aberrant secretion of growth-factors acting in autocrine loops or engage stromal cells and cells of the innate immunity in paracrine growth factor loops. Activating mutations in growth factor receptors or intracellular signaling molecules may fuel cancer cell growth and proliferation. **2)** Molecules that normally block cell cycle progression or stimulate terminal differentiation of cells may be inactivated to make the cancer cells insensitive to anti-growth signals. **3)** Loss of pro-apoptotic regulators (e.g p53), inactivation of apoptose effector molecules or increased anti-apoptotic signaling in the cancer cell enable evasion of apoptois and inhibit DNA repair. **4)** The natural shortening of chromsomal endings (telomeres) in each round of cell division is avoided by activation of telomerase, an enzyme that maintains the telomers and contributes to limitless replicative potential of a cancer cell. **5)** As a tumor expands beyond 1-2 cubic millimeters hypoxia limits its growth. Both cancer cells and tumor infiltrating immune cells stimulate formation of a new vascular tree by disrupting the balance between angiogenetic inducers and inhibitors. **6)** Cancer cells may attain the ability to migrate and invade into surrounding tissue, enter lymphatic vessels or the bloodstream and settle at distant sites forming metastases. **7)** Tumors often show high rates of glycolysis and lactate production in the presence of oxygen (aerobic glycolysis, the Warburg effect). Lactate dehydrogenase facilitates glycolysis in the lack of oxygen. Lactate produced in hypoxic areas of a tumor can and fuel oxidative fosforylation in oxygenated tumor regions (ref Bayley and Devilee 2012) **8-9)** Even though cancer cells deviate profoundly in behavior and phenotype from normal cells, they escape detection and destruction by the immune system. This is accomplished by secretion of immunomodulatory compounds and recruitment of immunosuppressive cells. In addition, the tumor infiltrating T-cells, B-cells, NK-cells and macrophages create a tumor promoting inflammatory environment. **10)** Genetic instability in tumor cells results in an increased mutational rate and acquisition of genomic aberrations which endow tumor cells with new properties and contribute to tumor progression.

Two major categories of genes are involved in cancer development. Oncogenes with gain of function promote tumor growth, while tumor suppressor genes promote tumor growth by loss of function. Oncogenes are activated by various mutational events targeting one allele and act dominantly. Tumor suppressor genes are recessive and inactivation requires mutations targeting both alleles.

Aberrant expression of oncogenes and tumor suppressor genes is achieved by a variety of genetic and epigenetic changes. Mutations at the nucleotide or base pair level, gain or loss of genomic DNA, chromosomal rearrangements such as translocations, insertions and inversions, hyper-or hypomethylation of genes and alterations in chromatin structure may all lead to altered expression or altered activity of the targeted gene and its protein product (Vaux *et al*, 1988;Slamon *et al*, 1987;Symonds *et al*, 1994;Kinzler & Vogelstein, 1996;Lengauer *et al*, 1998;Hanahan & Weinberg, 2000;Hanahan & Weinberg, 2011;Suva *et al*, 2013). In addition, alternative splicing of mRNA and post-translational protein modifications may also contribute to increased or decreased function of proteins (Maciejewski & Padgett, 2012;McCabe *et al*, 2012). During the last decade we have gained knowledge about the regulatory role of microRNAs in transcription and translation, and there is evidence for a role of altered microRNA expression in carcinogenesis (He *et al*, 2005;Lovat *et al*, 2011;Landau & Slack, 2011).

The recognition and repair of DNA damage is attenuated in cancer. The mechanisms for DNA repair and accurate chromosomal segregation at cell division may be overridden by the high proliferation rate and loss of normal cell cycle control. The genes governing chromosomal integrity, DNA replication and repair may also themselves be targeted by inactivating mutations or epigenetic silencing, resulting in an increased mutational rate and genomic instability (Lord & Ashworth, 2012). The various genetic aberrations observed in cancer cells and the aberrations observed at the genomic level in particular, collectively reflect the degree of genomic instability. Genomic instability contributes to genetic diversity among the tumor cells and cells with improved fitness establish new clones leading to disease progression. This is reflected on the clinical level, as a high degree of genomic instability correlates to worse prognosis in several cancer types (Russnes *et al*, 2010;Walther *et al*, 2008;Pretorius *et al*, 2009;Bakhoum *et al*, 2011).

At the chromosomal level the degree of genomic instability can be determined by quantifying the number of chromosomal translocations and other balanced rearrangements (inversions, insertions), the number of genomic gains and losses (amplifications and homo- and heterozygous deletions, respectively) and acquired uniparental disomies (in which one parental allele is lost while the other parental allele is duplicated).

Screening of cancer genomes by deep sequencing has shown that individual cancers may contain thousands of mutations, of which the majority is not essential for tumor progression (Vogelstein *et al*, 2013). The driver mutations provide the necessary traits to overcome intrinsic

control mechanisms and constraints in the microenvironment and are thus selectively advantageous. Passenger mutations may result from the same mutational mechanisms, but do not contribute to expansion of the tumor and are selectively neutral (e.g. mutations in protein coding genomic regions that do not lead to a change of aminoacid or mutations targeting genes that are usually not transcribed in tumor cells such as hemoglobin, albumin or collagen in carcinoma cells).

Development of phenotypic and genotypic heterogeneity during clonal expansion of tumor cells has been well documented in adenocarcinoma of the colon, in which the various mutated genes promoting progression from benign adenoma to invasive carcinoma have been characterized (Vogelstein *et al*, 2013;Hanahan & Weinberg, 2000). The high prevalence of premalignant clonal expansions (adenomas, dysplasia, expansions of atypical cells etc) compared to cancer rates indicate that microenvironmental and immunological factors naturally constrain tumor growth (Greaves & Maley, 2012).

In addition to cancer cells, neoplastic lesions contain various amounts of immune inflammatory cells, endothelial cells, pericytes, fibroblasts, tissue stem cells and progenitor cells. The composition of the tumor microenvironment is associated with prognosis for many cancer types, indicating a crucial role for tumor growth, progression and metastasis. On the one hand, tumor development and progression may be constrained by the immune system. For instance, genetically engineered mice lacking B- and T-lymphocytes (RAG2-deficient mice), showed enhanced development of various cancers. The finding indicated a role for lymphocytes in preventing cancer. Furthermore, the incidence of lymphomas and some solid tumors is increased in immunosuppressed patients. More compelling, infiltration of cytotoxic T-cells and NK-cells is associated with improved outcome in colon, lung and ovarian cancer as well as in lymphomas, indicating an anti-tumor immune response (Grivennikov *et al*, 2010). Tumor infiltrating B-and T-lymphocytes may recognize tumor antigens, and immune responses against cancer can be induced and give long-lasting remissions (Motz & Coukos, 2013).

Then again, cancer cells employ various mechanisms to avoid detection and destruction by the immune system. The expression of MHC class I is often low in cancer cells, preventing presentation of tumor antigens to antigen presenting cells (Motz & Coukos, 2013). Cancer cells often secrete immunosuppressive factors or immune-modulatory compounds such as TGFβ (Lippitz, 2013). Furthermore, by secreting chemokines, cancer cells may recruit immunosuppressive cells, such as T-regulatory cells and myeloid-derived suppressor cells into the tumor microenvironment. A high content of tumor associated macrophages (TAMs) correlates with inferior outcome in several cancer types, indicating a key role for TAMs in promoting tumor growth and progression (Grivennikov *et al*, 2010). Furthermore, certain types of chronic inflammation predispose for cancer development, exemplified by the increased risk for colon cancer in patients with inflammatory bowel disease. In an established tumor, the immune cells and stromal cells are engaged in a complex interplay with the tumor cells, and

their reciprocal signaling creates a microenvironment in favor of tumor expansion. The stromal and inflammatory cells provide growth factors and survival factors fueling tumor growth. They secrete enzymes that influence the extracellular matrix and facilitate invasion of tumor cells in neighboring tissue and promote metastasis to distant sides. Expression of angiogenic factors such as VEGF and FGF by tumor cells or angiogenic signals produced by TAMs promotes aberrant vascularization of the tumor. In addition, TAMs promote the release of reactive oxygen species (ROS) with mutagenic effect both in tumor cells and in the stromal cells (Hanahan & Weinberg, 2000;Hanahan & Weinberg, 2011;Greaves & Maley, 2012;Lippitz, 2013;Grivennikov *et al*, 2010). In summary, the cellular composition and the cytokine profile of the tumor microenvironment may tip the balance either in favor of anti-tumor immunity or in favor of tumor progression. Various treatment strategies targeting the tumor microenvironment have been developed during the last decades, for a review see for instance G. Motz and G. Coukous 2013 (Motz & Coukos, 2013).

In lymphomas, the microenvironment also plays a complex role in tumor development and progression (Dave *et al*, 2004;Lenz *et al*, 2008b;Cardesa-Salzmann *et al*, 2011;de Jong & Fest, 2011). For follicular lymphoma, this will be briefly discussed in sections below. For more detailed reviews of the impact of the microenvironment in lymphomas, see for instance J.G Gribben 2010 and P. Amé-Thomas 2013 for follicular lymphoma and Y. Liu 2013 for Hodgkin's lymphoma (Gribben, 2010;Ame-Thomas & Tarte, 2013;Liu *et al*, 2013). The lymphomas recapture features of their normal counterparts; B- and T-lymphomcytes. In the malignant transformation, the mechanisms governing normal lymphocyte development and activation are subverted to promote tumor growth. Thus, the molecular mechanisms regulating normal lymphocyte development and differentiation are important keys to the understanding of lymphomagenesis.

The immune system

The immune system has evolved to provide protection against disease-causing microorganisms. Demonstrating its importance for survival, an innate immune system is present in species at almost every level of phylogenesis (Cooper & Herrin, 2010). Innate immunity provides the rapid, first line defense against a diversity of invading viruses, bacteria, fungi and parasites. The adaptive immune system, present in vertebrates, provides a highly specific secondary line of defense and involves the generation of immunological memory (Cooper & Herrin, 2010).

Innate immunity

The innate immune system is composed of both specialized cells (phagocytes) and humoral effector molecules (complement system). Phagocytes recognize, engulf and digest pathogenic

microorganisms. The complement system interacts with microorganisms to directly kill them as well as to promote phagocytosis. The early innate immune response is evoked by the recognition of a pathogen by receptors on the cell surface of macrophages residing in the skin and mucosa. These receptors, termed pattern recognition receptors (PRRs), are germ line encoded and constitute a limited repertoire of sensors for pathogenic molecular patterns. Signaling PRRs activates macrophages to engulf (phagocytosis) and kill pathogens as well as to secrete inflammatory cytokines and chemokines. Inflammation of an infected or damaged tissue in turn leads to recruitment of the phagocytic neutrophil granulocytes and antimicrobial proteins such as the complement factors. The inflammatory response serves to deliver effector molecules and cells to the site of infection, to prevent further spread of infection by coagulation in microvasculature and to promote tissue repair.

Like the macrophages, dendritic cells residing in peripheral tissues constantly sample their microenvironment and become activated by the binding of a pathogen to PRRs. Engulfed pathogens are processed and presented to the cells of the adaptive immune system, and dendritic cells are crucial for the triggering and shaping of the following antigen-specific response (Janeway CA, 2005).

Adaptive immunity

The effector cells of the adaptive immune system are the B-and T-lymphocytes. Activation of B- and T-cells depends on a complex interplay with antigen presenting cells, such as dendritic cells, and results in a highly specific and flexible immune response. The B- and T-cells carry surface receptors with a broad repertoire of antigen specificities, enabling the recognition of any potential pathogen (Bonilla & Oettgen, 2010;Cooper & Herrin, 2010). The variable regions of B- and T-cell antigen receptors are encoded in gene clusters containing multiple gene segments. During B-and T-cell development these gene segments are cut and spliced in a myriad of combinations, introducing combinatorial diversity in the repertoire of antigen receptors. Additional variability is achieved by the introduction of random mutations in the splice regions between the recombined gene segments (mutational diversity). Each B- and T-cell are thus equipped with receptors with unique antigen specificity.

Activated B-lymphocytes differentiate into antibody secreting plasma cells providing humoral immunity mainly against extracellular pathogens/antigens. Activated T-lymphocytes regulates cellular immunity by the action of different T-cell subsets. Subset of CD4 positive T-cells may either support B-lymphocyte differentiation or stimulate macrophages and the subset of CD8 positive cytotoxic T-cells which recognize and kill cells infected by intracellular pathogens.

Following the initial immune response, immunological memory is established by the long-term survival of B- and T-lymphocytes carrying antigen-specific receptors (Bonilla &

Oettgen, 2010). These memory B- and T-cells are able to elicit a more rapid and efficient immune response to the pathogen at a second encounter.

The lymphatic system, composed of the lymphoid organs, provides the environment for lymphocyte development and activation (Bonilla & Oettgen, 2010). B- and T-lymphocytes originate from haematopoietic precursors in the bone marrow and develop in the primary lymphoid organs; the bone marrow and thymus, respectively. Supported by stromal cells of the primary lymphoid organs, lymphocytes that produce functional antigen receptors mature, while those with self-reactive receptors are eliminated. Thus, development of autoimmunity is avoided (Janeway CA, 2005). Mature B- and T-cells circulate in the peripheral blood and populate the secondary lymphoid organs; the lymph nodes, mucosa associated lymphatic tissue (MALT) and spleen. Antigens from peripheral tissues are drained into the lymphatic fluid and travel to the lymph nodes or MALT, while antigens entering the blood are captured in the spleen. Under the influence of signals from the innate immune system, a finely tuned humoral and cellular immune response is raised by the B- and T-cells, respectively. Antibody secreting plasmacells developed from B-cells migrate to the bone marrow or lamina propria of mucosa, while activated T-cells may travel to the sites of infection to kill pathogens or activate macrophages (Janeway CA, 2005;Bonilla & Oettgen, 2010).

B-cell development and assembly of the B-cell receptor

B-lymphocytes are derived from common lymphocyte progenitor cells in the bone marrow (BM), and commitment to B-cell lineage is controlled by a set of transcription factors including PU.1, IKAROS, E2A, EBF, PAX5 and IRF8 (Janeway CA, 2005;LeBien & Tedder, 2008;Bonilla & Oettgen, 2010). In the progenitor B-cell (pro-B cell) the assembly of the B-cell receptor (BCR) is initiated by recombination of the immunoglobulin (Ig) gene segments. First, the immunoglobulin heavy chain (IgH) is assembled by recombination of gene segments from four regions on chromosome 14: The variable region (V) containing 51 segments, the diversity region (D) containing 27 segments, the joining region (J) containing 6 segments and one constant (Cμ) region (Figure 2). The recombination of Ig gene segments is mediated by the enzyme complex RAG1/RAG2 which introduces DNA double strand breaks between the respective gene segments. One randomly picked gene segment from each of the regions V, D and J are then joined by the DNA repair mechanism termed non-homologous end-joining. The resulting combination of gene segments is unique for each developing B-cell. Following transcription and translation, the variable region of the Ig heavy chain (IgVH) is formed and eventually makes part of the antigen recognition site of the BCR. Antigen receptor diversity is also obtained by the action of the enzyme TdT, which randomly adds or deletes nucleotides (N-nucleotides) in the junctions between the recombined gene segments. If recombination results in the production of a functional IgH that is expressed on the cell surface, the B-cell receives stimulatory signals and

continues to the pre-B stage. At this stage a surrogate light chain is transcribed from the two genes VpreB and λ5 and expressed together with the IgH, thus forming a pre-B cell receptor (Janeway CA, 2005;LeBien & Tedder, 2008).

In pre-B cells the immunoglobulin light chains (IgL) are assembled from any one of two gene loci; the κ-locus on chromosome 2 or the λ-locus on chromosome 22. A recombination of the κ-gene is first attempted by splicing one of 31-35 V segments with one of 5 J segments and the C segment. If this fails to produce a light chain, the λ-gene (containing 29-33 V segments, 4-5 J segments and one C segment) undergoes the same process. Upon successful assembly, two light chains are combined with two heavy chains in a heterotetramer and a complete IgM-molecule is expressed on the surface of the immature B cell. Interaction with self-molecules present in the bone marrow environment then determines the fate of the immature lymphocyte. B-cells expressing IgM with the capacity to bind self-molecules are eliminated by apoptosis, while non-self-reacting cells receive signals for survival and maturation. The rearrangement of BCR gene loci combined with the negative selection of auto-reactive B-cells results in mature, circulating B-cells with a tremendous repertoire of BCR-specificities and the capacity to recognize almost any foreign antigen. The mature, naïve B-cells expressing both surface IgM and IgD then leave the BM to circulate in the peripheral blood, primary follicles and the follicle mantle zones of the lymph nodes and MALT (Janeway CA, 2005;LeBien & Tedder, 2008).

In addition to the different steps in BCR-assembly, the individual stages of B-cell development are characterized by differential expression of surface markers and intracellular markers representing i.e. the growth factor receptors, cytokine receptors and co-receptors, signal transducing molecules, enzymes and transcription factors. For instance, surface expression of CD38 characterize the pro-B and pre-B cells in the BM as well as the fully diffentiated plasma cell. The classical B-cell markers CD19 and CD20 are expressed from the late pro-B stage and through all stages of B-cell life, until they are lost in the terminal differentiation of plasma cells. The expression of lymphocyte markers can be detected by various laboratory methods such as Western blotting, immunohistochemical staining, flowcytometry with immunostaining and real time PCR and gene expression analyses (Hystad *et al*, 2007;Shaffer *et al*, 2001) and is used for both diagnostic and research purposes to distinguish developmental and differential stages of lymphocytes.

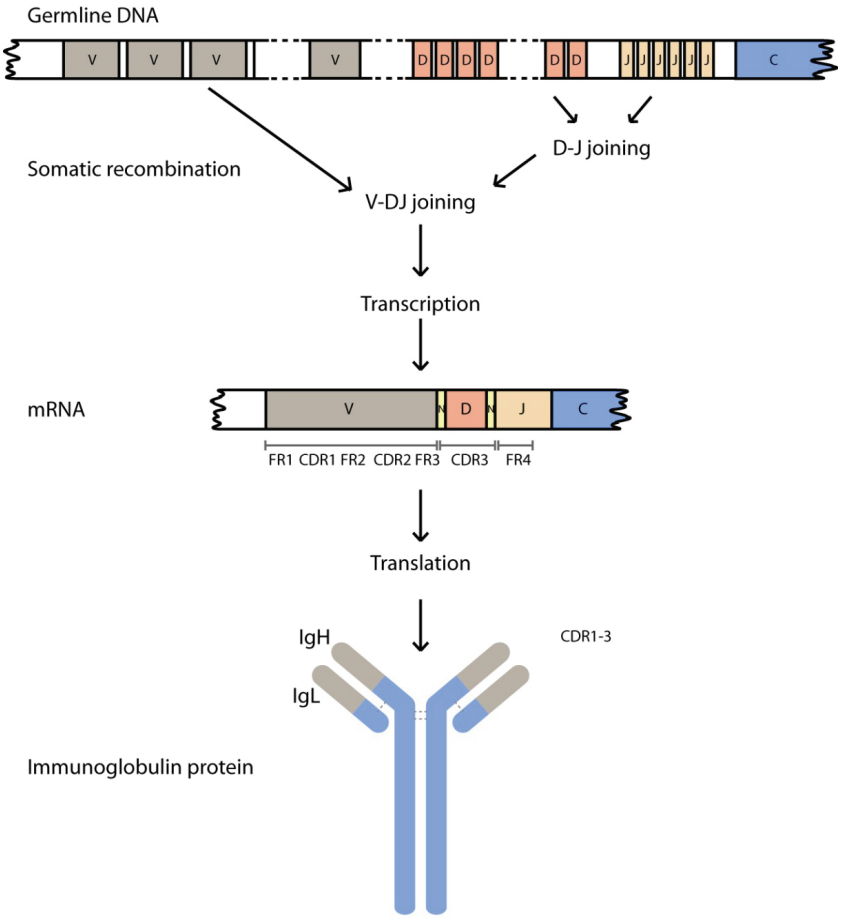

Figure 2. The IGH gene cluster is located on chromosome 14q32 and contains about 40 V-segments, 25 D-segments and six J-segments as shown on the figure. In B-cell development somatic recombination of one randomly picked V, D and J segment takes place. In addition, the IGH locus contains a large cluster of C genes encoding the constant regions of the immunoglobulin protein that determines the antibody isotype. CDR1-3 (complementarity-determining regions 1-3) represent the hypervariable regions of the immunoglobulin transcript. These are flanked by FR1-4 (framework regions 1-4) which show less sequence variability.

T-cell development

Common lymphocyte precursor cells originating from the bone marrow populate the thymus and start differentiation and proliferation under the influence of IL-1, IL-7 and TNF. Commitment to the T-cell lineage is controlled by the transcription factor Notch-1 among others, which induce expression of genes regulating T-cell receptor (TCR) assembly (Bonilla & Oettgen, 2010). In the thymic cortex the immature T-cells express TdT and carry out rearrangement of TRC-genes in a manner similar to the BCR assembly in B-cell development. The TCR of the αβ T-cells are assembled from α- and β-chain loci (gene names TCA@ and TRB@) on chromosome 14 and 7, respectively. The immature T-cells express the membranous markers CD1a, CD3, CD5 and CD7. As they move towards the thymic medulla, maturing T-cells express TCR in complex with CD3 and they are double positive for CD4 and CD8. Thymic epithelial cells express both MHC class I and MCH class II molecules presenting self-peptides to the maturing T-cells. Low avidity binding to the complexes of MHC and self-peptide leads to positive selection of the maturing T-cells. T-cells with low avidity binding to MHC I become CD8+ single positive. T-cells with low avidity binding to MCH II become CD4+ positive (Bonilla & Oettgen, 2010). No binding and high avidity binding of the TCR to complexes of MHC and self-antigen result in elimination of the T-cell. Thus, development of functional T-cells without self-reactivity is secured (Janeway CA, 2005).

The production of mature T-cells mainly takes place during embryonic development and childhood. After puberty the thymus atrophies. In adulthood, the population of T-cells is maintained by long-lived T-cells, division of mature T-cells and a low production of new T-cells. The mature T-cells recirculate in the blood and secondary lymphoid organs. The CD8 positive T-cells can recognize peptides from intracellular pathogens bound to MHC class I molecules of tissue cells. This triggers activation and differentiation into cytotoxic effector T-cells which efficiently kill the infected tissue cell. The CD4 positive T-cells, or T-helper cells, can recognize peptides bound to MHC class II molecules. These peptides originate either from pathogens in intracellular vesicles of macrophages and dendritic cells or from extracellular bacteria and toxins ingested and processed by antigen presenting cells. Depending on the type of antigen the T helper cells elicit either a cellular immune response by activating macrophages (T helper 1 cells; Th1 cells) or a humoral immune response by activating B-cells (T helper 2 cells; Th2 cells). The Th1 secrete INF-γ which trigger infected macrophages to destroy intracellular microorganisms, while the Th2 cells secrete B-cell growth factors such as IL-4 and IL-5.

In addition to the cytotoxic T-cells and T-helper cells there are other subsets of T-cells with more specialized functions and limited distribution. The Follicular T-helper cells (FTH) reside in lymph nodes and the spleen where they take part in B-cell activation and formation of the germinal center. They express Bcl6 and CD10 as the germinal center B-cells, in addition to CD4, CD57 and PD-1. FTHs produce the chemokine CXCL13 causing induction and proliferation

of follicular dendritic cells (FDCs), which in turn express CXCR5 (receptor for CXCL13) facilitating the migration of B- and T-cells into the germinal center.

The T-regulatory-cells (Tregs; CD4 and CD25 positive T-cells) arise in the thymus. A stronger binding of CD4 positive T-cells to a complex of self-derived peptides and MHC class II molecules induces expression of FOXP3. This gene encodes a transcription factor which directs differentiation of the T-cell into a T regulatory cell. Tregs are able to inhibit activated T- and B-cell as well as antigen presenting cells and can thus shut off or suppress immune responses. The Tregs are important for limitations of autoimmune responses and prevention of autoimmune diseases. In cancer, Tregs can suppress immune responses to tumor and thus promote tumor growth. As previously mentioned, the infiltration of Tregs in the tumor is associated with poor prognosis in several types of cancer (Janeway CA, 2005;Bonilla & Oettgen, 2010).

Subsets of both B- and T-cells possess antigen receptors of limited diversity. The B-1 cells carry the surface marker CD5, proliferate in and populate the peritoneal and pleural cavities, and react mainly to polysaccharide antigens (certain bacteria). They generate IgM independently of helper T-cells and do not produce immunological memory (LeBien & Tedder, 2008). The TCR of the γδ T-cells originate from γ- and δ-chain loci on chromosome 7 and 14, respectively. The γδ T-cells do not recycle in secondary lymphoid organs, but populate the gastrointestinal epithelium and skin. They express TCRs of limited diversity and recognize antigen presented by non-classical MHC-molecules in the CD1-family. The B-1 cells and γδ T-cells are often referred to as “innate-like lymphocytes” (Janeway CA, 2005).

Activation and differentiation of B-cells

The activation and differentiation of B-cells result in production of antigen specific antibodies that can neutralize pathogens or mark (opsonize) them for phagocytosis or complement binding. Circulating, naïve B-cells encounter antigens as they pass through the secondary lymphoid organs (Natkunam, 2007;Bonilla & Oettgen, 2010). The antigen-induced activation of B-cells which takes place in the specialized environment of the lymph node (Figure 3), spleen or MALT is termed the germinal center reaction.

The first signal for B-cell activation is cross-linkage of two surface B-cell receptors (BCRs) by antigen binding. The majority of antibody responses are T-cell dependent and co-stimulatory signals for B-cell activation are delivered by antigen-specific T helper-cells. The complex of antigen bound to BCR is internalized and processed so that antigen peptides can be exposed on MHC class II molecules on the surface of the B-cell. Thus, B-cells can act as antigen-presenting cells for T-helper cells engaging them in reciprocal activation. Activated T helper-cells express CD40 ligand (CD154) that directly engages CD40 on B-cells. CD40 belongs to the TNF-receptor family and downstream signaling promotes B-cell growth and immunoglobuline class-switching. The T-helper cells also secrete cytokines such as IL-4 which induces B-cell proliferation. The B-

cells migrate from the T-cell zone into the primary follicle where they populate the follicular dendritic cell (FDC) meshwork, proliferate and form a secondary follicle (Figure 3 and 4). Resting naïve B-cells without specificity for the present antigen are displaced to the mantle zone of the follicle.

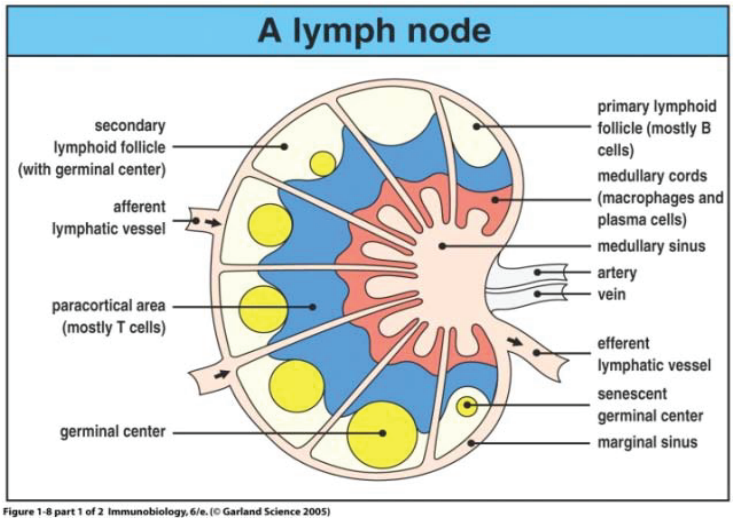

Figure 3. The architecture of a lymph node (Immunobiology 6th edition. Janeway, Travers, Walport, Shlomchic. Garland Science 2005). Naïve B-cells enter the lymph node through the high endothelial venules in the medullary cords. B-cells that bind antigen become activated at the border between the T-cell (blue) and the B-cell (yellow) areas. Activated B-cells then migrate into a primary follicle, proliferate and form a germinal center.

The dark zone of the secondary follicle is packed with centroblasts (Figure 4). These are rapidly dividing B-cells undergoing somatic hypermutation (SHM) of the variable regions of the BCR gene loci; detailed below. In the central light zone B-cells with mutated BCRs convert to the smaller non-dividing centrocytes and intermingle the FDC, the follicular T-helper cells and macrophages. B-cells expressing BCRs with high affinity for antigens bound to the surface of FDCs receive survival signals, while B-cells attaining low-affinity BCRs following SHM are eliminated by apoptosis and engulfed by the follicular macropghages. The cytokines produced by antigen specific T-helper cells stimulate Ig class-switch recombination; detailed below.

Ultimately the antigen specific B-cells differentiate into plasma cells or memory B-cells that leave the germinal center (Janeway CA, 2005;Natkunam, 2007;Swerdlow SH, 2008).

Certain antigens activate naïve B-cells in the absence of T helper-cells. Bacterial polysaccharides are made up of repetitive structures which can cause extensive cross-binding of BCRs on the B-cell surface and thus provide a strong signal for activation. Other antigens, such as bacterial lipopolysaccharide (LPS) bind both to BCRs and Toll-like receptors expressed on B-cells providing co-stimulatory signals for B-cell proliferation (McGettrick & O'Neill, 2007).

In centroblasts, the variable regions of heavy and light chain Ig genes (IgVH and IgVKappa or IgVLamda) are subject to somatic hypermutation (SHM). The process of SHM is induced by action of BCL6 which functions as a transcriptional repressor. BCL6 repress transcription of TP53 and other genes regulating the DNA-damage response. The attenuated DNA-damage response then allows DNA double strand breaks which are necessary for SHM.

During SHM the enzyme AID deaminates cytosine residues to uracil in the variable regions of the Ig genes. The base excision repair or mis-match repair pathways are then triggered to replace uracil. Simultaneous DNA replication by error-prone polymerases introduces mutations. The resulting characteristic pattern of mutations leads to diversification of the Ig gene sequences and thus an altered configuration of the antigen binding site of the BCR (Natkunam, 2007;Klein & la-Favera, 2008;LeBien & Tedder, 2008).

Following SHM the B-cells are prone to apoptosis as expression of the anti-apoptotic BCL-2 protein is shut down and the NFκB pathway is inactive. Only B-cells with high affinity binding of surface BCR to antigen are rescued from apoptosis and re-express Bcl2, while cells with low affinity BCR undergo apoptosis and are engulfed by the germinal center macrophages. The early primary immune response is characterized by the secretion of IgM antibodies with low antigen affinity. B-cells selected for further differentiation undergo class switch directed by cytokines and co-stimulatory signals provided by the T helper cells and FDCs present in the microenvironment. In class switch the rearranged and hypermutated IgVH gene is joined to a new constant C-region. Thus, the isotype of the produced immunoglobuline molecule is switched from IgM to IgG, IgA or IgE. There are nine classes of heavy chains; IgM, IgD, IgA1-2, IgG1-4, IgE with various ability to neutralize or opsonize pathogens and to activate the complement system. The combination of cytokines and co-stimulatory signals provided by FDCs and Th2- or Th1-cells determines the antibody class which is tailored for the type of antigen to be eliminated (Klein & la-Favera, 2008). Most commonly, high affinity IgG and IgA are produced which characterize the late primary and secondary immune responses. Interaction of B-cells with FDC through CD23 and CD40 ligand binding, leads to expression of the transcription factor IRF4/MUM1 and down regulation of Bcl-6 in the activated B-cells. This initiates terminal differentiation into plasma cells (Figure 4). Plasma cells enter the peripheral blood and home to BM or the mucosa of the digestive tract. The cytoplasm usually contains IgG or IgA, repectively, and there is no longer surface expression of immunoglobuline/BCR. Some become long lived

plasma cells in BM niches, contributing to humoral immunological memory (Janeway CA, 2005;LeBien & Tedder, 2008).

Following the germinal center reaction, some of the activated B-cells differentiate into long-lived memory B-cells (Figure 4). Memory B-cells express BCRs with somatically hypermutated antigen binding sites and IgG, IgA or IgE heavy-chains. They circulate in peripheral blood and populate follicular marginal zones in lymph nodes, spleen and MALT. Post germinal center memory B-cells tend to home to the sites where they first encountered antigen; B-cells arising from MALT return there and B-cells arising from lymph nodes return to nodes and BM (Janeway CA, 2005;Heyzer-Williams & Heyzer-Williams, 2005;Kalia *et al*, 2006).

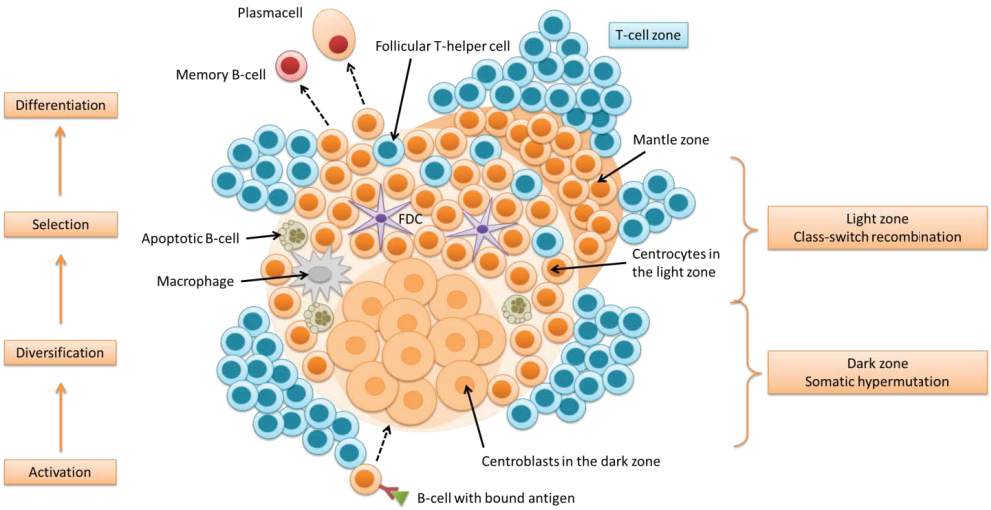

Figure 4. The germinal center reaction. Detailed in the main text.

BCR signaling

The BCR of a naïve B-cell is constructed of membrane bound IgM linked to Igα and Igβ chains (CD79a and CD79b). IgM recognizes and binds antigen on the B-cell surface, while Igα and Igβ initiate the intracellular signaling cascade which ultimately results in the differentiation of the B-cell into an antibody producing plasma cell or memory cell. The signaling units of the BCR, the Igα and Igβ chains, have tyrosin residues on their cytoplasmatic tails (immunoreceptor tyrosine-based activation motifs = ITAMs). Cross linking of surface IgMs by antigen binding leads to phosphorylation of these tyrosin residues by Src family kinases (Fyn, Blk, Lyn in B-cells). Syk tyrosin kinase recognizes and binds to these phosphorylated tyrosines and activates downstream intracellular targets: (i) Activation of phospholipase C (PLC-γ) via the adaptor protein BLNK and recruitment of Bruton tyrosin kinase (Btk). PLC-γ cleaves PI2P into DAG and PI3 which in turn results in activation of the transcription factors NFκB via Protein kinase C (PKC) and NFAT via Calcineurin, respectively. (ii) Binding of GEFs (guanine-nucleotide exchange factors) to the receptor complex activates small G-proteins (Ras and Rac) and downstream MAPkinase cascades. This finally activates the transcription factor JUN (Janeway CA, 2005;Kurosaki *et al*, 2010) (Figure 5).

At least three surface molecules are closely associated with the BCR. CD21 binds antigens tagged with the complement fragment C3d and cluster with BCR and CD19 and CD81. The cytoplasmatic tail of CD19 is then phosphorylated enabling it to bind Src family kinases and recruit PI 3-kinase. The co-receptor complex CD19/CD21/CD81 enhances BCR-signaling many-fold.

The target genes of NFκB, NFAT and JUN collectively prevent apoptosis, stimulate proliferation and initiate the differentiation of B-cells into antibody secreting plasma cells (Kurosaki *et al*, 2010;Janeway CA, 2005). Mature circulating B-cells depend on the surface expression of BCR for survival also in the absence of antigen and it is thought that this is due to a “tonic” BCR-signaling. However, the exact mechanism for the “tonic” BCR-signaling is largely unknown.

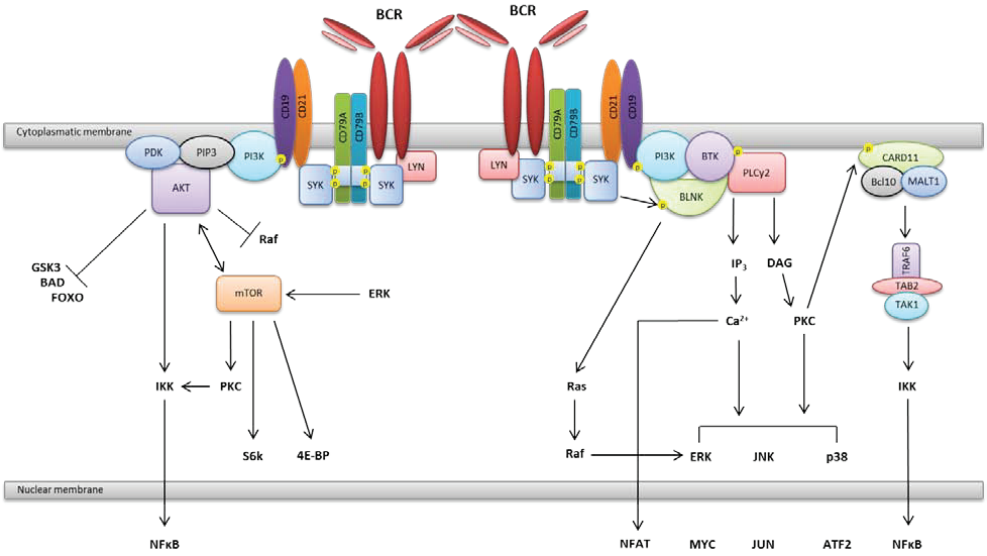

Figure 5. B-cell receptor signaling. Cross-linkage of two surface receptors by antigen activates a cascade of intracellular signaling that regulates the proliferation and differentiation of B-cells in the germinal center reaction. Detailed in the main text.

Activation of T-cells

As previously outlined, the CD4 positive T-cells comprise the T-helper cells, including the Th1-cells, Th2-cells and the subset of follicular T-helper cells as well as the T-regulatory cells (Tregs). T helper-cells are activated by recognition of antigen-peptide bound to MHC II on antigen presenting cells (APCs). APCs are present in a large number in skin and mucosa and actively sample extracellular proteins by phagocytosis and endocytosis. APCs are activated by innate immune stimuli which induce MHC II expression and stimulate migration of APCs to a regional lymph node where the encounter with T-helper cells takes place (Bonilla and Oettgen 2009). Activated Th1-cells secrete cytokines such as INFγ and TNFα which in turn stimulate macrophages to kill intracellular pathogens by increased NO production. Activated Th2 cells secrete IL-4 and other cytokines which support B-cell proliferation and differentiation (Janeway CA, 2005).

Cytotoxic T-cell expresses CD8 and their TCRs recognize foreign peptide bound to self MHC I of an infected cell. The binding of an activated cytotoxic T-cell to an antigen-MHC class I complex results in a directed release of cytotoxic granules. The granules contain perforin and granzymes which activates apoptosis in the infected target cell. In addition, engagement of Fas on target cell by Fas-ligand on the T-cell induces apoptosis of the infected cell (Janeway CA, 2005).

The T-cell response also results in immunological memory. There are two main subsets of memory T-cell derived both from CD4 and CD8 positive T-cells; TEM (effector memory) and TCM (central memory). TEM cells are located in the periphery; in the mucosa of the airway and gut and in the liver, while the TCM cells home to lymph nodes and tonsils (Kalia *et al*, 2006).

Introduction to lymphomas

Malignant lymphomas are tumors of immune cells in the lymphoid tissues, primarily the lymph nodes. The two main groups are Hodgkin's lymphomas and non-Hodgkin's lymphomas. The non-Hodgkin's lymphomas (NHLs) comprise a heterogeneous group of diseases caused by neoplastic proliferation of B- or T-lymphocytes. The wide spectrum of clinical presentations span from small localized lesions causing minimal symptoms to bulky lesions and disseminated tumor growth accompanied by threatened organ function and leading to severe reduction in the patient's general condition. The WHO classification of 2008 subdivides the NHLs into a plethora of distinct entities based on the combination of clinical information, histological and morphological features of the tumor tissue, phenotyping of tumor cells by immunological methods as well as detection of genetic abnormalities in tumor cells (Swerdlow SH, 2008). The lymphoma types and subtypes require different treatments and patient outcome varies markedly.

The two most common types of NHL are diffuse large B-cell lymphoma and follicular lymphoma, each of them accounting for about 30% of all new NHL cases in Europe and America. Both types are thought to arise from germinal center B lymphocytes in the lymph node. FL affects the middle-aged and elderly population and exhibits a variable clinical course with a median survival of at least 10 years. Initially, FL is often responsive to immunotherapy, chemotherapy and/or radiotherapy. However, the vast majority of patients experience relapses and progression to treatment resistant disease or transformation to higher-grade lymphoma with poor treatment outcome. Disease onset is more acute in DLBCL than in FL and DLBCL progress more rapidly. However, fifty to sixty percent of patients achieve durable remissions and there are few disease specific deaths beyond 5 years following treatment with immuno-chemotherapy regimens containing antracyclines. Among the remainder of patients with incomplete treatment response or early relapse a proportion can be cured by high dose chemotherapy followed by autologous stem cell support to rescue bone marrow function.

The heterogeneous outcome observed both in FL and DLBCL has motivated the search for prognostic and predictive markers. The reliable identification of patients at low or high risk may then provide a rationale for differentiated treatment with an optimized balance between toxicity and benefit. Furthermore, a detailed knowledge about molecular mechanisms underlying different disease phenotypes may reveal targets for new treatment strategies.

The advent of new methods for whole genome analyses during the two last decades has offered possibilities for large scale screening of tumor genomes in the search for genetic markers for prognosis. The extensive data produced from large scale genetic analyses have also generated hypotheses for disease mechanisms and identified potential treatment targets. Testing of such hypotheses in cancer cell lines and mouse models for cancer have provided new insight to tumor biology and resulted in development of new therapeutic agents for several cancer types.

As the focus of this thesis is on FL, the following sections describe in some detail clinical features, histopathology and molecular pathology of FL. Descriptions of other lymphoma entities are included to create a broader context for certain aspects of FL.

Classification of lymphomas

The medical and scientific history of lymphomas demonstrates the power of systematic observation and classification in biological research. The discovery and characterization of lymphoma subgroups hand in hand with advances in molecular biology and genetics has led to development of more specific treatment strategies followed by significant improvement in survival of lymphoma patients.

The history of lymphomas began in 1832 with Thomas Hodgkin's publication "On Some Morbid Appearances of the Absorbant Glands and the Spleen" (Aisenberg, 2000). In this publication Thomas Hodgkin made the distinction between inflammatory processes and the condition that would later be recognized as Hodgkin's disease (first by Wilks in 1865).

Sternberg and Reed (in 1898 and 1902, respectively) defined the microscopic features of the neoplastic cells in Hodgkin's disease, which later were designated Reed-Sternberg cells (RS-cells). However, Hodgkin's disease was not recognized as a lymphoid neoplasm and renamed Hodgkin's lymphoma until the 1990s when the development of PCR (polymerase chain reaction) with primers for IgH gene loci and techniques for micro dissection of RS-cells proved that these were clonal B-cells (Jaffe *et al*, 2008).

The first classification of lymphomas was proposed in 1966 by Rappaport in USA and mainly distinguished the nodular from the diffuse growth pattern of malignant cells (Rappaport, 1966). A more comprehensive classification was developed in the Working Formulation which also stratified lymphomas based on the clinical outcome (low-grade, intermediate grade, and high-grade) (The Non-Hodgkin's Lymphoma Pathologic Classification Project, 1982). In contrast,

the Kiel classification of 1974 used in Europe, categorized lymphomas according to their hypothetical counterparts in the scheme of normal lymphocyte differentiation (Lennert, 1978;Lennert, 1992). The different classification schemes and treatment traditions made it difficult to compare clinical studies from centers across the Atlantic. The REAL classification proposal of 1994 resulted from a series of meetings between European and American haematopathologists and represented a new paradigm in lymphoma management (Harris *et al*, 1994). Based on published data and consensus among lymphoma experts distinct disease entities were defined from a constellation of features: morphology, immunophenotype, gentic abnormalities and clinical information. Some broad categories had to be made because of limitations in current knowledge (i.e DLBCL with a range of morphological variants) thereby pointing out fields in the need of further study. The WHO Classification of 2001 was a continuation of this work that also pointed out the necessity of differential treatment of separate lymphoma entities. A revision was published in 2008 which emphasized the need for predictors of prognosis in the common lymphomas, such as DLBCL, FL and peripheral T-cell lymphomas (Swerdlow SH, 2008;Jaffe *et al*, 2008).

As opposed to carcinomas, malignant lymphomas can very rarely be cured by surgery. Shortly after the detection of x-ray and the radioactive radium isotope, radiation was tested as treatment for several cancer diseases, including HL (Easson & Russell, 1963). Different schedules for radiotherapy in HL were developed during the first half of 1900, and by the 1960s extensive radiotherapy became the standard treatment for HL (Kaplan & Rosenberg, 1966;Rosenberg *et al*, 1972). The development of chemotherapeutic agents started with the use of the mustard gas derivate nitrogen mustard during World War II. Its activity against HL and chronic leukemia was reported by Gilman and Philips and others in 1946 (GILMAN & PHILIPS, 1946). The benefit of nitrogen mustard in HL stood out and fuelled the discovery, development and clinical testing of new agents and combinational chemotherapy regimens. As of today, a high proportion of HL patients are cured by either chemotherapy alone or in combination with limited radiotherapy and the focus is to reduce long term adverse effects of treatment while maintaining the excellent survival figures.

NHL has lagged behind HL in classification and treatment results. The WHO classifications of 2001 and 2008 represent important milestones for the development of subtype specific treatment protocols (Swerdlow SH, 2008). Especially during the last two decades we have seen substantial improvements in outcome of NHL. This has been achieved by the combination of improved diagnostic precision, development of intensified chemotherapy regimens hand-in-hand with improved supportive case and during the last decade molecular targeted therapy.

Epidemiology of lymphomas

Most epidemiological studies have focused on the non-Hodgkin's lymphomas (NHL) as a whole and comprehensible epidemiological surveys of the individual subgroups have naturally lagged behind the refinement of lymphoma classification. Ninety percent of lymphoid neoplasms occurring worldwide are mature B-cell neoplasms, and these are most common in the developed countries of Western Europe, USA, New Zealand and Australia. FL and DLBCL are the most common subtypes, accounting for 60 % of NHLs in these countries (Swerdlow SH, 2008). Generally, the incidence rate of NHLs is rising worldwide, more in Western developed countries than in Africa and Asia (Muller *et al*, 2005). In the USA the age-adjusted rates of NHL increased by 3-4% annually until the 1990s, when incidence rates stabilized, showing a 1-2% annually increase thereafter (Figure 5) (SEER, NCI, April 2010, accessed 04.09.2010). A similar trend has been observed in many European countries (Bosetti *et al*, 2008). In Norway, the NHL incidence rate has increased steadily by 2-3% annually since the 1970s (Figure 6) (The Norwegian Cancer Registry, 2008, date of data retrieval 27.08.2010). Increasing NHL-incidences has been observed in both sexes, the age groups above and below 65 and all ethnic groups living in Western Countries. NHLs in general, are more frequent among males than females and occur most frequently among elderly persons, with increases in age-specific incidence especially after the age of 55 (Muller *et al*, 2005;Morton *et al*, 2006).

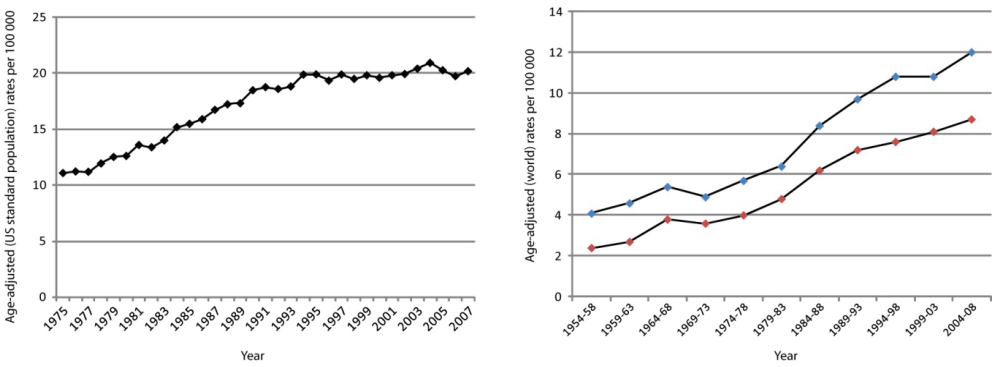

Figure 6. Incidence of non-Hodgkin's lymphoma in the United States (left panel; US standard population) and Norway (right panel; world standard population). In the right panel, incidence rates are shown separately for men (blue marks) and women (red marks).

The Cancer Registry of Norway has collected national data about cancer incidence and prevalence since 1951. All physicians are instructed by law to notify the registry about new cancer cases. This combined with the unique personal identification number used in Norway secures a high degree of data completeness. Figure 7 and the table below are based on data from the Cancer Registry of Norway and show the national incidence and prevalence of FL, respectively (date of data retrieval 03.09.2013).

		Number of years after primary diagnosis		
	Number of patients alive per 31.12.2011	**<1**	**1-4**	**5-9**
Men	607	90	290	227
Women	723	102	336	285
Both sexes	1330	192	626	512

Table 1. Prevalence of FL in Norway.

Data scarcity in cancer registries of less developed countries in Asia and Africa limits the analysis of global distribution patterns of the major NHL subtypes. Nevertheless, some trends are notable. FL is relatively more common in the United States (US) than in Europe, accounting for ≈30% and ≈20% of NHLs, respectively, while in Africa and Asia FL is rare. Endemic Burkitt's lymphoma caused by Ebstein-Barr virus (EBV) and enhanced by malaria is the most common of NHLs in Africa. The overall frequencies of NHL is lower in Asia than in the US and Europe. On the other hand, primarily extranodal disease and peripheral T-cell NHL associated with EBV and human T-cell leukemia/lymphoma virus type 1 (HTLV-1) is relatively more frequent in Asians (Muller *et al*, 2005;Swerdlow SH, 2008).

Increasing incidence rates can only partly be explained by factors such as improved cancer reporting, changed lymphoma classification, more sensitive diagnostic techniques and an increasing proportion of elderly people in the populations of Western Countries. The causes underlying a large proportion of additional NHL cases remain unknown. In the 1980s and 1990s, AIDS-associated lymphomas contributed to the increased NHL-incidence, particularly in young adult males, and a decreasing incidence in this group during the more recent years is due to the introduction of effective anti-retroviral therapy (HAART)(Fisher & Fisher, 2004;Morton *et al*, 2006). In addition to HIV infection, other conditions causing immunosuppression have been linked to NHL risk. Immunosuppressive treatment for autoimmune diseases and following organ transplantation as well as congenital immunodeficiencies predispose patients for NHL (Fisher & Fisher, 2004;Muller *et al*, 2005). Autoimmune diseases such as rheumatoid arthritis, Sjøgren's

disease and celiac disease are also associated with increased NHL-risk, independent of immunosuppressive treatment, indicating a role for persistent inflammatory activity in the etiology of NHL. Chronic inflammation caused by viral infections has also been linked to some types of NHL. Besides its role in endemic Burkitt's lymphoma, EBV is present in NHLs associated with immunosuppression; in AIDS-patients and in organ-transplant recipients. Other well documented examples are the association of adult T-cell lymphoma with HTLV-1 infection in endemic areas of Asia as well as the association of Helicobacter pylori infection with gastric NHLs (Fisher & Fisher, 2004;Muller *et al*, 2005). Although exposure to environmental agents such as agricultural pesticide, hair dye and UV radiation has been linked to NHL-risk, it is unclear to what extent such factors contribute to the increase in NHL-incidence (Fisher & Fisher, 2004;Muller *et al*, 2005).

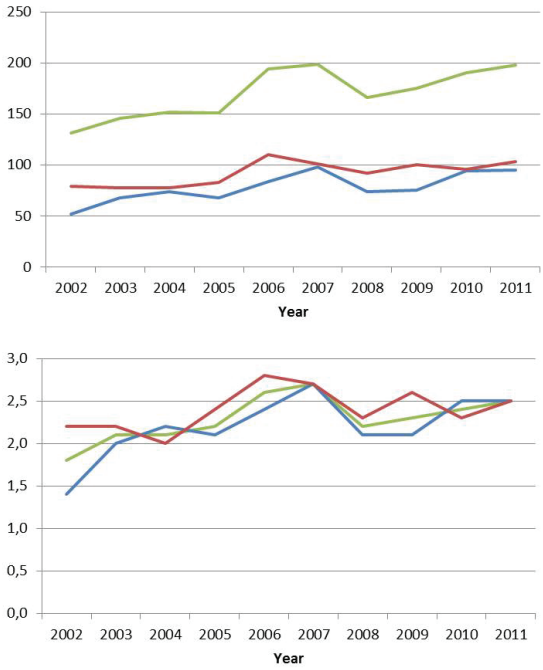

Figure 7. Incidence of follicular lymphoma in Norway 2002-2011. Data from the Norwegian Cancer Registry. Upper panel: Absolute numbers for both sexes (green), men (blue) and women (red). Lower panel: Age adjusted incidence rate, new cases per 100 000 person years, world standard population (Doll 1966) 2002-2011 for both sexes (green), men (blue) and women (red).

Generally, NHL mortality rates rose up to the mid-1990s. For the last decade declining morality rates have been observed in the countries of Western Europe and the US (Bosetti *et al*, 2008). Also, improved survival from NHL has been reported from cancer registries and population-based studies in Western countries (Sehn *et al*, 2005;Luminari *et al*, 2007;Keegan *et al*, 2009) (Figure 2). This trend is mainly attributable to improved treatment outcome of mature B-cell lymphomas by the introduction of the monoclonal anti-CD20 antibody rituximab in 1997 (Sehn *et al*, 2005;Molina, 2008;Keegan *et al*, 2009). A similar trend has not been observed for the peripheral T-cell lymphomas (Abouyabis *et al*, 2008). The use of high-dose therapy with autologous stem-cell support for patients with refractory and relapsing DLBCL may to a moderate extent also contribute to the improved outcome. Data from the lymphoma data base at our institution shows the improvement in outcome for patients with indolent non-Hodgkin's lymphomas (Figure 8).

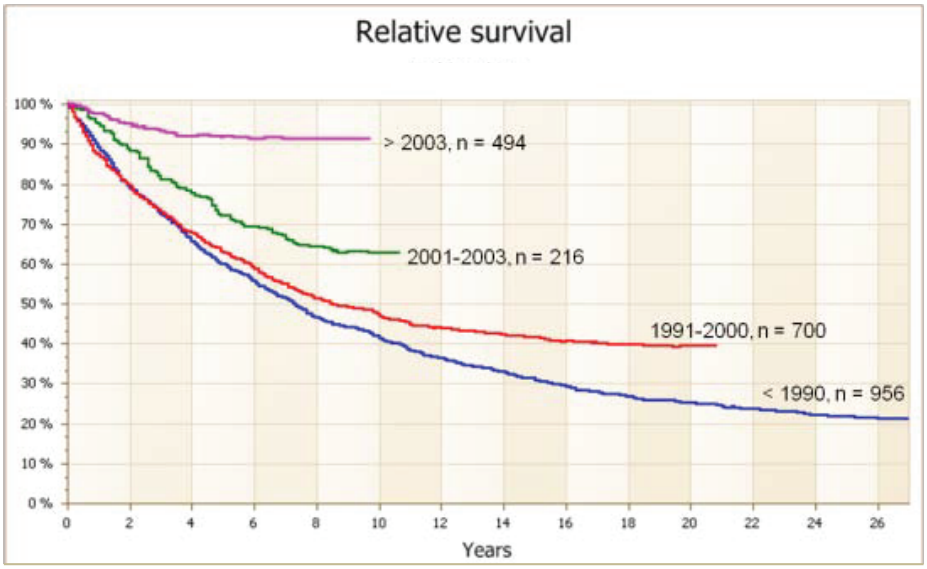

Figure 8. Relative survival of patients with indolent non-Hodgkin's lymphomas according to the year of primary diagnosis. Data from the lymphoma data base at Oslo University Hospital, the Norwegian Radium Hospital.

Histopathology of FL

In 1925, Brill et al described patients with enlarged lymph nodes and spleen, pathologically characterized by proliferation of lymphoid follicles and he was the first to recognize FL as a distinct disease entity (Aisenberg, 2000; van & Schouten, 2007). Additional cases were reported during the following years also describing the progression to large-cell neoplasm (van & Schouten, 2007; Jaffe *et al*, 2008; Swerdlow SH, 2008).

The malignant B-cells of FL are organized in a follicular growth pattern resembling the secondary follicle of a reactive germinal center, hence the name follicular lymphoma. The follicles are densely packed, often lacking mantle zones. Unlike the B-cells of a reactive germinal center, the centrocytes and centroblasts of FL are not polarized into light and dark zones, but reside and proliferate intermingled. FL cells are positive for B-cell associated antigens such as CD19, CD20, CD22 and CD79a as well as for germinal center B-cell markers such as CD10 and Bcl-6. CD5 and CD43 immunostaining is negative in FL, as opposed to chronic lymphatic leukemia. In contrast to B-cells of a normal germinal center, FL cells are positive for the anti-apoptotic protein BCL2 in most cases (Figure 9). The aberrant expression of BCL2 is due to the presence of the chromosomal translocation t(14;18) which will be described in detail in the section “Molecular pathology of lymphomas”.

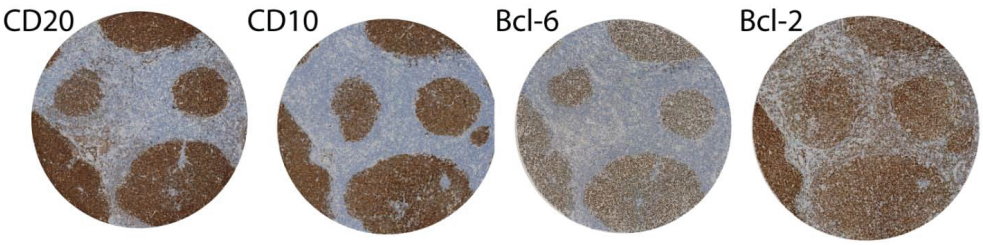

Figure 9. Immunohistochemical staining of follicular lymphoma showing that the malignant B-cells are positive for the B-cell marker CD20, the germinal center B-cell markers CD10 and Bcl-6. In addition, the malingnat B-cells of follicular lymphoma show aberrant expression of the anti-apoptotic protein Bcl-2.

There is a close relationship between FL-cells and the meshwork formed by the follicular dendritic cells as visualized by CD23 and CD21 immunostaining. In addition, the neoplastic follicles contain follicular T helper cells positive for CD4 and CD57. The interfollicular areas are composed of variable elements of T-cells, macrophages, micro vessels and fibroblasts (Figure 10).

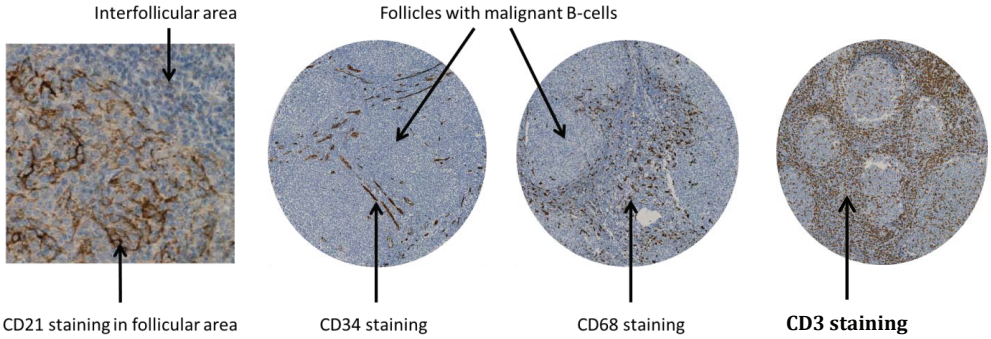

Figure 10. Immunohistochemical staning with markers for cells present in the microenvironment of follicular lymphoma. Anti-CD21 is a marker for Follicular Dendritic cells and the staining shows how their elongated cytoplasmatic processes form a network around the malignant B-cells. Anti-CD34 is a marker for endothelial cells and the staining shows how microvessels are mainly located tin the inter follicular area. Anti CD68 is a marker for macrophages that mostly reside in the interfollicular area with a few macrophages also present within the neoplastic follicle. CD3 is a pan T-cell marker and the staining shows the location of T-cells both inter- and intrafollicularily.

FLs are graded from 1 to 3a or 3b according to the content of centroblasts as determined by counting of 10 high power fields (40xobjective and 18 mm field of view). FL grade 1-2 are grouped together in the latest revision of the WHO classification and is dominated by centrocytes, containing up to 15 centroblasts per high power field. FL grade 3 is subdivided into 3a and 3b, the former containing >15 centroblasts/hpf and the latter showing solid sheets of centroblasts (Swerdlow SH, 2008). Although this method shows poor reproducibility, it has

clinical relevance as the higher grades of FL, particularily FL grade 3b, shows a more aggressive clinical behavior.

Upon higher-grade transformation the follicular growth pattern is lost and larger, blast-like cells growing in diffuse areas take over, commonly showing the histological features of Diffuse Large B-Cell Lymphoma (DLBCL) (Figure 11), or more rarely a Burkitt-like lymphoma harboring chromosomal rearrangements of both BCL2 and MYC. Areas of FL may be retained adjacent to the diffuse areas in a composite lymphoma.

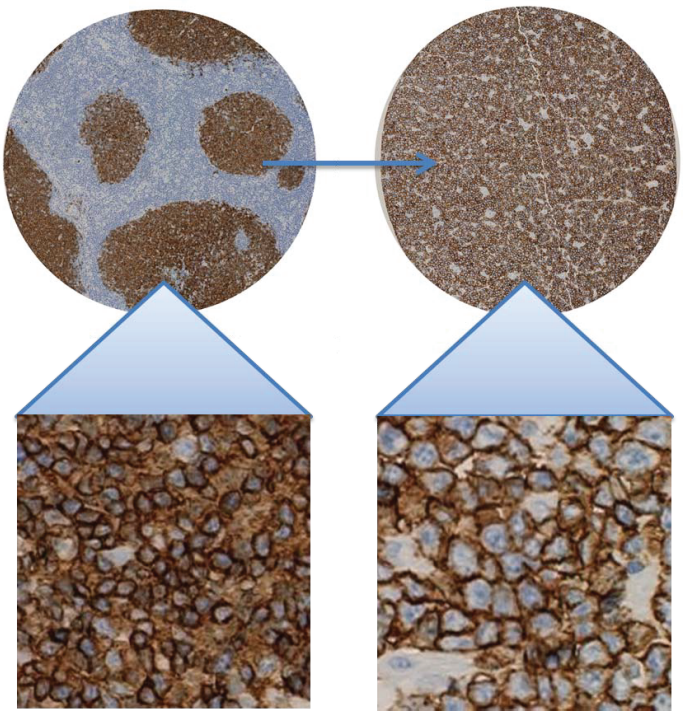

Figure 11. Transformation from FL to DLBCL. Two sections of paraffin-embedded tumor tissue obtained from the same patient at the time of primary diagnosis (left) and at the time of transformation (right). Immunohistochemical staining with anti-CD20 antibodies shows the different distribution of malignant B-cells before and after transformation. The follicular growth pattern in the primary follicular lymphoma sample is lost in the sample showing DLBCL. In the primary sample the majority of tumor cells within a neoplastic follicle morphologically resemble centrocytes, while in the transformed sample shows large, blast-like cells. The magnification is the same in the two square shaped detail images, clearly showing the differences in cell size in FL and DLBCL.

Clinical features and treatment of FL

The Norwegian national treatment recommendations below are given according to the national guidelines, which are generally in line with the referenced international recommendations (http://helsedirektoratet.no/publikasjoner/nasjonalt-handlingsprogram-med-retningslinjer-for-diagnostikk-behandling-og-oppfolging-av-maligne-lymfomer/Sider/default.aspx).

Patients most commonly present with moderately enlarged peripheral lymph nodes. The clinical symptoms and signs often develop gradually; lymph node enlargement may have been modest or occult for a long time before clinical recognition, and FL is commonly disseminated at the time of diagnosis. Symptoms may also arise from enlargement of deep nodes, mostly infradiaphragmatic, which threaten normal organ function causing hydronephrosis, intestinal obstruction, compression of vena cava inferior or cholestatsis. Primary mediastinal or spleen involvement is rare. The patient's general status is commonly preserved and a minority of patients has B-symptoms (night sweats, persisting fever and unexplained weight loss). Bone marrow involvement is found in 50-60% of cases at diagnosis. Less common presentation forms are extranodal FL, FL limited to the gastro-intestinal tract or to skin, FL in situ and pediatric FL.

Follicular lymphoma is staged according to the modified Ann Arbor classification (see for instance (Gospodarowicz, 2009)) based on results from the clinical examination, a CT-scan including neck, thorax, abdomen and pelvis, blood sampling and a bone marrow biopsy. PET-CT has limited additional value, as most cases are classified as disseminated disease by conventional staging methods, but may be useful to confirm localized disease prior to planning of curative radiotherapy.

The Follicular Lymphoma Prognostic Index (FLIPI) is made up of the following five clinical criteria (Solal-Celigny *et al*, 2004); age ≥ 60 years, Ann Arbor stage III-IV, hemoglobin level < 12 g/L, involved lymph node regions > 4 and serum LDH≥ upper reference value. By the se criteria, patients can be allocated to low (0-1 criterion), intermediate (2 criteria) and high risk (3-5 criteria) groups with significantly different survival. Each risk group comprises roughly one third of the patients at primary diagnosis. FLIPI and other clinical factors with prognostic value probably reflect different biological features of the disease (and its interaction with the host) already at primary diagnosis. However, patients with limited extent of disease combined with aggressive features and patients with advanced extent disease and indolent course may be misclassified in low and high risk groups, respectively. The FLIPI-score is considered for stratification of treatment for individual patients, but its poor specificity and sensitivity in predicting the disease course has limited its use in clinical studies and in translational research.

The choice of treatment strategy is based on an individual evaluation taking into account the patient's age, general health and degree of affliction. The FLIPI score is used to assess

disease extent and aggressiveness. No studies have shown any survival benefit from early treatment initiation in asymptomatic patients and no studies have shown survival benefit from early administration of intensified treatment regimens (Ghielmini *et al*, 2013). Patients with a low FLIPI-score who are in a good condition with diminutive symptoms can be observed untreated ("watch and wait"). Eventually, disease progression with declining general condition, uncomfortable lymph node enlargement, signs of bone marrow failure, B-symptoms or organ complications necessitate treatment. In younger patients and patients presenting with bulky tumor or intermediate to high FLIPI-score at diagnosis treatment is usually initiated early. Less than one third of patients have limited disease (stage I-II) at disease presentation. If involved lymph nodes can be included in one small to medium sized radiation field, radiotherapy (2Gy x 12) with curative intention is the treatment of choice. Nevertheless, half of these patients experience relapse and subsequently receive chemo-immunotherapy.

The standard first line therapy for most patients consists of a combination of immunotherapy with the anti-CD20 antibody rituximab and chemotherapy: bendamustine or a combination of cyclophosphamide, vincristine and prednisolone with or without doxorubicine (commonly abbreviated CHOP or COP, respectively). Rituximab monotherapy in first line is also widely used today, especially in cases where it is desirable to postpone chemotherapy. Maintenance rituximab treatment has been shown to confer improved progression free survival. Chlorambucile, which also may be combined with immunotherapy, is an alternative for elderly patients, but may be detrimental for the bone marrow function and increase the risk for secondary myeloid neoplasia and for inadequate bone marrow harvest yield for patients eligible for autologous bone marrow transplantation at a later stage. Treatment response is usually very good and in the majority of patients a CR or a good PR is achieved after 6 courses (4 months of treatment). At relapse of follicular lymphoma, different treatment options can be chosen based on an individual evaluation. If a good and long-lasting response to rituximab (R), R-Bendamustine or R-COP was obtained in first line, the same or the alternative regimen may be chosen. Maintenance rituximab immunotherapy is recommended if not given up front. In the case of more aggressive disease various chemotherapeutic agents have documented effect in FL; bendamustine, COP, CHOP and fludarabine, all in combination with rituximab are commonly used also at relapse. Trofosfamid is an alternative for patients with reduced tolerance for the toxic effects of combinatorial regimens. For patients relapsing after a shorter period or who do not respond, high dose therapy with autologous stem cell support (HD-ASCT) may be the treatment of choice (Ghielmini *et al*, 2013;Montoto *et al*, 2013).

The median overall survival in FL was reported to be 7-12 years in the pre-rituximab era. The classical survival curve in FL drops in an almost linear fashion demonstrating the heterogeneous course and lethal outcome of the disease. Common causes of death are treatment resistant FL, higher-grade transformation or inability to tolerate further treatment.

The advent of rituximab in the late 1990'ies, a monoclonal humanized antibody directed against the B-cell marker CD20 has revolutionized the treatment of B-cell lymphomas (see for instance (Sehn *et al*, 2005)). In Norway, rituximab became part of the routine treatment of follicular lymphoma and DLBCL in the beginning of the second millennium. The use of rituximab together with improvements in diagnostics of FL and the treatment of transformed FL have resulted in improved survival. However, recent data still indicate that FL relapses and remains incurable (Salles, 2007;Conconi *et al*, 2012).

Definition and treatment of transformed lymphoma

Transformation to an aggressive and often treatment resistant phenotype occurs in various proportions of patients in several of the mature B-cell neoplasms and is associated with short survival. In FL, transformation has been reported to occur in 10-70% of patients, depending on the diagnostic method (histological examination, clinical criteria or detected at autopsy), the observation time and the frequency of biopsies taken during the course of disease (Acker *et al*, 1983;Garvin *et al*, 1983;Horning & Rosenberg, 1984;Bastion *et al*, 1997;Gine *et al*, 2006;Montoto *et al*, 2007;Al-Tourah *et al*, 2008;Conconi *et al*, 2012). The probability of transformation seems to be constant at least during the first 5-10 years of observation, and after 10 years of observation approximately 30 % of patients have experienced transformation, resulting from a transformation rate of approximately 3 % per year. Whether or not the transformation rate declines in patients remaining at risk thereafter has not been clear and the existing data are conflicting (Montoto et al., 2007; Al-Tourah et al., 2008). Neither the timing of treatment onset (immediately following diagnosis of FL versus "watch and wait"), nor the intensity of the primary treatment regimen influenced the incidence of transformation according to most previous studies (Acker et al 1983, S Horning et al 1984, Yuen et al 1995, Al-Tourah et al., 2008; Conconi et al., 2012). Then again, a recent study reported significantly lower transformation rate in patients who received rituximab in monotherapy as first line treatment compared to patients that had been observed untreated initially. This indicates a reduced transformation rate of FL in the rituximab era (Link *et al*, 2013).

Before the WHO 2008 classification discriminated between FL grade 3a and – grade 3b, grade 3 was considered more aggressive than grade 1 and 2, and was treated like DLBCL at many centers. Thus, follicular lymphomas with increasing grade from 1 and 2 to grade 3 were by many clinicians considered as a transformation. In the beginning of the millennium data supporting a subdivision between FL grade 3a and 3b was published (Ott *et al*, 2002), and more recent studies have confirmed that FL grade 3b represents a separate entity with different molecular features (Piccaluga *et al*, 2008;Horn *et al*, 2011) accounting for≈ 5 % of FLs. The clinical significance of distinguishing between FL grade 3a and 3b has been a matter of debate as studies assessing survival (OS and PFS) according to FL grade have shown conflicting results

(Shustik *et al*, 2011). A recent population based study with long term follow-up data confirmed the aggressive clinical course of FL grade 3b (Wahlin *et al*, 2012). About 50% of patients FL grade 3b relapsed and died within 5 years, whereas ≈ 75% of patients with grade 1-3a were still alive 5 years after diagnosis. Thereafter the OS curve for FL 3b showed a plateau, indicating that a proportion of these patients were cured, while the OS curve for FL grade 1-3a showed a steady decline. The prognostic significance of FL grading seems to depend on treatment, as the OS was better for the patients with FL grade 3a than FL grade 1-2 when treated with rituximab monotherapy in first line (Wahlin *et al*, 2012;Wahlin *et al*, 2013). Based on the study by Ott et al (Ott *et al*, 2002) and other publications during the first decade of 2000 the definition of transformation was limited to the progression from FL of any grade to DLBCL. Then again, the prognostic significance of progression from FL grade 1-2 to grade 3a or b at relapse has been poorly studied.

Obtaining a representative biopsy is sometimes difficult as the site of transformation may be challenging to access surgically. The appearance of a least one of the following clinical characteristics in a patient with a history of FL, is associated with the same outcome as for patients with histologically verified transformation; rapid, discordant tumor growth, involvement of unusual extranodal site, new B symptoms, hypercalcemia or sudden rise in LDH-level (Al-Tourah *et al*, 2008). The diagnosis of transformed lymphoma should in such cases be based on these clinical findings and treatment planned accordingly.

In a large Canadian population based study of more than 300 cases of FL with observation times exceeding 15 years (median 109 months) (Al-Tourah *et al*, 2008) the 10 year survival rates were 36% and 75% for the patients with and without transformation, respectively. In the same retrospective analysis median survival following transformation was only 1.7 years. Traditionally, patients with transformed non-Hodgkin's lymphoma (t-NHL) have received salvage treatment with combination chemotherapy regimens like CHOP, alternatively dexamethasone, cytarabine and cisplatin (DHAP), ifosfamide, carboplatin and etoposide (ICE) or etoposide, ifosfamide and methotrexate (VIM) in cases where doxorubicin has to be avoided (elderly, patients with heart disease or patients that already received doxorubicin-containing regimens for their FL). Despite remission rates (CR) of up to 40%, the median overall survival obtained with these treatment lines was only in the range of 7 months to 1.7 years (Yuen *et al*, 1995;Bastion *et al*, 1997;Gine *et al*, 2006;Montoto *et al*, 2007;Al-Tourah *et al*, 2008). Then again, a subgroup of patients in CR after salvage treatment obtained long-term survival (Yuen *et al*, 1995). Studies prior to 1999 (the opening year of the study presented in Paper 2) had shown longer survival in patients with localized transformation, no previous chemotherapy, no B symptoms, a normal LDH-level as well as in patients attaining CR after salvage chemotherapy (Yuen *et al*, 1995;Bastion *et al*, 1997). On the other hand, it was known that post-transformation survival did not dependent on the interval from primary diagnosis to transformation (Yuen *et al*, 1995).

In a tumor biological perspective; the extent of disease, increased levels of LDH and beta2-microglobulin can be considered as markers for the proliferation rate of the tumor, while the attainment of partial remission (PR) or CR is a marker for chemo-sensitivity. Thus, bringing more patients into CR following transformation may improve the long-term outcome. High-dose chemotherapy with autologous stem cell support (HD-ASCT) is the standard treatment for recurrent and primarily treatment resistant DLBCL. This was established following the Parma-study (Philip *et al*, 1995) in which 215 patients with relapsed intermediate grade (n=163) and high grade (n=52) non-Hodgkin's lymphoma were first treated by two courses of conventional chemotherapy (DHAP=dexamethasone, cytarabine, cisplatin). The patients who responded (n=109) were randomized to treatment with high-dose chemotherapy followed by autologous bone marrow transplantation (n=55) or to continue conventional chemotherapy treatment (n=54). The rates of event-free survival and overall survival were significantly higher in patients receiving HD-ASCT. This formed a rationale for testing of HD-ASCT in various disease stages of FL (Al *et al*, 2012;Schaaf *et al*, 2012), and particularly in transformed FL. Retrospective studies of limited size (Foran *et al*, 1998;Friedberg *et al*, 1999;Chen *et al*, 2001;Williams *et al*, 2001;Andreadis *et al*, 2005;Sabloff *et al*, 2007;Hamadani *et al*, 2008) indicated a beneficial effect of HD-ASCT in transformed FL, as will be discussed below.

In HD-ASCT chemotherapy and / or total body radiation (TBI) is administrated in doses exceeding the tolerance of the hematopoietic stem cells with the aim to completely eradicate the neoplastic lymphoid cells. In advance, the patient's own hematopoietic stem cells are mobilized harvested from peripheral blood. The autologous stem cells are re-infused intravenously following HD-ASCT and re-populate the bone marrow restoring hematopoiesis. Only patients with disease response to immuno-chemotherapy will benefit from HD-ASCT. Patients achieving at least a PR following 2-3 courses of an induction regimen are eligible.

Hematopoietic CD34+ stem cells are efficiently mobilized from the bone marrow to peripheral blood by combining chemotherapy and G-CSF. From the majority (90%) of patients the required amount of CD34+ cells (> 2.0 x 10^6 CD34+ cells/kg), is collected by one or more days of aphaeresis. A high number (> 5 x 10^6 CD34+ cells/kg) of re-infused stem cells is associated with faster hematological recovery, less bleeding- and infections complications and improved outcome following HD-ASCT.

Even following HD-ASCT follicular lymphoma and transformed lymphoma show a notorious tendency to relapse. For relapses of the transformed component the prognosis is grave and there are no established treatment options. For relapses of the indolent component radio-immunotherapy can induce long-lasting remissions (Witzig *et al*, 2002b;Witzig *et al*, 2002a;Vose *et al*, 2000;Kaminski *et al*, 2001;Illidge & Morschhauser, 2011). Allogeneic stem cell transplantation with reduced intensity conditioning (RIC-allo) has given long-term remissions in younger, selected patients (Mortensen *et al*, 2012;Khouri, 2011).

Molecular pathology of lymphomas

The lymphoid neoplasms (leukemias and lymphomas) are tumors originating from clonal proliferation of B-cells and T/NK-cells at various stages of development and differentiation. The malignant lymphocytes often recapture features of their normal counterpart, an observation that has led to the “frozen stage hypothesis” of lymphoma development (Figure 11). .

The initial step in lymphomagenesis is often chromosomal translocations occurring at different stages of lymphocyte development, leading to oncogene activation. This is supported by the observations that recurrent translocations are the most frequently genomic aberrations in many lymphoma subtypes and some cases of lymphoma carry a translocation as the only detected genomic aberration. The first recurrent translocations to be recognized were the t(14;18) in FL and t(8;14) in Burkitt’s lymphoma. Specific translocations are closely associated with the different lymphoma entities (Table 2, next page).

Lymphoma entity	Translocation	Frequency	Genes	Oncogenetic mechanism
B-lymphoblastic leukemia/lymphoma	t(9;22)(q34;q11.2)		ABL and BCR	Fusion protein; constitutively activated tyrosin kinase
	rearrangement of 11q23	6%	MLL	Various fusion proteins; disruption of MLL complex and inappropriate expression of HOX genes
	t(12;21)(p13;q22)	20-50%	TEL and AML (ETV6/RNX1)	Fusion protein; function largely unknown
	t(5;14)(q31;q32)		IL3 and IGH	Over-expression of IL3
	t(1;19)(q23;p13.3)		PBX and E2A(TCF3)	Fusion protein; leading to inappropriate expression of HOX genes
Burkitt's lymphoma	t(8;14)(q24;q32) or variants with translocation to light chain loci (2p12 or 22q11)	100%	C-MYC and IGH	Over expression of the transcription factor Myc leading to increased proliferation
DLBCL	Rearrangement of 3q27,	30%	BCL6	Over expression of the transcriptional repressor Bcl-6
	t(14;18)(q32;q21),	20-30%	IGH and BCL2	Over expression of the anti-apoptotic protein Bcl-2
	rearrangement of 8q24	10%	C-MYC	Over expression of the transcription factor Myc leading to increased proliferation
Follicular lymphoma	t(14;18)(q32;q21) or variants with translocation to light chain loci (2p12 or 22q11)	90%	IGH and BCL2	Over expression of the anti-apoptotic protein Bcl-2
Mantel cell lymphoma	t(11;14)(q13;q32) or variants with translocation to light chain loci (2p12 or 22q11	95%	CCND1 and IGH	Over expression of the cell cycle regulator cyclin D1
Extra nodal Marginal zone lymphoma (MALT)	t(11;18)(q21;q21)	pulm/gast	API2 and MALT1	Fusion protein with MALT1 activity leading to constitutive activation of the NF-κB pathway
	t(1;14)(p22;q32)		BCL10 and IGH	Over expression of Bcl-10, involved in activation of the NF-κB pathway
	t(14;18)(q32,q21)	oc ad/orb	IGH and MALT1	Over expression of MALT1 which activates the NF-κB pathway
	t(3;14)(p14;q32)	thyr/skin/oc ad/orb	FOXP1 and IGH	Over expression of the transcription factor FOXP1
Anaplastic large cell lymphoma	t(2;5)(p23;q35) and other rearrangements of 2p32	90%	ALK and NPM	Fusion protein; constitutively active ALK, a receptor tyrosin kinase

Table 2. Lymphoma entities and associated translocations.

Primary oncogenetic aberrations

The physiological DNA modifications taking place during B-cell development and activation put B-cells at risk for genomic aberrations and point mutations. The recombination of V(D)J segments at the pro-B and pre-B stages of development, the somatic hypermutation (SHM) and class-switch recombination (CSR) occurring in germinal center B-cells all involve DNA double strand breaks (DSBs). These physiological breaks can persist over several cell divisions and erroneous repairing of the DSBs may lead to formation of chromosomal translocations. Indeed, the majority of recurrent translocations in lymphomas involve one physiologic DSB of an immunoglobulin loci and one pathologic DSB of a proto-oncogenes (Tsai & Lieber, 2010). The proto-oncogene thus comes under control of the active immunoglobulin promoter, resulting in oncogene activation by over-expression.

The translocation t(14;18) characterizes 80-90% of follicular lymphomas and a proportion of diffuse large B-cell lymphomas. Yunis et al (Yunis *et al*, 1982) were the first to report of an association between FL and the t(14;18). A few years later the breakpoints on chromosome 14 and 18 were cloned (Tsujimoto *et al*, 1984). The physiologic break at IgVH-locus is mediated by RAG1/RAG2 and may occur during VDJ rearrangement in pro-B stage of development. Two studies (Raghavan *et al*, 2004;Raghavan *et al*, 2005) showed involvement of RAG1/RAG2 also in the breakpoint formation at the BCL2-locus. A specific DNA structure in the major breakpoint cluster of BCL2 was shown to be the basis for fragility. More recently it has been shown that the pathologic break occurs in three breakpoint clusters that are CpG sites within or proximal to the BCL2-gene. This indicates that off-site involvement of both AID and RAG1/RAG2 may cause DSB in the BCL2-locus (Tsai & Lieber, 2010).

During the 1980'ies it was shown that the t(14;18) leads to expression of a BCL2-immunoglobulin chimeric RNA in resting B-cells (Nunez *et al*, 1989). A few years earlier, the BCL2 protein and its expression in non-Hodgkins B-cell lymphomas bearing the t(14;18) had been described (Tsujimoto & Croce, 1986;Ngan *et al*, 1988) and finally the anti-apoptotic function of BCL2 was described by Hockenbery D et al (Hockenbery *et al*, 1990). As a consequence of the translocation, the BCL2 locus on chromosome 18 comes under control of the IgH promoter on chromosome 14, resulting in over-expression of the anti-apoptotic protein BCL2. As opposed to normal germinal center B-cells which are prone to apoptosis due to suppression of BCL2, the clonal B-cells carrying BCL2 escape apoptosis in the germinal center independently of antigenic specificity and survive to circulate for a long time. In FL cells there is ongoing SHM of the variable region of immunoglobuline heavy chain gene (IGVH), and this indicates that FL originates from the germinal center B-cells (Bahler *et al*, 1991;Hardianti *et al*, 2004;Klein & la-Favera, 2008).

Some cases of FL harbor the translocations t(2;18) and t(18;22) in which the BCL2 genes is juxtaposed to the kappa or lamda lighr chain locus respectively. These variants are also

associated with BCL2 over-expression. About 10% of FLs are negative for translocations involving BCL2. These are morphologically indistinguishable from the t(14;18)positive cases. Most of translocation negative cases are BCL2 negative at the protein level, although some cases may be negative due to mutated BCL2 and crippled antibody epitope. Some t(14;18)-negative cases show up-regulation of alternative anti-apoptotic proteins such as Mcl-1 or Bcl-X. Translocations involving BCL6 at 3q27 are more common in t(14;18)-negative cases, resulting in over-expression of the BCL6 oncogene. The t(14;18)-negative cases commonly show higher levels of Ki67 and lower CD10-expression. They have the phenotype of late germinal center B-cells, showing protein expression of IRF4/MUM1, a marker for activated B-cells and plasma cell differentiation (Leich *et al*, 2009). FL grade 3b more commonly harbors a BCL6 rearrangement and lacks the t(14;18), and is today considered a biologically distinct subtype of FL sharing many features with *de novo* DLBCL (Horn *et al*, 2011).

DLBCL corresponds to germinal center B-cells and B-cells that are about to start plasma cell differentiation (also termed activated B-cells). A proportion of diffuse large B-cell lymphoma harbors translocations involving 3q27, the locus for BCL6. Translocations partners are various, frequently involving the Ig loci or other genes that are constitutively expressed in B-cells. The mechanism by which BCL6 promotes lymphomagenesis is not fully understood. It is thought that over-expression of BCL6 convey insensitivity to DNA damage and thus support genomic instability. In addition, high levels of BCL6 may block the differentiation to plasma cells (Basso & la-Favera, 2012).

Burkitt's lymphoma shares features with germinal center B-cells; they are positive for BCL6 and have mutated IGV genes. The hallmark translocation of Burkitt's lymphoma, the t(8;14), is induced by AID and brings the MYC-oncogene on chromosome 8 under control of the Ig heavy chain locus on chromosome 14. Alternatively, the MYC-oncogene is brought under control of the Ig light chains on chromosome 2 or 22. The resulting over-expression of the MYC-protein drives proliferation of the clonal B-lymphocytes (Molyneux *et al*, 2012).

Marginal zone lymphomas of mucosa associated lymphoid tissue (MALT), splenic and nodal type resemble the memory B-cells derived from and homing to extranodal, splenic or nodal tissues. MALT lymphomas are associated with the translocation t(11;18) resulting in the fusion gene API2/MALT1. The protein product of this fusion gene has been shown to mediate constitutive activation of the non-canonical NF-kB pathway (Rosebeck *et al*, 2011).

Mantle cell lymphoma corresponds to CD5+ naïve B-cells. The t(11;14) is present in large proportion of MCLs and results in over expression of the protein cyclin D1 which is a central regulator of cell cycle progression from G2- to M-phase. Variant translocations involving the same oncogene in combination with either one of the light chain loci on chr 2 and 22 for kappa and lambda, respectively, are also seen (Swerdlow SH, 2008).

Plasma cell myeloma corresponds to the bone marrow homing plasma cell. About 40% of multiple myelomas (myelomatosis) harbor translocations involving the immunoglobuling

heavy chain locus at 14q32 and a heterogeneous set of oncogenes as translocation partners. A large proportion of tumors (≈80%) are dependent on NFκB signaling for survival (Swerdlow SH, 2008).

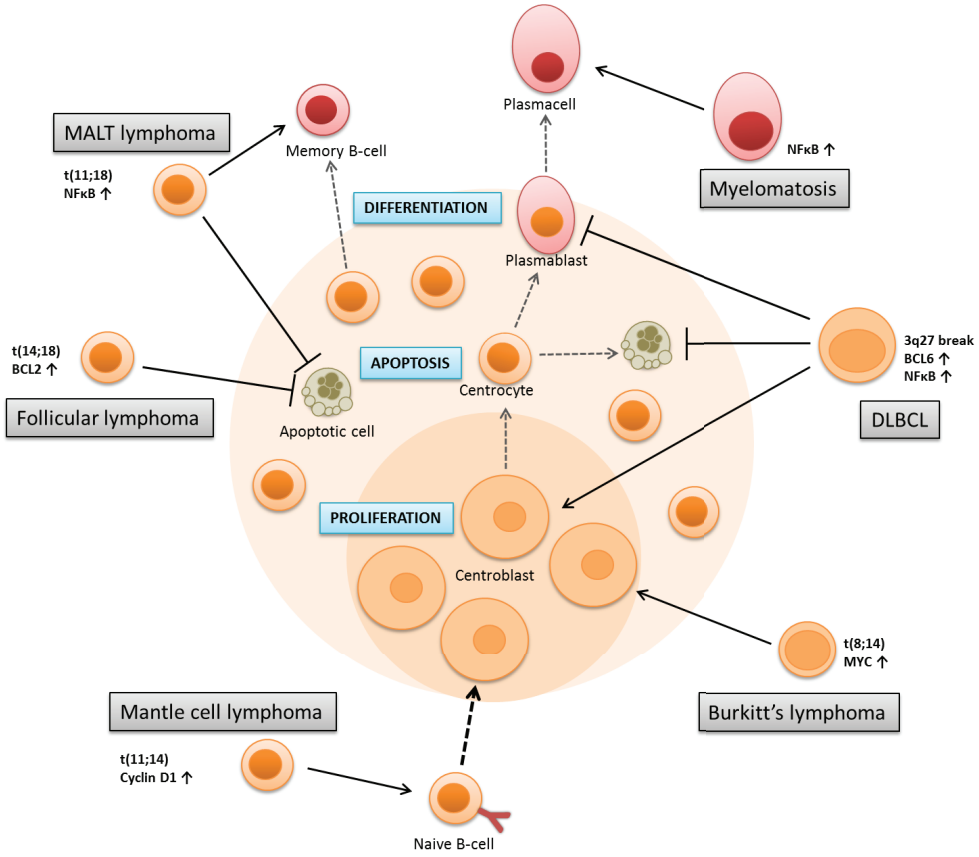

Figure 12. The dark and light orange circles represent the dark and light zones of a germinal center. The cell of origin for each of the B-cell lymphoma subtypes is illustrated and the hallmark genetic lesions contributing to malignant transformation is marked for each subtype.

For some lymphoma entities the “frozen stage hypothesis” cannot be so easily applied. For CLL the cell of origin is poorly defined. Tumor cells in CLL express CD5 and CD23 and two main molecular subgroups have been identified; 50 % bearing mutated and 50% bearing un-mutated Ig variable regions. Mantle zone B-cells, marginal zone B-cells and the human equivalent of B-1 cells have been proposed as the cell of origin. Neither does the neoplastic B-cell of hairy cell leukemia seem to have a normal counterpart. More recently, we have become aware of lineage heterogeneity and lineage plasticity; i.e. observations of FL transformation to histiocytic neoplasms and development of clonally related histocytic neoplasms from lymphoblastic B-cell and T-cell neoplasms challenge the “frozen stage hypothesis” (Jaffe *et al*, 2008). Furthermore, some lymphomas have characteristics of more than one type such as “B-cell lymphoma, unclassifiable, with features intermediate between DLBCL and classical HL”, often referred to as “Grey zone lymphoma”, and “B-cell lymphoma unclassifiable, with features intermediate between DLBCL and Burkitt lymphoma”. The latter is also referred to as “double-hit lymphomas” as they harbor translocations of both BCL2 and MYC (Aukema *et al*, 2011).
The T-cell neoplasms corresponds to innate and adoptive immunity T-cells. NK-cell lymphomas and γδ T-cell lymphomas, arise predominantly in extra-nodal sites. T-cell lymphomas originating from αβ T-cells are mainly of nodal origin. Generally, specific genetic abnormalities associated with T-cell lymphomas are not as well characterized as for the B-cell lymphomas. Most cases of anaplastic large cell lymphomas harbor the translocation t(2;5) or variants and express ALK, while hepatosplenic T-cell lymphoma is associated with an iso-chromosome of arm 7q (Swerdlow SH, 2008).

Secondary oncogenetic aberrations

Although the characteristic chromosomal translocations may provide targeted B-cells with growth advantages, they are rarely sufficient to cause clonal expansion and lymphomagenesis. Mice bearing the BCL2 translocation develop hyperplasia of polyclonal mature B-cells, but fail to develop lymphoma (McDonnell *et al*, 1989). Furthermore, small populations of circulating t(14;18) positive lymphocytes are frequently found in the healthy adult and elderly population (Limpens *et al*, 1991), at a much higher incidence than that of FL (Dolken *et al*, 1996;Rabkin *et al*, 2008) indicating that the translocation itself is not sufficient for lymphomagenesis. Lymphomas commonly harbor secondary genomic aberrations, and recurrent chromosomal gains and losses have been associated with different lymphoma entities, with different phenotypes within entities and with treatment outcome. For instance, Burkitt’s lymphoma commonly exhibits a simple karyotype and loss of chromosome arm 13q is associated with inferior overall survival (Molyneux *et al*, 2012). In contrast, DLBCL and FL often have more

complex karyotypes with several chromosomal gains and losses, reflecting a higher degree of genomic instability.

The mapping of secondary genomic aberrations in FL started in the 1970'ies. Using conventional karyotyping techniques, Yunis et al and others discovered that most FLs are hyperdiploid and profound aneuploidy is rare (Yunis *et al*, 1987). Several of the chromosomal gains and losses were recurrently observed in karyotypes from different tumors. Structural rearrangements in addition to t(14;18) also occur in FL, although these are more heterogeneous. The pattern of recurrent imbalances has later been confirmed and refined in several studies using techniques with higher genomic resolution. The most frequently observed aberrations occur in 10-50% of cases and comprise gains located on 1q, 2p, 6p, 7, 12/12q, 18, 22 and X, while recurrent deletions are located on 1p36, 6q, 9p, 10q and 13q (see for instance (Yunis *et al*, 1987;Horsman *et al*, 2001;Bouska *et al*, 2013). Some of the recurrent chromosomal gains and losses have been associated with patient survival in FL (see for instance (Tilly *et al*, 1994;Viardot *et al*, 2002;Cheung *et al*, 2008;Johnson *et al*, 2008). The aberrations commonly involve large chromosomal segments comprising hundreds or thousands of genes. Thus, target genes and potential drivers of lymphomagenesis in FL have been difficult to identify.

New techniques (SNP arrays) enabled whole genome screening for loss of heterozygosity (also called acquired uniparental disomy; aUPD) in which one of the parental alleles is lost while the other is duplicated resulting in a balanced genomic aberration. In FL, aUPD recurrently targets chromosomal arms 6p, 16p, 12q, 1p36, 10q and 6q, and some of these have been associated with survival and transformation (Fitzgibbon *et al*, 2007;O'Shea *et al*, 2009;Bouska *et al*, 2013).

In lymphomas oncogenic mutations may arise from aberrant SHM targeting genes other than the variable regions of heavy and light chain Ig genes. In a proportion of DLBCLs the pattern of accumulated mutations in the BCL6 gene reveals that the enzyme AID is involved. The aberrant SHM leads to increased expression of the transcription repressor BCL6 which promote lymphomagenesis (Basso & la-Favera, 2012). It has been shown that AID also targets other oncogenes such as MYC, RHOH, PIM1, PAX5, IRF4, BCL7A and BCL2 in DLBCL and other germinal center derived B-cell lymphomas, including FL. Aberrant SHM also contributes to inactivation of tumor-suppressor genes such as CD95 and SOCS1 in FL and other B-cell lymphomas (Pasqualucci *et al*, 2008;Mottok *et al*, 2009;Jiang *et al*, 2012).

Expression of the B-cell receptor (BCR) is a consistent feature of B-cell lymphomas and the last decade of research has produced evidence for a role for BCR-signaling in lymphomagenesis. The mechanisms underlying aberrant BCR-signaling in lymphomas vary (Rickert, 2013). Cases of localized gastric marginal zone lymphoma which are associated with H.pylori infection can be cured by antibiotic eradication of the pathogen, indicating that antigen engagement of the BCR may fuel lymphoma development (Bayerdorffer *et al*, 1995). For CLL there is indirect evidence for stimulation of BCR by an antigen as one third of CLL cases express

stereotypic BCRs that are reactive to self-antigens (Murray *et al*, 2008;Rickert, 2013). BCR-signaling is also involved in the pathogenesis of subsets of DLBCL (Lenz & Staudt, 2010). The role of BCR-signaling in FL poorly understood. This will be discussed below ("Gene dosage effect and potential cancer driver genes in FL").

Results from genome wide gene expression studies support and expand the cell of origin model in non-Hodgkin's lymphomas (Alizadeh *et al*, 2000;Shipp *et al*, 2002;Lossos *et al*, 2004). The lymphoma entities can be distinguished by different gene expression profiles resembling the profiles of normal B-cells at different stages of differentiation (Shaffer *et al*, 2001;Shaffer *et al*, 2006). For instance, FLs show rather homogeneous genome wide expression profiles resembling the profile of germinal center B-cells, while DLBCLs are more heterogeneous and fall into three molecularly distinct subgroups which are undistinguishable in the microscope; the germinal center B-cell like (GCB) subtype, the activated B-cell (ABC) subtype and the primary mediastinal large B-cell lymphoma (PMLBCL) (Alizadeh *et al*, 2000;Wright *et al*, 2003). These molecular subgroups are associated with distinct clinical characteristics and different response to treatment. Whereas the majority of patients with GCB-DLBCL can be cured by R-CHOP-like regimens, 50% of patients with the ABC subtype die of lymphoma (Lenz *et al*, 2008b). The transcriptional program of GCB-DLBCL resembles B-cells at the stage of the germinal center reaction. For instance, there is ongoing SHM and usually expression of switched immunoglobulin classes (most commonly IgG). The GCB-DLBCLs are associated with over-expression of BCL6 and translocations or mutations of the BCL6 gene are frequent (Lenz & Staudt, 2010). The gene expression profile of ABC-DLBCL have similarities with B-cells that are about to leave the germinal center and differentiate into plasma cells. ABC-DLBCLs express plasma cell differentiation markers like XBP1. However, inactivation of PRDM1 by different mechanisms blocks the terminal differentiation into plasma cells (Lenz & Staudt, 2010). The NFκB pathway is constitutively active, and inhibition of the pathway induces apoptosis in ABC-DLBCL derived cell lines (Davis *et al*, 2001). The mechanisms for constitutive NF-kB activation involve activating mutations in mediators of BCR-signaling and TLR-signaling (Lenz *et al*, 2008a;Davis *et al*, 2010;Ngo *et al*, 2011). The GCB- and ABC-subtypes of DLBCL also show different frequencies of primary and secondary chromosomal aberrations. The t(14;18), gain of 2p15-16 and 12 are more frequent in GCB-DLBCL, while ABC-DLBCL have frequent gains of 3, 18q and loss of 6q (Bea *et al*, 2005;Tagawa *et al*, 2005;Chen *et al*, 2006;Lenz *et al*, 2008c). Primary mediastinal large B-cell lymphomas (PMLBCL) have frequent gain of chromosome 9 and share similarities with Hodgkin's lymphoma nodular sclerosis both in terms of molecular features and clinical characteristics (Alizadeh *et al*, 2000;Rosenwald *et al*, 2003).

Although the genome wide gene expression profile of FL reflects its origin from germinal center B-cells, genes encoding cell cycle regulator proteins, transcription factors such as PAX-5 and ID-2 and several proteins involved in microenvironment interaction (cytokines, cytokine receptors and cell adhesion molecules) are differentially expressed in FL and germinal center

derived B-cells (Husson *et al*, 2002;Piccaluga *et al*, 2008). The interaction with cells present in the tumor microenvironment is vital for the malignant B-cells of FL and FL-cells are difficult to culture in vitro. The composition of the FL microenvironment is associated with clinical outcome. A combination of two gene expression signatures reflecting the presence of T-cells and macrophages (immune response 1 and immune response 2) in the tumor, predicted FL survival (Dave *et al*, 2004). Several studies based on immunohistochemical and (phospho-)flow cytometry markers for different immune reported variations in the composition of the FL microenvironment with correlations to survival (see for instance (Farinha *et al*, 2005;Wahlin *et al*, 2007;Canioni *et al*, 2008;Taskinen *et al*, 2008;Farinha *et al*, 2010;Ame-Thomas *et al*, 2012;Myklebust *et al*, 2013). Results are however, inconsistent and the prognostic significance of tumor infiltrating immune cells seems to be treatment dependent (Salles, 2007;de Jong & Fest, 2011;Gribben, 2010).

Recent studies applying modern DNA deep sequencing techniques on FL genomes, detected frequent mutations in genes regulating histone modifications. EZH2 catalyze methylation of the histone 3 (H3K27) and thereby contributes to repressed transcription in loci associated with methylated H3K27. The result is epigenetic silencing of anti-proliferative genes in germinal center B-cells. Activating mutations in EZH2 is present in 20-30 % of FL cases (Morin *et al*, 2010;Velichutina *et al*, 2010;Yap *et al*, 2011;Bödör *et al*, 2013). MLL2 is also frequently targeted by inactivating mutations in FL. This histone methyltransferase with specificity for H3K4 and regulates transcription of developmental genes (Morin *et al*, 2011). Furthermore, the acetyltransferase CREBBP is frequently inactivated by mutations in FL (Green *et al*, 2013). The high frequency of mutations targeting genes involved in histone modifications indicate that epigenetic regulation of gene expression is an early event in FL lymphomagensis.

The thesis papers

A brief summary of the four included papers is given below.

Paper 1

The frequencies and pattern of cytogenetic aberrations occurring secondary to t(14;18)(q32;q21) in a series of 149 non-Hodgkin's B-cell lymphomas were examined. The cases were retrieved from the archives at the Department for Medical Genetics at The Norwegian Radium Hospital. Cases with complete karyotype data based on G-banding and complete clinical information were selected for the analyses. Among the 149 samples 112 showed FL and 37 DLBCL. Five of the FL samples showed FL grade 3b, three of which were obtained at primary diagnosis. Fifteen of the DLBCL samples originated from patients with a previous history of FL

(transformed from FL) and the remaining 22 were from patients with *de novo* DLBCL. Secondary aberrations were observed in 140 of the samples (94%) with a median number of 6 aberrations per sample. Losses from chromosome arms 1p, 6q and trisomy 7 occurred most frequently in all samples. Losses from Xp, 1q, 14q, monosomy 16 and trisomy 12 were more frequent in *de novo* DLBCL and primary FL3b than in FL grade 1-3a, while loss from 6q was more frequent in FL grade 1-3a. This indicates that subsequent to t(14;18)(q32;q21) the clonal evolution follows different paths in FL and *de novo* DLBCL. Loss from 1p was more frequent in FL grade 3a than in grade 1-2 (p=0.049). Comparing FL-samples obtained before and after treatment, inversion of chromosome arm 6p and loss from 6q were more frequent after treatment (p=0.038 and p=0.011, respectively), indicating a selective advantage upon chemotherapy challenge for tumor cell clones bearing these aberrations. Comparing the FL samples (n=107) to the samples showing transformation (including 15 DLBCLs and 3 FL grade 3b from patients with previous FL grade 1-3a), loss from 14q, monosomy 15, trisomy 16 and trisomy 22 were more frequent in the transformed lymphoma samples (p=0.049, p=0.044, p=0.018 and p=0.04, respectively). This indicates that transformation is associated with clonal evolution and the appearance of certain secondary aberrations. The total number of secondary aberrations did not differ significantly in the two groups. No aberrations varied significantly in frequency among FL biopsies originating from patients showing histologic transformation during the observation time (n=33) and those without histologic transformation (n=74). In FL grade 1-3a, the presence of 6 or more aberrations and trisomy 21 correlated with inferior survival (p=0.0051 and p=0.0095, respectively). Gain of chromosome X was of borderline significance for inferior survival (p=0.067).

Paper 2

Forty-seven patients with histologically verified transformation from indolent B-cell lymphoma to DLBCL from five Norwegian centers were included between January 1999 and June 2004. All patients had previously received chemotherapy and the median time from primary diagnosis to inclusion was 41 months (range, 6-212). Only five had received rituximab prior to inclusion. The majority of patients responded to induction chemotherapy and 30 (63%) were treated by HD-ASCT. Eighteen patients (60%) achieved CR, 7 (23%) PR and 5 (10%) had progressive disease following HD-ASCT. Median follow-up time for patients alive was 75 months. PFS rates at 2 and 5 years were 50% (95% CI 0.32-0.68) and 32% (95% CI 0.18-0.46), respectively, while OS rates at 2 and 5 years were 73% (95% CI 0.57-0.89) and 47% (95% CI 0.29-0.65), respectively. In all, 13 patients relapsed after HD-ASCT. Median OS was only 10 months for the 17 patients not eligible for HD-ASCT. However, long term remission and survival was observed in three of these patients following either prolonged treatment with the induction regimen or radiation therapy. In vitro purging of the autologous stem cell graft was performed at one of the study centers. Patients

receiving CD34+ enriched/B-cell depleted grafts (n=10) had inferior PFS and a trend for inferior OS compared to patients receiving non-purged grafts (Log Rank 0.025 and 0.151, respectively). Patients with poor mobilization of stem cells to peripheral blood had inferior progression free survival compared to those with uncomplicated leukapheresis (p=0.025). Patients who had received 2 or more chemotherapy courses prior to inclusion had inferior PFS and OS (p=0.015 and p=0.022, respectively). In conclusion, the recommendation of HD-ASCT for patients with chemo-sensitive transformed lymphoma is reinforced by the study. However, the effect of HD-ASCT needs to be assessed in patients who have received rituximab for indolent lymphoma prior to transformation. The study confirms that ex vivo purging of the autologous stem cell graft by current methods is not beneficial for patient survival.

Paper 3

The aim of the study was to examine the genetics of clonal evolution in FL and to identify genetic alterations associated with disease progression. DNA copy number alterations (CNAs) were assessed by arrayCGH in 100 biopsies from 44 patients diagnosed with t(14;18)-positive FL. In addition, the patterns of somatic hypermutations in the IgVH-loci were analyzed in samples from 20 of the patients. Complete and long-term clinical information was available for all patients. Nineteen patients developed histologically verified transformation to DLBCL. Eight additional patients showed clinical criteria for higher grade transformation as defined by Al-Thourah et al (2008). The remaining 17 patients showed no signs of transformation.

In initial biopsies, the most frequently recurring regions of chromosomal gain were Xp11q21 (57%), 1q22 (50%) and 18p11q21 (41%), while chromosomal loss occurred most frequently in 6q23q25 (27%), 1p36 (23%) and 13q34 (18%). A different pattern of recurring chromosomal imbalances were observed in initial biopsies from the cases with and without subsequent transformation; gains of chromosome 5, chromosome arms 2p and 3q were exclusively observed in the initial biopsies of FL from patients with transformation. In addition, gains of 12q13 and chromosome 21 were more frequent in FL-biopsies from cases with subsequent transformation (25% vs 13% and 25% vs 6%, respectively). Gain of X in male patients was the strongest predictor of inferior survival, gain of 2p, 3q, 5p, 5q and 12q were also associated with inferior survival. Thus, regions of gain associated with transformation and inferior survival showed considerable overlap.

In 27 of the 39 cases with two or more biopsies, one or more of CNAs present in initial biopsies were absent in later relapse biopsies and in 24 of these new CNAs also appeared. This alteration in CNA profiles with time may reflect a parallel evolution of tumor cell clones. In 9 cases, only the appearance of new CNAs was observed in relapse samples. In 3 cases the CNA profiles were without changes. In the 12 latter cases, the addition of new CNAs or unchanged CNA profiles may reflect a linear evolutionary path. Genealogical trees generated from the

patterns of SHM in IgVH-loci confirmed the frequent occurrence of parallel clonal evolution in the successive FL-biopsies.

The level of CNAs as measured by the CNA index, showed a trend for increasing values in the last biopsies compared to the initial biopsies. However, the CNA index showed considerable variation with time at the individual level, also consistent with a parallel evolution of tumor cell clones.

In conclusion, this study confirms the extensive intraclonal heterogeneity in FL cells and demonstrates that the evolutionary path of tumor cell clones is traceable at the whole genome level. Parallel evolution of tumor cell clones is common and indicates the existence of a FL precursor cell that may survive repeated treatment and give rise to clonally related, but genetically different relapse clone. This hypothesis is supported by the observation that the degree of genomic instability (as measured by the CNA index) varies considerably in successive FL-biopsies.

Paper 4

The study is based on the same sample material as in Paper 3, consisting of 100 sequential fresh frozen biopsies from 44 patients diagnosed with follicular lymphoma positive for the translocation t(14;18). In addition to the whole-genome copy number data described above, we obtained gene expression profiles from 81 of the samples originating from 41 patients. Analysis of the correlation between gene expression and DNA copy number indicated that a large number of genes are subject to gene dosage effect in FL. Genes with Pearson correlation between copy number and expression exceeding 0.4 and differential expression ($p<0.05$) in samples with gain (or in samples with loss) compared to samples with normal copy number were defined as cis-associated genes and selected for further analyses. By these stringent selection criteria, we identified 698 cis-associated genes. Interestingly, 23% of the cis-associated genes reside on chromosome X and the gain of X is associated with inferior survival in FL. An independent set of 191 FL samples with gene expression data and corresponding conventional CGH data from 131 samples was used to validate our findings. The 698 cis-genes also showed high positive correlations between copy number and gene expression in the independent set of 131 FL samples. Pathway analyses revealed that the molecular network most enriched for these cis-associated genes included 14 mediators and regulators in the NF-κB signaling pathway. Importantly, 12 of these 14 genes also showed cis-association between copy number and gene expression in the independent data set. For each of these 14 NF-κB linked cis-genes, the correlated NF-κB target genes were identified. This resulted in 14 different signatures of associated NF-κB target genes and corresponding gene expression scores were defined by averaging the gene expression in each signature. The correlations between NF-κB linked cis-genes and target genes were positively and significantly associated in our data set and the

independent set of 131 FL samples. The corresponding signature scores showed similar distribution patterns in the two data sets. For six of the NF-κB linked cis-genes (*BTK, IGBP1, IRAK1, ROCK1, TMED7-TICAM2 and TRIM37*) the corresponding target gene scores were significantly associated with transformation. The strongest predictors for transformation were the *IRAK1*- and *TRIM37* associated signature scores, high scores being associated with transformation. In particular, the predictive role of *IRAK1*, *BTK* and *IGBP1* associated scores, suggests involvement of TLR- and BCR-signaling in transformation of FL.

Discussion of material and methods

Material in Paper 1; selection of tumor samples and patients

The study presented in Paper 1 was a retrospective analysis designed to characterize secondary chromosomal aberrations occurring in lymphomas carrying the translocation t(14;18)(q32;q21).

Among the 149 included samples, 112 showed FL and 37 DLBCL. Five of the FL samples showed FL grade 3b, three of which were obtained at primary diagnosis. Among the samples showing FL grade 1-3a, 47 were obtained at primary diagnosis. Fifteen of the DLBCL samples originated from patients with a previous history of FL (transformed from FL) and the remaining 22 were from patients with *de novo* DLBCL. These numbers of DLBCL samples deviate from those given in Paper 1, as two FL 3b samples were lumped together with the transformed DLBCLs and three with the *de novo* DLBCLs (Paper 1 page 457). In retrospect, the grouping of transformed DLBCL and *de novo* DLBCL together (also with FL3b) in some of the analyses for subtype comparisons and clinical associations is not justified, as these are biological distinct entities and have different clinical courses. It also seems more correct to leave the FL grade 3b out of the analyses.

As karyotyping requires the preparation of cell suspensions from fresh tumor tissue and culturing of tumor cells in vitro, not all specimens obtained for diagnostic purposes could be karyotyped. A bias for larger samples obtained from peripheral nodes is likely. In addition, especially FL may be difficult to culture in vitro and a bias for examining tumor cell clones that are less dependent on the microenvironment of the lymph node may be introduced.

Clinical information was retrieved from the lymphoma data base at our institution in which data about all referred lymphoma patients have been collected prospectively since 1980. Our center is a regional referral center for lymphomas and has been the largest in the Nordic countries for a long time. Thus, the clinical database contains data from a relatively unselected patient population. Although many of the patients referred to our center are included in clinical studies, selected cases for this study were subject to different treatment regimens. Furthermore, the trends in lymphoma treatment changed during the 1990s (for instance less use of chlorambucile in FL and advent of rituximab for both DLBCL and FL).

The number of included *de novo* DLBCL is somewhat lower than expected. According to our clinical database more than 700 patients have been treated for DLBCL since 1980, and 20-30% of these are expected to harbor the translocation t(14;18). As diagnostic samples often are needle biopsies of retroperitoneal masses it may not have been sufficient material for karyotyping in many of the DLBCL-cases. The few *de novo* DLBCL cases hamper robust clinical correlations in this group.

A relatively high number of FLs were included and the analyses of these confirmed previous findings such as the distribution of FLIPI-score, the impact of FLIPI-score on survival, the inferior survival in FL-patients with subsequent transformation, the association between inferior survival and a high total number of chromosomal aberrations, as well as the association between inferior survival and gain of X. In addition, we observed that gain of 21 is associated with inferior survival in FL.

As follicular lymphoma comprises the largest group of cases in Paper 1 and is the main focus in Paper 2-4, the following discussion of results from Paper 1 will focus this lymphoma entity.

Material in Paper 2; patient inclusion

The study presented in Paper 2 was designed as a prospective national multicenter study to make the patient sample as representative as possible for patients younger than 65 years. According to national guidelines all patients that are diagnosed with transformed FL should be referred to one of the four University Hospitals with facilities for ASCT. All four centers performing ASCT in Norway participated in the study. In clinical practice today, the upper age limit for HD-ASCT is not as absolute and patients aged above 65 may also be offered HD-ASCT if they are in good condition with minimal co-morbidity. As FL with transformation is a relatively uncommon disease it was not possible to perform a randomized study including patients in a control group. Instead, data were compared to published historical data. Patients were included at start of salvage chemotherapy for transformed FL so that the prognosis for the whole cohort of patients was available. In addition, this allowed unbiased comparison of outcome for those who were eligible for HD-ASCT and those who were not.

The study was planned to include 100 patients. Assuming a 70% response rate to salvage chemotherapy, 100 patients will give a 95% confidence interval (CI) of +/- 9%. If the 70 patients completing HD-ASCT have a three year OS of 50% and PFS of 30%, the CI will be +/- 12% and 11%, respectively. Due to the introduction of rituximab in the salvage treatment of patients with transformed B-cell non-Hodgkins lymphomas, the study was closed before this number was reached. The early closing results in wider 95% confidence intervals than expected for the estimated survival rates at 2 and 5 years, thus the conclusions are less robust than they would have been with more patients.

Originally, a total of 68 patients were included between January 1999 and July 2004 based on the diagnosis of either DLBCL or FL grade 3 at study entry. They all received induction chemotherapy with the intention to test chemosensitivity prior to HD-ASCT. Forty-four of the 68 went on to HD-ASCT. Data from 47 of the 68 patients were analyzed and reported in Paper 2. Four cases were excluded from the final analyses due to altered diagnosis from DLBCL at study entry to diffuse FL at central review. The remainder of excluded patients had the diagnosis FL grade 3 at study entry or at central review.

The clinical relevance of FL grading has been debated since the 1980ies. The present system for grading as defined in the latest WHO classification, had been linked to patient outcome in a number of studies prior to 1999 (Martin *et al*, 1995) showing inferior outcome for patients presenting with FL grade 3 at diagnosis compared to patients with FL grade 1-2. On the basis of these studies many centers, including ours, treated patients with FL grade 3 with intensified chemotherapy regimens more like the treatment for DLBCL. Progression from FL grade 1-2 in a primary biopsy to grade 3 at relapse was by many clinicians considered as higher grade transformation and treated thereafter.

During the inclusion time of our study data supporting a subdivision between FL grade 3a and 3 b was published, as discussed in the above section "Treatment of transformed lymphoma".

In conclusion, the 47 patients included in the analyses presented in Paper 2 are likely to be highly representative for the population of patients with histologic transformation younger than 65 years treated in the pre-rituximab era.

Material in Paper 3 and 4; selection of samples and patients

The studies presented in Paper 3 and 4 were retrospective analyses designed to detect genetic alterations occurring in the relapse of FL and correlate these to clinical outcome. Along with the detailed and long-term clinical follow-up data, we believe that this tumor sample material is unique in representing FL in a longitudinal perspective and well suited for addressing the molecular dynamics of this disease.

For 100 biopsies collected between 1987 and 2005 originating from 44 patients there was sufficient material for DNA extraction and arrayCGH analyses (Paper 3). For 81 of these biopsies originating from 41 of the same patients, there was also sufficient material for mRNA extraction and gene expression analyses (Paper 4).

Type of data	Number of biopsies per patient				Total number of patients
	1	2	3	4	
DNA copy number	5	24	13	2	44
Gene expression	8	27	5	1	41

Table 3. Data sets used in Paper 3 and 4. The four middle columns show how many of the patients that were represented by DNA copy number data and gene expression data from 1, 2, 3 and 4 biopsies.

Karyotypic data were available from 89 of the biopsies and showed the presence of t(14;18)(q32;q21) in at least one biopsy from each of the 44 patients. The karyotypes of the first biopsy from 40 of the patients were included in the study presented in Paper 1.

In theory, the selection of serial biopsies of FL may introduce a bias for patients with prolonged survival. Patients with transformation were, however, well represented with their respective FL-biopses in our material, even if few biopsies showing transformation to DLBCL were included. Furthermore, we have practiced liberal indications for biopsy at progression or relapse of FL at our institution. The detailed clinical information retrieved from the lymphoma database and from individual patient files was of great value in this study and the distribution of clinical parameters indicate that the included patients are representative for the population of FL patients diagnosed in the pre-rituximab era. We therefore believe that in practice this bias is minor.

The patients were treated according to national guidelines. The treatment of FL patients is based on individual evaluation and especially for relapses the patients had received heterogeneous treatment. For instance, 14 patients received HD-ASCT; nine due to transformation to DLBCL and 5 due to progression to FL grade 3a (which was treated more aggressively than FL grade 1-2 prior to 2005). In all, 23 patients received rituximab during the observation time; 3 at initial treatment and 20 at relapse. The proportion of patients receiving rituximab was equal among patients with and without transformation (≈50 % in both groups). There was no difference in overall survival between patients receiving and not receiving rituximab. Theoretically, differential treatment may influence the clonal selection and molecular features of the tumors and may thus complicate interpretation of clinical correlations. The vast majority of FL patients today receive rituximab both in first line treatment and as part of the treatment at relapse. Prognostic markers identified in patients in pre-rituximab era may not be

directly applicable to patients today and the findings in our studies need to be validated in new patient cohorts.

In the planning of this study, we decided to focus on the genetics in relapsing FL and archived biopsies showing transformation to DLBCL were omitted, except for 9 cases where the histology had been ambiguous prior to the review performed for this study. In retrospect, it becomes clear that inclusion of as many transformed biopsies as possible would have been of value to obtain a deeper insight to the clonal diversity in FL as well as the biology of transformation. This will be addressed in following studies.

Choice of method for quantification of DNA copy number (Paper 3 and 4)

Karyotyping by chromosome banding was introduced by the Swedish geneticist Torbjörn Caspersson and his colleagues in 1970. In human cytogenetics G-banding is a commonly used technique in which metaphase chromosomes are stained with Giemsa. This results in transverse dark bands in heterochromatic and A-T-rich regions and light bands in the G-C-rich euchromatic regions. Each chromosome can then be identified by a unique pattern of transverse bands. G-banding was used to obtain the cytogenetic data presented in Paper 1. The method allows detection of both chromosomal imbalances and structural aberrations at a resolution of 5-8 Mb. The method is time-consuming and the preparation and accurate interpretation of the G-banded metaphase spreads requires long experience. In tumor genomes with complex karyotypes the exact chromosomal origin of displaced genomic material can be difficult to determine. Multicolor-FISH may then be useful to examine more closely selected cases and was applied for a subset of samples in Paper 1.

Array comparative genomic hybridization (arrayCGH) is a powerful tool for simultaneous quantification of DNA copy number at thousands (or even millions) of genomic loci. Several microarray technologies are available (see below). Among other things, the source of the probes, the distribution and density of probes per gene or chromosome differs substantially between platforms. Some major technologies for high throughput copy number assessment are:

- **BAC/PAC arrays:** The probes are DNA constructs cloned in E.coli by the use of bacterial plasmids; F-plasmid and P1-plasmid for the cloning of Bacterial Artificial Chromosomes (BAC) and P1-derived Artificial Chromosomes (PAC), respectively. The probes are spotted in grids onto glass slides which serve as the platform for competitive hybridization of differently labeled sample DNA and reference DNA (Figure 12). The probes represent the human genome at variable intervals; the early arrays contained ≈3500 probes with the average length of 150-350 kb at intervals of approximately 1 Mb along the human genome. In modern arrays≈32000 probes are placed in a tiling pa th along the interrogated genome enabling the detection of smaller genomic lesions (theoretically down to the size of 50k) (Solinas-Toldo *et al*, 1997;Pinkel *et al*,

1998;Snijders *et al*, 2001;Fiegler *et al*, 2003;Ishkanian *et al*, 2004). The BAC/PAC arrays returns the average DNA copy number from the cells in the sample relative to a normal reference. Balanced chromosomal rearrangements are not detected. In chromosomal CGH, a spread of normal human metaphase chromosomes is used as the platform for hybridization instead of BAC clones printed on glass. This method preceded the development of array based technologies.

- **cDNA arrays:** The probes are complementary DNA synthesized from mature mRNA by the use of the enzyme reverse transcriptase. Thus, cDNA probes represent the coding regions of the human genome and the arrays are suited for gene expression profiling. However, they have also been widely used to interrogate DNA copy number; see for instance (Pollack *et al*, 2002).

- **Oligo arrays:** The probes are long (60 mer) or short (25 mer) oligonucleotides; see for instance (Bignell *et al*, 2004), which are synthesized in situ on the array platform (which may be either a chip or beads). The arrays may contain millions of probes representing both coding and non-coding regions of the human genome, including SNPs (single nucleotide polymorphisms) and CNPs (copy number polymorphisms).

- **SNP arrays:** Neither of the platforms above allows detection of balanced genomic rearrangements, loss of heterocygozity/acquired uniparental disomy (aUPD) or the determination of ploidy or tumor cell percentage. SNP arrays are a special type of oligo arrays that focus on SNP loci, or a combination of SNP loci and other loci. For each SNP locus, both alleles are measured, thus generating allele specific copy number data. This allows the estimation of tumor cell percentage, ploidy and identification of aUPD (Van *et al*, 2010). Such arrays were originally designed for SNP genotyping of germline DNA, but are currently widely used to examine somatically acquired genomic lesions.

In this thesis (Paper 3 and 4), in-house BAC/PAC arrays were used to obtain DNA copy number data from the serial biopsies of FL. The arrays were produced in a core facility at our institution (Meza-Zepeda *et al*, 2006) and contained ≈4500 probes printed in quadruplicate representing the human genome at intervals of approximately 1 Mb. The tumor DNA and a commercially available sex matched reference DNA were labeled with Cy3 and Cy5, respectively, and co-hybridized to the slides. Following scanning of the slides, the images were segmented, raw data was filtered and log2-ratios were calculated using GenePix 6.0 Simple.

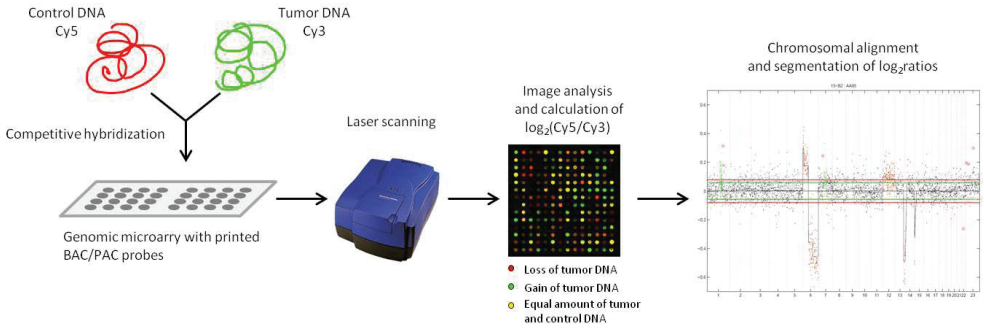

Figure 13. Array comparative genomic hybridization (arrayCGH) workflow. ArrayCGH exploits the intrinsic property of DNA to denature to single strands upon heating which hybridize to restore a double stranded helix upon cooling. Tumor DNA and a sex matched reference DNA are labeled with fluorescent Cy3 and Cy5, respectively. In cycles of heating and cooling the sample and reference DNA compete to hybridize with the complementary DNA of the BAC and PAC probes on the glass slide. After washing the slides are scanned and the relative fluorescent intensities are analyzed.

Gene-expression analysis (Paper 4)

The Affymetrix HG U133 Plus 2.0 Gene Chip® was applied to obtain gene expression profiles for 81 of the serial biopsies. The array contains short oligonucleotide probes (25-mer), synthesized *in situ* on the array, representing more than 47 000 gene transcripts, including > 38 000 well-characterized genes. On the array there is also a set of control probes representing sequences for quality control assessment (hybridization control, poly-A controls, normalization controls and housekeeping genes). Each sample is labeled individually and hybridized to a single array. The Gene Chip® is a single-color array and the estimated gene expression level of each transcript is reported as a single fluorescent intensity (not a ratio) (Quackenbush, 2006).

To map the expression probes to the respective genomic position we applied the annotation file (HG-U133_Plus_2.na27.annot.csv) supplied by Affymetrix and selected the probes with unique Entrez ID and an exact genomic location. The original set of 51k expression probes was thus reduced to 21k.

Choice of statistical methods (Paper 3 and 4)

The Cy3/Cy5-ratios (Figure 12) from arrayCGH are usually first log2-transformed and normalized and then segmented in order to determine the genomic loci where the copy number is altered. In this thesis, simple mean local normalization was applied to the log2-transformed arrayCGH measurements. Each subgrid of the array is then normalized separately. The intensity ratio of each probe spot is multiplied with a factor equal to the ratio of the mean dye intensities in all spots in the respective subgrid (Beheshti *et al*, 2003).

Several methods for copy number segmentation exist, including circular binary segmentation (Venkatraman & Olshen, 2007), aCGHsmooth (Picard *et al*, 2005) and piecewise constant fitting (PCF) (Nilsen *et al*, 2012). In this thesis, PCF was used for all segmentations. This method fits a piecewise constant function to the copy number profile by minimizing a penalized least squares criterion of the form $C = E + \gamma P$, where E is the goodness of fit to the data (represented by a sum of squared residuals) and P is the number of segments in a solution. The parameter $\gamma>0$ determines the trade-off between the two terms, and was set low in our analyses to provide a sensitive detection of breakpoints. PCF is implemented in the Bioconductor package *copynumber* and offers various options, including an algorithm for segmentation of a single array at a time (pcf) and an algorithm for simultaneous segmentation of several arrays (multipcf), thus resulting in a common set of breakpoints across the arrays. Both algorithms were used in this thesis.

The result of the copy number segmentation is a set of break points, or equivalently a division of the genome into a number of segments, each with constant copy number. For each segment, the estimated copy number level is the average log2Ratio value for all probes on that segment. To call copy number aberrations, a threshold $\theta>0$ is defined. All genomic loci with estimated copy number level below $-\theta$ are called as losses, while all loci with estimated copy number level above θ are called as gains. The parameter θ controls the trade-off between sensitivity and specificity of the copy number calling. As θ increases, fewer segments are called as aberrant. This results in a decrease in sensitivity (fewer real aberrations are called) and an increase in specificity (fewer false aberrations are called). In practice, the appropriate selection of θ depends on the platform used and the array quality. Furthermore, it depends on what biological features in the sample one wants to detect. In particular, in a sample consisting of a mixture of cells with different genetic constitution the signal from each cell subpopulation will be smaller. As previously discussed, the relative proportion of tumor cells versus cells with normal copy number differs between FL biopsies. To capture copy number aberrations also in samples with low tumor cell percentage, the threshold θ was set to relatively high sensitivity in Paper 3 and 4. Comparison of copy number calls in sequential biopsies with variable tumor cell percentage from the same individual confirmed that the chosen threshold was adequate (data not shown).

To measure the total extent of copy number alterations in a sample, we defined a score (CNA-index) that counts the number of aberrant probes, weighing each by the magnitude of aberration. This was used as a marker for genomic instability. The presence of balanced genomic aberrations other than t(14;18) and aUPDs also occur in FL and reflect genomic instability. These are not detected by the arrayCGH, and are therefore not counted for by the CNA-index. The CNA-index may be influenced by the number of tumor cells relative to the normal cells in the sample material. There may be a bias for higher CNA-index in samples with high tumor cell content relative to infiltrating normal cells, although this was tested and found to be minimal and we believe the CNA-index correlates well with genomic instability.

To correlate DNA copy number data derived from the BAC/PAC arrays containing 3091 probes and the gene expression data from 21k annotated genes, the PCF-values were interpolated so that each expression probe was matched to a corresponding PCF-value. Thousands of the genes represented on the gene expression array lie in genomic regions that are not covered by a probe on the arrayCGH. PCF estimates DNA copy number from several adjacent BAC probes and thus, provide robust copy number estimates for all genes within an aberrant (or normal) genomic segment. Genomic breakpoints were assumed to be placed in the middle between the flanking BAC probes and PCF segments were interpolated. Thus, for genes located close to genomic breakpoints, the estimate for copy number may be inaccurate.

To examine the presence of known oncogenes and tumor suppressor genes among the 698 cis-genes we performed pathway analyses in Ingenuity Systems Pathway Analysis tool (IPA). This is one of several tools available for pathway analysis and is based on a statistical gene set enrichment analysis. Gene set enrichment analyses have increased power to detect effects on gene set level and facilitates the interpretation of results from high-throughput analyses of gene expression. In such analyses, the genes of interest are compared to predefined sets of genes to investigate functional relationships. The predefined gene sets can be molecular interaction networks or biological functional categories of genes. The IPA tool is based on a manually curated knowledge database (Ingenuity Knowledge Database®).

Comparisons of survival in different groups of patients were carried out using Kaplan-Meier curves and log-rank tests. Differences in CNA-indexes were tested by variance component models. The impact of chromosomal gains or losses on survival was assessed by Cox's proportional hazards regression. For each biopsy, one or more copy number features are observed and related to survival. In this thesis, multipcf was used to define these features, and univariate Cox regression was then used to assess the association between survival and each feature individually. The association between gene-expression scores as defined in Paper 4 and transformation was assessed by Student's t-test and logistic regression.

General discussion of results and future perspectives

Treatment of transformed FL

Higher grade transformation is associated with a dramatic reduction in overall survival times, as shown in Paper 1 and 3. In the Canadian population based study by Al-Tourah et al (Al-Tourah *et al*, 2008) the 10 year survival rates for patients with and without transformation rates were 36% and 75 %, respectively, which is similar to our findings. In the same retrospective study the median survival following transformation was only 1.7 years. The majority of patients included in Paper 1, 3 and 4 was treated in the pre-rituximab era and received CHOP-like regimens. The poor outcome of transformed FL following treatment by standard chemotherapy regimens motivated the study presented in Paper 2. The effect of HD-ASCT for patients with transformation from indolent B-cell lymphoma to DLBCL was assessed in a multicenter prospective study.

A high response rate to induction chemotherapy was achieved and 63% of included patients were treated by HD-ASCT. The response rate to HD-ASCT exceeded 80% (CR and PR). PFS rates at 2 and 5 years were 50% (95% CI 0.32-0.68) and 32% (95% CI 0.18-0.46), respectively, while OS rates at 2 and 5 years were 73% (95% CI 0.57-0.89) and 47% (95% CI 0.29-0.65), respectively. Prior to 1999, the year of the opening of this clinical study, a few limited sized retrospective studies had indicated that a subgroup of patients with chemo-sensitive disease obtains long-term survival following HD-ASCT for transformed indolent B-cell lymphoma. The prospective phase II trial presented in Paper 2 confirmed the benefit of HD-ASCT in the group of patients with chemo-sensitive disease. In particular, we showed that many of the patients that obtained partial remission from induction treatment were brought into complete remission by HD-ASCT. The possible benefit of HD-ASCT is assessed with comparison to historical data (Yuen *et al*, 1995;Bastion *et al*, 1997;Gine *et al*, 2006;Montoto *et al*, 2007;Al-Tourah *et al*, 2008). Ideally, a randomized study with conventional treatment as the standard would give a more definite answer, but has not been performed due to the rarity of the condition.

The study was initiated and conducted before rituximab became part of the standard treatment for follicular lymphoma. Only five patients had received rituximab prior to study entry and Rituximab was not a part of the induction regimen. Four patients received one course of Rituximab before stem cell harvesting for in vivo purging of the stem cell graft. Interestingly, these four patients were among those with the longest survival following HD-ASCT, indicating that addition of rituximab in salvage regimens improves outcome of HD-ASCT in rituximab naïve patients. In a recent study by the Canadian Blood and Marrow Transplant group the 5-year PFS and OS were 55% and 65 %, respectively (Villa *et al*, 2013). In this study all patients received rituximab as part of the induction treatment prior to HD-ASCT, which may explain the

superiority of their results. The effect of rituximab is difficult to evaluate however, as the number of patients receiving rituximab for FL prior to transformation was not reported. In a recent study from Holland, the patients who had been treated with rituximab previous to transformation and HD-ASCT had inferior survival compared to patients that were rituximab-naïve at transformation and HD-ASCT (Ban-Hoefen *et al*, 2011).

There was no early treatment-related mortality and no patients developed secondary myelodysplastic syndrome. The study confirmed the safety of HD-ASCT for patients with transformed follicular lymphoma younger than 65 years. The procedure related mortality rate has dropped substantially from the mid 1980'ies (10-15%) till today (approximately 1%). This drop is most probably due to **(i)** a shorter cytopenia duration after introduction of blood derived rather than bone marrow derived haematopoietic stem cell support, **(ii)** better patient selection and **(iii)** improved supportive care during aplasia. In an unpublished series of all 711 Norwegian lymphoma patients undergoing HD-ASCT since 1987 until 2008, 10 year overall survival is 55%, ranging from 45% to 75% for the various entities. For many of the entities, there are few deaths beyond 5 years after HD-ASCT, indicating that a high percentage of these patients are cured from their disease.

The survival of patients with indolent B-cell lymphoma has improved substantially during recent years. Data from our institution clearly demonstrates this positive trend (Figure 8) which is in line with published data from international centers (Fisher *et al*, 2005;Swenson *et al*, 2005;Hiddemann *et al*, 2007;Hiddemann *et al*, 2005). The improved progression free and overall survival is largely due to the introduction of rituximab in the treatment of B-cell lymphomas. Rituximab has been used in combination with chemotherapy for more than 10 years in Norway. It is still not approved by the Medical Agencies in Norway as monotherapy in first line, although some studies have shown good responses and long-lasting remissions in patients with mild symptoms and low tumor burden (Colombat *et al*, 2012). First line rituximab monotherapy is currently being compared to novel combination regimens in recent and ongoing clinical trials, i.e. Nordic lymphoma group (NLG) trials M39065 (ref), ML16865: rituximab versus rituximab + interferon (ClinicalTrials.gov Identifier: NCT01609010); SAKK/NLG: rituximab versus rituximab + lenalidomide (ClinicalTrials.gov Identifier: NCT01145495).

Whether or not HD-ASCT remains a good treatment approach for patients with transformed indolent B-cell lymphoma in the rituximab era needs to be addressed. We have recently performed a retrospective study investigating the outcome for patients with transformed indolent B-cell lymphoma in the present treatment era, including all patients referred to our institution with this diagnosis between 2005 and 2011 (Blaker YN et al, accepted in Leukemia and Lymphoma). A total of 42 patients aged 18-65 were considered for HD-ASCT in this period, all received induction chemotherapy including rituximab and 28 completed HD-ASCT. The patients that were rituximab-naïve prior to study entry had significantly better OS after HD-ASCT than the patients who had already received rituximab (n=11 versus n=17,

p=0.039 in Log rank test), in line with results from a recently published smaller study by Ban-Hoefen et al, 2012. The overall survival following HD-ASCT in our recent patient cohort was similar to the OS observed in Paper 2, indicating that HD-ASCT remains an efficient and safe treatment option for these patients. Whether or not prior rituximab treatment contributes to poorer induction response needs to be addressed in larger patient cohorts. Does previous treatment with ritiximab impact chemo-sensitivity as it does in relapsed *de novo* DLBCL (Gisselbrecht *et al*, 2010)? Novel approaches to achieve higher induction response rates in rituximab treated patients may improve outcome following HD-ASCT; for instance the replacement of rituximab with ofatumumab (Czuczman *et al*, 2012) the addition of lenalidomide (Czuczman *et al*, 2011), radioimmunotherapy (Gopal *et al*, 2007) or inhibitors of Bcl-2, PI3-kinase and BTK (Advani *et al*, 2013;Sawas *et al*, 2011;Young & Staudt, 2013).

Secondary chromosomal aberrations in FL and association to survival and transformation

The tumor genome of FL is commonly hyperdiploid with low level gains recurrently involving 1q, 2p, 5q, 6p, 7, 8q24, 12q, 17q, 18, 21 and X and recurrent losses involving 1p, 6q and 13q. The pattern of recurrent gains and losses as identified by arrayCGH in initial FL samples (Paper 3) and by karyotypic data (Paper 1) were in line with the previous studies (Yunis *et al*, 1987;Hoglund *et al*, 2004;d'Amore *et al*, 2008;Horsman *et al*, 2001;Leich *et al*, 2009).

Recent studies using high resolution SNP arrays have confirmed the frequency pattern of copy number alterations in FL (Bouska *et al*, 2013). This study also identified small aberrations at the peaks of the larger commonly aberrant regions and these small aberrations pinpoint a few candidate target genes. Studies using SNP array also enables detection of acquired uniparental disomy (aUPD) which commonly involve 1p36, 6p21, 12q21-q24 and 16p13 (Fitzgibbon *et al*, 2007;Ross *et al*, 2007;O'Shea *et al*, 2009;Cheung *et al*, 2010;Bouska *et al*, 2013). The aUPDs of 6p21, 12q and 16p13 overlap with genomic regions that are commonly targeted by copy number gain, while 1p36 is frequently targeted by loss in FL, suggesting a selection in favor of a particular genotype in these regions. In the study by the Canadian group from 2012 (Cheung *et al*, 2012), aUPDs in these four regions were frequently observed in cases of t(14;18) positive FLs with a simple karyotype (harboring none or only one secondary aberration detected by karyotyping). This suggests that aUPDs are early genomic events in the pathogenesis of FL.

In Paper 1 and 3 we assessed the association between secondary genomic aberrations and overall survival. Although the patient materials in the two studies partly overlap, the results are somewhat different. Possible explanations for this are (i) more patients with FL were included in Paper 1 than in Paper 3 (107 vs 44), (ii) copy number alterations were detected at a higher genomic resolution in Paper 3 than in Paper 1 (arrayCGH versus karyotyping) and (iii) the definition of transformation differs in the two studies; while only histological transformation is

considered in Paper 1, cases with clinical signs of transformation and histological transformation are grouped together in Paper 2.

In Paper 3 gain of X in male patients was identified as the strongest predictor of inferior outcome. More specifically, high amplitude (pcf value) of the most commonly gained segment of X (chromosomal arm Xp) was associated with increased risk of death. In Paper 1 the association between an extra copy of X and inferior survival was only borderline significant. A higher frequency of gain of X was found in Paper 3 than in Paper 1 and this may be due to the more sensitive detection copy number aberrations by arrayCGH compared to karyotyping. The prognostic significance of gain of X has previously been shown in a study by Johnson et al 2008 (Johnson *et al*, 2008). In addition, gain of X was found to be associated with poor outcome in a study by Høglund et al 2004, but only in the univariate analysis. Viardot et al 2002 found a trend for poorer survival in patients with gain of Xq (Viardot *et al*, 2002). Many studies of genomic alterations in FL do not report clinical data and some of the CHG and SNP array studies do not report data on chromosome X (Cheung *et al*, 2008;Schwaenen *et al*, 2009;O'Shea *et al*, 2009). One reason for the latter may be the need for sex matched reference DNA to identify CNAs involving the sex chromosomes. Another challenge especially with chromosomal CGH is the relatively large proportion of heterochromatine on chromosome X, which may cause unspecific hybridization. Even when using reliable probe sets specific for chromosome X and a sex matched reference, the copy number values of chromosome X are sex biased, as gain of one copy results in a relatively higher LogRatio in male samples than in female samples. Whether the tumor biological consequence of gain of X differs in female and male patients remains unknown. Interestingly, in a study examining the methylation status of chromosome X, it was shown that the extra copy of chromosome X is active in male tumor samples while it can be either active or inactive in female samples (McDonald *et al*, 2000). We believe that our findings related to the gain of X are reliable and of importance for the deeper understanding of FL pathogenesis. Interestingly, a very recent study of high resolution copy number data in FL and transformed FL confirms that gain of X predicts patient outcome in FL (Bouska *et al*, 2013).

In addition to gain of X, gain of 2p, 3q, 5p, 5q and 12q were found to be more weakly associated with inferior survival in Paper 3. Comparing initial FL samples from cases with and without subsequent transformation, we also observed that gain of 2p, 3q and 5 were exclusively present in the transformation group, while gain of 12q and chr 21 occurred more frequently in the this group than in the group without transformation. Gains of 2p, 3q, 5 and 12q have been associated with aggressive clinical and histopathological features in previous studies (Yunis *et al*, 1987;Cheung *et al*, 2008;Johnson *et al*, 2008;Schwaenen *et al*, 2009;Hough *et al*, 2001). In a recent study by Bouska et al (Bouska *et al*, 2013) using SNP array the associations between gain of 3q, transformation and inferior survival was confirmed. Gain of chromosome 3 is associated with the more aggressive ABC-subtype of DLBCL and not the GCB-subtype (Bea *et al*, 2005;Tagawa *et al*, 2005). The different patterns of recurring chromosomal imbalances in initial

biopsies from cases with and without subsequent transformation, indicates divergent evolutionary pathways and different pathogenic mechanisms in the FLs that remain FL and the FLs that are prone to higher-grade transformation. The transformation-associated aberrations each occur in small subsets of patients, suggesting that the mechanisms for transformation are heterogeneous. This is consistent with previous findings in studies including gene-expression studies of paired pre- and post transformation samples (Martinez-Climent *et al*, 2003;Elenitoba-Johnson *et al*, 2003;Lossos *et al*, 2002;Davies *et al*, 2007). In Paper 1, trisomy 21 was associated with inferior survival in FL and no specific aberrations occurred with different frequencies in FL biopsies from patients with or without subsequent transformation. The association between gain of 2p, 3q and 5 was not assessed in Paper 1, as these aberrations occurred in less than 10% of cases.

No regions of genomic loss were significantly associated with FL survival or transformation risk. Others have reported associations between inferior survival and loss of 1p36, 6q21q24, 6q25q27 and 17p (Tilly *et al*, 1994;Viardot *et al*, 2002;Lestou *et al*, 2003;Hoglund *et al*, 2004;Cheung *et al*, 2008;d'Amore *et al*, 2008). In the recent study by the LLMPP cooperation loss of 6q predicted outcome in a large series of FL and transformed FL samples (Bouska *et al*, 2013).The reason for lack of significant associations between losses and survival in Paper 3 may be the low number of patients.

In Paper 1, the degree of genomic instability was assessed by counting the number of different breakpoints and the number of gains/losses of entire chromosomes. An increasing number of aberrations were associated with inferior survival. In Paper 3 we defined a marker for genomic instability, the CNA-index, and there was a trend for poorer survival with increasing CNA-index in initial FL samples, although not statistically significant. While the method applied in Paper 1 also counts balanced chromosomal aberrations (translocations and inversions), these are not detected by arrayCGH and not counted by the CNA-index. On the other hand, marker chromosomes were counted only as one aberration in Paper 1 and may contain DNA originating from more than one chromosome, while unbalanced rearrangements involving marker chromosomes would be detected by the methods applied in Paper 3. Several previous studies using various methods for quantification of genomic instability have reported significant association between the number of genomic aberrations and inferior survival in FL (Tilly *et al*, 1994;Ott *et al*, 2002;Viardot *et al*, 2002;d'Amore *et al*, 2008) and this was confirmed in the recent study by the LLMPP cooperation (Bouska *et al*, 2013).

Clonal evolution in follicular lymphoma and evidence for a FL progenitor cell

Examination of copy number profiles and patterns of SHM in sequential FL samples (Paper 3) revealed that tumor cell clones in relapse samples are unlikely to be direct descendants of tumor cell clones present in preceding samples. Among the 39 cases with multiple biopsies, 36

showed changes in copy number profiles and CNA-index with time, consistent with the extensive clonal heterogeneity observed in FL (d'Amore *et al*, 2008). In the majority of cases with multiple biopsies (27 of 39) the early and late samples harbored unique as well as common copy number aberrations. Nine cases showed acquisition of additional copy number aberrations at relapse, while the three remainder cases were without clear changes. Even though there was a trend for increasing CNA-index in the late FL samples, several cases showed lower CNA-index in samples obtained late in the course of disease. The CNA-index showed a striking variability both within individual cases and across the whole set of samples. The variable CNA-index as well as the patterns of unique and shared CNAs, indicate that tumor cell clones present at different time points in FL arise by divergent evolution from a common ancestor cell. Other studies using karyotypic data, chromosomal CGH data and SNP array data have reported similar findings comparing paired samples of primary FL and relapses showing either FL or transformed lymphoma (DLBCL) (Johnson *et al*, 2008;d'Amore *et al*, 2008;Martinez-Climent *et al*, 2003;Fitzgibbon *et al*, 2007).

Analysis of the DNA sequence in rearranged IGH loci in a subset of sequential samples confirmed a clonal relationship (Paper 3). In addition, the analysis of somatic hypermutations in the IGVH region showed that early and late samples harbor unique as well as common SHMs, suggesting that tumor cell clones dominating in different serial samples originate from a less-mutated common ancestor clone. Similar findings were reported in previous studies with sequence data from IGV loci and the swicht-region of IGH (Aarts *et al*, 2001;Bognar *et al*, 2005;Adam *et al*, 2007;Matolcsy *et al*, 1999;Ruminy *et al*, 2008;Carlotti *et al*, 2009).

Previous studies have addressed the question if genomic aberrations arise by distinct evolutionary pathways in FL. In the studies by Høglund et al (Hoglund *et al*, 2004) and d'Amore et al (d'Amore *et al*, 2008), copy number aberrations were classified as early or late events in the pathogenesis of FL. Both studies reported that extra copies of, chromosomes 7, X and der(18)t(14;18) as well as loss of chromosomal arm 6q were frequent early events in the development of FL. Other aberrations were however, also observed as early events, indicating that the evolutionary pathways of FL development are heterogeneous.

Clonal heterogeneity in FL can also be observed in simultaneously obtained samples from a lymph node and other anatomical sites such as the bone marrow or peripheral blood. It has been shown that the different tumor cell clones traffic in both directions between peripheral nodes and the bone marrow (Ruminy *et al*, 2008;Aarts *et al*, 2001;Oeschger *et al*, 2002;Wartenberg *et al*, 2013). It has been proposed that FL originates from a t(14;18) positive cell that has entered a reactive germinal center and that FL cells migrate early to the bone marrow which may function as a protective niche during chemotherapy (Wartenberg *et al*, 2013).

Recent studies using phosphorylation specific probes and cell sorting of FL cell suspensions by flowcytometry have shown that BCR-signaling was not inducible in FL cell clones

emerging at relapse as opposed to the potent inducible signaling in FL cells in samples obtained prior to treatment. The finding indicates that FL cells may become independent of BCR-signaling for survival. These relapse clones were also negative for the B-cell marker CD20 (Irish *et al*, 2010). The loss of CD20 expression represents a clinical challenge as it correlates with resistance to rituximab treatment (Hiraga *et al*, 2009). The observed phenotypic changes in signaling capacity and surface marker expression are consistent with the clonal diversity in FL.

Advances in high-throughput sequencing technology have enabled whole exome sequencing of FL cell subpopulations. In combination with analyses of SHM of the IGVH, findings from deep sequencing confirm that tumor cell subpopulations are genetically divergent and support the hypothetical evolution from a common FL progenitor cell. Based on the subclonal representation of somatically acquired mutations, early versus late mutational events could be identified in FL (Green *et al*, 2013). Mutations targeting genes encoding histone-modifying proteins (EZH2, CREBBP and MLL2) represent early genetic events in the development of FL (Morin *et al*, 2010;Green *et al*, 2013) and may thus characterize the FL progenitor cell.

Even though the copy number profiles varied in sequential biopsies, there were only small increases in overall CNA frequencies in the latest FL samples compared to the initial samples. In Paper 1, FL samples obtained from *different* patients who had or had not received treatment were compared to assess the impact of treatment on genetic evolution. Isochromosome 6p and loss of 6q were more frequent in samples from treated patients. A weak trend for increasing loss of 6q was also present in samples obtained after treatment in the serial biopsies (Paper 3, supplemental figure 1 A). The number of aberrations in samples from treated and untreated patients did not differ significantly between the two groups (Paper 1). Results from both studies indicate that few systematic changes in aberration frequencies take place in the relapse of FL. This is in line with a previous study by d'Amore et al 2008, in which cytogenetic data from 418 t(14;18)-positive FL samples originating from 360 patients showed that the frequencies of gains and losses were similar in different cell clones.

While there are small changes in aberration frequencies in primary compared to relapsed FL, we observed significant differences comparing samples of DLBCL transformed from FL (n=15) with samples of FL grade 1-3a (n=107) in Paper 1. Loss from 14q, monosomy 15, trisomy 16 and 22 were more frequent among the transformed samples. The total number of aberrations did not differ between the two groups of samples. Other studies comparing cytogenetic data from paired samples of FL and samples showing transformed FL (DLBCL) indicate that higher-grade transformation is associated with acquisition of a heterogeneous set of genomic aberrations. DLBCL samples often have more complex genotypes than the FL counterpart and some CNAs are more common in the transformed lymphoma; for instance gain/rearrangements of 3q27 (BCL6 locus), gain of 12q, 7q, 18q and Xq and loss of 1p36, 6q, 9p21, 13q, 17p (Johnson *et al*, 2008;Martinez-Climent *et al*, 2003;Nagy *et al*, 2000;Hough *et al*, 2001;Fitzgibbon *et al*, 2007).

The histological grade of FL may change at relapse. Among the 14 patients in Paper 3 with biopsies showing FL grade 3a, 10 had additional biopsies showing FL grade 1-2. To investigate whether or not the shift in histological grade is associated with clonal evolution, we compared the frequencies of copy number aberrations in FL grade 1-2 and FL grade 3a (Paper 1 and 3). We observed a higher incidence of loss from chromosomal arm 1p, including 1p36, in FL grade 3a, in line with previous findings (Horsman *et al*, 2001;Ott *et al*, 2002). In Paper 3, an increased incidence of gain of chromosome 5 in FL grade 3a was especially prominent. Genomic instability, as quantified by the CNA-index, was significantly higher in FL grade 3a samples, in line with other studies (Horsman *et al*, 2001;Ott *et al*, 2002;Mohamed *et al*, 2001;d'Amore *et al*, 2008). In contrast to this, the number of aberrations as counted in Paper 1 was not significantly different in FL grade 1-2 and FL grade 3a. Loss of 1p36, the degree of genomic instability and grading of FL have all been associated with prognosis in FL, suggesting that FL grade 3a may represent a more aggressive presentation of FL (Lestou *et al*, 2003) and others). On the other hand, results from other studies contradict a prognostic significance of FL grade (Shustik *et al*, 2011) and references therein). The clinical significance of FL grading may however, depend on treatment as a recent study by Whalin et al (Wahlin *et al*, 2013) show better overall survival in patients with FL grade 3a than in grade 1-2 when treated with rituximab monotherapy.

In summary, there are several lines of evidence for a parallel evolution of tumor cell clones in FL. We have shown that clonal evolution in FL is traceable at a whole genome level as well as by the mutational patterns of the IGH locus. Our findings confirmed that parallel evolution of tumor cell clones contribute to genetic diversification in FL and support the hypothesis of a FL progenitor cell. One may speculate that slowly proliferating FL progenitor cells escape chemotherapy treatment and give rise to relapse. Parallel evolution and trafficking of tumor cell clones explains how genetically different tumor clones may dominate at different times and different anatomical sites creating different morphologic and histologic appearances, and showing different interactions with the microenvironment, different proliferation capacity and different treatment sensitivity. Clonal heterogeneity in time and space may account for the different results reported in studies of associations between genetic aberrations and patient outcome in FL. Nevertheless, we and others have shown that specific secondary aberrations are significantly associated with the risk of transformation and/or with inferior survival. One may speculate that these aberrations, *upon appearance*, endow tumor cells with the propensity to transform and/or resist treatment. The existence of a FL progenitor cell has implications for the development of new treatment strategies in FL. In order to be curative, a treatment needs to combine drugs targeting features of the FL progenitor cells with drugs targeting derived subclones which may be present simultaneously. To try characterizing the FL progenitor cell more closely, it would be interesting to perform whole-genome deep sequencing as well as high resolution assessment of DNA copy number in new patient cohort with serial FL samples.

Gene dosage effect in FL and identification of cancer driver genes

In Paper 4, we showed that gene expression levels correlate positively with DNA copy number levels for a majority of genes within aberrant regions, indicating that a gene dosage effect deregulate transcription in FL. Applying strict selection criteria we singled out 698 genes showing a high correlation between gene expression and copy number (cis-genes).

Previous integrative analyses of gene expression and copy number data in for instance DLBCL, mantle cell lymphoma and other cancers such as breast cancer, proved the presence of a gene dosage effect in tumors with chromosomal imbalances. Closer examinations of the genes that are subject to gene dosage effect have identified key drivers of oncogenesis (Lenz *et al*, 2008c;Hartmann *et al*, 2010;Pollack *et al*, 2002;Akavia *et al*, 2010).

In FL, few integrative analyses of gene expression and copy number profiling have been performed (Martinez-Climent *et al*, 2003;Schwaenen *et al*, 2009;Leich *et al*, 2009). The studies report that low proportions of genes within aberrant genomic regions show consistent expression levels. The number of cis-genes identified in our study is also low considering the large genomic regions that are recurrently targeted by gains and losses in FL. High level amplifications are rarely seen in FL. Thus the gene dosage effect may be more subtle in FL than in cancers with higher degrees of genomic instability. Importantly, FL samples contain variable amounts of T-cells, macrophages, endothelial cells and fibroblasts in addition to the malignant B-cells. The signals from these normal cells attenuate signals from malignant B-cells in arrayCHG analyses and create “biological noise” in gene expression analyses. Indeed, gene expression profiles of tumor infiltrating immune cells rather than that of FL cells predicted patient outcome in the landmark study by Dave SS et al (Dave *et al*, 2004). The tumor specific molecular mechanisms behind progression and transformation of FL may on the other hand be masked by the effect of the microenvironment.

As copy number alterations are specific for the malignant B-cells in FL, the 698 cis-genes are likely to represent tumor specific transcriptional activity. In addition, the recurrent copy number aberrations are likely to represent genetic alterations of selective advantage for the lymphoma cells. We thus hypothesized that these genes may be of importance for the development and progression of follicular lymphoma.

The study by Martinez-Climent included expression and copy number data paired samples of FL and DLBCL originating from 10 patients. The findings were too heterogeneous to make a comprehensible comparison to the results presented in Paper 4. Both Schwaenen et al (2009) and Leich et at (2009) report similar patterns of recurrent genomic gains and losses as we do in Paper 3. In the study by Schwaenen et al (2009) expression levels of only 28 annotated genes located in consensus regions of gains or losses were reported to be significantly correlated to copy number. Nine of these (32%) were also present in the list of 698 cis-genes (*MAP3K7, SNX3, KIAA0274/FIG4, CRSP3/MED23, VNN2, TBPL1, FLJ11712/RNASEH2B, ACAA2*

and *SMAD2*). The study does not report data from chromosome X where a majority (23%) of cis-genes were located. In Leich *et al* (2009) the frequencies of aberrations are lower than in our data set, probably due to the lower resolution of conventional CGH compared to array CGH. Leich *et al* (2009) reported significant associations between gene expression and copy number levels for 2465 probe sets located within 195 minimally altered chromosomal regions. This data set was used for validation of our findings. Interestingly, most of the 698 cis-genes identified in Paper 4, also show high, positive correlations between expression and copy number in the Leich data set.

The molecular network most significantly enriched for cis-genes contained genes associated with B-cell receptor- and Toll like receptor-signaling including mediators and regulators in the NF-kB pathway. The pathway analysis indicated that expression levels of 14 genes that are involved in NF-kB-activation are influenced by copy number in FL. For these 14 genes, we identified a subset of correlated NF-kB target genes and these were used to calculate a corresponding gene expression score. For six of these genes (*BTK, IGBP1, IRAK1, ROCK1, TMED7-TICAM2* and *TRIM37*) the scores were significantly associated with transformation of FL.

The protein encoded by *IRAK1* mediates Toll- like receptor (TLR) signaling down-stream of the adaptor protein MyD88. TLRs recognize pathogen-associated molecular patterns (PAMPs) such as bacterial lipopolysaccharides (TLR4), bacterial flagellin (TLR5), viral double stranded RNA (TLR3), as well as bacterial or viral unmethylated CpG containing DNA (TLR9) and single stranded RNA (TLRs 7 and 8). Endogenous substances released at cellular stress and tissue destruction (i.e heat shock proteins and fibronectin) may also activate TRLs. Down-stream effects of TLR signaling include activation of transcription factors NF-kB, AP-1 and IRFs. Their role in initiation of the innate immune response is well described (Janeway CA, 2005). TLRs are expressed on both B- and T lymphocytes and modulate the adaptive immune responses. In a study by Ngo VN et al (2011) it was shown that 30% ABC-DLBCL cases harbor activating MyD88 mutations that promote constitutive NF-kB activation. This finding has contributed to an expanding research focusing on TLR-singaling in B-cell malignancies (Chiron *et al*, 2008;Harsini *et al*, 2013).

TRIM37 encodes a ubiqutinin ligase in the TRIM-familiy which compises several proto-oncogenes (ref). To the best of our knowledge, the role of TRIM37 in lymphocytes or lymphoma has not been investigated. Interestingly, a combination of the NF-kB target gene scores for *IRAK1* and *TRIM37* resulted in the best separation of FL cases with and without transformation.

The probe for *TMED7-TICAM2* represents a read through transcription of the neighboring genes *TMED7* and *TICAM2.* Several splice variants of the transcript exist. *TICAM2* encodes an adaptor molecule down-stream of TLR4 and mediates NF-kB activation (also known as TRAM)(refs as above), while the protein TMED7 has been shown to inhibit TLR4 signaling (Doyle *et al*, 2012). Further investigation is needed to determine the splice variants that are present in FL and what role the different variants may play in promoting transformation.

The kinase BTK is activated by BCR-signaling and mediates NF-kB activation. There is substantial evidence for involvement of BCR-signaling in the pathogenesis of ABC-DLBCL, marginal zone lymphoma, chronic lymphatic leukemia (CLL) and Burkitt's lymphoma (Young & Staudt, 2013). Although several observations indicate a role of BCR in FL-pathogenesis, the exact mechanisms for BCR-involvement are poorly understood. FL invariably express BCR and even cases relapsing after anti-idiotype antibody treatment are BCR-positive, indicating involvement of BCR in development and survival FL cells. The translocation t(14;18) always involves the unproductive IgH allele which may be isotype switched, while the productive allele remains IgM, indicating that sustained IgM surface expression is of importance for FL cells (Young & Staudt, 2013;Kuppers, 2005). In flowcytometry studies using probes specific for phosphorylated molecules, inducible BCR-signaling was more potent in FL B-cells than in normal B-cells. However, in FL cell clones emerging at relapse, BCR-signaling was not inducible, indicating an acquired independency of BCR-signaling for FL cell survival (Irish *et al*, 2010). Nevertheless, signaling in the pathways regulating B-cell proliferation may be maintained by other mechanisms working downstream of the BCR. It would be interesting to study closer the consequences of BTK over-expression or knock-out for signaling, survival and proliferation of FL-cells. Whether or not the higher expression score of the associated NF-kB target genes results from BTK over-expression needs to be further investigated. Recent clinical trials support a role of BTK in FL and other B-cell malignancies; treatment with ibrutinib, a BTK-inhibitor, induces remission in patients with relapsed and refractory B-cell malignancies, including follicular lymphoma (Advani *et al*, 2013).

The protein IGBP1 was first found to be associated with the CD79a subunit of the B-cell receptor (Inui *et al*, 1995) and later also identified as a broadly expressed regulatory subunit of protein phosphatase 2A (Chuang *et al*, 2000). Over-expression of IGBP1 is associated with inferior outcome of small cell lung carcinoma, which may indicate that its role as an oncogene is independent of its role in BCR-signaling in lymphoma (Sakashita *et al*, 2011). Whether the role for IGBP1 in transformation of FL is associated with activation of NF-kB or other oncogenetic pathways needs to be further investigated.

ROCK1 encodes a protein kinase with ubiquitous tissue distribution. It is known to regulate cellular adhesion, migration and invasion. *ROCK1* has been shown to be over-expressed in cancer and associated with metastasis and inferior outcome. To the best of our knowledge, a role in B-cells or lymphoma has not been investigated (Patel *et al*, 2013).

The average expression of all NF-kB target genes was not associated with transformation. Comparison of the average transcription level of NF-kB target genes across subtypes of B-cell lymphoma showed lower levels in FL than in DLBCL, and especially compared to the ABC subtype in which NF-kB signaling is constitutive active. Whether or not the subsets of NF-kB target genes that predicted transformation reflects a general NF-kB activation in FL is uncertain. One may speculate that NF-kB activation takes place in a subclone of FL and that the

gene expression signals produced from such a subclone is partly masked by other FL clones as well as by the bystander T-cells and macrophages. It is also possible that the activation of NF-kB target gene subsets is due to the action of other transcription factors with partially overlapping target gene sets.

The molecular mechanisms underlying transformation from FL to DLBCL have been studied by comparing tumor samples before and after transformation using various methods (reviewed in (Bernstein & Burack, 2009) and others). Elevated expression of c-MYC and other genes promoting proliferation, inactivation of TP53 and CDKN2A, dysregulation of p38-MAPK, mutations of BCL6 and composition of the tumor microenvironment have all been implicated in subsets of cases with transformation (Elenitoba-Johnson *et al*, 1998;Lossos *et al*, 2002;Martinez-Climent *et al*, 2003;Akasaka *et al*, 2003;Elenitoba-Johnson *et al*, 2003;Davies *et al*, 2005;Davies *et al*, 2007;Glas *et al*, 2007). More recently, an expression signature (LPS score) has been shown to predict transformation and survival in FL (Gentles *et al*, 2009). The LPS is derived from three gene modules; two modules with genes typically expressed in embryonic stem cells and one stromal cell module. The LPS score did not predict transformation in our data set. This may be due to the lower number of patients in our study than in Gentles et al. On the other hand, higher expression of the *MYC*-signature as defined by Lossos et al (2002) in tumor samples (FL and DLBCL) was significantly associated with transformation in our data set. Then again, the *MYC*-signature score in FL-samples did *not* predict transformation in our data set, indicating that increased expression of the *MYC*-signature reflects increased proliferation in transformed tumors rather than being the cause of transformation.

In Paper 4, we showed that transformation can be predicted based on the expression level of subsets of NF-kB target genes in biopsies showing FL. Of note, a high signature score was found in FL samples preceding transformation, but not always in the diagnostic specimen. This is in line with the clonal evolution and heterogeneity observed in FL. The prediction of transformation based on molecular features in FL samples will thus require a liberal practice for performing biopsies.

In future projects, we will seek to validate the predictive significance of the NF-kB target gene signatures in a patient cohort treated in the rituximab era. A recent study from the University of Iowa/Mayo Clinic, reports reduced transformation rates in the present treatment era (Link *et al*, 2013). Thus, studies of risk factors for outcome in FL will require prolonged observation times and larger patient cohorts. Results from such a study may then motivate the development of a test to assess transformation risk.

It would also be of interest to examine closer the functional role of some of the cis-genes follicular lymphoma; especially *BTK, IGBP1, IRAK1, ROCK1, TMED7-TICAM2* and *TRIM37,* in order to identify new treatment targets in FL.

References

Aarts,W.M., Bende,R.J., Bossenbroek,J.G., Pals,S.T., & van Noesel,C.J.M. (2001) Variable heavy-chain gene analysis of follicular lymphomas: subclone selection rather than clonal evolution over time. *Blood*, **98**, 238-240.

Abouyabis,A.N., Shenoy,P.J., Lechowicz,M.J., & Flowers,C.R. (2008) Incidence and outcomes of the peripheral T-cell lymphoma subtypes in the United States. *Leuk.Lymphoma*, **49**, 2099-2107.

Acker,B., Hoppe,R.T., Colby,T.V., Cox,R.S., Kaplan,H.S., & Rosenberg,S.A. (1983) Histologic conversion in the non-Hodgkin's lymphomas. *Journal of Clinical Oncology*, **1**, 11-16.

Adam,P., Schoof,J., Hartmann,M., Schwarz,S., Puppe,B., Ott,M., Rosenwald,A., Ott,G., & Muller-Hermelink,H.K. (2007) Cell migration patterns and ongoing somatic mutations in the progression of follicular lymphoma. *Cytogenet.Genome Res.*, **118**, 328-336.

Advani,R.H., Buggy,J.J., Sharman,J.P., Smith,S.M., Boyd,T.E., Grant,B., Kolibaba,K.S., Furman,R.R., Rodriguez,S., Chang,B.Y., Sukbuntherng,J., Izumi,R., Hamdy,A., Hedrick,E., & Fowler,N.H. (2013) Bruton Tyrosine Kinase Inhibitor Ibrutinib (PCI-32765) Has Significant Activity in Patients With Relapsed/Refractory B-Cell Malignancies. *Journal of Clinical Oncology*, **31**, 88-94.

Aisenberg,A.C. (2000) Historical review of lymphomas. *Br.J Haematol.*, **109**, 466-476.

Akasaka,T., Lossos,I.S., & Levy,R. (2003) BCL6 gene translocation in follicular lymphoma: a harbinger of eventual transformation to diffuse aggressive lymphoma. *Blood*, **102**, 1443-1448.

Akavia,U.D., Litvin,O., Kim,J., Sanchez-Garcia,F., Kotliar,D., Causton,H.C., Pochanard,P., Mozes,E., Garraway,L.A., & Pe'er,D. (2010) An integrated approach to uncover drivers of cancer. *Cell*, **143**, 1005-1017.

Al,K.M., de,A., Jr., Guyatt,G.H., Kuruvilla,J., & Crump,M. (2012) Autologous stem cell transplantation in follicular lymphoma: a systematic review and meta-analysis. *J Natl.Cancer Inst.*, **104**, 18-28.

Al-Tourah,A.J., Gill,K.K., Chhanabhai,M., Hoskins,P.J., Klasa,R.J., Savage,K.J., Sehn,L.H., Shenkier,T.N., Gascoyne,R.D., & Connors,J.M. (2008) Population-Based Analysis of Incidence and Outcome of Transformed Non-Hodgkin's Lymphoma. *Journal of Clinical Oncology*, **26**, 5165-5169.

Alizadeh,A.A., Eisen,M.B., Davis,R.E., Ma,C., Lossos,I.S., Rosenwald,A., Boldrick,J.C., Sabet,H., Tran,T., Yu,X., Powell,J.I., Yang,L., Marti,G.E., Moore,T., Hudson,J., Jr., Lu,L., Lewis,D.B., Tibshirani,R., Sherlock,G., Chan,W.C., Greiner,T.C., Weisenburger,D.D., Armitage,J.O., Warnke,R., Levy,R.,

Wilson,W., Grever,M.R., Byrd,J.C., Botstein,D., Brown,P.O., & Staudt,L.M. (2000) Distinct types of diffuse large B-cell lymphoma identified by gene expression profiling. *Nature*, **403**, 503-511.

Ame-Thomas,P., Le Priol,J., Yssel,H., Caron,G., Pangault,C., Jean,R., Martin,N., Marafioti,T., Gaulard,P., Lamy,T., Fest,T., Semana,G., & Tarte,K. (2012) Characterization of intratumoral follicular helper T cells in follicular lymphoma: role in the survival of malignant B cells. *Leukemia*, **26**, 1053-1063.

Ame-Thomas,P. & Tarte,K. (2013) The yin and the yang of follicular lymphoma cell niches: Role of microenvironment heterogeneity and plasticity. *Semin.Cancer Biol.*

Andreadis,C., Schuster,S.J., Chong,E.A., Svoboda,J., Luger,S.M., Porter,D.L., Tsai,D.E., Nasta,S.D., Elstrom,R.L., Goldstein,S.C., Downs,L.H., Mangan,P.A., Cunningham,K.A., Hummel,K.A., Gimotty,P.A., Siegel,D.L., Glatstein,E., & Stadtmauer,E.A. (2005) Long-term event-free survivors after high-dose therapy and autologous stem-cell transplantation for low-grade follicular lymphoma. *Bone Marrow Transplant.*, **36**, 955-961.

Aukema,S.M., Siebert,R., Schuuring,E., van Imhoff,G.W., Kluin-Nelemans,H.C., Boerma,E.J., & Kluin,P.M. (2011) Double-hit B-cell lymphomas. *Blood*, **117**, 2319-2331.

Bahler,D.W., Campbell,M.J., Hart,S., Miller,R.A., Levy,S., & Levy,R. (1991) Ig VH gene expression among human follicular lymphomas. *Blood*, **78**, 1561-1568.

Bakhoum,S.F., Danilova,O.V., Kaur,P., Levy,N.B., & Compton,D.A. (2011) Chromosomal instability substantiates poor prognosis in patients with diffuse large B-cell lymphoma. *Clin Cancer Res.*, **17**, 7704-7711.

Ban-Hoefen,M., Kelly,J.L., Bernstein,S.H., Liesveld,J., Constine,L., Becker,M., Milner,L., Phillips,G., & Friedberg,J.W. (2011) High-dose therapy and autologous stem cell transplant for transformed non-Hodgkin lymphoma in the rituximab era. *Leukemia & Lymphoma*, **53**, 830-835.

Basso,K. & la-Favera,R. (2012) Roles of BCL6 in normal and transformed germinal center B cells. *Immunol.Rev.*, **247**, 172-183.

Bastion,Y., Sebban,C., Berger,F., Felman,P., Salles,G., Dumontet,C., Bryon,P.A., & Coiffier,B. (1997) Incidence, predictive factors, and outcome of lymphoma transformation in follicular lymphoma patients. *Journal of Clinical Oncology*, **15**, 1587-1594.

Bayerdorffer,E., Neubauer,A., Rudolph,B., Thiede,C., Lehn,N., Eidt,S., & Stolte,M. (1995) Regression of primary gastric lymphoma of mucosa-associated lymphoid tissue type after cure of Helicobacter pylori infection. MALT Lymphoma Study Group. *Lancet*, **345**, 1591-1594.

Bea,S., Zettl,A., Wright,G., Salaverria,I., Jehn,P., Moreno,V., Burek,C., Ott,G., Puig,X., Yang,L., Lopez-Guillermo,A., Chan,W.C., Greiner,T.C., Weisenburger,D.D., Armitage,J.O., Gascoyne,R.D., Connors,J.M., Grogan,T.M., Braziel,R., Fisher,R.I., Smeland,E.B., Kvaloy,S., Holte,H., Delabie,J.,

Simon,R., Powell,J., Wilson,W.H., Jaffe,E.S., Montserrat,E., Muller-Hermelink,H.K., Staudt,L.M., Campo,E., & Rosenwald,A. (2005) Diffuse large B-cell lymphoma subgroups have distinct genetic profiles that influence tumor biology and improve gene-expression-based survival prediction. *Blood*, **106**, 3183-3190.

Bernstein,S.H. & Burack,W.R. (2009) The incidence, natural history, biology, and treatment of transformed lymphomas. *Hematology.Am.Soc.Hematol.Educ.Program.*, 532-541.

Bignell,G.R., Huang,J., Greshock,J., Watt,S., Butler,A., West,S., Grigorova,M., Jones,K.W., Wei,W., Stratton,M.R., Futreal,P.A., Weber,B., Shapero,M.H., & Wooster,R. (2004) High-resolution analysis of DNA copy number using oligonucleotide microarrays. *Genome Res.*, **14**, 287-295.

Blaker Y.N, Eide M.B, Liestøl K, Lauritzsen G.F, Kolstad A, Fosså A, Smeland E.B, Holte H, (2013) High dose chemotherapy with autologous stem cell transplantation for patients with transformed B-cell non-Hodgkin lymphoma in the rituximab era. Leukemia and Lymphoma, in press.

Bognar,A., Csernus,B., Bodor,C., Reiniger,L., Szepesi,A., Toth,E., Kopper,L., & Matolcsy,A. (2005) Clonal selection in the bone marrow involvement of follicular lymphoma. *Leukemia*, **19**, 1656-1662.

Bonilla,F.A. & Oettgen,H.C. (2010) Adaptive immunity. *J Allergy Clin Immunol.*, **125**, S33-S40.

Bosetti,C., Levi,F., Ferlay,J., Lucchini,F., Negri,E., & La,V.C. (2008) Incidence and mortality from non-Hodgkin lymphoma in Europe: the end of an epidemic? *Int.J Cancer*, **123**, 1917-1923.

Bouska,A., McKeithan,T.W., Deffenbacher,K.E., Lachel,C., Wright,G.W., Iqbal,J., Smith,L.M., Zhang,W., Kucuk,C., Rinaldi,A., Bertoni,F., Fitzgibbon,J., Fu,K., Weisenburger,D.D., Greiner,T.C., Dave,B.J., Gascoyne,R.D., Rosenwald,A., Campo,E., Rimsza,L.M., Delabie,J., Jaffe,E.S., Braziel,R.M., Connors,J.M., Staudt,L.M., & Chan,W.C. (2013) Genome-wide copy number analyses reveal genomic abnormalities involved in transformation of follicular lymphoma. *Blood*.

Bödör,C., Grossmann,V., Popov,N., Okosun,J., Riain,C., Tan,K., Marzec,J., Araf,S., Wang,J., Lee,A.M., Clear,A., Montoto,S., Matthews,J., Iqbal,S., Rajnai,H., Rosenwald,A., Ott,G., Campo,E., Rimsza,L.M., Smeland,E.B., Chan,W.C., Braziel,R.M., Staudt,L.M., Wright,G., Lister,T.A., Elemento,O., Hills,R., Gribben,J.G., Chelala,C., Matolcsy,A., Kohlmann,A., Haferlach,T., Gascoyne,R.D., & Fitzgibbon,J. (2013) EZH2 mutations are frequent and represent an early event in follicular lymphoma. *Blood*, **122**, 3165-3168.

Canioni,D., Salles,G., Mounier,N., Brousse,N., Keuppens,M., Morchhauser,F., Lamy,T., Sonet,A., Rousselet,M.C., Foussard,C., & Xerri,L. (2008) High Numbers of Tumor-Associated Macrophages Have an Adverse Prognostic Value That Can Be Circumvented by Rituximab in Patients With Follicular Lymphoma Enrolled Onto the GELA-GOELAMS FL-2000 Trial. *Journal of Clinical Oncology*, **26**, 440-446.

Cardesa-Salzmann,T.M., Colomo,L., Gutierrez,G., Chan,W.C., Weisenburger,D., Climent,F., Gonzalez-Barca,E., Mercadal,S., Arenillas,L., Serrano,S., Tubbs,R., Delabie,J., Gascoyne,R.D., Connors,J.M.,

Mate,J.L., Rimsza,L., Braziel,R., Rosenwald,A., Lenz,G., Wright,G., Jaffe,E.S., Staudt,L., Jares,P., Lopez-Guillermo,A., & Campo,E. (2011) High microvessel density determines a poor outcome in patients with diffuse large B-cell lymphoma treated with rituximab plus chemotherapy. *Haematologica*, **96**, 996-1001.

Carlotti,E., Wrench,D., Matthews,J., Iqbal,S., Davies,A., Norton,A., Hart,J., Lai,R., Montoto,S., Gribben,J.G., Lister,T.A., & Fitzgibbon,J. (2009) Transformation of follicular lymphoma to diffuse large B-cell lymphoma may occur by divergent evolution from a common progenitor cell or by direct evolution from the follicular lymphoma clone. *Blood*, **113**, 3553-3557.

Chen,C.I., Crump,M., Tsang,R., Stewart,A.K., & Keating,A. (2001) Autotransplants for histologically transformed follicular non-Hodgkin's lymphoma. *Br J Haematol.*, **113**, 202-208.

Chen,W., Houldsworth,J., Olshen,A.B., Nanjangud,G., Chaganti,S., Venkatraman,E.S., Halaas,J., Teruya-Feldstein,J., Zelenetz,A.D., & Chaganti,R.S. (2006) Array comparative genomic hybridization reveals genomic copy number changes associated with outcome in diffuse large B-cell lymphomas. *Blood*, **107**, 2477-2485.

Cheung,K.J., Delaney,A., Ben-Neriah,S., Schein,J., Lee,T., Shah,S.P., Cheung,D., Johnson,N.A., Mungall,A.J., Telenius,A., Lai,B., Boyle,M., Connors,J.M., Gascoyne,R.D., Marra,M.A., & Horsman,D.E. (2010) High resolution analysis of follicular lymphoma genomes reveals somatic recurrent sites of copy-neutral loss of heterozygosity and copy number alterations that target single genes. *Genes Chromosomes Cancer*, **49**, 669-681.

Cheung,K.J., Rogic,S., Ben-Neriah,S., Boyle,M., Connors,J.M., Gascoyne,R.D., & Horsman,D.E. (2012) SNP analysis of minimally evolved t(14;18)(q32;q21)-positive follicular lymphomas reveals a common copy-neutral loss of heterozygosity pattern. *Cytogenet.Genome Res.*, **136**, 38-43.

Cheung,K.J., Shah,S.P., Steidl,C., Johnson,N., Relander,T., Telenius,A., Lai,B., Murphy,K.P., Lam,W., Al-Tourah,A.J., Connors,J.M., Ng,R.T., Gascoyne,R.D., & Horsman,D.E. (2008) Genome-wide profiling of follicular lymphoma by array comparative genomic hybridization reveals prognostically significant DNA copy number imbalances. *Blood*, **113**, 137-148.

Chiron,D., Bekeredjian-Ding,I., Pellat-Deceunynck,C., Bataille,R., & Jego,G. (2008) Toll-like receptors: lessons to learn from normal and malignant human B cells. *Blood*, **112**, 2205-2213.

Chuang,E., Fisher,T.S., Morgan,R.W., Robbins,M.D., Duerr,J.M., Vander Heiden,M.G., Gardner,J.P., Hambor,J.E., Neveu,M.J., & Thompson,C.B. (2000) The CD28 and CTLA-4 receptors associate with the serine/threonine phosphatase PP2A. *Immunity.*, **13**, 313-322.

Colombat,P., Brousse,N., Salles,G., Morschhauser,F., Brice,P., Soubeyran,P., Delwail,V., Deconinck,E., Haioun,C., Foussard,C., Sebban,C., Tilly,H., Thieblemont,C., Bergougnoux,L., Lazreg,F., & Solal-Celigny,P. (2012) Rituximab induction immunotherapy for first-line low-tumor-burden follicular lymphoma: survival analyses with 7-year follow-up. *Annals of Oncology*, **23**, 2380-2385.

Conconi,A., Ponzio,C., Lobetti-Bodoni,C., Motta,M., Rancoita,P.M., Stathis,A., Moccia,A.A., Mazzucchelli,L., Bertoni,F., Ghielmini,M., Cavalli,F., & Zucca,E. (2012) Incidence, risk factors and outcome of histological transformation in follicular lymphoma. *Br.J Haematol.*, **157**, 188-196.

Cooper,M.D. & Herrin,B.R. (2010) How did our complex immune system evolve? *Nat.Rev.Immunol.*, **10**, 2-3.

Czuczman,M.S., Vose,J.M., Witzig,T.E., Zinzani,P.L., Buckstein,R., Polikoff,J., Li,J., Pietronigro,D., Ervin-Haynes,A., & Reeder,C.B. (2011) The differential effect of lenalidomide monotherapy in patients with relapsed or refractory transformed non-Hodgkin lymphoma of distinct histological origin. *Br.J Haematol.*, **154**, 477-481.

Czuczman,M.S., Fayad,L., Delwail,V., Cartron,G., Jacobsen,E., Kuliczkowski,K., Link,B.K., Pinter-Brown,L., Radford,J., Hellmann,A., Gallop-Evans,E., DiRienzo,C.G., Goldstein,N., Gupta,I., Jewell,R.C., Lin,T.S., Lisby,S., Schultz,M., Russell,C.A., & Hagenbeek,A. (2012) Ofatumumab monotherapy in rituximab-refractory follicular lymphoma: results from a multicenter study. *Blood*, **119**, 3698-3704.

d'Amore,F., Chan,E., Iqbal,J., Geng,H., Young,K., Xiao,L., Hess,M.M., Sanger,W.G., Smith,L., Wiuf,C., Hagberg,O., Fu,K., Chan,W.C., & Dave,B.J. (2008) Clonal Evolution in t(14;18)-Positive Follicular Lymphoma, Evidence for Multiple Common Pathways, and Frequent Parallel Clonal Evolution. *Clinical Cancer Research*, **14**, 7180-7187.

Dave,S.S., Wright,G., Tan,B., Rosenwald,A., Gascoyne,R.D., Chan,W.C., Fisher,R.I., Braziel,R.M., Rimsza,L.M., Grogan,T.M., Miller,T.P., LeBlanc,M., Greiner,T.C., Weisenburger,D.D., Lynch,J.C., Vose,J., Armitage,J.O., Smeland,E.B., Kvaloy,S., Holte,H., Delabie,J., Connors,J.M., Lansdorp,P.M., Ouyang,Q., Lister,T.A., Davies,A.J., Norton,A.J., Muller-Hermelink,H.K., Ott,G., Campo,E., Montserrat,E., Wilson,W.H., Jaffe,E.S., Simon,R., Yang,L., Powell,J., Zhao,H., Goldschmidt,N., Chiorazzi,M., & Staudt,L.M. (2004) Prediction of survival in follicular lymphoma based on molecular features of tumor-infiltrating immune cells. *N.Engl.J.Med.*, **351**, 2159-2169.

Davies,A.J., Lee,A.M., Taylor,C., Clear,A.J., Goff,L.K., Iqbal,S., Cuthbert-Heavens,D., Calaminici,M., Norton,A.J., Lister,T.A., & Fitzgibbon,J. (2005) A limited role for TP53 mutation in the transformation of follicular lymphoma to diffuse large B-cell lymphoma. *Leukemia*, **19**, 1459-1465.

Davies,A.J., Rosenwald,A., Wright,G., Lee,A., Last,K.W., Weisenburger,D.D., Chan,W.C., Delabie,J., Braziel,R.M., Campo,E., Gascoyne,R.D., Jaffe,E.S., Muller-Hermelink,K., Ott,G., Calaminici,M., Norton,A.J., Goff,L.K., Fitzgibbon,J., Staudt,L.M., & Andrew,L.T. (2007) Transformation of follicular lymphoma to diffuse large B-cell lymphoma proceeds by distinct oncogenic mechanisms. *Br.J.Haematol.*, **136**, 286-293.

Davis,R.E., Brown,K.D., Siebenlist,U., & Staudt,L.M. (2001) Constitutive nuclear factor kappaB activity is required for survival of activated B cell-like diffuse large B cell lymphoma cells. *J Exp.Med*, **194**, 1861-1874.

Davis,R.E., Ngo,V.N., Lenz,G., Tolar,P., Young,R.M., Romesser,P.B., Kohlhammer,H., Lamy,L., Zhao,H., Yang,Y., Xu,W., Shaffer,A.L., Wright,G., Xiao,W., Powell,J., Jiang,J.K., Thomas,C.J., Rosenwald,A., Ott,G., Muller-Hermelink,H.K., Gascoyne,R.D., Connors,J.M., Johnson,N.A., Rimsza,L.M., Campo,E., Jaffe,E.S., Wilson,W.H., Delabie,J., Smeland,E.B., Fisher,R.I., Braziel,R.M., Tubbs,R.R., Cook,J.R., Weisenburger,D.D., Chan,W.C., Pierce,S.K., & Staudt,L.M. (2010) Chronic active B-cell-receptor signalling in diffuse large B-cell lymphoma. *Nature*, **463**, 88-92.

De Jong.D. & Fest,T. (2011) The microenvironment in follicular lymphoma. *Best.Pract.Res.Clin Haematol.*, **24**, 135-146.

Dolken,G., Illerhaus,G., Hirt,C., & Mertelsmann,R. (1996) BCL-2/JH rearrangements in circulating B cells of healthy blood donors and patients with nonmalignant diseases. *Journal of Clinical Oncology*, **14**, 1333-1344.

Doyle,S.L., Husebye,H., Connolly,D.J., Espevik,T., O'Neill,L.A., & McGettrick,A.F. (2012) The GOLD domain-containing protein TMED7 inhibits TLR4 signalling from the endosome upon LPS stimulation. *Nat Commun.*, **3**, 707.

Easson,E.C. & Russell,M.H. (1963) Cure of Hodgkin's Disease. *Br.Med J*, **1**, 1704-1707.

Elenitoba-Johnson,K.S., Gascoyne,R.D., Lim,M.S., Chhanabai,M., Jaffe,E.S., & Raffeld,M. (1998) Homozygous deletions at chromosome 9p21 involving p16 and p15 are associated with histologic progression in follicle center lymphoma. *Blood*, **91**, 4677-4685.

Elenitoba-Johnson,K.S., Jenson,S.D., Abbott,R.T., Palais,R.A., Bohling,S.D., Lin,Z., Tripp,S., Shami,P.J., Wang,L.Y., Coupland,R.W., Buckstein,R., Perez-Ordonez,B., Perkins,S.L., Dube,I.D., & Lim,M.S. (2003) Involvement of multiple signaling pathways in follicular lymphoma transformation: p38-mitogen-activated protein kinase as a target for therapy. *Proc.Natl.Acad.Sci.U.S.A*, **100**, 7259-7264.

Farinha,P., Masoudi,H., Skinnider,B.F., Shumansky,K., Spinelli,J.J., Gill,K., Klasa,R., Voss,N., Connors,J.M., & Gascoyne,R.D. (2005) Analysis of multiple biomarkers shows that lymphoma-associated macrophage (LAM) content is an independent predictor of survival in follicular lymphoma (FL). *Blood*, **106**, 2169-2174.

Farinha,P., Al-Tourah,A., Gill,K., Klasa,R., Connors,J.M., & Gascoyne,R.D. (2010) The architectural pattern of FOXP3-positive T cells in follicular lymphoma is an independent predictor of survival and histologic transformation. *Blood*, **115**, 289-295.

Fiegler,H., Carr,P., Douglas,E.J., Burford,D.C., Hunt,S., Scott,C.E., Smith,J., Vetrie,D., Gorman,P., Tomlinson,I.P., & Carter,N.P. (2003) DNA microarrays for comparative genomic hybridization based on DOP-PCR amplification of BAC and PAC clones. *Genes Chromosomes Cancer*, **36**, 361-374.

Fisher,R.I., LeBlanc,M., Press,O.W., Maloney,D.G., Unger,J.M., & Miller,T.P. (2005) New treatment options have changed the survival of patients with follicular lymphoma. *Journal of Clinical Oncology*, **23**, 8447-8452.

Fisher,S.G. & Fisher,R.I. (2004) The epidemiology of non-Hodgkin's lymphoma. *Oncogene*, **23**, 6524-6534.

Fitzgibbon,J., Iqbal,S., Davies,A., O'Shea,D., Carlotti,E., Chaplin,T., Matthews,J., Raghavan,M., Norton,A., Lister,T.A., & Young,B.D. (2007) Genome-wide detection of recurring sites of uniparental disomy in follicular and transformed follicular lymphoma. *Leukemia*, **21**, 1514-1520.

Foran,J.M., Apostolidis,J., Papamichael,D., Norton,A.J., Matthews,J., Amess,J.A., Lister,T.A., & Rohatiner,A.Z. (1998) High-dose therapy with autologous haematopoietic support in patients with transformed follicular lymphoma: a study of 27 patients from a single centre. *Ann.Oncol*, **9**, 865-869.

Friedberg,J.W., Neuberg,D., Gribben,J.G., Mauch,P., Anderson,K.C., Soiffer,R.J., Takvorian,T., Fisher,D.C., Schlossman,R., Jallow,H., Kuhlman,C., Ritz,J., & Freedman,A.S. (1999) Autologous bone marrow transplantation after histologic transformation of indolent B cell malignancies. *Biol Blood Marrow Transplant.*, **5**, 262-268.

Garvin,A.J., Simon,R.M., Osborne,C.K., Merrill,J., Young,R.C., & Berard,C.W. (1983) An autopsy study of histologic progression in non-Hodgkin's lymphomas. 192 cases from the National Cancer Institute. *Cancer*, **52**, 393-398.

Gentles,A.J., Alizadeh,A.A., Lee,S.I., Myklebust,J.H., Shachaf,C.M., Shahbaba,B., Levy,R., Koller,D., & Plevritis,S.K. (2009) A pluripotency signature predicts histologic transformation and influences survival in follicular lymphoma patients. *Blood*, **114**, 3158-3166.

Ghielmini,M., Vitolo,U., Kimby,E., Montoto,S., Walewski,J., Pfreundschuh,M., Federico,M., Hoskin,P., McNamara,C., Caligaris-Cappio,F., Stilgenbauer,S., Marcus,R., Trneny,M., Dreger,P., Montserrat,E., & Dreyling,M. (2013) ESMO Guidelines consensus conference on malignant lymphoma 2011 part 1: diffuse large B-cell lymphoma (DLBCL), follicular lymphoma (FL) and chronic lymphocytic leukemia (CLL). *Ann.Oncol*, **24**, 561-576.

Gilman,A. & Philips,F.S. (1946) The biological actions and therapeutic applications of the B-chloroethyl amines and sulfides. *Science*, **103**, 409-415.

Gine,E., Montoto,S., Bosch,F., Arenillas,L., Mercadal,S., Villamor,N., Martinez,A., Colomo,L., Campo,E., Montserrat,E., & Lopez-Guillermo,A. (2006) The Follicular Lymphoma International Prognostic Index (FLIPI) and the histological subtype are the most important factors to predict histological transformation in follicular lymphoma. *Ann.Oncol.*, **17**, 1539-1545.

Gisselbrecht,C., Glass,B., Mounier,N., Singh,G.D., Linch,D.C., Trneny,M., Bosly,A., Ketterer,N., Shpilberg,O., Hagberg,H., Ma,D., Briere,J., Moskowitz,C.H., & Schmitz,N. (2010) Salvage regimens

with autologous transplantation for relapsed large B-cell lymphoma in the rituximab era. *Journal of Clinical Oncology*, **28**, 4184-4190.

Glas,A.M., Knoops,L., Delahaye,L., Kersten,M.J., Kibbelaar,R.E., Wessels,L.A., van,L.R., van Krieken,J.H., Baars,J.W., Raemaekers,J., Kluin,P.M., van't Veer,L.J., & de,J.D. (2007) Gene-expression and immunohistochemical study of specific T-cell subsets and accessory cell types in the transformation and prognosis of follicular lymphoma. *J.Clin.Oncol.*, **25**, 390-398.

Gopal,A.K., Rajendran,J.G., Gooley,T.A., Pagel,J.M., Fisher,D.R., Petersdorf,S.H., Maloney,D.G., Eary,J.F., Appelbaum,F.R., & Press,O.W. (2007) High-Dose [131I]Tositumomab (anti-CD20) Radioimmunotherapy and Autologous Hematopoietic Stem-Cell Transplantation for Adults Γëѐ 60 Years Old With Relapsed or Refractory B-Cell Lymphoma. *Journal of Clinical Oncology*, **25**, 1396-1402.

Gospodarowicz,M.K. (2009) Hodgkin's lymphoma--patient's assessment and staging. *Cancer J*, **15**, 138-142.

Greaves,M. & Maley,C.C. (2012) Clonal evolution in cancer. *Nature*, **481**, 306-313.

Green,M.R., Gentles,A.J., Nair,R.V., Irish,J.M., Kihira,S., Liu,C.L., Kela,I., Hopmans,E.S., Myklebust,J.H., Ji,H., Plevritis,S.K., Levy,R., & Alizadeh,A.A. (2013) Hierarchy in somatic mutations arising during genomic evolution and progression of follicular lymphoma. *Blood*, **121**, 1604-1611.

Gribben,J.G. (2010) Implications of the tumor microenvironment on survival and disease response in follicular lymphoma. *Curr.Opin.Oncol*, **22**, 424-430.

Grivennikov,S.I., Greten,F.R., & Karin,M. (2010) Immunity, inflammation, and cancer. *Cell*, **140**, 883-899.

Hamadani,M., Benson,D.M., Jr., Lin,T.S., Porcu,P., Blum,K.A., & Devine,S.M. (2008) High-dose therapy and autologous stem cell transplantation for follicular lymphoma undergoing transformation to diffuse large B-cell lymphoma. *Eur J Haematol.*, **81**, 425-431.

Hanahan,D. & Weinberg,R.A. (2000) The hallmarks of cancer. *Cell*, **100**, 57-70.

Hanahan,D. & Weinberg,R.A. (2011) Hallmarks of cancer: the next generation. *Cell*, **144**, 646-674.

Hardianti,M.S., Tatsumi,E., Syampurnawati,M., Furuta,K., Saigo,K., Nakamachi,Y., Kumagai,S., Ohno,H., Tanabe,S., Uchida,M., & Yasuda,N. (2004) Activation-induced cytidine deaminase expression in follicular lymphoma: association between AID expression and ongoing mutation in FL. *Leukemia*, **18**, 826-831.

Harris,N.L., Jaffe,E.S., Stein,H., Banks,P.M., Chan,J.K., Cleary,M.L., Delsol,G., De Wolf-Peeters,C., Falini,B., Gatter,K.C., & . (1994) A revised European-American classification of lymphoid neoplasms: a proposal from the International Lymphoma Study Group. *Blood*, **84**, 1361-1392.

Harsini,S., Beigy,M., khavan-Sabbagh,M., & Rezaei,N. (2013) Toll-like receptors in lymphoid malignancies: Double-edged sword. *Crit Rev.Oncol Hematol.*

Hartmann,E.M., Campo,E., Wright,G., Lenz,G., Salaverria,I., Jares,P., Xiao,W., Braziel,R.M., Rimsza,L.M., Chan,W.C., Weisenburger,D.D., Delabie,J., Jaffe,E.S., Gascoyne,R.D., Dave,S.S., Mueller-Hermelink,H.K., Staudt,L.M., Ott,G., Bea,S., & Rosenwald,A. (2010) Pathway discovery in mantle cell lymphoma by integrated analysis of high-resolution gene expression and copy number profiling. *Blood*, **116**, 953-961.

He,L., Thomson,J.M., Hemann,M.T., Hernando-Monge,E., Mu,D., Goodson,S., Powers,S., Cordon-Cardo,C., Lowe,S.W., Hannon,G.J., & Hammond,S.M. (2005) A microRNA polycistron as a potential human oncogene. *Nature*, **435**, 828-833.

Heyzer-Williams,L.J. & Heyzer-Williams,M.G. (2005) Antigen-specific memory B cell development. *Annu.Rev.Immunol.*, **23**, 487-513.

Hiddemann,W., Buske,C., Dreyling,M., Weigert,O., Lenz,G., & Unterhalt,M. (2007) Current management of follicular lymphomas. *Br.J Haematol.*, **136**, 191-202.

Hiddemann,W., Buske,C., Dreyling,M., Weigert,O., Lenz,G., Forstpointner,R., Nickenig,C., & Unterhalt,M. (2005) Treatment Strategies in Follicular Lymphomas: Current Status and Future Perspectives. *Journal of Clinical Oncology*, **23**, 6394-6399.

Hiraga,J., Tomita,A., Sugimoto,T., Shimada,K., Ito,M., Nakamura,S., Kiyoi,H., Kinoshita,T., & Naoe,T. (2009) Down-regulation of CD20 expression in B-cell lymphoma cells after treatment with rituximab-containing combination chemotherapies: its prevalence and clinical significance. *Blood*, **113**, 4885-4893.

Hockenbery,D., Nunez,G., Milliman,C., Schreiber,R.D., & Korsmeyer,S.J. (1990) Bcl-2 is an inner mitochondrial membrane protein that blocks programmed cell death. *Nature*, **348**, 334-336.

Hoglund,M., Sehn,L., Connors,J.M., Gascoyne,R.D., Siebert,R., Sall,T., Mitelman,F., & Horsman,D.E. (2004) Identification of cytogenetic subgroups and karyotypic pathways of clonal evolution in follicular lymphomas. *Genes Chromosomes.Cancer*, **39**, 195-204.

Horn,H., Schmelter,C., Leich,E., Salaverria,I., Katzenberger,T., Ott,M.M., Kalla,J., Romero,M., Siebert,R., Rosenwald,A., & Ott,G. (2011) Follicular lymphoma grade 3B is a distinct neoplasm according to cytogenetic and immunohistochemical profiles. *Haematologica*, **96**, 1327-1334.

Horning,S.J. & Rosenberg,S.A. (1984) The natural history of initially untreated low-grade non-Hodgkin's lymphomas. *The New England Journal of Medicine*, **311**, 1471-1475.

Horsman,D.E., Connors,J.M., Pantzar,T., & Gascoyne,R.D. (2001) Analysis of secondary chromosomal alterations in 165 cases of follicular lymphoma with t(14;18). *Genes Chromosomes.Cancer*, **30**, 375-382.

Hough,R.E., Goepel,J.R., Alcock,H.E., Hancock,B.W., Lorigan,P.C., & Hammond,D.W. (2001) Copy number gain at 12q12-14 may be important in the transformation from follicular lymphoma to diffuse large B cell lymphoma. *Br.J.Cancer*, **84**, 499-503.

Husson,H., Carideo,E.G., Neuberg,D., Schultze,J., Munoz,O., Marks,P.W., Donovan,J.W., Chillemi,A.C., O'Connell,P., & Freedman,A.S. (2002) Gene expression profiling of follicular lymphoma and normal germinal center B cells using cDNA arrays. *Blood*, **99**, 282-289.

Hystad,M.E., Myklebust,J.H., Bo,T.H., Sivertsen,E.A., Rian,E., Forfang,L., Munthe,E., Rosenwald,A., Chiorazzi,M., Jonassen,I., Staudt,L.M., & Smeland,E.B. (2007) Characterization of early stages of human B cell development by gene expression profiling. *J Immunol.*, **179**, 3662-3671.

Illidge,T. & Morschhauser,F. (2011) Radioimmunotherapy in follicular lymphoma. *Best.Pract.Res.Clin Haematol.*, **24**, 279-293.

Inui,S., Kuwahara,K., Mizutani,J., Maeda,K., Kawai,T., Nakayasu,H., & Sakaguchi,N. (1995) Molecular cloning of a cDNA clone encoding a phosphoprotein component related to the Ig receptor-mediated signal transduction. *J Immunol.*, **154**, 2714-2723.

Irish,J.M., Myklebust,J.H., Alizadeh,A.A., Houot,R., Sharman,J.P., Czerwinski,D.K., Nolan,G.P., & Levy,R. (2010) B-cell signaling networks reveal a negative prognostic human lymphoma cell subset that emerges during tumor progression. *Proceedings of the National Academy of Sciences*, **107**, 12747-12754.

Ishkanian,A.S., Malloff,C.A., Watson,S.K., DeLeeuw,R.J., Chi,B., Coe,B.P., Snijders,A., Albertson,D.G., Pinkel,D., Marra,M.A., Ling,V., MacAulay,C., & Lam,W.L. (2004) A tiling resolution DNA microarray with complete coverage of the human genome. *Nat Genet*, **36**, 299-303.

Jaffe,E.S., Harris,N.L., Stein,H., & Isaacson,P.G. (2008) Classification of lymphoid neoplasms: the microscope as a tool for disease discovery. *Blood*, **112**, 4384-4399.

Janeway CA. Immunobiology. Travers, P, Walport, M, and Shlomchik, MJ. The immune system in health and disease. 2005. New York and London, Garland Science, Taylor and Francis Group.
Ref Type: Generic

Jiang,Y., Soong,T.D., Wang,L., Melnick,A.M., & Elemento,O. (2012) Genome-wide detection of genes targeted by non-Ig somatic hypermutation in lymphoma. *PLoS.ONE.*, **7**, e40332.

Johnson,N.A., Al-Tourah,A., Brown,C.J., Connors,J.M., Gascoyne,R.D., & Horsman,D.E. (2008) Prognostic significance of secondary cytogenetic alterations in follicular lymphomas. *Genes Chromosomes Cancer*, **47**, 1038-1048.

Kalia,V., Sarkar,S., Gourley,T.S., Rouse,B.T., & Ahmed,R. (2006) Differentiation of memory B and T cells. *Curr.Opin.Immunol.*, **18**, 255-264.

Kaminski,M.S., Zelenetz,A.D., Press,O.W., Saleh,M., Leonard,J., Fehrenbacher,L., Lister,T.A., Stagg,R.J., Tidmarsh,G.F., Kroll,S., Wahl,R.L., Knox,S.J., & Vose,J.M. (2001) Pivotal study of iodine I 131 tositumomab for chemotherapy-refractory low-grade or transformed low-grade B-cell non-Hodgkin's lymphomas. *Journal of Clinical Oncology*, **19**, 3918-3928.

Kaplan,H.S. & Rosenberg,S.A. (1966) Extended-field radical radiotherapy in advanced Hodgkin's disease: short-term results of 2 randomized clinical trials. *Cancer Res.*, **26**, 1268-1276.

Keegan,T.H., McClure,L.A., Foran,J.M., & Clarke,C.A. (2009) Improvements in survival after follicular lymphoma by race/ethnicity and socioeconomic status: a population-based study. *Journal of Clinical Oncology*, **27**, 3044-3051.

Khouri,I.F. (2011) Allogeneic stem cell transplantation in follicular lymphoma. *Best.Pract.Res.Clin Haematol.*, **24**, 271-277.

Kinzler,K.W. & Vogelstein,B. (1996) Lessons from hereditary colorectal cancer. *Cell*, **87**, 159-170.

Klein,U. & Ia-Favera,R. (2008) Germinal centres: role in B-cell physiology and malignancy. *Nat Rev.Immunol.*, **8**, 22-33.

Kuppers,R. (2005) Mechanisms of B-cell lymphoma pathogenesis. *Nat Rev Cancer*, **5**, 251-262.

Kurosaki,T., Shinohara,H., & Baba,Y. (2010) B cell signaling and fate decision. *Annu.Rev.Immunol.*, **28**, 21-55.

Landau,D.A. & Slack,F.J. (2011) MicroRNAs in mutagenesis, genomic instability, and DNA repair. *Semin.Oncol*, **38**, 743-751.

LeBien,T.W. & Tedder,T.F. (2008) B lymphocytes: how they develop and function. *Blood*, **112**, 1570-1580.

Leich,E., Salaverria,I., Bea,S., Zettl,A., Wright,G., Moreno,V., Gascoyne,R.D., Chan,W.C., Braziel,R.M., Rimsza,L.M., Weisenburger,D.D., Delabie,J., Jaffe,E.S., Lister,A., Fitzgibbon,J., Staudt,L.M., Hartmann,E.M., Mueller-Hermelink,H.K., Campo,E., Ott,G., & Rosenwald,A. (2009) Follicular lymphomas with and without translocation t(14;18) differ in gene expression profiles and genetic alterations. *Blood*, **114**, 826-834.

Lengauer,C., Kinzler,K.W., & Vogelstein,B. (1998) Genetic instabilities in human cancers. *Nature*, **396**, 643-649.

Lennert,K. Malignant Lymphomas Other than Hodgkin's disease. 1978. New York, Springer-Verlag. Ref Type: Generic

Lennert,K. Histopathology of Non-Hodgkin's Lymphomas. 2nd ed. 1992. New York, Springer-Verlag. Ref Type: Generic

Lenz,G., Davis,R.E., Ngo,V.N., Lam,L., George,T.C., Wright,G.W., Dave,S.S., Zhao,H., Xu,W., Rosenwald,A., Ott,G., Muller-Hermelink,H.K., Gascoyne,R.D., Connors,J.M., Rimsza,L.M., Campo,E., Jaffe,E.S., Delabie,J., Smeland,E.B., Fisher,R.I., Chan,W.C., & Staudt,L.M. (2008a) Oncogenic CARD11 mutations in human diffuse large B cell lymphoma. *Science*, **319**, 1676-1679.

Lenz,G. & Staudt,L.M. (2010) Aggressive lymphomas. *The New England Journal of Medicine*, **362**, 1417-1429.

Lenz,G., Wright,G., Dave,S.S., Xiao,W., Powell,J., Zhao,H., Xu,W., Tan,B., Goldschmidt,N., Iqbal,J., Vose,J., Bast,M., Fu,K., Weisenburger,D.D., Greiner,T.C., Armitage,J.O., Kyle,A., May,L., Gascoyne,R.D., Connors,J.M., Troen,G., Holte,H., Kvaloy,S., Dierickx,D., Verhoef,G., Delabie,J., Smeland,E.B., Jares,P., Martinez,A., Lopez-Guillermo,A., Montserrat,E., Campo,E., Braziel,R.M., Miller,T.P., Rimsza,L.M., Cook,J.R., Pohlman,B., Sweetenham,J., Tubbs,R.R., Fisher,R.I., Hartmann,E., Rosenwald,A., Ott,G., Muller-Hermelink,H.K., Wrench,D., Lister,T.A., Jaffe,E.S., Wilson,W.H., Chan,W.C., & Staudt,L.M. (2008b) Stromal gene signatures in large-B-cell lymphomas. *The New England Journal of Medicine*, **359**, 2313-2323.

Lenz,G., Wright,G.W., Emre,N.C., Kohlhammer,H., Dave,S.S., Davis,R.E., Carty,S., Lam,L.T., Shaffer,A.L., Xiao,W., Powell,J., Rosenwald,A., Ott,G., Muller-Hermelink,H.K., Gascoyne,R.D., Connors,J.M., Campo,E., Jaffe,E.S., Delabie,J., Smeland,E.B., Rimsza,L.M., Fisher,R.I., Weisenburger,D.D., Chan,W.C., & Staudt,L.M. (2008c) Molecular subtypes of diffuse large B-cell lymphoma arise by distinct genetic pathways. *Proc.Natl.Acad.Sci.U.S.A*, **105**, 13520-13525.

Lestou,V.S., Gascoyne,R.D., Sehn,L., Ludkovski,O., Chhanabhai,M., Klasa,R.J., Husson,H., Freedman,A.S., Connors,J.M., & Horsman,D.E. (2003) Multicolour fluorescence in situ hybridization analysis of t(14;18)-positive follicular lymphoma and correlation with gene expression data and clinical outcome. *Br.J.Haematol.*, **122**, 745-759.

Limpens,J., de,J.D., van Krieken,J.H., Price,C.G., Young,B.D., van Ommen,G.J., & Kluin,P.M. (1991) Bcl-2/JH rearrangements in benign lymphoid tissues with follicular hyperplasia. *Oncogene*, **6**, 2271-2276.

Link,B.K., Maurer,M.J., Nowakowski,G.S., Ansell,S.M., Macon,W.R., Syrbu,S.I., Slager,S.L., Thompson,C.A., Inwards,D.J., Johnston,P.B., Colgan,J.P., Witzig,T.E., Habermann,T.M., & Cerhan,J.R. (2013) Rates and outcomes of follicular lymphoma transformation in the immunochemotherapy era: a report

from the University of Iowa/MayoClinic Specialized Program of Research Excellence Molecular Epidemiology Resource. *Journal of Clinical Oncology*, **31**, 3272-3278.

Lippitz,B.E. (2013) Cytokine patterns in patients with cancer: a systematic review. *Lancet Oncol*, **14**, e218-e228.

Liu,Y., Sattarzadeh,A., Diepstra,A., Visser,L., & van den,B.A. (2013) The microenvironment in classical Hodgkin lymphoma: An actively shaped and essential tumor component. *Semin.Cancer Biol.*

Lord,C.J. & Ashworth,A. (2012) The DNA damage response and cancer therapy. *Nature*, **481**, 287-294.

Lossos,I.S., Alizadeh,A.A., Diehn,M., Warnke,R., Thorstenson,Y., Oefner,P.J., Brown,P.O., Botstein,D., & Levy,R. (2002) Transformation of follicular lymphoma to diffuse large-cell lymphoma: alternative patterns with increased or decreased expression of c-myc and its regulated genes. *Proc.Natl.Acad.Sci.U.S.A*, **99**, 8886-8891.

Lossos,I.S., Czerwinski,D.K., Alizadeh,A.A., Wechser,M.A., Tibshirani,R., Botstein,D., & Levy,R. (2004) Prediction of survival in diffuse large-B-cell lymphoma based on the expression of six genes. *The New England Journal of Medicine*, **350**, 1828-1837.

Lovat,F., Valeri,N., & Croce,C.M. (2011) MicroRNAs in the pathogenesis of cancer. *Semin.Oncol*, **38**, 724-733.

Luminari,S., Cesaretti,M., Rashid,I., Mammi,C., Montanini,A., Barbolini,E., Bellei,M., Pennese,E., Sirotti,M.A., Marcheselli,L., Partesotti,G., Bari,A., Maiorana,A., Bonacorsi,G., & Federico,M. (2007) Incidence, clinical characteristics and survival of malignant lymphomas: a population-based study from a cancer registry in northern Italy. *Hematol.Oncol*, **25**, 189-197.

Maciejewski,J.P. & Padgett,R.A. (2012) Defects in spliceosomal machinery: a new pathway of leukaemogenesis. *Br.J Haematol.*, **158**, 165-173.

Martin,A.R., Weisenburger,D.D., Chan,W.C., Ruby,E.I., Anderson,J.R., Vose,J.M., Bierman,P.J., Bast,M.A., Daley,D.T., & Armitage,J.O. (1995) Prognostic value of cellular proliferation and histologic grade in follicular lymphoma. *Blood*, **85**, 3671-3678.

Martinez-Climent,J.A., Alizadeh,A.A., Segraves,R., Blesa,D., Rubio-Moscardo,F., Albertson,D.G., Garcia-Conde,J., Dyer,M.J., Levy,R., Pinkel,D., & Lossos,I.S. (2003) Transformation of follicular lymphoma to diffuse large cell lymphoma is associated with a heterogeneous set of DNA copy number and gene expression alterations. *Blood*, **101**, 3109-3117.

Matolcsy,A., Schattner,E.J., Knowles,D.M., & Casali,P. (1999) Clonal evolution of B cells in transformation from low- to high-grade lymphoma. *Eur.J Immunol.*, **29**, 1253-1264.

McCabe,M.T., Ott,H.M., Ganji,G., Korenchuk,S., Thompson,C., Van Aller,G.S., Liu,Y., Graves,A.P., Della,P.A., III, Diaz,E., LaFrance,L.V., Mellinger,M., Duquenne,C., Tian,X., Kruger,R.G., McHugh,C.F., Brandt,M., Miller,W.H., Dhanak,D., Verma,S.K., Tummino,P.J., & Creasy,C.L. (2012) EZH2 inhibition as a therapeutic strategy for lymphoma with EZH2-activating mutations. *Nature*, **492**, 108-112.

McDonald,H.L., Gascoyne,R.D., Horsman,D., & Brown,C.J. (2000) Involvement of the X chromosome in non-Hodgkin lymphoma. *Genes Chromosomes Cancer*, **28**, 246-257.

McDonnell,T.J., Deane,N., Platt,F.M., Nunez,G., Jaeger,U., McKearn,J.P., & Korsmeyer,S.J. (1989) bcl-2-immunoglobulin transgenic mice demonstrate extended B cell survival and follicular lymphoproliferation. *Cell*, **57**, 79-88.

McGettrick,A.F. & O'Neill,L.A. (2007) Toll-like receptors: key activators of leucocytes and regulator of haematopoiesis. *Br.J Haematol.*, **139**, 185-193.

Meza-Zepeda,L.A., Kresse,S.H., Barragan-Polania,A.H., Bjerkehagen,B., Ohnstad,H.O., Namlos,H.M., Wang,J., Kristiansen,B.E., & Myklebost,O. (2006) Array comparative genomic hybridization reveals distinct DNA copy number differences between gastrointestinal stromal tumors and leiomyosarcomas. *Cancer Res.*, **66**, 8984-8993.

Mohamed,A.N., Palutke,M., Eisenberg,L., & Al-Katib,A. (2001) Chromosomal analyses of 52 cases of follicular lymphoma with t(14;18), including blastic/blastoid variant. *Cancer Genet.Cytogenet.*, **126**, 45-51.

Molina,A. (2008) A decade of rituximab: improving survival outcomes in non-Hodgkin's lymphoma. *Annu.Rev.Med.*, **59**, 237-250.

Molyneux,E.M., Rochford,R., Griffin,B., Newton,R., Jackson,G., Menon,G., Harrison,C.J., Israels,T., & Bailey,S. (2012) Burkitt's lymphoma. *Lancet*, **379**, 1234-1244.

Montoto,S., Corradini,P., Dreyling,M., Ghielmini,M., Kimby,E., Lopez-Guillermo,A., Mackinnon,S., Marcus,R.E., Salles,G., Schouten,H.C., Sureda,A., & Dreger,P. (2013) Indications for hematopoietic stem cell transplantation in patients with follicular lymphoma: a consensus project of the EBMT-Lymphoma Working Party. *Haematologica*, **98**, 1014-1021.

Montoto,S., Davies,A.J., Matthews,J., Calaminici,M., Norton,A.J., Amess,J., Vinnicombe,S., Waters,R., Rohatiner,A.Z., & Lister,T.A. (2007) Risk and clinical implications of transformation of follicular lymphoma to diffuse large B-cell lymphoma. *Journal of Clinical Oncology*, **25**, 2426-2433.

Morin,R.D., Johnson,N.A., Severson,T.M., Mungall,A.J., An,J., Goya,R., Paul,J.E., Boyle,M., Woolcock,B.W., Kuchenbauer,F., Yap,D., Humphries,R.K., Griffith,O.L., Shah,S., Zhu,H., Kimbara,M., Shashkin,P., Charlot,J.F., Tcherpakov,M., Corbett,R., Tam,A., Varhol,R., Smailus,D., Moksa,M., Zhao,Y., Delaney,A., Qian,H., Birol,I., Schein,J., Moore,R., Holt,R., Horsman,D.E., Connors,J.M., Jones,S., Aparicio,S., Hirst,M., Gascoyne,R.D., & Marra,M.A. (2010) Somatic mutations altering EZH2

(Tyr641) in follicular and diffuse large B-cell lymphomas of germinal-center origin. *Nat Genet*, **42**, 181-185.

Morin,R.D., Mendez-Lago,M., Mungall,A.J., Goya,R., Mungall,K.L., Corbett,R.D., Johnson,N.A., Severson,T.M., Chiu,R., Field,M., Jackman,S., Krzywinski,M., Scott,D.W., Trinh,D.L., Tamura-Wells,J., Li,S., Firme,M.R., Rogic,S., Griffith,M., Chan,S., Yakovenko,O., Meyer,I.M., Zhao,E.Y., Smailus,D., Moksa,M., Chittaranjan,S., Rimsza,L., Brooks-Wilson,A., Spinelli,J.J., Ben-Neriah,S., Meissner,B., Woolcock,B., Boyle,M., McDonald,H., Tam,A., Zhao,Y., Delaney,A., Zeng,T., Tse,K., Butterfield,Y., Birol,I., Holt,R., Schein,J., Horsman,D.E., Moore,R., Jones,S.J.M., Connors,J.M., Hirst,M., Gascoyne,R.D., & Marra,M.A. (2011) Frequent mutation of histone-modifying genes in non-Hodgkin lymphoma. *Nature*, **476**, 298-303.

Mortensen,B.K., Petersen,S.L., Kornblit,B., Andersen,P.K., Braendstrup,P., Andersen,N.S., Sengelov,H., & Vindelov,L. (2012) Single-institution long-term outcomes for patients receiving nonmyeloablative conditioning hematopoeitic cell transplantation for chronic lymphocytic leukemia and follicular lymphoma. *Eur.J Haematol.*, **89**, 151-159.

Morton,L.M., Wang,S.S., Devesa,S.S., Hartge,P., Weisenburger,D.D., & Linet,M.S. (2006) Lymphoma incidence patterns by WHO subtype in the United States, 1992-2001. *Blood*, **107**, 265-276.

Mottok,A., Renne,C., Seifert,M., Oppermann,E., Bechstein,W., Hansmann,M.L., Kuppers,R., & Brauninger,A. (2009) Inactivating SOCS1 mutations are caused by aberrant somatic hypermutation and restricted to a subset of B-cell lymphoma entities. *Blood*, **114**, 4503-4506.

Motz,G.T. & Coukos,G. (2013) Deciphering and reversing tumor immune suppression. *Immunity.*, **39**, 61-73.

Muller,A.M., Ihorst,G., Mertelsmann,R., & Engelhardt,M. (2005) Epidemiology of non-Hodgkin's lymphoma (NHL): trends, geographic distribution, and etiology. *Ann.Hematol.*, **84**, 1-12.

Murray,F., Darzentas,N., Hadzidimitriou,A., Tobin,G., Boudjogra,M., Scielzo,C., Laoutaris,N., Karlsson,K., Baran-Marzsak,F., Tsaftaris,A., Moreno,C., Anagnostopoulos,A., Caligaris-Cappio,F., Vaur,D., Ouzounis,C., Belessi,C., Ghia,P., Davi,F., Rosenquist,R., & Stamatopoulos,K. (2008) Stereotyped patterns of somatic hypermutation in subsets of patients with chronic lymphocytic leukemia: implications for the role of antigen selection in leukemogenesis. *Blood*, **111**, 1524-1533.

Myklebust,J.H., Irish,J.M., Brody,J., Czerwinski,D.K., Houot,R., Kohrt,H.E., Timmerman,J., Said,J., Green,M.R., Delabie,J., Kolstad,A., Alizadeh,A.A., & Levy,R. (2013) High PD-1 expression and suppressed cytokine signaling distinguish T cells infiltrating follicular lymphoma tumors from peripheral T cells. *Blood*, **121**, 1367-1376.

Nagy,M., Balazs,M., Adam,Z., Petko,Z., Timar,B., Szereday,Z., Laszlo,T., Warnke,R.A., & Matolcsy,A. (2000) Genetic instability is associated with histological transformation of follicle center lymphoma. *Leukemia*, **14**, 2142-2148.

Natkunam,Y. (2007) The biology of the germinal center. *Hematology.Am.Soc.Hematol.Educ.Program.*, **2007**, 210-215.

Ngan,B.Y., Chen-Levy,Z., Weiss,L.M., Warnke,R.A., & Cleary,M.L. (1988) Expression in non-Hodgkin's lymphoma of the bcl-2 protein associated with the t(14;18) chromosomal translocation. *The New England Journal of Medicine*, **318**, 1638-1644.

Ngo,V.N., Young,R.M., Schmitz,R., Jhavar,S., Xiao,W., Lim,K.H., Kohlhammer,H., Xu,W., Yang,Y., Zhao,H., Shaffer,A.L., Romesser,P., Wright,G., Powell,J., Rosenwald,A., Muller-Hermelink,H.K., Ott,G., Gascoyne,R.D., Connors,J.M., Rimsza,L.M., Campo,E., Jaffe,E.S., Delabie,J., Smeland,E.B., Fisher,R.I., Braziel,R.M., Tubbs,R.R., Cook,J.R., Weisenburger,D.D., Chan,W.C., & Staudt,L.M. (2011) Oncogenically active MYD88 mutations in human lymphoma. *Nature*, **470**, 115-119.

Nilsen,G., Liestol,K., Van,L.P., Moen Vollan,H.K., Eide,M.B., Rueda,O.M., Chin,S.F., Russell,R., Baumbusch,L.O., Caldas,C., Borresen-Dale,A.L., & Lingjaerde,O.C. (2012) Copynumber: Efficient algorithms for single- and multi-track copy number segmentation. *BMC.Genomics*, **13**, 591.

Nunez,G., Seto,M., Seremetis,S., Ferrero,D., Grignani,F., Korsmeyer,S.J., & la-Favera,R. (1989) Growth- and tumor-promoting effects of deregulated BCL2 in human B-lymphoblastoid cells. *Proc.Natl.Acad.Sci.U.S.A*, **86**, 4589-4593.

O'Shea,D., O'Riain,C., Gupta,M., Waters,R., Yang,Y., Wrench,D., Gribben,J., Rosenwald,A., Ott,G., Rimsza,L.M., Holte,H., Cazier,J.B., Johnson,N.A., Campo,E., Chan,W.C., Gascoyne,R.D., Young,B.D., Staudt,L.M., Lister,T.A., & Fitzgibbon,J. (2009) Regions of acquired uniparental disomy at diagnosis of follicular lymphoma are associated with both overall survival and risk of transformation. *Blood*, blood-2008.

Oeschger,S., Br+ñuninger,A., K++ppers,R., & Hansmann,M.L. (2002) Tumor cell dissemination in follicular lymphoma. *Blood*, **99**, 2192-2198.

Ott,G., Katzenberger,T., Lohr,A., Kindelberger,S., Rudiger,T., Wilhelm,M., Kalla,J., Rosenwald,A., Muller,J.G., Ott,M.M., & Muller-Hermelink,H.K. (2002) Cytomorphologic, immunohistochemical, and cytogenetic profiles of follicular lymphoma: 2 types of follicular lymphoma grade 3. *Blood*, **99**, 3806-3812.

Pasqualucci,L., Bhagat,G., Jankovic,M., Compagno,M., Smith,P., Muramatsu,M., Honjo,T., Morse,H.C., III, Nussenzweig,M.C., & la-Favera,R. (2008) AID is required for germinal center-derived lymphomagenesis. *Nat Genet.*, **40**, 108-112.

Patel,R.A., Liu,Y., Wang,B., Li,R., & Sebti,S.M. (2013) Identification of novel ROCK inhibitors with anti-migratory and anti-invasive activities. *Oncogene*.

Philip,T., Guglielmi,C., Hagenbeek,A., Somers,R., Van Der Lelie,H., Bron,D., Sonneveld,P., Gisselbrecht,C., Cahn,J.Y., Harousseau,J.L., Coiffier,B., Biron,P., Mandelli,F., & Chauvin,F. (1995) Autologous Bone

Marrow Transplantation as Compared with Salvage Chemotherapy in Relapses of Chemotherapy-Sensitive Non-Hodgkin's Lymphoma. *The New England Journal of Medicine*, **333**, 1540-1545.

Picard,F., Robin,S., Lavielle,M., Vaisse,C., & Daudin,J.J. (2005) A statistical approach for array CGH data analysis. *BMC.Bioinformatics.*, **6**, 27.

Piccaluga,P.P., Califano,A., Klein,U., Agostinelli,C., Bellosillo,B., Gimeno,E., Serrano,S., Sole,F., Zang,Y., Falini,B., Zinzani,P.L., & Pileri,S.A. (2008) Gene expression analysis provides a potential rationale for revising the histological grading of follicular lymphomas. *Haematologica*, **93**, 1033-1038.

Pinkel,D., Segraves,R., Sudar,D., Clark,S., Poole,I., Kowbel,D., Collins,C., Kuo,W.L., Chen,C., Zhai,Y., Dairkee,S.H., Ljung,B.M., Gray,J.W., & Albertson,D.G. (1998) High resolution analysis of DNA copy number variation using comparative genomic hybridization to microarrays. *Nat Genet*, **20**, 207-211.

Pollack,J.R., Sorlie,T., Perou,C.M., Rees,C.A., Jeffrey,S.S., Lonning,P.E., Tibshirani,R., Botstein,D., Borresen-Dale,A.L., & Brown,P.O. (2002) Microarray analysis reveals a major direct role of DNA copy number alteration in the transcriptional program of human breast tumors. *Proc.Natl.Acad.Sci.U.S.A*, **99**, 12963-12968.

Pretorius,M.E., Waehre,H., Abeler,V.M., Davidson,B., Vlatkovic,L., Lothe,R.A., Giercksky,K.E., & Danielsen,H.E. (2009) Large scale genomic instability as an additive prognostic marker in early prostate cancer. *Cell Oncol*, **31**, 251-259.

Quackenbush,J. (2006) Microarray analysis and tumor classification. *The New England Journal of Medicine*, **354**, 2463-2472.

Rabkin,C.S., Hirt,C., Janz,S., & Dolken,G. (2008) t(14;18) Translocations and risk of follicular lymphoma. *J Natl.Cancer Inst.Monogr*, 48-51.

Raghavan,S.C., Swanson,P.C., Ma,Y., & Lieber,M.R. (2005) Double-strand break formation by the RAG complex at the bcl-2 major breakpoint region and at other non-B DNA structures in vitro. *Mol.Cell Biol.*, **25**, 5904-5919.

Raghavan,S.C., Swanson,P.C., Wu,X., Hsieh,C.L., & Lieber,M.R. (2004) A non-B-DNA structure at the Bcl-2 major breakpoint region is cleaved by the RAG complex. *Nature*, **428**, 88-93.

Rappaport,H. "Atlas of tumor pathology". Tumors of the hematopoietic system. 241-243. 1966. Washington, DC: Armed Forces Institute of Pathology.
Ref Type: Generic

Rickert,R.C. (2013) New insights into pre-BCR and BCR signalling with relevance to B cell malignancies. *Nat Rev.Immunol.*, **13**, 578-591.

Rosebeck,S., Madden,L., Jin,X., Gu,S., Apel,I.J., Appert,A., Hamoudi,R.A., Noels,H., Sagaert,X., Van,L.P., Baens,M., Du,M.Q., Lucas,P.C., & lister-Lucas,L.M. (2011) Cleavage of NIK by the API2-MALT1 fusion oncoprotein leads to noncanonical NF-kappaB activation. *Science*, **331**, 468-472.

Rosenberg,S.A., Moore,M.R., Bull,J.M., Jones,S.E., & Kaplan,H.S. (1972) Combination chemotherapy and radiotherapy for Hodgkin's disease. *Cancer*, **30**, 1505-1510.

Rosenwald,A., Wright,G., Leroy,K., Yu,X., Gaulard,P., Gascoyne,R.D., Chan,W.C., Zhao,T., Haioun,C., Greiner,T.C., Weisenburger,D.D., Lynch,J.C., Vose,J., Armitage,J.O., Smeland,E.B., Kvaloy,S., Holte,H., Delabie,J., Campo,E., Montserrat,E., Lopez-Guillermo,A., Ott,G., Muller-Hermelink,H.K., Connors,J.M., Braziel,R., Grogan,T.M., Fisher,R.I., Miller,T.P., LeBlanc,M., Chiorazzi,M., Zhao,H., Yang,L., Powell,J., Wilson,W.H., Jaffe,E.S., Simon,R., Klausner,R.D., & Staudt,L.M. (2003) Molecular diagnosis of primary mediastinal B cell lymphoma identifies a clinically favorable subgroup of diffuse large B cell lymphoma related to Hodgkin lymphoma. *J Exp.Med*, **198**, 851-862.

Ross,C.W., Ouillette,P.D., Saddler,C.M., Shedden,K.A., & Malek,S.N. (2007) Comprehensive Analysis of Copy Number and Allele Status Identifies Multiple Chromosome Defects Underlying Follicular Lymphoma Pathogenesis. *Clinical Cancer Research*, **13**, 4777-4785.

Ruminy,P., Jardin,F., Picquenot,J.M., Parmentier,F., Contentin,N., Buchonnet,G., Tison,S., Rainville,V., Tilly,H., & Bastard,C. (2008) S{micro} mutation patterns suggest different progression pathways in follicular lymphoma: early direct or late from FL progenitor-cells. *Blood*.

Russnes,H.G., Vollan,H.K., Lingjaerde,O.C., Krasnitz,A., Lundin,P., Naume,B., Sorlie,T., Borgen,E., Rye,I.H., Langerod,A., Chin,S.F., Teschendorff,A.E., Stephens,P.J., Maner,S., Schlichting,E., Baumbusch,L.O., Karesen,R., Stratton,M.P., Wigler,M., Caldas,C., Zetterberg,A., Hicks,J., & Borresen-Dale,A.L. (2010) Genomic architecture characterizes tumor progression paths and fate in breast cancer patients. *Sci.Transl.Med*, **2**, 38ra47.

Sabloff,M., Atkins,H.L., ce-Bruckler,I., Bredeson,C., Fergusson,D., Genest,P., Hopkins,H., Hutton,B., Mcdiarmid,S., & Huebsch,L.B. (2007) A 15-year analysis of early and late autologous hematopoietic stem cell transplant in relapsed, aggressive, transformed, and nontransformed follicular lymphoma. *Biol.Blood Marrow Transplant.*, **13**, 956-964.

Sakashita,S., Li,D., Nashima,N., Minami,Y., Furuya,S., Morishita,Y., Tachibana,K., Sato,Y., & Noguchi,M. (2011) Overexpression of immunoglobulin (CD79a) binding protein1 (IGBP-1) in small lung adenocarcinomas and its clinicopathological significance. *Pathol.Int.*, **61**, 130-137.

Salles,G.A. (2007) Clinical features, prognosis and treatment of follicular lymphoma. *Hematology.Am.Soc.Hematol.Educ.Program.*, 216-225.

Sawas,A., Diefenbach,C., & O'Connor,O.A. (2011) New therapeutic targets and drugs in non-Hodgkin's lymphoma. *Curr.Opin.Hematol.*, **18**, 280-287.

Schaaf,M., Reiser,M., Borchmann,P., Engert,A., & Skoetz,N. (2012) High-dose therapy with autologous stem cell transplantation versus chemotherapy or immuno-chemotherapy for follicular lymphoma in adults. *Cochrane.Database.Syst.Rev.*, **1**, CD007678.

Schwaenen,C., Viardot,A., Berger,H., Barth,T.F., Bentink,S., Dohner,H., Enz,M., Feller,A.C., Hansmann,M.L., Hummel,M., Kestler,H.A., Klapper,W., Kreuz,M., Lenze,D., Loeffler,M., Moller,P., Muller-Hermelink,H.K., Ott,G., Rosolowski,M., Rosenwald,A., Ruf,S., Siebert,R., Spang,R., Stein,H., Truemper,L., Lichter,P., Bentz,M., & Wessendorf,S. (2009) Microarray-based genomic profiling reveals novel genomic aberrations in follicular lymphoma which associate with patient survival and gene expression status. *Genes Chromosomes Cancer*, **48**, 39-54.

Sehn,L.H., Donaldson,J., Chhanabhai,M., Fitzgerald,C., Gill,K., Klasa,R., MacPherson,N., O'Reilly,S., Spinelli,J.J., Sutherland,J., Wilson,K.S., Gascoyne,R.D., & Connors,J.M. (2005) Introduction of combined CHOP plus rituximab therapy dramatically improved outcome of diffuse large B-cell lymphoma in British Columbia. *Journal of Clinical Oncology*, **23**, 5027-5033.

Shaffer,A.L., Rosenwald,A., Hurt,E.M., Giltnane,J.M., Lam,L.T., Pickeral,O.K., & Staudt,L.M. (2001) Signatures of the immune response. *Immunity.*, **15**, 375-385.

Shaffer,A.L., Wright,G., Yang,L., Powell,J., Ngo,V., Lamy,L., Lam,L.T., Davis,R.E., & Staudt,L.M. (2006) A library of gene expression signatures to illuminate normal and pathological lymphoid biology. *Immunol.Rev.*, **210**, 67-85.

Shipp,M.A., Ross,K.N., Tamayo,P., Weng,A.P., Kutok,J.L., Aguiar,R.C., Gaasenbeek,M., Angelo,M., Reich,M., Pinkus,G.S., Ray,T.S., Koval,M.A., Last,K.W., Norton,A., Lister,T.A., Mesirov,J., Neuberg,D.S., Lander,E.S., Aster,J.C., & Golub,T.R. (2002) Diffuse large B-cell lymphoma outcome prediction by gene-expression profiling and supervised machine learning. *Nat Med*, **8**, 68-74.

Shustik,J., Quinn,M., Connors,J.M., Gascoyne,R.D., Skinnider,B., & Sehn,L.H. (2011) Follicular non-Hodgkin lymphoma grades 3A and 3B have a similar outcome and appear incurable with anthracycline-based therapy. *Annals of Oncology*, **22**, 1164-1169.

Slamon,D.J., Clark,G.M., Wong,S.G., Levin,W.J., Ullrich,A., & McGuire,W.L. (1987) Human breast cancer: correlation of relapse and survival with amplification of the HER-2/neu oncogene. *Science*, **235**, 177-182.

Snijders,A.M., Nowak,N., Segraves,R., Blackwood,S., Brown,N., Conroy,J., Hamilton,G., Hindle,A.K., Huey,B., Kimura,K., Law,S., Myambo,K., Palmer,J., Ylstra,B., Yue,J.P., Gray,J.W., Jain,A.N., Pinkel,D., & Albertson,D.G. (2001) Assembly of microarrays for genome-wide measurement of DNA copy number. *Nat Genet*, **29**, 263-264.

Solal-Celigny,P., Roy,P., Colombat,P., White,J., Armitage,J.O., rranz-Saez,R., Au,W.Y., Bellei,M., Brice,P., Caballero,D., Coiffier,B., Conde-Garcia,E., Doyen,C., Federico,M., Fisher,R.I., Garcia-Conde,J.F., Guglielmi,C., Hagenbeek,A., Haioun,C., LeBlanc,M., Lister,A.T., Lopez-Guillermo,A., McLaughlin,P., Milpied,N., Morel,P., Mounier,N., Proctor,S.J., Rohatiner,A., Smith,P.,

Soubeyran,P., Tilly,H., Vitolo,U., Zinzani,P.L., Zucca,E., & Montserrat,E. (2004) Follicular lymphoma international prognostic index. *Blood*, **104**, 1258-1265.

Solinas-Toldo,S., Lampel,S., Stilgenbauer,S., Nickolenko,J., Benner,A., Dohner,H., Cremer,T., & Lichter,P. (1997) Matrix-based comparative genomic hybridization: biochips to screen for genomic imbalances. *Genes Chromosomes Cancer*, **20**, 399-407.

Suva,M.L., Riggi,N., & Bernstein,B.E. (2013) Epigenetic reprogramming in cancer. *Science*, **339**, 1567-1570.

Swenson,W.T., Wooldridge,J.E., Lynch,C.F., Forman-Hoffman,V.L., Chrischilles,E., & Link,B.K. (2005) Improved Survival of Follicular Lymphoma Patients in the United States. *Journal of Clinical Oncology*, **23**, 5019-5026.

Swerdlow SH. WHO classification of Tumors of Haematopoietic and Lymphoid Tissues (ed 4th). Campo, E., Harris, N. L., Jaffe, E. S., Pileri, S. A., Stein, H., Thiele, J, and Vardiman, J. W. 2008. Lyon, IARC Press.

Symonds,H., Krall,L., Remington,L., Saenz-Robles,M., Lowe,S., Jacks,T., & Van,D.T. (1994) p53-dependent apoptosis suppresses tumor growth and progression in vivo. *Cell*, **78**, 703-711.

Tagawa,H., Suguro,M., Tsuzuki,S., Matsuo,K., Karnan,S., Ohshima,K., Okamoto,M., Morishima,Y., Nakamura,S., & Seto,M. (2005) Comparison of genome profiles for identification of distinct subgroups of diffuse large B-cell lymphoma. *Blood*, **106**, 1770-1777.

Taskinen,M., Karjalainen-Lindsberg,M.L., & Leppa,S. (2008) Prognostic influence of tumor-infiltrating mast cells in patients with follicular lymphoma treated with rituximab and CHOP. *Blood*, **111**, 4664-4667.

The Non-Hodgkin's Lymphoma Pathologic Classification Project (1982) National Cancer Institute sponsored study of classifications of non-Hodgkin's lymphomas: summary and description of a working formulation for clinical usage. The Non-Hodgkin's Lymphoma Pathologic Classification Project. *Cancer*, **49**, 2112-2135.

Tilly,H., Rossi,A., Stamatoullas,A., Lenormand,B., Bigorgne,C., Kunlin,A., Monconduit,M., & Bastard,C. (1994) Prognostic value of chromosomal abnormalities in follicular lymphoma. *Blood*, **84**, 1043-1049.

Tsai,A.G. & Lieber,M.R. (2010) Mechanisms of chromosomal rearrangement in the human genome. *BMC.Genomics*, **11 Suppl 1**, S1.

Tsujimoto,Y. & Croce,C.M. (1986) Analysis of the structure, transcripts, and protein products of bcl-2, the gene involved in human follicular lymphoma. *Proc.Natl.Acad.Sci.U.S.A*, **83**, 5214-5218.

Tsujimoto,Y., Finger,L.R., Yunis,J., Nowell,P.C., & Croce,C.M. (1984) Cloning of the chromosome breakpoint of neoplastic B cells with the t(14;18) chromosome translocation. *Science*, **226**, 1097-1099.

van,B.K. & Schouten,H. (2007) Follicular lymphoma: a historical overview. *Leuk.Lymphoma*, **48**, 232-243.

Van,L.P., Nordgard,S.H., Lingjaerde,O.C., Russnes,H.G., Rye,I.H., Sun,W., Weigman,V.J., Marynen,P., Zetterberg,A., Naume,B., Perou,C.M., Borresen-Dale,A.L., & Kristensen,V.N. (2010) Allele-specific copy number analysis of tumors. *Proc.Natl.Acad.Sci.U.S.A*, **107**, 16910-16915.

Vaux,D.L., Cory,S., & Adams,J.M. (1988) Bcl-2 gene promotes haemopoietic cell survival and cooperates with c-myc to immortalize pre-B cells. *Nature*, **335**, 440-442.

Velichutina,I., Shaknovich,R., Geng,H., Johnson,N.A., Gascoyne,R.D., Melnick,A.M., & Elemento,O. (2010) EZH2-mediated epigenetic silencing in germinal center B cells contributes to proliferation and lymphomagenesis. *Blood*, **116**, 5247-5255.

Venkatraman,E.S. & Olshen,A.B. (2007) A faster circular binary segmentation algorithm for the analysis of array CGH data. *Bioinformatics.*, **23**, 657-663.

Viardot,A., Moller,P., Hogel,J., Werner,K., Mechtersheimer,G., Ho,A.D., Ott,G., Barth,T.F., Siebert,R., Gesk,S., Schlegelberger,B., Dohner,H., & Bentz,M. (2002) Clinicopathologic correlations of genomic gains and losses in follicular lymphoma. *J.Clin.Oncol.*, **20**, 4523-4530.

Villa,D., Crump,M., Panzarella,T., Savage,K.J., Toze,C.L., Stewart,D.A., MacDonald,D.A., Buckstein,R., Lee,C., Alzahrani,M., Rubinger,M., Foley,R., Xenocostas,A., Sabloff,M., Muccilli,A., Chua,N., Couture,F., Larouche,J.F., Cohen,S., Connors,J.M., Ambler,K., Al-Tourah,A., Ramadan,K.M., & Kuruvilla,J. (2013) Autologous and Allogeneic Stem-Cell Transplantation for Transformed Follicular Lymphoma: A Report of the Canadian Blood and Marrow Transplant Group. *Journal of Clinical Oncology*, **31**, 1164-1171.

Vogelstein,B., Papadopoulos,N., Velculescu,V.E., Zhou,S., Diaz,L.A., Jr., & Kinzler,K.W. (2013) Cancer genome landscapes. *Science*, **339**, 1546-1558.

Vose,J.M., Wahl,R.L., Saleh,M., Rohatiner,A.Z., Knox,S.J., Radford,J.A., Zelenetz,A.D., Tidmarsh,G.F., Stagg,R.J., & Kaminski,M.S. (2000) Multicenter phase II study of iodine-131 tositumomab for chemotherapy-relapsed/refractory low-grade and transformed low-grade B-cell non-Hodgkin's lymphomas. *Journal of Clinical Oncology*, **18**, 1316-1323.

Wahlin,B.E., Sundstrom,C., Sander,B., Christensson,B., Jeppsson-Ahlberg,A., Hjalmarsson,E., Holte,H., Ostenstad,B., Brown,P.D., Smeland,E.B., & Kimby,E. (2013) Higher World Health Organization grades of follicular lymphoma correlate with better outcome in two Nordic Lymphoma Group trials of rituximab without chemotherapy. *Leuk.Lymphoma*.

Wahlin,B.E., Yri,O.E., Kimby,E., Holte,H., Delabie,J., Smeland,E.B., Sundstrom,C., Christensson,B., & Sander,B. (2012) Clinical significance of the WHO grades of follicular lymphoma in a population-based cohort of 505 patients with long follow-up times. *Br.J Haematol.*, **156**, 225-233.

Wahlin,B.E., Sander,B., Christensson,B., & Kimby,E. (2007) CD8+ T-Cell Content in Diagnostic Lymph Nodes Measured by Flow Cytometry Is a Predictor of Survival in Follicular Lymphoma. *Clinical Cancer Research*, **13**, 388-397.

Walther,A., Houlston,R., & Tomlinson,I. (2008) Association between chromosomal instability and prognosis in colorectal cancer: a meta-analysis. *Gut*, **57**, 941-950.

Wartenberg,M., Vasil,P., Bueschenfelde,C.M.z., Ott,G., Rosenwald,A., Fend,F., & Kremer,M. (2013) Somatic hypermutation analysis in follicular lymphoma provides evidence suggesting bidirectional cell migration between lymph node and bone marrow during disease progression and relapse. *Haematologica*, **98**, 1433-1441.

Williams,C.D., Harrison,C.N., Lister,T.A., Norton,A.J., Blystad,A.K., Coiffier,B., Taghipour,G., Schmitz,N., & Goldstone,A.H. (2001) High-Dose Therapy and Autologous Stem-Cell Support for Chemosensitive Transformed Low-Grade Follicular Non-Hodgkin's Lymphoma: A Case-Matched Study From the European Bone Marrow Transplant Registry. *Journal of Clinical Oncology*, **19**, 727-735.

Witzig,T.E., Flinn,I.W., Gordon,L.I., Emmanouilides,C., Czuczman,M.S., Saleh,M.N., Cripe,L., Wiseman,G., Olejnik,T., Multani,P.S., & White,C.A. (2002a) Treatment with ibritumomab tiuxetan radioimmunotherapy in patients with rituximab-refractory follicular non-Hodgkin's lymphoma. *Journal of Clinical Oncology*, **20**, 3262-3269.

Witzig,T.E., Gordon,L.I., Cabanillas,F., Czuczman,M.S., Emmanouilides,C., Joyce,R., Pohlman,B.L., Bartlett,N.L., Wiseman,G.A., Padre,N., Grillo-Lopez,A.J., Multani,P., & White,C.A. (2002b) Randomized controlled trial of yttrium-90-labeled ibritumomab tiuxetan radioimmunotherapy versus rituximab immunotherapy for patients with relapsed or refractory low-grade, follicular, or transformed B-cell non-Hodgkin's lymphoma. *Journal of Clinical Oncology*, **20**, 2453-2463.

Wright,G., Tan,B., Rosenwald,A., Hurt,E.H., Wiestner,A., & Staudt,L.M. (2003) A gene expression-based method to diagnose clinically distinct subgroups of diffuse large B cell lymphoma. *Proc.Natl.Acad.Sci.U.S.A*, **100**, 9991-9996.

Yap,D.B., Chu,J., Berg,T., Schapira,M., Cheng,S.-W.G., Moradian,A., Morin,R.D., Mungall,A.J., Meissner,B., Boyle,M., Marquez,V.E., Marra,M.A., Gascoyne,R.D., Humphries,R.K., Arrowsmith,C.H., Morin,G.B., & Aparicio,S.A.J.R. (2011) Somatic mutations at EZH2 Y641 act dominantly through a mechanism of selectively altered PRC2 catalytic activity, to increase H3K27 trimethylation. *Blood*, **117**, 2451-2459.

Young,R.M. & Staudt,L.M. (2013) Targeting pathological B cell receptor signalling in lymphoid malignancies. *Nat Rev.Drug Discov.*, **12**, 229-243.

Yuen,A.R., Kamel,O.W., Halpern,J., & Horning,S.J. (1995) Long-term survival after histologic transformation of low-grade follicular lymphoma. *Journal of Clinical Oncology*, **13**, 1726-1733.

Yunis,J.J., Frizzera,G., Oken,M.M., McKenna,J., Theologides,A., & Arnesen,M. (1987) Multiple recurrent genomic defects in follicular lymphoma. A possible model for cancer. *The New England Journal of Medicine*, **316**, 79-84.

Yunis,J.J., Oken,M.M., Kaplan,M.E., Ensrud,K.M., Howe,R.R., & Theologides,A. (1982) Distinctive chromosomal abnormalities in histologic subtypes of non-Hodgkin's lymphoma. *The New England Journal of Medicine*, **307**, 1231-1236.

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