# Epigenetic modifications in lymphoma: Relevance for pathogenesis and treatment

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# Directory

# 1. Summary

Lymphomas comprise a very heterogeneous group of haematological cancers that originate from B or T lymphocytes, also known as B and T cells. For a long time, the malignant transformation of B and T cells was mainly associated with genomic alterations, but within the last decade epigenetic modifications were recognized as additional important factors in the pathogenesis of almost all lymphoma entities. Epigenetic mechanisms like DNA methylation and histone modifications regulate gene expression without altering the underlying DNA sequence. Normally, these processes of gene expression regulation are involved in organ development or cell proliferation. In cancer cells, epigenetic alterations are frequently caused by mutations in chromatin- and/or DNA methylation pattern-modifying enzymes. As a consequence, the accessibility of DNA for transcription factors and thus the respective gene expression is altered. The analysis and understanding of these epigenetic modifications in lymphoma cells is of great clinical relevance since epigenetic modifications can be pharmacologically reversed by using so-called “epidrugs” like DNA methylation inhibitors and histone deacetylation (HDAC) inhibitors. The restoration of the physiological epigenetic landscape might stop uncontrolled cellular proliferation and is thus thought to be a further therapeutic option. However, the determination of biomarkers is essentially required to identify patients who will benefit from treatment with epidrugs.

Therefore, the aim of this thesis was (i) to further unravel the role of epigenetic modifications in the pathogenesis of lymphoma and (ii) the identification of markers, which allow a stratification of lymphoma patients eligible for epigenetic therapy with HDAC inhibitors.

Regarding (i) the involvement of epigenetic modifications in the pathogenesis of anaplastic large cell lymphoma (ALCL) was in focus. ALCL shows a significant repression of the T-cell expression program despite its T-cell origin. This study identified that mainly two epigenetic mechanisms are involved in the process: (a) epigenetic activation of suppressors of lineage fidelity such as ID2 leading to the down-regulation of T-cell-specific genes and (b) epigenetic silencing of important T-cell transcription factors. Concerning (ii) this study determines the Src tyrosine kinase FGR as a factor mediating resistance to HDAC inhibitor suberoylanilide hydroxamic acid (SAHA) in B-cell lymphoma. Thus, expression of FGR appears to be a promising marker to stratify patients who benefit from SAHA treatment.

Taken together, this thesis provides additional evidence that epigenetic modifications play an essential role in the pathogenesis of lymphoma and that the modulation of these epigenetic modifications by epidrugs represents a promising therapeutic option for a subset of lymphoma patients.

# 2. Zusammenfassung

Lymphome umfassen eine sehr heterogene Gruppe von hämatologischen Krebserkrankungen, die von B- oder T-Lymphozyten – auch B- und T-Zellen genannt – abstammen. Lange Zeit wurden genomische Aberrationen und Mutationen als Hauptursache der malignen Transformation von B- und T-Zellen angenommen. Im letzten Jahrzehnt zeigte sich jedoch, dass auch epigenetische Modifikationen in der Pathogenese fast aller Lymphomentitäten eine wichtige Rolle spielen. Epigenetische Mechanismen wie die Methylierung der DNA und Modifikationen an Histonproteinen regulieren die Genexpression ohne die zugrundeliegende DNA-Sequenz zu verändern. Diese genregulatorischen Prozesse sind normalerweise an der Organentwicklung oder der Zellproliferation beteiligt. In Krebszellen entstehen epigenetische Veränderungen häufig durch Mutationen in Chromatin- und/oder DNA-Methylierungsmuster-modifizierenden Enzymen. Als Folge wird die Zugänglichkeit der DNA für Transkriptionsfaktoren und damit die jeweilige Genexpression verändert. Die Analyse und das Verständnis dieser epigenetischen Veränderungen in Lymphomzellen sind von großer klinischer Relevanz, da diese pharmakologisch mit Hilfe sogenannter „Epidrugs", wie DNA-Methylierungs- und Histondeacetylase (HDAC)-Inhibitoren, rückgängig gemacht werden können. Die Wiederherstellung der physiologischen epigenetischen Landschaft könnte das unkontrollierte Zellwachstum stoppen und somit als zusätzliche Therapieoption für Lymphompatienten dienen. Jedoch ist das Bestimmen von Biomarkern dringend erforderlich, um Patienten zu identifizieren, die von einer Behandlung mit Epidrugs profitieren würden.

Das Ziel dieser Arbeit war es daher, (i) die Rolle epigenetischer Modifikationen bei der Entstehung von Lymphomen besser zu verstehen und (ii) Marker zu identifizieren, die eine Stratifizierung von Lymphompatienten ermöglichen, die für eine epigenetische Therapie mit HDAC-Inhibitoren geeignet sind.

Bezüglich (i) stand die Beteiligung von epigenetischen Veränderungen in der Pathogenese von anaplastisch großzelligen Lymphomen (ALCL) im Fokus. Charakteristisch für ALCL ist die signifikante Herunterregulation des T-Zell-Phänotyps trotz ihrer T-Zell-Abstammung. Diese Arbeit zeigt, dass hauptsächlich zwei epigenetische Mechanismen an diesem Prozess beteiligt sind: (a) die epigenetische Aktivierung von Suppressoren wie ID2, die zu einer Herunterregulierung der T-Zell-spezifischen Genen führt, und (b) die epigenetische Stilllegung wichtiger T-Zell-Transkriptionsfaktoren. Im Hinblick auf (ii) konnte die Src Tyrosinkinase FGR als Faktor bestimmt werden, der die Resistenz gegenüber dem HDAC Inhibitor SAHA (suberoylanilide hydroxamic acid) in B-Zell-Lymphomen vermittelt. Die Expression von FGR stellt demnach einen vielversprechenden Marker dar, um Lymphompatienten zu identifizieren, die von einer SAHA Behandlung profitieren könnten.

Zusammengefasst liefert diese Arbeit zusätzliche Hinweise darauf, dass epigenetische Modifikationen eine wesentliche Rolle bei der Entstehung von Lymphomen spielen und dass die Modulation dieser epigenetischen Modifikationen durch Epidrugs für einige Lymphompatienten sehr vielversprechend sein kann.

# 3. Introduction

Lymphomas represent a heterogeneous group of haematological cancers that affect T and B cells. The initiation of malignant processes in T and B cells finally leading to lymphoma are thought to derive to a large extend from genomic alterations such as translocations, chromosomal gains and losses as wells as mutations in context-specific oncogenes or tumour suppressors (Dalla-Favera et al. 1982; Davis et al. 2001; Iqbal et al. 2007; Morris et al. 1994; Pasqualucci et al. 2006; Taub et al. 1982; Weiss et al. 1987; Zelenetz et al. 1991). However, within the last decade it has become increasingly evident that changes in the epigenetic landscape, i.e. changes in chromatin and methylation patterns, are also frequently detectable in lymphomas (Cairns et al. 2012; Couronne et al. 2012; Dukers et al. 2004; Lohr et al. 2012; Marquard et al. 2008; Marquard et al. 2009; Morin et al. 2010; Morin et al. 2011; Odejide et al. 2014; Pasqualucci et al. 2011a; Raaphorst et al. 2000). The frequent discovery of epigenetic modifications in lymphoma cells provides the rationale for the use of so-called “epidrugs”, which inhibit or activate disease-associated epigenetic proteins as an additional or alternative treatment option for lymphoma patients (Ivanov et al. 2014).

## 3.1 The role of B and T lymphocytes in the immune system

The human immune system can be defined as an interactive network of cells and molecules with specialized roles in defending the body against pathogens. There are two fundamentally different types of immune response: (i) the innate (natural) immune response and the (ii) adaptive (acquired) immune response. The innate immune response occurs unspecific as a first line defence against invading pathogens, whereas the adaptive immune is highly specific and improves on repeated exposure to a given pathogen afforded by immunological memory (Delves and Roitt 2000a).

Phagocytic cells (neutrophils, monocytes, and macrophages), inflammatory mediators releasing cells (basophils, mast cells, and eosinophils), and natural killer (NK) cells are mainly involved in the innate immune response. These cells are effective in combating many pathogens, but they have a limited and invariable repertoire of receptors to recognize pathogens (Janeway and Medzhitov 2002).

The adaptive immune response is responsible to more effectively fight the wide range of pathogens, which overcome the defences of the innate immune system. Lymphocytes (B and T cells) have evolved to recognize a great variety of different antigens from viruses, bacteria, and other disease-causing organisms as well as malignant cells.

In consequence of their distinct types of antigen receptors, B and T cells have different roles in the adaptive immune system (Delves and Roitt 2000a).

The main feature of B cells is the expression of specific antigen-binding immunoglobulin (Ig) proteins, also known as antibodies. Membrane-bound antibodies on the B-cell surface are part of the B-cell receptor (BCR) complex. Antibodies consist of two identical immunoglobulin heavy chains (IgH) and two identical immunoglobulin light chains (IgL) that are linked by disulfide bonds. The variable antigen binding-region (V region) at the N-terminal of each chain varies extremely between different antibody molecules, whereas the constant region (C region) at the C-terminal consists of only a few subtypes and is responsible for activating different effector mechanisms (Edelman 1973; Porter 1973). Secreted antibodies are able to bind specifically pathogens in the extracellular spaces of the body and recruit other cells (e.g. phagocytes) to eliminate the pathogen, whereas antigen binding at the V region of the BCR complex leads to B-cell activation, clonal expansion and creation of antibodies with higher affinity by somatic mutations in the Ig genes (Delves and Roitt 2000a).

The main function of T cells is the identification and elimination of cells, which are infected by intracellularly multiplying pathogens. Compared to B cells, T cells have antigen-recognition molecules, which are exclusively membrane-bound proteins. T-cell receptors (TCRs) consist of α/β (vast majority) or γ/δ heterodimer that also contain V and C regions. In contrast to the BCR, TCRs are not able to bind antigens directly. They recognize short peptide fragments of protein antigens processed by the ubiquitin system, which are present by the major histocompatibility complex (MHC) molecules on the cell surface (Delves and Roitt 2000a; Meuer et al. 1984; Schlossman 1972).

Lymphocytes are capable of producing about $10^{15}$ different antibody variable regions (B cells) and a comparable number of TCR variable regions (Delves and Roitt 2000a). This is achieved by somatic rearrangements of their variable (V), diversity (D) and joining (J) gene segments of the TCR and the immunoglobulin genes during B- and T-cell development known as V(D)J recombination. By random selection and combination of one V, one D and one J gene segment, this process is able to generate the huge number of different antigen binding regions (Tonegawa 1983).

### *3.1.1* T- and B-cell developmental stages

T cells arise from progenitor lymphoid cells in the bone marrow and mature during migration through the thymus to become either CD4+ or CD8+ single positive cells via double negative (DN) (CD4- and CD8-) and double positive (DP) (CD4+ and CD8+) thymocytes. TCR V(D)J gene rearrangements take place in the thymus and structural differences allow the distinction of αβ- (vast majority of all T cells) and γδ-T cells (Raulet et al. 1985). After activation of the TCR by antigen binding, T cells proliferate and differentiate into single positive (SP) (CD4+/CD8- or CD4-/CD8+) effector or memory T cells. T cells with non-functional TCRs, low antigen affinity or self-reactive TCRs die by apoptosis (Delves and Roitt 2000a; Murphy et al. 2008) (Figure 1).

B cells develop in the bone marrow, where development progresses through the progenitor B-cell, pre-B-cell and immature B-cell stages. During this differentiation, IgH and IgL V(D)J gene rearrangements result in the generation and surface expression of membrane-bound antibodies capable of binding antigen. These mature naive B cells circulate in the blood, populate primary lymphoid follicles and are activated by antigens that fit to their antibody. Antigen-exposed B cells migrate into the center of primary follicles, proliferate and form together with the follicular dendric cell (FDC) meshwork a germinal center (GC). GC is a specialized microenvironment in which B-cell proliferation, somatic hypermutation, and selection for affinity of antigen binding occur (Tarlinton 1998). Somatic hypermutation in the IgH and IgL region genes and class switch recombination are introduced during the GC reaction to improve antigen affinity (Jacob et al. 1991; Lorenz and Radbruch 1996). Following an immune response, antigen-specific B cells develop into either plasma (antibody-secreting) cells or memory B cells (Delves and Roitt 2000a; Murphy et al. 2008) (Figure 1).

Mature lymphocytes either circulate in the blood and the lymph or remain in lymphoid organs. Lymphoid organs are composed of lymphocytes and non-lymphoid cells and can be divided in primary and secondary lymphoid organs. The primary lymph organs are the bone marrow and the thymus, where lymphocytes are generated. In the secondary lymph organs mature and naïve lymphocytes are maintained to initiate adaptive immune responses. Secondary lymph organs comprise the lymph nodes, the spleen and the mucosal lymphoid tissue of e.g. the nasal and respiratory tract, the gut and the urogenital tract (Murphy et al. 2008; Delves and Roitt 2000b).

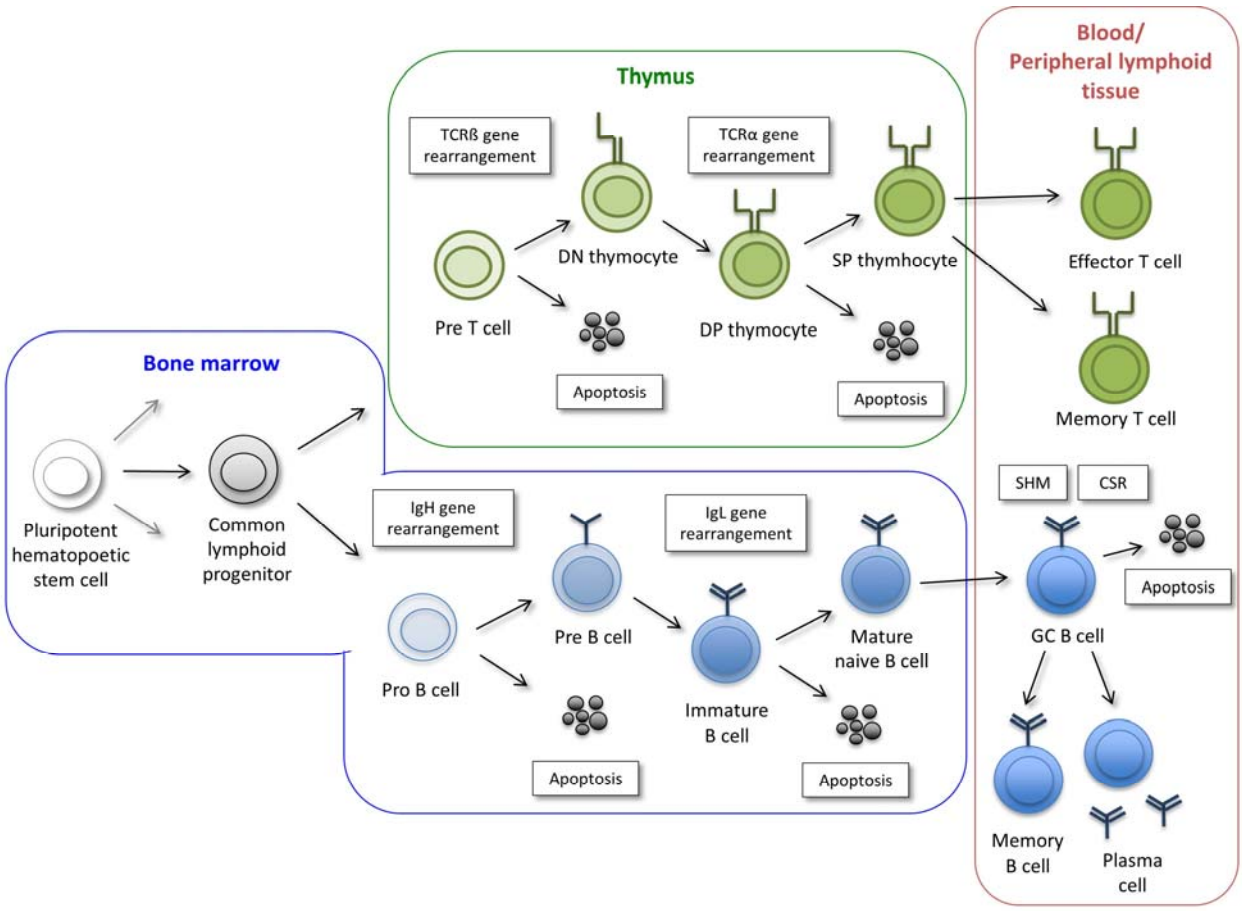


**Figure 1: Overview of T- and B-cell development.** Amongst others, pluripotent hematopoietic stem cells in the bone marrow give rise to a common lymphoid progenitor, from which both T and B cells develop. T-cell maturation occurs in the thymus, where they develop from double negative (DN) (CD4- and CD8-) into double positive (DP) (CD4+ and CD8+) thymocytes and finally to either CD4+ or CD8+ single positive (SP) thymocytes. During this maturation TCRß and TCRα gene rearrangement take place (in addition to TCRγ/δ; not shown). T cells with non-functional TCRs, with low antigen affinity or with self-reactive TCRs are eradicated by apoptosis. In the blood/peripheral lymphoid tissue, antigen binding leads to the differentiation into SP effector or memory T cells. B-cell development proceeds through a progenitor (pro) B cell, pre B cell and immature B-cell stages. Rearrangements of the IgH and IgL gene segments lead to the generation and surface expression of membrane-bound antibodies capable of binding antigen. Cells with functional and not self-reactive antibodies differentiate into naive mature B cells, whereas the remainder die by apoptosis. Mature naive B cells activated by matching antigens expand within germinal centers (GCs) and modify their Ig genes by the introduction of point mutations (somatic hypermutations (SHM)) and class switch recombination (CSR) to generate high affinity antibodies. Selected B cells develop further into either antibody-secreting plasma cells or memory B cells.

## 3.2 Lymphoma

B-cell and T-cell lymphoid neoplasms are clonal tumours of mature B or T cells at various stages of differentiation. Lymphomas are classified according to the WHO classification of lymphoid neoplasms (Swerdlow et al. 2008).

In many aspects, B- and T-cell lymphomas appear to recapitulate stages of normal differentiation, so they can be classified according to the corresponding to their physiological equivalents. Based on morphological features, two major groups of malignant lymphomas have been distinguished: classical Hodgkin lymphoma (cHL) and non-Hodgkin lymphoma (NHL) (Swerdlow et al. 2008) (Figure 2).

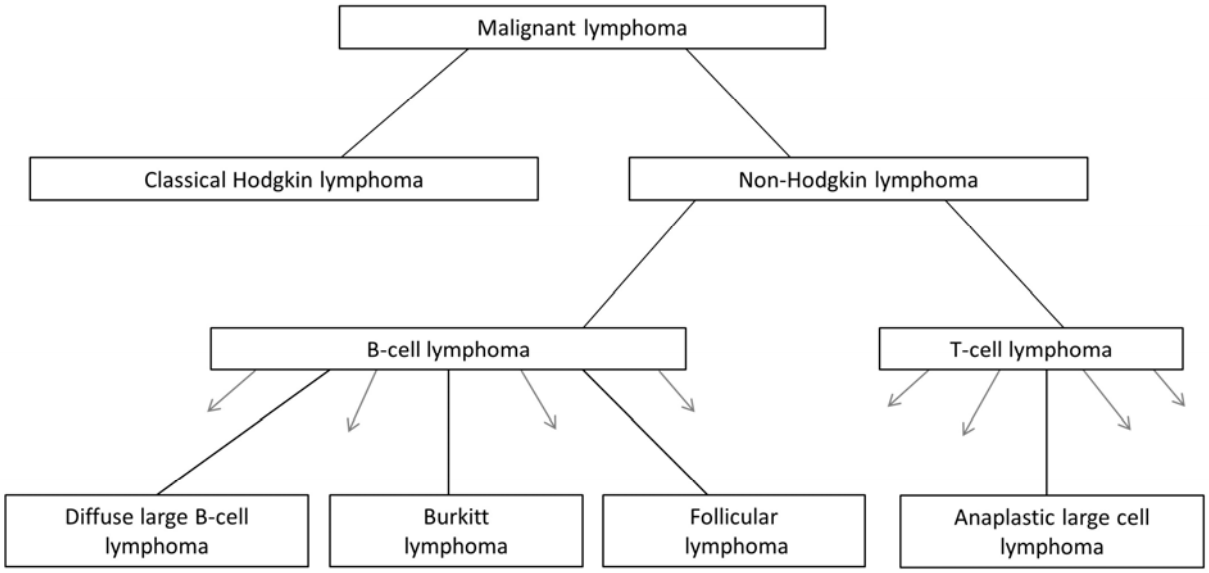


**Figure 2: Simplified classification of malignant lymphoma**. Based on histological features, malignant lymphoma can be classified into classical Hodgkin- and non-Hodgkin lymphoma. Classical Hodgkin lymphoma is characterized by large mono-nuclear Hodgkin and multi-nuclear Reed-Sternberg cells – the tumour cells of this disease, which usually represent less than 5% of all cells. Non-Hodgkin lymphomas (NHL) comprise a large group of morphologically, immunophenotypically or genetically distinct lymphomas derived from mature B or T cells. Depicted are only those NHL entities, which are of interest for this thesis: Diffuse large B-cell lymphoma, follicular lymphoma, Burkitt lymphoma (all B-NHL) and anaplastic large cell lymphoma (T-NHL).

### *3.2.1* Classical Hodgkin lymphoma

Classical Hodgkin lymphoma (cHL) is a B-cell-derived lymphoma that accounts for approximately 10% of all lymphomas and has an annual incidence in developed regions (e.g. the United States of America (USA) and Europe (EU)) of 2–3 new cases/100.000 (Torre et al. 2015).

Histologically, cHL consists of mono-nuclear Hodgkin (H) cells and multi-nuclear Reed-Sternberg (RS) tumour cells, which comprise less than 5% of all cells. Although HRS cells represent a clonal expansion of cells that originate from a single GC B cell, they infrequently – and usually weakly – express typical B-cell genes such as the CD19, CD20 and CD79a antigens and B-cell transcription factors such as OCT2, BOB1, PU.1 and PAX5 (Foss et al. 1999; Hummel et al. 1995; Jundt et al. 2002; Marafioti et al. 2000; Stein et al. 2001) (Figure 3B). However, the expression of CD30 (TNFRSF8), a member of the tumour necrosis factor (TNF) receptor super family usually only expressed by activated B-cells is very constant feature which is present in virtually all HRS cells of all cHL cases (Durkop et al. 1992; Fonatsch et al. 1992) (Figure 3A). In normal lymphoid tissue CD30 expression is restricted to few activated T and B cells. Thus, the consistent CD30 expression of HRS tumour cells implies an important role for CD30 in their pathogenesis (Chiarle et al. 1999).

More than 90% of cHL patients can be cured by a combination of chemotherapy and – for advanced disease stages – with additional radiation therapy. However, there is small subgroup of cHL patients who are therapy refractory or relapse with the disease. In addition, secondary malignancies or cardiovascular diseases are the consequence of the treatment-related toxicity, which eventually reduce the survival for a number of patients who are cured from the cHL (Armitage 2010; Stathis and Younes 2015).

### *3.2.2* Non-Hodgkin lymphoma

Compared to cHL, non-Hodgkin lymphoma (NHL) originating from mature B or T cells represents a very morphological and molecular heterogeneous disease entity comprising several lymphoma subtypes. NHL have incidence rates over 10/100.000 in the USA, Australia and Europe. Lower rates of less than 5/100.000 are reported in Central America and parts of Africa (Torre et al. 2015). Interestingly, the vast majority of NHL originates from B cells (Swerdlow et al. 2008).

There are clinically aggressive and indolent forms of NHL. Aggressive lymphomas develop rapidly, and treatment is needed to start immediately, whereas indolent lymphomas grow very slowly and patients may not need to start treatment at an early stage of the disease (Jaffe 1986).

For this study the T-cell derived anaplastic large cell lymphoma (ALCL) as well as B-cell derived diffuse large B-cell lymphoma (DLBCL), follicular lymphoma (FL) and Burkitt lymphoma (BL) are of interest and thus subsequently described in more detail.

#### 3.2.2.1 Anaplastic large cell lymphoma

Anaplastic large cell lymphoma (ALCL) is a T-cell lymphoma and accounts for approximately 25% of all T-cell lymphomas and 2–8% of all NHL (A clinical evaluation of the International Lymphoma Study Group classification of non-Hodgkin's lymphoma. The Non-Hodgkin's Lymphoma Classification Project 1997). ALCL was first described in 1985 by Stein and colleagues (Stein et al. 1985).

The molecular hallmark of ALCL is the usually absent expression of TCRs despite the presence of fully rearranged TCR genes. In line, ALCL often lacks specific T-cell markers such as CD3 and ZAP70, which reminds to the missing B-cell marker expression in cHL (Bonzheim et al. 2004) (Figure 3D). Another concordance with cHL is the very characteristic expression of CD30 (Chiarle et al. 1999; Stein et al. 1985) (Figure 3C).

A subset of ALCL patients express the chimeric protein nucleophosmin-anaplastic lymphoma kinase (NPM-ALK) that results from a balanced t(2;5)(p23;q25) chromosomal translocation fusing the N-terminal region of nucleophosmin gene to the intra-cytoplasmatic portion of the ALK gene (Morris et al. 1994). Based on the expression of NPM-ALK and localisation in the body, three subtypes of ALCL have been identified: primary systemic anaplastic lymphoma kinase (ALK)-positive ALCL, primary systemic ALK-negative ALCL, and primary cutaneous ALK-negative ALCL (c-ALCL) (Swerdlow et al. 2008).

ALK-positive ALCL and c-ALCL have a favourable prognosis if treated with standard chemotherapy, while ALK-negative ALCL has a more unfavourable prognosis (Hapgood and Savage 2015).

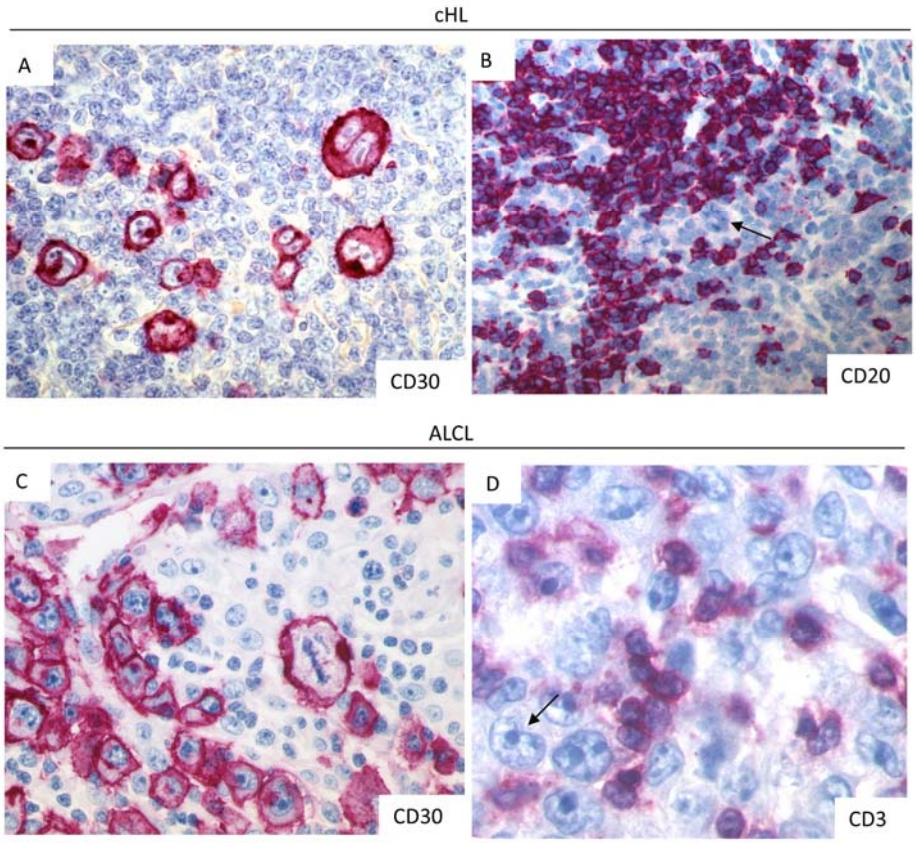


**Figure 3: CD30 expression and loss of lineage-specific markers in tumour cells of cHL and ALCL**. Tumour cells of cHL and ALCL are consistently CD30 positive (A, C), whereas they lack expression of lineage specific markers such as CD20 in cHL (B) or CD3 in ALCL (D) (arrows). Small reactive lymphocytes around the tumour cells express the lineage specific markers, but are CD30 negative. Original magnification x400 (A, B, C); 600x (D).

#### 3.2.2.2 Diffuse large B-cell lymphoma

Diffuse large B-cell lymphomas (DLBCL) represent the most common type of aggressive B-NHL and account for approximately 40% of all cases in adults (A clinical evaluation of the International Lymphoma Study Group classification of non-Hodgkin's lymphoma. The Non-Hodgkin's Lymphoma Classification Project 1997). Regarding morphology, biology, molecular features, and clinical presentation, DLBCL is very heterogeneous. Gene expression profiling studies revealed at least three molecular subtypes, which arise from B cells at different stages of differentiation: (i) GCB DLBCL, which derive from GC B cells, (ii) ABC DLBCLs, which derive from B cells that are related to activated B cells and which may show features of plasma cellular differentiation and (iii) primary mediastinal B-cell lymphoma, which seems to originate from a rare B-cell subpopulation in the thymus (Alizadeh et al. 2000; Monti et al. 2005; Rosenwald et al. 2003; Savage et al. 2003; Wright et al. 2003).

The DLBCL subtypes differ not only in their gene expression profile, but they also display an aberrant expression of different oncogenic signalling pathways. Up to 45% of GCB DLBCLs show a t(14;18)(q32;21) translocation juxtaposing the *BCL2* gene and the IgH locus and thereby leading to an overexpression of the anti-apoptotic BCL2 protein (Weiss et al. 1987; Zelenetz et al. 1991). The hallmark of ABC DLBCLs is the constitutive activation of the NF-κB pathway, which promotes proliferation and differentiation and suppresses apoptosis (Davis et al. 2001). Additionally, in about 30% of ABC DLBCLs genomic alterations are found in key genes of terminal B-cell differentiation such as *BLIMP/PRMD1b* and *BCL6* leading to BLIMP/PRMD1b down-regulation and overexpression of BCL6 (Iqbal et al. 2007; Mandelbaum et al. 2010; Pasqualucci et al. 2006). The distinction between the DLBCL subtypes is of major clinical importance, as they have significantly different survival rates after current standard therapy combing the anti-CD20 antibody Rituximab and chemotherapy (R-CHOP) (Nogai et al. 2011; Rosenwald et al. 2002). The majority of GCB DLBCL patients can be cured by standard therapy, whereas about 50% of ABC DLBCL patients succumb to their disease (Nogai et al. 2011; Sehn and Gascoyne 2015).

#### 3.2.2.3 Follicular lymphoma

Follicular lymphoma (FL) is a frequent indolent form of B-NHL accounting for approximately 20 – 30% of all NHLs (A clinical evaluation of the International Lymphoma Study Group classification of non-Hodgkin's lymphoma. The Non-Hodgkin's Lymphoma Classification Project 1997). The t(14;18)(q32;21) translocation leading to a BCL2 overexpression is a hallmark of FL since it is observed in up to 85–90% of FL cases (Weiss et al. 1987; Zelenetz et al. 1991).

FL patients have a variable clinical course with a median survival of 10 years. Approximately 30% of patients suffering from FL show transformation to a high-grade lymphoma, usually DLBCL, which is associated with a more unfavourable prognosis (Montoto et al. 2007; Acker et al. 1983).

#### 3.2.2.4 Burkitt lymphoma

Burkitt lymphoma (BL) is also an aggressive B-NHL (Dave et al. 2006). In 1958, the surgeon Dennis Burkitt first described BL as a tumour that is particularly prevalent in young male children in tropical Africa affecting the jaws, the abdomen, and endocrine organs (Burkitt 1958).

Currently, there are three subtypes of BL: (i) endemic BL, the African/South American variant almost exclusively affecting young male children and (ii) sporadic BL, which occur elsewhere (Swerdlow et al. 2008). Endemic and sporadic BL are mainly based on the geographical distribution of BL and differ in their association with the Epstein-Barr virus (EBV), which is present in almost all cases of endemic BL, but only in 10–20% of sporadic BL (Bellan et al. 2003).

Immunodeficient patients (e.g. patients with immunodeficiency virus (HIV) or after immunosuppression) are predisposed to NHL, including BL, which led to a third subtype of BL: (iii) immunodeficiency-related BL (Swerdlow et al. 2008).

The hallmark of all three subtypes of BL is a translocation t(8;14) juxtaposing the IgH or IgL locus to the *MYC* gene, which leads to a constitutive deregulation of the oncogenic transcription factor MYC due to the control of the fusion gene by the permanently active IG promoter (Dalla-Favera et al. 1982; Taub et al. 1982) (Figure 3).

Using intensive chemotherapy regimens, BL is curable in most cases (Yustein and Dang 2007). However, standard therapy is often not tolerated by older individuals (Schmitz et al. 2014).

## 3.3 Epigenetics

Epigenetics is defined as mitotically and meiotically heritable changes in gene expression that do not involve a change in the DNA sequence (Egger et al. 2004).

Eukaryotic DNA is compacted in the nucleus as chromatin, which consists of DNA and several proteins, mostly histone proteins. The basic repeating unit of chromatin – the nucleosome – is composed of four different histone proteins: H2A, H2B, H3 and H4, two of each kind. The nucleosome has about 146 base pairs (bp) of DNA wrapped around each histone octamer (Luger et al. 1997) (Figure 4).

There are basically two sets of epigenetic mechanisms, which are involved in the regulation of gene expression and constitute the epigenome in each cell: (i) post-translational modifications including acetylation, methylation, phosphorylation and ubiquitination of histones and (ii) DNA methylation (Figure 4). These mechanisms control different biological processes such as cell differentiation, proliferation, pre-mRNA processing, survival, genomic imprinting, and X chromosome inactivation (Inbar-Feigenberg et al. 2013; Mazzio and Soliman 2012).

### *3.3.1* Histone modifications

About 30 short chains of amino acids protrude from the histones. These ‘histone tails` are subjected to various post-translational modifications, which form a ‘histone code`. By defining the accessibility of the transcription machinery to genes and gating the accessibility of the genome to other machineries, such as repair and DNA replication, the ‘histone code` regulates chromatin function and thus determines gene expression patterns. Within the histone tails, lysine (K) and arginine (R) residues are the major sites of modifications (Figure 4). Reversible acetylation and methylation of the basic side chains of these amino acids are common (Bannister and Kouzarides 2011; Strahl and Allis 2000).

In 1963, histone acetylation was the first histone modification described and hyperacetylated histones were early associated with open chromatin formation and transcriptional activation (Phillips 1963; Allfrey et al. 1964; Pogo et al. 1966). Two groups of enzymes determine the pattern of histone acetylation: Histone acetyltransferases (HATs) and histone deacetylases (HDACs). HATs are responsible for acetylation of the ε-amino group of specific lysine (K) residues on histone (H) 3 and H4 and other target proteins, whereas HDACs deacetylate them (Tanner et al. 1999).

There are 17 human HATs, which are divided into at least five families based on the extent of sequence similarity in the catalytic HAT domain: HAT1 HATs, Gcn5/PCAF HATs, MYST HATs, SRC HATs and p300/CBP HATs. HAT families have distinct biological functions depending on their catalytic HAT domain (Marmorstein 2001).

Eighteen different HDAC isoforms have been described in humans, which are grouped into four classes based on phylogenetic analysis and sequence similarity to yeast factors: class I (HDAC1, 2, 3 and 8), class IIa (HDAC4, 5, 7, and 9), class IIb (HDAC6 and 10), class III (SIRT1, 2, 3, 4, 5, 6 and 7) and class IV (HDAC11). Class I, II and IV HDACs are Zn2+-dependent deacetylases, whereas class III HDAC-mediated deacetylation is dependent on NAD+ (de Ruijter et al. 2003).

HATs and HDACs can impact gene expression in two different ways: (i) by altering histone acetylation patterns, which modulate chromatin structure and its accessibility to transcriptional regulatory proteins, and (ii) by acetylating and affecting the activity of non-histone proteins that directly regulate transcription, including transcription factors and signalling molecules (Glozak et al. 2005).

Histone methylation can occur at arginine (R) and lysine (K) residues of H3 and H4 proteins (Figure 4). Arginine can either be mono- or dimethylated, whereas lysine can accept one, two or three methylgroups. Thus, methylation has a great combinatorial potential compared to other histone modifications (Grewal and Rice 2004; Santos-Rosa and Caldas 2005). In contrast to histone acetylation, which correlates mainly with transcriptional activation, histone methylation can lead to both transcriptional activation and inactivation depending on the modified residue and other simultaneous histone modifications (Santos-Rosa and Caldas 2005). Histone arginine methylation, which is catalysed by protein arginine methyltransferases (PRMTs), correlates with transcriptional activation of a variety of genes (Bedford and Richard 2005). Lysine methylation is catalysed by different enzymes which share a strong homology in the catalytic SET domain (**S**u(var), **E**nhancer-of-Zeste and **T**rithorax) (Jones and Gelbart 1993).

The consequences of lysine methylation are extremely diverse as it can lead to transcriptional activation or inactivation and even chromosome loss (Santos-Rosa and Caldas 2005). For example, methylation at H3K36 and H3K79 are related to transcriptional activation (Li et al. 2003; van Leeuwen et al. 2002), whereas methylation at H3K9 and H3K27 are signals for transcriptional repression and stable epigenetic silencing (Cao et al. 2002; Kirmizis et al. 2004; Kuzmichev et al. 2002).

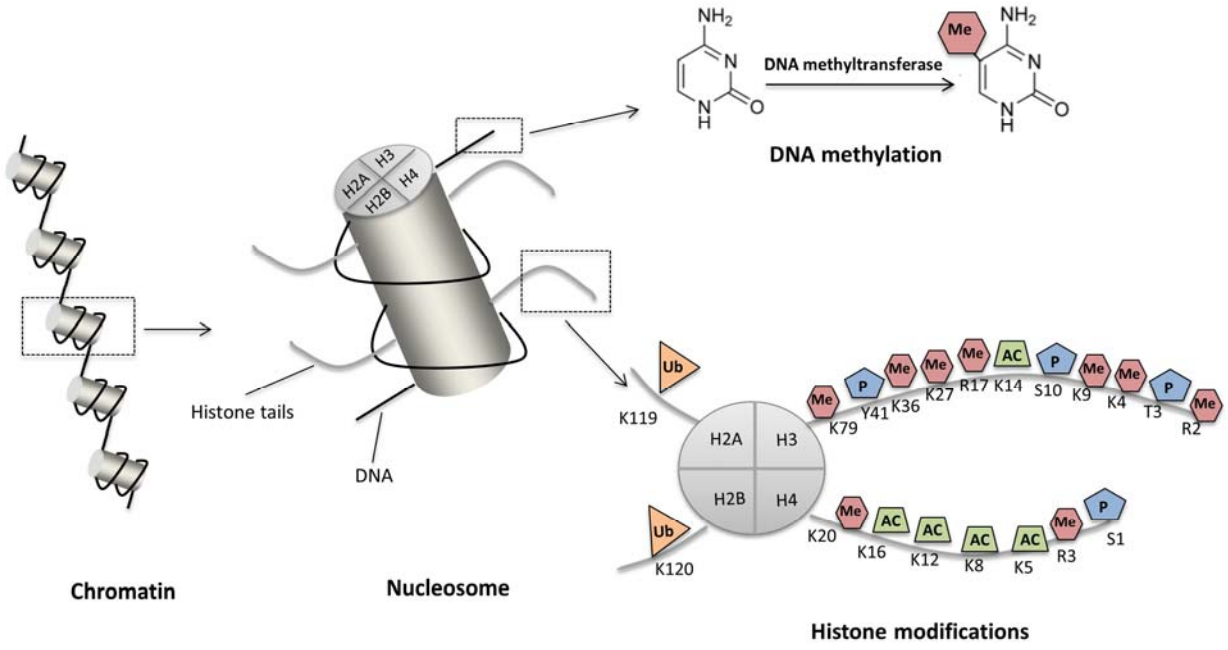


**Figure 4: DNA methylation and histone modifications.** Eukaryotic DNA is compacted in the nucleus as chromatin, which consists of DNA and mainly histone proteins. The nucleosome – the basic repeating unit of the chromatin – is composed of four different histone proteins: H2A, H2B, H3 and H4, two of each kind. About 146 bp of DNA are wrapped around each histone octamer. DNA methylation occurs at position 5 in the pyrimidine ring of cytosines of CpG dinucleotides and is mediated by DNA methyltransferases. The histone tails are subject to a variety of post-translational modifications, including methylation (Me), acetylation (Ac), phosphorylation (P) and ubiquitination (U). K = lysine, Y = tyrosine, R = arginine, S = serine, T = threonine.

### *3.3.2* DNA methylation

Methylation of DNA occurs at position 5 in the pyrimidine ring of cytosines of CpG dinucleotides and is mediated by two types of DNA methyltransferases (DNMTs): *de novo* and maintenance methyltransferases. DNMTs catalyse the covalent addition of a methyl group from S-adenosyl-methionine to the C5 position of cytosine mainly in CpG islands (Simon et al. 1978) (Figure 4).

*De novo* methyltransferases DNMT3A and DNMT3B establish DNA methylation patterns during early development, whereas the maintenance methyltransferase DNMT1 propagates the methylation patterns with extreme fidelity by reproducing patterns of methylated and unmethylated CpG sites between cell generations (Bestor 1992; Hsieh 1999; Okano et al. 1999). Chromatin structure is regulated and stabilized by CpG methylation since it is responsible for the accessibility of the transcription machinery to the regulatory regions of DNA. In general, CpG methylation near the transcription start site inhibits gene expression, whereas a low level or lack of CpG methylation in the promoter region of genes is correlated with active gene expression (Saxonov et al. 2006).

## 3.4 Epigenetic modifications in lymphoma

For long time cancer – including lymphoma – has been considered to be a disease caused by the accumulation of genomic alterations. However, there is a rapidly expanding list of reported epigenetic modifications in lymphoma cells, which reprogram the epigenome and lead to sustained proliferation and tumour progression.

Activating point mutations in the histone methyltransferase (HMT) *enhancer of zeste homolog 2* (*EZH2*) were found in up to 22% of GCB DLBCL and 7% of FL (Morin et al. 2010). EZH2 is part of the polycomb repressive complex (PRC2), which is responsible for trimethylation of H3K9 (H3K27me3) (Bracken and Helin 2009). This gain-of-function mutation promotes H3K27 trimethylation and leads to the inhibition of tumour-suppressor genes (McCabe et al. 2012; Morin et al. 2010). Strong expression of EZH2 was also observed in other NHL, including ALCL (Eckerle et al. 2009).

Loss-of-function mutations were observed in *MLL2* in about 90% of FL and 30% of DLBCLs (Lohr et al. 2012; Morin et al. 2011; Pasqualucci et al. 2011b). MLL2 is a trithorax HMT and is responsible for the methylation of H3K4 (Shilatifard 2008). In the mutated cases, the SET domain of *MLL2* was disturbed by truncation and frameshift mutations leading to protein inactivation and reduction of H3K4 methylation levels (Lohr et al. 2012; Morin et al. 2011; Pasqualucci et al. 2011b).

Mutations in genes coding for the HATs CREBBP and EP300 and HAT recruiting protein MEF2B lead to a disturbed balance of histone modifications (Lohr et al. 2012; Morin et al. 2011; Pasqualucci et al. 2011a). About 40% of DLBCL and FL cases displayed deletions and/or somatic mutations in the HAT-coding domain of CREBBP or EP300 (Pasqualucci et al. 2011a).

Recurrent point mutations in the MEF2 domain were seen in approximately 15% of FL and 13% of GCB DLBCL cases (Morin et al. 2011).

Additionally, several HDACs (1, 2, and 6) were overexpressed in DLBCL and several T-cell lymphomas, which can lead to DNA compaction and a repressive chromatin state (Marquard et al. 2008; Marquard et al. 2009).

Epigenetic modifications are not only seen in chromatin-regulating proteins, changes in DNA methylation patterns were observed as well. Both hypomethylation and hypermethylation of DNA was linked to the pathogenesis of multiple cancers including lymphomas (Esteller et al. 2001).

Mutations of epigenetic genes that result in abnormal DNA methylation such as *IDH2*, *TET2* and *DNMT3A* were mainly found in several types of T-cell lymphoma (Cairns et al. 2012; Couronne et al. 2012; Lemonnier et al. 2012; Odejide et al. 2014). Isocitrate dehydrogenase 2 (IDH2) is responsible for the conversion of isocitrate to alpha-ketoglutarate. Mutations in *IDH2* result in the constitutive production of 2-hydroxyglutarate (2HG), which inhibits TET2, a DNA methylase. Consequently, mutations in both *IDH2* and *TET2* cause gene silencing via DNA hypermethylation (Losman and Kaelin 2013; Ward et al. 2010).

Taken together, somatic mutations in epigenetic enzymes, a shift towards a repressed chromatin state and changes in DNA methylation patterns seem to be connected with malignant processes in NHL (Hassler et al. 2013) (Figure 5A).

Epigenetic modifications that affect DNA methylation patterns and chromatin state were also discovered in HRS cells of cHL. Frequent hypermethylation leading to transcriptional repression was observed in several tumour-suppressor genes (e.g. *CDKN2C*, *CHEK29*), the pro-apoptotic *IGSF4* and the B-cell transcription factor *KLF4* (Guan et al. 2010; Kato et al. 2004; Sanchez-Aguilera et al. 2004; Murray et al. 2010). Additionally, PcG proteins (BMI1, RING1, EED, YY1 and EZH2) are up-regulated in cHL (Dukers et al. 2004; Raaphorst et al. 2000).

The most interesting is the involvement of epigenetic modifications in the down-regulation of the B-cell phenotype in HRS cells in cHL. Analyses of DNA methylation revealed that many genes specifically methylated in HRS cells compared to normal B cells and other B-cell derived lymphomas are important components for B-cell function such as CD79B, BOB1, and SYK (Ammerpohl et al. 2012; Doerr et al. 2005; Ushmorov et al. 2006). Additionally, transcriptionally inactivating hypoacetylation at histone H3 and H3K27 trimethylation of many B-cell genes was described (Seitz et al. 2011). Histone acetylation and DNA demethylation of B-cell lines induced gene expression features of HRS cells, which indicates that the up-regulation of B-cell unspecific genes in HRS cells is also mediated by epigenetic modifications (Ehlers et al. 2008).

## 3.5 Epidrugs

The frequent discovery of epigenetic modifications in lymphoma provides the rationale for the use of epidrugs, which inhibit or activate disease-associated epigenetic proteins for ameliorating or curing patients (Ivanov et al. 2014).

Currently, inhibitors of HDACs and DNA methylation were in focus in many *in vitro* and *in vivo* studies and some are already approved by the U.S. Food and Drug Administration (FDA) and/or the European Medicines Agency (EMA) for cancer treatment. Besides these two classes, there are several other classes of epidrugs (e.g. histone acetyltransferase inhibitors, histone methyltransferase inhibitors), but none of them are approved for cancer treatment yet (Di Costanzo et al. 2014). The focus of this study lies on inhibitors of HDACs and DNA methylation.

### *3.5.1* Histone deacetylase inhibitors

HDAC inhibitors are able to interact with the catalytic domain of class I, II and IV HDACs to block the substrate recognition ability of these enzymes (Finnin et al. 1999; Finnin et al. 2001). Chemically, HDAC inhibitors can be divided into four groups: short-chain fatty acids, hydroxamic acids, cyclic tetrapeptides and benzamides. Some HDAC inhibitors are specific to one or two classes of HDACs (e.g. depsipeptide and MS-275), while others (trichostatin A (TSA), SAHA) inhibit the whole spectrum of HDACs (except class III HDACs) (Di Costanzo et al. 2014).

Thus, HDAC inhibitors can directly induce modifications in the cancer cell epigenome resulting in restoration of relevant gene expression and changes in non-histone proteins that consequently lead to growth arrest, promotion of differentiation and induction of apoptosis (Mai et al. 2005) (Figure 5). Compared to cancer cells, normal cells are relatively resistant to HDAC inhibitors (Qiu et al. 2000).

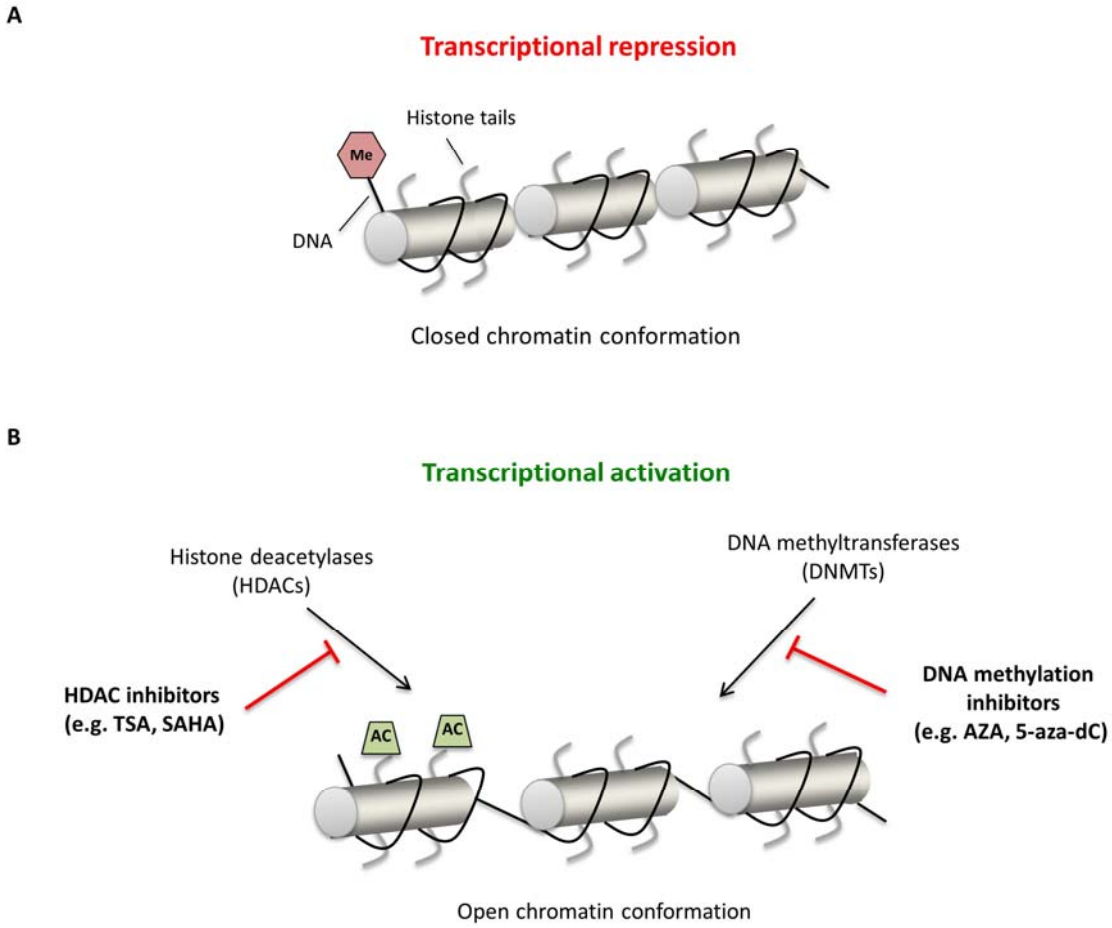


**Figure 5**: **Regulation of gene expression by inhibitors of DNA methylation and HDACs**. Gene expression is regulated by a combination of DNA methylation and histone modifications present in promoter regions. Mutations in chromatin- and/or DNA methylation-modifying enzymes lead to a disturbed epigenetic landscape that alters chromatin structures and thus DNA accessibility for DNA binding proteins. **A:** DNA methylation and deacetylated histone proteins lead to epigenetic silencing of gene expression due to a closed chromatin conformation. Since tumour suppressor genes are often silenced by a closed chromatin conformation, epigenetics appears to play a central role in tumourgenesis. **B:** DNA methylation inhibitors (e.g. AZA and 5-aza-dC) and/or HDAC inhibitors (e.g. TSA and SAHA) are able to open the chromatin conformation which results in the re-expression of silenced genes. The restoration of the epigenetic landscape is thought to be an alternative treatment option for lymphoma patients.

### *3.5.2* DNA methylation inhibitors

Several substances are established for the inhibition DNA methylation. Based on their structure and mode of action, they can be classified into (i) non-nucleoside analogues and (ii) nucleoside analogues. Non-nucleoside analogues (e.g. hydralazine and procainamide) inhibit DNA methylation by directly binding to the catalytic region of the DNMTs (Segura-Pacheco et al. 2003). Nucleoside analogues, e.g. azacytidine (AZA) and 5-aza-2´-deoxycytidine (5-aza-dC), have a modified cytosine ring that is attached to either a ribose or deoxyribose moiety.

Metabolization by kinases converts the nucleosides into nucleotides for incorporation into DNA and/or RNA (Bouchard and Momparler 1983; Jones and Taylor 1980). DNA methylation is inhibited when the compounds are incorporated into DNA, where they deplete DNA methyltransferases and induce replication-dependent DNA hypomethylation, which finally leads to the reactivation of silenced genes (Figure 5).

### *3.5.3* Clinical use of epidrugs in lymphoma

Since both DNA methylation inhibitors and HDAC inhibitors show antitumour activity in *vivo* and *vitro*, they had a rapid phase of clinical development in lymphoma as monotherapy or in combination with other anticancer drugs.

The DNA methylation inhibitors AZA (Vidaza®) and 5-aza-dC (decitabine, Dacogen®) are approved by the FDA and the EMA for the treatment of two haematological malignancies: (i) myelodysplastic syndrome (MDS) and (ii) acute myeloid leukemia (AML) (Kim et al. 2015; Steensma 2015). The expectation that these drugs would also be effective in malignant lymphomas was only partially achieved as demonstrated in clinical trials involving various types of lymphomas including DLBCL and cutaneous T-cell lymphoma (CTCL) (Blum et al. 2010; Stewart et al. 2009).

SAHA (vorinostat, Zolina®) was the first HDAC inhibitor approved in 2006 by the FDA for the treatment of a malignancy, namely for CTCL (Mann et al. 2007). Besides SAHA, two additional HDAC inhibitors are approved for the treatment of T-cell lymphoma: (i) romidepsin (Istodax®) for CTCL and PTCL (including ALCL) and, (ii) belinostat (Beleodaq®) for PTCL (including ALCL) (Lee et al. 2015; VanderMolen et al. 2011). A fourth HDAC inhibitor, panobinostat (Farydak®), is approved for the treatment of multiple myeloma, a type of B-NHL (Fenichel 2015).

Several clinical studies were conducted in other lymphoma entities such as DLBCL, FL and cHL using HDAC inhibitors in mono- or combination therapies (Chen et al. 2015; Morschhauser et al. 2015; Ogura et al. 2014; Oki et al. 2013; Stathis et al. 2011; Watanabe et al. 2010; Younes et al. 2012). Overall, a quite heterogeneous clinical benefit has been observed in lymphoma patients treated with HDAC inhibitors ranging from complete remissions (CR) to no response. The underlying molecular mechanisms of resistance are not understood yet. However, to identify patients who potentially benefit from a treatment with HDAC inhibitors, biomarkers for prediction of clinical outcome are urgently needed.

# 4. Aim of this thesis

The frequent discovery of epigenetic modifications in lymphoma provides the rationale to consider pharmacological restoration by so-called epidrugs as a therapeutic option. However, the efficacy of this treatment option for lymphoma patients requires the understanding of (i) the complex epigenetic modifications in lymphoma contributing to their malignant transformation and/or progression and (ii) the underlying molecular mechanisms mediating epidrug resistance. Therefore, the aim of this thesis is to gain a better insight in the role of epigenetic modifications in the pathogenesis of lymphoma and the identification of markers, which allow a stratification of lymphoma patients eligible for epigenetic therapy with HDAC inhibitors.

# 5. Results

## 5.1 Publication 1: *Histone acetylation and DNA demethylation of T cells result in an anaplastic large cell lymphoma-like phenotype*

Authors:
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### *5.1.1* Synopsis

Anaplastic large cell lymphoma (ALCL) is a T-cell neoplasm, which was first described in 1985 by Stein and colleagues (Stein et al. 1985). Although ALCL was discovered 30 years ago, little is known about the pathogenesis of this disease (Eckerle et al. 2009; Fornari et al. 2009). A characteristic feature of anaplastic large cell lymphoma is the significant repression of the T-cell expression program despite its T-cell origin (Bonzheim et al. 2004; Foss et al. 1996).

The aim of this study was to elucidate whether epigenetic modifications are responsible for the loss of the T-cell phenotype in ALCL, which is thought to be closely related with the malignant transformation. Therefore, ALCL cell lines (n = 4) and for comparison T-cell lines with a typical T-cell phenotype (n = 4) were treated with two epigenetic modifiers (the DNA methylation inhibitor 5-aza-dC and the HDAC inhibitor TSA) to evoke DNA demethylation and histone acetylation, respectively. Affymetrix GeneChips were used to generate global gene expression profiles from treated and untreated cell lines and differentially expressed genes were evaluated by real-time reverse transcriptase (RT) PCR and Western blot analysis.

Combined DNA demethylation and histone acetylation of ALCL cells were not able to reconstitute their T-cell phenotype. Instead, the same treatment induced in T cells (i) an up-regulation of ALCL-characteristic genes (e.g. *ID2*, *LGALS1*, *c-JUN*), and (ii) an almost complete extinction of their T-cell phenotype including *CD3*, *LCK* and *ZAP70*. Additional analysis of H3K27 trimethylation by chromatin immunoprecipitation revealed a suppression of important T-cell transcription factors (*GATA3*, *LEF1, TCF1*) in T cells very similar to the situation in ALCL cells. Interestingly, this is in line with their absence in primary tumour specimens as demonstrated by immunohistochemistry.

Taken together, epigenetically activated suppressors (e.g. *ID2*) contribute to the down-regulation of the T-cell expression program in ALCL, which is maintained by H3K27 trimethylation.

### *5.1.2* Own contribution in publication 1

| | | |
|---|---|---|
| **I Study design** | General study design | 20% |
| | Development of 5-aza-dC/TSA treatment protocol | 50% |
| | Development of bisulfite sequencing protocol | 50% |
| **II Experimental** | Cultivation and treatment of cell lines | 100% |
| | RNA isolation and microarray analysis | 100% |
| | Real-time RT PCR analysis | 100% |
| | DNA isolation and global quantification of methylated DNA | 100% |
| | Western blot analysis | 100% |
| | Chromatin immunoprecipitation | 100% |
| | Bisulfite conversion, polymerase chain reaction, TOPO TA cloning and sequencing | 100% |
| **III Data analysis** | Microarray data analysis | 70% |
| | Enrichment analysis of biological annotations | 70% |
| **IV Publication** | Data compilation | 50% |
| | Manuscript writing | 80% |

## 5.2 Publication 2: A novel approach to detect resistance mechanisms reveals FGR as a factor mediating resistance to the HDAC inhibitor SAHA in B-cell lymphoma

Authors:
**Maria Joosten**, Sebastian Ginzel, Christian Blex, Dmitri Schmidt, Michael Gombert, Cai Chen, René Martin Linka, Olivia Gräbner, Anika Hain, Burkhard Hirsch, Anke Sommerfeld, Anke Seegebarth, Uschi Gruber, Corinna Maneck, Langhui Zhang, Katharina Stenin, Henrik Dieks, Michael Sefkow, Carsten Münk, Claudia D. Baldus, Ralf Thiele, Arndt Borkhardt, Michael Hummel, Hubert Köster, Ute Fischer, Mathias Dreger, Volkhard Seitz

Published in:
**Molecular Oncology** (*in press*)

DOI: http://dx.doi.org/10.1016/j.molonc.2016.06.001

### *5.2.1* Synopsis

Histone deacetylase (HDAC) inhibitors, including suberoylanilide hydroxamic acid (SAHA), have shown promising clinical response rates in hematological malignancies and solid tumours. Clinical studies on relapsed or refractory B-cell lymphomas revealed that around 30% of patients receiving SAHA as a single agent achieved partial or complete remission (Kirschbaum et al. 2011; Ogura et al. 2014; Watanabe et al. 2010). For the remaining 70% non-responders, it is essential not to lose time with ineffective epigenetic treatment and to avoid unnecessary side-effects.

In this study, we hypothesized (i) that proteins which directly interact with SAHA are involved in SAHA response and (ii) that the mutation status of these direct SAHA targets might be of relevance for successful SAHA response. To identify the molecular mechanisms leading to SAHA resistance a novel approach was designed that combines **d**rug **e**fficacy **t**esting with **e**xome and **c**aptured **t**arget analysis (DETECT).

SAHA efficacy was tested in 26 B-cell lymphoma cell lines using flow cytometry (Annexin V/ propidium iodide staining (PI)). SAHA-interacting proteins were determined by Capture Compound Mass Spectrometry (CCMS) in SAHA resistant and SAHA sensitive cell lines (Fischer et al. 2011; Koster et al. 2007). In addition, whole exome sequencing was employed to access the impact of mutations in SAHA-interacting proteins. For validation expression analysis and knock-out experiments were performed.

Using our novel DETECT approach, the direct binding of the Src tyrosine kinase Gardner-Rasheed feline sarcoma viral (v-fgr) oncogene homolog (FGR) to SAHA was demonstrated for the first time. Interestingly, specific capturing of FGR by the SAHA capture compound (SAHA-CC) occurred only

in SAHA resistant cell lines. In line with this, FGR expression was significantly higher in SAHA resistant cell lines.

As functional proof, CRISP/Cas9 mediated knock-out of *FGR* in SAHA resistant cell lines increased SAHA sensitivity. *In silico* analysis of published data derived from B-cell lymphoma patients (n=1200) showed a wide range of differential FGR expression, which could be used to stratify patients for SAHA treatment.

Taken together, the comprehensive analysis of SAHA-interacting proteins reveals FGR as a factor, which is involved in SAHA resistance in B-cell lymphoma. Furthermore, expression of FGR might be a promising factor to stratify patients, who clinically benefit from SAHA treatment.

### *5.2.2* Own contribution in publication 2

| | | |
|---|---|---|
| **I Study design** | General study design | 50% |
| | Determination of B-cell lymphoma cell line panel | 100% |
| | Development of the SAHA treatment protocol | 100% |
| | Development of protein extraction protocol for CCMS | 50% |
| **II Experimental** | Human cell lines and cell culture | 100% |
| | Treatment with HDAC inhibitor SAHA and SAHA efficacy testing | 100% |
| | Protein extraction and Western blot analyses | 100% |
| | Cellular protein extraction for CCMS | 50% |
| | RNA isolation, cDNA synthesis and real-time RT PCR | 100% |
| | Whole-exome sequencing | 20% |
| | *In silico* FGR expression and mutation analysis in B-cell lymphoma patients | 50% |
| **III Data analysis** | Dose-response curve analysis | 100% |
| | Integrated network analysis | 20% |
| **IV Publication** | Data compilation | 30% |
| | Manuscript writing | 70% |

# 6. Discussion

## 6.1 Epigenetic modifications play an important role in the pathogenesis of lymphoma

In the last decades, a lot of new “omics-“ technologies were developed and established that elucidate molecular mechanisms involved in the pathogenesis of lymphoma. Beside genomic alterations, epigenetic modifications were frequently detected in lymphoma. Especially, B-cell-derived lymphomas such as DLBCL and FL show numerous mutations in epigenetic genes, such as *EZH2* and *MLL2* (Lohr et al. 2012; Morin et al. 2011; Pasqualucci et al. 2011b). These mutations in epigenetic regulators lead to a disturbed epigenome in the tumour cells and thus contribute to the malignant transformation and/or progression of B cells. However, the exact mechanisms by which mutations in chromatin modifiers promote lymphomagenesis are still unknown (Lunning and Green 2015).

As previously demonstrated, epigenetic modifications contribute to the repression of the B-cell phenotype of cHL (Ammerpohl et al. 2012; Doerr et al. 2005; Ehlers et al. 2008; Ushmorov et al. 2006; Seitz et al. 2011). cHL is characterized by a minority (less than 5% of all cells) of large mononuclear HRS cells embedded in an abundant and heterogeneous cellular infiltrate (Swerdlow et al. 2008). HRS cells lack or weakly express specific B-cell markers such as CD20, OCT2, BOB1, PU.1 and PAX5 in the vast majority of cases and several studies showed that epigenetic modifications play an important role in the down-regulation of these genes (Ammerpohl et al. 2012; Doerr et al. 2005; Ehlers et al. 2008; Ushmorov et al. 2006; Seitz et al. 2011). A very similar phenotype can be evoked in B-cell lines by DNA methylation and histone acetylation consisting of both, the up-regulation of cHL typical genes and the extinction of the B-cell expression program (Ehlers et al. 2008). Interestingly, several suppressors of lineage fidelity such as ID2 were in the list of genes up-regulated by this epigenetic treatment suggesting that the loss of the B-cell program is mediated by indirect mechanisms and not a direct consequence of the epigenetic treatment (Ehlers et al. 2008; Seitz et al. 2011).

ALCL shows several similarities with cHL, although they derive from mature T cells as indicated by the presence of fully rearranged TCR genes. Beside cytomorphological features and the consistent expression of the TNF receptor CD30, a down-regulation and/or (partial) loss of the T-cell expression program is frequently observed in ALCL cells comparable to the extinction of the B-cell phenotype in cHL (Bonzheim et al. 2004; Foss et al. 1996).

Although ALCL was discovered more than 30 years ago, little is known about the pathogenesis of this disease – especially regarding the cause for the repression of the T-cell expression (Stein et al. 1985). The striking parallels between cHL and ALCL led to the hypothesis that epigenetic modifications are also involved in the repression of the T-cell phenotype in ALCL.

Therefore this study was undertaken to clarify the role of epigenetic modifications for the loss of the T-cell phenotype in ALCL. To this end, T- and ALCL-cell lines (four cell lines of each disease entity) were treated with 5-aza-dC and TSA to evoke global DNA demethylation and histone acetylation in the cells. Gene expression analysis of untreated and 5-aza-dC/TSA treated cells revealed that this epigenetic treatment is unable to restore the T-cell phenotype of ALCL tumour cells (Joosten et al. 2013). This is very much in harmony with previous findings of our group that epigenetic treatment of HRS cells is not able to rescue the B-cell phenotype of cHL (Ehlers et al. 2008). In contrast, the same treatment applied to T cells that express the entire T-cell program led to the down-regulation of several genes (such as *CD3*, *LCK*, *ZAP70* and *LAT)* known to be involved in TCR signalling (Joosten et al. 2013). Most of these molecules are components of the proximal signal cascade and are thus essentially required for TCR signalling (Exley et al. 1991; Marie-Cardine and Schraven 1999). Our observation derived from treated T-cell lines is in harmony with previous studies, which found an absent or reduced expression of these TCR-signalling associated genes in primary ALCL cases (Bonzheim et al. 2004). In addition to our finding of down-regulation of the T-cell phenotype, 5-aza-dC/TSA treated T cells showed also an up-regulation of ALCL-characteristic genes (Joosten et al. 2013). Strikingly, the inhibitor of DNA binding 2, dominant negative helix-loop-helix protein (*ID2)* was the most up-regulated gene. ID2 belongs to the inhibitor of differentiation family of helix-loop-helix transcription factors. Members of this gene family are not capable of binding directly to DNA, but they exert their activity through interaction with other helix-loop-helix transcription factors, preventing them from binding to DNA (Massari and Murre 2000). Through this mechanism, ID proteins have a dominant-negative effect on the transcription of lineage-specific genes (Mathas et al. 2006; Perk et al. 2005). Our data revealed that the up-regulation of *ID2* after 5-aza-dC/TSA treatment was combined with a demethylation of the *ID2* promoter. In line with this, the *ID2* promoter in primary ALCL cases was also demethylated (Joosten et al. 2013). Thus ID2 could play a central role in the suppression of the T-cell phenotype in ALCL, which is further supported by the fact that ectopic overexpression of ID2 in T-cells resulted in the down-regulation of T-cell genes (Mathas et al. 2009).

A further very interesting finding of our data revealed that the promoters of important T-cell transcription factors (i.e. *GATA3*, *TCF1* and *LEF1*) were silenced in ALCL cell lines by H3K27 trimethylation, which is in agreement with the absence of these transcription factors in primary ALCL cases (Joosten et al. 2013). The lack of these transcription factors prevents the full development of the T-cell phenotype (Kuo and Leiden 1999).

Polycomb group proteins such as EZH2 and RYBP mediate H3K27 trimethylation (Bracken and Helin 2009). Interestingly, the RING1 and YY1 binding protein (RYBP) was up-regulated in our 5-aza-dC/TSA treated T cells and is strongly up-regulated in the tumour cells of primary cHL and ALCL cases but not in their lymphoid bystander cells (Joosten et al. 2013). The exact role of RYBP in this context is not fully understood yet. As described previously, the tumour cells of both, ALCL and cHL, show an overexpression of EZH2, which appears to be also involved in H3K27 trimethylation mediated suppression of lineage specific transcription factors (Eckerle et al. 2009; Dukers et al. 2004; Raaphorst et al. 2000). This finding could also be an explanation why 5-aza-dC/TSA treatment is not able to restore the cell-type phenotype in ALCL and cHL cell lines. The DNA methylation inhibitor 5-aza-dC is not able to prevent the methylation of H3K27 and thus cannot restore the expression of lineage specific genes.

Taken together, the same two epigenetic mechanisms seem to be involved in the repression of the lineage specific expression programs in ALCL and cHL: (i) epigenetic activation of suppressors of lineage fidelity such as ID2 leads to the down-regulation of lineage-specific genes and (ii) additional silencing through H3K27 trimethylation of important transcription factors prevents the reestablishment of the cell-type characteristic expression program (Joosten et al. 2013).

The full understanding of the molecular events leading to lymphoma would pave the way for new, targeted and less toxic treatment options. Since epigenetic modifications are reversible compared to genetic alterations, they are ideally suited to fulfil this task.

## 6.2 HDAC inhibitors showed promising results in a subset of lymphoma patients

The pharmacological restoration of epigenetic regulation balance by using epidrugs represents one of the hopes for new strategies in lymphoma treatment. Especially two classes of epidrugs were in focus: (i) HDAC inhibitors and (ii) inhibitors of DNA methylation.

The DNA methylation inhibitors AZA and 5-aza-dC showed promising results in patients with MDS and AML (Steensma 2015; Kim 2015), but failed in the vast majority of lymphoma patients (Blum et al. 2010; Stathis et al. 2011; Stewart et al. 2009).

More favourable results were achieved using HDAC inhibitors in lymphoma treatment. SAHA (vorinostat, Zolina®) is the most clinically established HDAC inhibitor and was approved in 2006 by the FDA for the treatment of advanced forms of CTCL, where SAHA showed objective response rates (ORR) of up to 30% (Olsen et al. 2007). Clinical trials with SAHA in relapsed or refractory B-cell lymphoma patients also revealed ORR of up to 30% including complete remissions (CR). In particular, FL patients showed the most favourable responses to SAHA monotherapy with ORR of up to 47% (Kirschbaum et al. 2011; Ogura et al. 2014). Additionally, preclinical data revealed promising results in BL (Richter-Larrea et al. 2010).

Romidepsin – a further HDAC inhibitor – is approved for the treatment of relapsed or refractory PTCL and CTCL. Clinical trials with CTCL patients showed ORR of up to 35% with CR of 6% (Piekarz et al. 2009; Whittaker et al. 2010). In PTCL, ORR was 25%, including 15% CR. Within this study ALK-negative ALCL showed ORR of 24% including 19% CR (Coiffier et al. 2012).

The HDAC inhibitor belinostat is also approved for the treatment of relapsed or refractory PTCL with ORR 26% including 10 % CR. In this study, ALK-negative ALCL had 15.3% ORR, whereas ALK-positive ALCL showed no response (O'Connor et al. 2015). In clinical trials with CTCL patients 14% ORR was achieved (Foss et al. 2015).

Although they are not approved for the treatment of lymphoma, the HDAC inhibitors panobinostat and abexinostat also showed favourable results in clinical trials in a subset of patients with cHL and FL (DeAngelo et al. 2013; Evens et al. 2015; Morschhauser et al. 2015).

Taken together, clinical responses in lymphoma patients treated with HDAC inhibitors are quite heterogeneous ranging from complete remissions to no response.

Like all anticancer agents, HDAC inhibitors were also associated with side effects such as anaemia, fatigue, diarrhea, nausea and dehydration (Coiffier et al. 2012; DeAngelo et al. 2013; Evens et al. 2015; Foss et al. 2015; Morschhauser et al. 2015; Piekarz et al. 2009; Whittaker et al. 2010). In order to avoid unsuccessful treatment and to prevent unnecessary side effects, it would be highly desirable to identify those patients who benefit from this treatment option. Thus, predictive biomarkers are urgently needed to identify those patients.

## 6.3 Stratified medicine – is it possible to predict the response to HDAC inhibitors?

Traditionally, cancer patients were treated with the type of therapy that has shown the highest statistical efficacy for a particular type of cancer. Due to the rapidly increasing alternative and more targeted treatment options there is a paradigm shift in cancer therapy. More and more cancer patients are tested for one or more biomarkers to determine the optimal targeted treatment strategies for each individual patient (Diamandis et al. 2010). This combination of a therapeutic and a predictive biomarker that targets a patient subpopulation for treatment is called “stratified medicine” (Trusheim et al. 2011). Most often a biomarker is a characteristic gene alteration or particular protein (over-) expression, which is utilized as a target for a respective drug and which indicates the efficacy of a respective drug (Dietel et al. 2015).

Detection of several biomarkers is already clinical routine (molecular pathology), e.g. the determination of *KRAS* mutation status in colorectal cancer. One therapeutic option in the treatment of advanced colorectal cancer is the use of antibodies that mediate a blockade of the epidermal growth factor receptor (EGFR). In this example, only patients without *KRAS* mutations benefit from EGFR-targeting antibodies like cetuximab and panitumumab. KRAS is an intracellular downstream component in the EGFR signalling cascade and mutations in this gene lead to a constitutional activation of EGFR, which might abolish the effect of an up-stream inhibition of EGFR (Amado et al. 2008; Dietel et al. 2015; Karapetis et al. 2008).

Several mechanisms for HDAC inhibitor resistance in lymphoma and thus potential predictive biomarkers were described including changes in (i) apoptosis pathways (Ierano et al. 2013), (ii) activation of NF-κB (Dai et al. 2005), (iii) DNA repair (Fotheringham et al. 2009; Khan et al. 2010), and (iv) overexpression of STAT signalling pathway genes (Fantin et al. 2008). However, the exact molecular mechanism mediating HDAC resistance in lymphoma is still poorly understood and all predictive biomarkers failed when applied clinically and thus none is approved yet (Treppendahl et al. 2014; Stimson and La Thangue 2009).

## 6.4 DETECT - a novel approach to unravel drug resistance mechanisms

Understanding the underlying molecular mechanisms in drug resistance is essential to find potential predictive biomarkers. We assume that drug response is mainly based on drug target proteins and their mutation status. Therefore, we designed the DETECT approach, which combines drug efficacy testing with whole-exome sequencing and captured target analysis for the detection of factors involved in drug resistance.

In this study, we hypothesized that SAHA resistance is mediated by direct SAHA binding partners and thus we used DETECT to unravel SAHA resistance mechanisms in a panel of 26 B-cell lymphoma cell lines.

SAHA efficacy testing revealed 11 SAHA resistant, 3 intermediate and 12 sensitive B-cell lymphoma cell lines, which nicely reflects the occurrence of responding and non-responding B-cell lymphoma patients. Both BL and DLBCL cell lines were present in SAHA sensitive and resistant groups. However, overall the BL cell lines showed the higher $IC_{50}$ values and thus were more resistant to SAHA compared to the DLBCL cell lines.

Using CCMS, 315 SAHA-interacting proteins were identified. Integrating SAHA efficacy and the combined bioinformatics analysis of CCMS data and whole exome sequencing data revealed a protein network containing mainly membrane-associated Src tyrosine kinases (FGR, HCK, LYN) and G-proteins linked to nucleus-associated HDAC complexes through the STAT pathway. Within this network, the Scr tyrosine kinase FGR showed the highest and most significant CCMS enrichment in resistant cell lines and also FGR mutations occurred only in SAHA resistant cells (n = 3/11). This encouraged us to study the role of FGR in SAHA resistance in more detail.

First we provided evidence that SAHA-FGR binding is direct and specific. Furthermore, we showed that the SAHA binding site at FGR must be different to the binding site of the known FGR inhibitor dasatinib, which blocks the catalytic pocket (Karaman et al. 2008). However, the exact SAHA-FGR binding site remains unclear.

To further validate the role of FGR for SAHA resistance, FGR expression analyses were performed in all 26 B-cell lymphoma cell lines. In line with the CCMS data, FGR expression was significantly higher in all SAHA resistant cell lines compared to SAHA sensitive cell lines.

To gain more understanding of the functional role of FGR of SAHA resistance, we evaluated the effect of *FGR* knock-out in SAHA resistant cell lines with high FGR expression. *FGR* knock-out led to an increase in SAHA sensitivity in FGR wild-type cell lines, whereas a *FGR* mutated cell line showed no difference in SAHA sensitivity. We postulated that this cell line acquired mechanisms to circumvent a possible altered FGR function.

In the context of B-cell lymphoma, the direct interaction of SAHA with FGR is of special interest, since FGR is a membrane-associated non-receptor tyrosine kinase and member of the Src-family kinases (SFKs). C-SRC, YES, LYN, FYN, HCK, BLK and YRK also belong to SFKs and they are all key mediators in the B-cell receptor (BCR) signalling transduction cascade (Saijo et al. 2003).

Activated SFKs phosphorylate cytoplasmic domains of BCR components, which finally leads to the activation of transcription factors such as signal transducers and activators of transcription (STATs) (Dal Porto et al. 2004). Interestingly, a higher expression of activated (phosphorylated) STAT1, STAT3, and STAT5 was previously described as a factor involved in SAHA resistance in lymphoma, and FGR is involved in phosphorylation of STATs (Schreiner et al. 2002; Fantin et al. 2008).

To test the feasibility using FGR analysis to stratify B-cell lymphoma patients eligible for SAHA treatment, FGR expression and mutation status was analyzed *in silico* by using publically available data sets. The data derived from 1200 B-cell lymphoma patients showed a wide range of FGR expression but FGR mutations were a very rare event in cases with available sequence information (n = 1/413). Therefore, FGR mutations seem to be negligible, whereas FGR expression might be a promising factor to stratify patients, who have a clinical benefit from SAHA treatment.

SAHA belongs to the class of hydroxamate HDAC inhibitors, which also includes the HDAC inhibitors panobinostat, belinostat and abexinostat. Since these HDAC inhibitors also showed favorable results in a subset of lymphoma patients, it would be interesting if FGR expression could also be a potential predictive biomarker for the treatment with other hydroxamate HDAC inhibitors (DeAngelo et al. 2013; Evens et al. 2015; Foss et al. 2015; Morschhauser et al. 2015; O'Connor et al. 2015). However, to get a predictive biomarker that can be used in patient care, the correlation between FGR expression and HDAC inhibitor resistance needs to be validated in a prospective clinical trial. Importantly, such studies should be extended to other lymphoma entities (e.g. ALCL and cHL) and also other types of cancer (e.g. glioblastoma), where a subset of patients also showed promising response to this kind of treatment (Galanis et al. 2009; Coiffier et al. 2012; DeAngelo et al. 2013; Morschhauser et al. 2015; O'Connor et al. 2015; Evens et al. 2015).

Overall, the DETECT approach is a promising strategy to identify drug resistance mechanisms and to determine biomarkers for stratified treatment modalities. However, there are also limitations in this approach since prodrugs such as AZA and 5-aza-dC are metabolized in the body after administration in order to be converted into a pharmacologically active drug (Momparler and Derse 1979).

## 6.5 Conclusion and Perspectives

In addition to genomic alterations, numerous epigenetic modifications were detectable in lymphoma cells that lead to a shift towards a repressed chromatin state and to changes in DNA methylation patterns contributing to their malignant transformation and/or progression.

The molecular events leading to the T-cell-derived ALCL were unknown for a long time. This study shows that epigenetic modifications are involved in the repression of the T-cell phenotype of ALCL – which is thought to occur together with the malignant transformation – comparable to the situation in B-cell derived cHL. In both lymphoma entities (i) epigenetic activation of suppressors of lineage fidelity leads to the down-regulation of lineage-specific genes and (ii) additional silencing of important transcription factors through H3K27 trimethylation prevents the re-establishment of the cell-type characteristic expression program.

The understanding of epigenetic modifications in lymphoma cells would pave the way for targeted treatment alternatives employing epidrugs. Such new treatment approaches are urgently needed, especially for lymphoma patients who do not response to or relapse after standard therapy. The HDAC inhibitor SAHA showed promising results in a subset of lymphoma patients, however the majority is resistant to SAHA treatment. Understanding the molecular mechanisms leading to SAHA resistance is essential to determine predictive biomarkers that help to identify patients eligible for this treatment option. Our novel DETECT approach revealed that the direct SAHA binding protein FGR is a factor mediating SAHA resistance in B-cell lymphoma and that expression of FGR might be a promising factor to stratify patients, who have a clinical benefit from SAHA treatment.

Taken together, this thesis provides clear and additional evidence that epigenetic modifications are key players in lymphomagenesis and that epigenetic therapies are very promising for lymphoma patients with a respective molecular make-up.

In addition to epidrug monotherapy, their combination with other anticancer agents might even improve the general future treatment situation. HDAC inhibitors were shown to sensitize DLBCL and BL tumour cells against standard chemotherapy, which provides the possibility to lower chemotherapy doses in order to limit toxicities and adverse effects (Ageberg et al. 2013; Dos Santos Ferreira et al. 2012). The combination of DNA methylation and HDAC inhibitors also showed synergistic effects resulting in increased anti-tumour activity (Kalac et al. 2011; Stathis et al. 2011).

Furthermore, epidrugs with more specific targets could also be of great interest. Recently, inhibitors of the histone methyltransferase EZH2 (e.g. EPZ-6438) were in focus for the treatment of lymphoma patients. Especially in DLBCL and FL, these inhibitors showed promising results (Knutson et al. 2014). Regarding our data, ALCL patients may also benefit from targeted treatment with EZH2 inhibitors.

All in all, the elucidation of the complex role of epigenetic modifications in lymphomagenesis will be essential to improve the use of epidrugs for lymphoma treatment. Moreover, predictive biomarkers are urgently needed to identify those lymphoma patients eligible for treatment with these promising treatment options.

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# 8. Appendix

## 8.1 List of abbreviations

| | |
|---|---|
| 5-aza-dC | 5-aza-2'-deoxycytidine |
| ABC DLBCL | activated B-cell DLBCL |
| Ac | acetylation |
| ALCL | anaplastic large cell lymphoma |
| ALK | anaplastic lymphoma kinase |
| ALL | acute lymphoblastic leukemia |
| AML | acute myeloid leukemia |
| AZA | azacytidine |
| B cell | B lymphocyte |
| BCR | B-cell antigen receptor |
| BL | Burkitt lymphoma |
| bp | base pair(s) |
| C | cytosine |
| C-ALCL | primary cutaneous ALCL |
| CC | capture compound |
| CCMS | Capture Compund Mass Spectometry |
| CD | cluster of differentiation |
| cHL | classical Hodgkin lymphoma |
| CpG islands | DNA region where a cytosine nucleotide occurs next to a guanine nucleotide (—C—phosphate—G—) |
| CR | complete remission |
| C region | constant region |
| CSR | class switch recombination |
| CTCL | cutaneous T cell lymphoma |
| DETECT | **d**rug **e**fficacy **t**esting with **e**xome and **c**aptured **t**arget analysis |
| DLBCL | diffuse large B-cell lymphoma |
| DN thymocyte | double negative thymocyte |
| DNA | desoxyribonucleic acid |
| DNMT | DNA methyltransferase |
| DP thymocyte | double positive thymocyte |
| EBV | Epstein-Barr virus |
| EMA | European Medicines Agency |
| *et al.* | and others (lat. *et alii*) |

| | |
|---|---|
| EZH2 | enhancer of zeste homolog 2 |
| FDA | U.S. Food and Drug Administration |
| FDC | follicular dendric cell |
| FGR | Gardner-Rasheed feline sarcoma viral (v-fgr) oncogene homolog |
| FL | follicular lymphoma |
| GC | germinal center |
| GCB DLBCL | germinal center B-cell DLBCL |
| H | histone |
| HAT | histone acetyltransferase |
| HDAC | histone deacetylase |
| HIV | human immunodeficiency virus |
| HMT | histone methyltransferase |
| HRS cells | Hodgkin-Reed-Sternberg cells |
| $IC_{50}$ | half-maximal inhibitory concentration |
| ID2 | inhibitor of differentiation 2 |
| Ig | immunoglobulin |
| IgH | immunoglobulin heavy chain |
| IgL | immunoglobulin light chain |
| K | lysine |
| MDS | myelodysplastic syndrome |
| Me | methylation |
| MHC | major histocompatibility complex |
| mRNA | messenger-RNA |
| n | number |
| $NAD^+$ | nicotinamide adenine dinucleotide |
| NF-κB | nuclear factor 'kappa-light-chain-enhancer' of activated B-cells |
| NHL | non-Hodgkin lym phoma |
| NK cells | natural killer cells |
| NPM-ALK | nucleophosmin-anaplastic lymphoma kinase |
| ORR | objective response rate |
| P | phosphorylation |
| PCR | polymerase chain reaction |
| PI | propidium iodide |
| PRC2 complex | polycomb repressive complex |
| PRMT | protein arginine methyltransferases |

| | |
|---|---|
| PTCL | peripheral T-cell lymphoma |
| R | arginine |
| R-CHOP | combination of anti-CD20 antibody **R**ituximab and **C**yclophosphamid, **H**ydroxydaunorubicin (Doxorubicin), Vincristin (**O**ncovin®) and **P**redniso(lo)n |
| RNA | ribonucleic acid |
| RT PCR | reverse transcription PCR |
| S | serine |
| SAHA | suberoylanilide hydroxamic acid |
| SET domain | **S**u(var), **E**nhancer-of-Zeste and **T**rithorax domain |
| SFK | Src-family kinases |
| SHM | somatic hypermutation |
| SP T cell | single positive T cell |
| STATs | signal transducers and activators of transcription |
| T | threonine |
| T cell | T lymphocyte |
| TCR | T-cell antigen receptor |
| TNF | tumour necrosis factor |
| TSA | trichostatin A |
| U | ubiquitination |
| USA | United States of America |
| V(D)J recombination | recombination of variable (V), diversity (D) and joining (J) gene segments of the TCR and the immunoglobulin genes |
| V region | variable region |
| $Zn^{2+}$ | zinc |

## 8.2 Publications, Patents, Conference talks and Awards

**Publications**

- **M. Joosten**, S. Ginzel, C. Blex, D. Schmidt, M. Gombert, C. Chen, R.M. Linka, O. Gräbner, A. Hain, B. Hirsch, A. Sommerfeld, A. Seegebarth, U. Gruber, C. Maneck, L. Zhang, K. Stenin, H. Dieks, M. Sefkow, C. Münk, C.D. Baldus, R. Thiele, A. Borkhardt, M. Hummel, H. Köster, U. Fischer, M. Dreger, V. Seitz. ***A novel approach to detect resistance mechanisms reveals FGR as a factor mediating resistance to the HDAC inhibitor SAHA in B-cell lymphoma.*** Molecular Oncology. In press. doi: 10.1016/j.molonc.2016.06.001

- **M. Joosten**, V. Seitz, K. Zimmermann, A. Sommerfeld, E. Berg, D. Lenze, U. Leser, H. Stein, M. Hummel. ***Histone acetylation and DNA demethylation of T cells result in an anaplastic large cell lymphoma-like phenotype***. Haematologica. 2013 Feb;98(2):247-54. doi: 10.3324/haematol.2011.054619

- I. Eriksson, **M. Joosten**, K. Roberg, K. Ollinger. ***The histone deacetylase inhibitor trichostatin A reduces lysosomal pH and enhances cisplatin-induced apoptosis.*** Experimental Cell Research. 2013 Jan 1;319(1):12-20. doi: 10.1016/j.yexcr.2012.10.004

- V. Seitz, P.E. Thomas, K. Zimmermann, U. Paul, A. Ehlers, **M. Joosten**, Dimitrova L, Lenze D, Sommerfeld A, Oker E, Leser U, Stein H, Hummel M. ***Classical Hodgkin's lymphoma shows epigenetic features of abortive plasma cell differentiation***. Haematologica. 2011 Jun;96(6):863-70. doi: 10.3324/haematol.2010.031138

**Patents**

- **M. Joosten**, S. Ginzel, C.D. Baldus, A. Borkhardt, M. Hummel, H. Köster, U. Fischer, M. Dreger, V. Seitz; ***"Method for Hydroxamic Acid based HDAC Inhibitor Response Prediction";*** patent number: EP15194040.0

**Conference talks**

- **M. Joosten**, V. Seitz, L. Dimitrova, A. Sommerfeld, E. Oker, E. Berg, H. Stein, M. Hummel. **Die Rolle epigenetischer Modifikationen bei der Auslöschung des T-Zell-Phänotyps im anaplastisch großzelligen Lymphom;** 95. Jahrestagung der Deutschen Gesellschaft für Pathologie (DGP), Leipzig, Germany, 2011

- **M. Joosten**, M. Hummel, V. Seitz, H. Stein. **Demethylierung und Acetylierung von T-Zellen führt zu einem ALCL-ähnlichen Phänotyp;** 94. Jahrestagung der Deutschen Gesellschaft für Pathologie (DGP), Berlin, Germany, 2010

**Awards**

- Katharina-Heinroth-Preis 2011

## 8.3 Selbstständigkeitserklärung (Declaration of Authorship)

Hiermit erkläre ich, dass ich die vorgelegte Arbeit selbst verfasst und keine weiteren als die aufgeführten Quellen sowie Hilfsmittel in Anspruch genommen habe. Die Dissertation wurde in dieser oder anderer Form keiner anderen Prüfungsbehörde vorgelegt.

Berlin, Februar 2016 Maria Joosten