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Preclinical evaluation of histone deacetylase inhibitors for epigenetic therapy of diffuse large B-cell lymphoma

Von Silja Leiber

aus Goch

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Aus dem veterinärwissenschaftlichen Department der Tierärztlichen Fakultät

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Lehrstuhl für Bakteriologie und Mykologie

Arbeit angefertigt unter der Leitung von:

Univ.-Prof. Dr. Reinhard K. Straubinger, Ph.D.

Angefertigt an der Klinik für Hämatologie, Onkologie und Klinischen Immunologie,
Universitätsklinikum Düsseldorf

Mentor: Univ.-Prof. Dr. med. Sascha Dietrich

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Korreferent:	Univ.-Prof. Dr. Johannes Hirschberger

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List of Abbreviations

ABC-DLBCL	*activated B-cell diffuse large B cell lymphoma*
AID	*activation-induced cytidine deaminase*
ATP	*adenosine triphosphate*
AUC	*area under curve*
B cell	*B lymphocyte*
BCL2	*B cell lymphoma*
BCR	*B cell receptor*
BSA	*bovine serum albumine*
CD	*cluster of differentiation*
CREBBP	*CREB binding protein*
CTG	*Cell Titer Glo*
DLBCL	*diffuse large B cell lymphoma*
DMSO	*dimethyl sulfoxide*
DNA	*desoxyribonucleic acid*
DPBS	*dulbecco's phosphate buffered saline*
DSCL	*diffuse small cell lymphoma*
EDTA	*ethylenediaminetetraacetic acid*
EIF4G3	*Eukaryotic Translation Initiation Factor 4 Gamma 3*
EMBL	*European Molecular Biology Laboratory*
EZH2	*Enhancer of zeste homolog 2*
FACS	*fluorescence activated cell sorting*
FBS	*fetal bovine serum*
FDA	U.S. Food and Drug Administration
FL	*follicular lymphoma*
FSC	*forward scatter*
GC	*germinal center*
GCB-DLBCL	*germinal center B-cell diffuse large B cell lymphoma*
GFP	*green fluorescent protein*
GGCT	*Gamma-Glutamylcyclotransferase*
HAT	*histone acetylase*
HDAC	*histone deacetylase*
HDACi	*histone deacetylase inhibitor*
HIST1H1D	*Histone Cluster 1 H1 Family Member D*
HTS	*high-throughput drug screen*
Ig	*immunoglobuline*
IgV	*variable regions of the immunoglobulin gene*

IL-10	*Interleukin-10*
IL-6	*Interleukin-6*
IMDM	*Iscove's Modified Dulbecco's Medium*
KMT2D	*lysine methyltransferase 2D*
MAD	*median absolute deviation*
MBC	*memory B cells*
MCL	*mantel cell lymphoma*
MDM2	*mouse double minute 2 homolog*
MHC-I	*major histocompatibility complex class I*
NAD	*nicotinamide adenin dinucleotide*
NF-kB	*nuclear factor 'kappa-light-chain-enhancer' of activated B-cells*
NHL	*non-Hodgkin lymphoma*
P/S	*penicillin / streptomycin*
PB	*peripheral blood*
PBMC	*peripheral blood mononuclear cells*
PD-1	*programmed death-1*
PD-L1	*programmed death ligand-1*
PD-L2	*programmed death ligand-2*
PI	*propidium iodide*
PMBL	*primary mediastinal B-cell lymphoma*
R-CHOP	*rituximab, cyclophosphamide, doxorubicin, vincristine, and prednisone*
RASAL1	*RAS Protein Activator Like 1*
RNA	*ribonucleic acid*
RPMI 1640	*Roswell Park Memorial Institute 1640 Medium*
SGK1	*serine/threonine-protein kinase 1*
SHM	*somatic hypermutation*
Sir	*sirtuin*
SSC	*side scatter*
STAT3	*signal transducer and activator of transcription 3*
T cell	*T lymphocyte*
TME	*tumor microenvironment*
UKHD	*Universitätsklinikum Heidelberg*

1. Introduction

1.1. B-cell developement

B-cells are an essential component of the adaptive immune system, primarily responsible for producing B-cell receptors (BCRs) which are specific to particular antigens and allow B-cells to recognize and bind to pathogens (Cano & Lopera, 2013). Upon cognate antigen interaction and T cell-dependent stimulation, activated B cells can participate in germinal center (GC) reactions within the B cell follicles of secondary lymphoid organs. GC reactions involves the proliferation of B cells, somatic hypermutation (SHM), and selection in specialized regions of the GC, all of which serve to improve BCR affinity (Cyster & Allen, 2019; MacLennan, 1994; Rauschmeier et al., 2021). B cells that maturate in the GC reaction can carry out class switch recombination and acquire activation-induced cytidine deaminase (AID)-dependent mutations of their immunoglobulin genes. B cells can leave the GC reaction as long-lived memory B cells or antibody secreting plasmacells (Vieira & Rajewsky, 1990).

Memory B cells are classified based on their GC maturation experience. They are categorized into two main types: IgM memory B cells (IgM MBCs) and IgG-, IgA- or IgE- class switched memory B cells (IgG/IgA/IgE MBCs). IgM MBCs generally have on average lower IgV mutation frequencies in their BCR genes compared to IgG/IgA/IgE MBCs. This is because IgM MBCs develop earlier in the GC reaction, undergoing fewer rounds of somatic hypermutation (SHM). IgM MBCs are more likely to participate in secondary GC reactions upon reencounter of cognate or structurally similar antigen (Seifert et al., 2015; Y. Shi et al., 2003). In contrast, IgG/IgA/IgE MBCs, which have undergone class switch recombination, tend to differentiate into plasma cells more readily and possess highly Ig-mutated, high-affinity BCRs (Budeus et al., 2015; Seifert & Küppers, 2009)

However, class switching and SHM can lead to somatic mutations that contribute to malignant transformation. B-cell lymphomas, a group of hematological cancers, arise from such transformed B-cells, most frequently originating from germinal center or post-germinal center B cells (Lossos et al., 2000).

1.2. B-cell lymphomas

Lymphomas represent a heterogenous group of lymphoid cancers and exhibit a wide range of clinical behaviors and outcomes (Jiang et al., 2017; SH et al., 2017; Shankland et al., 2012). Hodgkin lymphomas make up around 10% of all diagnosed lymphomas, while non-Hodgkin lymphomas (NHL) represent the vast majority (90%) of all lymphoma diagnoses (W. Cai et al., 2021; Sedeta et al., 2022). In general, lymphomas are categorized based on the specific type of cell of origin or normal counterpart, reflecting the complexity due to the varied immune functions of lymphocytes across different lineages and differentiation stages (Jiang et al., 2017), and these various subtypes differ often significantly in their progression and response to treatment (Shankland et al., 2012).

1.2.1. Diffuse Large B-cell Lymphoma

Diffuse large B-cell lymphoma (DLBCL) is the most common type of non-Hodgkin lymphoma, encompassing a heterogeneous group of diseases with varied clinical presentations, outcomes, and molecular characteristics (Alaggio et al., 2022; Pan et al., 2022; Sehn & Gascoyne, 2015; Takahara et al., 2023). DLBCL is primarily classified into two subtypes: activated B-cell (ABC-DLBCL) and germinal center B-cell (GCB-DLBCL) (Figure 1) (Y. Liu & Barta, 2019; Richards et al., 2013). Both are characterized by Ig mutations in their BCR genes (Chapuy et al., 2018; Takahara et al., 2023).

Specifically, DLBCLs exhibiting high SHM levels in the immunoglobulin heavy chain variable region genes are associated with an increased presence of immunoglobulin-derived neoantigens, enhancing their immunogenicity (Xu-Monette et al., 2019). Neoantigens are derived from tumor-specific genetic alterations and play an essential role in tumor immunology as they are recognized as foreign antigens by immune cells (Schumacher & Schreiber, 2015). Nevertheless, a significant proportion of DLBCL subtypes exhibits reduced expression of major histocompatibility complex class I (MHC-I), as a prevalent immune escape mechanism in approximately half of these lymphomas (Fangazio et al., 2021). Moreover, DLBCLs often show high activity of activation-induced cytidine deaminase (AID), potentially leading to heightened immunogenicity through additional neoantigens (Chapuy et al., 2018; Takahara et al., 2023).

ABC-DLBCL represents an aggressive lymphoma subtype (Roschewski et al., 2020). It is distinguished by a high frequency of IgV gene mutations and an activated cellular phenotype that resembles post-GC immunoblasts (Seifert et al., 2013). ABC-DLBCL is marked by chronically active B-cell receptor signaling, resulting in persistent activation of the NF-κB pathway, ABC-DLBCL are driven by mutations in genes such as *CD79, CARD11, MYD88,* and *TNFAIP3* (Alizadeh et al., 2000; Davis et al., 2001, 2010; Seifert et al., 2013). This signaling cascade results in the continuous production of cytokines, including interleukin-6 (IL-6) and IL-10, which further promote the expression of genes important for B cell survival (Lam et al., 2008; Schmitt et al., 2021).

GCB-DLBCL is a subtype of DLBCL characterized by distinct genetic and molecular features, setting it apart from other forms of DLBCL. GCB-DLBCL is known for its activation of the PI3K signaling pathway, which is often driven by tonic BCR signaling. It frequently harbors alterations in genes, such as *EZH2*, *CREBBP* or *SGK1*, as well as *BCL2* translocations (Chapuy et al., 2018; Compagno et al., 2009; Davis et al., 2010; Grondona et al., 2018; Schmitt et al., 2021; Schmitz et al., 2018).

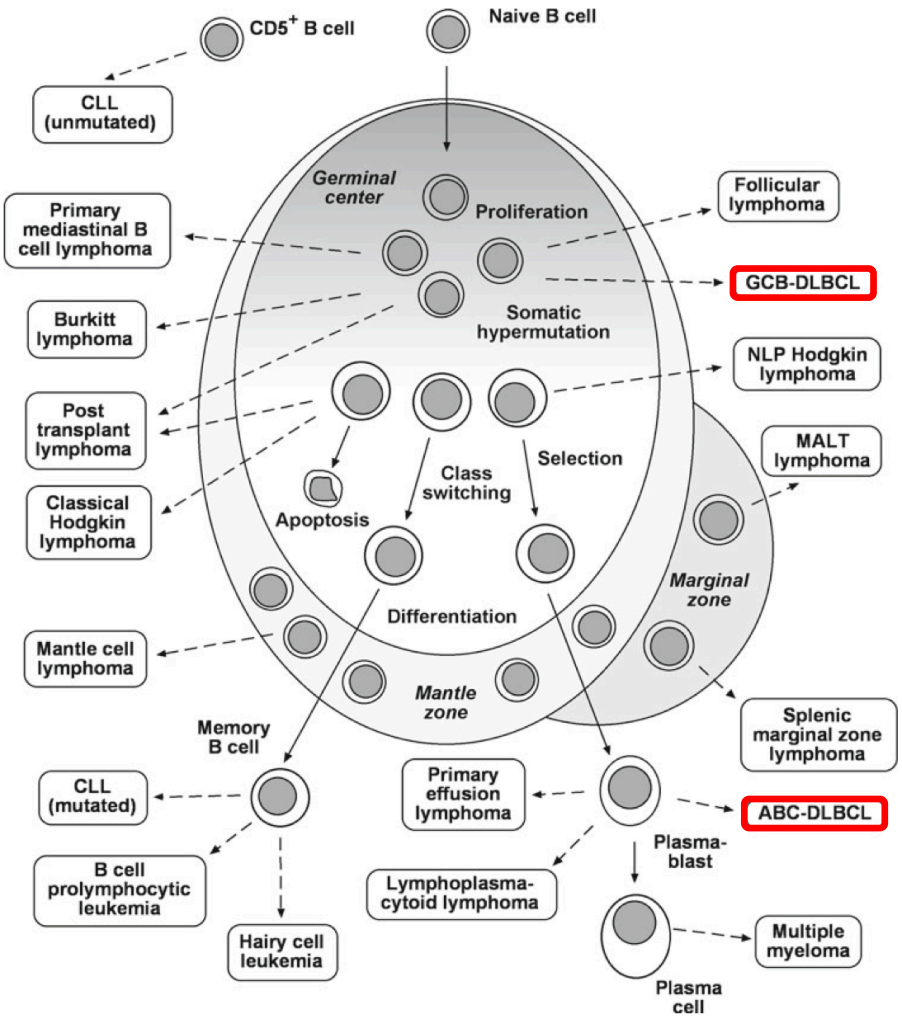

Figure 1: Germinal center reaction and cellular origin of human B cell lymphomas. The key stages of mature B-cell differentiation within the germinal center and their connection to lymphomagenesis are illustrated. Antigen-activated B cells first expand extensively within the dark zone, where they initiate somatic hypermutation (SHM). B cells with improved antigen affinity due to changes in the immunoglobulin variable (IgV) genes are selected within the light zone. Positively selected cells will undergo repeated cycles of mutation, proliferation and selection before they differentiate into memory B cells or plasmablasts. A certain number of germinal center B cells also undergo class switch recombination. The origins of most lymphomas can be traced back to cells at these germinal or post-germinal center stages (modified after Seifert et al., 2013).

The heterogeneity of DLBCL not only complicates its diagnosis and classification but also raises significant challenges in treatment. DLBCL is a common type of non-Hodgkin lymphoma, accounting for about 30-58% of NHL cases in the EU and 25-35% in the US (Dulac III et al., 2013; Kanas et al., 2022). Approximately one-third of DLBCL patients experience relapse or are refractory to standard treatment (Frontzek et al., 2022; Xu-Monette et al., 2019).

DLBCL can be divided into four stages based on the extent of the disease. Stage one is classified through only one single lymph node or a group of lymph nodes in the same spot being affected. Stage two includes the involvement of two or more lymphatic areas on the same side of the diaphragm, mainly in the upper part of the patient's body. These stages are also known as limited or Ann Arbor stages I and II. Stage three is classified through the infiltration of lymphatic regions on both sides of the diaphragm. Stage four is considered the most severe stage, in which not only lymphatic areas but also organs such as the liver, spleen, or bone marrow are infiltrated. These stages are also called advanced stages or Ann Arbor stage III or IV (Cheson et al., 2014; Jones, 2020; Mamgain et al., 2022; Morley-Jacob & Gallop-Evans, 2012; Rojek & Smith, 2022). Survival rates for DLBCL can differ depending on the stage of the disease. Patients who receive curative treatment have an overall five-year survival rate of approximately 65,3% (Harrysson et al., 2021). However, for patients with relapsed or refractory DLBCL, the five-year overall survival rate is lower, ranging from 11-26% depending on the timing of relapse (Harrysson et al., 2018).

Current treatment options are variable, with different treatment modalities, efficiencies, and potential side effects. R-CHOP (rituximab, cyclophosphamide, doxorubicin, vincristine, and prednisone) remains the standard first-line treatment for DLBCL(Sehn & Gascoyne, 2015). While it is known to be the first treatment option, it continues to pose significant challenges (Modi et al., 2021). Common side effects include myelosuppression, infection risks, and cardiovascular events, particularly in older patients (Doraiswamy et al., 2021). Targeted therapies have expanded the treatment landscape for DLBCL. CAR T-cell therapy, which

targets CD19, has shown promise in treating relapsed/refractory DLBCL. Clinical trials for CAR T-cell therapies such as ZUMA-1 (NCT02348216), JULIET (NCT02445248), and TRANSCEND (NCT03483103) have reported substantial efficacy in refractory DLBCL, leading to durable remissions in a significant portion of patients, although it is associated with significant risks, including cytokine release syndrome (CRS) and neurotoxicity (Abramson et al., 2020; Neelapu et al., 2017; Schuster et al., 2019; Sehgal et al., 2019; Shouse & Herrera, 2021). Bispecific antibodies have been introduced to manage relapsed or refractory B-cell lymphomas, including DLBCL. These antibodies engage two different antigens, typically one on the cancer cell and one on T-cells, to bring the immune cells into proximity with the cancer cells, thereby promoting their destruction (Hutchings, 2023). Bispecific T-cell engagers (BiTE), like blinatumomab, link CD3-positive T cells to CD19-positive B cells. In a phase 2 study, blinatumomab achieved a 43% overall response rate in relapsed/refractory DLBCL patients. Adverse events include neurological symptoms such as tremors, encephalopathy, and aphasia, which need careful management during treatment (Viardot et al., 2016, p. 2).

1.3. Epigenetic regulatory mechanisms in tumorgenesis

In tumorigenesis, especially in DLBCL, not only genomic alterations play a role but also epigenetic regulatory mechanisms that promote tumor cell survival (Baylin & Jones, 2011; Feinberg et al., 2016; Hanahan & Weinberg, 2011; S. V. Sharma et al., 2010).

Epigenomics is the study of the complete set of epigenetic modifications across a cell's genetic material. These modifications don't alter the DNA sequence but can significantly impact gene activity and expression (K. C. Wang & Chang, 2018). Key types of epigenomic modifications include DNA methylation and histone modification (Berger et al., 2009; Kouzarides, 2007; Mann & Bartolomei, 2002; Waddington, 2012). Epigenomics sheds light on how gene expression is regulated across various cells and tissues, and how these patterns shift in response to environmental factors, disease, and development (Jirtle & Skinner, 2007).

Recent studies underscore the impact of the tumor microenvironment on tumor epigenetics (Lin et al., 2020). Epigenetic changes can lead to abnormal cell behavior and tumorigenesis. Hypomethylation, for instance, can lead to genomic instability and the activation of oncogenes (Sheaffer et al., 2016; Van Tongelen et al., 2017). On the other hand, hypermethylation of CpG islands within promoter regions of tumor suppressor genes can result in their silencing, contributing to uncontrolled cell growth (Esteller, 2002; Y. Li & Tollefsbol, 2010). For example, BCL-6 and MYC are genes involved in the pathogenesis of DLBCL. BCL6 is essential for maintaining the germinal center B-cell phenotype, which is crucial for normal B-cell development and function. Dysregulation of BCL6 can occur through various mechanisms, including mutations, translocations, and epigenetic modifications such as promoter hypermethylation, which leads to the downregulation of its tumor suppressor activities and a poor prognosis (Yang & Green, 2019). Similarly, MYC plays a significant role in the pathogenesis of aggressive lymphomas. MYC overexpression, often due to translocations, leads to increased cellular proliferation and survival, and when co-occurring with BCL2 or BCL6 rearrangements, it is associated with a poorer prognosis and aggressive disease behavior (Karube & Campo, 2015).

1.3.1. Histone modifications

Chromatin architecture is fundamental to genomic organization, playing an essential role in the compaction and organization of DNA within the eukaryotic nucleus (Misteli, 2020). Nucleosomes, consisting of about 146 base pairs of DNA wrapped around core histone octamers (H2A, H2B, H3, H4), alongside the linker histone H1, mediate DNA packaging (Bannister & Kouzarides, 2011; Khorasanizadeh, 2004; Kimura, 2013).

The reversible modifications of histones, such as acetylation and methylation, are crucial for the dynamic regulation of chromatin states, influencing gene expression, DNA repair, and replication (Kouzarides, 2007; Voss & Hager, 2014). These modifications serve not only as a mechanism for structural adjustment but also for signaling within the cellular machinery, affecting chromatin-protein interaction and regulating its function across different cellular processes (Vaughan et al., 2021).

The range of histone modifications creates a detailed system that determines chromatin structure and accessibility. This system is essential for ensuring genome stability and enabling cells to respond to environmental changes (Chen et al., 2012; Nightingale et al., 2006). Understanding the complex regulation mechanisms of histones offers insights into the cellular mechanisms that discriminate between health and disease, hence, systematically interfering with such mechanisms – e.g., by inhibition of histone deacetylases, represents potential therapeutic options in cancer (Cheng et al., 2019; Kelly et al., 2010).

1.3.1.1. Role of histone deacetylases

Histone acetyltransferases (HATs) catalyze the transfer of acetyl groups from acetyl-coenzyme A to the ε-amino group of lysine residues on histones, neutralizing the lysine's positive charge (Berndsen & Denu, 2008; Fragou et al., 2011). This neutralization blocks histone interaction with the negatively charged DNA backbone, facilitating transcriptional activation by making DNA more accessible to transcription factors (Chueh et al., 2015). In contrast, histone deacetylases (HDACs) remove acetyl groups from histones, resulting in chromatin condensation and subsequent transcriptional repression (Kuo & Allis, 1998; Verdin & Ott, 2015) (Figure 2). This dynamic regulation by HATs and HDACs extends beyond histones, affecting various non-histone proteins involved in critical cellular functions such as the cell cycle, DNA damage repair, and autophagy (Y. Luo & Li, 2020; Narita et al., 2019; Shu et al., 2023).

The HDAC enzyme family is categorized into four distinct classes based on sequence homology to yeast orthologs (Emiliani et al., 1998; Ververis et al., 2013). Class I HDACs, including HDAC1, HDAC2, HDAC3, and HDAC8, are comparable to the yeast Rpd3 protein (Emiliani et al., 1998). These enzymes are primarily nuclear, ubiquitously expressed across different tissues, and have been implicated in transcriptional repression as well as the development and progression of various cancers such as classical Hodgkin’s lymphoma (Adams et al., 2010; E. Seto & Yoshida, 2014). Class II HDACs are divided into Class IIa (HDAC4, HDAC5, HDAC7, and HDAC9) and Class IIb (HDAC6 and HDAC10), exhibiting tissue-specific expression and differential localization within the cell, which can be

modulated by phosphorylation (Hsu et al., 2017; Montgomery et al., 2007). The sirtuin family, or Class III HDACs (SIRT1-7), stands apart from other classes by requiring Nicotinamide adenine dinucleotide (NAD^+) for their deacetylase activity and shares sequence similarities with the yeast Sir2 protein, suggesting a unique regulatory mechanism distinct from zinc-dependent HDACs (North & Verdin, 2004). Finally, Class IV of the HDAC family is uniquely represented by HDAC11, which exhibits homology with both Class I and II HDACs, positioning it as a bridge within the HDAC superfamily (Ropero & Esteller, 2007; M.-Q. Shi et al., 2024). HDAC11 plays a significant role in metabolic regulation. The enzyme's involvement in metabolic pathways highlights a broader range of functions beyond its traditional roles in histone deacetylation and transcriptional repression. This points to its significant influence on cellular metabolism and energy balance (Chen et al., 2022; Ropero & Esteller, 2007).

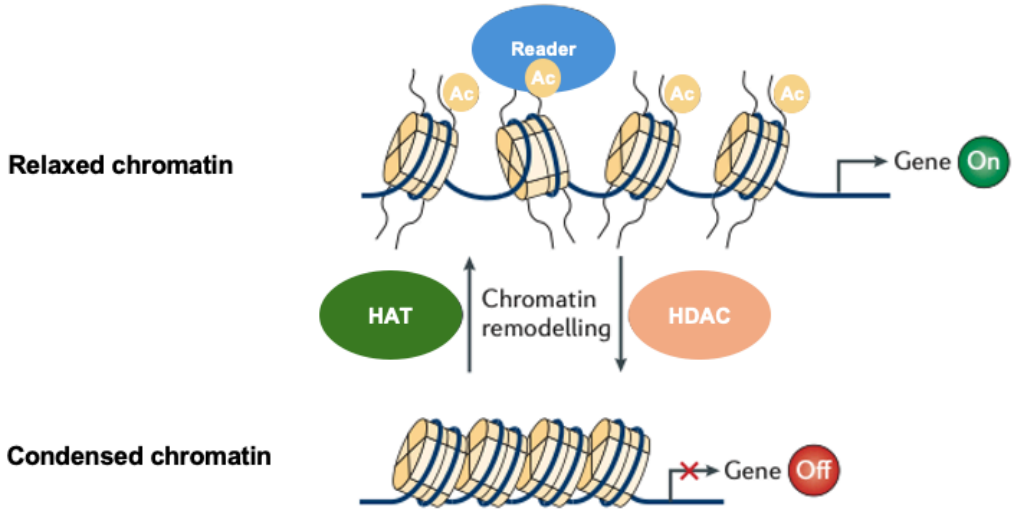

Figure 2: Mechanisms of chromatin remodeling and gene expression regulation. This figure illustrates the dynamic process of chromatin remodeling and its impact on gene expression. The upper part of the figure shows relaxed chromatin, where histone acetylation (Ac) by histone acetyltransferases (HAT) results in an open chromatin structure, allowing gene transcription to occur (Gene On). Acetyl groups are recognized by reader proteins that promote transcriptional activation. The lower part illustrates condensed chromatin, where histone deacetylases (HDAC) remove acetyl groups, leading to a more compact chromatin structure and repression of gene transcription (Gene Off) (modified after Verdin & Ott, 2015).

1.3.1.2. Histone deacetylase inhibitors

HDAC inhibitors (HDACis) are capable of inducing cancer cell cycle arrest, promoting apoptosis, and influencing various signaling pathways involved in tumor progression (Marks & Xu, 2009; Zhang & Zhong, 2014). HDACi have emerged as promising therapeutic agents in oncology, offering a novel approach to cancer treatment by modulating the chromatin landscape of cancer cells (Y. Li & Seto, 2016; Shanmugam et al., 2022). HDACi can correct the abnormal chromatin modifications observed in cancer cells (Shanmugam et al., 2022; Verza et al., 2020). By inhibiting HDAC activity, these compounds increase the acetylation of histones, leading to a more accessible chromatin structure and the reactivation of genes involved in cell cycle arrest, apoptosis, and suppression of tumor growth (Lane & Chabner, 2009; Y. Li & Seto, 2016). Additionally, HDACi can modulate non-histone proteins such as immunosuppressant program death ligand-1 (PD-L1) and programmed death ligand-2 (PD-L2) on the cell surface of tumor cells (Knox et al., 2019; Ray et al., 2018).They are categorized into four main classes based on their structural features and specificity towards HDAC enzymes (H.-J. Kim & Bae, 2011; Park & Kim, 2020) (Table 1).

Table 1: HDACi classes and their corresponding targets.

HDACi class	Target enzymes
Class I	HDAC1, HDAC2, HDAC3, HDAC8
Class IIa	HDAC4, HDAC5, HDAC7, HDAC9
Class IIb	HDAC6, HDAC10
Class III	SIRT1, SIRT2, SIRT3, SIRT4, SIRT5, SIRT6, SIRT7
Class IV	HDAC11

Group I HDACi target Class I HDAC enzymes. Located primarily in the nucleus, these enzymes significantly impact gene expression through the deacetylation of histones and non-histone proteins (Glozak et al., 2005). Group I inhibitors are particularly effective in inducing apoptosis and differentiation in cancer cells (Bolden et al., 2006).

Group II HDACi are divided into groups IIa and IIb, targeting Class II HDAC enzymes that shuttle between the nucleus and cytoplasm. These inhibitors play a crucial role in modulating not only histone acetylation but also acetylation of non-histone proteins (p53, STAT3, NF-kB, MDM2) thereby regulating gene expression, migration, and angiogenesis in cancer cells (Bradner et al., 2010; Shukla & Tekwani, 2020; Wagner et al., 2010).

Group III HDACi (sirtuin inhibitors) target class III HDAC enzymes (Sirtuins) (Bursch et al., 2024). These NAD^+-dependent enzymes are involved in various cellular processes, including aging, transcription, and DNA repair (Carafa et al., 2016). Inhibitors in this group have been shown to alter cancer cell metabolism and enhance sensitivity to chemotherapy and radiation therapy (Minucci & Pelicci, 2006; Zhao et al., 2022).

Group IV HDACi specifically target HDAC11, a Class IV enzyme. Although this group is the least understood, studies have started to uncover how HDAC11 can influence immune responses, indicating that targeting this enzyme might be beneficial for cancer treatments (S.-S. Liu et al., 2020; Martin et al., 2018; West & Johnstone, 2014).

1.3.1.3. Histone deacetylase inhibitors in lymphoma treatment

HDAC inhibitors such as vorinostat, romidepsin, belinostat, and panobinostat have been approved by the U.S. Food and Drug Administration (FDA) for various therapeutic uses in anti-tumor therapies. These inhibitors are primarily used for treating hematological malignancies like cutaneous T-cell lymphoma and multiple myeloma (Imai et al., 2019; Mottamal et al., 2015; San-Miguel et al., 2014). Additionally, other countries have also approved HDACi for similar global uses. For instance, chidamide (CS055/HBI-8000) is approved in China for the treatment of peripheral T-cell lymphoma (M. Zhu et al., 2024). These inhibitors are primarily used for their ability to induce cell cycle arrest and apoptosis in cancer cells by

modulating gene expression through epigenetic mechanisms (Mrakovcic et al., 2019; Ning et al., 2012).

HDAC inhibitors have also become an essential part of combination cancer therapies. Studies have shown that combining HDAC inhibitors with other therapeutic compounds can enhance efficacy and reduce resistance (Mazzone et al., 2017; P. Sharma et al., 2017). Panobinostat, a pan-HDACi was tested in a notable study in combination with rituximab, an anti-CD20 monoclonal antibody, in patients with relapsed or refractory DLBCL. The study found that the combination therapy had an overall response rate of 30%, with responses noted in both patient groups (Assouline et al., 2016; Taylor & Lindorfer, 2007). Another example involves the combination of HDAC6 inhibitors with CD47 blockade, which significantly enhances anti-tumor immunity. HDAC6 inhibition has been found to modulate macrophage phenotypes and boost the efficacy of CD47-targeted therapies, facilitating the phagocytosis of cancer cells by macrophages (Gracia-Hernandez et al., 2024). Similarly, tacedinaline (CI-994), a class I HDAC inhibitor, targets both intrinsic tumor growth and leptomeningeal spread in MYC-driven medulloblastoma while also enhancing the tumor's vulnerability to anti-CD47-induced macrophage phagocytosis via NF-kB-TGM2-mediated tumor inflammation. This combined strategy highlights the potential of epigenetic modulation to improve the efficacy of immunotherapy in aggressive cancers. (Marquardt et al., 2023).

1.4. Aim of the thesis

The primary aim of this dissertation was to evaluate the antitumor effects of a set of newly synthesized Histone Deacetylase Inhibitors (HDACi) on Diffuse Large B-Cell Lymphoma (DLBCL) in vitro and to establish advanced models for assessing drug responses. This included the evaluation of cytotoxic effects on non-neoplastic cells, such as peripheral blood mononuclear cells (PBMCs) from healthy donors and stromal cell lines, as well as a comparative analysis of existing proteomics and whole-genome sequencing data from the utilized cell lines. A promising drug candidate was identified for further in vitro evaluation, including cell cycle analyses using fluorescence-activated cell sorting (FACS).

Additionally, co-culture systems were employed to assess the interactions between stable GFP-labeled lymphoma cells and their microenvironment, specifically stromal cells derived from patient lymph nodes.

2. Material and Methods

2.1. Cell lines and culture conditions

In this study, 24 lymphoma cell lines were used (Table 7), encompassing diffuse large B-cell lymphoma (DLBCL), mantle cell lymphoma (MCL), and follicular lymphoma (FL). Detailed information regarding these cell lines and their culture conditions is outlined in Tables 7 and 8. All cell lines were cultured at 37°C with 5% CO_2. Suspension cells were passaged at dilutions ranging from 1:2 to 1:6 using the appropriate growth medium every two to three days. Adherent cells were passaged with 0.05% Trypsin (Gibco) at dilutions between 1:3 and 1:5. For cryopreservation, cells were sedimented (5 min at 300 × g), resuspended in a freezing solution composed of fetal bovine serum (FBS; Sigma-Aldrich) with 10% dimethyl sulfoxide (DMSO; AppliChem), at a density of 2.5 to 3 $\times 10^6$ cells per mL. and stored at -80°C in a freezing container (Life Technologies) for a minimum of 24 hours, cooling the samples at a rate of approximately -1°C/min.

2.2. Preparation of library plates

This study utilized an HDACi library consisting of 41 inhibitors (Table 6), arranged on 384-well plates (Corning) with staurosporine (MedChemExpress) serving as a positive control compound. Drugs were dispensed using the D300e Tecan digital dispenser and a previously created dispensing protocol made with D300e control (version 3.4.3). Each inhibitor was dispensed in singlicate across five concentrations, ranging from 0.00433 to 10 µM on a logarithmic scale, enabling evaluation across a broad concentration spectrum. All wells were standardized to the maximum DMSO concentration of 0.1%, with 15 wells containing only DMSO and 12 wells were filled with staurosporine at a concentration of 25 µM. The outer two rows and columns were excluded, and the inhibitors were distributed in a randomized manner.

2.3. Screening of cell lines with inhibitor libraries

Half an hour prior screening, the library plates were removed from the -80°C storage and allowed to equilibrate to room temperature. This step is crucial to prevent cells from experiencing cold shock while seeding. The viability of cells in DMSO post-incubation was evaluated.

Two automated pipetting systems utilized: the Multidrop Combi (Life Technologies), a peristaltic pump-based dispenser, and the Cybio Well Vario (Analytic Jena), a robot equipped with automated simultaneous tips-based pipetting. To assess the efficiency and accuracy of each system, two cell lines were seeded onto separate 384 well plates using the predetermined optimal cell numbers. This assay provided a quantitative measure of cell viability, allowing for a direct comparison of the performance between the Multidrop Combi and Cybio Well Vario pipetting systems.

2.4. Cell seeding using Multidrop Combi

Prior to cell seeding, the tubes of the Multidrop Combi were thoroughly washed to eliminate any potential contaminants. Initially, the tubes were rinsed with PBS. Subsequently, the tubes were washed twice with a 0.1% MICRO-90 solution in water, allowing the solution to act for 2.5 minutes during each wash. Following this, the MICRO-90 was removed by washing the tubes twice with 80% ethanol. To ensure the removal of any residual solution that could be harmful to the cells, a final wash with water was performed. For cell seeding, a cell suspension was prepared in a 50 mL tube according to cell number validation. The pre-printed drug plate was then placed in the designated position, and the seeding protocol was executed.

2.5. Cell seeding using Cybio Well Vario

The seeding protocol from CyBio Composer was utilized to seed cells into pre-printed drug plates. Before cell seeding, the tips were washed with 80% ethanol using the CyBio washing station. After the washing process was completed, the pre-prepared cell suspension was placed in the system's reservoir. Subsequently, the cell suspension was automatically pipetted into the drug plates. After seeding, the plates were removed from the system by

hand, and the tip tray was washed again with 80% ethanol. A final wash with water was performed to remove any residual ethanol.

2.6. CellTiter-Glo luminescent cell viability assay

To measure cell viability, the CellTiter-Glo Luminescent Cell Viability Assay (Promega) was utilized. The CellTiter-Glo reagent was prepared following the manufacturer's instructions and then diluted 1:1 with Dulbecco's Phosphate Buffered Saline (DPBS, Gibco). 30 µL of the reagent was dispensed into each well of the 384-well plates. This was achieved using the Cybio Well Vario for multiple plates simultaneously or a Rainin 8-channel multipipette (Mettler Toledo) for single plates. After dispensing, the plates were incubated for 30 min at room temperature in the dark. Luminescence was measured using the Spark 10M microplate reader (TECAN), providing quantitative data on cell viability for each condition tested.

2.7. Density gradient centrifugation

Peripheral blood mononuclear cells (PBMCs) were isolated using density gradient centrifugation. Peripheral blood (PB) samples were layered over 15 mL of lymphocyte separation medium Pancoll (Pan Biotech), a polysaccharide solution with a density of 1.077 g/mL, and sedimented for 35 min at 18°C. This procedure effectively separates PBMCs from serum and erythrocytes. PBMCs were washed twice with DPBS containing 0.5% w/v bovine serum albumin fraction V (BSA; Pan Biotech; PBS/BSA) and sedimented for 5 min at 300 × g.

2.8. Lentivirus transduction

Lentivirus transduction was performed to stably label DLBCL cell lines with green fluorescent protein (GFP). Two transduction methods were employed to ensure the best labeling. Cells should be in the exponential phase while performing transduction.

2.9. Lentivirus transduction using Lenti-X^{TM} Transduction Sponge

In a 24-well plate (Greiner), target cells were incubated with 20 µL lentivirus within a sponge for 24 h at 37°C to enhance transduction. After incubation, the transduced cells were released from the sponge using 1 mL of release

buffer (TAKARA) that depolymerized the alginate. Eluted cells were washed and replated for expansion over the next 72 hours. The efficiency of the transduction was assessed using fluorescence microscopy (Keyence) and fluorescence-activated cell sorting (FACS, BD).

2.10. Lentivirus transduction using Polybrene

Target cells were incubated with 20 µL of lentivirus and Polybrene (Sigma-Aldrich) diluted 1:1000 in a 15 mL tube. The cell suspension was then sedimented at 800 × g for 30 minutes at 32 °C. Following centrifugation, the virus-containing medium was aspirated, and each cell pellet was resuspended in the appropriate medium. The resuspended cells were transferred to a 24-well culture plate and incubated for 72 hours. Transduction efficiency was evaluated using fluorescence microscopy and FACS.

2.11. Flow cytometry

Cell aliquots ranging from 2.5 $\times 10^5$ to 5 $\times 10^5$ cells in 50 to 100 µL of PBS/BSA were prepared. Cells were stained with 2 uL of directly labeled antibodies (Table 9). After an incubation for 20 min at 4°C in the dark, cells were washed twice with 4 mL of PBS/BSA (400 × g, 5 min, 18°C). The analyses were performed using the BD FACSymphony A1 (Becton Dickinson Bioscience). Flow cytometry data was analyzed with FlowJo and FACS Diva (Becton Dickinson Bioscience) software.

Table 2: Antibodies used in flow cytometry analyses

Specificity and Conjugate	Host	Clone	Manufacturer	Article Number
Anti-human CD19-BV711	Mouse	SJ25C1	BioLegend	363022
Anti-human CD20-PerCy5.5	Mouse	2H7	BD Biosciences	560736
Anti-human PD-L1-BV605	Mouse	29E.2A3	BioLegend	329724
Anti-human PD-1-APC	Mouse	EH12.2H7	BioLegend	329908

2.12. Cell cycle analysis

To evaluate the impact of HDACi (e.g. YAK477) on cycle arrest and apoptosis induction, cells were treated with 2 µM of YAK477 or DMSO as a control for 72 h. Following treatment, cells were fixed with ethanol (Otto Fischer) and stained with propidium iodide (PI; Life Technologies) to identify cell cycle phases such as G0/G1, S, and G2/M. Initially, a minimum of 1×10^6 cells were harvested and transferred into a 2 mL tube (Eppendorf). After sedimentation at 400 g for 5 min, supernatant was discarded, and cells were washed with ice-cold DPBS. Subsequently, cells were added to 900 µL of 70% ethanol while vortexing to prevent clumping. Cells were fixed by incubating at -20°C for 2 h. Post-fixation, cells were sedimented at 500 × g for 5 min, supernatant was carefully discarded, and cells were washed with DPBS. To ensure selective desoxyribonucleic acid (DNA) staining, 100 µL of DPBS containing RNase [100 µg/mL] (Merck) were added. 150 µL of PI [50 µg/mL] (Life Technologies) were added, and cells were stained overnight at 4°C. The analyses were performed using FACS.

2.13. Live cell imaging

Live-cell imaging was conducted using the Incucyte® S3 Live-Cell Analysis System (Sartorius) to evaluate the co-culture assay. The experiment specifically focused on the interaction between a DLBCL cell line and stromal cells derived from patient lymph nodes. A DLBCL cell line (e.g. OCI-Ly7), labeled with GFP, was used along with a non-fluorescent stromal cell line (e.g. LNSC 177). Stromal cells were seeded into wells of a 96-well plate according to their validated cell number in 50 uL IMDM. Once the stromal cells were adherent, the medium was carefully discarded, and 100 uL of fresh medium containing DLBCL cells was added. Following a 15- minute incubation at room temperature, the plate was placed in the Incucyte, which captured two images per well every four hours over a total period of 92 hours. For comparative analyses, DLBCL cells were also plated in monoculture.

2.14. Data analysis

Graphs for cell number validation were created with GraphPad Prism 9 (version 9.03) (GraphPad Software). Analyses of drug screen data on the bioinformatic level were performed by Dr. Junyan Lu at UKHD and Thomas Naake, EMBL. Their names and contributions are mentioned in the respective places. The proteomic profiles of lymphoma cell lines were analyzed using proDA, a statistical method designed for differential protein expression analysis. Flowcytometric data was analyzed using Flowjo (version 10.10) (BD Bioscience). Live cell imaging data was analyzed using the green object count per image normalized to time point zero in Sartorius Basic Analyzer.

3. Results

3.1. Validation of cell numbers highlights growth variability in lymphoma cell lines

It is crucial to validate the appropriate seeding densities for each cell line individually to ensure the best outcomes in subsequent HTS. The cell numbers need to be high enough to produce a measurable ATP signal, but not leading to overcrowding, which would negatively impact cell viability. To determine the optimal seeding density, initial cell numbers ranging from 1,000 to 12,000 cells per well were tested for each cell line. After 72 hours of incubation, ATP levels were assessed using the CellTiter-Glo assay. The results indicated that lymphoma cell lines on average exhibited optimal growth when seeded between 5,000 and 8,000 cells per well (Table 10).

Table 3: Cell number validation results for lymphoma cell lines.

Entity	Cell line	Cell number for HTS
ABC-DLBCL	HBL-1	8,000
ABC-DLBCL	OCI-Ly3	3,500
ABC-DLBCL	OCI-Ly18	6,000
ABC-DLBCL	Riva (RI-1)	7,000
ABC-DLBCL	SU-DHL-2	2,500
ABC-DLBCL	TMD-8	3,500
ABC-DLBCL	U-2932	3,000
ABC-DLBCL	WSU-DLCL2	6,000
DSCL	WSU-FSCCL	7,500
FL	SC-1	10,000
GCB-DLBCL	Farage	8,000
GCB-DLBCL	K422	6,000
GCB-DLBCL	OCI-Ly1	6,000
GCB-DLBCL	OCI-Ly2	6,000
GCB-DLBCL	OCI-Ly7	5,500
GCB-DLBCL	OCI-Ly8	5,500
GCB-DLBCL	Pfeiffer	5,000
GCB-DLBCL	SU-DHL-4	7,500
GCB-DLBCL	SU-DHL-5	5,500
GCB-DLBCL	SU-DHL-8	12,000

MCL	HBL-2	7,000
MCL	JEKO-1	10,000
MCL	Z-138	8,000
PMBCL	U-2940	9,000
Stroma	LNSC177	5,000

The linear regression range was used to identify the optimal cell numbers representing exponential growth (Figure 3). The growth patterns varied substantially across different lymphoma subtypes. Specifically, at lower seeding densities (1,000 to 3,000 cells per well), most of the cell lines exhibited suboptimal growth, detectable by lower luminescence readings. ABC-DLBCL cell lines, such as OCI-Ly3, U-2932, and SUDHL-2 reached their growth plateau more quickly compared to GCB-DLBCL cell lines like Farage, K422, and OCI-Ly2. This difference in growth dynamics necessitated the adjustment of seeding densities to ensure optimal growth conditions for each cell line. Notably, some cell lines required higher initial densities to achieve similar growth characteristics. For instance, non-DLBCL cells, including SC-1, HBL-2, and U-2940, exhibited robust growth even at higher seeding numbers. Beyond the optimal seeding range, a decrease in luminescence was observed.

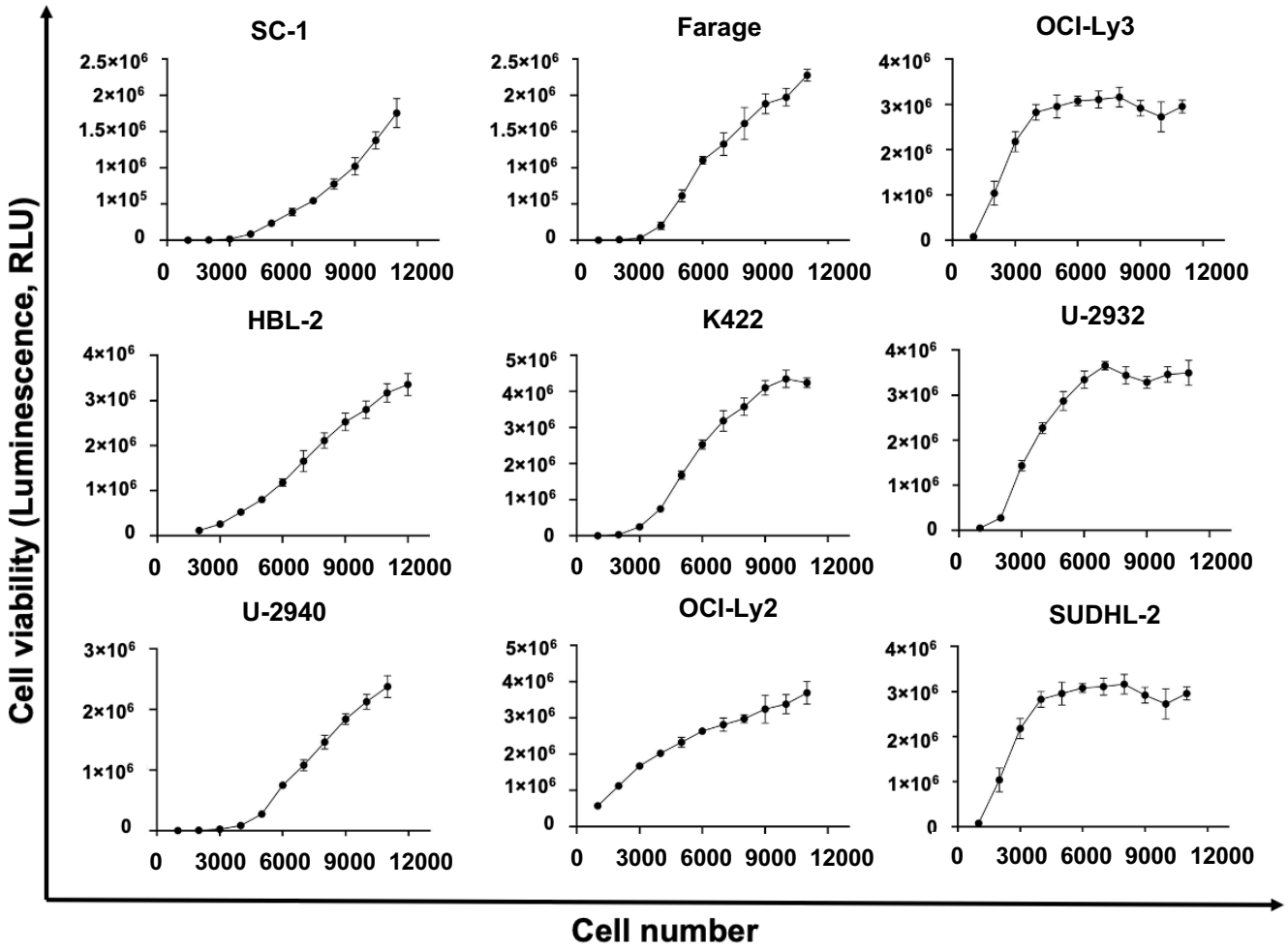

Figure 3: Correlation between cell number and viability across different cell lines. Examples for luminescence readings (Relative Light Units, RLU) from the CellTiter-Glo Assay (ranging from 1×10^6 to 6×10^6), representing ATP levels and cell viability, are plotted against the number of cells initially seeded per well for different lymphoma cell lines (1000-12000 on y axis).

3.2. Comparison of our two automated pipetting systems

To identify a suitable pipetting method for the upcoming high-throughput drug screen, the performance of two different pipetting systems, the Cybio Well Vario and the Multidrop Combi, was compared.

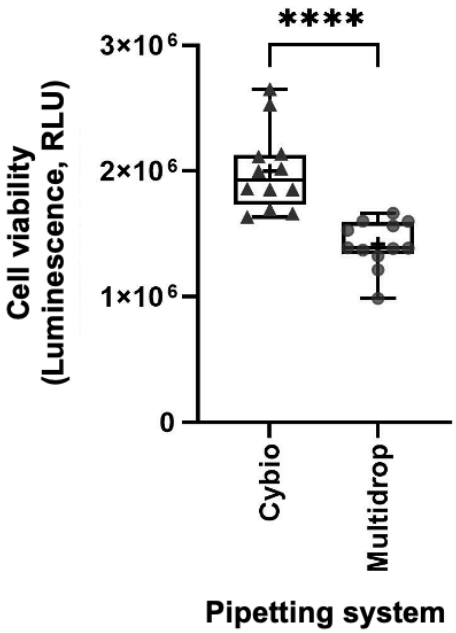

Figure 4: Comparison of cell viability between Cybio and Multidrop DMSO control groups. Cell viability was assessed using a luminescence assay (RLU), with higher luminescence values indicating greater cell viability. Statistical evaluation was done using students t-test (****$p < 0.0001$).

The Cybio method exhibited significantly higher luminescence among the cell lines tested compared to the Multidrop instrument, indicating better cell viability after using the Cybio instrument (Figure 4). Based on these results, the Cybio Well Vario system was chosen for screening cell lines in this study.

3.3. Viability assessment of tumor cell lines treated with HDAC inhibitors

The initial screening of the HDACi library, consisting of 41 newly synthesized and modified compounds, was conducted on 18 tumor cell lines, mainly GCB- and ABC-DLBCL, and 3 peripheral blood mononuclear cell (PBMC) samples from healthy donors. To ensure the robustness of the data, quality control steps were implemented, including staurosporine (25 µM) as a positive control and 15 wells with DMSO 0.1% as a negative control. Cell lines exhibiting inconsistent viability in DMSO control wells were excluded from the analysis (U-2932, HBL-2, OCI-Ly8). Furthermore, drugs demonstrating excessive toxicity to non-cancerous PBMCs (viability below 0.8 post-treatment) were also excluded. Cell viability results (Figure 5) illustrate the impact of various HDAC inhibitors on different cell lines. The drugs are arranged from left to right based on their cytotoxic potential, with those on the left showing highest reduction of cell viability. Many class I/II inhibitors showed a high effect on cell viability. To focus on effective drugs, those with a median absolute deviation (MAD) of less than 0.05 and median viability greater than 0.9 were excluded from the analysis. To evaluate the overall effect of HDACi on the tested cell line cohort, the area under curve (AUC) for each drug and sample was calculated using the trapezoidal rule (Junyan Lu).

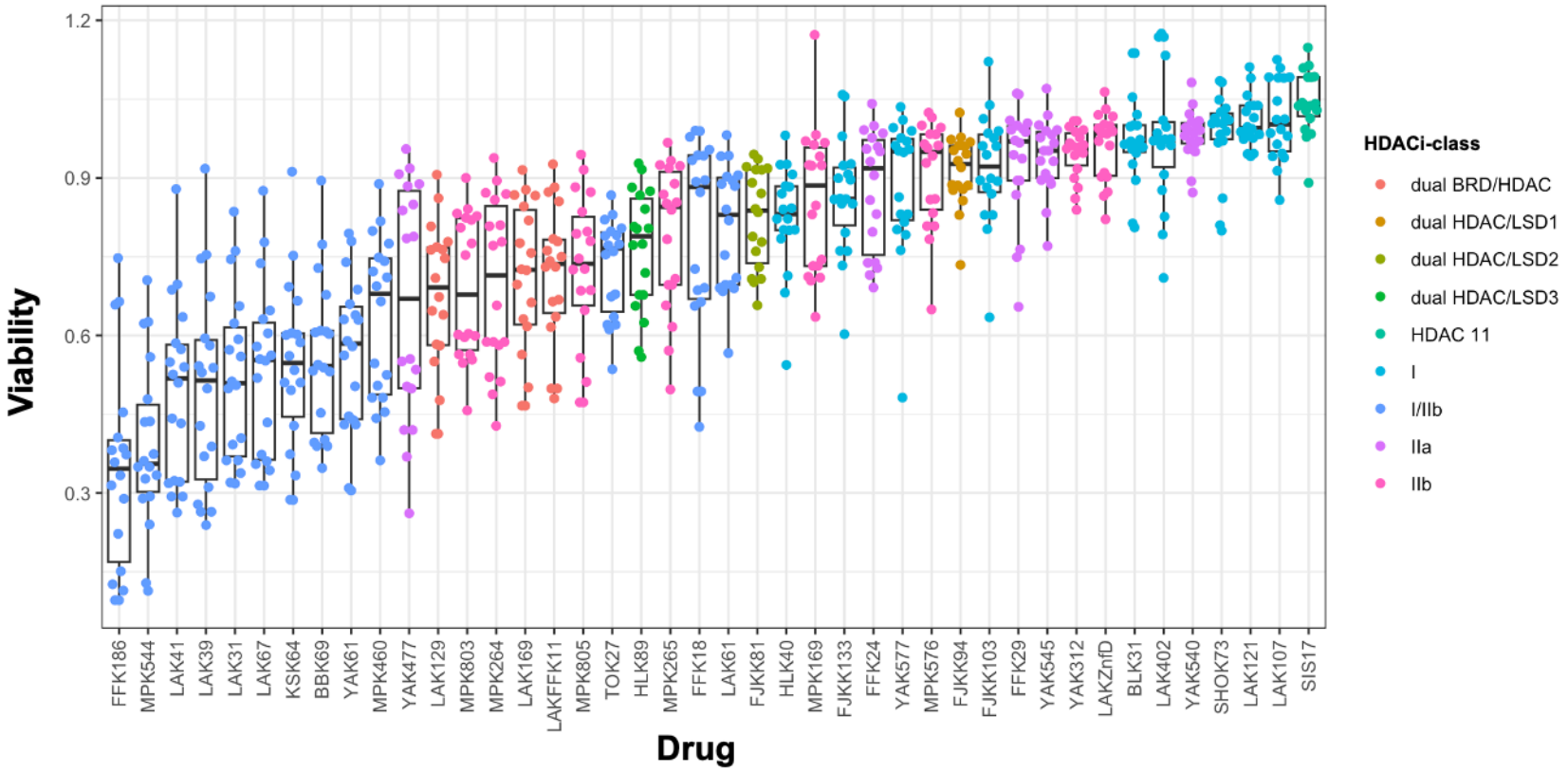

Figure 5: Effect of different HDAC inhibitors on cell viability. Box plot illustrating the viability of multiple cell lines treated with various HDAC inhibitors (HDACi) following DMSO normalization. HDAC inhibitors are arranged from left to right in order of decreasing efficiency, with those on the left demonstrating the strongest targeted effects and those on the right showing reduced responsiveness. Individual dots correspond to distinct cell lines and three samples of PBMCs from healthy donors, with colors distinguishing the respective HDACi classes. This figure was prepared by Dr. J. Lu, UKHD.

After quality control three cell lines that showed inconsistent viability in the DMSO control were excluded. Nine compounds were excluded that showed high toxicity on non-cancerous cells (PBMC viability < 0.8): *MPK544, FFK186, LAK41, YAK61, LAK31, KSK64, HLK89, LAK67,* and *BBK69.* Furthermore, 12 compounds were excluded that had less effect on lymphoma cell lines (MAD <0.05 and median viability > 0.9): *FJKK94, FJKK103, YAK545, YAK312, FFK29, LAK402, BLK31, LAKZnfD, YAK540, SHOK73, LAK121,* and *SIS17*. In summary, we used 23 HDACi compounds and 15 cell lines for further analysis. This comprehensive evaluation ensured that only those drugs with significant anti-cancer activity and minimal toxicity to normal cells were considered for further studies.

3.4. Correlation of genetic mutations and HDAC inhibitor response in DLBCL cell lines

The responsiveness of selected lymphoma cell lines to the selected HDACi revealed two distinct response clusters: six cell lines classified as resistant (response cluster 1), and nine cell lines classified as sensitive (response cluster 2). No significant correlation was found between the responsiveness to HDACi and the subclasses of DLBCL cell lines (Figure 6).

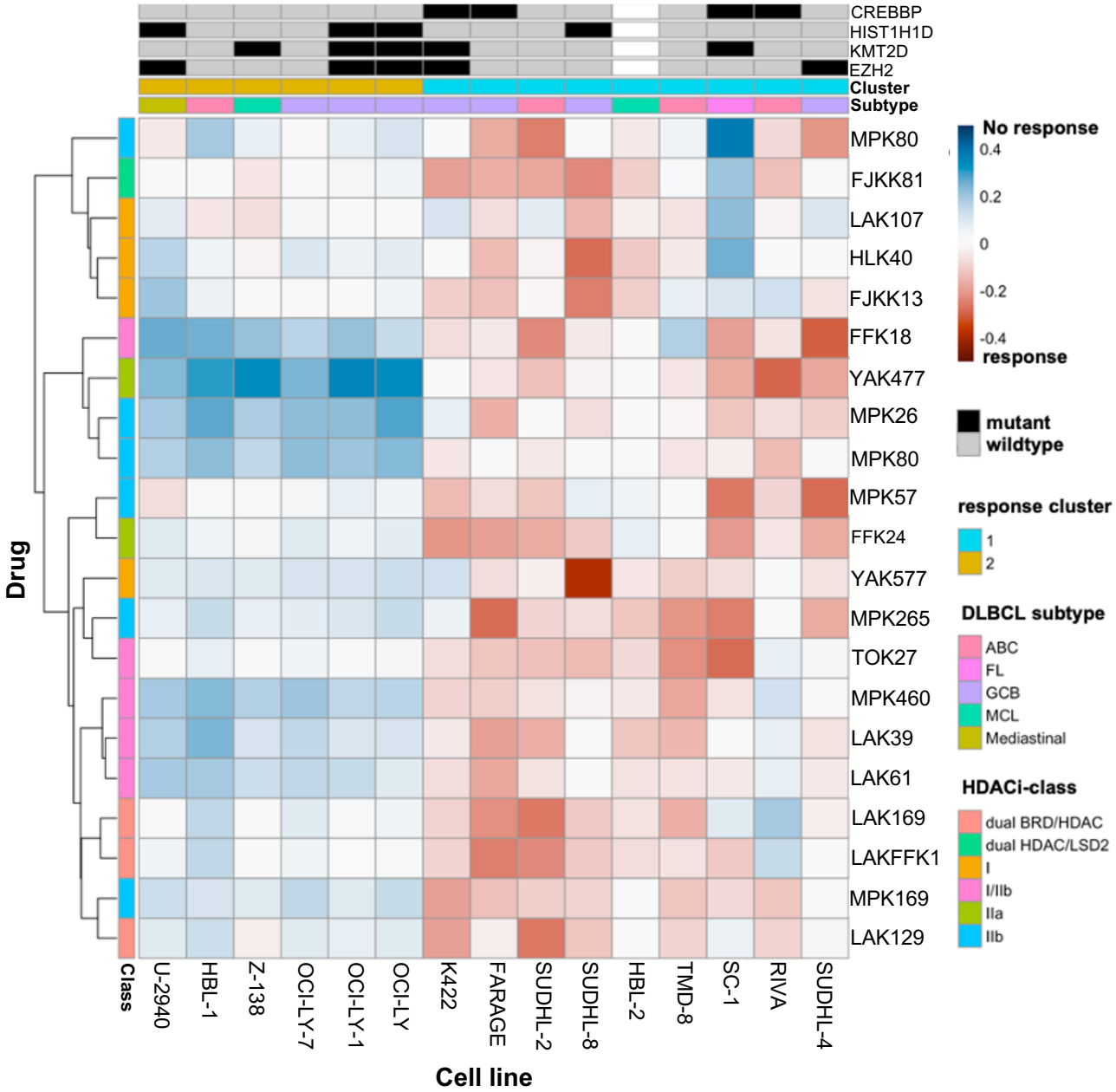

Figure 6: Lymphoma cell line responses to HDAC Inhibitors correlated with predominant gene mutations detected by whole genome sequencing. The heatmap displays selected cell lines, along with information on the four most prevalent genes identified through whole genome sequencing and their responsiveness to specific HDAC inhibitors (x-axis). The y-axis enumerates the HDACi and their respective classes. The color gradient within the matrix denotes

the response levels, with different hues representing varying degrees of sensitivity (red) or resistance (blue) to each HDAC inhibitor. This Figure was prepared by Dr. J. Lu, UKHD.

Four genetic mutations showed a correlation with response to the HDAC inhibitors: CREB Binding Protein (CREBBP), Enhancer of Zeste Homolog 2 (EZH2), Lysine Methyltransferase 2D (KMT2D) and Histone Cluster 1 H1 Family Member D (HIST1H1D). CREBBP mutations were exclusively observed in sensitive cell lines, suggesting a potential link between these mutations and increased sensitivity to HDACi. Conversely, mutations in EZH2 and KMT2D were slightly less common in the sensitive cluster, and HIST1H1D mutations were also less prevalent among the sensitive cell lines. This genetic and response profiling provides insight into the potential molecular mechanisms influencing the responsiveness of DLBCL cell lines to HDAC inhibitors, highlighting the potential for deeper molecular investigations and eventually, targeted therapeutic strategies.

3.5. YAK477 as a potential drug target for CREBBP mutant lymphomas

While comparing the responses between sensitive and resistant cell lines, we identified the class IIa HDAC inhibitor, YAK477, which showed the most significantly different targeted effect (Figure 7).

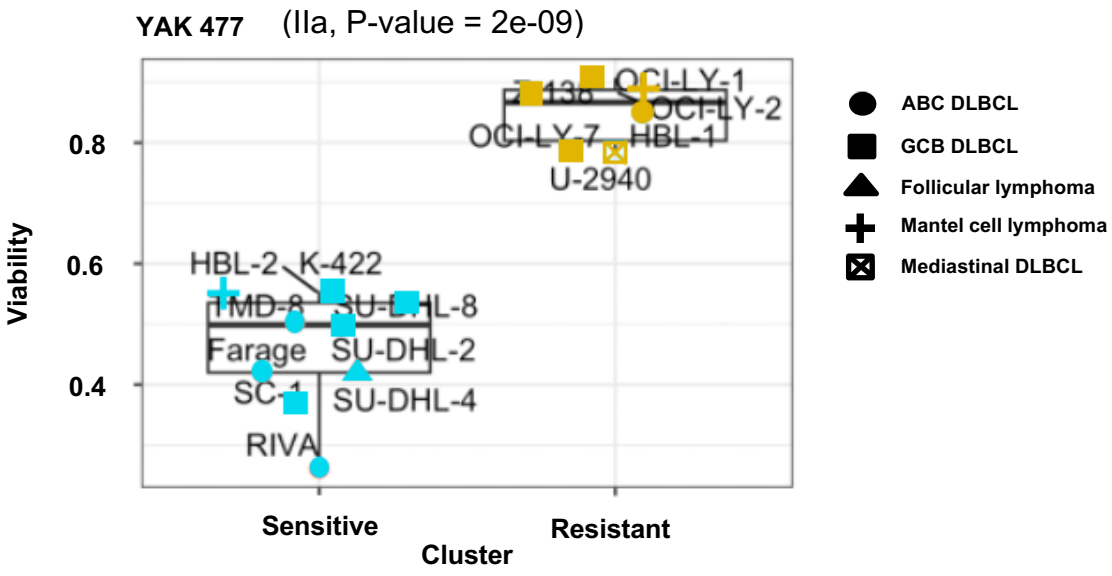

Figure 7: Effect of YAK477 on different lymphoma entities. Scatter plot showing the viability of various DLBCL subtypes and other lymphoma cell lines in

response to YAK477 treatment. The x-axis categorizes cell lines into sensitive and resistant clusters, while the y-axis represents cell viability. This figure was modified after J. Lu, UKHD.

The dose-response curve (Figure 8) demonstrates that YAK477 targets CREBBP mutant lymphoma cell lines more effectively than CREBBP wild-type lymphoma cell lines. Additionally, YAK477 shows no significant cytotoxic effects on non-cancerous cells, such as PBMCs and stroma cells.

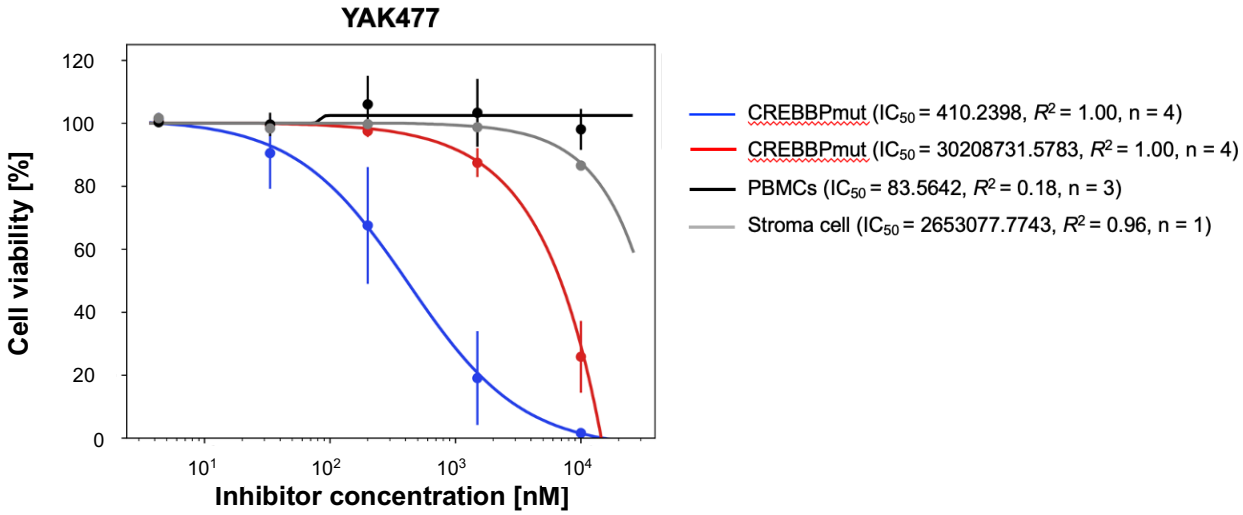

Figure 8: Dose-response curve of YAK477 on CREBBP mutant and wild-type cells such as non-cancerous cells. Shown are dose-response curves illustrating the effect of YAK477 on cell viability, focusing on CREBBP mutant (blue) and CREBBP wild-type (red) cells. Non-cancerous cell lines, including PBMC (black) and stromal cells (gray) are also shown for comparison. Cell viability is plotted as a percentage against the logarithmic scale of inhibitor concentration (nM). The IC50 values, goodness of fit (R2), and the number of replicates (n) are provided for each condition, indicating the concentration at which YAK477 reduces cell viability by 50%.

These findings suggest that YAK477 is a promising candidate for selectively targeting CREBBP mutant DLBCL, with minimal impact on normal cells *in vitro*.

3.6. Correlation between proteomic profiles and HDAC inhibitor sensitivity in lymphoma cell lines

The proteomic profiles of lymphoma cell lines were analyzed to identify correlations between protein expression and sensitivity to HDAC inhibitors (Figure 9). Initially, proteins were sorted based on their raw p-values. Proteins with a raw p-value below 0.05 were considered significant and selected for further downstream analysis, resulting in a focus on 298 proteins. Subsequently, these proteins were ranked according to their p-values, from the lowest (< 0.01) to the highest (0.05). The four most significantly expressed proteins, selected for further evaluation were Eukaryotic Translation Initiation Factor 4 Gamma 3 (EIF4G3), RAS Protein Activator Like 1 (RASAL1), Insulin-like Growth Factor 2 mRNA Binding Protein 1 (IGF2BP1), and Gamma-Glutamylcyclotransferase (GGCT). These proteins were identified as the most predominant markers associated with sensitivity to HDAC inhibitors.

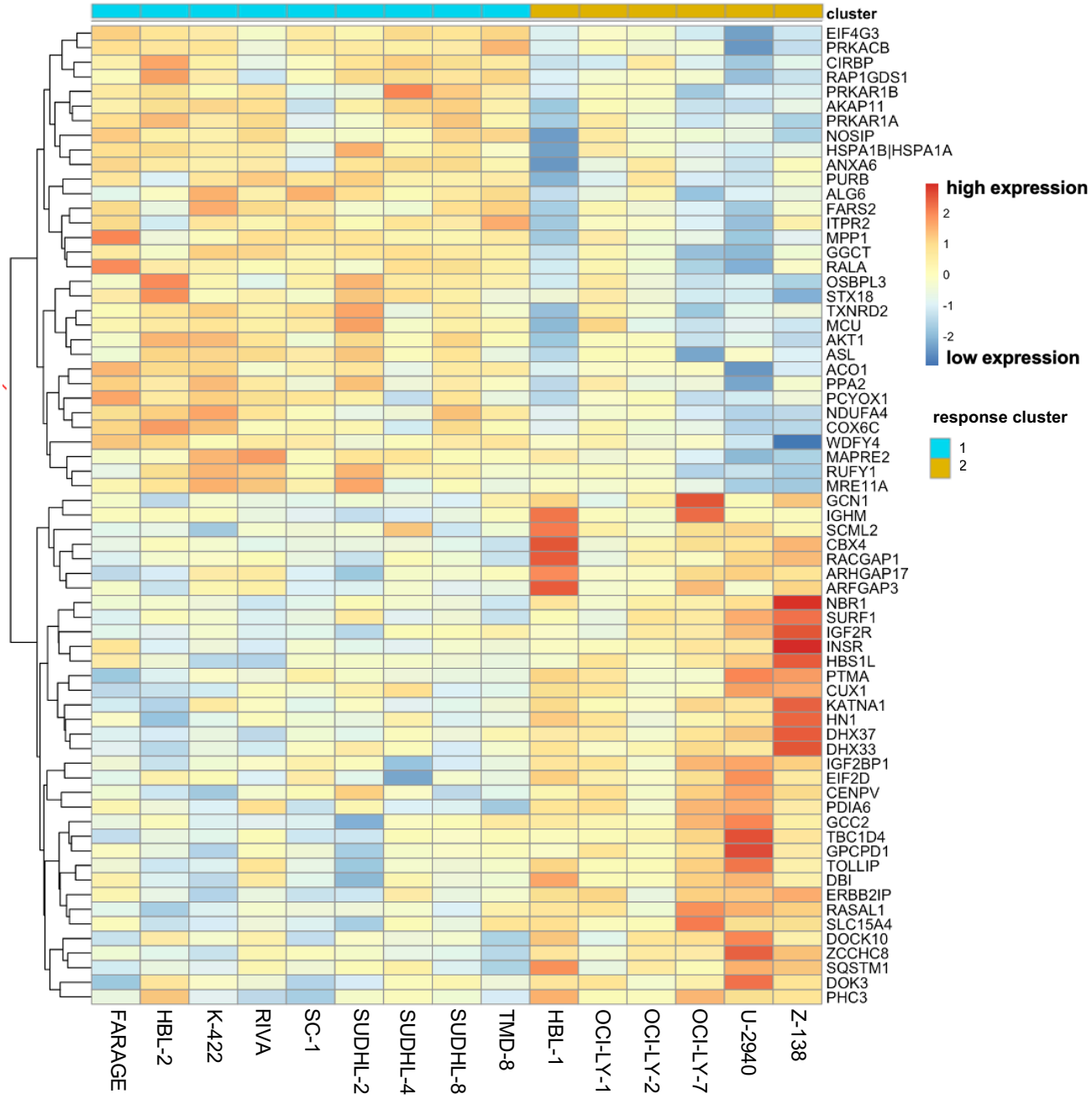

Figure 9: Proteomic profiling of cell lines in response to HDAC inhibitors. The heatmap displays proteomic data from selected cell lines, categorized into a sensitive group (response cluster 1) and a resistant group (response cluster 2), in response to HDAC inhibitors (x-axis). The y-axis lists the most significant proteins identified through proteomic analysis. This figure was prepared by T. Naake, EMBL.

High expression levels of EIF4G3 and GGCT were predominantly found in the sensitive cluster, indicating a potential link between these proteins and increased sensitivity to HDAC inhibitors. Conversely, RASAL1 and IGF2BP1 were upregulated in the resistant cluster, suggesting their association with reduced sensitivity to HDAC inhibitors (Figure 10).

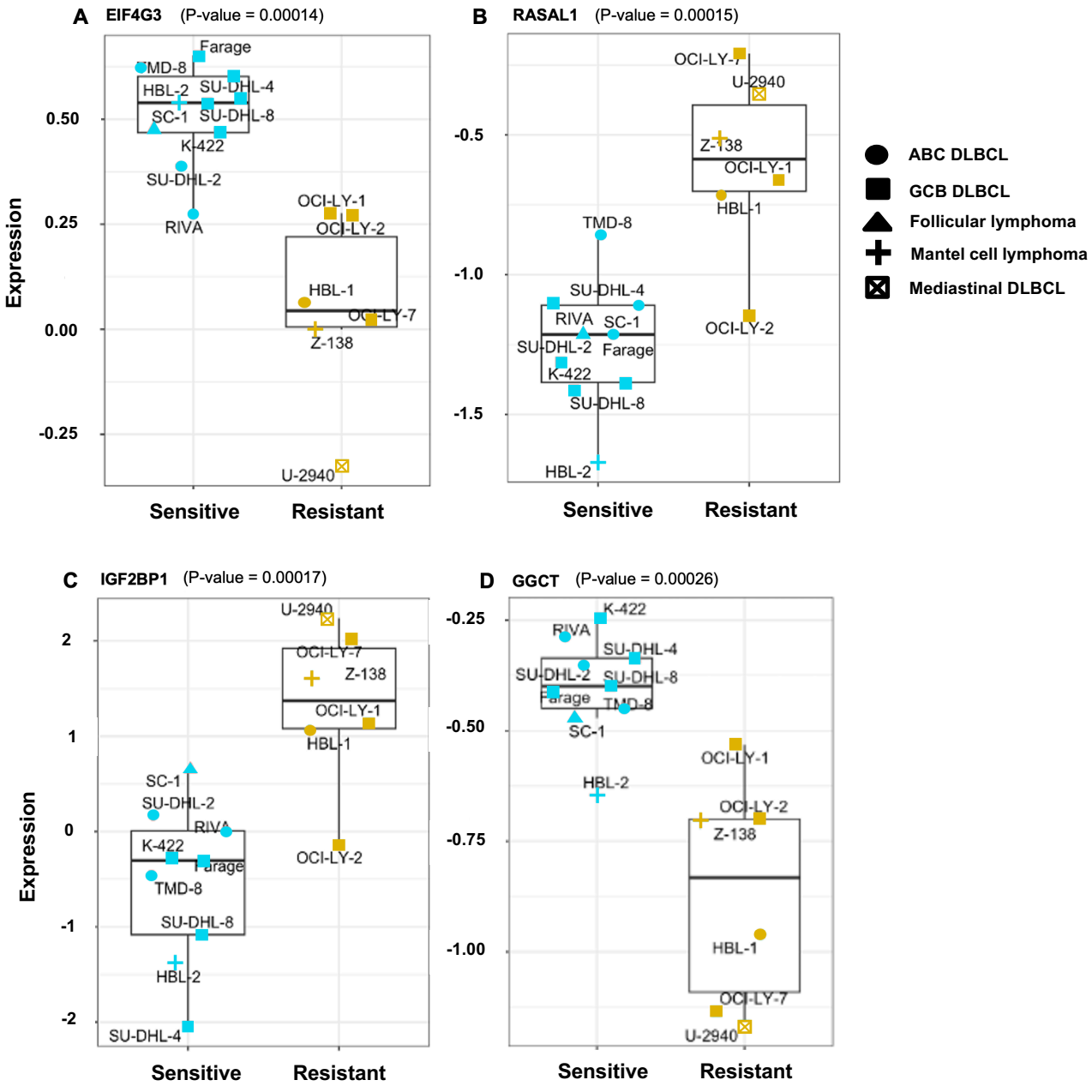

Figure 10: Expression Levels of Highly Significant Proteins in Lymphoma Subtypes. Expression levels of four proteins with the highest significance in distinguishing between sensitive and resistant lymphoma clusters. EIF4G3 (A) and GGCT (D) are highly expressed in sensitive cell lines (blue) and RASAL1 (B) and IGF2BP1 (C) are highly expressed in resistant cell lines (yellow). Different lymphoma subtypes are represented by distinct shapes. This figure was modified after T. Naake, EMBL.

The pathway enrichment analysis revealed significant differences in the regulation of various pathways between HDACi resistant and sensitive lymphoma cell lines. In resistant cell lines, several pathways were upregulated, as indicated by the red bars. Notably, the "HATs Acetylate Histones" pathway was significantly upregulated ($p < 0.01$), suggesting increased histone acetylation activity may contribute to resistance mechanisms. Another crucial upregulated pathway in resistant cell lines is the "Reactome Membrane Trafficking," which may play a role in cellular processes that reduce the efficacy of HDAC inhibitors.

On the other hand, pathways downregulated in resistant cell lines (indicated by blue bars) included "KEGG Apoptosis" ($p < 0.01$), which underscores the reduced apoptotic activity in these cells, potentially contributing to their survival despite HDACi treatment (Figure 11).

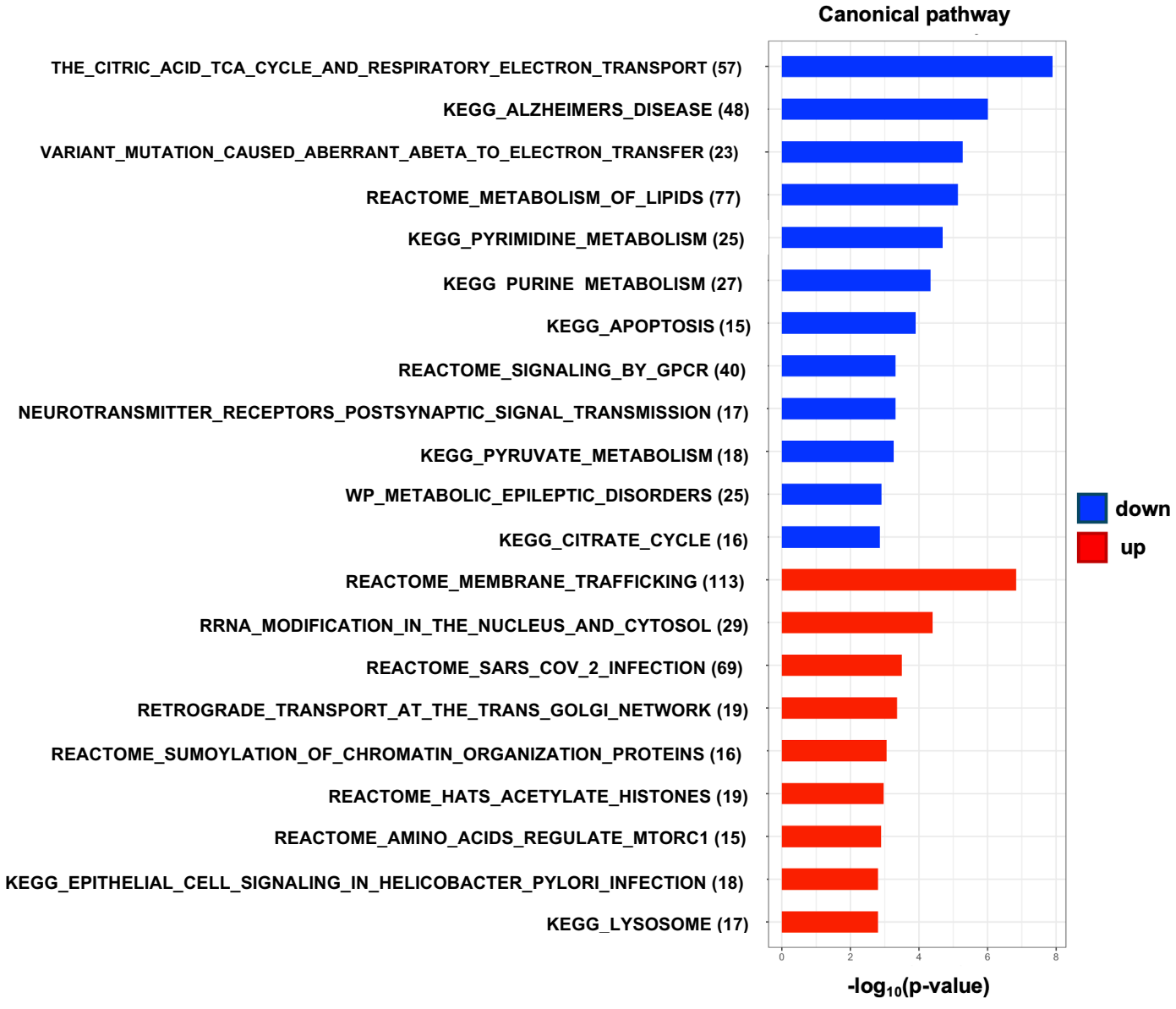

Figure 11: Canonical pathway analysis from proteomics data of DLBCL cell lines. Bar chart illustrating the canonical pathways identified from proteomics analysis of DLBCL cell lines, which were tested in the drug screen. Pathways are listed on the vertical axis, and the horizontal axis represents the -log10(p-value) indicating the statistical significance of each pathway. The bars are color-coded to show the direction of regulation, with blue indicating downregulated pathways and red indicating upregulated pathways in resistant cell lines. This figure was prepared by T. Naake, EMBL.

3.7. Impact of YAK477 on cell cycle distribution over time

Flow cytometry analysis was performed to assess the impact of YAK477 treatment on cell proliferation over five days of treatment. Via event gating single cells were identified and evaluated.

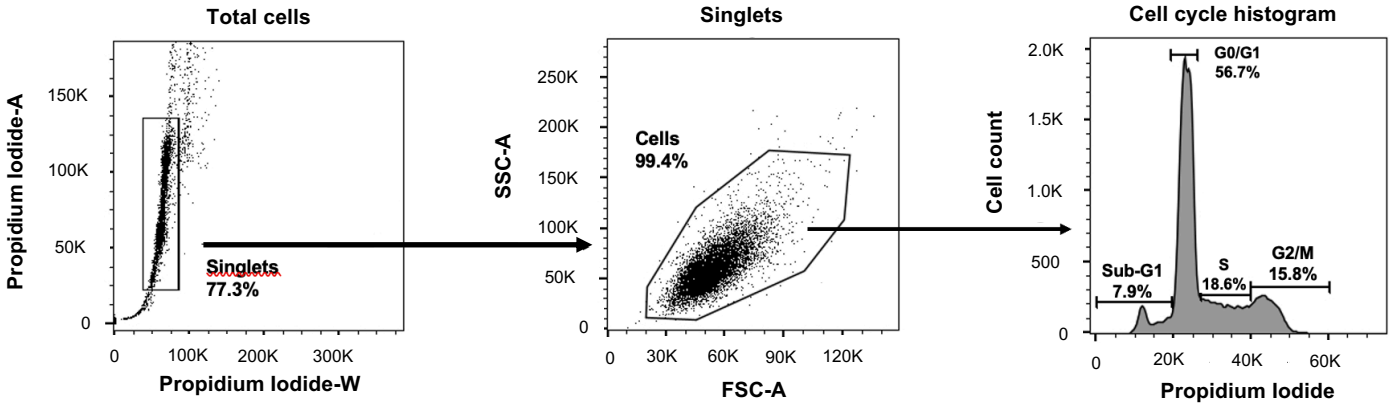

Figure 12: Flow cytometric cell cycle analysis with propidium iodide. The gating strategy for single cells using PI staining is shown. This gate was applied to the scatter plot to gate out obvious debris. For analysis of the cell cycle, a PI histogram plot was applied to detect sub-G1 cells, G0/G1 phase, S phase, and G2/M phase.

The histogram was evaluated for the cell cycle phases G1, S, G2 and mitosis. sub-G1 phase was considered as dead cells according to the Nicoletti assay.

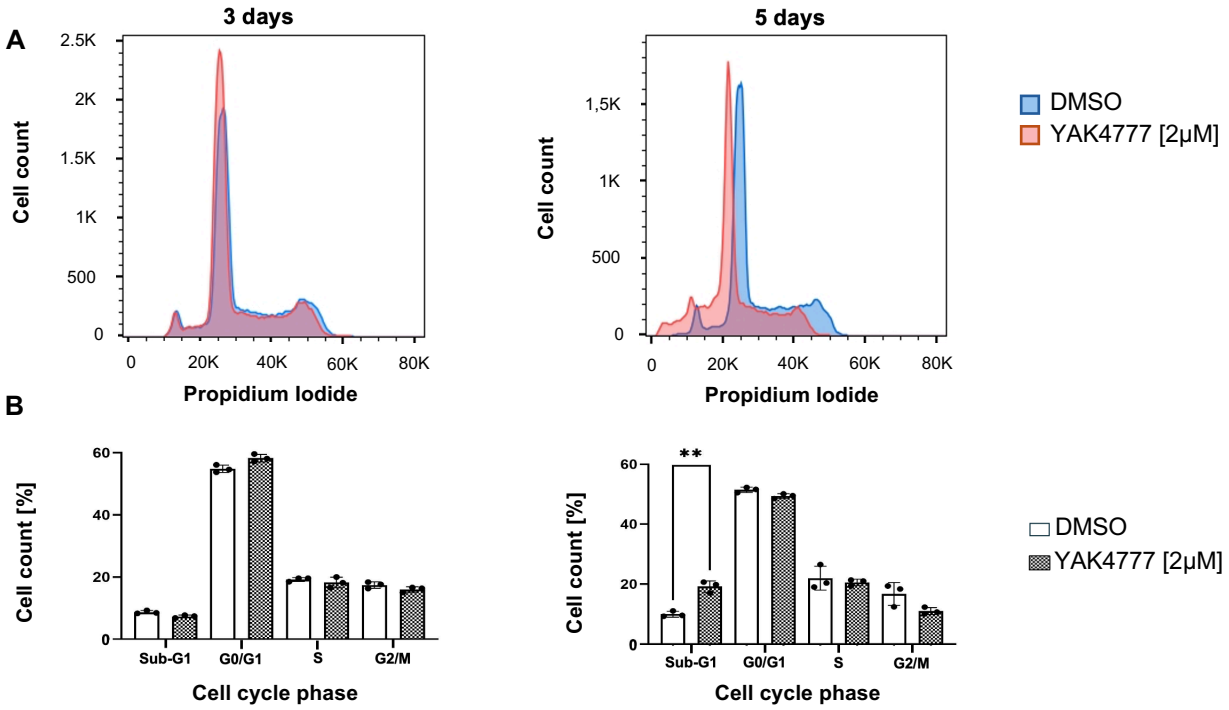

Figure 13: Impact of YAK477 on cell cycle distribution in DLBCL cells over three and five days. Overlay histograms (A) comparing the cell cycle distribution of DLBCL cells treated with YAK477 (blue) and DMSO control (red). The x-axis represents propidium iodide staining intensity, reflecting DNA content, while the y-axis shows the cell count. Peaks in the histograms correspond to different cell cycle phases. And bar graphs (B) showing cell cycle distribution of DLBCL cells (Riva) treated with 2µM YAK477 compared to DMSO control at two time points. Cell cycle phases, encompassing sub-G1, G0/G1, S, and G2/M phases are displayed along with the percentage of cell count in each phase. The left panel depicts the distribution after 3 days of treatment, whereas the right panel shows the distribution after 5 days. Statistical significance is based on the unpaired student t-test (p-value **p<0.01).

After three days, the cell cycle histograms as well es the bar graphs show no statistically significant difference in the distribution of cell cycle phases between the DMSO control and YAK-477-treated group (Figure 13). After five days of treatment, YAK477-treated cells showed a significant increase in the sub-G1 phase population compared to the control group, indicating a higher number of apoptotic cells. These findings suggest that YAK477 induces apoptosis in a time-dependent manner.

3.8. Establish GFP-labeled DLBCL cell lines for conducting co-culture assay

Prior to labeling, a cell number validation for transduction was conducted in a 24-well plate to ensure the highest number of viable cells for the procedure. Cells were seeded at densities of 10 $\times 10^6$, 5 $\times 10^6$ and 1 $\times 10^6$ and incubated for 72 hours. Following incubation, cell number and viability were assessed using trypan blue exclusion. Cells seeded at 10 $\times 10^6$ showed a slight decrease in cell number with a viability of 56%. Cells seeded at 5 $\times 10^6$ exhibited a modest increase in cell number (5.4 $\times 10^6$) with a viability of 72.2%. Cells seeded at 1 $\times 10^6$ more than doubled in number after 72 hours (to 2.75 $\times 10^6$) with a mean viability of 75.5%, indicating promising growth and high viability. Based on these results, an initial seeding density 1 $\times 10^6$ cells was selected for transduction.

Two different transduction protocols were evaluated to enhance transduction efficiency in lymphoma cell lines. The transduction process was carried out using the TAKARA Transduction Sponge in comparison to an in-house standard transduction protocol with Polybrene. After a 72-h cultivation period post-transduction, fluorescence microscopy and flow cytometry were used to evaluate transduction efficiency. Cell viability was assessed using trypan blue exclusion. Fluorescence microscopy revealed a strong GFP signal, particularly in cells forming clusters (Figure 14). The mean cell viability was observed to be 68% after Takara transduction, while 54% viability was observed with polybrene transduction.

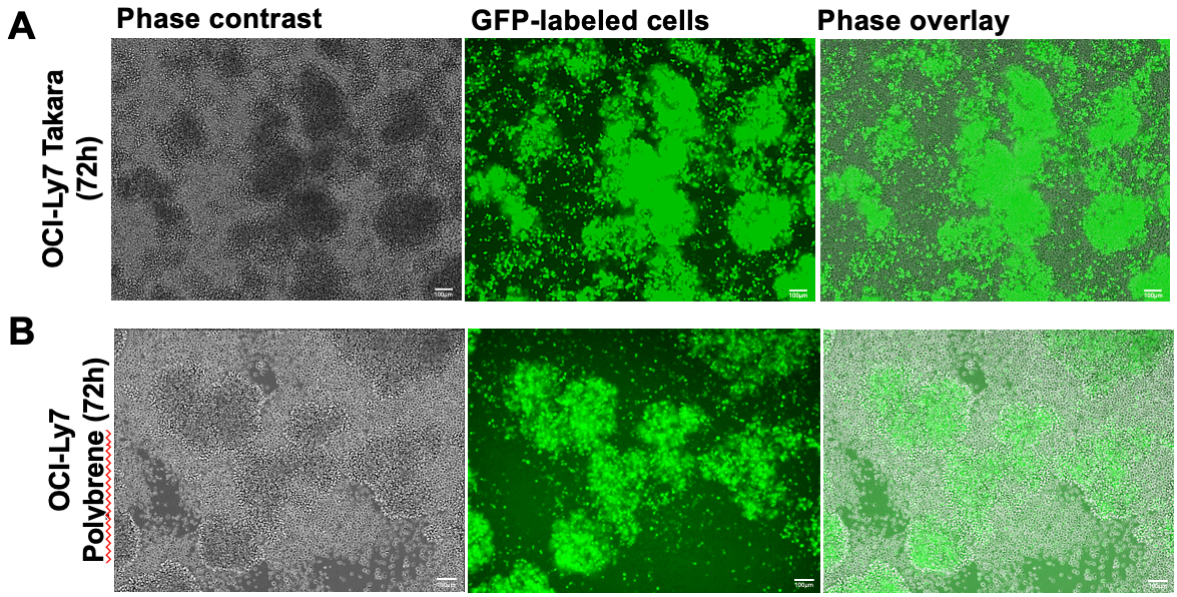

Figure 14: Visualization of OCI-Ly7 cells after GFP transduction. Representative images of OCI-Ly7 cells after 72 hours of incubation following GFP transduction using Takara Sponge (A) and Polybrene (B), captured with phase contrast microscopy. The left panel shows the cells under phase contrast microscopy, highlighting their overall morphology and clustering. The middle panel displays GFP-labeled OCI-Ly7 cells, emphasizing the fluorescence intensity of the labeled cells. The right panel provides an overlay of the phase contrast and GFP images, offering a combined view of GFP-cell distribution and clustering within the sample. All images were captured at 10× magnification, with scale bars indicating 100 µm.

Flow cytometry was employed to quantify the efficiency of transduction. The first row of plots corresponds to the Takara kit, while the second row represents the polybrene method. GFP-positive cells were quantified, revealing a transduction efficiency of 44.2% with the Takara kit and 26.1% with the Polybrene method (Figure 15).

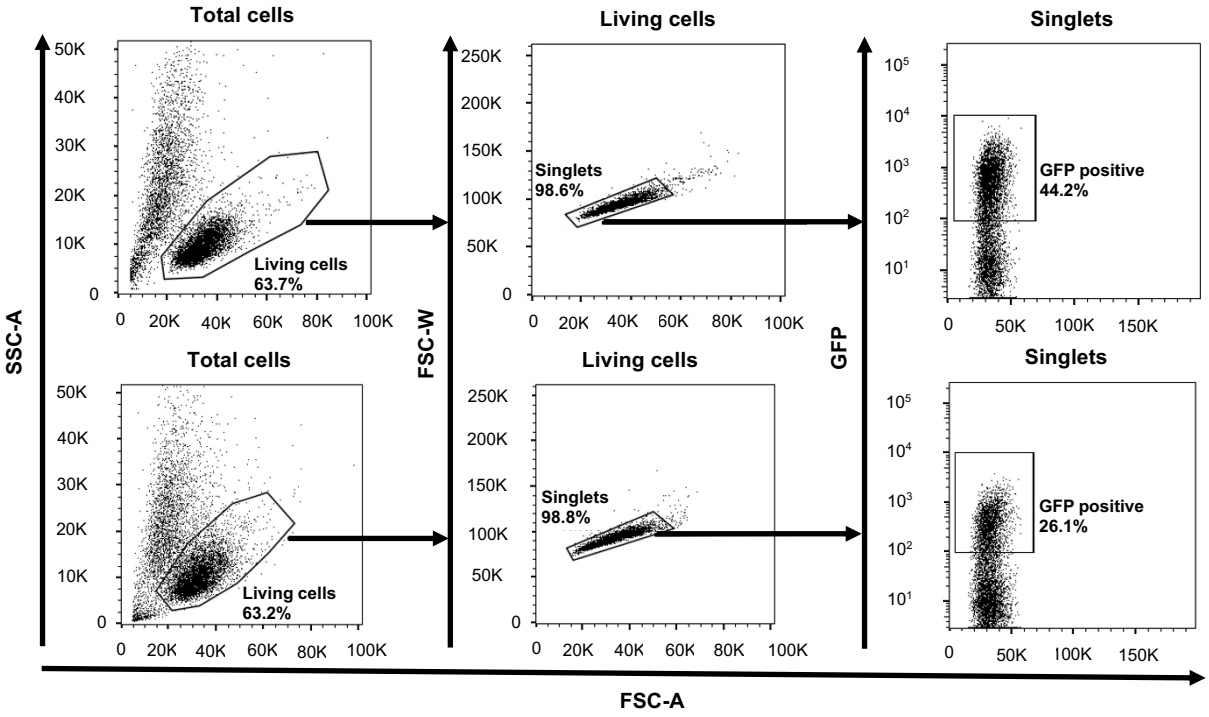

Figure 15: Flow cytometric analysis of GFP transduction efficiency in an DLBCL cell line before flow cytometric cell sorting. The initial gating strategy identified living cells after (63.7%) and singlet cells (98.6%). Among the singlet cells, 44.2% were GFP-positive. Post-sorting, living cells comprised 92.6% of the population, with singlets accounting for 98.9%. After flow cytometric cell sorting and a brief incubation period, the percentage of GFP-positive singlet cells increased to 98.3%.

The results from fluorescence microscopy and flow cytometry demonstrate both, that the Takara kit provided higher cell viability, as well as higher transduction efficiency compared to the traditional polybrene method. Therefore, the Takara kit was chosen for subsequent experiments. In the next step, the transduced cells were sorted for GFP to enrich the population with predominantly GFP-labeled cells (Figure 16).

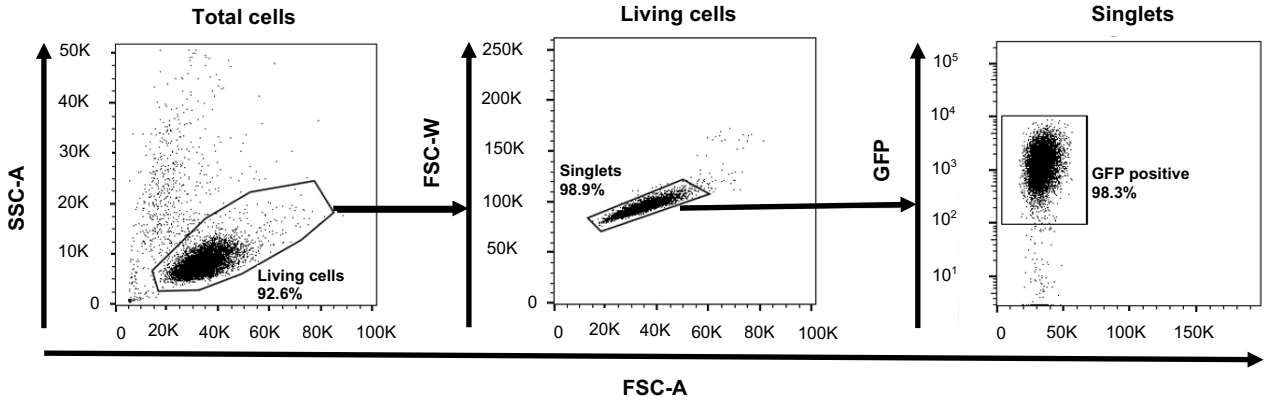

Figure 16: Flow cytometric analysis of GFP signal after flow cytometric cell sorting. Post-sorting, living cells comprised 92.6% of the population, with singlets accounting for 98.9%. After flow cytometric cell sorting and a brief incubation period, the percentage of GFP-positive singlet cells increased to 98.3%.

After cells were sorted for their GFP signal, 98% GFP-positive cells were identified.

3.9. Growth kinetics of DLBCL cells in co-culture with stromal cells under various conditions

The growth kinetics of OCI-Ly7 cells, which are known to be resistant to HDAC inhibitors, were analyzed under different conditions to understand the impact of the microenvironment represented by stromal cells. The experimental setup included OCI-Ly7 cells in monoculture and OCI-Ly7 cells co-cultured with stromal cells.

Fluorescence microscopy images taken at 0-, 52-, and 92-hours post-treatment showed minimal GFP signal and cell proliferation in OCI-Ly7 cells (Figure 17). The only noticeable change was the formation of clusters. In contrast, the presence of stromal cells in co-culture significantly boosted the proliferation of OCI-Ly7 cells. Using the cell-by-cell analysis model from Incucyte, we observed a significant increase in the number of cells in the co-culture condition.

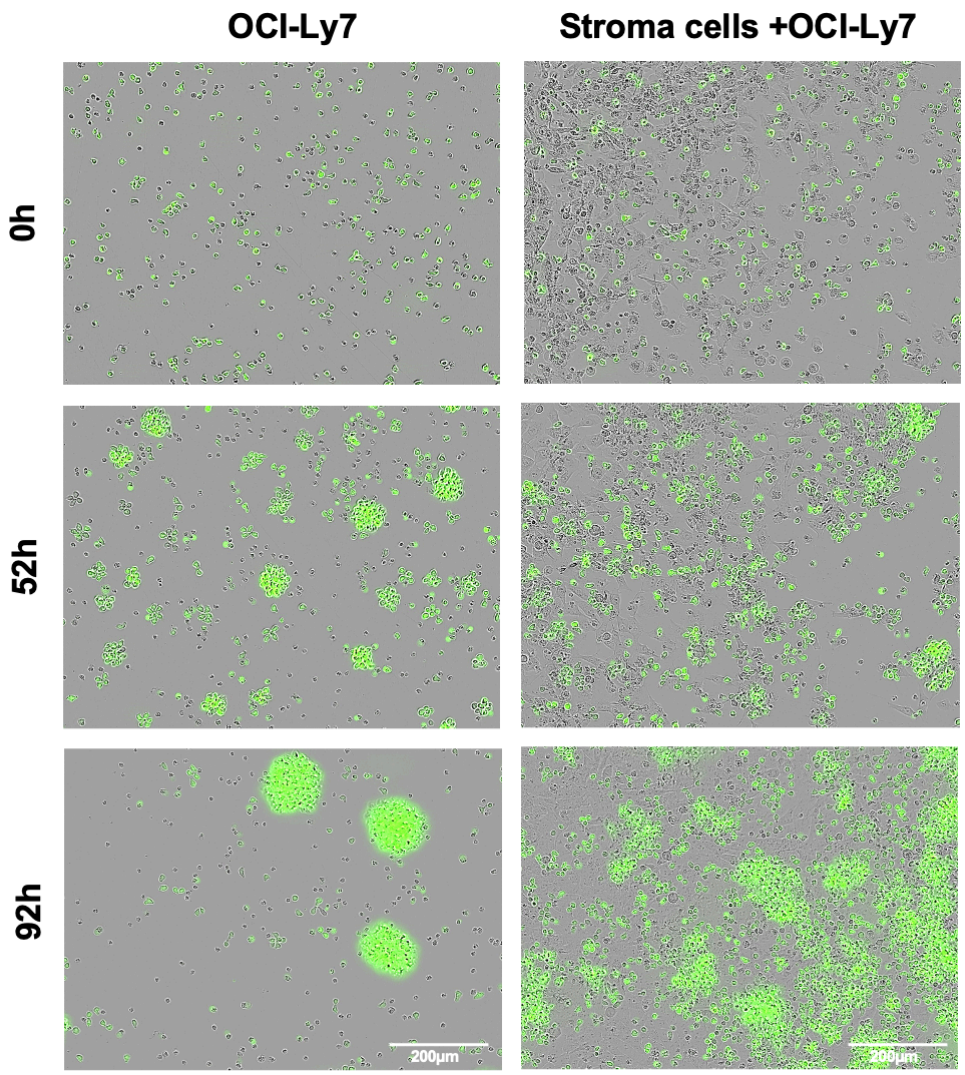

Figure 17: Live cell imaging of DLBCL cell lines under monoculture and co-culture conditions. Live cell imaging was performed to evaluate OCI-Ly7 cells and their co-culture with stromal cells under two different conditions over a time course of 0, 52, and 92 hours. The conditions include untreated OCI-Ly7 cells and co-culture of OCI-Ly7 with stromal cells. GFP labeling was used to visualize the OCI-Ly7 cells, with green fluorescence indicating GFP-positive cells. Images were taken at 20× magnification. Scale bars represent 200 µm.

The growth kinetics graph, based on live cell imaging data, further supports these observations. OCI-Ly7 cells grown in monoculture displayed minimal growth over time. However, when co-cultured with stromal cells, OCI-Ly7 cells showed a significant increase in relative cell count (Figure 18).

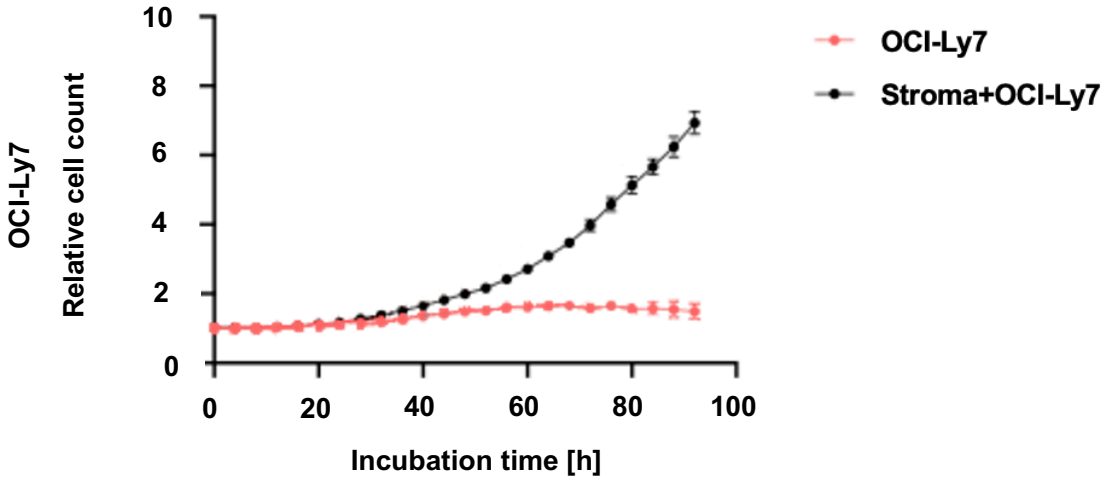

Figure 18: Analysis of OCI-Ly7 cell proliferation under various co-culture conditions over 92 hours, derived from live cell imaging data. Growth curves showing the relative cell count of GFP-labeled OCI-Ly7 cells over 92 hours under four different conditions: monoculture of OCI-Ly7 (red) and co-culture with non-cancerous stroma cells (black). The x-axis represents the incubation time in hours, while the y-axis represents the relative cell count of OCI-Ly7 cells.

These results underscore the significant role of the tumor microenvironment in modulating growth dynamics.

4. Discussion

Our findings revealed that certain HDAC inhibitors, notably YAK477, show potential in targeting DLBCL cell lines, especially those harboring CREBBP mutations. These results underscore the potential of epigenetic therapies in the treatment of lymphoma.

4.1. High-throughput drug screening and whole genome sequencing

This study showed that many HDAC inhibitors, especially class I/II inhibitors, significantly reduced cell viability in DLBCL cell lines. This finding aligns with previous studies demonstrating the efficacy of HDAC inhibitors in inducing cell cycle arrest and apoptosis in various cancers (Y. Cai et al., 2013). YAK477, a Class IIa HDAC inhibitor showed toxic efficacy, in particular on CREBBP-mutant DLBCL cell lines. This result points to a potential use in personalized cancer treatment. CREBBP mutations disrupt acetylation processes, making cells more vulnerable to deacetylase inhibition (Hellwig et al., 2020; Juskevicius et al., 2017). Our results align with other studies that have found CREBBP mutant cancers to be more sensitive to HDAC inhibitors, highlighting YAK477's potential as a treatment (Ednersson et al., 2020; Greenawalt et al., 2017; Juskevicius et al., 2017).

Moreover, previous studies have showed that mutations in CREBBP, along with EP300 mutations, are linked to poorer outcome in overall survival, progression-free survival, and event-free survival for DLBCL patients (Genta et al., 2022; Juskevicius et al., 2017). CREBBP mutations are more frequently found in relapsed or refractory DLBCL compared to primary cases, suggesting an additional role in resistance to standard treatments (Greenawalt et al., 2017). Additionally, the functional role of CREBBP in regulating transcription and chromatin remodeling further suggests that CREBBP mutations render DLBCL cases more accessible to HDAC inhibition (Juskevicius et al., 2017; Lee et al., 2014).

Additionally, genes such as EZH2, KMT2D, and HIST1H1D were slightly less common in sensitive cell lines. EZH2, a histone methyltransferase involved in gene silencing, had reduced expression in sensitive cell lines (Simon & Lange, 2008). Lower expression might be

linked to a more open chromatin state, making the cells more vulnerable to HDAC inhibition (K. H. Kim & Roberts, 2016). KMT2D, another histone methyltransferase, is frequently mutated in DLBCL, and its lower expression in sensitive cells might indicate a reduced capacity for methylation-mediated gene silencing, enhancing the efficacy of HDAC inhibitors (Zhang et al., 2015). HIST1H1D encodes a histone H1 variant involved in chromatin compaction (Yusufova et al., 2021). Lower levels of HIST1H1D might also contribute to a more relaxed chromatin structure, facilitating the action of HDAC inhibitors (Fyodorov et al., 2018).

Class IIa HDAC inhibitors, including YAK477, primarily target enzymes like HDAC4, HDAC5, HDAC7, and HDAC9, which play key roles in deacetylating non-histone proteins. This process influences various cellular activities, such as differentiation and apoptosis (G. Li et al., 2020; Milazzo et al., 2020). Selective inhibitors in this class, like MC1568, have been observed to promote apoptosis without significantly disrupting the cell cycle. It was found to have minimal impact on cell cycle progression but amplified the apoptotic response when combined with other cancer treatments (G. Wang et al., 2012). YAK540, another selective inhibitor in this class, triggers apoptosis by upregulating pro-apoptotic genes and activating caspase pathways, while leaving the cell cycle largely unaffected (Bollmann et al., 2022). To gain a deeper understanding of YAK477's mechanism, further studies involving apoptosis assays and autophagy markers are necessary.

4.2. Protein expression and sensitivity

The study identified specific proteins associated with sensitivity and resistance to HDAC inhibitors in DLBCL. GGCT and EIF4G3 were found to be highly expressed in sensitive cell lines, while RASAL1 and IGF2BP1 being highly expressed in resistant cell lines.

Gamma-glutamyl cyclotransferase (GGCT) is known to promote tumor growth by regulating glutathione metabolism, and its high expression is generally linked to poor prognosis in various cancers (Kageyama et al., 2015). However, in this study elevated levels of GGCT in diffuse large B-cell lymphoma (DLBCL) cell lines sensitive to HDACi were found. This

sensitivity may be mediated through GGCT's interaction with the p53 pathway, a vital regulator of cell cycle and apoptosis (Lee et al., 2014). HDAC and SIRT proteins typically reduce p53 activity, promoting cancer cell survival under oxidative stress conditions. HDAC inhibitors promote acetylation of histones and non-histone proteins, resulting in a more relaxed chromatin structure, and with this, activate tumor suppressor genes, which may also include p53 (Juan et al., 2000; J. Luo et al., 2000, 2001; Vaziri et al., 2001). The presence of functional p53 enhances the apoptotic effects of HDACi, underscoring the importance of this pathway for the inhibitors' effectiveness (Fröhlich et al., 2016; J. Luo et al., 2001; Mrakovcic et al., 2017, 2019). EIF4G3 (Eukaryotic Translation Initiation Factor 4 Gamma 3) plays a crucial role in the initiation of protein synthesis. This factor acts as a scaffold for assembling other initiation factors, facilitating the binding of mRNA to the ribosome (Marcet-Palacios et al., 2011; Prévôt et al., 2003). High expression of EIF4G3 in sensitive cell lines might suggest that enhanced translation initiation is important for HDAC inhibitors' efficacy, potentially by increasing the production of pro-apoptotic proteins (Hayashi et al., 2000). circEIF4G3 has been found to suppress gastric cancer progression by inhibiting β-catenin and promoting δ-catenin ubiquitin degradation. This suggests that EIF4G3 could be involved in regulating cell growth and apoptosis (Zang et al., 2022).

In contrast, RASAL1 (RAS Protein Activator Like 1) and IGF2BP1 (Insulin Like Growth Factor 2 MRNA Binding Protein 1) are associated with resistance. RASAL1 negatively regulates RAS signaling, which is involved in cell proliferation and survival (Chang et al., 2019; Jin et al., 2017; Meng et al., 2017). In HDACi-resistant DLBCL cell lines, RASAL1 expression is upregulated, enhancing survival pathways and reducing the efficacy of HDAC inhibitors. Studies have shown that HDAC inhibitors such as trichostatin A and vorinostat can induce apoptosis in various DLBCL cell lines, but resistance is often mediated through changes in signaling pathways including the RAS/ERK pathway, where RASAL1 plays a big role. This regulation is critical in maintaining cell survival despite HDACi treatment (Chen et al., 2014; Z. Huang et al., 2017; Qiao et al., 2012; M. Seto et al., 2011). IGF2BP1 (Insulin-like growth factor 2 mRNA-binding

protein 1) is known to stabilize mRNAs that encode growth-promoting genes. This stabilization enhances the translation of these mRNAs, thereby promoting cell proliferation and survival. This process has been observed in various cancers where IGF2BP1 is overexpressed (X. Huang et al., 2018; Singh et al., 2023; T.-Y. Zhu et al., 2023). By stabilizing these mRNAs, IGF2BP1 also contributes to resistance to apoptosis (Du et al., 2021).

4.3. Canonical pathway analysis

Canonical pathway analysis showed that particular pathways were differently expressed on protein level in sensitive and resistant cell lines. Notably, apoptosis pathways were downregulated, while the histone acetyltransferases (HATs) pathway was upregulated in the resistant cell lines. Pathways related to apoptosis were significantly underrepresented in resistant cell lines, indicating that these cells are less responsive to apoptosis-inducing agents, like HDAC inhibitors. Apoptosis is essential for programmed cell death in response to cellular stress and damage (Bertheloot et al., 2021). By downregulating this pathway, resistant cells can avoid the cytotoxic effects of HDAC inhibitors, leading to drug resistance. The reduction in apoptosis pathway activation may indicate that resistant cells can continue to survive and proliferate even when exposed to drugs that normally induce cell death (Bolden et al., 2006; Fulda, 2009). In contrast, the HATs pathway, which involves the addition of acetyl groups to histones and results in a relaxed chromatin structure that promotes gene activation, was upregulated in resistant cells. This upregulation suggests that resistant cells have a greater ability to activate transcription, which could result in the expression of genes that enhance cell survival and proliferation. The increased acetylation state may help resistant cells sustain high levels of gene expression necessary for growth and resistance to HDAC inhibitors (Bolden et al., 2006; Roth et al., 2001).

4.4. Co-culture systems

This study showed that OCI-Ly7 cells, a GCB-DLBCL cell line, exhibited different growth phenotypes when cultured alone compared to when co-cultured with stromal cells. showed increased growth when co-cultured with stromal cells compared to when grown in monoculture. This aligns with research indicating that stromal cells can protect lymphoma cells from drug-induced cell death, suggesting that treatments must target both the tumor and its surrounding environment (Cader et al., 2013; Duś-Szachniewicz et al., 2022).

The tumor microenvironment (TME) plays a crucial role in determining how tumors responsiveness to treatment and hence, influences clinical outcome (Q. Wang et al., 2023). Previous data indicate that soluble factors released by tumor or stromal cells can contribute to microenvironment-mediated drug resistance. Additionally, the interaction between tumor cells and stromal fibroblasts or elements of the extracellular matrix can diminish the effectiveness of therapies (Wu & Dai, 2017). Moreover, the TME can affect the immune response, either promoting or inhibiting tumor growth, which further complicates treatment strategies (Tuccitto et al., 2019). The development of combination therapies targeting both tumor cells and the TME has shown promise in overcoming drug resistance. For example, combining nanoparticles designed to modulate the TME with traditional chemotherapeutic agents can significantly improve drug delivery and efficacy (Amini et al., 2019). This highlights the importance of considering the TME's role in crafting effective cancer treatments.

4.5. Future directions: exploring combinational therapies

The varying levels of CD20, CD19, PD-L1, and PD-1 expression in the different B-cell lymphoma cell lines (Figure 19) suggest opportunities for further research into personalized combination therapies. Although this study didn't focus on exploring treatment synergies, the differences in expression patterns emphasize the need to consider these markers in developing targeted therapies.

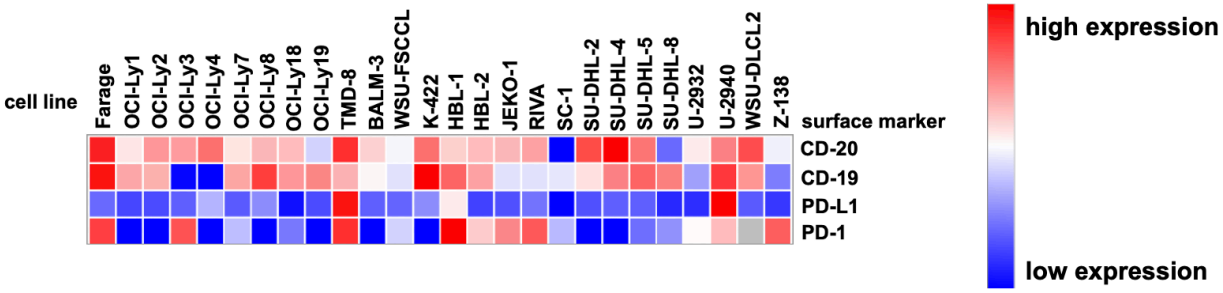

Figure 19: Expression levels of four surface markers across B-cell lymphoma cell lines. Heatmap showing the expression levels of CD20, CD19, PD-L1, and PD-1 surface markers across various B-cell lymphoma cell lines. The color gradient shows expression levels, where red indicates high expression and blue indicates low expression.

For example, the integration of HDAC inhibitors (HDACi) with immune checkpoint inhibitors, such as PD-1 or PD-L1 blockades, has shown promise in other cancer types (Sui et al., 2018). Previous studies have demonstrated that such combinations can enhance antitumor immune responses, leading to improved outcomes. Specifically, the combination of HDACi with PD-1/PD-L1 inhibitors has been found to upregulate PD-L1 expression, thereby increasing the efficacy of PD-1 blockade therapies in several cancer models (e.g., melanoma, colon cancer) (Bissonnette et al., 2016; Woods et al., 2015). In B-cell lymphomas, HDAC3 inhibition has been found to upregulate PD-L1 expression, enhancing the efficiency of anti-PD-L1 therapy and leading to tumor regression (Deng et al., 2019). A study involving Hodgkin lymphoma patients demonstrated that the combination of vorinostat, an HDACi, with pembrolizumab, a PD-1 inhibitor, led to a significant overall response rate (Collins, 2023; P. Sharma et al., 2017). It would be valuable to investigate whether similar combinational approaches could yield beneficial effects. Moreover, the expression data presented in this study could serve as a foundation for selecting patients who might benefit most from such strategies. Future studies should aim to validate these combinations in preclinical models of B-cell lymphoma and explore the potential for clinical translation.

4.6. Shortcomings

I recognize several limitations in my study that should be considered when interpreting the results. My study included a relatively small sample size of 24 lymphoma cell lines. Selection bias is a potential limitation, as the cell lines were chosen based on availability and existing data. This could affect the external validity of my findings. Studies involving a more diverse and representative selection of cell lines would enhance the generalizability of the results. Moreover, larger sample sizes are necessary to validate these findings and assess the effectiveness of the HDAC inhibitors we tested, particularly YAK477 and its effect on CREBBP mutant lymphomas. The preclinical nature of my study, which was conducted entirely in vitro, restricts the ability to directly apply the findings to clinical settings. In vitro conditions cannot fully mimic the complexity of the tumor microenvironment and patient-specific factors present in vivo. Additionally, the study was limited by funding and time constraints, which prevented me from exploring combinational therapies that could potentially enhance the effectiveness of HDACi. Investigating these combinations might offer a more comprehensive understanding of treatment strategies and their effectiveness in clinical settings.

These limitations might have influenced my findings by potentially introducing bias and affecting the generalizability of my results. However, the study also has several strengths, such as using a newly developed HDACi library and detailed genetic and proteomic analyses, which support the validity of my conclusions. In conclusion, while acknowledging these limitations, my study offers valuable insights into the potential of HDAC inhibitors, especially YAK477, for treating diffuse large B-cell lymphoma and contributes to the existing body of knowledge. Addressing these limitations in future research will further enhance our understanding and improve the generalizability of the findings.

4.7. Contributions to veterinary research

The study of HDAC inhibitors in human medical research has significant implications for veterinary medicine, particularly in understanding diseases like DLBCL, which affect both humans and animals such as canines. DLBCL is the most common type of non-Hodgkin lymphoma in both humans and canines, sharing similar clinical presentations and biological behaviors (Breen & Modiano, 2008; Marconato et al., 2013). The molecular characteristics and clinical behavior of DLBCL in dogs are remarkably similar to those in humans. For instance, both human and canine DLBCL show common genetic alterations and similar biological behaviors. An integrated analysis of exome and RNA sequencing data from canine DLBCL identified numerous genetic similarities with human DLBCL, including mutations in key oncogenic pathways such as MYC, TP53, and NF-kB signaling (Giannuzzi et al., 2022; Richards et al., 2011, 2013).

The clinical presentation and progression of DLBCL in canines closely resemble those observed in human patients. This includes similarities in tumor morphology, immunophenotype, and response to conventional therapies such as the CHOP regimen (Giannuzzi et al., 2022). Standard therapies like CHOP can be highly toxic, leading to severe side effects in both humans and animals (Sitzia et al., 1997; S.-L. Wang et al., 2016). HDAC inhibitors are already being investigated in veterinary medicine, showing promising results in animal models. A study on panobinostat revealed its potent antitumor effects in canine DLBCL, showing dose-dependent inhibitory effects on proliferation and inducing significant apoptosis in canine lymphoma cells (Dias et al., 2018).

The research on HDACi in human DLBCL not only advances human medicine but also significantly contributes to veterinary oncology by providing insights into similar diseases in animals. The parallels in disease pathology and treatment responses between humans and canines make HDAC inhibitors a promising area for developing safer and more effective therapies for DLBCL across species.

5. Summary

This dissertation investigates the preclinical evaluation of histone deacetylase inhibitors (HDACi) as a potential treatment for diffuse large B-cell lymphoma (DLBCL). DLBCL is the most common form of non-Hodgkin's lymphoma and is characterized by its heterogeneity in clinical presentations, outcomes and molecular features. The study focused on evaluating the cytotoxic effects of 41 newly synthesized HDAC inhibitors on a range of lymphoma cell lines. Further attention was paid to the identification of potential biomarkers for DLBCL response to the tested HDACi. In addition, the study investigated the importance of the tumor microenvironment (TME), which was represented here by stromal cells.

One of the most important findings of the study is the identification of YAK477 as a promising HDACi. YAK477 showed promising efficacy in reducing cell viability in DLBCL cell lines, especially those with CREBBP mutations. The study also provided valuable insights through proteomic profiling and identified high expression levels of EIF4G3 and GGCT in HDACi-sensitive cell lines. These proteins were found to be potential biomarkers for sensitivity to HDAC inhibitors. In contrast, proteins such as RASAL1 and IGF2BP1 were upregulated in resistant cell lines, indicating reduced sensitivity to HDAC inhibitors.

In addition, the tumor microenvironment appears to have a major impact on tumor development, as it can support tumor cell growth and distribution. GFP-labeled DLBCL cells incubated together with stromal cells showed increased growth in live cell microscopy.

6. Zusammenfassung

Diese Dissertation untersucht die präklinische Bewertung von Histondeacetylase-Inhibitoren (HDACi) als potenzielle Behandlungsmethode für das diffuse großzellige B-Zell-Lymphom (DLBCL). DLBCL ist die häufigste Form des Non-Hodgkin-Lymphoms und zeichnet sich durch seine Heterogenität in klinischen Präsentationen, Ergebnissen und molekularen Merkmalen aus. Die Studie konzentrierte sich auf die Bewertung der zytotoxischen Effekte von 41 neu synthetisierten HDAC-Inhibitoren an einer Reihe von Lymphomzelllinien. Ein weiteres Augenmerk galt der Identifizierung potenzieller Biomarker für das Ansprechen von DLBCL auf die getesteten HDACi. Weiterführend untersuchte die Studie die Bedeutung der Tumormikroumgebung (TME), welche hier durch Stromazellen dargestellt wurde.

Eine der wichtigsten Erkenntnisse der Studie ist die Identifizierung von YAK477 als vielversprechenden HDACi. YAK477 zeigte eine vielversprechende Wirksamkeit bei der Reduzierung der Zellviabilität in DLBCL-Zelllinien, insbesondere in solchen mit CREBBP-Mutationen. Die Studie lieferte auch wertvolle Erkenntnisse durch proteomisches Profiling und identifizierte hohe Expressionsniveaus von EIF4G3 und GGCT in HDACi-sensitiven Zelllinien. Diese Proteine erwiesen sich als potenzielle Biomarker für die Sensitivität gegenüber HDAC-Inhibitoren. Im Gegensatz dazu waren Proteine wie RASAL1 und IGF2BP1 in resistenten Zelllinien hochreguliert, was auf eine verminderte Sensitivität gegenüber HDAC-Inhibitoren hinweist.

Zudem scheint die Tumormikroumgebung einen großen Einfluss auf die Tumorentwicklung zu haben, da sie das Tumorzellwachstum und dessen Verteilung unterstützen kann. GFP markierte DLBCL Zellen, die zusammen mit Stromazellen inkubiert wurden, zeigten in der Lebendzellmikroskopie ein verstärktes Wachstum.

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References

Abramson, J. S., Palomba, M. L., Gordon, L. I., Lunning, M. A., Wang, M., Arnason, J., Mehta, A., Purev, E., Maloney, D. G., Andreadis, C., Sehgal, A., Solomon, S. R., Ghosh, N., Albertson, T. M., Garcia, J., Kostic, A., Mallaney, M., Ogasawara, K., Newhall, K., … Siddiqi, T. (2020). Lisocabtagene maraleucel for patients with relapsed or refractory large B-cell lymphomas (TRANSCEND NHL 001): A multicentre seamless design study. *The Lancet*, *396*(10254), 839–852. https://doi.org/10.1016/S0140-6736(20)31366-0

Adams, H., Fritzsche, F. R., Dirnhofer, S., Kristiansen, G., & Tzankov, A. (2010). Class I histone deacetylases 1, 2 and 3 are highly expressed in classical Hodgkin's lymphoma. *Expert Opinion on Therapeutic Targets*, *14*(6), 577–584. https://doi.org/10.1517/14728221003796609

Alaggio, R., Amador, C., Anagnostopoulos, I., Attygalle, A. D., Araujo, I. B. de O., Berti, E., Bhagat, G., Borges, A. M., Boyer, D., Calaminici, M., Chadburn, A., Chan, J. K. C., Cheuk, W., Chng, W.-J., Choi, J. K., Chuang, S.-S., Coupland, S. E., Czader, M., Dave, S. S., … Xiao, W. (2022). The 5th edition of the World Health Organization Classification of Haematolymphoid Tumours: Lymphoid Neoplasms. *Leukemia*, *36*(7), 1720–1748. https://doi.org/10.1038/s41375-022-01620-2

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., Lu, L., Lewis, D. B., Tibshirani, R., Sherlock, G., … Staudt, L. M. (2000). Distinct types of diffuse large B-cell lymphoma identified by gene expression profiling. *Nature*, *403*(6769), 503–511. https://doi.org/10.1038/35000501

Amini, M. A., Abbasi, A. Z., Cai, P., Lip, H., Gordijo, C. R., Li, J., Chen, B., Zhang, L., Rauth, A. M., & Wu, X. Y. (2019). Combining Tumor Microenvironment Modulating Nanoparticles with Doxorubicin to Enhance Chemotherapeutic Efficacy and Boost Antitumor Immunity. *JNCI: Journal of the National Cancer Institute*, *111*(4), 399–408. https://doi.org/10.1093/jnci/djy131

Assouline, S. E., Nielsen, T. H., Yu, S., Alcaide, M., Chong, L., MacDonald, D., Tosikyan, A., Kukreti, V., Kezouh, A., Petrogiannis-Haliotis, T., Albuquerque, M., Fornika, D., Alamouti, S., Froment, R., Greenwood, C. M. T., Oros, K. K., Camglioglu, E., Sharma, A., Christodoulopoulos, R., … Mann, K. K. (2016). Phase 2 study of panobinostat with or without rituximab in relapsed diffuse large B-cell lymphoma. *Blood*, *128*(2), 185–194. https://doi.org/10.1182/blood-2016-02-699520

Bannister, A. J., & Kouzarides, T. (2011). Regulation of chromatin by histone modifications. *Cell Research*, *21*(3), 381–395. https://doi.org/10.1038/cr.2011.22

Baylin, S. B., & Jones, P. A. (2011). A decade of exploring the cancer epigenome—Biological and translational implications. *Nature Reviews. Cancer*, *11*(10), 726–

734. https://doi.org/10.1038/nrc3130

Berger, S. L., Kouzarides, T., Shiekhattar, R., & Shilatifard, A. (2009). An operational definition of epigenetics. *Genes & Development*, *23*(7), 781–783. https://doi.org/10.1101/gad.1787609

Berndsen, C. E., & Denu, J. M. (2008). Catalysis and Substrate Selection by Histone/Protein Lysine Acetyltransferases. *Current Opinion in Structural Biology*, *18*(6), 682–689. https://doi.org/10.1016/j.sbi.2008.11.004

Bertheloot, D., Latz, E., & Franklin, B. S. (2021). Necroptosis, pyroptosis and apoptosis: An intricate game of cell death. *Cellular & Molecular Immunology*, *18*(5), 1106–1121. https://doi.org/10.1038/s41423-020-00630-3

Bissonnette, R. P., Rolland, A., Goodeneow, B., & Gillings, M. (2016). Abstract B108: The HDAC inhibitor HBI-8000 enhances immunotherapy with either PD-1 or PD-L1 blockade in the MC38 model of colon cancer. *Cancer Immunology Research*, *4*(11_Supplement), B108. https://doi.org/10.1158/2326-6066.IMM2016-B108

Bolden, J. E., Peart, M. J., & Johnstone, R. W. (2006). Anticancer activities of histone deacetylase inhibitors. *Nature Reviews. Drug Discovery*, *5*(9), 769–784. https://doi.org/10.1038/nrd2133

Bollmann, L. M., Skerhut, A., Asfaha, Y., Horstick, N., Hanenberg, H., Hamacher, A., Kurz, T., & Kassack, M. (2022). The Novel Class IIa Selective Histone Deacetylase Inhibitor YAK540 Is Synergistic with Bortezomib in Leukemia Cell Lines. *International Journal of Molecular Sciences*, *23*. https://doi.org/10.3390/ijms232113398

Bradner, J. E., West, N., Grachan, M. L., Greenberg, E. F., Haggarty, S. J., Warnow, T., & Mazitschek, R. (2010). Chemical phylogenetics of histone deacetylases. *Nature Chemical Biology*, *6*(3), 238–243. https://doi.org/10.1038/nchembio.313

Breen, M., & Modiano, J. F. (2008). Evolutionarily conserved cytogenetic changes in hematological malignancies of dogs and humans—Man and his best friend share more than companionship. *Chromosome Research: An International Journal on the Molecular, Supramolecular and Evolutionary Aspects of Chromosome Biology*, *16*(1), 145–154. https://doi.org/10.1007/s10577-007-1212-4

Budeus, B., Schweigle de Reynoso, S., Przekopowitz, M., Hoffmann, D., Seifert, M., & Küppers, R. (2015). Complexity of the human memory B-cell compartment is determined by the versatility of clonal diversification in germinal centers. *Proceedings of the National Academy of Sciences*, *112*(38), E5281–E5289. https://doi.org/10.1073/pnas.1511270112

Bursch, K. L., Goetz, C. J., & Smith, B. C. (2024). Current Trends in Sirtuin Activator and Inhibitor Development. *Molecules*, *29*(5), Article 5.

https://doi.org/10.3390/molecules29051185

Cader, F. Z., Vockerodt, M., Bose, S., Nagy, E., Brundler, M.-A., Kearns, P., & Murray, P. G. (2013). The EBV oncogene LMP1 protects lymphoma cells from cell death through the collagen-mediated activation of DDR1. *Blood*, *122*(26), 4237–4245. https://doi.org/10.1182/blood-2013-04-499004

Cai, W., Zeng, Q., Zhang, X., & Ruan, W. (2021). Trends Analysis of Non-Hodgkin Lymphoma at the National, Regional, and Global Level, 1990-2019: Results From the Global Burden of Disease Study 2019. *Frontiers in Medicine*, *8*, 738693. https://doi.org/10.3389/fmed.2021.738693

Cai, Y., Cui, W., Chen, W., Wei, P., Chi, Y., Zhang, P., Bi, R., & Zhou, X. (2013). The effects of a histone deacetylase inhibitor on biological behavior of diffuse large B-cell lymphoma cell lines and insights into the underlying mechanisms. *Cancer Cell International*, *13*(1), 57. https://doi.org/10.1186/1475-2867-13-57

Cano, R. L. E., & Lopera, H. D. E. (2013). Introduction to T and B lymphocytes. In *Autoimmunity: From Bench to Bedside [Internet]*. El Rosario University Press. https://www.ncbi.nlm.nih.gov/books/NBK459471/

Carafa, V., Rotili, D., Forgione, M., Cuomo, F., Serretiello, E., Hailu, G. S., Jarho, E., Lahtela-Kakkonen, M., Mai, A., & Altucci, L. (2016). Sirtuin functions and modulation: From chemistry to the clinic. *Clinical Epigenetics*, *8*(1), 61. https://doi.org/10.1186/s13148-016-0224-3

Chang, R.-X., Cui, A.-L., Dong, L., Guan, S.-P., Jiang, L.-Y., & Miao, C.-X. (2019). Overexpression of RASAL1 indicates poor prognosis and promotes invasion of ovarian cancer. *Open Life Sciences*, *14*(1), 133–140. https://doi.org/10.1515/biol-2019-0015

Chapuy, B., Stewart, C., Dunford, A. J., Kim, J., Kamburov, A., Redd, R. A., Lawrence, M. S., Roemer, M. G. M., Li, A. J., Ziepert, M., Staiger, A. M., Wala, J. A., Ducar, M. D., Leshchiner, I., Rheinbay, E., Taylor-Weiner, A., Coughlin, C. A., Hess, J. M., Pedamallu, C. S., … Shipp, M. A. (2018). Molecular subtypes of diffuse large B cell lymphoma are associated with distinct pathogenic mechanisms and outcomes. *Nature Medicine*, *24*(5), 679–690. https://doi.org/10.1038/s41591-018-0016-8

Chen, H., Cheng, Z.-Y., Pan, Y., Wang, Z., Liu, Y., & Zhang, J.-Q. (2014). RASAL1 influences the proliferation and invasion of gastric cancer cells by regulating the RAS/ERK signaling pathway. *Human Cell*, *27*(3), 103–110. https://doi.org/10.1007/s13577-014-0090-2

Chen, H., Fan, M., Pfeffer, L. M., & Laribee, R. N. (2012). The histone H3 lysine 56 acetylation pathway is regulated by target of rapamycin (TOR) signaling and functions directly in ribosomal RNA biogenesis. *Nucleic Acids Research*, *40*(14), 6534–6546. https://doi.org/10.1093/nar/gks345

Chen, H., Xie, C., Chen, Q., & Zhuang, S. (2022). HDAC11, an emerging therapeutic target for metabolic disorders. *Frontiers in Endocrinology*, *13*, 989305. https://doi.org/10.3389/fendo.2022.989305

Cheng, Y., He, C., Wang, M., Ma, X., Mo, F., Yang, S., Han, J., & Wei, X. (2019). Targeting epigenetic regulators for cancer therapy: Mechanisms and advances in clinical trials. *Signal Transduction and Targeted Therapy*, *4*(1), 1–39. https://doi.org/10.1038/s41392-019-0095-0

Cheson, B. D., Fisher, R. I., Barrington, S. F., Cavalli, F., Schwartz, L. H., Zucca, E., Lister, T. A., Alliance, Australasian Leukaemia and Lymphoma Group, Eastern Cooperative Oncology Group, European Mantle Cell Lymphoma Consortium, Italian Lymphoma Foundation, European Organisation for Research, Treatment of Cancer/Dutch Hemato-Oncology Group, Grupo Español de Médula Ósea, German High-Grade Lymphoma Study Group, German Hodgkin's Study Group, Japanese Lymphorra Study Group, Lymphoma Study Association, NCIC Clinical Trials Group, … United Kingdom National Cancer Research Institute. (2014). Recommendations for initial evaluation, staging, and response assessment of Hodgkin and non-Hodgkin lymphoma: The Lugano classification. *Journal of Clinical Oncology: Official Journal of the American Society of Clinical Oncology*, *32*(27), 3059–3068. https://doi.org/10.1200/JCO.2013.54.8800

Chueh, A. C., Tse, J. W. T., Tögel, L., & Mariadason, J. M. (2015). Mechanisms of Histone Deacetylase Inhibitor-Regulated Gene Expression in Cancer Cells. *Antioxidants & Redox Signaling*, *23*(1), 66–84. https://doi.org/10.1089/ars.2014.5863

Collins, G. P. (2023). Tackling PD1i resistance in Hodgkin lymphoma. *Blood*, *142*(16), 1333–1334. https://doi.org/10.1182/blood.2023021283

Compagno, M., Lim, W. K., Grunn, A., Nandula, S. V., Brahmachary, M., Shen, Q., Bertoni, F., Ponzoni, M., Scandurra, M., Califano, A., Bhagat, G., Chadburn, A., Dalla-Favera, R., & Pasqualucci, L. (2009). Mutations of multiple genes cause deregulation of NF-kB in diffuse large B-cell lymphoma. *Nature*, *459*(7247), 717–721. https://doi.org/10.1038/nature07968

Cyster, J. G., & Allen, C. D. C. (2019). B Cell Responses: Cell Interaction Dynamics and Decisions. *Cell*, *177*(3), 524–540. https://doi.org/10.1016/j.cell.2019.03.016

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. *The Journal of Experimental Medicine*, *194*(12), 1861–1874. https://doi.org/10.1084/jem.194.12.1861

Davis, R. E., Ngo, V. N., Lenz, G., Tolar, P., Young, R., Romesser, P. B., Kohlhammer, H., Lamy, L., Zhao, H., Yang, Y., Xu, W., Shaffer, A. L., Wright, G., Xiao, W., Powell, J., Jiang, J., Thomas, C. J., Rosenwald, A., Ott, G., … Staudt, L. M. (2010). Chronic Active B Cell Receptor Signaling in Diffuse Large B Cell Lymphoma. *Nature*,

463(7277), 88–92. https://doi.org/10.1038/nature08638

Deng, S., Hu, Q., Zhang, H., Yang, F., Peng, C., & Huang, C. (2019). HDAC3 Inhibition Upregulates PD-L1 Expression in B-Cell Lymphomas and Augments the Efficacy of Anti–PD-L1 Therapy. *Molecular Cancer Therapeutics*, *18*(5), 900–908. https://doi.org/10.1158/1535-7163.MCT-18-1068

Dias, J. N. R., Aguiar, S. I., Pereira, D. M., André, A. S., Gano, L., Correia, J. D. G., Carrapiço, B., Rütgen, B., Malhó, R., Peleteiro, C., Goncalves, J., Rodrigues, C. M. P., Gil, S., Tavares, L., & Aires-da-Silva, F. (2018). The histone deacetylase inhibitor panobinostat is a potent antitumor agent in canine diffuse large B-cell lymphoma. *Oncotarget*, *9*(47), 28586–28598. https://doi.org/10.18632/oncotarget.25580

Doraiswamy, A., Shah, M. R., & Bannerji, R. (2021). Immunotherapies Old and New: Hematopoietic Stem Cell Transplant, Chimeric Antigen Receptor T Cells, and Bispecific Antibodies for the Treatment of Relapsed/Refractory Diffuse Large B Cell Lymphoma. *Current Hematologic Malignancy Reports*, *16*(1), 72–81. https://doi.org/10.1007/s11899-021-00610-y

Du, Q.-Y., Zhu, Z.-M., & Pei, D.-S. (2021). The biological function of IGF2BPs and their role in tumorigenesis. *Investigational New Drugs*, *39*(6), 1682–1693. https://doi.org/10.1007/s10637-021-01148-9

Dulac III, E. J., Joy, K. A., Ndindjock, R., Coyle, K. B., & Wade, R. L. (2013). The Burden Of Illness and Prevalence In Diffuse Large B-Cell (DLBCL) and Follicular (FL) Lymphomas. *Blood*, *122*(21), 5619. https://doi.org/10.1182/blood.V122.21.5619.5619

Duś-Szachniewicz, K., Gdesz-Birula, K., & Rymkiewicz, G. (2022). Development and Characterization of 3D Hybrid Spheroids for the Investigation of the Crosstalk Between B-Cell Non-Hodgkin Lymphomas and Mesenchymal Stromal Cells. *OncoTargets and Therapy*, *15*, 683–697. https://doi.org/10.2147/OTT.S363994

Ednersson, S. B., Stern, M., Fagman, H., Nilsson-Ehle, H., Hasselblom, S., & Andersson, P.-O. (2020). TBLR1 and CREBBP as potential novel prognostic immunohistochemical biomarkers in diffuse large B-cell lymphoma. *Leukemia & Lymphoma*, *61*(11), 2595–2604. https://doi.org/10.1080/10428194.2020.1775216

Emiliani, S., Fischle, W., Van Lint, C., Al-Abed, Y., & Verdin, E. (1998). Characterization of a human RPD3 ortholog, HDAC3. *Proceedings of the National Academy of Sciences of the United States of America*, *95*(6), 2795–2800. https://doi.org/10.1073/pnas.95.6.2795

Esteller, M. (2002). CpG island hypermethylation and tumor suppressor genes: A booming present, a brighter future. *Oncogene*, *21*(35), 5427–5440. https://doi.org/10.1038/sj.onc.1205600

Fangazio, M., Ladewig, E., Gomez, K., Garcia-Ibanez, L., Kumar, R., Teruya-Feldstein, J., Rossi, D., Filip, I., Pan-Hammarström, Q., Inghirami, G., Boldorini, R., Ott, G., Staiger, A. M., Chapuy, B., Gaidano, G., Bhagat, G., Basso, K., Rabadan, R., Pasqualucci, L., & Dalla-Favera, R. (2021). Genetic mechanisms of HLA-I loss and immune escape in diffuse large B cell lymphoma. *Proceedings of the National Academy of Sciences of the United States of America*, *118*(22), e2104504118. https://doi.org/10.1073/pnas.2104504118

Feinberg, A. P., Koldobskiy, M. A., & Göndör, A. (2016). Epigenetic modulators, modifiers and mediators in cancer aetiology and progression. *Nature Reviews. Genetics*, *17*(5), 284–299. https://doi.org/10.1038/nrg.2016.13

Fragou, D., Fragou, A., Kouidou, S., Njau, S., & Kovatsi, L. (2011). Epigenetic mechanisms in metal toxicity. *Toxicology Mechanisms and Methods*, *21*(4), 343–352. https://doi.org/10.3109/15376516.2011.557878

Fröhlich, L. F., Mrakovcic, M., Smole, C., & Zatloukal, K. (2016). Molecular mechanism leading to SAHA-induced autophagy in tumor cells: Evidence for a p53-dependent pathway. *Cancer Cell International*, *16*, 68. https://doi.org/10.1186/s12935-016-0343-0

Frontzek, F., Karsten, I., Schmitz, N., & Lenz, G. (2022). Current options and future perspectives in the treatment of patients with relapsed/refractory diffuse large B-cell lymphoma. *Therapeutic Advances in Hematology*, *13*, 20406207221103321. https://doi.org/10.1177/20406207221103321

Fulda, S. (2009). Tumor resistance to apoptosis. *International Journal of Cancer*, *124*(3), 511–515. https://doi.org/10.1002/ijc.24064

Fyodorov, D. V., Zhou, B.-R., Skoultchi, A. I., & Bai, Y. (2018). Emerging roles of linker histones in regulating chromatin structure and function. *Nature Reviews. Molecular Cell Biology*, *19*(3), 192–206. https://doi.org/10.1038/nrm.2017.94

Genta, S., Ghilardi, G., Cascione, L., Juskevicius, D., Tzankov, A., Schär, S., Milan, L., Pirosa, M. C., Esposito, F., Ruberto, T., Giovanella, L., Hayoz, S., Mamot, C., Dirnhofer, S., Zucca, E., & Ceriani, L. (2022). Integration of Baseline Metabolic Parameters and Mutational Profiles Predicts Long-Term Response to First-Line Therapy in DLBCL Patients: A Post Hoc Analysis of the SAKK38/07 Study. *Cancers*, *14*(4), Article 4. https://doi.org/10.3390/cancers14041018

Giannuzzi, D., Marconato, L., Fanelli, A., Licenziato, L., De Maria, R., Rinaldi, A., Rotta, L., Rouquet, N., Birolo, G., Fariselli, P., Mensah, A. A., Bertoni, F., & Aresu, L. (2022). The genomic landscape of canine diffuse large B-cell lymphoma identifies distinct subtypes with clinical and therapeutic implications. *Lab Animal*, *51*(7), 191–202. https://doi.org/10.1038/s41684-022-00998-x

Glozak, M. A., Sengupta, N., Zhang, X., & Seto, E. (2005). Acetylation and deacetylation

of non-histone proteins. *Gene*, *363*, 15–23. https://doi.org/10.1016/j.gene.2005.09.010

Gracia-Hernandez, M., Yende, A. S., Gajendran, N., Alahmadi, Z., Li, X., Munoz, Z., Tan, K., Noonepalle, S., Shibata, M., & Villagra, A. (2024). Targeting HDAC6 improves anti-CD47 immunotherapy. *Journal of Experimental & Clinical Cancer Research*, *43*(1), 60. https://doi.org/10.1186/s13046-024-02982-4

Greenawalt, D. M., Liang, W. S., Saif, S., Johnson, J., Todorov, P., Dulak, A., Enriquez, D., Halperin, R., Ahmed, A., Saveliev, V., Carpten, J., Craig, D., Barrett, J. C., Dougherty, B., Zinda, M., Fawell, S., Dry, J. R., & Byth, K. (2017). Comparative analysis of primary versus relapse/refractory DLBCL identifies shifts in mutation spectrum. *Oncotarget*, *8*(59), 99237–99244. https://doi.org/10.18632/oncotarget.18502

Grondona, P., Bucher, P., Schulze-Osthoff, K., Hailfinger, S., & Schmitt, A. (2018). NF-κB Activation in Lymphoid Malignancies: Genetics, Signaling, and Targeted Therapy. *Biomedicines*, *6*(2), 38. https://doi.org/10.3390/biomedicines6020038

Hanahan, D., & Weinberg, R. A. (2011). Hallmarks of cancer: The next generation. *Cell*, *144*(5), 646–674. https://doi.org/10.1016/j.cell.2011.02.013

Harrysson, S., Eloranta, S., Ekberg, S., Enblad, G., Jerkeman, M., Hasselblom, S., Wahlin, B. E., Andersson, P.-O., & Smedby, K. E. (2018). Incidence and Outcome of Relapsed/Refractory Diffuse Large B-Cell Lymphoma (DLBCL) in a Population-Based Cohort of 3165 Patients in Sweden. *Blood*, *132*(Supplement 1), 2975. https://doi.org/10.1182/blood-2018-99-110672

Harrysson, S., Eloranta, S., Ekberg, S., Enblad, G., Jerkeman, M., Wahlin, B. E., Andersson, P.-O., & Smedby, K. E. (2021). Incidence of relapsed/refractory diffuse large B-cell lymphoma (DLBCL) including CNS relapse in a population-based cohort of 4243 patients in Sweden. *Blood Cancer Journal*, *11*(1), 1–10. https://doi.org/10.1038/s41408-020-00403-1

Hayashi, S., Nishimura, K., Fukuchi-Shimogori, T., Kashiwagi, K., & Igarashi, K. (2000). Increase in cap- and IRES-dependent protein synthesis by overproduction of translation initiation factor eIF4G. *Biochemical and Biophysical Research Communications*, *277 1*, 117–123. https://doi.org/10.1006/BBRC.2000.3637

Hellwig, M., Merk, D. J., Lutz, B., & Schüller, U. (2020). Preferential sensitivity to HDAC inhibitors in tumors with CREBBP mutation. *Cancer Gene Therapy*, *27*(5), 294–300. https://doi.org/10.1038/s41417-019-0099-5

Hsu, K.-C., Liu, C.-Y., Lin, T. E., Hsieh, J.-H., Sung, T.-Y., Tseng, H.-J., Yang, J.-M., & Huang, W.-J. (2017). Novel Class IIa-Selective Histone Deacetylase Inhibitors Discovered Using an in Silico Virtual Screening Approach. *Scientific Reports*, *7*(1), 3228. https://doi.org/10.1038/s41598-017-03417-1

Huang, X., Zhang, H., Guo, X., Zhu, Z., Cai, H., & Kong, X. (2018). Insulin-like growth factor 2 mRNA-binding protein 1 (IGF2BP1) in cancer. *Journal of Hematology & Oncology*, *11*(1), 88. https://doi.org/10.1186/s13045-018-0628-y

Huang, Z., Wang, R., Wei, G., Jiang, R., Zheng, Y., Tian, Y., Chen, B., Ye, C., Xue, M., & Yu, C. (2017). RAS protein activator-like 1 is functionally involved in hypoxia resistance in breast cancer cells by targeting hypoxia inducible factor-1α. *Oncology Letters*, *14*(3), 3839–3845. https://doi.org/10.3892/ol.2017.6648

Hutchings, M. (2023). The evolving therapy of DLBCL: Bispecific antibodies. *Hematological Oncology*, *41*(S1), 107–111. https://doi.org/10.1002/hon.3154

Imai, Y., Hirano, M., Kobayashi, M., Futami, M., & Tojo, A. (2019). HDAC Inhibitors Exert Anti-Myeloma Effects through Multiple Modes of Action. *Cancers*, *11*(4), 475. https://doi.org/10.3390/cancers11040475

Jiang, M., Bennani, N. N., & Feldman, A. L. (2017). Lymphoma classification update: T-cell lymphomas, Hodgkin lymphomas, and histiocytic/dendritic cell neoplasms. *Expert Review of Hematology*, *10*(3), 239–249. https://doi.org/10.1080/17474086.2017.1281122

Jin, W., Chen, L., Cai, X., Zhang, Y., Zhang, J., Ma, D., Cai, X., Fu, T., Yu, Z., Yu, F., & Chen, G. (2017). Long non-coding RNA TUC338 is functionally involved in sorafenib-sensitized hepatocarcinoma cells by targeting RASAL1. *Oncology Reports*, *37*(1), 273–280. https://doi.org/10.3892/or.2016.5248

Jirtle, R. L., & Skinner, M. K. (2007). Environmental epigenomics and disease susceptibility. *Nature Reviews. Genetics*, *8*(4), 253–262. https://doi.org/10.1038/nrg2045

Jones, S. J. (2020). *Characterization of environmental and genetic factors in multiple-case lymphoid cancer families* [University of British Columbia]. https://doi.org/10.14288/1.0390430

Juan, L. J., Shia, W. J., Chen, M. H., Yang, W. M., Seto, E., Lin, Y. S., & Wu, C. W. (2000). Histone deacetylases specifically down-regulate p53-dependent gene activation. *The Journal of Biological Chemistry*, *275*(27), 20436–20443. https://doi.org/10.1074/jbc.M000202200

Juskevicius, D., Jucker, D., Klingbiel, D., Mamot, C., Dirnhofer, S., & Tzankov, A. (2017). Mutations of CREBBP and SOCS1 are independent prognostic factors in diffuse large B cell lymphoma: Mutational analysis of the SAKK 38/07 prospective clinical trial cohort. *Journal of Hematology & Oncology*, *10*(1), 70. https://doi.org/10.1186/s13045-017-0438-7

Kageyama, S., Hanada, E., Ii, H., Tomita, K., Yoshiki, T., & Kawauchi, A. (2015). Gamma-Glutamylcyclotransferase: A Novel Target Molecule for Cancer Diagnosis and Treatment. *BioMed Research International*, *2015*, 345219.

https://doi.org/10.1155/2015/345219

Kanas, G., Ge, W., Quek, R. G. W., Keeven, K., Nersesyan, K., & Jon E. Arnason. (2022). Epidemiology of diffuse large B-cell lymphoma (DLBCL) and follicular lymphoma (FL) in the United States and Western Europe: Population-level projections for 2020–2025. *Leukemia & Lymphoma*, *63*(1), 54–63. https://doi.org/10.1080/10428194.2021.1975188

Karube, K., & Campo, E. (2015). *MYC* Alterations in Diffuse Large B-Cell Lymphomas. *Seminars in Hematology*, *52*(2), 97–106. https://doi.org/10.1053/j.seminhematol.2015.01.009

Kelly, T. K., De Carvalho, D. D., & Jones, P. A. (2010). Epigenetic Modifications as Therapeutic Targets. *Nature Biotechnology*, *28*(10), 1069–1078. https://doi.org/10.1038/nbt.1678

Khorasanizadeh, S. (2004). The Nucleosome: From Genomic Organization to Genomic Regulation. *Cell*, *116*(2), 259–272. https://doi.org/10.1016/S0092-8674(04)00044-3

Kim, H.-J., & Bae, S.-C. (2011). Histone deacetylase inhibitors: Molecular mechanisms of action and clinical trials as anti-cancer drugs. *American Journal of Translational Research*, *3*(2), 166–179.

Kim, K. H., & Roberts, C. W. M. (2016). Targeting EZH2 in cancer. *Nature Medicine*, *22*(2), 128–134. https://doi.org/10.1038/nm.4036

Kimura, H. (2013). Histone modifications for human epigenome analysis. *Journal of Human Genetics*, *58*(7), 439–445. https://doi.org/10.1038/jhg.2013.66

Knox, T., Sahakian, E., Banik, D., Hadley, M., Palmer, E., Noonepalle, S., Kim, J., Powers, J., Gracia-Hernandez, M., Oliveira, V., Cheng, F., Chen, J., Barinka, C., Pinilla-Ibarz, J., Lee, N. H., Kozikowski, A., & Villagra, A. (2019). Selective HDAC6 inhibitors improve anti-PD-1 immune checkpoint blockade therapy by decreasing the anti-inflammatory phenotype of macrophages and down-regulation of immunosuppressive proteins in tumor cells. *Scientific Reports*, *9*, 6136. https://doi.org/10.1038/s41598-019-42237-3

Kouzarides, T. (2007). Chromatin modifications and their function. *Cell*, *128*(4), 693–705. https://doi.org/10.1016/j.cell.2007.02.005

Kuo, M. H., & Allis, C. D. (1998). Roles of histone acetyltransferases and deacetylases in gene regulation. *BioEssays: News and Reviews in Molecular, Cellular and Developmental Biology*, *20*(8), 615–626. https://doi.org/10.1002/(SICI)1521-1878(199808)20:8<615::AID-BIES4>3.0.CO;2-H

Lam, L. T., Wright, G., Davis, R. E., Lenz, G., Farinha, P., Dang, L., Chan, J. W.,

Rosenwald, A., Gascoyne, R. D., & Staudt, L. M. (2008). Cooperative signaling through the signal transducer and activator of transcription 3 and nuclear factor-{kappa}B pathways in subtypes of diffuse large B-cell lymphoma. *Blood*, *111*(7), 3701–3713. https://doi.org/10.1182/blood-2007-09-111948

Lane, A. A., & Chabner, B. A. (2009). Histone deacetylase inhibitors in cancer therapy. *Journal of Clinical Oncology: Official Journal of the American Society of Clinical Oncology*, *27*(32), 5459–5468. https://doi.org/10.1200/JCO.2009.22.1291

Lee, S. H., Yoo, C., Im, S., Jung, J., Choi, H., & Yoo, J. (2014). Expression of Histone Deacetylases in Diffuse Large B-cell Lymphoma and Its Clinical Significance. *International Journal of Medical Sciences*, *11*, 994–1000. https://doi.org/10.7150/ijms.8522

Li, G., Tian, Y., & Zhu, W.-G. (2020). The Roles of Histone Deacetylases and Their Inhibitors in Cancer Therapy. *Frontiers in Cell and Developmental Biology*, *8*. https://doi.org/10.3389/fcell.2020.576946

Li, Y., & Seto, E. (2016). HDACs and HDAC Inhibitors in Cancer Development and Therapy. *Cold Spring Harbor Perspectives in Medicine*, *6*(10), a026831. https://doi.org/10.1101/cshperspect.a026831

Li, Y., & Tollefsbol, T. O. (2010). Impact on DNA methylation in cancer prevention and therapy by bioactive dietary components. *Current Medicinal Chemistry*, *17*(20), 2141–2151.

Lin, P., wen, D., Chen, G., Dang, Y., He, Y., & Yang, H. (2020). Predictive value of hypoxia, metabolism and immune factors for prognosis in hepatocellular carcinoma: A retrospective analysis and multicenter validation study. *Journal of Cancer*, *11*(14), 4145–4156. https://doi.org/10.7150/jca.41983

Liu, S.-S., Wu, F., Jin, Y.-M., Chang, W.-Q., & Xu, T.-M. (2020). HDAC11: A rising star in epigenetics. *Biomedicine & Pharmacotherapy*, *131*, 110607. https://doi.org/10.1016/j.biopha.2020.110607

Liu, Y., & Barta, S. K. (2019). Diffuse large B-cell lymphoma: 2019 update on diagnosis, risk stratification, and treatment. *American Journal of Hematology*, *94*(5), 604–616. https://doi.org/10.1002/ajh.25460

Lossos, I. S., Alizadeh, A. A., Eisen, M. B., Chan, W. C., Brown, P. O., Botstein, D., Staudt, L. M., & Levy, R. (2000). Ongoing immunoglobulin somatic mutation in germinal center B cell-like but not in activated B cell-like diffuse large cell lymphomas. *Proceedings of the National Academy of Sciences*, *97*(18), 10209–10213. https://doi.org/10.1073/pnas.180316097

Luo, J., Nikolaev, A. Y., Imai, S., Chen, D., Su, F., Shiloh, A., Guarente, L., & Gu, W. (2001). Negative Control of p53 by Sir2α Promotes Cell Survival under Stress. *Cell*, *107*(2),

137–148. https://doi.org/10.1016/S0092-8674(01)00524-4

Luo, J., Su, F., Chen, D., Shiloh, A., & Gu, W. (2000). Deacetylation of p53 modulates its effect on cell growth and apoptosis. *Nature*, *408*(6810), 377–381. https://doi.org/10.1038/35042612

Luo, Y., & Li, H. (2020). Structure-Based Inhibitor Discovery of Class I Histone Deacetylases (HDACs). *International Journal of Molecular Sciences*, *21*(22), 8828. https://doi.org/10.3390/ijms21228828

MacLennan, I. C. (1994). Germinal centers. *Annual Review of Immunology*, *12*, 117–139. https://doi.org/10.1146/annurev.iy.12.040194.001001

Mamgain, G., Singh, P. K., Patra, P., Naithani, M., & Nath, U. K. (2022). Diffuse large B-cell lymphoma and new insights into its pathobiology and implication in treatment. *Journal of Family Medicine and Primary Care*, *11*(8), 4151–4158. https://doi.org/10.4103/jfmpc.jfmpc_2432_21

Mann, M., & Bartolomei, M. (2002). Epigenetic reprogramming in the mammalian embryo: Struggle of the clones. *Genome Biology*, *3*, REVIEWS1003. https://doi.org/10.1186/gb-2002-3-2-reviews1003

Marcet-Palacios, M., Duggan, B., Shostak, I., Barry, M., Geskes, T., Wilkins, J., Yanagiya, A., Sonenberg, N., & Bleackley, R. (2011). Granzyme B Inhibits Vaccinia Virus Production through Proteolytic Cleavage of Eukaryotic Initiation Factor 4 Gamma 3. *PLoS Pathogens*, *7*, e1002447. https://doi.org/10.1371/journal.ppat.1002447

Marconato, L., Gelain, M. E., & Comazzi, S. (2013). The dog as a possible animal model for human non-Hodgkin lymphoma: A review. *Hematological Oncology*, *31*(1), 1–9. https://doi.org/10.1002/hon.2017

Marks, P. A., & Xu, W.-S. (2009). Histone Deacetylase Inhibitors: Potential in Cancer Therapy. *Journal of Cellular Biochemistry*, *107*(4), 600–608. https://doi.org/10.1002/jcb.22185

Marquardt, V., Theruvath, J., Pauck, D., Picard, D., Qin, N., Blümel, L., Maue, M., Bartl, J., Ahmadov, U., Langini, M., Meyer, F.-D., Cole, A., Cruz-Cruz, J., Graef, C. M., Wölfl, M., Milde, T., Witt, O., Erdreich-Epstein, A., Leprivier, G., … Mitra, S. (2023). Tacedinaline (CI-994), a class I HDAC inhibitor, targets intrinsic tumor growth and leptomeningeal dissemination in MYC-driven medulloblastoma while making them susceptible to anti-CD47-induced macrophage phagocytosis via NF-kB-TGM2 driven tumor inflammation. *Journal for Immunotherapy of Cancer*, *11*(1), e005871. https://doi.org/10.1136/jitc-2022-005871

Martin, M. W., Lee, J. Y., Lancia, D. R., Ng, P. Y., Han, B., Thomason, J. R., Lynes, M. S., Marshall, C. G., Conti, C., Collis, A., Morales, M. A., Doshi, K., Rudnitskaya, A., Yao, L., & Zheng, X. (2018). Discovery of novel N-hydroxy-2-arylisoindoline-4-

carboxamides as potent and selective inhibitors of HDAC11. *Bioorganic & Medicinal Chemistry Letters*, *28*(12), 2143–2147. https://doi.org/10.1016/j.bmcl.2018.05.021

Mazzone, R., Zwergel, C., Mai, A., & Valente, S. (2017). Epi-drugs in combination with immunotherapy: A new avenue to improve anticancer efficacy. *Clinical Epigenetics*, *9*, 59. https://doi.org/10.1186/s13148-017-0358-y

Meng, F., Zhang, W., & Wang, Y. (2017). RASAL1 inhibits HepG2 cell growth via HIF-2α mediated gluconeogenesis. *Oncology Letters*, *14*(6), 7344–7352. https://doi.org/10.3892/ol.2017.7123

Milazzo, G., Mercatelli, D., Di Muzio, G., Triboli, L., De Rosa, P., Perini, G., & Giorgi, F. M. (2020). Histone Deacetylases (HDACs): Evolution, Specificity, Role in Transcriptional Complexes, and Pharmacological Actionability. *Genes*, *11*(5), Article 5. https://doi.org/10.3390/genes11050556

Minucci, S., & Pelicci, P. G. (2006). Histone deacetylase inhibitors and the promise of epigenetic (and more) treatments for cancer. *Nature Reviews Cancer*, *6*(1), 38–51. https://doi.org/10.1038/nrc1779

Misteli, T. (2020). The self-organizing genome: Principles of genome architecture and function. *Cell*, *183*(1), 28–45. https://doi.org/10.1016/j.cell.2020.09.014

Modi, D., Potugari, B., & Uberti, J. (2021). Immunotherapy for Diffuse Large B-Cell Lymphoma: Current Landscape and Future Directions. *Cancers*, *13*(22), Article 22. https://doi.org/10.3390/cancers13225827

Montgomery, R. L., Davis, C. A., Potthoff, M. J., Haberland, M., Fielitz, J., Qi, X., Hill, J. A., Richardson, J. A., & Olson, E. N. (2007). Histone deacetylases 1 and 2 redundantly regulate cardiac morphogenesis, growth, and contractility. *Genes & Development*, *21*(14), 1790–1802. https://doi.org/10.1101/gad.1563807

Morley-Jacob, C., & Gallop-Evans, E. (2012). An update on lymphoma in children and young adults. *Paediatrics and Child Health*, *22*(3), 92–97. https://doi.org/10.1016/j.paed.2011.08.010

Mottamal, M., Zheng, S., Huang, T. L., & Wang, G. (2015). Histone Deacetylase Inhibitors in Clinical Studies as Templates for New Anticancer Agents. *Molecules*, *20*(3), 3898–3941. https://doi.org/10.3390/molecules20033898

Mrakovcic, M., Kleinheinz, J., & Fröhlich, L. F. (2017). Histone Deacetylase Inhibitor-Induced Autophagy in Tumor Cells: Implications for p53. *International Journal of Molecular Sciences*, *18*(9), 1883. https://doi.org/10.3390/ijms18091883

Mrakovcic, M., Kleinheinz, J., & Fröhlich, L. F. (2019). P53 at the Crossroads between Different Types of HDAC Inhibitor-Mediated Cancer Cell Death. *International*

Journal of Molecular Sciences, *20*(10), 2415. https://doi.org/10.3390/ijms20102415

Narita, T., Weinert, B. T., & Choudhary, C. (2019). Functions and mechanisms of non-histone protein acetylation. *Nature Reviews Molecular Cell Biology*, *20*(3), 156–174. https://doi.org/10.1038/s41580-018-0081-3

Neelapu, S. S., Locke, F. L., Bartlett, N. L., Lekakis, L. J., Miklos, D. B., Jacobson, C. A., Braunschweig, I., Oluwole, O. O., Siddiqi, T., Lin, Y., Timmerman, J. M., Stiff, P. J., Friedberg, J. W., Flinn, I. W., Goy, A., Hill, B. T., Smith, M. R., Deol, A., Farooq, U., … Go, W. Y. (2017). Axicabtagene Ciloleucel CAR T-Cell Therapy in Refractory Large B-Cell Lymphoma. *New England Journal of Medicine*, *377*(26), 2531–2544. https://doi.org/10.1056/NEJMoa1707447

Nightingale, K. P., O'Neill, L. P., & Turner, B. M. (2006). Histone modifications: Signalling receptors and potential elements of a heritable epigenetic code. *Current Opinion in Genetics & Development*, *16*(2), 125–136. https://doi.org/10.1016/j.gde.2006.02.015

Ning, Z.-Q., Li, Z.-B., Newman, M. J., Shan, S., Wang, X.-H., Pan, D.-S., Zhang, J., Dong, M., Du, X., & Lu, X.-P. (2012). Chidamide (CS055/HBI-8000): A new histone deacetylase inhibitor of the benzamide class with antitumor activity and the ability to enhance immune cell-mediated tumor cell cytotoxicity. *Cancer Chemotherapy and Pharmacology*, *69*(4), 901–909. https://doi.org/10.1007/s00280-011-1766-x

North, B. J., & Verdin, E. (2004). Sirtuins: Sir2-related NAD-dependent protein deacetylases. *Genome Biology*, *5*(5), 224.

Pan, Y.-B., Wang, W., Cai, H.-K., Zhang, J., Teng, Y., Xue, J., Zhu, M., & Luo, W.-D. (2022). Integrative analysis of a necroptosis-related gene signature of clinical value and heterogeneity in diffuse large B cell lymphoma. *Frontiers in Genetics*, *13*. https://doi.org/10.3389/fgene.2022.911443

Park, S.-Y., & Kim, J.-S. (2020). A short guide to histone deacetylases including recent progress on class II enzymes. *Experimental & Molecular Medicine*, *52*(2), 204–212. https://doi.org/10.1038/s12276-020-0382-4

Prévôt, D., Darlix, J.-L., & Ohlmann, T. (2003). Conducting the initiation of protein synthesis: The role of eIF4G. *Biology of the Cell*, *95*(3–4), 141–156. https://doi.org/10.1016/s0248-4900(03)00031-5

Qiao, F., Su, X., Qiu, X., Qian, D., Peng, X., Chen, H., Zhao, Z., & Fan, H. (2012). Enforced expression of RASAL1 suppresses cell proliferation and the transformation ability of gastric cancer cells. *Oncology Reports*, *28*(4), 1475–1481. https://doi.org/10.3892/or.2012.1920

Rauschmeier, R., Reinhardt, A., Gustafsson, C., Glaros, V., Artemov, A. V., Dunst, J.,

Taneja, R., Adameyko, I., Månsson, R., Busslinger, M., & Kreslavsky, T. (2021). Bhlhe40 function in activated B and TFH cells restrains the GC reaction and prevents lymphomagenesis. *Journal of Experimental Medicine*, *219*(2), e20211406. https://doi.org/10.1084/jem.20211406

Ray, A., Das, D. S., Song, Y., Hideshima, T., Tai, Y.-T., Chauhan, D., & Anderson, K. C. (2018). Combination of a novel HDAC6 inhibitor ACY-241 and anti-PD-L1 antibody enhances anti-tumor immunity and cytotoxicity in multiple myeloma. *Leukemia*, *32*(3), 843–846. https://doi.org/10.1038/leu.2017.322

Richards, K. L., Motsinger-Reif, A. A., Chen, H.-W., Fedoriw, Y., Fan, C., Nielsen, D. M., Small, G. W., Thomas, R., Smith, C., Dave, S. S., Perou, C. M., Breen, M., Borst, L. B., & Suter, S. E. (2013). Gene profiling of canine B-cell lymphoma reveals germinal center and postgerminal center subtypes with different survival times, modeling human DLBCL. *Cancer Research*, *73*(16), 5029–5039. https://doi.org/10.1158/0008-5472.CAN-12-3546

Richards, K. L., Motsinger-Reif, A., Chen, H.-W., Fedoriw, Y. D., Fan, C., Nielsen, D. M., Thomas, R., Smith, C., Dave, S. S., Perou, C. M., Breen, M., & Suter, S. E. (2011). Characterizing Canine Lymphoma As a Potential Large Animal Model of Human Diffuse Large B-Cell Lymphoma. *Blood*, *118*(21), 5193. https://doi.org/10.1182/blood.V118.21.5193.5193

Rojek, A. E., & Smith, S. M. (2022). Evolution of therapy for limited stage diffuse large B-cell lymphoma. *Blood Cancer Journal*, *12*(2), 1–8. https://doi.org/10.1038/s41408-021-00596-z

Ropero, S., & Esteller, M. (2007). The role of histone deacetylases (HDACs) in human cancer. *Molecular Oncology*, *1*(1), 19–25. https://doi.org/10.1016/j.molonc.2007.01.001

Roschewski, M., Phelan, J. D., & Wilson, W. H. (2020). Molecular Classification and Treatment of Diffuse Large B-cell Lymphoma and Primary Mediastinal B-cell Lymphoma. *Cancer Journal (Sudbury, Mass.)*, *26*(3), 195–205. https://doi.org/10.1097/PPO.0000000000000450

Roth, S. Y., Denu, J. M., & Allis, C. D. (2001). Histone acetyltransferases. *Annual Review of Biochemistry*, *70*, 81–120. https://doi.org/10.1146/annurev.biochem.70.1.81

San-Miguel, J. F., Hungria, V. T. M., Yoon, S.-S., Beksac, M., Dimopoulos, M. A., Elghandour, A., Jedrzejczak, W. W., Günther, A., Nakorn, T. N., Siritanaratkul, N., Corradini, P., Chuncharunee, S., Lee, J.-J., Schlossman, R. L., Shelekhova, T., Yong, K., Tan, D., Numbenjapon, T., Cavenagh, J. D., … Richardson, P. G. (2014). Panobinostat plus bortezomib and dexamethasone versus placebo plus bortezomib and dexamethasone in patients with relapsed or relapsed and refractory multiple myeloma: A multicentre, randomised, double-blind phase 3 trial. *The Lancet. Oncology*, *15*(11), 1195–1206. https://doi.org/10.1016/S1470-

2045(14)70440-1

Schmitt, A., Xu, W., Bucher, P., Grimm, M., Konantz, M., Horn, H., Zapukhlyak, M., Berning, P., Brändle, M., Jarboui, M.-A., Schönfeld, C., Boldt, K., Rosenwald, A., Ott, G., Grau, M., Klener, P., Vockova, P., Lengerke, C., Lenz, G., … Hailfinger, S. (2021). Dimethyl fumarate induces ferroptosis and impairs NF-κB/STAT3 signaling in DLBCL. *Blood*, *138*(10), 871–884. https://doi.org/10.1182/blood.2020009404

Schmitz, R., Wright, G. W., Huang, D. W., Johnson, C. A., Phelan, J. D., Wang, J. Q., Roulland, S., Kasbekar, M., Young, R. M., Shaffer, A. L., Hodson, D. J., Xiao, W., Yu, X., Yang, Y., Zhao, H., Xu, W., Liu, X., Zhou, B., Du, W., … Staudt, L. M. (2018). Genetics and Pathogenesis of Diffuse Large B-Cell Lymphoma. *The New England Journal of Medicine*, *378*(15), 1396–1407. https://doi.org/10.1056/NEJMoa1801445

Schumacher, T. N., & Schreiber, R. D. (2015). Neoantigens in cancer immunotherapy. *Science (New York, N.Y.)*, *348*(6230), 69–74. https://doi.org/10.1126/science.aaa4971

Schuster, S. J., Bishop, M. R., Tam, C. S., Waller, E. K., Borchmann, P., McGuirk, J. P., Jäger, U., Jaglowski, S., Andreadis, C., Westin, J. R., Fleury, I., Bachanova, V., Foley, S. R., Ho, P. J., Mielke, S., Magenau, J. M., Holte, H., Pantano, S., Pacaud, L. B., … Maziarz, R. T. (2019). Tisagenlecleucel in Adult Relapsed or Refractory Diffuse Large B-Cell Lymphoma. *New England Journal of Medicine*, *380*(1), 45–56. https://doi.org/10.1056/NEJMoa1804980

Sedeta, E., Ilerhunmwuwa, N., Wasifuddin, M., Uche, I., Hakobyan, N., Perry, J., Aiwuyo, H., Abowali, H., & Avezbakiyev, B. (2022). Epidemiology of Non-Hodgkin Lymphoma: Global Patterns of Incidence, Mortality, and Trends. *Blood*, *140*(Supplement 1), 5234–5235. https://doi.org/10.1182/blood-2022-158830

Sehgal, A. R., Godwin, J., Pribble, J., Wang, L., Thorpe, J., & Hildebrandt, G. C. (2019). Lisocabtagene Maraleucel (liso-cel) for Treatment of Second-Line Transplant Noneligible (TNE) Relapsed/Refractory (R/R) Aggressive Non-Hodgkin Lymphoma (NHL): Initial Results from the PILOT Study. *Blood*, *134*(Supplement_1), 2882. https://doi.org/10.1182/blood-2019-127658

Sehn, L. H., & Gascoyne, R. D. (2015). Diffuse large B-cell lymphoma: Optimizing outcome in the context of clinical and biologic heterogeneity. *Blood*, *125*(1), 22–32. https://doi.org/10.1182/blood-2014-05-577189

Seifert, M., & Küppers, R. (2009). Molecular footprints of a germinal center derivation of human IgM+(IgD+)CD27+ B cells and the dynamics of memory B cell generation. *The Journal of Experimental Medicine*, *206*(12), 2659–2669. https://doi.org/10.1084/jem.20091087

Seifert, M., Przekopowitz, M., Taudien, S., Lollies, A., Ronge, V., Drees, B., Lindemann,

M., Hillen, U., Engler, H., Singer, B. B., & Küppers, R. (2015). Functional capacities of human IgM memory B cells in early inflammatory responses and secondary germinal center reactions. *Proceedings of the National Academy of Sciences of the United States of America*, *112*(6), E546-555. https://doi.org/10.1073/pnas.1416276112

Seifert, M., Scholtysik, R., & Küppers, R. (2013). Origin and pathogenesis of B cell lymphomas. *Methods in Molecular Biology (Clifton, N.J.)*, *971*, 1–25. https://doi.org/10.1007/978-1-62703-269-8_1

Seto, E., & Yoshida, M. (2014). Erasers of histone acetylation: The histone deacetylase enzymes. *Cold Spring Harbor Perspectives in Biology*, *6*(4), a018713. https://doi.org/10.1101/cshperspect.a018713

Seto, M., Ohta, M., Ikenoue, T., Sugimoto, T., Asaoka, Y., Tada, M., Mohri, D., Kudo, Y., Ijichi, H., Tateishi, K., Otsuka, M., Hirata, Y., Maeda, S., Koike, K., & Omata, M. (2011). Reduced expression of RAS protein activator like-1 in gastric cancer. *International Journal of Cancer*, *128*(6), 1293–1302. https://doi.org/10.1002/ijc.25459

SH, S., E, C., NL, H., ES, J., SA, P., H, S., & J, T. (2017). *WHO Classification of Tumours of Haematopoietic and Lymphoid Tissues*. https://publications.iarc.fr/Book-And-Report-Series/Who-Classification-Of-Tumours/WHO-Classification-Of-Tumours-Of-Haematopoietic-And-Lymphoid-Tissues-2017

Shankland, K. R., Armitage, J. O., & Hancock, B. W. (2012). Non-Hodgkin lymphoma. *Lancet (London, England)*, *380*(9844), 848–857. https://doi.org/10.1016/S0140-6736(12)60605-9

Shanmugam, G., Rakshit, S., & Sarkar, K. (2022). HDAC inhibitors: Targets for tumor therapy, immune modulation and lung diseases. *Translational Oncology*, *16*, 101312. https://doi.org/10.1016/j.tranon.2021.101312

Sharma, P., Hu-Lieskovan, S., Wargo, J. A., & Ribas, A. (2017). Primary, Adaptive, and Acquired Resistance to Cancer Immunotherapy. *Cell*, *168*(4), 707–723. https://doi.org/10.1016/j.cell.2017.01.017

Sharma, S. V., Lee, D. Y., Li, B., Quinlan, M. P., Takahashi, F., Maheswaran, S., McDermott, U., Azizian, N., Zou, L., Fischbach, M. A., Wong, K.-K., Brandstetter, K., Wittner, B., Ramaswamy, S., Classon, M., & Settleman, J. (2010). A chromatin-mediated reversible drug-tolerant state in cancer cell subpopulations. *Cell*, *141*(1), 69–80. https://doi.org/10.1016/j.cell.2010.02.027

Sheaffer, K. L., Elliott, E. N., & Kaestner, K. H. (2016). DNA hypomethylation contributes to genomic instability and intestinal cancer initiation. *Cancer Prevention Research (Philadelphia, Pa.)*, *9*(7), 534–546. https://doi.org/10.1158/1940-6207.CAPR-15-0349

Shi, M.-Q., Xu, Y., Fu, X., Pan, D.-S., Lu, X.-P., Xiao, Y., & Jiang, Y.-Z. (2024). Advances in targeting histone deacetylase for treatment of solid tumors. *Journal of Hematology & Oncology*, *17*(1), 37. https://doi.org/10.1186/s13045-024-01551-8

Shi, Y., Agematsu, K., Ochs, H. D., & Sugane, K. (2003). Functional analysis of human memory B-cell subpopulations: IgD+CD27+ B cells are crucial in secondary immune response by producing high affinity IgM. *Clinical Immunology*, *108*(2), 128–137. https://doi.org/10.1016/S1521-6616(03)00092-5

Shouse, G., & Herrera, A. F. (2021). Advances in Immunotherapy for Diffuse Large B Cell Lymphoma. *BioDrugs*, *35*(5), 517–528. https://doi.org/10.1007/s40259-021-00491-w

Shu, F., Xiao, H., Li, Q.-N., Ren, X.-S., Liu, Z.-G., Hu, B.-W., Wang, H.-S., Wang, H., & Jiang, G.-M. (2023). Epigenetic and post-translational modifications in autophagy: Biological functions and therapeutic targets. *Signal Transduction and Targeted Therapy*, *8*(1), 1–23. https://doi.org/10.1038/s41392-022-01300-8

Shukla, S., & Tekwani, B. L. (2020). Histone Deacetylases Inhibitors in Neurodegenerative Diseases, Neuroprotection and Neuronal Differentiation. *Frontiers in Pharmacology*, *11*, 537. https://doi.org/10.3389/fphar.2020.00537

Simon, J. A., & Lange, C. A. (2008). Roles of the EZH2 histone methyltransferase in cancer epigenetics. *Mutation Research*, *647*(1–2), 21–29. https://doi.org/10.1016/j.mrfmmm.2008.07.010

Singh, V., Walter, V., Elcheva, I., Imamura Kawasawa, Y., & Spiegelman, V. S. (2023). Global role of IGF2BP1 in controlling the expression of Wnt/β-catenin-regulated genes in colorectal cancer cells. *Frontiers in Cell and Developmental Biology*, *11*. https://doi.org/10.3389/fcell.2023.1236356

Sitzia, J., North, C., Stanley, J., & Winterberg, N. (1997). Side effects of CHOP in the treatment of non-Hodgkin's lymphoma. *Cancer Nursing*, *20*(6), 430.

Sui, H., Ma, N., Wang, Y., Li, H., Liu, X., Su, Y., & Yang, J. (2018). Anti-PD-1/PD-L1 Therapy for Non-Small-Cell Lung Cancer: Toward Personalized Medicine and Combination Strategies. *Journal of Immunology Research*, *2018*(1), 6984948. https://doi.org/10.1155/2018/6984948

Takahara, T., Nakamura, S., Tsuzuki, T., & Satou, A. (2023). The Immunology of DLBCL. *Cancers*, *15*(3), 835. https://doi.org/10.3390/cancers15030835

Taylor, R. P., & Lindorfer, M. A. (2007). Drug Insight: The mechanism of action of rituximab in autoimmune disease—the immune complex decoy hypothesis. *Nature Clinical Practice Rheumatology*, *3*(2), 86–95. https://doi.org/10.1038/ncprheum0424

Tuccitto, A., Shahaj, E., Vergani, E., Ferro, S., Huber, V., Rodolfo, M., Castelli, C., Rivoltini,

L., & Vallacchi, V. (2019). Immunosuppressive circuits in tumor microenvironment and their influence on cancer treatment efficacy. *Virchows Archiv*, *474*(4), 407–420. https://doi.org/10.1007/s00428-018-2477-z

Van Tongelen, A., Loriot, A., & De Smet, C. (2017). Oncogenic roles of DNA hypomethylation through the activation of cancer-germline genes. *Cancer Letters*, *396*, 130–137. https://doi.org/10.1016/j.canlet.2017.03.029

Vaughan, R. M., Kupai, A., & Rothbart, S. B. (2021). Chromatin Regulation through Ubiquitin and Ubiquitin-like Histone Modifications. *Trends in Biochemical Sciences*, *46*(4), 258–269. https://doi.org/10.1016/j.tibs.2020.11.005

Vaziri, H., Dessain, S. K., Eaton, E. N., Imai, S.-I., Frye, R. A., Pandita, T. K., Guarente, L., & Weinberg, R. A. (2001). hSIR2SIRT1 Functions as an NAD-Dependent p53 Deacetylase. *Cell*, *107*(2), 149–159. https://doi.org/10.1016/S0092-8674(01)00527-X

Verdin, E., & Ott, M. (2015). 50 years of protein acetylation: From gene regulation to epigenetics, metabolism and beyond. *Nature Reviews Molecular Cell Biology*, *16*(4), 258–264. https://doi.org/10.1038/nrm3931

Ververis, K., Hiong, A., Karagiannis, T. C., & Licciardi, P. V. (2013). Histone deacetylase inhibitors (HDACIs): Multitargeted anticancer agents. *Biologics : Targets & Therapy*, *7*, 47–60. https://doi.org/10.2147/BTT.S29965

Verza, F. A., Das, U., Fachin, A. L., Dimmock, J. R., & Marins, M. (2020). Roles of Histone Deacetylases and Inhibitors in Anticancer Therapy. *Cancers*, *12*(6), 1664. https://doi.org/10.3390/cancers12061664

Viardot, A., Goebeler, M.-E., Hess, G., Neumann, S., Pfreundschuh, M., Adrian, N., Zettl, F., Libicher, M., Sayehli, C., Stieglmaier, J., Zhang, A., Nagorsen, D., & Bargou, R. C. (2016). Phase 2 study of the bispecific T-cell engager (BiTE) antibody blinatumomab in relapsed/refractory diffuse large B-cell lymphoma. *Blood*, *127*(11), 1410–1416. https://doi.org/10.1182/blood-2015-06-651380

Vieira, P., & Rajewsky, K. (1990). Persistence of memory B cells in mice deprived of T cell help. *International Immunology*, *2*(6), 487–494. https://doi.org/10.1093/intimm/2.6.487

Voss, T. C., & Hager, G. L. (2014). Dynamic regulation of transcriptional states by chromatin and transcription factors. *Nature Reviews Genetics*, *15*(2), 69–81. https://doi.org/10.1038/nrg3623

Waddington, C. H. (2012). The epigenotype. 1942. *International Journal of Epidemiology*, *41*(1), 10–13. https://doi.org/10.1093/ije/dyr184

Wagner, J. M., Hackanson, B., Lübbert, M., & Jung, M. (2010). Histone deacetylase

(HDAC) inhibitors in recent clinical trials for cancer therapy. *Clinical Epigenetics*, *1*(3–4), 117–136. https://doi.org/10.1007/s13148-010-0012-4

Wang, G., He, J., Zhao, J., Yun, W., Xie, C., Taub, J. W., Azmi, A., Mohammad, R. M., Dong, Y., Kong, W., Guo, Y., & Ge, Y. (2012). Class I and Class II Histone Deacetylases Are Potential Therapeutic Targets for Treating Pancreatic Cancer. *PLOS ONE*, *7*(12), e52095. https://doi.org/10.1371/journal.pone.0052095

Wang, K. C., & Chang, H. Y. (2018). Epigenomics—Technologies and Applications. *Circulation Research*, *122*(9), 1191–1199. https://doi.org/10.1161/CIRCRESAHA.118.310998

Wang, Q., Shao, X., Zhang, Y., Zhu, M., Wang, F. X. C., Mu, J., Li, J., Yao, H., & Chen, K. (2023). Role of tumor microenvironment in cancer progression and therapeutic strategy. *Cancer Medicine*, *12*(10), 11149–11165. https://doi.org/10.1002/cam4.5698

Wang, S.-L., Lee, J.-J., & Liao, A. T. (2016). Comparison of efficacy and toxicity of doxorubicin and mitoxantrone in combination chemotherapy for canine lymphoma. *The Canadian Veterinary Journal*, *57*(3), 271–276.

West, A. C., & Johnstone, R. W. (2014). New and emerging HDAC inhibitors for cancer treatment. *The Journal of Clinical Investigation*, *124*(1), 30–39. https://doi.org/10.1172/JCI69738

Woods, D. M., Sodré, A. L., Villagra, A., Sarnaik, A., Sotomayor, E. M., & Weber, J. (2015). HDAC Inhibition Upregulates PD-1 Ligands in Melanoma and Augments Immunotherapy with PD-1 Blockade. *Cancer Immunology Research*, *3*(12), 1375–1385. https://doi.org/10.1158/2326-6066.CIR-15-0077-T

Wu, T., & Dai, Y. (2017). Tumor microenvironment and therapeutic response. *Cancer Letters*, *387*, 61–68. https://doi.org/10.1016/j.canlet.2016.01.043

Xu-Monette, Z. Y., Xiao, M., Au, Q., Padmanabhan, R., Xu, B., Hoe, N., Rodríguez-Perales, S., Torres-Ruiz, R., Manyam, G. C., Visco, C., Miao, Y., Tan, X., Zhang, H., Tzankov, A., Wang, J., Dybkær, K., Tam, W., You, H., Bhagat, G., … Young, K. H. (2019). Immune Profiling and Quantitative Analysis Decipher the Clinical Role of Immune-Checkpoint Expression in the Tumor Immune Microenvironment of DLBCL. *Cancer Immunology Research*, *7*(4), 644–657. https://doi.org/10.1158/2326-6066.CIR-18-0439

Yang, H., & Green, M. R. (2019). Epigenetic Programing of B-Cell Lymphoma by BCL6 and Its Genetic Deregulation. *Frontiers in Cell and Developmental Biology*, *7*. https://doi.org/10.3389/fcell.2019.00272

Yusufova, N., Kloetgen, A., Teater, M., Osunsade, A., Camarillo, J. M., Chin, C. R., Doane, A. S., Venters, B. J., Portillo-Ledesma, S., Conway, J., Phillip, J. M., Elemento, O.,

Scott, D. W., Béguelin, W., Licht, J. D., Kelleher, N. L., Staudt, L. M., Skoultchi, A. I., Keogh, M.-C., … Melnick, A. M. (2021). Histone H1 loss drives lymphoma by disrupting 3D chromatin architecture. *Nature*, *589*(7841), 299–305. https://doi.org/10.1038/s41586-020-3017-y

Zang, X., Jiang, J., Gu, J., Chen, Y., Wang, M., Zhang, Y., Fu, M., Shi, H., Cai, H., Qian, H., Xu, W., & Zhang, X. (2022). Circular RNA EIF4G3 suppresses gastric cancer progression through inhibition of β-catenin by promoting δ-catenin ubiquitin degradation and upregulating SIK1. *Molecular Cancer*, *21*, 141. https://doi.org/10.1186/s12943-022-01606-9

Zhang, J., Dominguez-Sola, D., Hussein, S., Lee, J.-E., Holmes, A. B., Bansal, M., Vlasevska, S., Mo, T., Tang, H., Basso, K., Ge, K., Dalla-Favera, R., & Pasqualucci, L. (2015). Disruption of KMT2D perturbs germinal center B cell development and promotes lymphomagenesis. *Nature Medicine*, *21*(10), 1190–1198. https://doi.org/10.1038/nm.3940

Zhang, J., & Zhong, Q. (2014). Histone deacetylase inhibitors and cell death. *Cellular and Molecular Life Sciences*, *71*(20), 3885–3901. https://doi.org/10.1007/s00018-014-1656-6

Zhao, Q., Zhou, J., Li, F., Guo, S., Zhang, L., Li, J., Qi, Q., & Shi, Y. (2022). The Role and Therapeutic Perspectives of Sirtuin 3 in Cancer Metabolism Reprogramming, Metastasis, and Chemoresistance. *Frontiers in Oncology*, *12*, 910963. https://doi.org/10.3389/fonc.2022.910963

Zhu, M., Han, Y., Gu, T., Wang, R., Si, X., Kong, D., Zhao, P., Wang, X., Li, J., Zhai, X., Yu, Z., Lu, H., Li, J., Huang, H., & Qian, P. (2024). Class I HDAC inhibitors enhance antitumor efficacy and persistence of CAR-T cells by activation of the Wnt pathway. *Cell Reports*, *43*(4), 114065. https://doi.org/10.1016/j.celrep.2024.114065

Zhu, T.-Y., Hong, L.-L., & Ling, Z.-Q. (2023). Oncofetal protein IGF2BPs in human cancer: Functions, mechanisms and therapeutic potential. *Biomarker Research*, *11*(1), 62. https://doi.org/10.1186/s40364-023-00499-0

Appendix

Table 4: Devices used in this work.

Device	Distributors
All-in-one Fluorescence Microscope BZ-X series	Keyence Deutschland GmbH
BD FACSymphony A1	BD Biosciences, Heidelberg, Germany
Centrifuge 5425 R	Eppendorf, Hamburg, Germany
Centrifuge Megafuge ST1R Plus	Life Technologies GmbH, Darmstadt, Germany
Counting chamber, Neubauer improved	Brand Scientific GmbH, Wertheim, Germany
Cybio Well vario – Multichannel Automated Pipetting System	Analytik Jena GmbH 6 Co. KG, Jena, Germany
D300e Digital Dispenser	Tecan Group LTd., Männedorf, Switzerland
Eppendorf Multipette E3x	Eppendorf, Hamburg, Germany
EVE™ Automatic cell counter	NanoEntek, Inc., Seoul, Korea
Heracell™ 150i CO_2 - Incubator	Life Technologies GmbH, Darmstadt, Germany
Herasafe™ 2025 Class II Biological Safety Cabinet	Life Technologies GmbH, Darmstadt, Germany
Incucyte® S3 Live-Cell Analysis System	Sartorius AG, Göttingen, Germany
Multidrop™ Combi Reagent Dispenser	Life Technologies GmbH, Darmstadt, Germany
Pipet-Lite Multi Pipette L8-300XLS+	Mettler-Toledo GmbH, Erftstadt, Germany
Pipetboy accu-jet S	Brand Scientific GmbH, Wertheim, Germany
Primovert Light microscope	Carl Zeiss AG, Oberkochen, Germany
Spark 10M Multimode microplate reader	Tecan Group LTd., Männedorf, Switzerland
Vortex-Genie 2	Scientific Industries, Inc., New York, USA

Table 5: Software used in this work.

Software	Distributors
BD FACSDiva	BD Biosciences, Heidelberg, Germany
CyBio Composer	Analytik Jena GmbH+Co. KG
D300e control (version 3.4.3)	Tecan Group LTd., Männedorf, Switzerland
FILLit for Multidrop Combi	Life Technologies GmbH, Darmstadt, Germany

FlowJo (version 10.10)	BD Biosciences, Heidelberg, Germany
GraphPad Prism 9 (version 9.03)	Graphpad Software (San Diego, USA)
Microsoft Office	Microsoft (Redmond, USA)
Spark control	Tecan Group LTd., Männedorf, Switzerland

Table 6: Consumables used in this work.

Consumables	Distributor	Catalog number #
12 well Cell Culture Plate	Greiner Bio-One, Kremsmünster, Austria	665180
150 mL Polystyrene Reservoir	INTEGRA Biosciences GmbH, Biebertal, Germany	6318
150 mL Reservoir base	INTEGRA Biosciences GmbH, Biebertal, Germany	6301
24 well Cell Culture Plate	Greiner Bio-One, Kremsmünster, Austria	662160
384 well Cell Culture Plate	Corning, New York, USA	734-4104
6 well Cell Culture Plate	Greiner Bio-One, Kremsmünster, Austria	639160
96 well Cell Culture Plate	Greiner Bio-One, Kremsmünster, Austria	651160
BD Vacutainer	Becton Dickinson GmbH, Heidelberg, Germany	368861
Cell culture flasks 25 cm^2	Greiner Bio-One, Kremsmünster, Austria	690175
Cell culture flasks 75 cm^2	Greiner Bio-One, Kremsmünster, Austria	658175
CyBio TipTray 384/60 µL	Fluotics LTD., New York, USA	736-0997
D4+ casette	Tecan Group LTd., Männedorf, Switzerland	30097371
Mr. Frosty freezing container	Life Technologies GmbH, Darmstadt, Germany	5100-0001
Multidrop tubing	Life Technologies GmbH, Darmstadt, Germany	24072670 24073295
Nunc Cryo Tubes 1 ml	Life Technologies GmbH, Darmstadt, Germany	377224
Parafilm® M	Sigma-Aldrich Chemie GmbH, Taufkirchen, Germany	P7543
Pipette tips 10 µl, 20 µl,	Biozym Scientific GmbH,	VT0400, VT0220,

100 µl, 200 µl, 1,000 µl	Hessisch Oldendorf, Germany	VT0230, VT0038, VT0370
Safe-Lock Tubes 1.5 ml	Eppendorf, Hamburg, Germany	0030 120.086
Safe-Lock Tubes 2.0 ml	Eppendorf, Hamburg, Germany	0030 120.094
Serological pipettes 5 ml, 10 ml, 25 ml	Sarstedt AG 6 Co. KG, Nümbrecht, Germany	86.1253.001, 86.1254.001, 86.1685.001
T8+ casette	Tecan Group LTd., Männedorf, Switzerland	30097370
Tubes 0.5 ml	Eppendorf, Hamburg, Germany	3217305
Tubes 15 ml, graduated, sterile	Greiner Bio-One, Kremsmünster, Österreich	188 271-N
Tubes 50 ml, graduated, sterile	Greiner Bio-One, Kremsmünster, Österreich	227 261
White 384 well plates, sterile	Corning, New York, USA	734-4104
White 96 well plate, sterile	Greiner Bio-One, Kremsmünster, Austria	651160

Table 7: Chemicals, reagents and kits used in this work.

Chemicals, reagents and kits	Distributor	Catalog number #
Bovine Serum Albumin (BSA)	PAN Biotech, Aidenbach, Germany	P06-1391500
CellTiter-Glo Luminescent Cell Viability Assay	Promega, Walldorf, Germany	G7573
CellTiter-Glo luminescent viability assay	Promega, Walldorf, Germany	G7573
Dimethyl Sulfoxide for cell culture (DMSO)	AppliChem GmbH, Darmstadt, Germany	A3672
Dulbecco's phosphate buffered saline (DPBS)	Life Technologies GmbH, Darmstadt, Germany	14190-250
Ethanol 80%	Otto Fischer GmbH & Co. KG, Saarbrücken, Germany	PZN11193404
Heat Inactivated Fetal Bovine Serum (FBS)	Sigma-Aldrich, Taufkirchen, Germany	F9665
Iscove's Modified Dulbecco's Medium (IMDM) Medium	Life Technologies GmbH, Darmstadt, Germany	12440-053
L-Glutamine (200nM)	Life Technologies GmbH, Darmstadt, Germany	25030-024
Lenti-X Transduction	Takara Bio Europe SAS, Saint-	631478

Sponge	Germain-en-Laye, France	
MICRO-90	International products coorporation, Burlington, USA	M-9050-12
Pancoll human	PAN Biotech, Aidenbach, Germany	P04-60500
Penicillin (10.000 U/mL)-Streptomycin (10 mg/mL) (P/S)	Life Technologies GmbH, Darmstadt, Germany	15140-122
Polybren Infektions- / Transfektionsreagenz	Sigma-Aldrich, Taufkirchen, Germany	TR-1003-G
Propidium Iodide (100mg) (PI)	Life Technologies GmbH, Darmstadt, Germany	P1304MP
Roswell Park Memorial Institute (RPMI) 1640 Medium	Life Technologies GmbH, Darmstadt, Germany	22400-089
Staurosporine (10mM)	MedChemExpress EU, Sollentuna, Sweden	HY-1541
Trypan Blue solution 0.4%	Sigma-Aldrich, Taufkirchen, Germany	T8154
Trypsin-EDTA (0.05%), phenol red	Life Technologies GmbH, Darmstadt, Germany	25300-096

Table 8: The Inhibitor library used in this work was provided by Prof. Thomas Kurz.

Inhibitor	Isoform preference	Inhibitor	Isoform preference
LAK 402	Class I	**FFK 24**	Class IIa
FJKK 103	Class I	**FFK 29**	Class IIa
FJKK 133	Class I	**YAK 540**	Class IIa
HLK 40	Class I	**YAK 545**	Class IIa
LAK 107	Class I	**YAK 477**	Class IIa
LAK 121	Class I	**MPK 169**	Class IIb
SHOK 73	Class I	**MPK 576**	Class IIb
BLK 31	Class I	**MPK 265**	Class IIb
YAK 577	Class I	**MPK 264**	Class IIb
LAK 67	Class I/II	**MPK 803**	Class IIb
KSK 64	Class I/II	**MPK 805**	Class IIb
MPK 544	Class I/II	**YAK 312**	Class IIb
LAK 31	Class I/II	**LAKZnfD**	Class IIb
LAK 41	Class I/II	**LAK 129**	Dual BRD/HDAC-inhibitors

LAK 61	Class I/II	**LAK 169**	Dual BRD/HDAC-inhibitors
LAK 39	Class I/II	**LAK-FFK 11**	Dual BRD/HDAC-inhibitors
YAK 61	Class I/II	**FJKK 94**	Dual HDAC/LSD1 inhibitors
FFK 186	Class I/II	**FJKK 81**	Dual HDAC/LSD1 inhibitors
MPK 460	Class I/II	**HLK 89**	Dual HDAC/LSD1 inhibitors
BBK 69	Class I/II	**SIS17**	HDAC 11
TOK 27	Class I/II		

Table 9: Overview of cell lines used in this work.

Cell line	Entity	Catalog number	Culture condition	Suspension / adherent
Farage	GCB-DLBCL	CRL-2630	M1	suspension
HBL-1	ABC-DLBCL	T8204-ABM	M1	suspension
HBL-2	MCL	T8205-ABM	M1	suspension
JEKO-1	MCL	CRL-3006	M1	suspension
K422	GCB-DLBCL	K422	M1	suspension
OCI-Ly1	GCB-DLBCL	ACC 722	M2	suspension
OCI-Ly2	GCB-DLBCL		M2	suspension
OCI-Ly3	ABC-DLBCL	ACC 761	M1	suspension
OCI-Ly7	GCB-DLBCL	ACC 688	M2	suspension
OCI-Ly8	GCB-DLBCL		M1	suspension
OCI-Ly18	ABC-DLBCL	ACC 699	M1	suspension
Pfeiffer	GCB-DLBCL	CRL-2632	M1	suspension
Riva (RI-1)	ABC-DLBCL	ACC 585	M1	suspension
SU-DHL-2	ABC-DLBCL	CRL-2956	M1	suspension
SU-DHL-4	GCB-DLBCL	CRL-2957	M1	suspension
SU-DHL-5	GCB-DLBCL	ACC 571	M1	suspension
SU-DHL-8	GCB-DLBCL	CRL-2961	M1	suspension
SC-1	FL	ACC 558	M1	suspension
TMD-8	ABC-DLBCL		M1	suspension
U-2932	ABC-DLBCL	ACC 633	M1	suspension
U-2940	PMBL	ACC 634	M1	suspension
WSU-FSCCL	DSCL	ACC 612	M1	suspension
WSU-DLCL2	ABC-DLBCL	ACC 575	M1	suspension

Z-138	MCL	CRL-3001	M2	suspension
LNSC177	Stroma		M1	adherent

Table 10: Overview of media composition.

Entry	Medium	Supplements
M1	RPMI 1640	1% L-Glutamine, 1% P/S, 10% FBS
M2	IMDM	1% L-Glutamine, 1% P/S, 10% FBS

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