Chapter 1

General Introduction
1. Introduction

Historically, lymphoid malignancies have been divided into 2 major categories, Hodgkin lymphomas (HL) and Non-Hodgkin lymphomas (NHL). HLs and the majority of NHLs are derived from germinal center (GC) B-cells, which are prone to malignant transformation because of their extensive physiologic DNA editing and rapid proliferation. The most common NHL in adults, diffuse large B-cell lymphoma (DLBCL), is largely derived from GC or post-GC B-cells. Additional less common large B-cell lymphoma (LBCL) subtypes include entities with unique cellular composition – T-cell/histiocyte-rich large B-cell lymphoma (TCRBCL) – or specific clinical presentations and sites of involvement – primary central nervous system lymphoma (PCNSL), primary testicular lymphoma (PTL) and primary mediastinal B-cell lymphoma (PMBL). Emerging data suggests that LBCL subtypes and HLs have characteristic genetic signatures comprised of shared and unique alterations. In LBCLs and HLs, certain molecular abnormalities limit the efficacy of an anti-tumor immune response. The overarching goal of this thesis to delineate specific genetic features of select LBCL subtypes, including PCNSL, PTL, PMBL and TCRBCL, and HL, with an emphasis on targetable genetic bases of immune evasion.

In the introduction, salient features of normal and malignant GC B-cells will be reviewed. The LBCL subtypes, PCNSL, PTL, PMBL and TCRBCL, will be compared and contrasted with the more common systemic DLBCLs and select features of HLs will be described. Finally, essential aspects of normal and aberrant immune recognition and the consequences for lymphoid malignancies will be summarized.

1.1. The Germinal Center

Most B-cell lymphomas are derived from GC B-cells or from post-GC B-cells, indicating the important role of the GC in their pathogenesis.\textsuperscript{1-3} In physiological conditions, GCs are formed upon recognition of an antigen by a mature naïve B-cell. The antigen-exposed B-cells will rapidly expand and aggregate into primary follicles to form GCs (Figure 1). To produce different subclasses of antibodies with distinct effector functions, B-cells undergo somatic hypermutation (SHM) and class switch recombination (CSR) accompanied by rapid cellular proliferation (Figure 1). This rapid proliferation rate and errors in SHM and CSR, can cause malignant transformation of B-cells.\textsuperscript{4} As a result, the malignant B-cells in many lymphoid malignancies share molecular features of normal GC and post-GC B-cells.\textsuperscript{4}
1.2. Non-Hodgkin Lymphomas – DLBCL and LBCL subtypes

NHLs are identified and classified by morphologic features, clinical presentation and specific sites of involvement. DLBCL is the most common LBCL entity, and additional, less common, LBCL subtypes have been identified. Certain LBCL subtypes are characterized by distinct histologic features – TCRBCL – or defined locations – PCNSL, PTL and PMBL. The characteristics of DLBCL provide a framework to more fully understand the different LBCL subtypes and therefore warrant consideration.

1.2.1. Transcriptionally defined DLBCL subtypes

DLBCLs exhibit multiple low-frequency genetic alterations, including chromosomal rearrangements, somatic mutations and copy number alterations (CNAs). Classification systems based on transcriptional profiles have been developed to capture the heterogeneity of this disease.

1.2.1.1. Cell-of-origin classification

The cell-of-origin (COO) classification distinguishes DLBCL subsets that share characteristics with normal B-cell subsets and include: GC B-cell (GCB)- and activated B-cell (ABC)-type DLBCLs. Certain genetic alterations are more frequently found in the COO subtypes.
**GCB-type DLBCLs.** GCB-type DLBCLs have more frequent chromosomal translocations involving *MYC* and *BCL2* in 10% and 40%, respectively. In addition, alterations in the histone methyl transferase *EZH2* and Ga13 signaling components, regulating cell motility, have been described.

**ABC-type DLBCLs.** ABC-type DLBCLs have a similar transcriptional profile as B-cells that are differentiating to a plasmablastic stage and harbor alterations of Toll-like receptor (TLR) and B-cell receptor (BCR) signaling components that result in constitutive NF-κB activation. This pathway is critical for proliferation, differentiation and survival of lymphoid cells. TLR signaling activates the NF-κB pathway by utilizing the adaptor protein MyD88 (Figure 2, left panel). MyD88 associates with the Toll-Interleukin receptor domain of TLRs and upon stimulation with ligand, IRAKs are recruited that associate with TRAF6. A complex is formed that activates the IKK complex, which in turn activates NF-κB (Figure 2, left panel). Alterations in *MYD88* are seen in 29% of ABC-type DLBCLs. In addition, ABC-type DLBCLs rely on oncogenic BCR signaling for their survival.

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**Figure 2. TLR and BCR signaling pathways.** Left panel: MyD88 associates with TLRs and recruits IRAK4. IRAK4 phosphorylates IRAK1, which in turn activates TRAF6 that then forms a complex with TAK1, TAB1 and TAB2. The IKK complex, consisting of IKKα, IKKβ and IKKγ, is activated, activating NF-κB. Right panel: upon binding of antigen to the BCR, a CD79A/B heterodimer is formed. SYK is recruited and phosphorylated, which recruits BLNK that coordinates phosphorylation and activation of BTK and PLCγ2. Subsequently, PKCβ is activated, which phosphorylates CARD11 and NF-κB is activated. A20 functions as a negative regulator of NF-κB activation. Signaling through the BCR pathway also activates the MAPK and PI3K pathways. Stars indicate common genetic alterations in components of the TLR/BCR signaling pathways in ABC-type DLBCLs.
BCR signaling can also activate the NF-κB pathway, among other pathways. Upon binding of antigen to the BCR, the CD79A/B heterodimer recruits SYK, followed by recruitment of BLNK that coordinates phosphorylation and activation of BTK and PLCγ2. Subsequently, PKCβ is activated, after which CARD11 is phosphorylated, leading to activation of NF-κB (Figure 2, right panel). A20 (also known as TNFAIP3) functions as a negative regulator of NF-κB activation (Figure 2, right panel). ABC-type DLBCLs have mutations in multiple components of this signaling pathway. Loss of A20 by mutations or deletions leads to loss of inhibition of NF-κB signaling, more commonly in ABC-type DLBCLs. Also, alterations in CARD11 are seen in 10% of ABC-type DLBCLs. In addition, 18% of ABC-type DLBCLs have mutations in CD79A or CD79B, causing chronic active BCR signaling. Of interest, co-occurrence of MYD88 and CD79B mutations is seen in 43% of ABC-type DLBCLs.

1.2.1.2. Comprehensive consensus cluster classification

An alternative classification scheme delineates DLBCLs solely on differences in the transcriptional signature of specific tumor subsets and includes: BCR, oxidative phosphorylation (OxPhos) and host-response (HR) tumors. BCR DLBCLs have increased expression of multiple components of the BCR signaling pathway and activation of the BCR signaling and survival pathway. These tumors are sensitive to targeted inhibition of proximal BCR pathway components, including SYK and PI3K. OxPhos DLBCLs have a distinct metabolic signature and primarily rely on oxidative phosphorylation and fatty acid oxidation, as opposed to glycolysis, for their survival. HR DLBCLs are defined by an inflammatory/immune cell infiltrate. This subtype includes most cases diagnosed as TCRBCL, see section 1.2.2.

1.2.1.3. CNA-associated signature of decreased p53/cell cycle

In addition to these transcriptionally defined classification schemes, a recent study identified a comprehensive DNA-based signature of perturbed p53/cell cycle pathway in DLBCLs. Cell cycle progression is controlled by cyclin-dependent kinases (CDKs) that phosphorylate the RB proteins, releasing E2F transcription factors that initiate cell cycle progression. The tumor suppressor gene CDKN2A, coding for the proteins p16INK4a and p14ARF, inhibits CDKs. Using an integrative approach of copy number (CN) and gene expression data, followed by pathway enrichment, this study showed that ~66% of primary DLBCLs had CNAs of multiple p53/cell cycle components (“complex” DLBCLs), whereas only 16% of patients had TP53 somatic mutations. The broader CNA-associated signature provided the genetic basis for increased cellular proliferation and genomic instability, was linked to inferior outcome and amenable to targeted therapy with either CDK inhibitors or bromodomain and extraterminal protein (BET)-inhibitors.
1.2.2. LBCL with distinct histologic features
TCRBCL is a morphologically defined subtype of DLBCL that accounts for <10% of all DLBCLs.1 Patients frequently present with advanced stage disease involving lymph nodes, liver, spleen and bone marrow.1,19,23 This disease has a unique cellular composition and is characterized by scattered malignant large B-cells embedded in small lymphocytes and histiocytes.24 Despite this brisk immune infiltrate, anti-tumor immune responses are ineffective.19

1.2.3. LBCL subtypes with unique sites of involvement
While DLBCLs often involve multiple nodal and extranodal sites, certain LBCL subtypes, including PCNSL, PTL and PMBL present as localized masses in specific extranodal organs.1 PCNSL and PTL are commonly grouped and studied together as DLBCLs occurring in “immune privileged” sites, because the testes and central nervous system (CNS) are considered to be immune sanctuary sites with physiological barriers limiting the access of immune cells.25-27 However, recent data indicates that the physiological barrier between blood and brain or testis is often perturbed in cancer.28,29

1.2.3.1. Primary central nervous system lymphoma
PCNSL accounts for <1% of all NHLs and 2-3% of all brain tumors.1 PCNSL primarily occurs in elderly patients and often presents as an infiltrative mass of Epstein-Barr Virus (EBV) negative tumor cells.29,30 EBV positive PCNSLs are seen infrequently in immunocompetent patients.29 PCNSLs have a phenotype consistent with GC exposed B-cells and have an angiotropic growth pattern, where lymphoma cells accumulate around blood vessels, possibly disrupting the integrity of the blood-brain barrier.29,31 Current first-line treatment of PCNSLs consists of high-dose methotrexate, often in association with rituximab or other agents.29 Nonetheless, nearly 50% of patients with PCNSL relapse within 2 years of diagnosis and one third of patients have primary refractory disease.29

1.2.3.2. Primary testicular lymphoma
PTL accounts for 1-2% of all NHLs and 3-9% of testicular cancers and is the most common testicular malignancy in men older than 60 years.28 Most patients present with localized disease, but the disease often relapses to additional extranodal sites, such as the CNS, skin, pleura and contralateral testis.28 PTLs are currently treated with R-CHOP chemotherapy. However, almost 50% of patients with PTL progress following induction therapy, frequently with CNS or contralateral testicular involvement.28,32
1.2.3.3. **Genetic alterations in PCNSL and PTL**

Subsets of genetic alterations that are described in DLBCL are also found in PCNSL and PTL, although they occur with different frequencies.\(^{25-27,33,34}\) In previous array comparative genomic hybridization studies, loss of 9p21.3, containing the tumor suppressor gene *CDKN2A*, has been described, as well as gains of 19q13 in PTL.\(^{26,33}\) In addition, by qPCR and Sanger sequencing, frequent somatic mutations in *MYD88* and *CD79B* were found in PCNSL and PTL (Figure 2).\(^{27,34}\) Furthermore, losses of the human leukocyte antigen (HLA) loci and gains of 9p24.1 have been described in PCNSL and PTL, see section 1.4.\(^{25,26}\)

1.2.3.4. **Primary Mediastinal Large B-cell Lymphoma**

PMBL accounts for 2-4% of NHLs and occurs predominantly in young adults.\(^1\) Patients present with a localized bulky mediastinal mass. PMBL is thought to originate from post-GC thymic B-cells in the mediastinum.\(^{35}\) PMBL is a distinct LBCL subtype that differs from DLBCL, but shares certain clinical and genetic features with classical Hodgkin lymphoma (cHL, see section 1.3). CN gain of chromosome 2p16.1, containing the NF-κB transcription factor, *REL*, and chromosome 9p24.1 gains have been shown in 50% and 65% of PMBLs, respectively.\(^{36-38}\) The 9p24.1 locus contains a number of genes, of which the demethylase *JMJD2C*, *JAK2* and the PD-1 ligands: *CD274 (PD-L1)* and *PDCD1LG2 (PD-L2)* have been identified as putative driver alterations of this genetic lesion.\(^{36,37}\) (see also section 1.4.).

1.3. **Hodgkin Lymphoma**

HL accounts for ~30% of all lymphomas and is derived from B-cells that share characteristics with antigen-exposed mature GC B-cells.\(^{39}\) This disease is defined by small numbers of malignant cells within an extensive immune cell infiltrate.

1.3.1. **Clinical features of HL**

HL is classified into cHL and nodular lymphocyte-predominant HL. Ninety-five percent of all HLs are cHL and this group is further subdivided into the morphologic subtypes: nodular sclerosis, mixed cellularity, lymphocyte-depleted and lymphocyte-rich.\(^1,39\) PMBL and the nodular sclerosis subtype of cHL have similar clinical profiles, mainly affecting adolescent and young adults.\(^1\) EBV infection is found in about 40% of cHL patients, most frequently in the mixed cellularity and lymphocyte-depleted subtypes.\(^{39}\) Risk stratification and associated treatment decisions are made on the Ann Arbor staging system, that is also used in NHLs. Patients are grouped into early stage I/II disease or advanced stage III/IV disease (Table 1). Early stage patients are further subdivided into favorable and unfavorable risk groups by the presence or absence of unfavorable factors.\(^{40}\) The latter includes: bulky disease either by a
mediastinal mass ratio of greater than 0.33 or a single node or nodal mass that is 10 cm or greater in diameter and the presence of B symptoms that include unexplained weight loss of more than 10% of body weight, unexplained fevers or drenching night sweats. In advanced stage patients, the International Prognostic Score helps to predict prognosis and is defined by the number of adverse prognostic factors: age ≥45, male, serum albumin <40 g/L, hemoglobin <10.5 g/dL, stage IV disease, leukocytosis and lymphocytopenia.

The most commonly used chemotherapy regimen in cHL is ABVD, which is a twice-weekly regimen of doxorubicin (Adriamycin), bleomycin, vinblastine, and dacarbazine. The weekly alternating Stanford V regimen, which consists of doxorubicin, vinblastine, mechlorethamine, vincristine, bleomycin, etoposide and prednisone, is equally effective. These chemotherapy regimens are often given in combination with involved field radiation. Generally, patients respond very well to these regimens, however, treatment failure is still seen in about 10%; 20-30% of patients with recurrent disease respond, but relapse after initial treatment. The treatment for patients with relapsed/refractory disease consists of high-dose chemotherapy followed by an autologous stem cell transplant (ASCT). Also, an immunotoxin directed against CD30 on malignant Hodgkin Reed Sternberg (HRS) cells, brentuximab vedotin (BV), has demonstrated efficacy in relapsed/refractory disease. A recent study showed that a subset of relapsed/refractory cHL patients treated with BV have stayed in remission for 5 years.

1.3.2. Genetic alterations in cHL
Frequent genetic alterations found in HRS cells involve family members of the JAK-STAT and NF-κB pathways. REL amplifications are described in 50% of cHLS and mutations in several other members of the NF-κB pathway are also reported, resulting in constitutive NF-κB activity in HRS cells. A striking shared genetic feature of cHL and PMBL is CN gain of chromosome 9p24.1 with increased expression of JAK2 and the PD-1 ligands, PD-L1 and PD-L2 (see section 1.4.).

<table>
<thead>
<tr>
<th>Stage</th>
<th>Definition</th>
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<tbody>
<tr>
<td>I</td>
<td>Involvement of a single lymph node region or lymphoid structure</td>
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<tr>
<td>II</td>
<td>Involvement of two or more lymph node regions on the same side of the diaphragm</td>
</tr>
<tr>
<td>III</td>
<td>Involvement of lymph node regions or structures on both sides of the diaphragm</td>
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<tr>
<td>IV</td>
<td>Involvement of extranodal sites</td>
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Table 1. Ann Arbor staging system
1.4. Role of the immune system in lymphoid malignancies

1.4.1. Normal immune response

A key event for an effective immune response is the expansion of effector T-cells after recognition of a target antigen. This requires the formation of a specific interaction between antigen presenting cells (APCs) and a T-cell. Major histocompatibility complex (MHC) proteins bind to foreign antigens and present them on the cell surface. In humans, MHC proteins are also referred to as HLA molecules. There are two classes of MHC molecules; MHC class I and MHC class II. MHC class I is a trimolecular complex consisting of a heavy chain, peptide antigen and the \( \beta_2 \)-microglobulin (\( \beta_2 \text{M} \)) light chain, which is necessary for the correct cellular sorting of MHC class I to the plasma membrane (Figure 3A). The MHC class I complex is present on the plasma membrane of all nucleated cells and plays an important role in the presentation of antigenic peptides derived from the degradation of endogenous proteins. After degradation by the proteasome, the antigens are loaded onto MHC class I molecules in the endoplasmatic reticulum (ER), after which the complex is transported to the cell membrane (Figure 3A).\(^{55}\) CD8\(^+\) cytotoxic T-cells recognize these peptides and destroy the target cell that presents them.\(^{55}\)

Exogenous proteins are degraded by endolysosomal enzymes and loaded onto MHC class II molecules (Figure 3B). These MHC class II molecules have been assembled in the ER and are transported to a special endosome, often referred to as the MHC class II compartment. Here, class II-associated invariant chain peptide (CLIP) is removed after which antigen can bind.\(^{55}\) Transcription of the MHC class II locus is controlled by a multi-protein complex containing CIITA, the master regulator of MHC class II expression.\(^{56}\)

Recognition of the antigen presented by MHC class II to CD4\(^+\) T-cells can result in activation of CD4\(^+\) T helper (T\(_H\)) cells, which, in turn, assist in activating T- and B-cells.\(^{57}\) T\(_H\) cells are generally classified into T\(_H\)\(^1\) and T\(_H\)\(^2\) lineages. T\(_H\)\(^1\) cells produce IFN-\( \gamma \) and are important for cellular immunity, while T\(_H\)\(^2\) cells produce IL-4, IL-5 and IL-13 and play an important role in humoral immunity and allergic responses.\(^{58}\) Additional CD4\(^+\) T-cell subsets, including T\(_H\)\(^{17}\), critical for protection against extracellular bacteria, T\(_{reg}\)\(^{61}\) regulating immune tolerance, and T\(_{FH}\) cells, important for selection of B-cells (see section 1.1), can be activated, depending on the cytokine milieu.\(^{58}\) In addition to T\(_H\) subsets, the presence and activation of CD4\(^+\) cytotoxic T-cells has been shown in the context of viral infection and anti-tumor immune responses.\(^{59-61}\) These cytotoxic CD4\(^+\) T-cells may directly kill the target cells presenting the antigen.\(^{59-61}\)
The interaction between T-cells and APCs is a balanced interplay between positive and negative signals. T-cells contain co-stimulatory and co-inhibitory receptors that direct T-cell function and determine T-cell fate following recognition of peptide-MHC complexes. These co-stimulatory and co-inhibitory signals are required to clear pathogens, while maintaining immune tolerance. CD28, a well-characterized co-stimulatory receptor, binds the co-stimulatory molecules, B7-1 (CD80) and B7-2 (CD86). This interaction results in expansion and differentiation of T-cells.

On the other hand, T-cell activation induces the expression of co-inhibitory receptors, such as CTLA-4 and PD-1. CTLA-4 is a cell surface molecule with homology to CD28. CTLA-4 also binds to CD80 and CD86 by which it can out-compete binding to the CD28 receptor, thereby serving as an important negative regulator. CTLA-4 knockout mice have an immense expansion of autoreactive T-cells, resulting in lymphoproliferative disease and death 3 to 4 weeks after birth.
PD-1 is a CD28 family member expressed on activated T-cells, B-cells, and myeloid cells. The PD-1 ligands, \textit{PD-L1} and \textit{PD-L2}, engage the PD-1 receptor on T-cells, inducing PD-1 signaling. Upon binding of the ligands to the receptor, the PD-1 cytoplasmic domain is phosphorylated and phosphatases, especially SHP2, are recruited. Subsequently, TCR proximal signaling molecules are dephosphorylated, leading to reversible inhibition of T-cell proliferation and activation, a state called T-cell exhaustion (Figure 4).\textsuperscript{67,68} The physiological role of this signaling pathway is to downregulate the immune response after elimination of disease and to limit the strength of an immune response to protect tissues from immune-mediated tissue damage.\textsuperscript{67}

1.4.2. Development of lymphomas in immunodeficient patients

The importance of an effective immune response to prevent lymphoma growth is demonstrated by the development of lymphoid or plasmacytic proliferations as a consequence of acquired immunodeficiency. These lymphomas occur predominantly in the setting of infection with the Human Immunodeficiency Virus (HIV) or following immunosuppressive therapy administered after organ transplantation\textsuperscript{1}. Immunodeficiency-related lymphoproliferative disorders (LPDs) are organized according to the background in which they arise or their immunodeficiency setting and form a heterogeneous group of diseases.\textsuperscript{1} The most common HIV-associated lymphomas include Burkitt lymphoma, DLBCL, primary effusion lymphoma, plasmablastic lymphoma and cHL.\textsuperscript{1} Post-transplant proliferative disorders (PTLDs) comprise a spectrum of B-cell proliferations, often indistinguishable from the subset of B-cell lymphomas occurring in immunocompetent individuals and include DLBCL,
Burkitt lymphoma, plasma cell myeloma, plasmacytoma-like lesions and cHL. The manifestations of these LPDs are highly variable. LPDs are frequently driven by viruses such as EBV, although other pathways might be involved in the pathogenesis as well.

1.4.3. Genetic bases of immune evasion in lymphoid malignancies
Emerging data suggests that certain lymphoid malignancies rely upon multiple genetic mechanisms to avoid an effective anti-tumor immune response.

1.4.3.1. Impairment of antigen presentation
One strategy for effective immune evasion is the impairment of antigen presentation by downregulation of MHC genes on the cell surface of tumor cells, resulting in less or no recognition of tumor cells by immune cells. Genetic losses of the MHC loci at 6p21 have been reported in a subset of systemic DLBCLs. Loss of MHC class II gene and protein expression has been associated with decreased tumor immunosurveillance and poor patient outcome in DLBCL. In addition, reduced expression of β₂M, caused by mutations or CN losses in the B2M gene, has been reported in both ABC- and GCB-type DLBCLs, and associated with reduced or complete loss of cell surface MHC class I expression. While loss of MHC class I and II might result in increased recognition of tumor cells by the innate immune system, it is of note that DLBCL harbors frequent mutations and CN loss of CD58. CD58 is the ligand for the CD2 receptor on T-cells and most natural killer (NK) cells and is required for their adhesion and activation. Thus, recognition of tumor cells by both NK cells and cytotoxic T-cells is impaired in a subset of DLBCLs.

PTLs and PCNSLs also harbor losses of both the MHC class I and MHC class II loci in more than half of the patients, resulting in lack of MHC expression. In addition, a small subset of the PCNSL and PTL patients exhibit B2M alterations.

In PMBL and cHL, decreased expression of MHC class II has been associated with an inferior survival. Translocations of the MHC class II transactivator, CIITA, contribute to this downregulation. In addition, loss of MHC class I expression has been shown in cHL; in a small set of flow-sorted HRS cells, B2M mutations were seen in 7/10 (70%) of the cHL patients and this was associated with loss of MHC class I expression.

1.4.3.2 PD-1 ligand deregulation
As previously mentioned, the PD-1 pathway plays an important role in T-cell exhaustion. Tumor cells can utilize this pathway to evade an effective immune
response. The underlying genetic mechanism for overexpression of the PD-1 ligands includes chromosome 9p24.1 genetic alterations, which contains the \( PD-L1 \) and \( PD-L2 \) loci (Figure 5). These CN alterations increase expression of the PD-1 ligands on tumor cells, which in turn, bind to the PD-1 receptor on T- cells and thereby induce a state of T-cell exhaustion (Figure 4, see section 1.4.1.). Additionally, the 5’ region of \( PD-L1 \) includes an interferon (IFN)–stimulated regulatory element/IFN-regulatory factor 1 (ISRE/IRF1) module and several degenerate STAT-binding sites. For this reason, activation of the JAK2/STAT pathway by CN changes of the 9p24.1 locus (Figure 5) causes further upregulation of PD-L1 expression.\(^{36}\)

EBV infection is another mechanism to upregulate PD-L1 expression.\(^{82}\) The EBV latent membrane protein 1 (LMP1) activates JAK-STAT signaling\(^{83}\), thereby increasing PD-L1 promoter activity. In addition, LMP1 triggers activity of the AP-1 component, cJUN.\(^{84}\) Tandem AP-1 binding sites have been described in the \( PD-L1 \) enhancer, and as a consequence, AP-1 components can increase PD-L1 expression.\(^{82}\)

### 1.5. Immune checkpoint blockade in lymphoid malignancies

Understanding the strategies that tumor cells use to evade an effective immune response may help guide targeted therapies. As noted above, the immune checkpoints CTLA-4 and PD-1 function to inhibit T-cell responses. Tumor cells hijack these physiological mechanisms to evade an effective anti-tumor immune response. Recent development of drugs that target immune checkpoint pathways has led to success in multiple hematological malignancies and solid tumors. CTLA-4 was the first immune checkpoint to be clinically targeted and showed clinical benefit in multiple tumor types.\(^{85-92}\)

The initial successes of CTLA-4 blockade have led to the search for drugs targeting other immune checkpoints. A number of antibodies targeting the PD-1 pathway have been developed.\(^{93}\) In solid tumors, targeting this pathway with PD-1 or PD-
L1 blocking antibodies resulted in durable responses and an acceptable safety profile.\textsuperscript{94-96} This led to studies evaluating anti-PD-1 as a therapeutic strategy in hematologic malignancies.\textsuperscript{97-99} Of great interest, a phase I/II study of anti-PD-1 with Nivolumab in 23 patients with heavily pretreated relapsed/refractory HL showed an overall response rate of 87%.\textsuperscript{100} In this study, all (10/10) evaluable patients had genetic alterations of 9p24.1/PD-L1/PD-L2 that resulted in increased expression of the PD-1 ligands.\textsuperscript{100} In a second phase Ib study using an independent antibody, Pembrolizumab, in relapsed/refractory HL, a similar response rate of 65% was reported.\textsuperscript{101}

1.6. Outline and aims of this thesis
The inferior responses to current empiric treatment regiments in PCNSL and PTL (see section 1.2.3.1. and 1.2.3.2.), prompted us to perform comprehensive comparative and integrative genomic analyses in these lymphoma subtypes to identify potentially targetable genetic alterations (chapter 2).\textsuperscript{102} These studies led to the discovery that the LBCL subtypes, PCNSL, PTL, PMBL and cHL, share the same targetable genetic feature; frequent CN gains of chromosome 9p24.1/PD-L1/PD-L2 and increased PD-1 ligand expression.

The genetic basis for PD-1 ligand overexpression in cHL might explain the high response rates of cHL to PD-1 blockade\textsuperscript{100} and for this reason, we developed a fluorescent \textit{in situ} hybridization (FISH) assay and examined 9p24.1/PD-L1/PD-L2 genetic alterations and PD-1 ligand expression by immunohistochemistry (IHC) in additional lymphoid malignancies, including TCRBCL (chapter 3) and immunodeficiency-associated LPDs (chapter 4)\textsuperscript{103} and showed that these lymphoid malignancies harbor frequent PD-1 ligand deregulation.

Given the compelling response rates of relapsed/refractory cHL to PD-1 blockade (see section 1.5.), we focused our next line of investigation on characterizing the incidence, nature and prognostic significance of 9p24.1/PD-L1/PD-L2 genetic alterations using FISH and associated PD-1 ligand expression using IHC in a uniformly treated cohort of primary cHLs with long-term follow-up (chapter 5).\textsuperscript{104} We demonstrated that 9p24.1/PD-L1/PD-L2 genetic alterations are a near-universal feature of cHL and that 9p24.1/PD-L1/PD-L2 amplification is associated with inferior progression-free survival (PFS) following standard induction therapy. We then examined the prevalence and type of 9p24.1/PD-L1/PD-L2 genetic alterations and PD-L1 expression and its association with best overall response (BOR) and PFS in patients who received PD-1 blockade using Nivolumab for relapsed/refractory cHL (chapter 6).\textsuperscript{105} Patients with high-level 9p24.1/PD-L1/PD-L2 genetic alterations and
increased PD-L1 expression had significantly better overall responses to Nivolumab and longer PFS.

In addition to frequent PD-1 ligand deregulation, a study in a small cohort of cHLs report frequent loss of β2M/MHC class I in cHL.\textsuperscript{81} We characterized the incidence and prognostic value of these potentially competing immune evasion strategies using FISH and IHC in a uniformly treated cohort of primary cHL patients. We explored protein expression of the antigen presentation components, β2M, MHC class I and MHC class II, in the primary cHL cohort with long-term follow-up and defined 9p24.1/PD-L1/PD-L2 genetic alterations (\textit{chapter 7}).\textsuperscript{106} In this study, we showed frequent impairment of β2M/MHC class I and/or MHC class II expression in cHL patients. Decreased/absent β2M/MHC class I expression was associated to a shorter PFS, independent of 9p24.1/PD-L1/PD-L2 genetic status.

In \textit{chapter 8}, the results of these studies are summarized and discussed, clinical implications are highlighted and future directions are outlined.

References


64. van der Merwe PA, Bodian DL, Daenke S, Linsley P, Davis SJ. CD80 (B7-1) binds both CD28 and CTLA-4 with a low affinity and very fast kinetics. *J Exp Med*. 1997;185(3):393-403.


74. Reichel J, Chadburn A, Rubinstein PG, et al. Flow sorting and exome sequencing reveal the oncogene of


