The molecular pathogenesis of primary mediastinal large B cell lymphoma

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Running head: Molecular pathogenesis of PMBCL

Keywords: Primary mediastinal large B cell lymphoma, molecular pathogenesis

Scientific Category: Lymphoid Neoplasia

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Abstract

Primary mediastinal large B cell lymphoma (PMBCL) is a recognized non-Hodgkin lymphoma entity with unique pathological, clinical and molecular characteristics distinct from those of other diffuse large B cell lymphomas. Immunohistochemical characterization and molecular studies strongly suggest that PMBCL is of germinal center or post-germinal center origin. Pivotal gene expression profiling work defined major deregulated pathway activities that overlap with Hodgkin lymphoma and prompted a more detailed analysis of candidate genes. In particular, the nuclear factor kappa B (NFκB) and the Janus Kinase-Signal Transducer and Activator of Transcription (JAK-STAT) signaling pathways are targeted by multiple genomic hits and constitutive activity of both pathways can be considered molecular hallmark alterations of PMBCL. Moreover, data are emerging giving unique insight into remodeling of the epigenome that affects transcriptional regulation of a multitude of genes. More recently, the tumor microenvironment of PMBCL has shifted into focus based on a number of gene perturbations altering expression of surface molecules that contribute to immune escape. These findings highlight the importance of immune privilege in the pathogenesis of PMBCL and suggest that disrupting crosstalk between the tumor cells and the microenvironment might be a rational new therapeutic target in conjunction with traditional treatment strategies.
Introduction

Primary mediastinal large B cell lymphoma (PMBCL) represents approximately 2-4% of all non-Hodgkin lymphomas.\textsuperscript{1} PMBCL is now a fully recognized entity in the World Health Organization (WHO) classification of lymphoid neoplasms. Previously, in the revised European-American classification of lymphoid neoplasms (REAL)\textsuperscript{2} and frequently in clinical practice, PMBCL was considered a subtype of diffuse large B cell lymphoma (DLBCL), arising in the mediastinum, reflected in study designs that did not distinguish PMBCL from DLBCL patients in earlier clinical trials. In 2001 the WHO classification listed PMBCL as a distinct entity. Currently, with distinct clinical and immunophenotypic features, and recent molecular characterization of PMBCL, there is now conclusive evidence that PMBCL represents a distinct clinicopathological entity.

Distinguishing PMBCL from other forms of DLBCL can be viewed as a paradigm for reclassification of cancer subtypes based on molecular features, as the molecular characterization of PMBCL highlighted an overlap with the nodular sclerosis (NS) subtype of classical Hodgkin lymphoma (cHL) sharing a number of genetic and gene expression features\textsuperscript{3,4}. Interestingly, this association had been long suspected based on similarities in clinical features and reports of sequential or composite occurrences of cHL and PMBCL\textsuperscript{5,6}. Functional analyses of both lymphomas confirmed that the malignant cells rely on the same principal signaling pathways, namely JAK-STAT and NFκB signaling, both providing these cells with a proliferative advantage\textsuperscript{7-9}. However, more recent lines of evidence also suggest that acquired immune privilege by the malignant cells contributes to
the cancer phenotype of PMBCL in a cooperative way.\textsuperscript{10,11} The specific composition of the inflammatory infiltrates discovered through histological study of primary PMBCL biopsies further supports the significance of the interactions of the malignant cells with the surrounding non-neoplastic immune cells. Unraveling the underlying genetic mechanism(s) of these tumor-microenvironment interactions might be important for future therapies targeting the immune privilege phenotype of the malignant cells.

From a clinical point of view, PMBCL patients treated with multi-agent chemotherapy appear to have a better survival compared to DLBCL based on retrospective analyses.\textsuperscript{12,13} However, the intensity of primary chemotherapy, the role of radiotherapy and the effectiveness of added rituximab are still the subject of debate and definitive conclusions are hampered by the paucity of prospective studies. Equally, meaningful biomarker studies to inform risk stratification and outcome prediction of PMBCL are largely unavailable.

With a number of important biological insights in PMBCL reported in the last 12 months, a critical review of the molecular pathogenesis of this disease was warranted. We will first focus on the pathological and clinical features of PMBCL to define in detail the disease entity in comparison to related lymphoid cancers. We will then characterize the genetic alterations and deregulated signaling pathways that underlie the pathogenesis and review the recent data that highlight the importance of the microenvironment. Finally, we will pay special attention to the potential synergy of the underlying genetic alterations that
cooperate to develop the full malignant phenotype, and briefly outline avenues for clinical translation of these novel findings.

**Pathology and Clinical features**

PMBCL characteristically presents with a bulky anterior mediastinal mass and affects predominantly female patients in their third or fourth decade of life (Figure 1). PMBCL can also occur in children and adolescents showing similar clinical and pathological characteristics with adult cases. In contrast to DLBCL, the bone marrow is rarely involved and the tumor extension is typically local, with pleural, pericardial effusions and superior vena cava obstruction as typical clinical complications in a significant number of patients. Systemic involvement beyond the thorax and Ann Arbor stage III are uncommon at initial diagnosis and the absence of infradiaphragmatic lymph node or bone marrow involvement was recommended as a prerequisite for diagnosis of PMBCL in the WHO classification, to exclude systemic DLBCL with secondary mediastinal involvement. However, particularly at relapse, the disease has a very high incidence of extranodal spread with a tendency to spare lymph nodes. Prospective clinical trials specifically enrolling PMBCL are lacking in the literature, in part because of small patient numbers and the recent recognition of PMBCL as a distinct entity. Therefore outcome studies and treatment recommendations are based on retrospective studies or subgroup analysis of randomized trials of DLBCL. As relapses after first-line therapies usually occur early and salvage therapies have been reported to have high failure rates,
successful primary treatment has been considered critical. In particular, more dose-intense regimens have been suggested and shown to be effective, especially in comparison to DLBCL\textsuperscript{12,19,20}. Consolidating radiation therapy has also been studied in non-comparator studies.\textsuperscript{21} However, it remains to be determined if the addition of rituximab immunotherapy is making radiation and high-dose therapies obsolete. Very recent reports of combining rituximab with dose-adjusted EPOCH have shown very promising results\textsuperscript{20}, and equally, PMBCL subgroup analysis of a randomized DLBCL trial (Mabthera International Trial group study/MINT) showed increased response rates and event-free survival in patients receiving rituximab that was similar to the benefit seen in DLBCL patients.\textsuperscript{13}

Of note, most of the prognostic factors that were established in DLBCL proved to be of limited value in PMBCL and individual clinical parameters such as poor performance status or elevated LDH have been inconsistently reported to be associated with treatment failure.\textsuperscript{12,22} Importantly, the age-adjusted IPI did not predict treatment outcome in most series.\textsuperscript{12,17} This lack of prognostic factors highlights the need for molecular biomarkers and an improved understanding of the underlying pathobiology.

Although PMBCL is a distinct clinicopathologic entity, the histomorphological features and phenotype often varies and underscores the relatedness to other B cell lymphomas, namely DLBCL and nodular sclerosis cHL.\textsuperscript{1} The histological appearance of PMBCL is characterized by sheets of medium-large sized cells containing abundant pale cytoplasm resembling other
large B cell lymphoma subtypes. Occasionally some neoplastic cells are multinucleated and resemble Hodgkin Reed Sternberg cells, and a fine background sclerosis, distinct from broad collagen bands seen in NS cHL, often leads to compartmentalization of the malignant cells (Figure 1). This relatedness of HL and PMBCL is also reflected by the revised WHO classification recognizing mediastinal grey zone lymphomas (GZL) that are defined as unclassifiable B cell lymphomas with features intermediate between DLBCL and cHL\textsuperscript{1,23}. Although histopathologic and genetic data are limited, recent molecular studies confirm the transitional nature of mediastinal GZL existing in the borderland between PMBCL and cHL\textsuperscript{24-26}. Figure 1 highlights the most prominent clinical, morphological and molecular features recognized in PMBCL, cHL, DLBCL and mediastinal GZL demonstrating the biological continuum of these B cell lymphomas. However, the main focus of this review is PMBCL and reference to the related entities will only be made if relevant to the molecular pathogenesis of PMBCL.

Based on immunophenotypic data and mutational analysis of various loci that are typical targets of somatic hypermutation (SHM), PMBCL has been suggested to be of germinal center or post-germinal center origin.\textsuperscript{27,28} The B cell immunophenotype shows general positivity for surface CD19, CD20, CD22, CD79a and frequent nuclear positivity of the transcriptional regulators BOB1, PU.1, OCT2, PAX5, BCL6 and IRF4\textsuperscript{27,29}. However, the mature B cell phenotype is incomplete as HLA class I and II molecules are expressed at varying levels and are often completely lacking and, equally, surface immunoglobulin (Ig) expression is frequently absent.\textsuperscript{30-32} Of note, most PMBCL cases also stain
positively for CD30 but the staining intensity is often weaker compared to HL or anaplastic large cell lymphoma\textsuperscript{33,34} (Figure 1). The malignant cells typically lack expression of CD15 and do not harbor latent EBV. SHM of the immunoglobulin (\textit{IG}) genes and \textit{BCL6} are considered reliable markers indicating transit of a B cell through the germinal center (GC) and, in agreement with the proposed (post-) germinal center origin of PMBCL, isotype-switched \textit{IG} genes and \textit{BCL6} gene mutations unequivocally show the pattern of SHM.\textsuperscript{28,35,36} Of note, the spatial distribution and targeted motifs of hypermutation in the \textit{BCL6} 5\` non-coding region were found to be distinct from DLBCL and FL suggesting specificity of the SHM process and a distinct clonal evolution in PMBCL. However, the significance of this finding remains unclear.\textsuperscript{37} Interestingly, activation-induced cytidine deaminase (\textit{AID}), a gene that plays a key role in SHM and class switch recombination (CSR), has been shown to be constitutively expressed in PMBCL and the PMBCL-derived cell line KARPAS-1106P\textsuperscript{38} showed ongoing SHM, but not CSR, involving \textit{IG} genes and \textit{BCL6}.\textsuperscript{39} Although, taken together, these data are consistent with a germinal center origin of PMBCL and migration into the thymus, another potential explanation is that PMBCL is derived from AID-expressing non-germinal center B lymphocytes residing in the medullary thymus.\textsuperscript{40}

**Genetic alterations and deregulated signaling pathways**

Since recognition of PMBCL as a distinct entity, the number of studies describing the specific molecular features and genetic underpinnings of the
disease is increasing. While earlier studies used target gene approaches, seminal genome-wide gene expression profiling work defined major deregulated pathway activities and boosted investigation of candidate genes, some of which overlapped with cHL. Table 1 summarizes the most recurrent gene alterations involved in the pathogenesis of PMBCL. Among the first studied genes was CDKN2A (p16/INK), which was found to be mutated or hypermethylated in a small number of cases. Other gene alterations encompass MYC rearrangements, MYC promoter sequence variations, which might be due to SHM, and coding sequence mutations in TP53. In contrast to DLBCL, BCL6 translocations are only sporadically reported. Of importance, genetic heterogeneity in some of these older studies almost certainly reflects the inclusion of cases of typical DLBCL with secondary mediastinal lymph node localization that may have little in common with PMBCL.

NFκB signaling pathway

The nuclear factor kappa B (NFκB) signaling pathway has been described as one of the most important dysregulated pathways in the malignant pathogenesis of B cell lymphomas, and of cHL and PMBCL in particular (for detailed review of HL see ref. 7). In normal B cell development the activity of the NFκB transcription factor complex is influenced and regulated by several extrinsic and intrinsic factors, of which stimulation through tumor necrosis factor (TNF) receptors play a major role for pathway activation (Figure 2). In PMBCL, early gene expression profiling (GEP) studies revealed that several genes of the
NFκB signaling pathway such as TNF family members, TNF receptor associated factors and NFκB complex members were overexpressed compared to DLBCL and that this gene expression pattern more closely resembled cHL\textsuperscript{3,41}. Subsequent studies confirmed overexpression of an NFκB activation signature and nuclear localization of NFκB transcription factor complexes in PMBCL-derived cell line KAPRAS1106P in comparison to the activated B cell (ABC) and germinal center B (GCB) type DLBCL\textsuperscript{9}. Moreover, small molecule inhibition of the IκB kinase (IKK) complex, which functions as an activator of NFκB signaling, was toxic to KARPAS1106P cells demonstrating dependence on this pathway\textsuperscript{44}. The underlying genetic causes for this overexpression and pathway activation are not yet fully understood. More recently, specific gene mutations and structural alterations of pathway genes have been described leading to constitutive activity. Chromosomal gains and amplifications of the REL gene locus on chromosome 2p16.1 have been consistently found in 50% or more cases using array comparative genomic hybridization (CGH) and fluorescence in-situ hybridization\textsuperscript{45-47}. Although amplification of the REL gene locus did not correlate with elevated mRNA levels, genomic amplification was associated with nuclear localization of REL protein indicating increased pathway activity in these cases. Other chromosomal imbalances involving NFκB regulators encompass amplification of BCL\textit{10} (1p22) and MALT\textit{1} (18q21)\textsuperscript{46}.

Very recently, TNFAIP3 (encoding A20) has been reported as a novel tumor suppressor gene in HL and PMBCL. The A20 protein acts as a ubiquitin-modifying enzyme that inhibits NFκB signaling downstream of TNFR receptor
engagement by interacting with RIP1, TRAF1 and TRAF2. Specifically, A20 adds polyubiquitins to RIP1 (NFκB essential modulator) targeting it for proteasomal degradation and thereby negatively regulates the IκB kinase (IKK) complex and NFκB activity. Destructive and biallelic gene mutations were found in 36% of PMBCL cases leading to constitutive NFκB activity. One study included cell line KARPAS 1106P that was found to be null for A20 protein expression and, importantly, earlier array CGH study of primary PMBCL cases revealed chromosomal deletion of the genomic TNFAIP3 locus on chromosome 6q23.3 in approximately 30% of patients. These data provide further evidence for complete inactivation of the gene in a significant proportion of cases. Somatic TNFAIP3 mutations and/or chromosomal 6q deletion have also been described in several other B cell malignancies such as mantle cell lymphoma (MCL), ABC type DLBCL, marginal zone B cell lymphoma (MZL), mucosa-associated lymphoid tissue (MALT) lymphomas and AIDS-related B cell lymphomas, highlighting the frequency and overall importance of TNFAIP3 in B cell lymphoma pathogenesis.

Moreover, unlike cHL, NFKBIA mutations were absent in PMBCL, suggesting a different mutational landscape of gene mutations in the two diseases leading to NFκB activation. Genome-wide discovery studies using next-generation sequencing will clarify the mutational landscape in the very near future.

JAK-STAT signaling
Similar to NFkB signaling, the Janus Kinase-Signal Transducer and Activator of Transcription (JAK-STAT) is a major regulator of gene transcription involved in cellular proliferation, apoptosis and angiogenesis and has been implicated in the pathogenesis of multiple malignancies including lymphoid cancers.\textsuperscript{57} In normal B cells the pathway is typically activated by interleukins or interferons binding to a variety of receptors, which lead to auto-phosphorylation of JAK molecules, subsequent STAT phosphorylation and translocation of STAT dimers into the nucleus where they act as DNA-binding transcription factors (\textbf{Figure 2}). In PMBCL, GEP revealed high levels of IL13 receptor expression and downstream effectors such as JAK2 and STAT1, indicating up-regulation of pathway genes.\textsuperscript{3,41,58} Moreover, constitutive STAT6 activation has been identified as a characteristic feature of PMBCL in comparison with DLBCL and, in contrast to cHL, this is not likely due to autocrine interleukin (IL-4, IL-13) production.\textsuperscript{8,59}

Genomic amplification of a subtelomeric region of chromosome 9 (9p24.1) involving several genes including JAK2 has been consistently reported in more than half of cases and can be considered as one of the hallmark genetic alterations in PMBCL.\textsuperscript{10,46,60,61} (\textbf{Table 1}) In these high-resolution copy number studies a minimally amplified region of approximately 3.5 Mb could be identified leading to co-amplification of the JAK2, CD274, PDCD1LG2 and JMJD2C genes that are likely to cooperate in PMBCL pathogenesis (\textbf{Table 2}). Wessendorf \textit{et al} further delineated a high-level amplicon of approximately 1.6Mb harboring the aforementioned genes.\textsuperscript{46} Focusing on JAK-STAT signaling, Green and
colleagues demonstrated a significant correlation between JAK2 genomic gains and mRNA expression\textsuperscript{10}.

Furthermore, $SOCS1$ (suppressor of cytokine signaling 1) has been identified as a recurrently mutated tumor suppressor gene in PMBCL. SOCS1 can be viewed as the key effector of a classical negative feedback loop in which expression of SOCS1 is induced by activated STATs, inhibits JAK phosphorylation and targets phosphorylated JAK for proteasomal degradation.\textsuperscript{62} In the MedB-1 mediastinal B cell line\textsuperscript{63} biallelic $SOCS1$ mutations were found abrogating the SOCS box function of the protein and leading to constitutive phosphorylation of JAK2 and STAT5.\textsuperscript{64} This constitutive pathway activation was abolished by ectopic wildtype overexpression of SOCS1 in MedB-1 cells demonstrating the tumor-suppressor gene function of SOCS1 in a PMBCL context. In a study of 20 patients with PMBCL, $SOCS1$ deletion mutations were detected in 45\% of cases. Notably, $SOCS1$ mutations and genomic JAK2 amplification were not mutually exclusive, suggesting an additive effect of these two alterations. Interestingly, $SOCS1$ mutations have been found to be consistent with aberrant SHM in nodular lymphocyte-predominant HL, PMBCL, and other germinal center-derived lymphomas. However, further study of the mutational patterns, specifically in PMBCL, is needed.\textsuperscript{65,66} Additionally, a copy number study revealed homozygous deletion of the $SOCS1$ locus in cell line KARPAS 1106P with similar functional consequences as in MedB-1.\textsuperscript{67} However, recurrent copy number losses of the $SOCS1$ locus could not be definitively detected by aCGH in a study of 37 PMBCL patients.\textsuperscript{46}
Recurrent somatic mutations of \textit{STAT6} have been described in 36% of PMBCL cases, contrasting with DLBCL, in which no mutations could be detected, adding further evidence that the JAK-STAT signaling pathway is the target of multiple genomic hits, specifically in PMBCL.\textsuperscript{68} Of note, concurrent \textit{SOCS1} gene alterations and \textit{JAK2} gene amplification were not significantly more prevalent in either the \textit{STAT6} mutated or non-mutated cases, further suggesting an additive effect of the signaling pathway hits. Investigating the \textit{STAT6} DNA binding domain, all gene mutations were found to be heterozygous including two mutations in MedB1 that were on the same allele (“CIS” configuration). Moreover, the mutational pattern of predominantly base pair transversions (A $\rightarrow$ C or G $\rightarrow$ T) suggested a genesis other than SHM. However, questions remain on the mechanistic level how the identified mutations in the \textit{STAT6} DNA binding domain contribute to the malignant phenotype. As mutated STAT6 protein appears to have diminished DNA binding properties and decreased ability to activate transcription in HEK293 cells, these results are unexpected given the overall pathway activation characteristic of PMBCL. Furthermore, expression of known STAT6-regulated target genes remained unaltered, which has led to the hypothesis that \textit{STAT6} mutations might be “passenger” mutations.\textsuperscript{69} Additional experiments using alternative \textit{in vitro} and \textit{in vivo} model systems are needed to clarify the functional role of these heterozygous mutations.

Histone-modification
Gene expression regulation and aberrant gene expression by histone modification in normal tissue development and carcinogenesis is increasingly recognized and altered regulation of the histone modification process itself by mutations in key enzymes have been described in the most current literature regarding B cell lymphomas. In PMBCL, specifically JAK2 and JMJD2C have been shown to be key players in histone 3 modifications essential to tumor cell proliferation and survival using an RNA interference screen. This finding was unique to PMBCL and HL cell lines with the 9p24 amplicon compared with DLBCL cell lines without 9p amplification, highlighting the specificity of this observation linked to overexpression of both genes. JMJD2C functions as a demethylase for the trimethylation mark at lysine 9 of histone 3 and impedes the recruitment of HP1α and heterochromatin formation. Furthermore, activated phospho-JAK2 cooperates in this process by phosphorylating tyrosine 41 of histone 3 and phosphorylation of HP1α, thereby inactivating HP1α and further contributing to increased formation of active chromatin. Interestingly, upon RNA interference with JAK2 and JMJD2C, MYC was identified as one of the most regulated genes targeted by altering the chromatin structure of the MYC locus. These studies for the first time give unique insight into remodeling of the PMBCL epigenome and open avenues for further investigations and potentially therapeutic intervention with JAK2 and JMJD2C as potential drug targets.

The tumor microenvironment of PMBCL
The number of lymphoma subtypes for which interactions of the malignant cells with non-neoplastic cells of the microenvironment are described is constantly increasing. However, the underlying genetic events harbored by the malignant cells that influence the immune microenvironment remain largely unknown. Only recently a handful of specific mechanisms have been described in PMBCL. By contrast, the microenvironment in cHL has been extensively studied and serves as a paradigm of tumor-microenvironmental interactions. In the following section we will review the most important microenvironment-related features of PMBCL known to date, including the composition of the cellular infiltrate, overexpression of surface molecules and the underlying genetic changes contributing to an immune escape phenotype of the malignant cells (Figure 3).

Downregulation of HLA class II expression

As a defining feature of the immunophenotype of PMBCL, early studies described reduced expression of major histocompatibility (MHC) class II genes in a substantial number of cases\(^\text{31}\). In subsequent work, decreased expression of MHC class II molecules has been consistently linked to inferior outcome\(^\text{32}\), similar to observations in DLBCL\(^\text{74,75}\) and cHL\(^\text{76}\). Furthermore, MHC class II loss was found to be correlated with a decreased numbers of infiltrating cytotoxic T cells in PMBCL\(^\text{77}\) and a similar correlation was previously described in DLBCL suggesting a possible mechanism of escape from immune surveillance\(^\text{78}\). Specifically, of 92 PMBCL cases, 65% were negative for HLA-DR protein.
Lack of HLA-DR expression correlated significantly with diminished numbers of cytotoxic T cells (CD8+, TIA1+), but not with CD68+ macrophages and both loss of HLA-DR expression and decreased TIA1+ cells were correlated with disease-specific and progression-free survival. In aggregate, these data provide the first evidence that immune escape by malignant cells following downregulation of HLA class II molecules might be an important part of the malignant phenotype. However, the exact functional link between HLA class II loss and reduced numbers of CD8+ cytotoxic T cells still needs to be established, as a CD8+ immune response is usually linked to MHC class I expression. Involvement of T helper cells to assist or modulate the cytotoxic T cell response as the final common pathway of cell-mediated death appears likely. Interestingly, the density of a distinct T cell subset, CD4+CD25+FOXP3+ cells (so-called Treg cells), has been found to have high variability (average FOXP3+ proportion 2.6%) in comparison to other B and T cell lymphomas, warranting further investigations focusing on the role of this T cell subset in the microenvironment of PMBCL.

The cause for downregulation of HLA class II in PMBCL and other lymphomas is still not well understood. Gene expression array data revealed a strong correlation between expression of the master transcriptional regulator of class II expression CIITA (MHC2TA) and HLA class II expression. These data suggest a distinct mechanism compared to immune-privileged site-associated B cell lymphomas, such as testicular or primary central nervous system (CNS) lymphomas, in which copy number loss as a result of deletion at the HLA locus
on chromosome 6p21.3 has been recurrently described in over 60% of cases.\textsuperscript{82} By contrast, in PMBCL, chromosomal loss of a distinct region on 6p21 harboring the MHC class I and II genes has only been seen in a small number of cases.\textsuperscript{46}

More evidence for an important role of \textit{CIITA} in the regulation of MHC class II expression in PMBCL comes from the recent finding of recurrent genomic \textit{CIITA} rearrangements\textsuperscript{11}. Evaluation of 263 B-cell lymphomas by fluorescence \textit{in situ} hybridization (FISH) demonstrated that genomic \textit{CIITA} breaks were recurrent in PMBCL (38%) and cHL (15%), but only rarely found in DLBCL (3%). In PMBCL these breaks were also linked to inferior disease-specific survival. Moreover, \textit{CIITA} was found as a promiscuous gene with various partners resulting in novel in-frame gene fusions and strikingly, in these cases with gene fusions, a cluster breakpoint region of 1.6kb in \textit{CIITA} intron 1 could be identified, disrupting normal \textit{CIITA} transcription of this allele. Interestingly, in some cases \textit{CIITA} breaks were shown to occur in more than one allele raising the possibility that \textit{CIITA} rearrangements might lead to complete loss of function. Of note, \textit{CIITA} coding sequence mutations have been described in a small number of DLBCL\textsuperscript{83}, but the functional consequences remain unclear. The \textit{CIITA} gene was initially found in studies of patients with bare lymphocyte syndrome (BLS, complementation group A), a rare autosomal recessive disease characterized by mutations in \textit{CIITA} leading to loss of MHC class II expression and clinical manifestations due to an immunodeficiency phenotype.\textsuperscript{84} Several groups found that CIITA functions as a transactivator in a complex of transcription factors (RFX, NFY, X2BP) that bind to class II MHC promoters.\textsuperscript{85} Previous studies also
describe deletion mutants that inhibit wild type CIITA function\textsuperscript{86-89}. Consistent with these findings \textit{in vitro} functional experiments in the cHL-derived cell line KM-H2 and DLBCL-derived cell line SUDHL-4 demonstrated that a specific \textit{CIITA} fusion (\textit{CIITA-FLJ27352}) suppresses HLA class II expression\textsuperscript{11}. These data suggest that, in addition to haploinsufficiency of CIITA, MHC class II expression may be suppressed in a dominant-negative manner (Figure 3). In summary, \textit{CIITA} breaks likely lead to loss of MHC class II expression and might explain, in part, the previously identified clinical correlations discussed above.

Overexpression of PD-1 ligands (\textit{PDL1/CD274, PDL2/PDCD1LG2})

The surface molecule PDCD1LG2 (CD273, PDL2) has been identified as a highly overexpressed PMBCL-specific gene by GEP. It is harbored in the common amplicon on chromosome 9p (see above)\textsuperscript{3,41,60} and a direct correlation between 9p copy number changes and mRNA expression of both PD-1 ligands, CD274 and PDCD1LG2, has been demonstrated by integrative analysis\textsuperscript{10}. Both \textit{CD274} and \textit{PDCD1LG2} have also been found as recurrent gene fusion partners of \textit{CIITA}\textsuperscript{11} and both genes are highly expressed under control of \textit{CIITA} promoter III by translocation t(9;16)(p24.1;p13.13) resulting in the formation of in-frame \textit{CIITA-CD274} and \textit{CIITA-PDCD1LG2} gene fusions, respectively.\textsuperscript{90,91} CD274 and PDCD1LG2 belong to the CD28 costimulatory receptor superfamily and regulate T cell activity by providing an additional signal to T cells via the PD1 receptor in conjunction with T-cell receptor signaling (Figure 3).\textsuperscript{92} In DLBCL-derived U2932 cells\textsuperscript{93} that do not express wildtype PD1 ligands, forced expression of the CIITA-
CD274 and CIITA-PDCD1LG2 fusions inhibits activation of Jurkat T cell in co-culture; a mechanism that is mediated by PD-1 receptor signaling. These observations were in agreement with findings using U2940, a cell line with PMBCL features that expresses high wildtype levels of PD-1 ligands, inducing anergy in co-cultured Jurkat T cells. A similar mechanism has been described for cHL, in which PD1 ligand overexpression has been shown to contribute to T cell exhaustion of the infiltrating T cells. These data demonstrate that PD-1 ligands can be overexpressed by either gene amplification or translocation and strongly suggest that in a subset of PMBCL the malignant B cells escape immunosurveillance by over-expression of surface molecules inactivating infiltrating effector T cells. Of note, elevated surface expression of galectin-1 (encoded by LGALS1) has been described in HL influencing the composition of the microenvironment towards increased T helper 2 and T regulatory cells, providing further support for the hypothesis that the malignant cells uniquely orchestrate their microenvironment in certain lymphoma subtypes.

Synergy of alterations and perspective

Similar to other cancers, the pathogenesis of PMBCL is presumably a multi-step process with accumulation of multiple genetic alterations. The JAK-STAT signaling pathway is illustrative, demonstrating the coincidence of multiple pathway hits that very likely cooperate in conferring an additive survival advantage to the malignant cells. However, very recent studies also provide evidence that multiple pathways can be affected by a single underlying event on
the genomic level.\textsuperscript{10,11,61} This process can be viewed as a comparably economical way of cancer cells gaining a survival advantage in contrast to accumulation of multiple independent events.

In the following section we will discuss the simultaneous consequences of unbalanced chromosome 16p13.13 rearrangements and chromosomal gain of the 9p24 amplicon (Table 2). Finally we will outline how the dependence of PMBCL on some of the previously described key pathways might be translated into novel therapeutic approaches.

Unbalanced 16p13.13 rearrangements

Based on cytogenetic studies of chromosomal rearrangements of the \textit{CIITA} locus (16p13.13), a substantial number of cases were found to harbor unbalanced rearrangements with loss of genetic material centromeric of the breakpoint.\textsuperscript{11} This finding is in keeping with previous reports describing deletions of the tumor-suppressor gene \textit{SOCS1} that closely maps to \textit{CIITA} on chromosome 16, leading to down-stream activation of JAK-STAT signaling\textsuperscript{67}. As these rearrangements coincide with disruptive \textit{CIITA} breaks and overexpression of \textit{CIITA} fusion partners, one can reasonably hypothesize that deletion of tumor suppressor genes such as \textit{SOCS1}, overexpression of oncogenes resulting from gene fusion and \textit{CIITA} loss of function might be concurrent consequences of a single genetic event. The functional consequences might be diverse as numerous \textit{CIITA} fusion partners have been described; however, it appears likely that at least in a subset of PMBCL cases a proliferative advantage through JAK-
STAT signaling is combined with an immune escape mechanism through downregulation of MHC class II and up-regulation of PD-1 ligands inducing T cell anergy (Figure 3).

Chromosomal gain of 9p24

As discussed above, amplification of a well-defined region on chromosome 9p24 has been identified in the majority of cases and can be considered as one of the genetic hallmark events of PMBCL pathogenesis. However, only recently have systematic approaches identified the target genes in the amplicon that are overexpressed and prove to be functionally most relevant. Remarkably, these studies did not identify a single target gene, but revealed that multiple genes are co-amplified and cooperate in the pathogenesis.10,61 Integrative study of copy number and mRNA expression level changes confirmed overexpression of JAK2 and PD-1 ligands in cases with 9p amplification and demonstrated that JAK2 further augments PD-1 ligand expression by transcriptional up-regulation.10 Moreover, JAK2, JMJD2C and RANBP6 were identified as critical genes required for proliferation and survival by an RNA interference screen, of which JAK2 and JMJD2D2 were shown to cooperate in modifying the epigenome in these lymphomas.61 In aggregate, increased JAK-STAT signaling through JAK2 amplification, PD-1 ligand surface expression sculpting a favorable microenvironment, and epigenetic modifications changing gene expression of a wide range of genes, such as MYC, are likely to cooperate to develop the full malignant phenotype of PMBCL.
Novel therapeutic approaches

Dose-intensified treatments have been suggested to improve clinical outcomes in patients with PMBCL in a retrospective study.\textsuperscript{20} However, no randomized clinical trials have been reported to date. With the exception of rituximab (NCT00001337), there are currently no registered trials that are actively recruiting (ClinicalTrials.gov, accessed April 26, 2011) that use targeted therapy approaches to provide an alternative to dose-escalation and to minimize treatment-related sequelae. As treatment recommendations are historically linked to DLBCL, the relative specificity of JAK-STAT\textsuperscript{8}, NF\textgreek{k}B pathway activation\textsuperscript{9}, overexpression of PD-1 ligands\textsuperscript{3} and the histone modifying genes JAK2 and JMJD2C\textsuperscript{61} stand out as the most rational targets for specifically tailored future therapies in PMBCL. \textit{In vitro} studies in MedB-1 cells demonstrate proof of principle that JAK pathway inhibition and reduction of STAT5 phosphorylation are toxic to the cells,\textsuperscript{58} raising hope that similar approaches of JAK-STAT pathway inhibition might be a promising approach. Similarly, NF\textgreek{k}B pathway inhibition has yielded promising results in preclinical models.\textsuperscript{44,97} Specifically, the histone deacetylase inhibitor vorinostat has also been described to specifically inhibit STAT6 phosphorylation in HL\textsuperscript{98}. Furthermore, targeting the tumor cell microenvironment interaction by inhibition of T cell accessory signaling might be very promising based on encouraging data in melanoma, in which a combined PD-1/CTLA-4 blockade led to increased infiltration of effector T cells promoting tumor cell kill.\textsuperscript{99} These data highlight the importance of host immunity and that
immune escape mechanisms of tumor cells can be targeted in conjunction with cytostatic therapy; a paradigm shift in treatment approaches that might be very fruitful in a subset of PMBCL and related lymphomas.

Acknowledgements: This work is supported by a postdoctoral fellowship of the Cancer Research Society (Steven E. Drabin fellowship) to CS. RDG is supported by a Canadian Institutes for Health Research grant (#178536) and a Program Project grant from the Terry Fox Foundation (#019001). We thank Dr David Scott for critical review of the manuscript.

Author Contributions

C.S and R.D.G wrote this review. The authors have no conflict of interest to declare.
References


83. Rimsza LM, Chan WC, Gascoyne RD, et al. CIITA or RFX coding region loss of function mutations occur rarely in diffuse large B-cell lymphoma cases
and cell lines with low levels of major histocompatibility complex class II expression. *Haematologica.* 2009;94(4):596-598.


### Tables

**Table 1:** Recurrent gene alterations involved in the pathogenesis of primary mediastinal B cell lymphoma. The primary reference (ref.) is given in brackets.

<table>
<thead>
<tr>
<th>Gene</th>
<th>Pathway/Function</th>
<th>Frequency of alteration in PMBCL</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>I. Copy number gain</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>REL</td>
<td>NFkB pathway</td>
<td>75% (ref. 45)</td>
</tr>
<tr>
<td>PDL1/PDL2</td>
<td>Induction of T cell exhaustion / apoptosis</td>
<td>63% (ref. 10)</td>
</tr>
<tr>
<td>JAK2</td>
<td>Interleukin / JAK-STAT pathway / Histone modification</td>
<td>63% (ref. 10)</td>
</tr>
<tr>
<td>JMJD2C</td>
<td>Histone modification</td>
<td>63% (ref. 61)</td>
</tr>
<tr>
<td><strong>II. Chromosomal translocation / rearrangement</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CIITA</td>
<td>Transcriptional regulation of HLA class II / Antigen presentation</td>
<td>38% (ref. 11)</td>
</tr>
<tr>
<td><strong>III. Coding sequence mutation</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SOCS1</td>
<td>Interleukin / JAK-STAT pathway</td>
<td>45% (ref. 64)</td>
</tr>
<tr>
<td>STAT6</td>
<td>Interleukin / JAK-STAT pathway</td>
<td>36% (ref. 68)</td>
</tr>
<tr>
<td>TNFAIP3</td>
<td>NFkB pathway</td>
<td>36% (ref. 4)</td>
</tr>
<tr>
<td>MYC</td>
<td>Transcriptional regulation / Chromatin remodeling</td>
<td>25% (ref. 42)</td>
</tr>
<tr>
<td>TP53</td>
<td>p53 pathway</td>
<td>13% (ref. 42)</td>
</tr>
<tr>
<td><strong>IV. Promotor hypermethylation</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>p16/INK</td>
<td>Cell cycle progression, p53 pathway</td>
<td>9% (ref. 42)</td>
</tr>
</tbody>
</table>
Table 2: Proposed synergy of genetic alterations

<table>
<thead>
<tr>
<th>Genomic event</th>
<th>Gene components</th>
<th>Proposed synergy</th>
</tr>
</thead>
<tbody>
<tr>
<td>Unbalanced CIITA rearrangement</td>
<td>SOCS1, CIITA, CIITA fusion partners (e.g. PDL1 and PDL2)</td>
<td>Impaired CIITA function, overexpression of fusion partners and deletion of tumor suppressor genes might be simultaneous consequences of a single unbalanced translocation event: 1) Proliferative advantage through increased JAK-STAT signaling in the malignant cells by deletion of SOCS1 cooperates with escape from immune surveillance by 2) overexpression of PD-1 ligands and 3) downregulation of HLA class II.</td>
</tr>
<tr>
<td>Chromosomal gain of 9p</td>
<td>JAK2, JMJD2C, PDL1, PDL2</td>
<td>Overexpression of JAK2, JMJD2C, PDL1 and PDL2 are linked as these genes are part of the same amplicon involving cytoband 9p24.1: 1) JAK2 overexpression increases JAK-STAT signaling promoting proliferation; 2) JAK2 transcriptionally augments PD-1 ligand expression inhibiting T cell activation in the tumor microenvironment and, 3) cooperates with JMJD2C to alter the epigenome in PMBCL by histone H3 modifications promoting proliferation and survival.</td>
</tr>
</tbody>
</table>
Figure legends

**Figure 1: Pathological and clinical features of PMBCL in comparison with related diseases.** Top panel: Representative hematoxylin/eosin (H&E) and immunohistochemical stains are shown. Positivity of CD20 and CD30 in PMBCL is characteristic, although not mandatory for the diagnosis of PMBCL. The H&E stain of PMBCL shows typical sheets of medium-sized to large cells containing abundant pale cytoplasm in a background of fine sclerosis. Of note, CD20 staining in NSHL is typically heterogeneous and CD30 is usually absent in DLBCL. Bottom panel: The typical clinical features of PMBCL are given and contrasted with related entities.

**Figure 2: Main deregulated signaling pathways in PMBCL.** The main activation cascades of JAK-STAT and NFκB signaling are shown. Alternative pathway activation exists. Known gene alterations leading to constitutive pathway activity in PMBCL are shown in color.

**Figure 3: Impact of genetic alterations on the tumor microenvironment.** The major mechanisms of 16p13.13 gene rearrangements leading to CIITA gene fusions and 9p24.1 amplification are depicted. These mechanisms involve downregulation of MHC class II and upregulation of PD-1 ligands leading to an imbalance of T cell receptor vs co-stimulatory signaling. Functional consequences for the tumor microenvironment encompass induction of T cell
anergy, apoptosis and skewed differentiation towards regulatory T cells. B in triangle indicates chromosomal break and ER = endoplasmic reticulum.
**Figure 1:**

<table>
<thead>
<tr>
<th>Pathological features</th>
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<tbody>
<tr>
<td>Hematoxylin and Eosin</td>
</tr>
<tr>
<td>CD20</td>
</tr>
<tr>
<td>CD30</td>
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</tbody>
</table>

<table>
<thead>
<tr>
<th>Clinical features</th>
</tr>
</thead>
<tbody>
<tr>
<td>Approximate median age</td>
</tr>
<tr>
<td>30 years</td>
</tr>
<tr>
<td>30 years</td>
</tr>
<tr>
<td>35 years</td>
</tr>
<tr>
<td>65 years</td>
</tr>
<tr>
<td>Gender predominance</td>
</tr>
<tr>
<td>female</td>
</tr>
<tr>
<td>male &gt; female</td>
</tr>
<tr>
<td>female</td>
</tr>
<tr>
<td>male ≥ female</td>
</tr>
<tr>
<td>Typical manifestation</td>
</tr>
<tr>
<td>supravacular LN / mediastinal</td>
</tr>
<tr>
<td>mediastinal</td>
</tr>
<tr>
<td>mediastinal / supravacular LN</td>
</tr>
<tr>
<td>nodal</td>
</tr>
<tr>
<td>Bone marrow involvement</td>
</tr>
<tr>
<td>uncommon</td>
</tr>
<tr>
<td>rare</td>
</tr>
<tr>
<td>rare</td>
</tr>
<tr>
<td>16%</td>
</tr>
</tbody>
</table>
Figure 2:

JAK-STAT signaling

Interleukin receptors

STAT6

JAK2

STAT6

STAT6

STAT6

STAT6

STAT dimers/oligomers

SOCS1

Canonical NFkB

Interleukin receptors

TNF receptor superfamily

RIP

TRAF

MEK

MAP3K14

NEMO

IKKa

IKKB

IKK complex

IkBa

IkBε

IkB complex

proteasomal degradation

Rel

NFkB complex

Alternative NFkB

A20

TRAF

IkBa

IkBε

p65

p100

p52

Transcriptional regulation:
- Inflammation
- Survival
- Cell proliferation
- Differentiation
Figure 3:

Proposed functional consequence

T cell receptor signaling

Co-stimulatory signaling

T cell anergy
Apoptosis
Differentiation towards T reg

T cell receptor

PD-1 molecules

MHC class II molecule

PD-1 ligands (CD273, CD274)

Cellular interface

PMBCL cell

ER

CIITA gene fusions

9p24.1 amplification
The molecular pathogenesis of primary mediastinal large B cell lymphoma

Christian Steidl and Randy D. Gascoyne