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Summary and discussion

In the WHO-EORTC classification, B-cell lymphomas primarily presenting in the skin are categorized as primary cutaneous marginal zone B-cell lymphoma (PCMZBL), primary cutaneous follicle center lymphoma (PCFCL) or primary cutaneous large B-cell lymphoma, leg type (PCLBCL-LT).[1] The three subtypes have their own clinicopathologic characteristics, but differentiation between PCFCL and PCLBCL-LT can sometimes be challenging. These two lymphoma subtypes generally show clinical differences in presentation, i.e. localized skin lesions on the head, in particular the scalp, or trunk for PCFCL, and skin lesions on one or both legs for PCLBCL-LT, but the distinction between the two entities is primarily based on specific tumor cell morphology, i.e. large cells with cleaved nuclei (centrocytes) in PCFCL and large cells with round nuclei (centroblasts and immunoblasts) in PCLBCL-LT.[1,2] However this distinction based on tumor cell morphology can become challenging when cases of PCFCL have a diffuse growth pattern and lack the follicular infiltrate that is distinct from the growth pattern of PCLBCL-LT. Accurately separating these two types of lymphoma has large implications for the patient, as PCFCL are treated with local radiotherapy (5-year overall survival (OS) 95%), but patients with PCLBCL-LT are preferentially treated with polychemotherapy, and still have an unfavorable clinical outcome (5-year OS survival 50%).[3]

Recent guidelines indicate that patients with PCLBCL-LT should be treated with R-CHOP, cyclophosphamide, doxorubicin, vincristine, and prednisone combined with monoclonal antibody rituximab, directed at CD20 at the surface membrane of B-lymphocytes.[4] However, due to the relatively high age at first presentation and concomitant comorbidities, not all patients are eligible for this type of chemotherapy. Furthermore, a proportion of patients receiving polychemotherapy still show an aggressive clinical course after initial response, leading to a challenge in further treatment strategies. Patients might therefore benefit from therapies that are less toxic and target more specifically the lymphoma cells, to reduce morbidity and increase survival. Molecular and/or genetic characterization of these tumors is thus warranted and might reveal better insight into the pathogenesis of the disease. These insights might eventually yield potential targets for therapy directed specifically at these targets.

A rare type of diffuse large B-cell lymphoma (DLBCL) presenting in the skin is methotrexate (MTX)-associated B-cell lymphoproliferative disorder (B-LPD). This lymphoma belongs to the group of 'iatrogenic immunodeficiency-associated lymphoproliferative disorders'[5] and develops in patients with autoimmune disorders (mainly rheumatoid arthritis) receiving chronic low-dose treatment with MTX. The clinicopathologic features of MTX-associated B-LPD first presenting in the skin are still ill-defined, as there are only sporadic reports in literature.[6-10] Differentiation from PCFCL and PCLBCL-LT is nonetheless important, as these lymphomas require a different therapeutic approach. It seems warranted to stop MTX treatment and follow a careful wait-and-see policy to evaluate potential remission before considering more aggressive therapies, as regression of MTX-associated lymphomas after abrogation of MTX treatment has been reported.[11]

The studies presented in this thesis investigated clinicopathologic and genetic aspects of PCFCL and PCLBCL-LT. The main aims of this thesis were (1) to define additional diagnostic markers that could aid in the differential diagnosis of PCFCL with a diffuse growth pattern from PCLBCL-LT, (2) to investigate molecular and genetic alterations in PCLBCL-LT underlying the pathogenesis of this type of lymphoma, and (3) to better define the group of MTX-associated B-LPD presenting in the skin, in order to find clinicopathologic and/or immunophenotypical characteristics that would allow rapid recognition of these lymphomas. In this final chapter, these issues will be discussed based on the data and observations described in the preceding chapters and in recent literature.

Differentiation between PCFCL and PCLBCL-LT

IgM immunohistochemistry

Gene expression profiling studies in PCFCL and PCLBCL-LT have demonstrated different gene expression profiles for both lymphoma subtypes, including higher expression of immunoglobulin mu heavy chain enhancer and constant region (*IGHM*) transcripts in PCLBCL-LT as compared to PCFCL.[12] In **chapter 2**, we explored whether these differences were also reflected at protein levels, and performed B-cell heavy and light chain characterization for 53 patients with PCFCL and 40 patients with PCLBCL-LT by immunohistochemistry on formalin-fixed and paraffin-embedded (FFPE) tumor biopsy specimens. All 40 cases of PCLBCL-LT consistently showed cytoplasmic staining for IgM, in 18 of them with coexpression of IgD. In contrast, only 5 of the PCFCL cases showed cytoplasmic staining for IgM and/or IgD. Not only PCLBCL-LT presenting on the leg, but also those cases presenting on the trunk or head, showed strong expression of IgM, implying that for lesions on these locations IgM expression might be used as an additional marker in differentiating between PCFCL and PCLBCL-LT in clinical pathology practice. However, the three PCFCLs that presented on the leg(s) also expressed IgM. Therefore, IgM expression does not seem to discriminate between both lymphoma subtypes when presenting on the leg. Recent studies confirmed our findings, demonstrating expression of IgM in 10 out of 10 cases of PCLBCL-LT and only 3 out of 30 cases of PCFCL.[13]

MicroRNA profiling

MicroRNAs can regulate gene expression of their target genes by being able to inhibit translational initiation by blocking ribosomes and they can also directly target mRNA at the 3 prime untranslated region leading to degradation of the molecule.[14] Higher expression of a certain microRNA might therefore silence a tumor suppressor gene, while loss of expression might cause oncogenes to be upregulated. Through these mechanisms, microRNAs are thought to contribute in oncogenesis (reviewed in [15]). In **chapter 3** we investigated microRNA profiles by performing high-throughput sequencing analysis on frozen tumor biopsies from 6 cases of PCFCL and 13 cases of PCLBCL-LT. Analogous to previous gene expression profiling studies,[12] we tried to cluster PCFCL and PCLBCL-LT based on their microRNome. In contrast to gene expression profiling, cluster analysis of the complete microRNA profile did not discriminate the lymphoma subtypes. As gene expression profiles of PCFCL and PCLBCL-LT were concordant with a germinal center B-cell (GCB) and activated B-cell (ABC), respectively, we tried to cluster the two cutaneous lymphoma subtypes by three different microRNA profiles of these two B-cell subtypes from literature.[16-18] These microRNA profiles were reported to be able to separate nodal ABC-type from GCB-type DLBCL, but we were not able to separate our two groups by one of these methods. Still, the extensive profiling method yielded 16 individual mature microRNAs that were differentially expressed between PCFCL and PCLBCL-LT. Single microRNA quantitative PCR (qPCR) was conducted for 11 of these 16 microRNAs on FFPE tumor biopsies of 20 additional cases (10 cases of PCFCL and 10 cases of PCLBCL-LT), confirming higher expression of miR-9-5p, miR-31-5p, miR-129-2-3p and miR-214-3p in PCFCL as compared to PCLBCL-LT. This technique can easily be performed on routine biopsy specimens. Especially the detection of miR-129-3p by RT-qPCR on FFPE tumor biopsies might be helpful in clinical settings, as this microRNA was not expressed in a detectable amount in PCLBCL-LT, but showed substantial expression in about half of the cases of PCFCL. This microRNA therefore seems a rather specific, yet not highly sensitive, marker for PCFCL. Remarkably, this miR-129-2-3p was also specifically expressed in substantial amounts in GCB-type nodal DLBCL as compared to ABC-type DLBCL in our RT-qPCR control group of 20 nodal DLBCLs, and therefore

seems a true germinal center-related microRNA.

Pathogenesis of primary cutaneous diffuse large B-cell lymphoma, leg type

Class switch recombination

In **chapter 2** we demonstrated consistent immunohistochemical cytoplasmic expression of IgM in 40 cases of PCLBCL-LT, with co-expression of IgD in about half of them. As genotyping showed that PCLBCL-LT have the profile of a post-germinal center, activated B-cell,[12] it is expected that class switch recombination (CSR) from IgM (and IgD) to IgG or IgA has occurred. Given this presumed germinal center experience, the absence of CSR in PCLBCL-LT is surprising and might indicate a defect in the CSR mechanism. Although deficiency of the RNA-editing enzyme activation-induced cytidine deaminase (AID) is associated with impairment of CSR,[19] relatively high levels of AID have been demonstrated in PCLBCL-LT,[20] so the absence of CSR cannot be explained by shortage of AID. The expression of IgM is not exclusive for PCLBCL-LT. Over 50% of nodal DLBCL also show immunohistochemical expression of IgM in the tumor cells,[21,22] and high levels of IgM RNA are more frequently encountered in ABC-type DLBCL than in GCB-type DLBCL.[23] IgM expression therefore seems ABC-type related, and is indeed also encountered in primary central nervous system (CNS) DLBCL, and primary testicular DLBCL, two types of extranodal DLBCL that are also of ABC-type.[24,25] In nodal ABC-type DLBCL as well as in primary CNS DLBCL it was suggested that CSR was impaired due to the high frequency of mutations in the switch regions of the constant chain, comprising both intra-Sμ and intra-Sγ deletion/recombination events,[26,27] but data concerning these switch regions are not available for PCLBCL-LT. Noteworthy, it was recently described that transcription factor forkhead box protein 1 (FOXP1) might also play a role in (blocking) CSR, as constitutional expression of FOXP1 led to impairment of switching from IgM to IgG1.[28] Indeed in PCLBCL-LT, immunohistochemical FOXP1 expression is consistently high, as was shown in **chapter 5**, although the exact underlying mechanism for this high expression is not yet understood.

Nuclear factor-kappa B

Aberrant constitutive activation of the nuclear factor-kappa B (NF-κB) signal transduction pathway has been implicated in tumor cell survival of nodal ABC-type DLBCL and other types of B-cell lymphomas. Studies on the molecular background of NF-κB pathway activation have demonstrated that mutations in multiple genes can cause deregulation of NF-κB signaling in nodal ABC-type DLBCL.[29] The genes most frequently affected by genetic aberrations are tumor necrosis factor, alpha-induced 3 (*TNFAIP3, A20)*, cluster of differentiation 79B (*CD79B*), caspase recruitment domain-containing protein 11 *(CARD11)*, and myeloid differentiation primary response gene 88 (*MYD88*), which all can contribute to constitutive activation of the NF-κB pathway, and in two-third of cases of nodal ABC-type DLBCL, one or more of these genes are affected.[30] In **chapter 4**, we investigated NF-κB activating genetic aberrancies in 10 cases of PCLBCL-LT. Tumor suppressor gene *TNFAIP3* was heterozygously deleted in 4 cases. No additional promoter hypermethylation was detected that would lead to epigenetic silencing of the non-deleted allele. A *CD79B* Y196 mutation was found in 2 cases in both DNA and cDNA. The coiled-coil domain of *CARD11* contained a D415E and a R423W mutation in 1 sample. At genomic level, the oncogenic *MYD88* L265P mutation was found in 4 cases, 3 of which showed the same mutation at transcriptional level. Combined, 7 out of 10 cases of PCLBCL-LT showed genetic alterations in genes that regulate NF-κB activation. Although estimation of the real proportion of alterations is difficult in this relatively small study co-

hort, for pathway activating mutations in *CD79B*, *MYD88*, and *CARD11* the percentages of tumors affected strikingly resemble those present in nodal ABC-DLBCL.[30] However, the homozygous deletion and/or epigenetic silencing of *TNFAIP3*, as is encountered in 23% of cases of nodal ABC-DLBCL,[30] was not detected in our cases of PCLBCL-LT. The data on the deletion of (part of) chromosome 6q, on which *TNFAIP3* is located, are in line with previous studies in PCLBCL-LT using conventional array comparative genomic hybridization[31] or fluorescent in situ hybridization.[32] Furthermore, other studies detected *MYD88* L265P mutations in 69% of PCLBCL-LT cases.[32,33]

Several molecules in the NF-κB pathway have already been selectively targeted in B-cell NHL *in vitro* and *in vivo* to explore potential new treatments strategies. For example, sotrastaurin can interfere with NF-κB pathway activation, by inhibition of PKC-β, which is required for CARD11-dependent activation of the NF-κB pathway.[34] Sotrastaurin was shown to be selectively toxic for *CD79*-mutant DLBCL in a mouse xenograft model[35] as compared to unmutated DLBCL, but only in presence of wild-type *CARD11*. An RNA interference screen revealed that a BCR signaling component, Bruton's tyrosine kinase (BTK), is essential for the survival of nodal ABC-type DLBCL with wild-type *CARD11* and in addition, knockdown of proximal B-cell receptor units, i.e. CD79B, was selectively toxic to wild-type *CARD11* nodal ABC-type DLBCL, but not to other lymphomas.[36] Furthermore, in a phase I trial, the BTK-inhibitor ibrutinib was well-tolerated and generated a clinical (partial) response in 40% of refractory nodal ABC-type DLBCL, both in patients with tumors bearing *CD79* mutations or a combination of *CD79* and *MYD88* mutations. However, patients with only *MYD88* mutations were refractory to this treatment, reflecting the B-cell receptor signaling-independency of these MYD88-mutated tumors.[37] The potency of ibrutinib in treating nodal ABC-DLBCL has recently been further explored, in a high throughput combinatorial screening study, yielding drugs that could cooperate with ibrutinib in killing ABC-DLBCL cells *in vitro*.[38] Together, these results imply the relevance of somatic mutations in *CD79B*, *CARD11* and *MYD88* in developing new treatments for nodal ABC-DLBCL, and potentially for the treatment of PCLBCL-LT. Especially the impact of ibrutinib in the treatment of (several types of) B-cell lymphomas seems promising in treating PCLBCL-LT patients who are not eligible for aggressive treatment or who quickly relapse after receiving polychemotherapy.[39] Therefore, particularly investigating the mutational status of *MYD88* in PCLBCL-LT might become of clinical relevance, as these patients will potentially not benefit from treatment with ibrutinib.

As 70% of PCLBCL-LT shows genetic aberrancies in NF-κB pathway-related genes, our data also emphasize the relevance of NF-κB signaling in this type of lymphoma. In nodal ABC-DLBCL, NF-κB signaling has been recognized as a key feature.[40] The lack of genetic aberrancies in the remaining 30% of our cases studied does not necessarily imply physiological NF-κB signaling in these cases, as activating mutations in pathway components are not the only pathophysiologic mechanism underlying constitutive signaling of this pathway. For example, hedgehog signaling is deregulated in DLBCL.[41] The dysregulation might partly be due to copy number gains involving smoothened (SMO), which is a signal transducer subunit of the hedgehog pathway.[42] Aberrant hedgehog signaling has been linked to NF-κB pathway activation in DLBCL through SMO, which is able to recruit G-protein subunits that lead to NF-κB pathway activation through PKC-β – CARD11 signaling.[43] Furthermore, several microRNAs have been implicated in NF-κB pathway activation. MiR-155 is able to downregulate IκB kinases, which will lead to enhanced NF-κB signaling.[44] MiR-21 blocks translation of phosphatase and tensin homolog (*PTEN*), which is a tumor suppressor inhibiting the activation of NF-κB by AKT signaling. Both microRNAs are frequently reported to be upregulated in nodal ABC-DLBCL as

compared to GCB-DLBCL, concordant with NF-κB being a feature of ABC-DLBCL. However, in our cohort explored in **chapter 3**, we did not encounter differences in expression levels of miR-155 and miR-21 between PCLBCL-LT and PCFCL. This might imply that these microRNAs do not contribute in aberrant NF-κB signaling in PCLBCL-LT, or that aberrant signaling is also a feature of PCFCL. This unclarity warrants for further investigation into this topic.

Transcription factors

In physiologic circumstances, B-cell lymphoma 6 (BCL6) is expressed in germinal center B-cells and downregulates Blimp1 expression, the protein encoded by of PR domain containing 1, with ZNF domain (*PRDM1*).[45] After a successful germinal center reaction, BCL6 is downregulated, leading to expression of Blimp1. This Blimp1 expression is sufficient to allow B-cells to undergo plasma cell maturation, and also induces other transcription factors, such as interferon regulatory factor-4 (IRF4), which are also part of the plasma cell program.[46] Furthermore, FOXP1 expression is inversely correlated to BCL6 expression, thereby occurring in a post germinal center stage, and FOXP1 is also able to upregulate the expression of Blimp1.[28] Noteworthy, the immunohistochemical profile of PCLBCL-LT concerning these transcription factors is inconsistent with the profile of activated B-cells, its presumed cell of origin, as BCL6 is expressed in more than half of the cases, IRF4 and FOXP1 are virtually always expressed by the tumor cells, and no substantial expression of Blimp1 is detected.[47] The activated B-cell, as normal counterpart of PCLBCL-LT, is arrested in differentiation in a transitional stage between germinal center B-cell and plasma cell.[12] In nodal DLBCL, several mechanisms have been described that might (in part) be responsible for this differentiation arrest, for example translocations involving *BCL6*,[48,49] deletion and mutations *PRDM1*.[48,50] Other molecules driving B-cells towards plasma cell differentiation include IRF4 and FOXP1, and sporadic genetic alterations, such as translocations involving these genes, also occur in nodal DLBCL.[51,52] In **chapter 5**, we investigated genetic and immunohistochemical alterations of these B-cell activation-related transcription factors in ten cases of PCLBCL-LT. We could not find evidence that *BCL6*, *FOXP1* and/or *IRF4* have an autonomous way of protein upregulation, as we did not encounter translocations or amplifications involving these genes. Moreover, the absence of Blimp1 protein by immunohistochemistry in PCLBCL-LT could not be explained by genetic silencing through homozygous deletion of the gene, or heterozygous deletion with concomitant missense or nonsense mutations. These alterations actually have been described in ABC-DLBCL, the nodal counterpart of PCLBCL-LT. Especially genetic inactivation of PRDM1 is frequently detected in ABC-DLBCL, occurring in approximately one-third of cases.[48,50] Translocations involving *IRF4* have only sporadically been described in nodal ABC-DLBCL[51] and translocations involving *FOXP1* are also infrequent, but might preferentially be detected in extranodal DLBCL.[52]

MYC

In the study described in **chapter 6**, in which we compared MTX-B-LPD presenting in the skin with PCFCL and PCLBCL-LT, we demonstrated that PCLBCL-LT were consistently positive for MYC immunohistochemistry, and more than 75% of the tumor cells showed nuclear staining in the 10 cases investigated. In nodal DLBCL, high percentages of MYC-positive tumor cells have been linked to *MYC* rearrangement, gain and/or amplification (53, 54). However, in PCLBCL-LT, data concerning translocations involving the *MYC* gene vary, from occurrence in sporadic cases[55] to approximately one-third of cases.[56] Reports of gains or amplification of the *MYC* gene have to our knowledge not been reported and were not found in the same cohort of 10 patients PCLBCL-LT, for which immunohistochemistry was reported, by using fine

tiling comparative genomic hybridization (FT-CGH) analysis (Koens et al, unpublished data). Above that, in this cohort, FT-CGH analysis did also not reveal evidence of translocations involving the *MYC* gene. MYC protein levels are therefore most likely upregulated by other mechanisms. MYC is under physiologic circumstances repressed by B-cell transcription factor Blimp-1 in post-germinal center B-cells.[57] This protein is however absent in PCLBCL-LT[47] and this absence might therefore contribute to high levels of MYC protein.

Methotrexate-associated B-cell lymphoproliferative disorder presenting in the skin

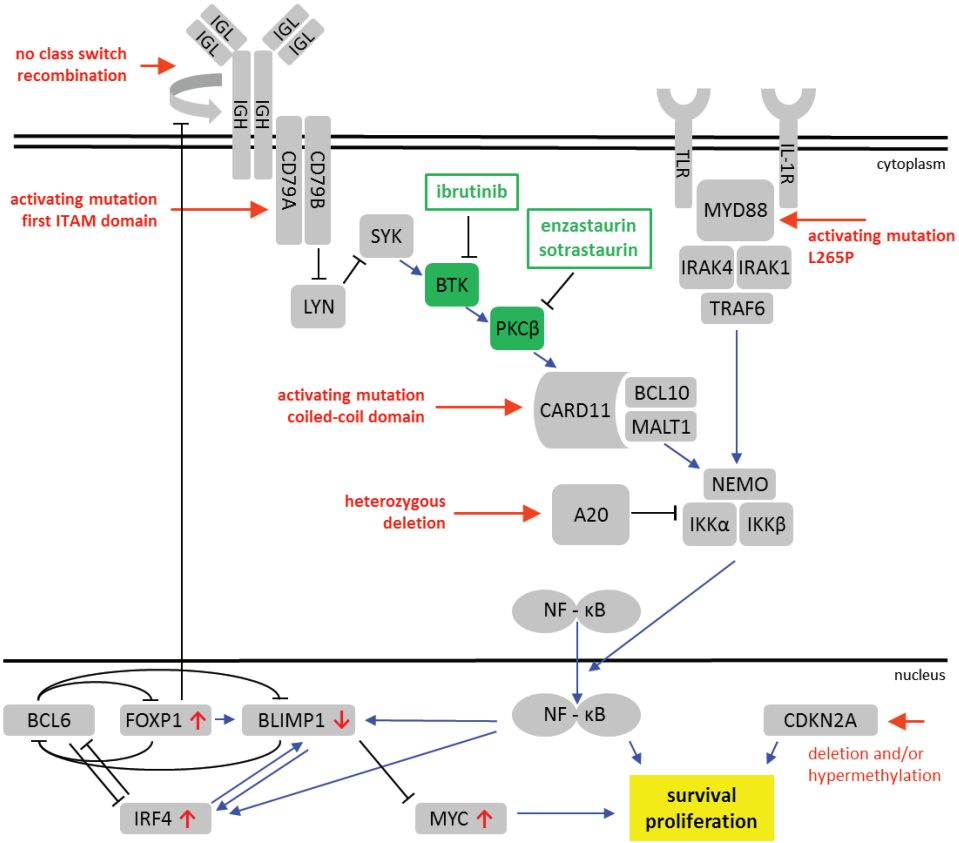
In **chapter 6** the clinicopathologic and phenotypical features of 10 patients with MTX-associated B-LPD first presenting in the skin were studied, including 5 Epstein Barr virus (EBV)-positive and 5 EBV-negative cases. 6 patients had skin-limited disease. Clinically, abrogation of MTX therapy resulted in a complete response in four cases and a partial response in another two. The 5-year disease-specific survival was 90%. MTX-associated B-LPD differed from PCFCL by the presence of ulcerating and/or generalized skin lesions, an infiltrate composed of centroblasts/immunoblasts rather than large centrocytes, reduced staining for CD79a, expression of BCL2, IRF4, FOXP1 in most cases, and monotypic intracytoplasmic immunoglobulin expression in half of the cases. EBV-positive MTX-associated B-LPD differed from PCLBCL-LT by the presence of ulcerating skin lesions, marked tumor cell polymorphism, reduced staining for CD79a, and expression of CD30 and EBV. EBV-negative cases showed morphological and immunophenotypical similarities to PCLBCL-LT, but differed by presentation with generalized skin lesions in 4 of 5 cases. The good clinical outcome and spontaneous disease regression that occurred after withdrawal of MTX in a considerable proportion of patients, underscores the importance of a careful wait-and-see policy before considering more aggressive therapies in patients with MTX-associated B-LPD of the skin.

Concluding remarks and perspectives

Through the different studies described in this thesis more knowledge is gained about the clinicopathologic (and genetic) aspects of PCFCL, PCLBCL-LT and MTX-associated B-LPD presenting in the skin. The results aid in better definition of these separate entities, and thereby generate leads for diagnosing these lymphomas in clinical practice. The differences in microRNA profile further support the notion that PCFCL and PCLBCL-LT are different molecular entities with different biological behavior. An up-to-date overview of clinicopathologic, immunophenotypical and molecular genetic features of PCFCL and PCLBCL-LT is given in Table 1. For PCLBCL-LT therapeutical improvement is much needed and delineation of molecular or genetic events in the development and/or progression of this type of lymphoma might yield potential leads for targeted therapy. Currently known potential genetic events and involved molecular pathways in PCLBCL-LT are depicted in Figure 1. In the light of potential treatments, the studies described in this thesis provide evidence that components of the NF-κB pathway might be interesting therapeutical targets. As targeted therapy based on specific molecular characteristics is extensively explored in nodal DLBCL,[58] it will be important to further investigate these specific molecular features in PCLBCL-LT, to find out whether these therapies might also be beneficial for patients with this type of lymphoma. The observed differences between MTX-associated B-LPD presenting in the skin and PCFCL and PCLBCL-LT might give clinicians clues for diagnosing this rare entity. However, it is still unknown which cases will spontaneously resolve after abrogation of MTX therapy and which will show progressive disease and therefore require more aggressive treatment. More studies on the

molecular and genetic background of MTX-associated B-LPD presenting in the skin are therefore required to better understand its biological behavior.

Figure 1. Molecular and genetic pathways and aberrations in PCLBCL-LT



The (previously) demonstrated aberrations are depicted in red of which the ones represented in bold were demonstrated in the studies described in this thesis. The green boxes represent a selection of therapeutical targets that are under current investigation in nodal DLBCL.

Table 1. Main clinicopathologic, immunophenotypical and molecular genetic features of PCFCL and PCLBCL-LT

	PCFCL	**PCLBCL-LT**
Clinical features		
- lesions	solitary or multiple tumors	solitary or multiple tumors
- site of primary presentation	head and/or trunk	one or both leg(s)
- cutaneous relapse	30%	69%
- nodal/visceral dissemination	10%	47%
- 5 year OS/DSS	87% / 95%	37% / 50%
- first choice treatment	local radiotherapy	R-CHOP
Histopathology		
- infiltrate	diffuse or (partly) follicular	diffuse
- B-cell morphology	centrocytes (cleaved)	centroblasts/immunoblasts round)
- T-cell admixture	abundant	sparse
Immunohistochemistry		
- B-cells		
* B-cell lineage markers	CD20+, CD79a+, PAX5+	CD20+, CD79a+, PAX5+
* germinal center markers	BCL2-, BCL6+, CD10-	BCL2+, BCL6+/-, CD10-
* activation markers	IRF4/MUM1-, FOXP1-	IRF4/MUM1+, FOXP1+
*** B-cell receptor**	**heavy/light chains- (intracytoplasmic)**	**IgM+, IgD+/-, monotypic light chain+ (intracytoplasmic)**
*** cell cycle regulator marker**	**MYC-**	**MYC+**
- background	CD21/CD35: (remnants of) follicular dendritic networks	CD21/CD35: no (remnants of) follicular dendritic networks
Molecular genetics		
- copy number alteration	amplification 2p16.1 region, deletion 14q11.2-q12	deletion 6q arm, deletion 9p21.3 region
- gene expression profiling	high expression SPINK2	high expression *IGHM*, *PIM1*, *PIM2*, *IRF4* and *OCT2*
- microRNA profiling	**miR-129-2-3p present in 50%**	**no miR-129-2-3p present**
- NF-κB pathway mutations	**not available**	**MYD88 mutations 40%, CD79B mutations 20%, CARD11 mutations 10%**

The characteristics represented in bold are results of the studies described in this thesis.

References

1. Willemze R, Jaffe ES, Burg G, et al. (2005) WHO-EORTC classification for cutaneous lymphomas. Blood 105: 3768-85.

2. Swerdlow SH, Campo E, Harris NL, et al. (2008) World Health Organisation Classification of Tumours of Haematopoietic and Lymphoid Tissues. In: Harris NL, ed. Mature B-cell neoplasms: IARC Press, Lyon: 179-267.

3. Senff NJ, Hoefnagel JJ, Jansen PM, et al. (2007) Reclassification of 300 primary cutaneous B-Cell lymphomas according to the new WHO-EORTC classification for cutaneous lymphomas: comparison with previous classifications and identification of prognostic markers. J Clin Oncol 25: 1581-7.

4. Senff NJ, Noordijk EM, Kim YH, et al. (2008) European Organization for Research and Treatment of Cancer and International Society for Cutaneous Lymphoma consensus recommendations for the management of cutaneous B-cell lymphomas. Blood 112: 1600-9.

5. Swerdlow SH, Campo E, Harris NL, et al. (2008) World Health Organisation Classification of Tumours of Haematopoietic and Lymphoid Tissues. In: Gaulard P, Swerdlow SH, Harris NL, et al., eds. Other iatrogenic immunodeficiency-associated lymphoproliferative disorders: IARC Press, Lyon: 350-1.

6. Verma S, Frambach GE, Seilstad KH, et al. (2005) Epstein--Barr virus-associated B-cell lymphoma in the setting of iatrogenic immune dysregulation presenting initially in the skin. J Cutan Pathol 32: 474-83.

7. Pfistershammer K, Petzelbauer P, Stingl G, et al. (2001) Methotrexate-induced primary cutaneous diffuse large B-cell lymphoma with an 'angiocentric' histological morphology. Clin Exp Dermatol 35: 59-62.

8. Tournadre A, D'Incan M, Dubost JJ, et al. (2001) Cutaneous lymphoma associated with Epstein-Barr virus infection in 2 patients treated with methotrexate. Mayo Clin Proc Mayo Clinic 76: 845-8.

9. Chai C, White WL, Shea CR, et al. (1999) Epstein Barr virus-associated lymphoproliferative-disorders primarily involving the skin. J Cutan Pathol 26: 242-7.

10. Rausch T, Cairoli A, Benhattar J, et al. (2013) EBV+ cutaneous B-cell lymphoproliferation of the leg in an elderly patient with mycosis fungoides and methotrexate treatment. APMIS 121: 79-84.

11. Rizzi R, Curci P, Delia M, et al. (2009) Spontaneous remission of "methotrexate-associated lymphoproliferative disorders" after discontinuation of immunosuppressive treatment for autoimmune disease. Review of the literature. Medi Oncol 26: 1-9.

12. Hoefnagel JJ, Dijkman R, Basso K, et al. (2005) Distinct types of primary cutaneous large B-cell lymphoma identified by gene expression profiling. Blood 105: 3671-8.

13. Demirkesen C, Tuzuner N, Esen T, et al. (2011) The expression of IgM is helpful in the differentiation of primary cutaneous diffuse large B cell lymphoma and follicle center lymphoma. Leukemia Res 35: 1269-72.

14. Bartel DP. (2004) MicroRNAs: genomics, biogenesis, mechanism, and function. Cell 116: 281-97.

15. Calin GA, Croce CM. (2006) MicroRNA signatures in human cancers. Nature Rev Cancer 6: 857-66.

16. Montes-Moreno S, Martinez N, Sanchez-Espiridion B, et al. (2011) miRNA expression in diffuse large B-cell lymphoma treated with chemoimmunotherapy. Blood 118: 1034-40.

17. Culpin RE, Proctor SJ, Angus B, et al. (2010) A 9 series microRNA signature differentiates between germinal centre and activated B-cell-like diffuse large B-cell lymphoma cell lines. Int J Oncol 37: 367-76.

18. Lawrie CH, Chi J, Taylor S, et al. (2009) Expression of microRNAs in diffuse large B cell lymphoma is associated with immunophenotype, survival and transformation from follicular lymphoma. J Cell Mol Med 13: 1248-60.

19. Muramatsu M, Kinoshita K, Fagarasan S, et al. (2000) Class switch recombination and hypermutation require activation-induced cytidine deaminase (AID), a potential RNA editing enzyme. Cell 102: 553-63.

20. Dijkman R, Tensen CP, Buettner M, et al. (2006) Primary cutaneous follicle center lymphoma and primary cutaneous large B-cell lymphoma, leg type, are both targeted by aberrant somatic hypermutation but demonstrate differential expression of AID. Blood 107: 4926-9.

21. Miyazaki K, Yamaguchi M, Suguro M, et al. (2008) Gene expression profiling of diffuse large B-cell lymphoma supervised by CD21 expression. Br J Haematol 142: 562-70.

22. Ogawa S, Yamaguchi M, Oka K, et al. (2004) CD21S antigen expression in tumour cells of diffuse large B-cell lymphomas is an independent prognostic factor indicating better overall survival. Br J Haematol 125: 180-6.

23. Wright G, Tan B, Rosenwald A, et al. (2003) A gene expression-based method to diagnose clinically distinct subgroups of diffuse large B cell lymphoma. Proc Natl Acad Sci USA 100: 9991-6.

24. Booman M, Douwes J, Glas AM, et al. (2006) Primary testicular diffuse large B-cell lymphomas have activated B-cell-like subtype characteristics. J Pathol 210: 163-71.

25. Camilleri-Broet S, Criniere E, Broet P, et al. (2006) A uniform activated B-cell-like immunophenotype might explain the poor prognosis of primary central nervous system lymphomas: analysis of 83 cases. Blood 107: 190-6.

26. Lenz G, Nagel I, Siebert R, et al. (2007) Aberrant immunoglobulin class switch recombination and switch translocations in activated B cell-like diffuse large B cell lymphoma. J Exp Med 204: 633-43.

27. Montesinos-Rongen M, Schmitz R, Courts C, et al. (2005) Absence of immunoglobulin class switch in primary lymphomas of the central nervous system. Am J Pathol 166: 1773-9.

28. Sagardoy A, Martinez-Ferrandis JI, Roa S, et al. (2013) Downregulation of FOXP1 is required during germinal center B-cell function. Blood 121: 4311-20.

29. Compagno M, Lim WK, Grunn A, et al. (2009) Mutations of multiple genes cause deregulation of NF-kappaB in diffuse large B-cell lymphoma. Nature 459: 717-21.

30. Ngo VN, Young RM, Schmitz R, et al. (2011) Oncogenically active MYD88 mutations in human lymphoma. Nature 470: 115-9.

31. Dijkman R, Tensen CP, Jordanova ES, et al. (2006) Array-based comparative genomic hybridization analysis reveals recurrent chromosomal alterations and prognostic parameters in primary cutaneous large B-cell lymphoma. J Clin Oncol 24: 296-305.

32. Pham-Ledard A, Prochazkova-Carlotti M, Andrique L, et al. (2014) Multiple genetic alterations in primary cutaneous large B-cell lymphoma, leg type support a common lymphomagenesis with activated B-cell-like diffuse large B-cell lymphoma. Mod Pathol 27: 402-11.

33. Pham-Ledard A, Cappellen D, Martinez F, et al. (2012) MYD88 somatic mutation is a genetic feature of primary cutaneous diffuse large B-cell lymphoma, leg type. J Invest Dermatol 132: 2118-20.

34. Sommer K, Guo B, Pomerantz JL, et al. (2005) Phosphorylation of the CARMA1 linker controls NF-kappaB activation. Immunity 23: 561-74.

35. Naylor TL, Tang H, Ratsch BA, et al. (2011) Protein kinase C inhibitor sotrastaurin selectively inhibits the growth of CD79 mutant diffuse large B-cell lymphomas. Cancer Res 71: 2643-53.

36. Davis RE, Ngo VN, Lenz G, et al. (2010) Chronic active B-cell-receptor signalling in diffuse large B-cell lymphoma. Nature 463: 88-92.

37. Wilson WH, Gerecitano JF, Goy A, et al. (2012) The Bruton's Tyrosine Kinase (BTK) Inhibitor, Ibrutinib (PCI-32765), Has Preferential Activity in the ABC Subtype of Relapsed/Refractory De Novo Diffuse Large B-Cell Lymphoma (DLBCL): Interim Results of a Multicenter, Open-Label, Phase 2 Study. Blood (ASH Annual Meeting Abstracts) 120: 4039.

38. Mathews Griner LA, Guha R, Shinn P, et al. (2014) High-throughput combinatorial screening identifies drugs that cooperate with ibrutinib to kill activated B-cell-like diffuse large B-cell lymphoma cells. Proc Natl Aacad Sci USA 111: 2349-54.

39. Gyory I, Fejer G, Ghosh N, et al. (2003) Identification of a functionally impaired positive regulatory domain I binding factor 1 transcription repressor in myeloma cell lines. J Immunol 170: 3125-33.

40. Davis RE, Brown KD, Siebenlist U, et al. (2001) Constitutive nuclear factor kappaB activity is required for survival of activated B cell-like diffuse large B cell lymphoma cells. J Exp Med 194: 1861-74.

41. Singh RR, Kim JE, Davuluri Y, et al. (2010) Hedgehog signaling pathway is activated in diffuse large B-cell lymphoma and contributes to tumor cell survival and proliferation. Leukemia 24: 1025-36.

42. Ramirez E, Singh RR, Kunkalla K, et al. (2012) Defining causative factors contributing in the activation of hedgehog signaling in diffuse large B-cell lymphoma. Leukemia Res 36: 1267-73.

43. Qu C, Liu Y, Kunkalla K, et al. (2013) Trimeric G protein-CARMA1 axis links smoothened, the hedgehog receptor transducer, to NF-kappaB activation in diffuse large B-cell lymphoma. Blood 121: 4718-28.

44. Ma X, Becker Buscaglia LE, Barker JR, et al. (2011) MicroRNAs in NF-kappaB signaling. J Mol Cell Biol 3: 159-66.

45. Shaffer AL, Yu X, He Y, et al. (2000) BCL-6 represses genes that function in lymphocyte differentiation, inflammation, and cell cycle control. Immunity 13: 199-212.

46. Sciammas R, Davis MM. (2004) Modular nature of Blimp-1 in the regulation of gene expression during B cell maturation. J lmmunol 172: 5427-40.

47. Hoefnagel JJ, Mulder MM, Dreef E, et al. (2006) Expression of B-cell transcription factors in primary cutaneous B-cell lymphoma. Mod Pathol 19: 1270-76.

48. Mandelbaum J, Bhagat G, Tang H, et al. (2010) BLIMP1 is a tumor suppressor gene frequently disrupted in activated B cell-like diffuse large B cell lymphoma. Cancer cell 18: 568-79.

49. Ye BH, Chaganti S, Chang CC, et al. (1995) Chromosomal translocations cause deregulated BCL6 expression by promoter substitution in B cell lymphoma. EMBO J 14: 6209-17.

50. Pasqualucci L, Compagno M, Houldsworth J, et al. (2006) Inactivation of the PRDM1/BLIMP1 gene in diffuse large B cell lymphoma. J Exp Med 203: 311-7.

51. Salaverria I, Philipp C, Oschlies I, et al. (2011) Translocations activating IRF4 identify a subtype of germinal center-derived B-cell lymphoma affecting predominantly children and young adults. Blood 118: 139-47.

52. Goatly A, Bacon CM, Nakamura S, et al. (2008) FOXP1 abnormalities in lymphoma: translocation breakpoint mapping reveals insights into deregulated transcriptional control. Mod Pathol 21: 902-11.

53. Green TM, Nielsen O, de Stricker K, et al. (2012) High levels of nuclear MYC protein predict the presence of MYC rearrangement in diffuse large B-cell lymphoma. Am J Surg Pathol 36: 612-9.

54. Valera A, Lopez-Guillermo A, Cardesa-Salzmann T, et al. (2013) MYC protein expression and genetic alterations have prognostic impact in patients with diffuse large B-cell lymphoma treated with immunochemotherapy. Haematologica 98: 1554-62.

55. Wiesner T, Streubel B, Huber D, et al. (2005) Genetic aberrations in primary cutaneous large B-cell lymphoma: a fluorescence in situ hybridization study of 25 cases. Am J Surg Pathol 29: 666-73.

56. Hallermann C, Kaune KM, Gesk S, et al. (2004) Molecular cytogenetic analysis of chromosomal breakpoints in the IGH, MYC, BCL6, and MALT1 gene loci in primary cutaneous B-cell lymphomas. J Invest Dermatol 123: 213-9.

57. Lin Y, Wong K, Calame K. (1997) Repression of c-myc transcription by Blimp-1, an inducer of terminal B cell differentiation. Science 276: 596-9.

58. Barton S, Hawkes EA, Wotherspoon A, et al. (2012) Are we ready to stratify treatment for diffuse large B-cell lymphoma using molecular hallmarks? The Oncologist 17: 1562-73.