Diffuse large B-cell lymphoma: The history, current view and new perspectives

Minireview

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The basic principles of lymphoma classification(s) in general have been widely evolving in a course of decades of years with the use of contemporary resources and recent cutting edges in hematooncology on a clinical, morphological and molecular level bring new possibilities not only in improvements of diagnostic and prognostic algorithms and also bear new opportunities in so called targeted and tailored strategies of lymphoma therapy. The pathogenesis and biologic behavior of lymphoproliferations and even lymphomas should be studied in a context of lymphocytic and (neoplastic) lymphoid stage and chronologic development. In a current more complex insight into lymphoproliferations we would like to describe huge heterogeneity of diffuse large B-cell lymphoma in relationship to mandatory WHO classification since 2008 and the next development of knowledge in this field with potential new influence on an advancement of both classification and therapy.

Key words: lymphoma, diffuse large B-cell lymphoma, classification, review

The history

Lymphoid neoplasms and classifications. The early beginning of lymphoma classification starts in the first half of 19th cent. with the pioneer works of sir Thomas Hodgkin and Rudolph Ludwig Carl Virchow and between the second half of 19th cent. and first half of 20th cent. many other types of lymphomas have been described. The first attempt of lymphoma classification has been proposed by Gall and F. B. Mallory but the clinical correlations have been designed by H. Rappaport and still is based only on the morphological, architectonic and cytologic, grounds. In London 1973 the first new immunologic insight has been presented by two groups – from Kiel, Germany under the supervision of K. Lennert and European Lymphoma Club and up-dated in 1988 and 1992 and sorting of lymphomas was based on their definition as anatomic and clinical entities, according to their B or T cell origin, and larger volume of neoplastic cells have been called transformed lymphoma cells in oposite to histiocytic cells. During 1980’s International Lymphoma Study Group (ILSG) has been grounded and this group proposed new classification named REAL (Revised European American Lymphoma) which was based also on lymphoma entities, sorting according to B and T cell origin, and precursor and mature morphology. In 1995 new executive committee was organized under the supervision WHO inviting ten hematopathologists to chair ten committees to propose new classification which was also discussed with hematologist and oncologist to get common consensus. This
up-dated sorting was based on Kiel and REAL classification with defined lymphoma entities and their variants that can be recognised by pathologist with the support of molecular pathologist or hematologist (morphologic, immunophenotypic and genotypic lymphoma features) and bear clinical relevance (clinical features)[9, 25]. WHO (2001, 2008) – and this new basic principles of lymphoma classification are encountered in last two WHO blue books dealing hematologic neoplasms and are based on complex of lymphoma features – architectural growth pattern, origin of neoplastic lymphoid cell defined upon morphological, immunophenotypic and molecular genetic analysis [1, 9, 25].

The current view

DLBCL introduction. Diffuse Large B-cell Lymphoma (DLBCL) constitutes app. 30-40% of non-Hodgkin lymphomas [48, 49] and current complex classification is presented in WHO “Blue book” (WHO 2008, tab. 1) [1]. DLBCL occur mostly in the elderly though they are not restricted to any age group and the median age is in the 7th decade [48, 49]. The usual tissue architecture both in nodal and extranodal topography is replaced by a diffuse infiltration and occasionally by a dispersed infiltration of large lymphoid cells of B-cell origin [1, 9, 25]. In extranodal localization the DLBCLs show permissive growth pattern at the borders and blood vessel invasion is usual [1, 9, 25]. And selective types of infiltration do exist e.g. DLBCL proliferation in the interfollicular areas and sinusoidal involvement of a lymph node or may also form a discrete nodules. The cell morphology and nuclear appearance are very variable from particular case to case, but typical features are well known [1, 7, 9, 25, 44]. The neoplastic lymphoid cell is large (usually twice the size of a reactive lymphocyte) with nucleus larger than that of reactive histiocyte [1]. And sometimes the neoplastic lymphoid cell is medium sized but is different in nuclear and cytoplasmic features from the medium sized B-cell lymphomas (Burkitt’s lymphoma, B-cell lymphoblastic lymphoma). Nucleoli are almost always conspicuous, multiple (centroblastic type) or solitary (immunoblastic type) and nuclei may also show highly variable morphology HRS-like, bizarre, multinucleated (anaplastic type). Mitotic activity is commonly easily identified and mitotic figures may be atypical. The highly variable histology and cytology of DLBCL is a reflection that this lymphoma form really several different and yet incompletely distinguishable entities. Background reactive cells are also very variable and known cases of DLBCL rich in T-cell, histiocytes and eosinophils exist [38]. DLBCLs express pan-B markers CD19, CD20, CD22, CD79a, PAX5 and may show variable positivity of these B-markers. Surface and/or cytoplasmic immunoglobulins can be demonstrated in app. 50-75% of DLBCL cases (usually IgM) and cases with plasmocytoid differentiation are more commonly positive for cytoplasmic immunoglobulins [1]. App. 10% of DLBCLs express CD5, 30% express CD10, 80% express Bcl-6 (independent of BCL-6 rearrangement), 75% express Bcl-2, 10% express CD30 [1, 7]. The proliferation fraction (Ki67) is usually more than 40% up to 95% [1, 7]. Rearrangement of the BCL-2 gene occurs in 20-30% of DLBCL [1, 7]. Reciprocal chromosomal translocation involving the 3q27 region is shown up to 30% of DLBCLs, BCL-6 rearrangement is detected in more than 30% of DLBCLs and its presence correlates strongly with an extranodal involvement. BCL-2 and BCL-6 rearrangements are mutually exclusive in DLBCLs. The translocation of the protooncogen c-MYC is detected in app. 10-20% of DLBCLs, and in app. 10-20% of DLBCL cases the RB gene, tumor suppressor gene, is inactivated. There is an extensive hunt (literary documented) for prognostic markers to separate DLBCLs with more favourable outcome from more aggressive tumors [44]. The proliferation rate and EBV status are still being considered prognostic markers. The potential prognostic and predictive factors in DLBCL can be evaluated on clinical and histomorphologic grounds with a contribution of an immunoprofile and molecular profile (WHO 2001, 2008) [1]. Few studies used an immunohistochemical expression detection for a stratification

Table 1. WHO classification 2008 [1]

1. Diffuse large B-cell lymphoma NOS
   - Common morphologic variants
     - Centroblastic
     - Immunoblastic
     - Anaplastic and other rare morphologic variants
   - Rare morphologic variants
   - Molecular subgroups
     - Germinal centre B-cell-like (GCB)
     - Activated B-cell-like (ABC)
   - Immunohistochemical subgroups
     - CD5-positive DLBCL
     - Germinal centre B-cell-like (GCB)
     - Non-germinal centre B-cell-like (non-GCB)

2. Diffuse large B-cell lymphoma subtypes
   - T-cell/histiocyte-rich large B-cell lymphoma
   - Primary DLBCL of the CNS
   - Primary cutaneous DLBCL, leg type
   - EBV-positive DLBCL of the elderly

3. Other lymphomas of large B cells
   - Primary mediastinal (thymic) LBCL
   - Intravascular large B-cell lymphoma
   - DLBCL associated with chronic inflammation
   - Lymphomatoid granulomatosis
   - ALK-positive LBCL
   - Plasmablastic lymphoma
   - Large B-cell lymphoma arising in HHV8-associated multicentric Castleman disease
   - Primary effusion lymphoma

4. Borderline cases
   - B-cell lymphoma, unclassifiable, with features intermediate between DLBCL and Burkitt lymphoma
   - B-cell lymphoma, unclassifiable, with features intermediate between DLBCL and classical Hodgkin lymphoma
of DLBCL [8, 50-52]. The most known is the “Hans Classifier” where a combination of CD10, Bcl-6 and MUM1/IRF4 is used to sort DLBCLs into two groups GCB-like, nonGCB-like with app. 80% concordance with gene expression profiling (GEP). A new immunostaining classifier of DLBCL that is sorting into GCB-like, nonGCB-like and unclassified subtype can be applied with app. 93% concordance with GEP [26] with the use of GCET1, CD10, Bcl-6, MUM1/IRF4 and FOXP1 [26]. Two major patterns of gene expression by gene array technology have been proposed [53, 54] dividing into prognostically significant subgroups in a Germinal centre B-cell-like (GCB) and an Activated B-cell-like (ABC) DLBCL. Expected 5-year over-all survival (OS) in GCB and ABC DLBCL is app. 70% and 39% resp [55]. First publication describing NFκB pathway is mentioned in 1986 [56] and comprises family of transcription factors (RelA, RelB, c-Rel, p105, p100, p50, p52) with an important role in a cell proliferation, antiapoptotic function and differentiation. NFκB signaling pathway is activated by numerous stimuli, including bacteria and viruses and is referred to as a central mediator of an immune response and controls the expression of many inflammatory cytokines, chemokines, immune receptors and cell surface adhesion molecules [11-22]. NFκB signaling pathway regulates survival of normal and malignant B-cells by controlling the expression of cell death regulatory genes [12, 13]. The extrinsic apoptotic pathway is triggered by engagement of tumor necrosis factor (TNF) family death receptors (TNFR1, TNFR6/FAS/CD95) and intrinsic apoptotic pathway is activated by translocation of proapoptotic BCL2 family members to the mitochondria with subsequent release of cytochrome c [11-22]. NFκB target genes enhance cell survival by modulating TNFα signaling, inhibiting FAS-mediated apoptosis and limiting the activity of proapoptotic BCL2 family members [11-22]. In functional analyses DLBCL cell lines with ABC-type signatures had high levels of NFkB activity and also increased sensitivity to NFκB inhibition that specifically implicating the NFκB survival pathway in ABC-type of DLBCL. Constitutive activation of NFκB signaling pathway may contribute to the lymphomagenesis also in DLBCL [13, 24], expression of NFκB proteins can be identified by immunohistochemistry with potential benefit from targeted anti-NFκB therapeutic approaches, e.g. bortezomib, rituximab [40].

**Germinal center-derived lymphomas.** Germinal center (GC) derived B-cells express GC B-cell expressed transcript-1 (GCET-1) that is also marker of subset of GC-derived lymphomas [3, 7]. An increased level of GCET-1 is shown in follicular lymphoma, nodular lymphocyte predominant Hodgkin lymphoma, Burkitt lymphoma, T-cell/histiocyte rich large B-cell lymphoma and subset of diffuse large B-cell lymphoma [3, 7]. GCET-1 is molecule that belongs to the family of serin-protease inhibitors (serin) and GCET-1 gene
is located on chromosome 14q32 [3]. During the stimulation of naive B-cell via CD40 signaling the GCET-1 expression is induced [3]. Follicular lymphoma shows up to 2.2 fold greater expression of GCET-1 according to a reactive lymph node [3]. Immunohistochemistry with the use of GCET-1 monoclonal antibody (mAb) reveals the cytoplasmic positivity of GCET-1 with granular feature and also with dot or perinuclear halo staining of centroblasts and large centrocytes [3]. No preference of staining with GCET-1 mAb was found between dark and light zone of GC, only scattered positive large B-cells were found in the mantle-zone [3]. Double CD10/Bcl-6 positive B-cells also demonstrated GCET-1 mAb positivity and double immunoenzymatic staining with MUM1/IRF4 and GCET-1 mAb showed mutually exclusive staining [3]. Only a minority of small B-cells showed GCET-1 mAb and MUM1/IRF4 double positivity [3]. BCL6 is protooncogen with function of transcriptional repressor and expression of BCL6 is detected in mature B-cells in the GC and is negative in post/non-GC B-cells and precursor B-cells. BCL6 is crucial protein for the development of the GC and for antigen dependent immunological response. The function of BCL6 is based on its interaction with Blimp-1 (α and β isoform) which is another transcriptional repressor with a key role in plasma cell differentiation [35-36]. Blimp-1 is transcriptional repressor of c-MYC and causes growth arrest in successful plasmacytic differentiation or on the other hand apoptotic death [35-36]. Deregulated expression of BCL6 may be important in lymphomagenesis due to repression of Blimp-1 function and its consequence of continued B-cell growth [35-36]. Follicular lymphoma (FL) grade 3B is from the morphological point of view very closed to DLBCL, but the prevalence of BCL6 rearrangements, t(3;14)(q27;q32) were detected in significant higher number of FL grade 3B with DLBCL component compared to "pure" nodal and extranodal DLBCL [23]. In general there is some genetic overlap between FL and DLBCL with BCL6 rearrangements encountered app. in 14% of all FLs and BCL2 rearrangements app. in 30% of all DLBCLs [23]. The t(14;18)(q32;q21) was detected in 80% of transformed FLs, in 12% de novo nodal DLBCLs and not found in primary extranodal DLBCL [23]. The level of BCL6 expression do not correlate with the presence of any BCL6 alteration [23]. FLs G3B also show declined immunoreactivity for CD10 and Bcl6 (37%, 44% resp.) [23]. The number of BCL6 immunopositive cells in 3q27 rearranged and non-rearranged FLs G3B with DLBCL component and DLBCLs is lower than in FL G1, G2, G3A with or without 3q27 alterations (here at least 93%) [23]. There is no difference in CD10 and BCL6 expression between BCL6 rearranged and non-rearranged FL G3B with DLBCL component or "pure" DLBCL which is indicating that BCL6 rearrangements are not significantly associated with the GC B-cell phenotype [23].

Prognostically favorable/unfavourable subgroups of DLBCL. DLBCL is a heterogeneous entity with patients exhibiting a wide range of outcomes and long-term survivors seem to be cured [1, 4, 7, 25, 26]. A number of features are of value in predicting overall survival and disease free survival, including age, LDH, performance status, disease stage, number of extranodal sites of the infiltration [1, 4, 7, 25, 26]. Multiple histologic subtypes and morphologic variants are recognized, a variety of molecular and genetic abnormalities are variably present [1, 7, 25, 26]. Gene-expression profiling (GEP) studies have identified at least 3 distinct molecular subtypes of DLBCL [1, 7, 26]. One with an expression profile similar to normal germinat center B-cells (GCB subtypes), one mimicking activated peripheral-blood B-cells (ABC subtypes) and a third, primary mediastinal large B-cell lymphoma (PMLBCL) displaying some molecular genetic similarities to Hodgkin lymphoma [1, 7, 26]. A small number of cases do not fit into any of these categories and have been designated as "unclassifiable" [1, 26]. The addition of rituximab to CHOP chemotherapy (R-CHOP) has led to marked improvement in survival and has called into question the significance of previously recognized prognostics markers [4, 7, 26]. The reassessment of IPI showed the remaining prediction of IPI, but indentified only 2 risks groups [1, 4, 7]. Redistribution of the IPI factors into revised IPI (R-IPI) provides a more clinically useful prediction of outcome [1, 4]. The R-IPI identifies 3 distinct prognostic groups with a very good (4 year PFS 94%, OS 94%), good (4 year PFS 80%, OS 79%), poor (4 year
PFS 94%, OS 94%) outcome [1, 4]. The IPI or R-IPI no longer indentifies a risk group with less than a 50% chance of survival [1, 4]. R-IPI is a clinically useful prognostic index that may help guide treatment planning and interpretation of clinical trials [1, 4]. And patients with diagnosis of GCB-like subtype of DLBCL have a better survival independent of IPI/R-IPI [1, 4, 7, 26].

**Intravascular large B-cell lymphoma.** This is very rare subtype of DLBCL with the most common involvement of skin and brain, and dissemination virtually to any organ [1, 7, 25, 44]. The typical localization of neoplastic cells is intravascular (capillaries) in pure type of IVLBCL but also double component type of this lymphoma exists with interstitial infiltration around the involved vessels [1, 7, 25, 44]. The lymphoma cells are very rarely seen in lymph nodes and cerebrospinal fluid [1]. Two major forms of clinical presentation exist, Western one are very rarely seen in lymph nodes and cerebrospinal fluid [1, 7, 25, 44]. The lymphoma cells are occasionally detected in peripheral blood [1]. The neoplastic cells express B-cell markers and coexpression of CD5 and CD10 may be seen [1]. The intravascular type of growth is due to defect in homing receptors/signal phenotypic lack of β1 integrin (CD29), ICAM-1 (CD54).

**EBV-associated lymphomas.** Only 10% of primary DLBCLs occurring in immunocompetent patient, most of them are elderly, are EBV positive (LMP, EBER), but majority of EBV positive DLBCLs are diagnosed in patients with immunodeficiency and significant number of secondary DLBCLs that result from the transformation of low grade B-cell lymphoma and are associated with EBV latent infection [1, 7, 25, 44]. Nearly all of DLBCL associated with chronic inflammation are also EBV positive [1, 7, 25]. This type of lymphoma is associated with longstanding pyothorax or also longstanding osteomyelitis, metal implants or chronic venous ulcers and have very aggressive course [1, 7]. The lymphoma cells show aberrant immunoprofile with expression of CD20, CD79a, CD3, CD4, CD43 with potential diagnostic confusion [1]. Another angiocentric lymphoproliferation, lymphomatoid granulomatosis (LyG), is associated with latent EBV infection and LyG Grade 3 is identified as DLBCL subtype [1, 7, 25]. EBV-positive DLBCL of the elderly (over 50 years) is another EBV positive DLBCL without prior lymphoma or immunodeficiency that is separately categorized due to worse clinical course and prognosis for the patients [1, 7, 25]. This type of DLBCL is more commonly presented as extranodal lymphoma. The EBV is usually detected by in situ hybridization – EBER ISH – and most cases are aslo LMP1 or LMP2 positive by immunohistochemistry [1, 25]. The supression of viral replication is an important pathogenetic event in Epstein-Barr virus associated lymphomas [35-37]. The physiological signals that drive normal B-cell differentiation towards plasma cells are absent in EBV-transformed cells [35-37]. BLIMP1α is a tumor suppressor transcription factor that is required for plasma cell differentiation and is activated in a subset of diffuse large B-cell lymphoma – ABC subtype [35-37]. BLIMP1α is encoded by PRDM1 gene and represses genetic programs associated with GC stages of B-cells, promotes cell cycle exit and prime plasma cells for apoptosis due to down-regulation of anti-apoptotic genes [35-37]. Expression of MYC, BCL6 and PAX5 is silenced and MUM1/IRF4 is induced by BLIMP1α [35-37]. LMP1 as EBV oncogene is alone capable to down-regulate BLIMP1α and partially disrupts the BLIMP1α transcriptional programme [35-37]. BLIMP1α is possible to induce expression of XBP-1, transcription factor that binds to BZLF-1 promoter and activates EBV lytic phase [35-37]. The replicative cycle of EBV is induced by expression of the immediate-early gene, BZLF-1, that is sufficient to activate downstream lytic genes [35-37]. BZLF-1 promoter is activated in memory cells only after differentiation of these cells into plasma cells that implicates the switch between latent and lytic cycle is intimately controled by factors of plasma cell differentiation [35-37]. The absence of such factors could be important pathogenetic tool for EBV-associated lymphomas. Cellular homologue of LMP1, CD40, can also supresses induction of EBV lytic cycle, directs the differentiation of memory B-cells and supresses plasma cell differentiation [35-37].

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Plasmablastic lymphoma. This is a very rare and aggressive subtype of DLBCL with app. 60% of cases showing EBV positivity and consists of immunoblast-like cells [1, 7, 25, 39, 44]. Plasmablastic lymphoma was originally described in the oral cavity and usually occurs in extranodal sites (orbit, sinonasal region, skin, bone, soft tissues and GIT) [1, 7, 25, 39, 44]. Neoplastic cells express CD138, MUM1/IRF4, CD79a (up to 85%), CD30, EMA, p63 and are usually negative for PAX5, CD20, CD56 and LCA (CD45) [1, 25, 39, 44]. The positivity of EBV EBER in situ hybridization is detected up to 75% of cases [1, 25, 44]. Very similar morphology and immunophenotype is detected in DLBCL arising in HHV8-associated multicentric Castleman disease [1, 25, 39, 44]. Both of them may appear in HIV infection settings and in general the immunodeficiency predisposes to their development [1, 25, 39, 44].

Primary mediastinal large B-cell lymphoma (PMLBCL). PMLBCL histogenesis is based on thymic medulla B-cell (thymic asteroid cells), constitutes app. up to 10% of all diffuse large B-cell lymphomas [1, 2, 7, 25, 44]. The highest incidence is in young females [1, 2, 44]. PMLBCL primarily originates in the anterior mediastinum and the neoplastic cells are unrelated to follicle centre or mantle zone cells [1, 2, 25, 44]. The PMBCL relapses and outside of the mediastinum, if outside than usually in the liver, gut and brain, very rarely infiltrates bone marrow and lymph nodes [1, 2, 24, 25, 44]. Typical morphologic feature of PMLBCL is fibrotic compartmentalisation into alveolar clusters and cords of diffuse proliferation of medium to large B-cells with polymorphic nuclei with vesicular or granular chromatin, with single or multiple nuclei and wide rim of clear or slightly basophilic cytoplasm [1, 2, 7, 25, 44]. In B5 and Zenker’s fixative the neoplastic cells lack a clear cytoplasm that is usually amphophilic and less abundant [25]. Less often the cells resemble immunoblasts or HRS-like cells [1, 2, 7, 25, 44]. The gene expression profile of PMLBCL is much more closer to classic Hodgkin lymphoma (cHL) than to diffuse large B-cell lymphoma, but PMLBCL lacks silencing of B-cell programme opposed to cHL [1, 2]. Like HRS cells in cHL, PMLBCL neoplastic cells show low expression levels of multiple B-cell signaling components and coreceptors, and show high expression levels of a cytokine pathway components, TNF family members and extracellular matrix elements intenified in cHL [2]. The main molecular characteristics of PMLBCL comprise p53 mutation, Bcl-2 and MAL overexpression, somatic mutation of IgVH, PAX-5, BCL-6 and c-MYC, constitutional activation of NFkappaB signaling pathway [2]. PMLBCL shows increased expression of the critical NFkB target and key inhibitor of FAS-mediated apoptosis and caspase-8, caspase-8 and FADD-like apoptosis regulator (CFLAR, c-FLIP) [2]. The ABC-like DLBCL bears more restricted NFkB target gene signature than PMLBCL [2]. In GCB-like DLBCL the c-REL amplification is more common than in other DLBCL subtypes and PMLBCL [2]. And the amplification of the c-REL locus is not the pathogenetic mechanism associated with NFkB activity in DLBCL subtypes and the additional gene(s) at 2p12-16 may contribute to the GCB-like subtype [1, 2]. The immunohistochemical profile includes positivity of CD30 (activated B-cell), CD79a, Bcl-6, MUM1/IRF4 (passed through the and are about to leave GC), MAL (lymphocyte signaling transduction) expression [1, 2]. Comparative genomic hybridization (CGH) shows gains in segments of the chromosome 9p, including amplification of the REL proto-oncogene and tyrosine kinase gene JAK2 [1, 2, 25]. Published series showed the 5-year survival app. 65% using CHOP and radiotherapy [2]. Any recurrence is almost always seen in the first 2 years with usually extranodal relapses (liver, GIT, kidney, ovary, adrenal gland, pancreas, CNS) [2].

Primary cutaneous DLBCL (PCLBCL), leg type. This is a type of DLBCL that usually arises on the legs in elderly patients and app. up to 20% arises in other sites than leg, and frequently disseminates to extracutaneous sites [1, 7, 25, 44]. The neoplastic cells form diffuse, monotonous infiltrate of centroblastic and immunoblastic morphology without epidermotropism and express CD20, CD79a, Ig (monotypic), usually Bcl-2, MUM1/IRF4 and Bcl-6, CD10 is negative in the most cases [1, 7, 25, 44].

ALK positive DLBCL. This type of lymphoma is very rare and shows strong cytoplasmic ALK positivity, usually sinusoidal growth pattern [1, 7, 25, 44, 44]. Only minority of cases have also nuclear a nucleolar ALK positivity [1, 25, 41]. Most patients present with advanced stage – stage III or IV [1, 25, 41]. Neoplastic cells resemble immunoblasts or plasmablasts and express EMA (strongly), CD138, IgA (usually), kappa or lambda, but without CD20, CD79a, CD30 (usually), CD3 positivity and shows weak positivity or negativity of LCA (CD45) [1, 25, 41]. The HHV8 encodes more than ten homologues to cellular genes providing proliferative and anti-apoptotic signaling. The neoplastic cells resemble immunoblasts or bear anaplastic morphology. PEL is associated with two Herpes viruses infection – HHV8 and EBV (co-infection) [1, 25]. The HHV8 encodes more than ten homologues to cellular genes providing proliferative and anti-apoptotic signaling. The neoplastic cells resemble immunoblasts, plasmablasts or even anaplastic and HRS-like cells, the cytoplasm is abundant and deeply basophilic [1, 25]. The immunoprofile is usually CD20 and Bcl-6 negative, CD79a mainly positive [1, 25]. The lymphoma cells express CD138, CD30 and EMA, and these findings are suggestive for (aberrant) plasma cell differentiation or potential plasma cell origin [1, 25]. But the surface and cytoplasmic immunoglobulins are usually absent [1]. An aberrant T-cell marker expression may occur. EBV LMP1 is usually negative even in a presence of positivity of EBER in situ hybridization and
LANA protein is mainly expressed (HHV8-associated latent protein) [1]. Regardless of the therapy the clinical course is usually progressive and most of the patients die in less than 6 months from a diagnosis [1]. PEL was first recognised in HIV positive patients in AIDS stage and is mainly or nearly exclusively diagnosed in this group of patients and also in patients after solid organ transplantation [1, 25].

**Primary DLBCL of the CNS.** This type of DLBCL is very rare and clinically distinctive lymphoma with very variable morphologic features that represents primary intracerebral or intraocular localization of infiltration with restricted homing of neoplastic cells to CNS, eye (and also testis) [1, 7, 25, 44]. Any type of systemic DLBCL with CNS involvement should be excluded and also those associated with any immunodeficiency [1]. Dissemination to other sites is very rare. The neoplastic cells are typically present perivascularly, usually resemble centroblasts and express B-cell markers, Bcl-6 in up to 80%, MUM1/IRF4 in up to 90% and CD10 only in up to 20% of cases [1, 44]. In up to 40% of this type of lymphoma the BCL6 translocation is detected [1]. The primary DLBCL of the CNS bears very poor prognosis [1, 44].

**The gray zone lymphomas between BL and DLBCL, cHL and DLBCL.** Biological subgroups of DLBCL are defined by gene expression profile into GC-like, ABC-like and the "third" subgroup [1, 5, 7, 44]. Nevertheless the data on reliability of the immunohistochemical classifiers to distinguish GCB-like and nonGCB-like DLBCL as well as in predicting outcome are contradictory. The one of the most common genetic abnormalities (app. 30% of cases) in DLBCL is chromosomal translocations affecting the band 3q27 where the BCL-6 gene is located [1, 5, 7, 25, 44]. Translocation occurs predominantly within the major translocation cluster of BCL-6 [5]. Translocation of a one of the three IG genes or variable non-IG genes with juxtaposition to the BCL-6 locus leading to promoter substitution and the disturbance of the BCL-6 autoinhibitory loop [5]. The translocation of the BCL-2 gene next to the IGH gene through t (14;18)(q32;q21) is detected up to 45% of GCB-like DLBCL and is absent in ABC-like subgroup [1, 5, 25, 44]. A Myc break has been observed in most studies up to 10% of patients with classical DLBCL and has been linked to complex karyotypes and a very unfavorable outcome [1, 5, 25, 44]. Mutation of TP53 has been associated with poor survival in some series of DLBCL [5]. The aberrant hypermutation may represent a major contributor to lymphomagenesis of DLBCL, several genes like Bcl6, PIM1, MYC, RhoH/TTF and PAX5 usually passed through somatic hypermutation in DLBCL [5, 7]. The hypermutable and hypermutated genes are susceptible to chromosomal translocations due to generated DNA double-strand breaks [5]. Ongoing IGHV somatic hypermutation has been more frequently detected in GCB-like subgroup of DLBCL [5]. ABC-like subgroup of DLBCL bears abnormalities in the regulation of the immunoglobulin class switch recombination that may predispose to chromosomal translocations [5]. Borderline cases of DLBCL are difficult category which reflects heterogeneity of DLBCL de novo types and transformed ones e.g. from FL [1, 5, 25, 44]. One type is B-cell lymphoma, unclassifiable, with features intermediate between diffuse large B-cell lymphoma and Burkitt lymphoma and the second one is B-cell lymphoma, unclassifiable, with features intermediate between diffuse large B-cell lymphoma and classical Hodgkin lymphoma. The neoplastic cells of the first type of the lymphoma are large and more polymorphic than typical cells in BL and show positivity for CD20, CD79a, CD10, Bcl-6, c-Myc and negativity for Bcl-2, Ki67 is higher than 95% [1, 5, 7, 25, 44]. Starrry sky appearance, many mitotic figures and apoptosis are typically present [1, 25, 44]. In many of these cases the c-MYC translocation to non-immunoglobulin genes is usually detected (up to 50%) and also other translocations are presented usually those involving BCL-2 (app. 15%) [1, 5, 7, 44]. Sometimes both MYC and BCL-2 translocation...
exist in so called “double hit” lymphomas or even with BCL-6 translocation in “triple hit” lymphomas [1, 5, 44]. Molecular profiling studies have shown distinctive gene signatures for typical BL and DLBCL [1, 5, 44]. The diagnosis of this type of B-cell lymphoma should not be made in cases of morphologically typical DLBCL with MYC rearrangement or in cases of typical BL without detected c-MYC rearrangement [1]. The second one borderline type of the B-cell lymphoma relates to the overlap between classical Hodgkin lymphoma (cHL) and mediastinal LBCL (PMLBCL). The most common site of involvement is located in the anterior mediastium and regional lymph nodes, in contrast to PMLBCL the non-lymphoid organs are rarely involved [1, 7, 25]. This type of lymphoma is typically composed of a confluent neoplastic cells in diffusely fibrotic stroma [1, 7, 25]. The significant number of neoplastic cells are large abnormal mononuclear and binuclear cells that resemble morphology of HRS cells or even lacunar cells and the whole architectonic features are similar to a PMLBCL [1, 7, 25]. Scattered eosinophils, histiocytes and lymphocytes may be present [1]. The lymphoma cells express CD20, CD79a, CD30, CD15, surface or cytoplasmic immunoglobulins are typically absent [1, 7, 25]. Bcl-6 is variably expressed and CD10 is usually negative [1]. In app. 20% of these cases of B-cell lymphoma the EBV latent infection can be detected [1].

Double-hit B-cell lymphomas. Approximately 40% of all B-cell lymphomas are characterized by the presence of a recurrent reciprocal chromosomal translocations [1, 6, 44]. In most cases an oncogene is deregulated by juxtaposition to an enhancer of the immunoglobulin loci, whereas promoter substitution or fusion of genes leading to fusion proteins are less frequent [1, 6]. Certain translocations are characteristic for a specific type of lymphoma and are often considered as neoplasms-initiating events [6]. Lymphomas with recurrent chromosomal breakpoints activating multiple oncogenes one of which being MYC are often referred to as “Double Hit” (DH) lymphomas [1, 6, 44]. DH lymphoma is a rather imprecise term because it is neither restricted to B-cell lymphoma nor does it exclude 2 translocations activating oncogenes other than MYC [6]. Nevertheless the term DH lymphoma is mostly used for mature B-cell lymphomas with chromosomal breakpoint affecting the MYC locus [1, 6, 44]. The term DH lymphoma may be used for all cases with characterized multiple recurrent breakpoints, dual/triple/quadruple [6]. The most common DH lymphomas are lymphomas with MYC(q824) and BCL2(18q21) – B-cell lymphoma unclassifiable with features intermediate between DLBCL and BL [1, 6, 44]. The Mitelman Database of Chromosome Aberrations in Cancer contains virtually all published cytogenetic data on a wide variety of neoplasms including B-cell lymphomas, app. 62% with BCL2/MYC, 16% with BCL2/BCL6/MYC, app. 10% with CCND1/MYC, app. 8% with BCL6/MYC, app. 2% with BCL3/MYC [6]. MYC is transcription factor controlling the expression of a large set of target genes involved in cell cycle regulation, metabolism, DNA repair, stress response and protein synthesis [6]. MYC exerts its function by dimerization with MAX and subsequent binding to specific consensus DNA sequences (CACG TG) called E-box [6]. MYC is involved in the regulation of micro-RNA expression, usually represses many micro-RNA [6]. MYC expression is lower in GC compared to naive and memory B-cells and this lower expression may protect against MYC induced genomic instability in the GC [6]. Genomic alterations of the MYC gene include chromosomal translocations, mutations affecting regulatory sequences and promoter regions and copy number increase [6]. The most chromosomal breakpoints that involve MYC and the IGH locus are mediated by activation-induced cytidine deaminase (AIDCA) and not by recombinase activating gene 1/2 (RAG1/2) [6].

New perspectives

New perspectives and horizons in lymphomas are in general still evolving on all already above listed levels (clinical, morphologic, molecular, biologic, both diagnostic and experimental). We would like to mention only few of them that are more close to morphology and molecular biology.

Lymphoma microenvironment. DLBCL is usually diagnosed in older patients and the decreased immunosurveillance may play a role in (not only) DLBCL pathogenesis due to impaired anti-tumor and anti-viral immunologic reaction. Very good experimental model for immunocompromised state related lymphoma is AIDS related DLBCL (AR-DLBCL). AR-DLBCL compared to sporadic DLBCL shows high angiogenesis, increased hyperproliferation, reduced count of CD4+ and FOXP3+ T-helper cells, increased activated cytotoxic T-cells and HIV-infected patients with few tumor associated macrophages had very poor outcome [43]. Very robust R-IPI independent prognosticator is (status of) circulating host immunity (reflecting intratumoral immune microenvironment) and flow cytometry performed on fresh diagnostic lymphoma tissue (DLBCL) revealed high and low risk group according to percentage of CD4+ infiltrating T-helper cells, under 23% and more or equal 23% of CD4+ T-cells resp. [45, 46].

The process of homing of lymphoid cells. The “homing” process of lymphoid cells is depending on coordinated lymphocyte migration and recirculation to the microenvironments that control their differentiation, survival, disperses the immunologic repertoire and targets effector lymphocytes to sites of antigenic insult. This “remarkable” tissue specificity has already been described and published in 70’s [29, 57-59]. The “virgin” lymphocytes show usually homogeneous recirculation through secondary lymphoid tissues but the memory and effector lymphocytes form distinct subgroups with tissue-selective/specific migratory capability to provide “appropriate” immune cells and locally effective immune reaction [10, 27-31]. As few as one in 100 thousand lymphocytes are specific for a single antigen and secondary lymphoid organs bear a principal function in bringing antigen-specific lymphocytes into a physical contact to an antigen or antigen presenting-cells [10, 27-31]. The central role in
<table>
<thead>
<tr>
<th>LBCL</th>
<th>Main histologic feature(s)</th>
<th>Immunoprofile</th>
<th>Genetic feature(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>DLBCL</td>
<td>Variable</td>
<td>Variable</td>
<td>Variable</td>
</tr>
<tr>
<td>Centroblastic variant</td>
<td>More than 90% of centroblasts</td>
<td>Variable</td>
<td>Variable</td>
</tr>
<tr>
<td>Immunoblastic variant</td>
<td>More than 90% of immunoblasts</td>
<td>Variable</td>
<td>Variable</td>
</tr>
<tr>
<td>Anaplastic variant</td>
<td>Large and very large cells with distinct anaplasia, HRS-like cells</td>
<td>Variable</td>
<td>Variable</td>
</tr>
<tr>
<td>Rare variants</td>
<td>Spindle-shaped, signet ring cells, etc.</td>
<td>Variable</td>
<td>Variable</td>
</tr>
<tr>
<td>GCB-like</td>
<td>Variable</td>
<td>Variable</td>
<td>GEP of GC B-cells</td>
</tr>
<tr>
<td>ABC-like</td>
<td>Variable</td>
<td>Variable</td>
<td>GEP of activated B-cells</td>
</tr>
<tr>
<td>CD5-positive</td>
<td>Variable</td>
<td>Usually de novo, only rarely arising from B-CLL/SLL, cyclin D1 negative</td>
<td>Variable</td>
</tr>
<tr>
<td>GCB-like</td>
<td>Variable</td>
<td>Immunophenotype acc. Hans´ classifier</td>
<td>Doesn´t correlate exactly with GEP</td>
</tr>
<tr>
<td>Non-GCB-like</td>
<td>Variable</td>
<td>Immunophenotype acc. Hans´ classifier</td>
<td>Doesn´t correlate exactly with GEP</td>
</tr>
<tr>
<td>THRLBCL</td>
<td>Scattered and single neoplastic B-cells, usually centroblasts and HRS-like cells, in a dominant “background”</td>
<td>Usually Bcl-6 positive, ETA and Bcl-2 variably positive</td>
<td>Variable</td>
</tr>
<tr>
<td>Primary of the CNS</td>
<td>Usually centroblasts characteristically in perivascular spaces</td>
<td>Usually express MUM1/IRF4 and Bcl-6, only minority CD10 positive</td>
<td>High load of ongoing somatic hypermutations, up to 40% with BCL-6 translocations</td>
</tr>
<tr>
<td>PCLBCL, leg type</td>
<td>Confluent sheets of centroblasts and immunoblasts</td>
<td>Strong expression of Bcl-2, MUM1/IRF4, usually Bcl-6 positive and CD10 negative</td>
<td>Amplification of the BCL-2 gene, translocation of c-MYC and BCL-6, GEP of ABC-like</td>
</tr>
<tr>
<td>EBV-positive of the elderly</td>
<td>Diffuse infiltration with effacement, large cells, centroblasts, HRS-like cells</td>
<td>MUM1/IRF4 usually positive, CD10 and Bcl-6 typically negative, variably CD30 positive, usually LMP1 positive</td>
<td>Variable</td>
</tr>
<tr>
<td>PMLBCL</td>
<td>Usually ass. with compartmentalizing alveolar fibrosis, medium to large cells with pale cytoplasm, HRS-like cells may be detected</td>
<td>CD30, CD23 and MUM1/IRF4 usually expressed, Bcl-6 and Bcl-2 variably expressed</td>
<td>High load of ongoing somatic hypermutations, GEP shares features with CHL, rearrangements of BCL-2, BCL-6 and c-MYC are rare</td>
</tr>
<tr>
<td>Intravascular</td>
<td>Intravascular localization of neoplastic B-cells in small and intermediate vessels including sinusoids</td>
<td>Expression of CD5 and CD10 may be detected</td>
<td>Few cases have been studied</td>
</tr>
<tr>
<td>ass. with chronic inflammation</td>
<td>Usually centroblasts/immunoblasts</td>
<td>In cases with plasmacytic differentiation loss of pan B-cell positivity is detected with expression of CD138 and MUM1/IRF4, LMP1 positive, CD30 variably expressed</td>
<td>Complex karyotypes, overexpression of IFI27, downregulation of HLA class I expression</td>
</tr>
<tr>
<td>LyG</td>
<td>Angiocentric and angiodestructive polymorphous lymphoid infiltrate, EBV-positive cells with immunoblast and HRS-like morphology, Grading acc. proportion of EBV-positive cells</td>
<td>CD30 variably expressed, CD15 negative, LMP1 positive</td>
<td>Demonstration of clonal rearrangement of immunoglobulin genes is more consistent in grade 2 and 3</td>
</tr>
<tr>
<td>ALK-positive</td>
<td>Immunoblasts, plasmablasts, multinucleated giant cells</td>
<td>Usually granular cytoplasmic ALK positivity, EMA and CD138 expression, CD30 usually negative</td>
<td>The most frequent is t(2;17)(p23;q21) with CTLC-ALK fusion protein</td>
</tr>
<tr>
<td>Plasmablastic</td>
<td>Immunoblasts, plasmablasts</td>
<td>Commonly positive for CD138, MUM1/IRF4, EMA, CD30, weak positivity of CD20, PAX5, usually negative for CD56, clgM positive, lambda light chain restriction, LANA-1 positive</td>
<td>Variable</td>
</tr>
<tr>
<td>arising in HHV8-ass. multicentric Castleman disease</td>
<td>Milieu MCD with neoplastic plasmablasts in mantle zones, scattered in interfollicular areas, in progression neoplastic cells form sheets</td>
<td>Lack pan B-cell markers positivity, usually positive for CD138, CD30, EMA</td>
<td>Microlymphomas may be monoclonal or polyclonal, unambiguous PI is immunoglobulin genes are unmutated, Immunoglobulin genes are clonally rearranged, T-cell receptor genes may be clonally rearranged</td>
</tr>
<tr>
<td>Primary effusion lymphoma</td>
<td>Serous effusion without tumor masses, immunoblasts, plasmablasts, anaplastic cells, HRS-like cells</td>
<td>Usually CD10 and Bcl-6 positive, Bcl-2 and MUM1/IRF4 variably positive or variable</td>
<td>Up to 50% with c-MYC translocation, up to 15% with BCL-2 translocation, both detected in double hit lymphoma, BCL-6 translocation is less frequent</td>
</tr>
<tr>
<td>B-UCL with features intermediate between DLBCL and BL</td>
<td>Medium to large neoplastic cells, starry sky pattern, prominent mitotic and apoptotic activity</td>
<td>Pan B-cell markers positive, PAX-5, CD30 and CD15 usually positive, Bcl-6 variably positive, CD10 negative</td>
<td>Specific genomic studies have not been performed</td>
</tr>
<tr>
<td>B-UCL with features intermediate between DLBCL and chL</td>
<td>Confluent growth with pleomorphic cells that show variable cyto logical appearance in a diffusely fibrotic stroma</td>
<td>Up to 50% with c-MYC translocation, up to 15% with BCL-2 translocation, both detected in double hit lymphoma, BCL-6 translocation is less frequent</td>
<td></td>
</tr>
</tbody>
</table>

lymphocytes movements inside lymphoid organs is layed on family of chemokines [10, 28, 30, 31]. The lymphocyte homing is multistep process that requires chemotaxis and cell adhesion [10, 27-31]. The lymphoma dissemination is usually conserved physiological behavior rather than a reflection of lymphoma progression [10]. The dissemination patterns often reflect basic rules of lymphocyte homing, explaining the strikingly tissue-specific dissemination, e.g. mucosal lymphomas, cutaneous lymphomas and multiple myeloma [10]. Understanding the molecular mechanisms underlying the homing behavior may provide novel targets for treatment fo lymphoma [10].

Specific recognition of foreign antigens and effective surveillance are the 2 mainstays of the body’s defense against microbial invasion [10, 28, 30, 31]. Evolution has created great antigen-receptor diversity and has equipped lymphocytes with exquisite motility and migratory properties to accomplish these tasks [10, 28, 30, 31]. As discovered more than 4 decades ago by Gowans and Knight, mature lymphocytes circulate, moving continuously from blood to tissue and back to the bloodstream again [28]. Within the tissues lymphocytes display a characteristic ameboid form of cell migration that represents a physically optimized migration mode which allows easy cell traffic toward and between different tissue compartments [10, 28]. Lymphocyte-endothelial recognition plays an important role in controlling the access of specialized lymphocyte subsets to particular tissues and thus influencing the nature of local immune and inflammatory responses [10, 27-31]. The homing of circulating lymphocytes is partly directed by high endothelial venules due to lymphocytes adhering and migration between blood and targeting tissues (tissue specific distribution) [10, 27-31]. At the molecular level the homing process is regulated by adhesion molecules (vascular adressins) in concert with chemokines [10, 27, 28, 30, 31]. Lymphocytes and endothelial cells specifically program their expression of adhesion molecules and chemokines / chemokine receptors, allowing lymphocytes to move selectively to specific functional compartments of an immune system [10, 27, 28, 30, 31]. The final distribution of neoplastic lymphocytes depends on the balance of entry , proliferation and retention [10, 30, 32, 33].

The molecular basis of lymphoma dissemination.

A number of clinical observations suggest that conserved homing programs mediate the dissemination of non-Hodgkin lymphoma, e.g. B-chronic lymphocytic leukemia/small lymphocytic lymphoma and mantle cell lymphoma usually show systemic dissemination at presentation whereas NHLs related to lymphocytes undergoing active proliferation and differentiation such as DLBCL and BL are often initially localized [10]. Tumor dissemination to sites of trauma and inflammation is regularly observed in lymphoma patients implying specific recruitment of tumor cells by locally produced chemokines and activated endothelium [10]. Extralodal lymphomas arising in the gut-associated lymphoid tissues or the skin show a strong preference to disseminate to mucosal sites and skin resp., they may eventually disseminate to the regional lymph nodes [10].

The trafficking pathways of T- and B-lymphocytes are markedly different reflecting the distinctive functions of T and B cells in the immune system [10, 28]. B-cell effector function depends primarily on antibodies produced by plasma cells [10]. These antibodies are solubilized in body fluids and hence can act at distance obviating the need for B cells to migrate to peripheral sites of antigenic insult [10, 27-31]. B-cell migration to the extralymphoid tissue sites occurs almost exclusively in the context of chronic inflammation driven by locally persistent antigen, e.g. Borrelia Burgdorferi, Helicobacter pylori, Sjögren disease [10]. This persistent antigen stimulation can lead to neoformation of lymphoid tissue with local expression of vascular adressins and chemokines that are physiologically expressed in organized lymphoid tissues [1, 10, 27-31]. At these site of chronic antigenic stimulation in the skin or other extra-lymphoid sites such as the stomach, intestinal mucosa or orbit B-cell lymphomas can arise – usually the marginal zone type and follicular lymphoma [1, 10]. B-cells consequently must adapt their homing signature to their specific maturational stage [10]. These maturation-dependent profiles are largely conserved in B-cell lymphomas and control their dissemination, e.g. naive B-cells coexpress the PLN (peripheral lymph node)-homing receptor L-selectin and the intestinal –homing receptor α4β7 [10]. Combined expression of these molecules has also been reported on a subset of mantle cell lymphomas (MCL), that generally lack somatic hypermutations in their immunoglobulin variable genes and therefore presumably derived from naive B-cells [10]. Expression α4β7 in MCL is associated with a clinical presentation known as malignant lymphomatous polyposis characterized by multifocal involve ment of the gastrointestinal tract as well as widespread lymph node involvement [10]. In addition to the adhesion profile of the MCL the chemokine receptor profile of these tumors that includes CCR7 and CXCR4 also allows wide dissemination to PLN and mucosal sites [10]. The intestinal homing receptor α4β7 is of the key importance for the homing of normal memory B-cells and plasmablast producing IgA to the marginal zone of the Peyer patches and the intestinal lamina propria resp. and malignant counterpart extranodal marginal zone B-cell lymphoma MALT type express α4β7 and coexpression of L-selectin remain this tumor localized and disseminated in mucosal topography [10]. Primary GIT follicular lymphomas also express α4β7 and are often IgA positive oposite to primary nodal type [10]. But in contrast DLBCL and Burkitt lymphoma in primary GIT localization do not express α4β7 [10]. The major chemoattractants for B-cells are CXCL12 and CXCL13, both of them are present on HEVs of lymph nodes and Peyer patches and are also present on HEV-like vessels at sites of lymphoid neogenesis (e.g. chronic inflammation – Borrelia Burgdorferi, Sjögren syndrome) [10]. CXCL12 guides CXCR4-positive lymphocytes to the GC dark zones whereas CXCL13 is produced by FDCs and attracts CXCR5 positive B-cells into the light zones [10]. The chemokine receptors CXCR5 and CXCR4
are widely expressed in B-cell neoplasms including B-CLL, HCL, MCL, MALT lymphomas, FL and DLBCL and these receptors can drive migration of lymphoma cells [10]. Ectopic chemokine expression at sites of chronic inflammation with lymphoid neogenesis presumably is a key factor in the selective homing of malignant B-cells to these sites [10]. Autocrine expression of CXCL13 has been reported in FL and primary central nervous system lymphomas [10]. Another molecule – JAM-C molecule (junctional adhesion molecule) is expressed on vascular endothelial cells and B-lymphocytes [30]. JAM-B is a ligand to JAM-C molecule (receptor) and is expressed on lymphatic endothelium [30]. JAM-C positive B-lymphocytes and lymphoid neoplasms are derived from marginal-zone in contrary to JAM-C negative B-lymphocytes and lymphoid neoplasms that originate from germinal center [30]. The antiadhesion antibodies anti-JAM-C reduce the migration of B-lymphocytes (JAM-C positive) and their malignant counterparts to bone marrow, spleen (the most remarkable) and lymph nodes, and long term using of this antibodies prevents engraftment of JAM-C positive B-lymphomas to spleen, lymph nodes and bone marrow [30]. The administration of JAM-C targeted antibodies is another new potential and tailored therapeutic strategy in blocking “dissemination” or neoplastic homing of B-lymphoid neoplasms expressing JAM-C adhesion molecule [30]. Very enigmatic lymphoproliferation is follicular lymphoma in situ (FLIS) with typical folliculotropism and no evidence of concomitant overt B-cell lymphoma at presentation or on follow-up in app. 40%-60% of cases [32]. The precursors of neoplastic follicular lymphoma cells are generated in a bone marrow due to aberrant recombination of VDJ genes with the deregulation of Bcl-2 expression in B-cell population [32]. The precursor/preneoplastic cells are harboring characteristic translocation t(14;18)(32;21), display only limited neoplastic/immortality potential and require additional hit(s) for completeneoplastic transformation [1, 32]. The reactive secondary follicles and GC are colonized by B-cells expressing CD10 and Bcl-2 these cells were also deced in patients with synchronously or metachronously diagnosed lymphomas and even clonally related to these lymphomas [32]. This suggests a phenomenon of follicular homing of precursor and/or lymphoma B-cells to the reactive follicles rather than “multifocal” primary evolution of neoplasm, neoplastic or preneoplastic field in the lymph nodes [32].

Very unique environment for B-cell homing is the liver with B-lymphocytes forming only 10% of all lymphocytic population and specialized hepatic sinusoidal endothelial cells where the classical adhesion with rolling is usually not detected and the initial tethering is only brief and selectin independent [33]. The patterns of B-cell lymphoma infiltration in a liver (sinusoidal, nodular, portal) reflect the homing mechanisms of malignant cells with distinct differences in molecular mechanisms of adhesion, crawling and transmigration [33]. The adhesion with following transmigration of non-neoplastic B-cells is mainly dependent on VCAM-1, VAP-1 and CLEVER-1 (also expressed on endothelium in lymphoid tissues) [33]. The ligands for CXCR3 and CXCR4 are expressed on inflamed hepatic sinusoids and these receptors contribute for chemokine mediated transendothelial migration of B-lymphocytes and their malignant counterparts [10, 33]. Very important is also the knowledge of the proliferation and cell division status of B-lymphoid cells because actively dividing cells are unable to transmigrate across endothelial lining [33]. The specific recruitment of the neoplastic B-cells to the liver is suggesting also the new potential therapeutic target(s) to prevent B-cell lymphoma dissemination to the liver [33].

The differentiation of B-lymphocytes into plasma cells is accompanied by coordinated change in chemokine receptor expression. CXCR5 and CCR7 are down-regulated resulting in loss of responsiveness to the B and T-zone chemokines CXCL13, CCL19, CCL21, and CXCX4, receptor for CXCL12 is up-regulated and its interaction is required for plasma cells homing to the BM, for recruitment and retention [10]. CXCL12 promotes transendothelial migration and induction of α4β1-mediated adhesion to VCAM-1 and fibronectin that are expressed by stromal cells [10]. The blocking antibody/ies that prevent interaction of α4β1-mediated adhesion disturb both chemoattractant induced adhesion/migration and also inhibit growth of MM cells [10]. Blocking integrin-mediated interactions between BM stromal cells and MM cells may also disturb the niche required for MM cells expansion [10]. The adhesion of human MM cells to BM stromal cells stimulates secretion of IL-6 that has potent proliferative and antiapoptotic effects [10]. The c-Maf oncogene besides promoting expression cyclin D2 also promotes expression of β7-integrins and enhancing MM adhesion to BM stromal cells and increasing VEGF production which in turn stimulates MM growth and survival as well as angiogenesis [10]. Cytokins including IL-6, HGF, and WNTs produced by BM stromal cells provide MM cells with proliferative and and survival signals required for their expansion. The α4β1 and α5β1 mediated adhesion to fibronectin can protect MM cells from drug induced apoptosis – a phenomenon called cell adhesion mediated drug resistance (CAM-DR) [10]. Because various chemokines and growth factors produced in the BM stimulate integrin mediated adhesion, these cytokines can contribute to resistance of MM cells to a treatment [10]. Hence integrins and their regulation by chemokines play a crucial role in the homing of MM cells to the BM and contribute to the expansion of the MM cells in the BM niche by mediating interaction with the BM stroma that via “outside-in signaling” generates growth and survival signals for the malignant plasma cells [10]. This is very good and still evolving model of lymphoma biology with the striking impact on a new potential strategies in the optimized targeted therapy [10]. Prospective new agents against integrins α4 (natalizumab) and α4β7 (MLN02) may play very important role in future for the treatment of lymphoid malignancies [10].

**New future in immunoprofiling.** The cell surface capture technology (CSC) is a mass spectrometry-based method,
that can identify cell surface glycoproteins including cluster of differentiation (CD) proteins [42]. CSC can be a part of systematic and quantitative analysis of differentially expressed cell surface proteins [42].

**Idiotype vaccination for B-cell lymphomas.** B-cell lymphomas have proven immunogenicity that can be used in the new immunotherapeutic strategy era (anti-tumor vaccines) due to tumor-specific immune response and even remission on the molecular level currently described in follicular lymphoma [60]. But the development of anti-lymphoma vaccines (individualized idiotypic vaccines) is still time-consuming and very expensive complex process [34].

**Conclusion**

In a brief the DLBCLs (and also lymphomas in general) show a huge and still evolving heterogeneity on a clinical, morphological and molecular diagnostic level and only tight and clearcut cooperation of the whole hematologic team (clinician, hematopathologist, molecular biologist and statistician) is a real benefit for correct diagnosis with a potential influence on (more and more) targeted and tailored therapy and also on a new insight on and design of experimental algorithms and useful new algorithms in translation medicine close to the real patient(s).

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