EUROPEAN HEMATOLOGY ASSOCIATION

Aggressive lymphoma - Section 1

The biological basis of aggressive lymphoma

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Take-home messages

- Aggressive mature B-cell lymphomas are a heterogeneous group of diseases with different clinical and biological features that may be related in part to the diverse pathogenic molecular mechanism.
- The identification of molecular subtypes and specific genetic alterations in aggressive DLBCL are clinical relevant and may guide therapeutic strategies.

Aggressive mature B-cell lymphomas are a heterogeneous group of diseases with different clinical and biological features.[1] The most common subtype accounting for approximately 80% is diffuse large B-cell lymphoma, not otherwise specified (DLBCL, NOS), a category that includes tumors that cannot be recognized in any of the other more specific entities. Other aggressive B-cell lymphomas are Burkitt lymphoma and different subtypes of large B-cell lymphomas that may be grouped in those originated in specific topographic sites, associated with EBV and/or HHV8 infection, and expressing a terminal B-cell differentiation phenotype among others. The updated WHO classification includes a provisional category of high grade B-cell lymphomas (HGBL) that highlights the relevance of *MYC* together with *BCL2* and/or *BCL6* translocations. The marked diversity of all these tumors is related to different pathogenic mechanisms.

Diffuse large B-cell lymphoma, NOS is very heterogeneous. One of the major advances understanding its diversity was the recognition of two molecular subtypes based on their gene expression profiling (GEP) related to the different cell of origin (COO) in germinal center B-cells (GCB) or activated B-cells (ABC).[2] In addition to the GEP these two molecular subtypes differ in the activation of different molecular pathways, profile of chromosomal alterations and somatic mutations (Table 1). These biological differences translate into different outcome of the patients with most of the studies showing worse prognosis for ABC than GCB-DLBCL. GCB tumors rely preferentially on the activation of the PI3K pathway whereas ABC tumors have a constitutive activation of the NFkB pathway through different mechanisms including the activation of the BCR signaling pathway. Genetic alterations in GCB include *BCL2* translocations, activating mutations in the histone modifier *EZH2*, loss of function mutations of *GNA13* that regulates the topographic location of germinal center B-cells and inactivating mutations of *TNFRSF14* that lead to a cell-autonomous activation of B cell proliferation. ABC tumors have frequent activating mutations in the BCR and TLR pathways including *CD79a, CARD11,* and *MYD88*, inactivating mutations in inhibitors of NFkB such as *TNFAIP3*, and mutations preventing the terminal B-cell differentiation of B-cells such as inactivating mutations of *PRDM1*.[2,3] In spite of these different alterations, GCB and ABC-DLBCL share also common alterations including alterations in *TP53*, histone modifiers (*CREBBP, KMT2D*), *FOXO1*, *BCL6* translocations and point mutations, and inactivating mutations in genes related to immunesurveillance (e.g. *B2M*). Given the interest in defining different therapeutic strategies the recognition of these GCB and ABC DLBCL molecular subtypes is now recommended in the clinical practice and can be performed using different immunohistochemical algorithms or gene expression based assays.[4,5]

Primary mediastinal large B-cell lymphoma (PMBL) usually presents in young females with a large mediastinal mass that may infiltrate surrounding structures. Gene expression profiling has identified a specific signature that may be useful to differentiate PMBL from DLBCL, NOS involving the mediastinum or to recognize these tumors in locations outside the thorax (Table 1).[6] The genetic profile differs from DLBCL, NOS with frequent translocations inactivating *CIITA*, and activation of the NFkB and JAK/STAT pathways due to several genetic alterations in regulatory genes such as *TNFAIP3* and *SOCS1* and *PTPN1*, respectively.[6]

Burkitt lymphoma (BL) is a well defined entity genetically characterized by *MYC* rearrangements. In the last years NGS have revealed the profile of somatic mutations with frequent mutations in *TCF3* and *ID3* that are very uncommon in DLBCL and lead to the activation of the PI3K pathway. Activating mutations of *CCND3* are found in 30% of the

Aggressive lymphoma - Section 1

Table 1. Distinctive molecular characteristics of aggressive B-cell lymphomas.

	GCB	ABC	PMBCL	HGBL-DH
Cell of origin	Germinal Ctr cell	Activated B cell	Thymic mature B cell	Germinal Ctr cell
Chromosomal alterations	BCL2-R BCL6-R MYC-R	3q, 18q, 19q gains 9p del BCL6-R MYC-R	9p24 gain/amp CIITA	MYC-R and BCL2 and/or BCL6-R
Somatic mutations	EZH2, GNA13, TNFRSF14	MYD88, CARD11, CD79B, A20, PRDM1	JAK, SOCS1, PTPN1, A20	EZH2, CREBBP, ID3, CCND3,
Altered pathways	PI3K GNA13	NFkB BCR	NFkB	JAK/STAT

R: rearrangements.

tumors. An unresolved issue is the existence of true BL without *MYC* translocation. Recent studies have identified cases with similar morphology and phenotype but negative for *MYC* rearrangements that have 11q alterations with proximal gains and telomeric losses. These cases have been named Burkitt-like lymphoma with 11q aberrations and have more frequent nodal presentation and complex karyotypes.[1] The information on these cases is still limited.

In the last years, the possibility to study MYC protein expression and gene alterations in routine cases has expanded the knowledge of *MYC* driven aggressive lymphomas. *MYC* rearrangements can be found in virtually all BL, 10-15% DLBCL, NOS, 50% of plasmablastic lymphomas and in around 50% of HGBL.[7] The updated 2016-WHO classification has considered the provisional category of HGBL with *MYC* and *BCL2* and/or *BCL6* rearrangements that includes all large B-cell lymphomas with these alterations, independently of their morphology.[1] These cases have been also named "double hit" HGBL (HGBL-DH).[8] Cases with blastoid morphology or with features intermediate between DLBCL and BL without translocations are considered HGBL, NOS. DLBCL with high expression of MYC and BCL2 protein without the double genetic hit alterations are called "dual-expressors" (DLBCL-DE).[1,9] This DE is considered an adverse prognostic factor but these tumors are not included in the HGBL category since the outcome does not seem so adverse. The biology of HGBL-DH is complex and not yet well understood.[8,9] The presence of *MYC* rearrangements is the determinant factor but there are several additional elements that modulate their biological relevance.[8-10] The association with *BCL2* translocations usually confers an adverse prognosis but the role of *BCL6* is still controversial.[9,10] Other modulators of *MYC* rearrangements are the translocated partner. IG-*MYC* rearrangements induce higher levels of MYC expression than non-IG partners and this may explain the worse prognosis of the former. The MYC and BCL2 protein expression levels may be also different in cases with translocation probably be due to additional phenomena such as amplification of the translocated allele, mutations of *MYC* or others. The cell context may also be a modifier with DLBCL cases having a better outcome than tumors with blastoid or DLBCL/BL intermediate morphology. The clinical features of the patients seem also important since patients with high-risk clinical features have more adverse evolution than patients with low-risk factors.[9,10] Mutations in other genes may also contribute to the aggressiveness of these tumors (Table 1).[11,12]

In summary, the increasing knowledge of the molecular pathogenesis of aggressive B-cell lymphoma is providing a better understanding of the clinical heterogeneity of these tumors and provide solid basis for new therapeutic approaches. The significance of the new HGBL-DH category is clinically relevant because most of the tumors have a very aggressive behavior and standard DLBCL treatments are considered insufficient. However, the particular relevance of some of these alterations, the possible relationship between the different molecular alterations, and how they should guide clinical intervention are still open questions that require further studies.

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Aggressive lymphoma - Section 1

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