Diffuse large B-cell lymphoma: classic and novel prognostic factors and their impact on therapeutic decisions

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A B S T R A C T

The International Prognostic Index, originally established to predict outcome of patients with aggressive lymphoma treated in the pre-rituximab era, has been confirmed to be a valid prognosticator for patients receiving rituximab, with the differences between the four risk groups (low, low-intermediate, high-intermediate and high) being smaller, yet significant compared to the pre-rituximab era. While many IPI risk groups have now a cure rate of over 80%, young high-risk patients and all elderly patients except for those with low risk fare worse, warranting further improvement. Apart from the IPI (and independent of it), there are other subsets of diffuse large B-cell lymphoma (DLBCL) that are characterized by criteria not included in the IPI or are too rare to be recognized in multivariable analyses. This applies to very old patients (>80 years), histological subgroups like DLBCL with immunoblastic or plasmablastic morphology, and Epstein-Barr (BV)-positive B-cell diffuse large B-cell lymphoma of the elderly, the germinal center *versus* the non-germinal center subgroups, DLBCL with MYC breakpoints (including double- and triple hit DLBCL), and expression of MYC together with BCL2 protein. Finally, patients presenting with skeletal involvement or developing central nervous system (CNS) involvement during the course of disease, represent a subpopulation with an almost always fatal course. Strategies to improve the outcome of these prognostically very poor subgroups will be discussed.

Learning goals

At the conclusion of this activity, participants should be able to:
- describe relevant clinical, morphological and molecular risk factors associated with a worse outcome in the rituximab era;
- select appropriate up-front therapy based upon the presence of certain risk factors;
- discuss treatment options for subgroups of DLBCL for which standard therapy is inappropriate.

Introduction

Prognostic factors are (usually) pre-therapeutically identifiable parameters of the tumor and/or the patient that affect the patient's outcome. They emerge and are valid only in the context of a given therapy and are likely to change with different therapies. Numerous factors that affect the prognosis of patients with diffuse large B-cell lymphomas (DLBCL) have been claimed in recent years, and very few have survived scrutiny. In the following review, we will discuss those risk factors which are valid in the rituximab era, i.e. under a treatment with CHOP[1] or CHOP-like chemotherapy in combination with the anti-CD20 antibody rituximab.

The International Prognostic Index

The International Prognostic Index (IPI) is the widely accepted prognostic factor index for patients with aggressive lymphomas. It was introduced by Shipp *et al.*[2,3] in the 1990s and was based on an individual case-based prognostic factor analysis of cyclophosphamide, doxorubicin, vincristine, and pred-nisone (CHOP)–like regimens[1] with overall survival (OS) as the end point. The IPI considered five factors: age (≤60 years *vs.* >60 years), lactate dehydrogenase (LDH) value (≤ upper limit of normal [ULN] *vs.* > ULN), performance status (Eastern Cooperative Oncology Group [ECOG] 0, 1 *vs.* >1), Ann Arbor stage (I/II *vs.* III/IV), and the number of extranodal involvements (0, 1 *vs.* >1). The age-adjusted IPI (aaIPI) for younger patients includes the factors LDH, performance status, and stage. The IPI score separates four prognostic groups based on the number of factors present (0, 1: low risk group; 2: low-intermediate risk group; 3: high-intermediate risk group; and 4, 5: high-risk group). The IPI has been widely used and reproduced to analyze various conventional, high-dose, and dose-dense regimens.[1,4-6] Recently, a major improvement in treatment outcome has been achieved by adding rituximab to CHOP-like regimens.[7-12] The revised IPI or "R-IPI" with only three risk groups as suggested by Sehn *et al.*[13] was based on only 365 patients treated with R-CHOP (rituximab plus CHOP) and this suffered initial technical problems (*e.g.* no method to protect against errors of misclassifying ordered risk strata due to its low statistical power, no multivariable model approach, no independent validation set) and did not

hold up to scrutiny when appropriately tested. Rituximab significantly improved treatment outcome within each IPI group resulting in a quenching of the Kaplan-Meier estimators. While the differences became smaller between the four risk groups under R-CHOP, the IPI retained its highly significant prognostic power with respect to all three end points and the ordering of the IPI groups remained valid, demonstrating that the IPI is still valid in the R-CHOP era.[14] In the Mega-CHOEP trial, young patients with aaIPI of 2 had a 3-year survival of 90%, and aaIPI of 3 73% after 8 x R-CHOEP-14.[11] Therefore, in young patients, only the high-risk group with a 3-year survival of less than 75% definitely represents a clinically relevant risk group, while for patients with aaIPI of 2 it will be difficult to achieve and demonstrate further improvement. Since both CHOP-14 and CHOEP-14 leave room for further toxicity, combinations with targeted therapies like bortezomib, lenalidomide or ibrutinib are currently being evaluated in this population of young patients with high-risk DLBCL.

The situation is different in elderly (age 61-80 years) DLBCL patients, with a 3-year overall survival of 88% for low-risk, 78% for low-intermediate, 67% for high-intermediate and 58% for the high-risk group,[10] all but the low-risk group have a high risk of failure and must be improved. The increased toxicity in elderly patients leaves little room for additional hematotoxicity, and strategies pursued include dose-dense application of rituximab, adding other CD20 monoclonal antibodies or antibodies directed against targets other than CD20, addition of lenalidomide to R-CHOP, or lenalidomide or enzastaurin for maintenance therapy.

Morphological subtype

Immunoblastic subtype

In a study of morphological and immunohistochemical biomarkers in elderly patients treated both with and without rituximab within the RICOVER-60 of the German High-Grade Non-Hodgkin Lymphoma Study Group (DSHNHL), immunoblastic morphology emerged as a robust, significantly adverse prognostic factor,[15] confirming a previous study in DBLC.[16] Patients with the immunoblastic subtype had a significantly lower CR/CRu and an inferior 3-year event-free survival (EFS) (P=0.013) and OS (54% $vs.$ 78%; P=0.004), while the survival curves for all other subtypes of DLBCL closely matched the curve of centroblastic lymphomas.[15] This also applies to primary mediastinal B-cell lymphoma, which did not differ from other DLBCL in the MInT trial when treated with rituximab.[17] In multivariate analysis adjusted for the factors of the IPI, the immunoblastic subtype was an independent predictor for EFS (relative risk [RR] 1.5; P=0.034) and OS (RR 1.7; P=0.007). So far no specific therapeutic approaches have been developed for immunoblastic DLBCL.

Plasmablastic subtype

This subtype has been recently characterized as an aggressive lymphoma, most frequently arising in the oral cavity of HIV-infected or elderly patients, with a male predominance. In the RICOVER-60, after a median fol-

low up of 72 months, 2 of 7 patients with plasmablastic subtype are alive in complete remission for more than six years, and the median overall survival of these patients was 13 months. In another series of 12 patients, 6 of whom were HIV-positive, 8 are alive after a median follow up of more than 11 months.[18-20] Obviously, the outcome of plasmablastic lymphomas is not as dismal as originally reported.

Age-related EBV-associated B-cell lymphoproliferative disorders

EBV-positive B-cell diffuse large B-cell lymphoma of the elderly (also known as senile EBV-associated B-cell lymphoproliferative disorder) is an EBV-positive clonal B-cell lymphoid proliferation that occurs in patients over 50 years of age and predominantly in elderly patients without any known immunodeficiency or prior lymphoma. It accounts for 8%-10% of DLBCL in Asia, and for 20%-25% of DLBCL in patients over 90 years of age. These patients are diagnosed at older age, present more often with elevated LDH, poor performance status, B symptoms, and frequent skin and lung involvement.[21] B symptoms and age over 70 years, but not IPI, appear to be reliable prognostic factors. Patients with 0, 1 or 2 of these risk factors have a median overall survival of 56, 25 and 9 months. The 5-year survival in a series of 96 patients was 25%.[21,22]

Age

Age is one of the strongest prognostic factors in the IPI. This is not only due to increasing comorbidities of elderly patients, but also because adverse biological features like the ABC-type and MYC breaks are enriched in the elderly population. While the IPI discriminates between patients aged 60 years or under and those over 60 years, a modification of the IPI, the IPI for elderly patients or E-IPI, was suggested using 70 instead of 60 years as a cut-off point to delineate older age as a risk factor.[23] However, the prognostic discrimination provided by the E-IPI for elderly DLBCL patients needs validation by other datasets. The results of the RICOVER-60 trial suggest that 75 years is a cut-off above which the outcome of patients with DLBCL shows the sharpest decline, with more therapy-associated deaths in this population and more primary progressions. Best results in patients over 80 years of age have been reported with a combination of rituximab and dose-reduced CHOP,[24] the 2-year survival rate of 59% representing an acceptable compromise between efficacy and toxicity, but further prospective trials in this population are badly needed.

The underrepresentation of patients over 70 years of age in studies designed for 'elderly' patients often prohibits meaningful multivariate analyses adjusting for higher age ranges. Even fewer prospective data are available for octogenarians or nonagenarians, even though this population of DLBCL patients is increasing fast. In a retrospective analysis of 205 NHL patients, most of them with DLBCL, who were treated at a single institution from one center, death was shown to be mainly due to lymphoma, justifying and warranting treatment of NHL patients over 80 years of age.[25]

Gender

Male gender is a negative prognostic factor in (elderly) patients treated with rituximab,[26] because female patients have a considerably higher benefit from the addition of rituximab to CHOP chemotherapy than male patients.[27] This is most likely due to the slower rituximab clearance in elderly females that results in higher serum levels, longer serum half-life elimination time and larger area under the curve data.[27] As a consequence, the DSHNHL performed the SEXIER-R CHOP-14 study with more than 250 elderly DLBCL patients, dosing female patients at standard 375 mg/m^2, and male patients at 500 mg/m^2. This resulted in slightly higher serum levels in elderly males compared to females. Efficacy data from this study will not be available until 2014.

A historical comparison of the RICOVER-60 results with the SMARTE-R-CHOP-14 study, a phase-II pharmacokinetic-based study with R-CHOP-14, in which 8 administrations of rituximab at standard dose were given dose-dense at the beginning with increasing intervals and the last application on Day 239, showed an improved outcome of elderly high-risk (IPI 3-5) patients with this extended rituximab exposure time.[28] This better outcome was due to a 20% improvement in 3-year PFS and OS of high-risk elderly males with their faster rituximab clearance who benefited more from the extended exposure time than females: indeed, with the SMARTE-R rituximab schedule, the differences between males and females disappeared.[29] The OPTIMAL>60 study is currently comparing 8 × 2-week administrations of rituximab with a pharmacokinetic-based schedule in elderly DLBLC patients in a randomized fashion.

No pharmacokinetic data are available for young DLBCL patients and results according to gender in young patients have not been published.

Bulky disease

Bulky disease was an independent risk factor in the MInT study in young patients with an aaIPI of 0 or 1 and bulky disease, despite the fact that nearly all patients with bulky disease had received radiotherapy to the respective area.[30,31] A comparison of MInT patients with aaIPI of 1 and patients with this aaIPI score in a French trial[32] in which bulky disease was also an independent risk factor and R-ACVBP was shown to be superior to 8 x R-CHOP-21, strongly suggests that 6 x R-CHOP-21 with radiotherapy to bulky disease is considerably better than 8 x R-CHOP-21 without radiotherapy. The comparison also suggests that 6 x R-CHOP-21 with radiotherapy is indeed equally effective as the more toxic R-ACVBP without radiotherapy. This led to the recommendation in the European Society for Medical Oncology (ESMO) 2012 guidelines[33] that either 6 x R-CHOP-21 with radiotherapy to bulky disease or R-ACVBP (without) should be given to young patients with aaIPI of 1. Moreover, the two arms without radiotherapy of the UNFOLDER study, which compares R-CHOP-14 with R-CHOP-21 in young patients with bulky disease and/or aaIPI of 1, with and without radiotherapy to bulky and extralymphatic disease, had to be closed after a planned interim analysis due to the pre-

defined superiority criteria of the two arms with radiotherapy (C Zwick *et al.,* personal communication, 2013). For elderly patients with bulky disease, the results of the RICOVER-noRX study also suggest a benefit of additional radiotherapy, at least in patients achieving a PR or less.[34] Whether radiotherapy to bulky disease can be skipped in patients with a negative PET scan after chemoimmunotherapy is currently under investigation.

Skeletal involvement

While skeletal involvement (whether localized or diffuse) was not a risk factor in the pre-rituximab era, it evolved as such when rituximab was given. Indeed, the addition of rituximab failed to improve the outcome of patients with skeletal involvement in the RICOVER-60 and MInT studies,[35] while radiotherapy to sites of skeletal involvement did. Therefore, for the time being, radiotherapy to sites of skeletal involvement is recommended.

CNS disease

Involvement of the central nervous system (CNS) is a serious and mostly fatal complication of DLBCL and remains to be so in the rituximab era. Risk models have been developed derived from analyses of prospective studies.[36-40] A multivariate analysis of elderly DLBCL patients treated with R-CHOP identified 3 independent risk factors for development of CNS disease: elevated LDH, >1 extranodal site, and ECOG performance status >1. Patients presenting with all three risk factors made up 4.8% of the 610 patients treated with R-CHOP and they had a 33.5% risk of developing CNS disease compared to only 2.8% in the remaining patients receiving R-CHOP.[40] While intrathecal prophylaxis with MTX appeared to have some effect on the incidence of CNS disease in patients not receiving rituximab, this prophylaxis had no effect in patients receiving R-CHOP in the RICOVER-60 trial or the MInT study. Several retrospective studies[41,42] suggest that intravenous high-dose methotrexate can reduce the incidence of CNS involvement in patients at increased risk. The DHNHL is currently evaluating intermediate-dose methotrexate (1.5 g/m^2) in elderly patients presenting with elevated LDH, ECOG over 1 and more than one extranodal site, which is given and well tolerated before the first and after the last cycle of R-CHOP.

The situation is less clear in younger patients for whom a group at significant risk for CNS involvement (elevated LDH plus advanced stage) develops CNS disease in only 6.5% of the cases.[40] A strategy to limit spinal tab to these 6.5% young patients and treat only those with signs of CNS involvement by sensitive flow cytometric analysis of spinal fluid and/or cranial NMR is currently being pursued by the German High-Grade Non-Hodgkin Lymphoma Study Group (DSHNHL) for young patients.

Other clinical presentations

Concordant bone marrow involvement (with large, but not with small cells) was shown in a retrospective register study to be a risk factor independent of the IPI,[43] as were

elevated serum levels of free light chains,[44] VEGF,[45] soluble IL-2 receptors[46] and interferon-inducible protein 10 (CXCL10)[47] as well as vitamin D,[48] and selenium.[49] Whether substitution of vitamin D or selenium can compensate the worse outcome of these patients still has to be shown.

Interim FDG-PET positivity

Early studies of DLBCL patients not (yet) receiving rituximab suggested that a PET after 1, 2, 3, or 4 cycles of CHOP was highly predictive for patient outcome.[50-53] However, this was not confirmed in larger and more recent studies of patients receiving rituximab[54-56] who showed a good negative predictive value (NPV) of approximately 80%, but a positive predictive value (PPV) of 33% or under. A French group reported that the reduction in SUV_{max} at the interim PET compared to the pre-therapy PET resulted in a much better predictive power (PPV 81%, NPV 75%) than a visual analysis.[57,58] However, using the French criteria for SUV_{max} reduction in a prospective study of 212 patients resulted in a PPV of 37% (U Dührsen, personal communication, 2010). Similar results were recently presented by the Groupe Ouest Est d'Etude des Leucémies et Autres Maladies du Sang (GOE-LAMS)[59] and an Italian[60] study. In summary, in the rituximab era, a positive interim PET appears to be unable to identify patients with high-risk DLBCL.

Molecular prognostic factors

Diffuse large B-cell lymphomas (DLBCL) constitute a heterogeneous category of aggressive lymphomas. Chromosomal instability and changes confer a worse prognosis,[61] and the expression of certain microRNAs[62] and proteins has been reported to be associated with a favorable (BCL6, CD10, HIF-1α, HLA-DR, IRF4/MUM1, LMO2; CD30) or an adverse (BCL2, CD5, indolamine 2,3-dioxygenase, high Ki-61, mutated p53, VEGFR2, Skp2) outcome. However, none of these reports have been confirmed in prospective studies. In contrast to single molecules, the analysis of the entire exome by gene expression profiling (GEP) studies identified three biologically and prognostically relevant subtypes of DLBCL: the activated B cell (ABC)-like DLBCL, the germinal center (GC)-like and the mediastinal large B-cell lymphoma[63,64] based on cell-of-origin (COO) gene signatures, with the activated B-cell (ABC) type being associated with an inferior outcome compared to the germinal center (GC) type.[64-66] ABC- and GC-like DLBCL differ with respect to the cell of origin, pathogenetic mechanisms and prognosis: the GC/non-GC was shown to be a prognostic factor independent of the IPI in patients treated with CHOP only, and the gene-expression-based model added to the predictive power of the IPI, and the IPI added to the predictive power of the gene-expression–based model in patients treated with CHOP plus rituximab.[64] Only the combined stromal-1/GC groups of patients fared significantly better than the ABC-type independent of the IPI.

DLBCL of the ABC type are characterized by NFkB activation that contributes to the high proliferative capacity of this subtype. Therefore, drugs interfering with this signaling pathway are attractive candidates for targeted therapy. A better response of the ABC-type to bortezomib,[67] lenalidomide[68] and the Bruton tyrosine kinase inhibitor ibrutinib[69] has been reported, but needs to be confirmed prospectively. In contrast, in relapsed DLBCL, the GC type had a better outcome with R-DHAP than with R-ICE chemoimmunotherapy in the CORAL study.[70] Nevertheless, for the time being, there is no justification for a differential treatment approach to GC and non-GC DLBCL outside prospective trials.

Because classical gene expression studies require fresh (-frozen) biopsy material, the impact of GEP on daily lymphoma practice is still rather limited, more than 12 years after Alizadeh *et al.*,'s pivotal publication.[65] Surrogate markers for the assignment to the ABC- and GC-like subtypes are warranted which are applicable to formalin-fixed-paraffin-embedded (FFPE) biopsies. However, the translation of complex GEP predictors into immunohistochemical algorithms such as the "Hans"[71] or "Choi"[72] classifiers that assign a COO subtype based on the expression of subtype-related proteins has been difficult, and prognostic and predictive accuracy of such algorithms have been shown to be quite variable, even in the hands of expert hematopathologists.[15,73-76] While immunohistology of FFPE was reported to allow the assignation of DLBCL to the GC- and non-GC subtype based on an algorithm using a limited number of antibodies suitable for FFPE biopsies,[71] a multivariate analysis by the Groupe D'Etude des Lymphomes de L'Adulte (GELA) confirmed that only the International Prognostic Index (IPI) and treatment arm influenced the outcome, but not the immunohistochemically assigned GC/non-GC phenotype.[77] Moreover, the Lunenburg consortium, made up of the most experienced hematopathologists worldwide observed unexpectedly highly variable results among the leading immunohistochemistry (IHC) laboratories in the world and very poor reproducibility in scoring for almost all markers.[78] Thus, it is not surprising that the largest TMA study performed to date in elderly DLBCL patients did not confirm the "Hans classifier",[15] the most popular algorithm used as surrogate for gene expression profiling. Whether novel algorithms show a better concordance with the GC/ABC subtyping by gene expression profiling, remains to be confirmed.[76] In summary, studies that evaluated the reliability of immunohistochemical algorithms as a surrogate for gene expression profiling yielded controversial results and studies that relied on immunohistochemistry for the assignment of GC and non-CC type DLBCL must be interpreted with caution. This also applies to immunohistochemical algorithms that tried to simulate a stromal-1[76] and stromal-2[79] signature, respectively. Besides these, multiple individual biomarkers as well as prognostic models incorporating several parameters have been evaluated in DLBCL using different techniques. Some of these models are based on mRNA expression by gene expression profiling or by real-time polymerase chain reaction (RT-PCR) and provide a quantitative measurement of gene expression.[80] Other bioprognostic models that have been proposed include a paraffin-based 6-gene prognostic model that distinguished low- and high-risk patients independent of the IPI,[81] and a 2-gene model based on an MYC and HLA-DR expression.[82] Recently, another 2-gene model based on the expression of LMO2 by the lymphoma cells and TNFRFS9 by the microenvironment has been published claiming to be an

independent factor for survival,[83] but none of these models have been confirmed in prospective studies, making it difficult to interpret their value. This also holds true for another 'bioprognostic marker' that was based on microvessel density, non-GCB subtype and low (<5%) expression of SPARC (secreted protein, acidic and rich in cysteine) in the stroma.[84] While all these novel bioprognostic markers are simplified compared to gene expression profiling, the technologies used in these models are not simple, standardized or commercially available, most likely precluding their widespread use.

c-myc breaks, double and triple hits

In many B-cell lymphomas, chromosomal translocations are biological and diagnostic hallmarks of the disease. A subset of these lymphomas has structural aberrations affecting the myc locus that is associated with a poor prognosis independent of clinical risk factors.[85] MYC-break positive DLBCL cases may also co-express high levels of BCL2, and up to half of these cases have a concurrent translocation involving BCL-2. These double-hit (DH) lymphomas are defined by a chromosomal breakpoint affecting the MYC/8q24 locus in combination with another recurrent breakpoint, *e.g.* a t(14;18)(q32;q21) involving BCL2. Recently, these lymphomas have been introduced as a novel category of lymphomas in the 2008 WHO classification[86] and were designated as "B cell lymphoma unclassifiable with features intermediate between DLBCL and Burkitt's lymphoma". DH lymphomas have been classified heterogeneously, but mostly as DLBCL, the majority having a GC phenotype and expressing BCL2. Patients with DH lymphomas often present with a poor prognosis profile including elevated LDH, bone marrow and CNS involvement, and a high IPI score. In a review of the published literature,[87] MYC breakpoints in general had a wide range of frequency (3-16%) and DH lymphomas a frequency of 0-12%. Of 689 MYC breakpoint-positive lymphomas, 47% were DH lymphomas, and from 804 cases diagnosed as DLBCL, 139 (17%) cases had an MYC breakpoint, demonstrating that MYC rearrangements in DLBCL are not rare. BCL2/MYC lymphomas form the vast majority of DH lymphomas (63%); BCL6/MYC DH lymphomas were relatively rare (8%) and triple-hit lymphomas involving MYC, BCL2 and BCL6 (16%) were, in fact, more frequent than BCL6/MYC DH. Other rarer forms of DH lymphomas involve MYC/CCND1, and MYC/BCL3. Most DH lymphomas have a GC phenotype with expression of CD10 and BCL6, a lack of MUM1/IRF, nearly always express BCL2 protein, and have a high Ki67/MIB1 proliferation rate. Therefore, aggressive lymphomas with co-expression of CD10, BCL6, BCL2 and high Ki67 proliferation index should always be checked for DH.

The DH DLBCL have been reported to have a dismal prognosis,[87,88] but a recent study from the GELA found no independent negative impact of MYC-double hits in contrast to MYC single hits[89] on survival. It has been suggested that an MYC translocation, with or without concurrent BCL2 translocation, was associated with inferior survival only, if MAC had immunoglobulin translocation partner gene.[90]

DH lymphomas show heterogeneous morphologies, the majority being morphologically classified as DLBCL. Of note, the category of "mature B cell neoplasms NOS", was in the past often called "Burkitt-like lymphoma"[91] and, therefore, often put with Burkitt's lymphoma. The median age at diagnosis of DH lymphomas ranges from 51-65 years and thus younger than in DLBCL,[87] but is rare in children. The bone marrow and CNS are frequently involved, and pleural effusions are often reported. DH lymphomas have a poor prognosis: both with CHOP and high-dose chemotherapy regimens the median survival is less than one year. The addition of rituximab appears to improve the outcome. However, even with rituximab the median survival rarely exceeds 1.5 years.[91-96] Whether regimens designed for and effective in Burkitt's lymphomas, that typically incorporate high-dose methotrexate such as the CODOX-M/IVAC regimen,[97] will improve the outcome of DH lymphomas still has to be shown. The rarity of DH lymphomas and their poor prognosis call for joint international efforts and prospective clinical phase II studies evaluating new chemotherapy regimens and targeted therapies for these prognostically poor DLBCL.

Expression of MYC and BCL2 proteins

While the prognostic impact of BCL2 and BCL6 breaks has been disputed,[85,98-102] there is a consensus that MYC translocations confer a worse prognosis in DLBCL patients treated with CHOP, both in combination with and without rituximab.[103,104]

In addition to translocations, MYC can also be deregulated by amplifications, mutations, or by microRNA-dependent mechanisms,[105-107] and it has recently been reported[108] that tumors with increased MYC protein expression have co-ordinate upregulation of MYC target genes, providing molecular confirmation of the IHC results. While MYC translocations can be detected by fluorescence *in situ* hybridization (FISH), FISH fails to detect MYC deregulation caused by mechanisms other than translocation. The recent availability of a robust monoclonal antibody (concordance for the ICH scoring was 94% for MYC[109]) that targets the N-terminus of the MYC protein has been shown to predict MYC rearrangements and has been validated for use in formalin-fixed paraffin-embedded (FFPE) tissues,[106] and allows for the study of large series of archived DLBCL samples for nuclear MYC protein expression by immunohistochemistry. Johnson and colleagues[109] found MYC translocations, high MYC mRNA and MYC protein expression in 11%, 11% and 33% of samples, respectively. In contrast to MYC translocations, which were observed in approximately 5% of the cases and had a median overall survival of less than one year, MYC protein expression was associated with an inferior progression-free and overall survival only when BCL2 protein was co-expressed. MYC/BCL2 protein co-expression was observed in 21% of the DLBCL cases, and the negative impact on prognosis remained significant after adjusting for the presence of high-risk features in a multivariable model that included elevated IPI score. The results of Johnson *et al.* confirm similar observations reported by Green and colleagues[110,111] and a German study confirmed the prognostic value of MYC/BC2 double-protein expression in a population treated uniformly within a prospective trial.[112] Since MYC protein expres-

sion is associated with MYC translocation, all MYC protein-positive patients should be tested for MYC translocations by FISH. MYC/BCL2 IHC was possible in 96% of the cases, demonstrating that the vast majority of FFPE tissue samples processed in the community are of satisfactory quality for this type of IHC.[110] However, while MYC IHC appears to be quite robust, it should be kept in mind that BCL2 IHC has been reported to be more variable, even among international experts in the field. In the pivotal validation study of IHC on tissue microarrays, the concordance rate was only 70%,[78] similar to that achieved by a group of German hematopathologists.[15] Data on how reproducible BCL2 IHC is in the community are not available. Therefore, for the time being, the diagnosis of MYC/BCL2 double-protein expressing DLBCL should be made only by internationally recognized hematopathologists.

With repeated and convincing evidence that patients with DLCBL co-expressing MYC and BCL2-proteins by IHC have a poor prognosis, the question arises as to which therapeutic strategies should be pursued for these patients. So far, there are no results from trials that specifically addressed MYC/BCL2 double protein-positive patients, but some information can be drawn from the analysis of DH lymphomas, since the two populations are overlapping. DH DLBCL do even worse than double protein-positive DLBCL when treated with R-CHOP,[103,104] and, in the case of failing during or after primary treatment, can rarely be salvaged by standard approaches like R-ICE or DHAP followed by high-dose BEAM and autologous stem cell transplantation.[113] The very obvious assumption that these patients should fare better with regimens that have been shown to work well in patients with Burkitt's lymphoma, could not be confirmed for the CODOX-M/IVAC regimen,[97] and the numbers of DH patients treated with aggressive regimens that included high-dose chemotherapy and autologous stem cell transplantation[94-96,114] are too small to allow for any conclusion. This also applies to a study from the NCI where MYC+ DLBCL had an event-free survival of 83% after four years with dose-adjusted EPOCH-R, by far the best treatment results reported for this subgroup of DLBCL.[115] Since patients with DH and MYC/BCL2 double-protein expression are rare, it can only be through international joint efforts that new therapeutic approaches for these patients can be tested and validated. With the ease and speed that these patients can now be identified by IHC, an important logistical obstacle has been eliminated. After the pathologists have paved the way, it is now up to clinical investigators to make use of this opportunity and develop better treatments for these patients.

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