Prognostic factors for diffuse large B-cell lymphoma in the R(X)CHOP era

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Background: The introduction of rituximab (R) to conventional CHOP chemotherapy for newly diagnosed diffuse large B-cell lymphoma (DLBCL) led to an unequivocal improvement in survival, establishing RCHOP as the standard of care. Still, nearly 40% of DLBCL patients will eventually die of relapsed disease. Efforts to improve outcomes by addition of new biologic agents (X) to the RCHOP backbone are underway. In this era of R(X)CHOP, it is imperative to develop prognostic and predictive markers, not only to identify patients who will suffer a particularly aggressive course, but also to accurately select patients for clinical trials from which they will most benefit.

Design: The following review was undertaken to describe prognostic factors in DLBCL, with emphasis on markers that are accurate, relatively available, and clinically applicable in 2014.

Results: The International Prognostic Index retains its validity in the era of RCHOP, although with limited ability to predict those with <50% chance of long-term survival. Gene expression profiling has provided novel insights into the biology of DLBCL and led to the development of immunohistochemistry (IHC) algorithms that are in routine practice. Identification of a ‘double-hit’ (DH) lymphoma by fluorescent in situ hybridization with aberrations involving MYC and/or BCL2 and BCL6 genes has important implications due to its extremely dismal prognosis with RCHOP. Other markers such as the absolute lymphocyte count (ALC), serum immunoglobulin free light chains, vitamin D levels, serum cytokines/chemokines, and imaging with positron emission tomography (PET) have all shown promise as future predictive/prognostic tests.

Conclusions: The future for new treatment options in DLBCL is promising with current clinical trials testing novel targeted agents such as bortezomib, lenalidomide, and ibrutinib as the ‘X’ in R(X)CHOP. Predictive factors are required to select and randomize patients appropriately for these trials. We envision the day when ‘X’ will be chosen based on the biological characteristics of the tumor.

Key words: lymphoma, non-Hodgkin, large cell, double hit, prognosis
introduction

Diffuse large B-cell lymphoma (DLBCL) is the most common type of non-Hodgkin lymphoma (NHL) in the world, and accounts for 30%–40% of all adult NHLs. The clinicopathologic and molecular genetic diversity of DLBCL is reflected in the 2008 WHO classification of lymphomas that describes more than 15 DLBCL subgroups based on distinct morphologic, biologic, immunophenotypic, and clinical parameters. Although potentially curable, ~40% of patients with DLBCL will die of relapsed or refractory disease. The standard of care for initial treatment of DLBCL is rituximab, cyclophosphamide, doxorubicin, vincristine, and prednisone on a 21-day schedule (RCHOP-21) for six cycles [1, 2]. Attempts to improve on RCHOP-21 by shortening the interval to 14 days have not been successful [3, 4].

We are now entering a new era where investigators are adding new drugs to standard RCHOP-21 (R(X)CHOP). Several trials have already been published including the addition of epatuzumab (ERCHOP) [5], bortezomib (RBCHOP) [6], and lenalidomide (R2CHOP) [7, 8]. Such new agents are designed to target specific oncogenic pathways, and have biological actions distinct from RCHOP. The plethora of new agents does present challenges because large randomized trials will be needed to prove the superiority of any new regimen over RCHOP. It is for this reason that new prognostic factors and predictive factors need to be developed based on the biology of DLBCL, so that such trials can be designed rationally and take into account specific predictive markers to select patients for specific R(X)CHOP combinations.

The following review will describe the prognostic factors in DLBCL (Table 1), with a particular focus on new prognostic markers that have been discovered and described since the International Prognostic Index (IPI) in 1993 [9]. We aimed to summarize key prognostic markers that have demonstrated efficacy, are relatively available to most physicians, and clinically relevant.

Table 1. Prognostic factors in diffuse large B-cell lymphoma

<table>
<thead>
<tr>
<th>Standard prognostic factors (recommended in all newly diagnosed DLBCL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. The International Prognostic Index (IPI)</td>
</tr>
<tr>
<td>2. Immunohistochemistry for cell of origin (e.g. Hans algorithm)</td>
</tr>
<tr>
<td>3. Imaging with positron emission tomography (PET)/CT scan</td>
</tr>
<tr>
<td>4. Fluorescent in situ hybridization (FISH) for MYC and BCL2 rearrangements</td>
</tr>
<tr>
<td>5. Absolute lymphocyte and absolute monocyte count</td>
</tr>
</tbody>
</table>

Provisional prognostic factors

1. Gene expression profiling for cell of origin
2. Serum immunoglobulin free light chains
3. Serum 25-hydroxy vitamin D levels
4. Serum cytokine/chemokines

*The provisional prognostic factors may not be routinely obtained at the present time due to lack of commercial availability OR lack of validation in independent cohorts.

the international prognostic index and the revised (r)-international prognostic index

The IPI continues to remain the most robust prognostic tool for DLBCL and is used in all clinical trials. Originally described in the prerituximab era, this model identified five factors to predict DLBCL survival: age >60, elevated serum lactate dehydrogenase (LDH), ECOG performance status ≥2, Ann Arbor stage III or IV, and number of involved extranodal sites ≥2. Four risk groups were identified that predicted 5-year survival rates of 73%, 51%, 43%, and 26%, respectively [9]. The age-adjusted IPI (aaIPI) was described for patients <60 years, and included LDH, performance status, and stage as risk factors with predicted 5-year survivals ranging from 32% to 83% for 0–3 risk factors, respectively [9, 10].

The introduction of the monoclonal anti-CD20 antibody rituximab demonstrated a consistent 15% absolute benefit in overall survival (OS) when added to conventional CHOP chemotherapy [1, 2, 11, 12]. This established R-CHOP as the new standard of care for newly diagnosed DLBCL. Sehn et al. [13] confirmed the validity of the IPI in the rituximab era in a cohort of 365 RCHOP-treated patients. However, the IPI was able to distinguish only two rather than the four risk groups in the original IPI. The authors proposed a revised IPI (R-IPI) by redistributing the IPI factors into three prognostic groups—the ‘very good’ group (0 risk factors), ‘good’ (1 or 2 factors), and ‘poor’ (3–5 factors). The 4-year OS was 94%, 79%, and 55% in the three groups, respectively. Subsequently, Ziepert et al. tested the validity of IPI in three large, prospective phase II/III trials: minT (Mab-TheraInternational Trial), RICOVER-60 (RCHOP for patients >60 years), and MegaCHOEP (dose-escalated cyclophosphamide, doxorubicin, vincristine, etoposide, and prednisone) [14]. Although the original IPI remains valid in the RCHOP era, it now has limited ability to predict patients who will suffer a particularly aggressive course, since even the ‘high-risk’ group has a 50% chance of 3-year event-free survival (EFS) (Table 2). This limitation of the IPI underscores the need to develop and validate new prognostic tools.

gene expression profiling and cell of origin

Gene expression profiling (GEP), a genomics tool that utilizes DNA microarray to assess gene expression, identifies at least three molecularly distinct forms of DLBCL—the germinal center B-cell (GCB), activated B-cell (ABC), and primary mediastinal B-cell subtypes [15]. While the GCB subtype frequently expresses CD10, LMO2, and BCL6 as seen in normal GCB, the hallmark of the ABC subtype is constitutive activation of genes related to the NF-κB pathway. The GCB subtype has a higher OS compared with the ABC subtype with R-CHOP-based treatment [76% versus 16% 5-year OS, respectively (P < 0.001)]. Several attempts were subsequently made to develop predictive models based on supervised analysis of individual genes that correlated with OS [16–20]. Such predictive models incorporated anywhere from 6 to 17 genes, and provided prognostic information independent of the IPI. Surprisingly, there was little
overlapping among the genes included in the individual predictive models, likely due to different composition of the microarrays and different algorithms used in building the models. In 2014, using standard RCHOP-21 in a nonprotocol situation, such gene-basedpredictive models have limited clinical utility in making treatment decisions for patients with newly diagnosed DLBCL. Several immunohistochemistry (IHC)-based algorithms have been developed as surrogates for prognostic information obtained from GEP. The commonly used Hans algorithm designates patients as GCB versus non-GCB based on the presence of three IHC markers: CD10, BCL6, and MUM1 [21]. The Choi algorithm includes additional immunostains such as GCET1 and FOXP1, and was designed to improve the accuracy of the Hans algorithm [22]. Several other IHC-based algorithms have been described [23–26]; however, there is no consensus regarding the best IHC algorithm in DLBCL. Due to its simplicity and high concordance with GEP results, the Hans algorithm remains one of the most frequently used algorithms in practice and in clinical trials where GEP is not being carried out.

molecular prognostic factors

MYC

First described in Burkitt lymphoma, translocations involving the MYC oncogene are known to be present in 5%–10% of DLBCLs [27–30]. The typical t(8;14)(q24;q32) juxtaposes the MYC gene in chromosome region 8q24 next to the immunoglobulin heavy chain (IgH) locus in chromosome region 14q32, leading to deregulation and overexpression of the MYC transcription factor. Other mechanisms such as translocation to non-Ig loci, mutations affecting the promoter region and copy number increase may also affect the MYC protein expression [31]. The significantly inferior 5-year progression-free survival (PFS) (31% versus 66%, P = 0.006) and OS (33% versus 72%, P = 0.016) in MYC-rearranged versus nonarranged DLBCL in a study by Savage et al. [30] was confirmed in other similar retrospective series [27, 28]. Not all of those studies have assessed the simultaneous presence of BCL2 and/or BCL6 translocations, calling into question whether MYC only translocations indeed confer an inferior prognosis in the absence of additional chromosomal breakpoints involving BCL2 or BCL6.

BCL2

BCL2, a potent antiapoptotic protein, was first discovered due to the involvement of the BCL2 gene in t(14;18) in follicular lymphoma. Approximately 47%–58% of DLBCL tumors overexpress the BCL2 protein. In the prerituximab era, BCL2 overexpression was found to be significantly associated with a higher relapse rate, worse disease-free and OS [32–34]. The introduction of rituximab to standard chemotherapy seems to ameliorate the negative prognostic impact of BCL2 overexpression [35], especially in the ABC subtype [36].

double-hit lymphoma

There is convincing recent evidence that the adverse prognostic impact of MYC translocation seems to increase several-fold in the presence of an additional chromosomal breakpoint involving the BCL2 or BCL6 loci. These DH lymphomas with dual translocations involving both MYC/8q24 and BCL2/18q21, or BCL6/3q27, as detected on fluorescent in situ hybridization (FISH), seem to have an extremely aggressive clinical course and poor response to standard chemotherapy. By far, the most studied type of DH B-cell lymphoma has concurrent MYC and BCL2 breaks, and is known to affect 5%–10% of DLBCLs. Previously classified as ‘Burkitt-like’ lymphoma, most of these lymphomas are now classified under a novel category of ‘B-cell lymphoma unclassifiable with features intermediate between DLBCL and BL’ in the 2008 WHO classification of lymphomas [37]. Patients with DH lymphomas often present with poor prognostic parameters, including elevated LDH, bone marrow and CNS involvement, and a high IPI score. With standard RCHOP chemotherapy, the survival in this group of patients is extremely dismal and often under a year (Table 3). It is unknown whether intensified chemotherapy regimens such as those used to treat Burkitt lymphoma will improve outcome in this subgroup.

With the recent availability of specific anti-MYC antibodies suitable for IHC, it is possible to study the prognostic effect of MYC and BCL2 protein overexpression. MYC overexpression has been shown to confer an adverse prognosis [54], and the prognostic impact is more pronounced with concurrent BCL2 overexpression [43]. About 20%–30% of DLBCL cases co-express MYC and BCL2 protein, which is a higher percentage compared with DH lymphomas detected by FISH. This suggests that mechanisms other than translocation may be responsible for the protein overexpression. In the report by Green et al. [39],

### Table 2. Treatment outcomes in diffuse large B-cell lymphoma (DLBCL)

<table>
<thead>
<tr>
<th>Number of risk factors</th>
<th>Risk group</th>
<th>5-year OS, % (without rituximab)</th>
<th>3-year OS, % (with rituximab)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 or 1</td>
<td>Low risk</td>
<td>73</td>
<td>91</td>
</tr>
<tr>
<td>2</td>
<td>Low-intermediate risk</td>
<td>51</td>
<td>81</td>
</tr>
<tr>
<td>3</td>
<td>High-intermediate risk</td>
<td>43</td>
<td>65</td>
</tr>
<tr>
<td>4 or 5</td>
<td>High risk</td>
<td>26</td>
<td>59</td>
</tr>
</tbody>
</table>

Risk factors: age >60 years; number of extranodal disease sites >1; serum lactate dehydrogenase (LDH) above normal EOGO performance status ≥2; Ann Arbor stage III or IV.

aOnly the last three risk factors are considered for the aaIPI.

bToo few numbers in the MegaCHOEP trial to provide a reliable estimate (Ziepert et al.).

cPercentages indicate 4-year OS.
DLBCLs with a protein DH score of 2, defined by high expression of both MYC (≥40%) and BCL2 (≥70%) protein, were significantly associated with a shorter PFS and OS, independent of the IPI and the cell of origin. Similar findings were confirmed by Johnson et al. where presence of concurrent MYC and BCL2 protein expression was associated with a significantly inferior 5-year OS of 36% versus 71% in those without MYC and BCL2 co-expression (P < 0.05) [43].

### Table 3. Incidence and outcomes of DLBCL with MYC and BCL2 rearrangement (double-hit lymphomas) detected by fluorescent in situ hybridization (FISH) or chromosome analysis in literature

<table>
<thead>
<tr>
<th>Study</th>
<th>N</th>
<th>MYC-R and BCL2-R (double hit), N</th>
<th>MYC-R and BCL6-R (double hit), N</th>
<th>MYC-R, BCL2-R and BCL6-R (triple hit), N</th>
<th>Treatment</th>
<th>Outcomes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Akyurek et al.</td>
<td>239</td>
<td>5</td>
<td>3</td>
<td>1</td>
<td>RCHOP (n = 145)</td>
<td>Median survival 9 months, 2-year OS 14%</td>
</tr>
<tr>
<td>Barrans et al.</td>
<td>245</td>
<td>26</td>
<td>10</td>
<td>7</td>
<td>RCHOP</td>
<td>Probability of 2-year survival 0.35 versus 0.61 in MYC-R versus MYC-nonR. Separate survival analysis of double and triple hit lymphomas not reported.</td>
</tr>
<tr>
<td>Green et al.</td>
<td>193</td>
<td>11</td>
<td></td>
<td></td>
<td>RCHOP</td>
<td>Median survival 13 months, 3-year OS 46%</td>
</tr>
<tr>
<td>Horn et al.</td>
<td>442</td>
<td>17</td>
<td>8</td>
<td>4</td>
<td>CHOP ± R</td>
<td>Too few double-hit lymphomas in the R-CHOP arm for analysis</td>
</tr>
<tr>
<td>Hu et al.</td>
<td>394</td>
<td>10</td>
<td>–</td>
<td>–</td>
<td>RCHOP</td>
<td>Markedly poor OS and PFS, &lt;20 months.</td>
</tr>
<tr>
<td>Johnson et al.</td>
<td>1260a</td>
<td>54</td>
<td>–</td>
<td>–</td>
<td>CHOP ± R, high-dose chemotherapy with SCT (n = 6)</td>
<td>Median OS &lt;6 months (1.4 years versus 0.4 years for R-CHOP versus CHOP; 3 months versus 1 month for SCT versus palliation)</td>
</tr>
<tr>
<td>Johnson et al.</td>
<td>307</td>
<td>14</td>
<td>–</td>
<td>–</td>
<td>RCHOP</td>
<td>5-year OS 27%</td>
</tr>
<tr>
<td>Kanungo et al.</td>
<td>–</td>
<td>14</td>
<td>–</td>
<td>–</td>
<td>Combination chemotherapy, SCT (n = 2)</td>
<td>Median survival 9 months</td>
</tr>
<tr>
<td>Kobayashi et al.</td>
<td>93</td>
<td>2</td>
<td>2</td>
<td>–</td>
<td>RCHOP</td>
<td>Median survival 162 days</td>
</tr>
<tr>
<td>Kojima et al.</td>
<td>100</td>
<td>1</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Le Gouill et al.</td>
<td>–</td>
<td>16</td>
<td>–</td>
<td>4</td>
<td>CHOP ± R or similar. autologous SCT (3), allogeneic SCT (2)</td>
<td>Median survival 5 months</td>
</tr>
<tr>
<td>Li S et al.</td>
<td>52a</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>R-CHOP/R-hyperCVAD/ CODOX/R-Fludarabine, Mitoxantrone, Dexamethasone</td>
<td>Median survival 18.6 months</td>
</tr>
<tr>
<td>Lin P et al.</td>
<td>52b</td>
<td>13</td>
<td>–</td>
<td>–</td>
<td>R-CHOP/R-hyperCVAD</td>
<td>Median survival &lt;18 months</td>
</tr>
<tr>
<td>Niitsu et al.</td>
<td>394</td>
<td>19</td>
<td>–</td>
<td>–</td>
<td>CHOP ± R, CycLOBEAP ± R</td>
<td>2-year OS 23%, 2-year PFS 0%</td>
</tr>
<tr>
<td>Savage et al.</td>
<td>137</td>
<td>3</td>
<td>–</td>
<td>–</td>
<td>R-CHOP</td>
<td>–</td>
</tr>
<tr>
<td>Snuderl et al.</td>
<td>–</td>
<td>20</td>
<td>–</td>
<td>–</td>
<td>CHOP ± R, R-ICE, R-EPOCH, CODOX-M/R-IVAC</td>
<td>Median survival 4.5 months</td>
</tr>
<tr>
<td>Tomita et al.</td>
<td>–</td>
<td>27</td>
<td>–</td>
<td>7</td>
<td>CHOP, CODOX-M/IVAC, HyperCVAD ± R</td>
<td>Median survival 6 months, 1-year OS 22%</td>
</tr>
<tr>
<td>Valera et al.</td>
<td>219</td>
<td>6</td>
<td>3</td>
<td>2</td>
<td>RCHOP, R-high-dose CHOP/R-ESHAP</td>
<td>Median OS 1.7 years</td>
</tr>
</tbody>
</table>

| Study included all non-Hodgkin lymphomas. |
| Study included only high-grade B-cell lymphoma, unclassifiable, with features intermediate between DLBCL and Burkitt lymphoma. |

MYC-R, MYC rearranged by FISH; BCL2-R, BCL-2 rearranged by FISH; BCL6-R, BCL-6 rearranged by FISH; SCT, stem-cell transplant.
It is reasonable to consider testing for MYC and BCL2 rearrangement by FISH for all newly diagnosed DLBCL. The dismal prognosis of DH lymphomas as defined by FISH with RCHOP-21 warrants the upfront use of more aggressive Burkitt-like chemotherapy regimens in eligible patients, or enrollment in clinical trials for DH lymphomas. At present, IHC for detecting overexpression of MYC or BCL2 is not routinely recommended since it is not known whether a positive result necessitates a change in front-line therapy.

**the absolute lymphocyte count, absolute monocyte count and the ALC/AMC ratio**

There is increasing evidence that tumor microenvironment and host immunity play an important role in lymphoma progression [16]. The absolute lymphocyte count (ALC), calculated from the complete blood count, is considered a surrogate for host immunity. In 2001, Porrata et al. described that early lymphocyte recovery (day +15 ALC) after autologous peripheral blood stem-cell transplant predicted superior survival in NHL [55]. Many subsequent retrospective studies have assessed the role of ALC at diagnosis in predicting outcomes of DLBCL. [56–65]. Although individual studies have used different ALC cutoffs ranging from 0.8 to 1.3 × 10⁹/l, most studies report that a higher ALC at the time of diagnosis is associated with an improved PFS and OS, independent of the IPI. These findings seem to hold true both the pre- and postrituximab era [58, 60, 65]. The ALC prognostic factor is independent of cell of origin (COO), and further stratifies clinical outcome within the GCB and non-GCB genotypes [62, 63]. Attempts have been made to devise a new prognostic scoring system that incorporates the ALC at diagnosis with the IPI. Cox et al. described a new trichotomous score (ALC/R-IPI) that classified patients into low risk (R-IPI very good or good ‘and’ ALC ≥0.84 × 10⁹/l), intermediate risk (R-IPI poor ‘or’ ALC ≥0.84 × 10⁹/l) and high-risk (R-IPI poor and ALC <0.84 × 10⁹/l) [66]. On multivariate analysis, this ALC/R-IPI score was the most powerful predictor in their cohort for PFS and OS.

In contrast to a high ALC being associated with a good prognosis, high levels of blood monocytes have just the opposite effect. Wilcox et al. described a dichotomous absolute monocyte count (AMC)/ALC prognostic score that stratified patients into three risk groups: low risk (AMC <630/ml and ALC >1000/ml), intermediate risk (AMC ≥630/ml or ALC ≤1000/ml) and high risk (AMC ≥630/ml and ALC ≤1000/ml) [65]. The AMC/ALC score remained an independent prognostic factor after adjusting for the IPI, and was able to identify a high-risk subset within the low or intermediate-risk IPI patients. The authors validated the AMC/ALC score in a new cohort of 99 patients with de novo DLBCL, and confirmed its independent prognostic value from the IPI as well as COO [62]. Li et al. used an inverse lymphocyte-to-monocyte (L/M) ratio at diagnosis in their study with similar results [59]. Thus, there are convincing data that ALC at diagnosis is an independent predictor of outcome from the IPI and cell of origin. It is a reliable, objective, and easily reproducible test that can be derived from a routine CBC. However, the optimal method of incorporating the ALC data into our existing scoring system, either with an absolute cutoff value for ALC or with L/M ratio has not been validated prospectively. The ALC may also have significance during routine surveillance after completion of immunotherapy. Development of lymphopenia during follow-up after RCHOP has been described as a risk factor for predicting relapse [67, 68], and the ALC at the time of first relapse has been described as an independent predictor of survival [69].

Systemic immune suppression in NHL may also result from altered monocyte phenotype in the peripheral blood [70]. The presence of immunosuppressive monocytes defined as those with a decreased expression of HLA-DR (CD14+HLA-DRlow– monocytes) is associated with higher-stage disease, more aggressive pathology, and faster rate of disease progression. This altered phenotype seems to affect immunity through multiple pathways, including altered signal transduction and cytokine production affecting the innate pathway, impaired dendritic cell differentiation affecting the adaptive immune response, and direct suppression via decreased T-cell proliferation.

**serum immunoglobulin free light chains**

The immunoglobulin free light chain (FLC) assay [FREELITE assay (The Binding Site, Ltd, Birmingham, UK)] detects κ and λ immunoglobulin light chains that are not attached to the IgH [71, 72]. FLC elevations can be polyclonal (increase in one or both light chains with a normal κ:λ ratio) or monoclonal (elevation of one FLC that results in an abnormal κ:λ ratio). The assay is quite useful in the diagnosis and management of patients with multiple myeloma and amyloidosis [73]. Polyclonal elevation of FLC also carries a negative prognosis and is associated with all-cause mortality and impaired renal function [74, 75]. Initial studies in 25 patients with DLBCL found the incidence of monoclonal FLC elevations at 8% [76]. Maurer et al. studied the prognostic relevance of elevated FLC in 259 newly diagnosed DLBCL patients and reported an elevated FLC in 32% [77]. Both monoclonal and polyclonal elevation in FLCs was significantly associated with inferior EFS and OS in the initial and validation cohorts (cohort 1: OS HR 3.16; P < 0.02; cohort 2: OS HR 3.74; P < 0.001). In multivariable analysis that included the five IPI components, elevated FLC (either monoclonal or polyclonal) was the strongest predictor of outcome. These findings were confirmed by Jardin et al. in their cohort of 409 DLBCL patients [78]. In addition, their study reported that an abnormal IgMk/IgMλ ratio also predicted inferior PFS (HR 1.54, P = 0.03). Serial measurement of FLCs may also have utility during follow-up for monitoring response to therapy, detecting minimal residual disease and identifying early relapse. Given that the serum FLC assay is a standardized, easily measurable biomarker, it is an attractive tool for prognostication if validated in future prospective cohorts.

**vitamin D**

Vitamin D is increasingly recognized for its effects on cellular differentiation, proliferation, angiogenesis, and apoptosis [79]. Low levels of circulating vitamin D have been associated with a higher risk of NHL [80, 81]. Conversely, higher levels of recreational sun exposure, which would be anticipated to increase vitamin D levels, have been associated with a lower risk of NHL.
Recent data suggest that low vitamin D levels predict an inferior prognosis in NHL [83, 84]. In a cohort of 983 newly diagnosed NHL patients, 44% were found to be vitamin D insufficient [25-hydroxvitamin D levels <25 ng/ml] with levels measured within 120 days of diagnosis [83]. After adjusting for IPI and treatment received, patients with vitamin D insufficiency had an inferior OS (HR 1.99). Moreover, low levels of 1,25-dihydroxyvitamin D, the physiologically active form of 25-hydroxvitamin D, also correlated with inferior OS, suggesting that tumor 1-α hydroxylase activity does not explain the 25-hydroxvitamin D associations. Similar findings were reported in a separate study of 359 DLBCL patients, wherein vitamin D deficiency predicted an inferior outcome in those treated with RCHOP. *In vitro* data suggested impaired rituximab-mediated cellular toxicity with low vitamin D levels [85]. These observational studies generate a hypothesis for future studies to determine whether vitamin D repletion would abrogate the inferior prognosis in the vitamin D insufficient patients.

**serum cytokines/chemokines**

Cytokines and chemokines are small secreted proteins that play an important role in B-cell activation, proliferation, and apoptosis, as well as in immune regulation. It is well known that an altered cytokine/chemokine milieu exists in DLBCL, either causing the lymphoma or arising as a result of the lymphoma itself. In a case–control study that assessed the levels of 30 cytokines in the pretreatment serum of 188 DLBCL and 400 control participants, significant differences were found in the cytokine expression profile in DLBCL patients compared with normal controls [86]. Abnormal cytokine levels seem to correlate with disease presentation as well as prognosis. Elevated pretreatment levels of serum interferon-inducible protein-10 (IP-10, CXCL10) were found to independently predict an inferior EFS (HR 1.99, \( P = 0.009\)) and OS (HR 1.93, \( P = 0.021\)) in 69 patients with DLBCL treated with RCHOP plus epratuzumab. These results were confirmed in a separate cohort of 185 patients treated with standard RCHOP [87]. Other studies have reported an association of high soluble interleukin-2 receptor (sIL-2R) levels with poor EFS and OS [88]. Thus, specific signatures of these easily measurable biomarkers may serve as useful predictive and prognostic tools in DLBCL in future.

**role of metabolic imaging with positron emission tomography**

The 18-fluorodeoxyglucose positron emission tomography (18FDG–PET) with computed tomography (CT) imaging (PET/CT) has proved to be highly sensitive in determining sites of disease in DLBCL. A FDG–PET/CT obtained at the time of diagnosis leads to a change in stage of disease in up to 20%–40% of patients, and leads to a change in the treatment in 5%–15% [89–93]. A positive PET/CT scan at the end of treatment is highly predictive for residual or recurrent disease, and is associated with an inferior PFS and OS [94–97]. The presence of an FDG-avid mass in such cases also helps determine the need for consolidation radiation at the end of chemotherapy [98, 99]. Thus, obtaining a FDG–PET/CT scan at diagnosis and at treatment completion for DLBCL has become the standard of care at most treatment centers. Some form of tumor imaging (PET, CT, or MRI) is recommended during treatment to confirm adequate response. The role of the midtreatment imaging technique being a PET/CT scan is controversial. Although early retrospective studies suggested a difference in outcome based on the results of an interval PET scan [100–102], subsequent prospective studies have not supported these findings [103, 104].

One of the strongest predictors of long-term survival after DLBCL treatment is the maintenance of an event-free status until a minimum of 24 months after initial diagnosis. Most patients who develop relapsed or recurrent disease do so within the first 12–24 months of diagnosis, and carry a significantly inferior prognosis despite salvage chemotherapy and stem-cell transplant. On the contrary, patients who are event-free at 24 months after diagnosis have an excellent prognosis with an OS similar to that of an age- and sex-matched general population [105]. The whole area of surveillance imaging in patients with DLBCL who are >24 months out and disease free is in flux. In a recent retrospective study of 537 patients with DLBCL who entered an observation phase after completion of immunotheraphy, a majority of the 109 patients who relapsed presented with clinical signs and symptoms of recurrence. Surveillance scanning detected DLBCL relapse before clinical manifestations in only 8 of 537 patients (1.5%) [106].

**future directions**

The prognostic factors described above indicate that outcomes in DLBCL are a composite measure of host characteristics (age and performance status in IPI), tumor burden (stage, extranodal sites, serum LDH in IPI, serum FLC levels), tumor biology (GEp, MYC, and BCL2 translocations), tumor microenvironment (vitamin D, altered ALC/AMC ratio, circulating monocytes, aberrant cytokines/chemokines), and response to therapy (PET/CT scan). While these prognostic factors help identify patients at high risk for disease relapse and death, there are little data at present to support deviation from RCHOP-21 for most high-risk patients in the nonstudy situation.

With improved understanding of tumor biology and driver pathways, we are at the brink of ushering in a new era of targeted therapy for DLBCL. Until the results of CALGB 50303 (phase III trial of RCHOP versus DA-EPOCH-R) are reported, RCHOP-21 continues to remain the standard of care for newly diagnosed DLBCL.

There are and will continue to be a number of R(X)CHOP clinical trials using the RCHOP backbone with the addition of ‘X’ novel agent. The design and selection of ‘X’ will be guided by strong scientific rationale with knowledge of pathways that are activated in specific DLBCL subsets, demonstration of activity in preclinical models, and phase I trials showing some inherent single-agent activity of the drug in relapsed DLBCL. An example of this concept is the development of specific agents targeting the NF-kB pathway which is known to be upregulated in the ABC subtype DLBCL. Lenalidomide, an immunomodulatory agent, demonstrated selective *in vitro* activity against ABC DLBCL cell lines [107]. Subsequent phase I and II clinical trials with lenalidomide monotherapy showed durable responses in...
relapsed/refractory NHL [108, 109], with significantly higher overall response rate (ORR) in ABC compared with GCB DLBCL (52.9% versus 8.7%, \(P = 0.006\)) [110]. This led to the development of R2CHOP (RCHOP + lenalidomide), which has now been shown to be safe in two phase I trials that included patients with both GCB and ABC subtypes [7, 8]. Further analysis of these trials is being carried out in order to analyze the association of lenalidomide response with DLBCL genotype [111]. In addition, a randomized phase II clinical trial of RCHOP versus R2CHOP is underway in the ECOG (ECOG 1412; NCT01856192) to learn whether or not R2CHOP can improve the survival of patients with both genotypes of DLBCL. A similar strategy is being tested with bortezomib. Furman et al. have reported the safety of combining bortezomib with standard RCHOP in patients with untreated DLBCL [112]. Based on in vitro evidence that bortezomib inhibits NF-κB activity in ABC DLBCL cell lines, bortezomib was combined with DA-EPOCH in patients with relapsed/refractory DLBCL [113]. Patients with ABC DLBCL had a significantly higher response and median OS compared with GCB DLBCL. A randomized phase III trial (ReMODL-B; NCT01324596) is underway in Europe that will test the hypothesis that RCHOP is better in patients with ABC-type DLBCL compared with GCB.

A multitude of novel agents with targets upstream of the NF-κB pathway, such as Bruton tyrosine kinase (BTK), SYK, PI3 K, mTOR, AKT, and PKCβ have shown promising activity in DLBCL. A summary of these agents is provided in Table 4. The discovery that chronic B-cell receptor signaling leads to NF-κB activation makes various components of this pathway attractive targets for drug inhibition. Ibrutinib, a BTK inhibitor, showed a 29% ORR in a phase I clinical trial in DLBCL [114]. A subsequent phase II clinical trial demonstrated preferential activity in ABC compared with GCB DLBCL, with 40% and 5% response rates, respectively (\(P = 0.01\)) [115]. Fostamatinib, a SYK inhibitor, also showed activity in relapsed DLBCL in a phase I/II study, and additional phase II trials are now underway [116].

In GCB DLBCL, there is a potential role for EZH2 inhibitors since these molecules have shown preclinical activity in the presence of EZH2 mutations that occur in 21% of GCB DLBCL [121]. Although MYC and BCL2 overexpressing tumors may have an inferior prognosis, it has been a challenge to develop small molecule inhibitors that target the MYC oncoprotein due to its protein structure. However, BCL2 inhibitors, such as ABT-199, when available, may have a role in BCL2 overexpressing GCB tumors.

In order to achieve the goal of individualized care plans for DLBCL patients, identification of predictive markers for these R(X)CHOP regimens will need to be initiated in the early phase of drug development for subsequent validation in phase III clinical trials. It is clearly an exciting time for the field of DLBCL and it is likely that the factors outlined in this review will become even more useful in the near future to not only guide prognostication but also choose the ‘X’ in R(X)CHOP.

**Table 4. Novel targeted agents under evaluation in DLBCL**

<table>
<thead>
<tr>
<th>Target</th>
<th>Drug</th>
<th>Subtype</th>
<th>Study phase</th>
<th>Preliminary results</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>BTK</td>
<td>Ibrutinib</td>
<td>ABC</td>
<td>Phase I</td>
<td>29% ORR</td>
<td>Advani et al. [114]</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Phase II</td>
<td>23% ORR (40% ABC versus 5% GCB)</td>
<td>Wilson et al. [115]</td>
</tr>
<tr>
<td>SYK</td>
<td>Fostamatinib</td>
<td>ABC</td>
<td>Phase II</td>
<td>22% ORR</td>
<td>Friedberg et al. [116]</td>
</tr>
<tr>
<td>PI3K</td>
<td>Idelalisib (GS 1101)</td>
<td>ABC</td>
<td>Preclinical</td>
<td>–</td>
<td>Lannutti et al. [117]</td>
</tr>
<tr>
<td>PKCβ</td>
<td>Enzastaurin</td>
<td>ABC</td>
<td>Phase I</td>
<td>14% ORR</td>
<td>Robertson et al. [118]</td>
</tr>
<tr>
<td></td>
<td>Enzastaurin + RCHOP</td>
<td>ABC</td>
<td>Phase II</td>
<td>80% ORR</td>
<td>Hainsworth et al. [119]</td>
</tr>
<tr>
<td>DNA methylation</td>
<td>Azacytidine + RCHOP</td>
<td>ABC</td>
<td>Phase I</td>
<td>91% ORR</td>
<td>Elstrom et al. [120]</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>Phase II</td>
<td>Ongoing</td>
<td>NCT01004991</td>
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<tr>
<td>EZH2</td>
<td>EZH2 inhibitor (GSK126)</td>
<td>GCB</td>
<td>Preclinical</td>
<td>–</td>
<td>McCabe et al. [121]</td>
</tr>
<tr>
<td>Immunomodulatory</td>
<td>Lenalidomide</td>
<td>ABC</td>
<td>Phase II</td>
<td>28% ORR</td>
<td>Witzig et al. [108]</td>
</tr>
<tr>
<td></td>
<td>Lenalidomide + RCHOP</td>
<td>ABC</td>
<td>Phase I</td>
<td>100% ORR</td>
<td>Nowakowski et al. [7]</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>Phase I</td>
<td>90% ORR</td>
<td>Chiappella et al. [8]</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Phase II</td>
<td>Ongoing</td>
<td>NCT01856192</td>
</tr>
<tr>
<td>InRex (NF-κB)</td>
<td>Bortezomib + RCHOP</td>
<td>ABC</td>
<td>Phase I</td>
<td>100% ORR</td>
<td>Furman et al. [112]</td>
</tr>
<tr>
<td></td>
<td>Bortezomib + DA-EPOCH</td>
<td>ABC</td>
<td>Phase II</td>
<td>83% ABC versus 13% GCB</td>
<td>Dulewicz et al. [113]</td>
</tr>
<tr>
<td>BCL2</td>
<td>ABT-199</td>
<td>GCB</td>
<td>Preclinical</td>
<td>–</td>
<td>Kang et al. [122]</td>
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<tr>
<td>mTORC1</td>
<td>Everolimus</td>
<td>Both</td>
<td>Phase II</td>
<td>30% ORR</td>
<td>Witzig et al. [123]</td>
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<td></td>
<td>Everolimus + RCHOP</td>
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<td>Phase II</td>
<td>Ongoing</td>
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<tr>
<td></td>
<td>Everolimus maintenance</td>
<td>Both</td>
<td>Phase III</td>
<td>Completed, results pending</td>
<td>NCT00790036</td>
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<tr>
<td>Anti-CD22</td>
<td>Epratuzumab + RCHOP</td>
<td>Both</td>
<td>Phase II</td>
<td>96% ORR</td>
<td>Micallef et al. [5]</td>
</tr>
</tbody>
</table>

*aGenotype most likely to benefit from preclinical data.

phase II clinical trial demonstrated preferential activity in ABC compared with GCB DLBCL, with 40% and 5% response rates, respectively (\(P = 0.01\)) [115]. Fostamatinib, a SYK inhibitor, also showed activity in relapsed DLBCL in a phase I/II study, and additional phase II trials are now underway [116].

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**disclosure**

The authors have declared no conflicts of interest.
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