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Molecular Classification and Treatment of Diffuse Large B-cell Lymphoma and Primary Mediastinal B-cell Lymphoma

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Abstract

Diffuse large B-cell lymphoma (DLBCL) encompasses a group of aggressive B-cell non-Hodgkin lymphomas with striking genetic heterogeneity and variable clinical presentations. Among these is primary mediastinal B-cell lymphoma (PMBL), which has unique clinical and molecular features resembling Hodgkin lymphoma. Treatment of DLBCL is usually curative, but identifiable subsets at highest risk for treatment failure may benefit from intensified chemotherapy regimens and/or targeted agents added to frontline therapy. Recent comprehensive genomic analyses have identified distinct genetic subtypes of DLBCL with characteristic genetic drivers and signaling pathways that are targetable. Immune therapy with chimeric antigen receptor T-cells and checkpoint inhibitors has revolutionized the treatment of relapsed or refractory disease, and antibody drug conjugates have weaponized otherwise intolerable cytotoxic agents. Ongoing clinical trials are further refining the specificity of these approaches in different genetic subtypes and moving them from the setting of recurrent disease to frontline treatment in high risk patient populations.

Keywords

diffuse large B-cell lymphoma; DLBCL; primary mediastinal B-cell lymphoma; PMBL; gene-expression profiling; genetic subtypes; targeted therapy; chimeric antigen receptor

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I. Introduction

Diffuse large B-cell lymphoma (DLBCL) comprises a group of aggressive B-cell lymphomas with underlying genetic diversity and variable clinical presentations. DLBCL is the most common B-cell non-Hodgkin lymphoma (NHL) accounting for 40% of lymphomas worldwide.[1] Rituximab, cyclophosphamide, doxorubicin, vincristine, and prednisone (R-CHOP) cures ~60% of DLBCL including in advanced stage. Patients with early progression or refractory disease have an overall survival (OS) of only 6 months and are often the focus of clinical trials testing novel strategies.[2]

The molecular heterogeneity of DLBCL was initially appreciated from gene-expression profiling (GEP) where subtypes were identified by cell-of-origin (COO): germinal center B-cell (GCB), activated B-cell (ABC) and primary mediastinal B-cell (PMBL) with 15–20% of cases unclassified.[3–5] The 2016 revision of the World Health Organization (WHO) classification recognized ABC DLBCL and GCB DLBCL as distinct molecular subtypes of DLBCL and introduced a new entity, high-grade B-cell lymphoma, defined by the presence of *MYC* and *BCL2* and/or *BCL6* rearrangements (HGBCL-DH/TH).[6] The COO phenotype can now be determined by the digital gene expression Lymph2X assay applied to formalin-fixed paraffin-embedded tissue.[7] HGBCL-DH/TH is not a distinct biologic entity, but identifies subsets of DLBCL at risk for treatment failure with R-CHOP.

Prospective studies have not clearly identified treatment approaches superior to R-CHOP, but high-risk subgroups may benefit from targeted agents added to R-CHOP or alternative chemotherapy regimens (Table 1). Unfortunately, the requirements of clinical trials presents a barrier to enrollment of high-risk patients resulting in unexpected biases and potentially limiting interpretation of results.[8] In this review, we describe molecular classifications of DLBCL and discuss lessons learned from frontline studies and targeted drug and immune therapies.

II. Pathologic classification of diffuse large B-cell lymphoma

Classification systems predicated on disease biology are mandatory for optimal clinical management and research. The WHO classification of lymphoid neoplasms incorporates input from pathologists, molecular geneticists, and clinicians to reach consensus regarding entities that share clinical, biologic and prognostic features.[9]

DLBCL encompasses clinically diverse entities that present in any organ. Indeed, certain DLBCL subtypes are categorized by anatomic location such as primary central nervous system lymphoma (PCNSL), primary cutaneous leg-type, and intravascular large B-cell lymphoma.[9] The COO classification identifies subtypes arising from B-cells at different developmental stages with distinct oncogenic mechanisms, reliance on different survival pathways, and differential outcomes.[4,10,11] GCB DLBCL arises from malignant B-cells with the gene expression program of normal germinal center B-cells including ongoing somatic hypermutation and CD10 expression.[5] ABC DLBCL arises from malignant B-cells with the gene expression program of post-germinal center or “activated” B-cells characterized by constitutive activation of the NF-κB pathway.[12,13] PMBL arises from a thymic B-cell and

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shares molecular features with nodular sclerosing Hodgkin lymphoma (NSHL) including activation of the NF-κB and JAK-STAT signaling pathways, and genetic alterations that promote immune evasion.[14–16] Up to 20% remain unclassified and rare histologic subtypes like T-cell/histiocyte-rich large B-cell lymphoma are not captured by COO classification.

Approximately 10–15% of patients with untreated DLBCL have a rearrangement of the *MYC* oncogene.[17] The 2016 WHO classification defined a new subset of high-grade DLBCL, irrespective of a high-grade morphology, based on the presence of rearranged *MYC* and *BCL-2* and/or *BCL-6*, and is present in approximately 8% of DLBCL.[6,18] Previously, these were called 'double-hit' lymphomas associated with a dismal prognosis and included both *de novo* DLBCL and transformed follicular lymphoma, which interjected imprecision given their different pathogenesis.[19,20]

III. Genetic subtypes of diffuse large B-cell lymphoma

Gene-expression profiling alone does not capture genetic heterogeneity, whereas next-generation sequencing studies of whole genome, whole exome, or transcriptome have unveiled the genetic landscape of DLBCL and further refined pathogenetic mechanisms.[21–23] Defining genetic events have been found exclusively within COO subtypes as well as across subtypes, and can further sub-classify DLBCL into unique genetic subtypes (Figure 1).[24] Further studies are needed to understand the clinical impact of these newly minted subtypes regarding the role of targeted therapy.

A landmark study used a multiplatform genomic approach on 574 cases of DLBCL incorporating gene expression profiling, exome, deep amplicon resequencing, and DNA copy number analysis to identify genetic subtypes; cases included ABC DLBCL (51.4%), unclassified (20%) and GCB DLBCL (28.6%).[25] The study developed molecular classification based on enrichment of driver mutations and signaling pathways. Using iterative statistical modeling, 4 distinct genetic subtypes of DLBCL were described. Each subtype shared genetic features, microenvironment gene expression signatures, and displayed differential clinical outcomes.[25] The four subtypes accounted for 47% of all cases and were named EZB (21.8%), BN2 (14.8%), MCD (8%), and N1 (2.1%).

EZB is associated with genetic events common amongst GCB DLBCL including *BCL2* translocations, *EZH2* mutations and *REL* amplifications. Other genetic alterations include inactivation of *TNFRSF14*, *CREBBP*, and *EP300*. EZB is almost exclusively GCB DLBCL (88%) with a minority ABC DLBCL (3%) or unclassified (9%). Overall, EZB is associated with the best prognosis, but has worse survival than other GCB DLBCL.[25] Rational targeted agents for EZB include inhibitors of EZH2 or BCL2 and inhibitors of B-cell receptor (BCR) and phosphatidylinositol 3-kinase (PI3K) signaling.

BN2 is enriched for *BCL6* fusions and alterations of the NOTCH pathway, including mutations or amplifications in *NOTCH2* and mutations in *SPEN* and *DTX1*.[25] Inactivating mutations of the NF-kB regulator *TNFAIP3* and mutations within the BCR signaling pathway, *PRKCB* and *BCL10* activate NF-κB. BN2 is found in ABC (41%), GCB (19%), and unclassified (40%) DLBCL cases. Within ABC DLBCL, BN2 is associated with the best

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prognosis of other genetic subtypes.[25] Rational targeted agents in BN2 include inhibitors of BCR or PI3K signaling and BCL2.

MCD is characterized by *MYD88*^{L265P} and/or *CD79B* mutations, which are found in 82% of cases and often together. Immune editing is another prominent feature as mutations or deletions of *HLA-A*, *HLA-B*, or *HLA-C* are seen in 76% of cases. MCD is almost exclusively ABC DLBCL (96%) and rarely GCB (1%) or unclassified (3%) DLBCL. MCD frequently involves extranodal sites, including the testes, breast and CNS, and has a poor prognosis.[25] Other recurrent mutations in MCD are seen in PCNSL including *PIM1*. The genetic profile of MCD indicates chronic active BCR signaling and susceptibility to inhibitors of Bruton's tyrosine kinase (BTK), highly active agents in PCNSL.[26–28] Other rational targets include PI3K, BCL2, and IRAK4.

N1 is characterized by *NOTCH1* mutations and most are ABC DLBCL (95%) with some unclassified (5%). N1 includes mutations in *IRF4* and *ID3* controlling B-cell differentiation. N1 is the least common genetic subtype of DLBCL (2.1%) and associated with the worst prognosis.[25] N1 has underlying chronic active BCR signaling and should be susceptible to BTK inhibitors.

Another landmark study performed an integrative genomic analysis of 304 cases of DLBCL.[29] Using a different computational method, this study identified recurrent mutations employing whole exome sequencing and incorporated associated structural variants and somatic copy number alterations to identify 5 distinct genetic subtypes of DLBCL based on consensus clustering.

Cluster 1 (C1) is characterized by structural variations in BCL6, mutations of *NOTCH2*, and mutations in NF-κB including *BCL10* and *TNFAIP3*: a genetic profile resembling BN2. Multiple genetic alterations in C1 allow for immune escape including structural variations of *PD-L1* and *PD-L2* and inactivating mutations of *B2M* and *CD70*.[29] Most C1 are ABC DLBCL with associated *MYD88* mutations, but the specific point mutation MYD88^{L265P} associated with chronic active BCR signaling does not occur in C1.[30] The prognosis of C1 is more favorable than Cluster 5 (C5), which is also frequently ABC DLBCL. Cluster 2 (C2) is characterized by mutations and deletions of chromosome 17p that lead to the biallelic inactivation of *TP53* associated with increased genomic instability. C2 does not specifically associate with a COO phenotype and has an overall intermediate prognosis.

Cluster 3 (C3) has a genetic profile characterized by mutations and translocations of *BCL2* and inactivating mutations of chromatin modifiers including *EZH2*, *EP300*, and *KMT2D*. Accordingly, C3 is almost always GCB DLBCL (95%). Cluster 4 (C4) is characterized by mutations in genes encoding for histones and associated with immune evasion, including *CD70*, *CD83*, and *CD58*. Other genetic features of C4 include mutations of *RHOA* and *GNA13* in the PI3K pathway as well as *BRAF* and *STAT3* in the JAK/STAT signaling pathway. C4 are usually GCB DLBCL with a better overall prognosis than C3. Cluster 5 (C5) is characterized by *MYD88*^{L265P} and *CD79B* mutations, gain of *18q*, and *PIM1* mutations. C5 is almost exclusively seen in ABC DLBCL (96%) and resemble the genetic

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subtype MCD. C5 also exhibits a propensity for extranodal sites, including the CNS and testis.[29] The prognosis of C5 is worse than other ABC DLBCL, including C1.

IV. Molecular hallmarks of primary mediastinal B-cell lymphoma

PMBL is a distinct subtype of DLBCL with characteristic clinical and pathologic features, including a gene expression pattern more closely resembling NSHL than GCB DLBCL or ABC DLBCL.[14,15,31] Diagnosis requires clinical correlation, but may be improved by the molecular classification assay, Lymph3Cx, that distinguishes PMBL from NSHL and other DLBCL subtypes from paraffin-embedded tissue.[32]

The molecular hallmarks of PMBL include constitutive activation of NF-κB and JAK-STAT along with genetic alterations that promote immune evasion (Figure 1).[33,34] Genetic mechanisms of NF-κB activation include copy gains of *REL* and inactivating mutations of *TNFAIP3* and *NFKBIE*, negative regulators of NF-κB.[35–37] Gain-of-function mutations in *STAT6* and *IL4R* and loss-of-function mutations in *PTPN1* further promote increased JAK/STAT signaling.[34,38–40] Amplifications of a region on chromosome 9p24 are observed in 70% of PMBL containing several key genes including *JAK2* that further increase signaling via the JAK/STAT pathway, and *PD-L1* and *PD-L2* which encode for ligands of the programmed cell death-1 (PD-1) pathway responsible for regulation of balance between T-cell activation and immune tolerance.[41–43] Structural variants of *PD-L1* and *PD-L2* have also been reported in PMBL including translocations, inversions, and deletions.[43] Another genetic mechanism of immune escape is recurrent unbalanced rearrangements of the major histocompatibility complex (MHC) class II transactivator (*CIITA*).[44] Alterations of *CIITA* contribute to immune evasion via overexpression of *PD-L2* and *PD-L2* and reduction of MHC class II expression.[44,45]

Two integrative genomic analyses characterized additional key pathogenic mechanisms in PMBL.[46,47] One study analyzed 95 cases of PMBL and incorporated gene expression profiling, whole exome sequencing, and DNA copy number analysis to investigate driver genetic alterations and relevant signaling pathways.[46] Mutations in genes associated with interferon response were observed in nearly half of cases, including *IRF1*, *IRF4*, *IRF8*, and *IRF2BP2*.[46] Another study analyzed 37 cases of PMBL using whole exome sequencing, associated structural variants, and somatic copy number alterations.[47] This study identified recurrent mutations in *IRF2BP2* (19%) which affects PD-L1 expression and mutations in *β2M* (32%) which encodes for the invariant chain of the MHC class I responsible for antigen presentation. Mutations associated with GCB DLBCL including *GNA13* and *EZH2* were also observed. Point mutations of *EZH2* were first reported exclusively in GCB DLBCL.[48] *EZH2* encodes a histone methyltransferase responsible for adding methyl groups to specific histones and gain-of-function mutations in *EZH2* contribute to immune evasion by increasing trimethylation of H3K27 and reducing expression of MHC class I and MHC class II.[49–51]

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V. Frontline therapy for diffuse large B-cell lymphoma

Anthracycline-based chemotherapy first cured DLBCL in the 1970s.[52] Cyclophosphamide, doxorubicin, vincristine, and prednisone (CHOP) became standard after a randomized study showed no difference compared to third-generation anthracycline-based chemotherapy regimens and less toxicity.[53] The anti-CD20 monoclonal antibody rituximab with CHOP improves survival for all subgroups and is an essential component of frontline therapy (Figure 3).[54,55] Indeed, R-CHOP is the *de facto* standard for most patients with DLBCL.

Standard treatment approach for low-risk DLBCL

R-CHOP is highly effective for low-risk DLBCL with acceptable toxicity. A randomized study in patients under age 60 with low-risk features showed rituximab with doxorubicin, cyclophosphamide, vindesine, bleomycin, and prednisone (R-ACVBP) improved the 3-year OS compared to R-CHOP (92% vs. 84%, P=0.007).[56] R-ACVBP use is limited by its associated hematologic toxicity. A landmark randomized study demonstrated that most patients with low-risk DLBCL are cured with only 4 cycles of chemotherapy (Table 1).[57] In this study, 592 patients under age 60 with early stage DLBCL and favorable features were randomized to receive either 6 cycles of standard R-CHOP or abbreviated R-CHOP for 4 cycles with 2 additional doses of rituximab. Consolidative radiotherapy was not planned. After a median follow-up of 66 months, the 3-year progression-free survival (PFS) after R-CHOP × 4 cycles was 96% (95% CI, 94–99) and noninferior to standard R-CHOP.[57] This study established R-CHOP × 4 as the standard of care in younger patients with early stage DLBCL and low-risk features.

Standard treatment approach for advanced stage DLBCL

Improving therapy beyond R-CHOP for advanced stage DLBCL has proven difficult as neither intensification with autologous stem cell transplantation (ASCT) nor intensifying the dosing schedule of R-CHOP improved outcomes.[58,59]

The infusional regimen dose-adjusted etoposide, doxorubicin, and vincristine with prednisone, cyclophosphamide and rituximab (DA-EPOCH-R) is highly effective for DLBCL.[60,61] The scientific rationale includes prolonged chemotherapy exposure to overcome resistance and pharmacodynamic dose adjustments based on the neutrophil nadir.[62] A multicenter phase 2 study in 69 patients with all molecular subtypes of DLBCL demonstrated a 5-year event-free survival (EFS) of 75% (95% CI, 63–84) after a median follow-up of 62 months.[63] Within molecular subtypes, the 5-year EFS was 94% (95% CI, 65–99) vs 58% (95% CI, 33–76)(P=0.008) for GCB DLBCL compared to non-GCB.[63] A randomized phase 3 study compared six cycles of DA-EPOCH-R to six cycles of R-CHOP in 524 patients with all DLBCL molecular subtypes (Table 1).[64] After a median follow-up of 5 years, no improvement was observed in 2-year PFS or OS with DA-EPOCH-R compared to R-CHOP 78.9% (95% CI, 73.8% to 84.2%) vs 75.5% (95% CI, 70.2% to 81.1) and 86.5% (95% CI, 82.3% to 91%) vs. 85.7% (95% CI, 81.4% to 90.2%), respectively. Tumor biopsies were required for molecular subtyping which delayed treatment a median of 21 days and inadvertently skewed the patient population more favorable.[8] In a post-hoc subgroup analysis, patients with high-risk International Prognostic Index (IPI) scores had significantly

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improved PFS with DA-EPOCH-R. However, this observation was underpowered, and the benefit of DA-EPOCH-R in high-risk subgroups remains unknown.

Obinutuzumab is a type II anti-CD20 monoclonal antibody that improves antibody-dependent cellular cytotoxicity compared to rituximab in xenograft models (Figure 3).[65] A randomized phase 3 study compared obinutuzumab with CHOP (O-CHOP) to R-CHOP in 1440 patients with untreated advanced stage DLBCL (Table 1).[66] After a median follow-up of 29 months, no differences in 3-year PFS after O-CHOP compared to R-CHOP 70% and 67%, respectively (hazard ratio (HR) 0.92, 95% CI, 0.76 to 1.11; P=0.39). GCB DLBCL had improved outcomes compared to ABC DLBCL/unclassified but this was independent of anti-CD20 monoclonal antibody used.[66]

VI. High-risk subsets of diffuse large B-cell lymphoma

Activated B-cell (ABC) DLBCL

ABC DLBCL has an inferior prognosis compared to GCB DLBCL and genetic mechanisms underlying intrinsic drug resistance is a research priority.[10] Normal B-cells express both antigen-specific BCR and receptors of the innate immune system, known as Toll-like receptors (TLR).[67] Dual expression allows response to various stimuli that then activates downstream transcription factors. The defining molecular hallmark of ABC DLBCL is constitutive activation of NF-κB, activated by a number of mutations.[68] Point mutations in the B-cell receptor subunit CD79B occur in 21% of ABC DLBCL cases and facilitate chronic active BCR signaling.[69] An alternative pathway of NF-κB activation occurs through MYD88, a signal adaptor for the TLRs. A single oncogenic point mutation, *MYD88*^{L265P}, occurs in 30% of ABC DLBCL, but is rarely observed in GCB DLBCL.[30] Further, biallelic inactivation of *TNFAIP3* occurs in 30% of ABC DLBCL and can coexist with mutations in *MYD88* and *CD79B*, suggesting a mechanism that enhances both BCR and TLR signaling pathways.[68]

Bortezomib targets NF-κB via degradation of the inhibitory protein IκBα. Patients with relapsed ABC DLBCL treated with bortezomib plus DA-EPOCH had a response rate of 83% compared to 13% for GCB DLBCL (p<0.001).[70] A randomized phase 3 study compared R-CHOP with bortezomib to R-CHOP alone in 1128 patients with untreated DLBCL with molecular profiling.[71] After a median follow-up of 29.7 months, no difference in PFS was noted in R-CHOP alone 70.1% (95% CI 65.0–74.7) compared to R-CHOP with bortezomib 74.3% (95% CI, 69.3–78.7); hazard ratio 0.86, p=0.28).[71]

Ibrutinib inhibits BTK, a tyrosine kinase associated with chronic active BCR signaling.[72] In a phase II study of ibrutinib in 70 patients with relapsed/refractory DLBCL a response rate of 40% was observed in ABC DLBCL compared to 5% in GCB DLBCL.[73] Mutational analysis showed that more than 60% of ABC DLBCL with a BCR mutation responded, whereas those patients that lacked a BCR mutation, but had a *MYD88* mutation, did not respond. However, 80% of patients with mutations in both *MYD88* and *CD79B* responded to ibrutinib. A recent study implemented a functional proteogenomic discovery pipeline in ibrutinib-responsive cell line models and discovered that the BCR can form a multiprotein ‘supercomplex’ with TLR9, MYD88, mTOR and NF-kB. This ‘My-T-BCR supercomplex’

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mediated NF-kB signaling and was present in biopsies from ibrutinib-responsive DLBCL patients and PCNSL.[74]

A randomized phase 3 study evaluated ibrutinib plus R-CHOP compared to placebo plus R-CHOP in 838 patients with untreated non-GCB DLBCL (Table 1).[75] After a median follow-up of 34.8 months, ibrutinib plus R-CHOP did not improve EFS in either the intention-to-treat population (HR 0.93), nor patients with confirmed ABC DLBCL (HR 0.95). Interestingly, in patients under age 60, the 3-year PFS and OS was improved in patients who received ibrutinib plus R-CHOP compared to placebo 75.4% (95% CI, 67–82) vs 64.6% (95% CI, 57–72) P=0.01) and 93.2% (95% CI, 88–96) vs 80.9% (95% CI, 74–86) P=0.01), respectively. In patients over age 60, the benefit of ibrutinib was offset by toxicities that caused patients to discontinue therapy, suggesting ibrutinib is beneficial in younger patients.

Lenalidomide is an immunomodulatory agent that binds cereblon and downregulates IRF4 through degradation of Ikaros transcription factors.[76] In a phase 2 study of 40 patients with relapsed/refractory DLBCL, lenalidomide demonstrated a response rate of 53% in non-GCB DLBCL compared to 8.7% in GCB DLBCL (P=0.004).[77] A phase 2 study in 64 patients with untreated DLBCL demonstrated lenalidomide plus R-CHOP (R2CHOP) achieved a high rate of complete remission.[78] Further, non-GCB patients treated with R2CHOP had improved outcomes compared to historical controls. Two prospective randomized trials tested R2CHOP in untreated DLBCL with recently reported results (Table 1).[79,80] In a randomized phase 2 study of 349 patients with all molecular subtypes, R2CHOP was compared to R-CHOP alone.[79] In this study, 94 (38%) patients had ABC DLBCL, 122 (50%) had GCB DLBCL, and 30 (12%) unclassified. After a median follow-up of 2.4 years, the PFS was improved in R2CHOP compared to R-CHOP alone (HR 0.67, 95% CI, 0.44–1.03).[79] In a phase 3 study, 570 patients with ABC DLBCL confirmed by Lymph2Cx were randomized to R2CHOP or placebo plus R-CHOP.[80] After a median follow-up of 27.1 months, no differences in PFS with R2CHOP compared to R-CHOP (HR=0.85, 95% CI, 0.63–1.14). In this study, a nonsignificant trend towards improved outcomes was reported in patients with high risk IPI scores treated with R2CHOP.

Taken together, prospective trials do not identify frontline treatment for ABC DLBCL better than R-CHOP alone, but the addition of ibrutinib or lenalidomide might benefit certain subsets. Another possibility is that lenalidomide synergizes with ibrutinib in ABC DLBCL. A recent study in 60 patients with untreated non-GCB DLBCL reported preliminary results for ibrutinib and lenalidomide added to frontline therapy.[81] In this study, patients received 2 cycles of ibrutinib, lenalidomide, and rituximab before receiving R-CHOP or DA-EPOCH-R. The response rate after the lead-in was 86% and the complete response rate after therapy was 95%. Further studies are needed to identify ABC DLBCL patients that benefit from targeted agents in frontline therapy.

High-risk subsets of GCB DLBCL

Up to 10–15% of patients with newly diagnosed DLBCL have an underlying *MYC* rearrangement and nearly half of these also harbor a rearrangement of *BCL2* and/or *BCL6* categorized as HGBCL-DH/TH and associated with poor prognosis.[19] HGBCL-DH/TH with *BCL2* rearrangements are all classified as GCB DLBCL.[82] A recent study analyzed RNA

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sequencing data from 157 patients with GCB DLBCL and established a gene expression signature (DHITsig) of 104 genes that identifies 27% of GCB DLBCL with a 5-year time to progression rate of 57% compared to 81% (HR 2.8, $P<0.001$) for other GCB DLBCL cases after R-CHOP.[82] The outcomes in this GCB DLBCL subgroup were similar to ABC DLBCL. The DHIT signature robustly identifies HGBCL-DH/TH with *BCL2* rearrangements, but they account for only 50% of the high-risk group. A follow-up study with whole genome sequencing demonstrated that high-risk GCB DLBCL by DHITsig may have cryptic rearrangements of *MYC* or *BCL2* not detectable by routine testing.[83] In another study of 400 patients with DLBCL, a molecular high grade (MHG) gene expression signature identified 83 (9%) patients with a 3-year PFS of only 37% (95% CI, 24–55) compared to 72% (95% CI, 68–77) for those without the signature.[84] Interestingly, 75 (90%) of the patients classified with the MHG signature were GCB DLBCL. Taken together, these studies identify high-risk GCB DLBCL cases with poor outcomes after R-CHOP.

The only prospective study in patients with *MYC* rearrangements is a multicenter prospective study of DA-EPOCH-R × 6 cycles in 53 patients with HGBCL-DH/TH (45%), HGBCL (19%), and DLBCL and *MYC* rearrangements (34%).[85] After a median follow-up of 55.6 months, the 4-year EFS was 71.0% (95% CI, 56–81) with an 4-year OS of 76.7% (95% CI, 63–86). No difference in outcome was observed between HGBCL-DH/TH and DLBCL with *MYC* rearrangements, but patients with high-risk IPI scores had an inferior outcome. Until further studies, patients with HGBCL-DH/TH or DLBCL and *MYC* rearrangements should be treated with regimens such as DA-EPOCH-R.

VII. Frontline therapy for primary mediastinal B-cell lymphoma

PMBL has unique biologic features and primarily presents as a bulky mediastinal mass in adolescents and young adults, predominantly females. Retrospective studies demonstrate high rates of treatment failure with R-CHOP without consolidative mediastinal radiotherapy.[86] Subgroup analyses of randomized trials show the 5-year PFS after R-CHOP is 75% with most patients receiving consolidative radiotherapy.[87] Mediastinal radiotherapy is associated with late toxicities including second malignancies and cardiovascular complications, so minimizing its use is critical. In a prospective phase II study, 51 patients with PMBL received six cycles of DA-EPOCH-R without any consolidative mediastinal radiotherapy.[61] After a median follow-up of 5 years, the EFS was 93% (95% CI, 81 to 98) and only 2 (4%) patients required mediastinal radiotherapy. Results of this study were updated including a cohort of consecutive PMBL patients from another institution. Overall, 93 patients were included with a median follow-up of 8.4 years and the 8-year EFS and OS were 90.6% (95% CI, 82–95) and 94.7% (95% CI, 86–98), respectively with 5 (5%) patients requiring mediastinal radiotherapy.[88] A retrospective study compared R-CHOP to DA-EPOCH-R and showed no significant difference in 2-year PFS or OS, but greater radiotherapy use with R-CHOP (59% vs. 13%, P<0.001).[89] DA-EPOCH-R is the standard of care for PMBL given its high rates of cure without use of radiotherapy. An important caveat with DA-EPOCH-R for PMBL is that some patients have positron emission tomography (PET) scans after therapy positive by conventional response criteria who do not relapse.[88]

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VIII. Therapeutic approach to relapsed or refractory DLBCL

The standard approach to relapsed or refractory DLBCL is salvage chemotherapy followed by autologous stem cell transplantation (ASCT), but this approach cures few patients in the rituximab era.[90] Patients refractory to chemotherapy or those who relapse after ASCT have a grim prognosis.[2]

Immunotherapy approaches for DLBCL and PMBL

Immune escape represents a major hallmark of some DLBCL, and multiple immunotherapy strategies promote effective immune recognition, activation, and cytotoxicity from effector cells in the tumor microenvironment, including T-cells and macrophages (Figure 4).

Chimeric antigen receptor (CAR) T-cell therapies target surface proteins on tumor cells and have revolutionized the salvage approach to DLBCL and PMBL. CARs are fusion proteins that include antigen-recognition moieties along with T-cell signaling domains.[91] In DLBCL and PMBL, the most effective antigen recognition domain is derived from monoclonal antibodies targeting CD19, and multiple CAR-19 agents have been tested in relapsed and refractory DLBCL.[92–94] CAR-T therapy is a multistep process and includes apheresis of T-cells, transfer of the gene encoding for the CAR into the genome of T-cells, expansion of CAR-T cells products *ex vivo*, and reinfusion of CAR-T cells after conditioning chemotherapy. The process introduces a delay of nearly 20 days and patients with highly aggressive tumors may not be candidates. Further, CAR-T infusions risk serious cytokine release syndrome and the potential for irreversible neurotoxicity.[95] Despite limitations, CAR-T therapy induces durable remissions in nearly 40% of patients infused, including patients refractory to chemotherapy.

Immune checkpoint inhibitors targeting PD-1 have excellent clinical activity in PMBL.[96] PD-1 blockade inhibits the signal that promotes T-cell senescence and restores anti-tumor T-cell activity. PD-1 inhibitors such as pembrolizumab and nivolumab are rational targeted agents for PMBL given its association with 9p24 amplification and genetic events that lead to overexpression of the PD-1 ligands, *PD-L1* and *PD-L2*. In two studies of 21 and 53 patients with relapsed and refractory PMBL treated with pembrolizumab, the overall response rate was 48% (95% CI, 26–70) and 45% (95% CI, 32–60), respectively. The median PFS was only 10.4 (95% CI, 3 to not yet reached) and 5.5 (95% CI, 3–12) months, respectively. Nivolumab was combined with the anti-CD30 antibody drug conjugate (ADC) brentuximab vedotin (BV) in 30 patients with relapsed/refractory PMBL.[97] After a median follow-up of 11.1 months, the overall response rate was 73% (95% CI, 54–88) and the median PFS had not been reached. Taken together, these studies show excellent activity of PD-1 inhibitors in PMBL, but the benefit of combination therapy is unknown since single agent activity of BV in PMBL is only 13%.[98]

Novel immunotherapy agents include magrolimab, a monoclonal antibody targeting CD47 being developed with rituximab in DLBCL. Lymphoma cells evade intrinsic phagocytic (“eat me”) signals via binding of CD47 to its ligand, signal regulatory protein alpha (SIRPα, a “don’t eat me” signal,) which is expressed on phagocytic cells including macrophages. Blocking the SIRPα-CD47 interaction with magrolimab, combined with additional “eat me”

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signals, can lead to phagocytosis. In a phase 1 study, the ORR of magrolimab with rituximab in relapsed/refractory DLBCL was 40% including a 33% rate of complete response.[99] Bispecific monoclonal antibodies are also being tested in DLBCL. Mosunetuzumab is a bispecific antibody that targets both CD3 on the surface of effector T-cells as well as CD20 on the surface of tumor cells thus bringing the effector and target in close proximity.[100] In a phase 1b study of 87 patients with relapsed/refractory DLBCL, the preliminary results were reported with an observed response rate of 35% and including a 19% rate of complete response.

Targeting intracellular survival pathways

Novel agents that target intracellular survival pathways are currently being investigated for the treatment of DLBCL (Figure 2). A critical lesson learned from early monotherapy clinical trials is that responses are frequently short in duration and resistance develops rapidly.[73] Most clinical trials now test targeted agents as combinations to prevent acquired resistance.

Ibrutinib and lenalidomide both demonstrate single agent activity in ABC DLBCL and synergize in preclinical models.[101] The combination of ibrutinib, lenalidomide, and rituximab (iR2) was tested in a phase 2 study of 89 patients with non-GCB DLBCL with a response rate was 47% (95% CI, 36–58) and a 28% rate of complete response.[102] The median PFS on this study was 5 months. Venetoclax is a second-generation inhibitor of BCL2 that also synergizes with both ibrutinib and lenalidomide in preclinical models.[103,104] In a phase 1 study, venetoclax was added to ibrutinib, lenalidomide, prednisone, and obinutuzumab (ViPOR).[105] This regimen demonstrated acceptable safety and the preliminary ORR in 13 patients with DLBCL was reported as 69%, including a 25% rate of complete response. Another important targetable survival pathway is the PI3K pathway, and copanlisib is a targeted inhibitor of both PI3K-α and PI3K-δ isoforms that has selective activity in ABC DLBCL.[106] Further, the combination of copanlisib with BCL-2 blockade was synergistic in preclinical models in specific genetically defined subgroups.[107]

Antibody-drug conjugates (ADC) use a monoclonal antibody to attach to a surface protein on the tumor cell and deliver a conjugated chemotherapy agent (Figure 3). Polatuzumab vedotin is an ADC containing a monoclonal antibody to CD79B conjugated to monomethyl auristatin E which disrupts cell division by preventing the polymerization of tubulin.[108] Polatuzumab vedotin was added to bendamustine and rituximab (BR) and compared to BR alone in a randomized study of 80 patients with relapsed or refractory DLBCL.[109] After a median follow-up of 22.3 months, patients who received polatuzumab vedotin had a higher rate of CR (40% v 17.5%; P = .026), longer median PFS (9.5 v 3.7 months; HR=0.36, 95% CI, 0.21 to 0.63; P < .001) and median OS (12.4 v 4.7 months; HR, 0.42; 95% CI, 0.24 to 0.75; P = .002) compared to BR alone.

IX. Conclusions and future directions

DLBCL is a genetically diverse disease that manifests with equal clinical diversity. Most patients with low-risk DLBCL are cured with R-CHOP, but high-risk subsets should be prioritized for clinical trials testing the addition of targeted agents or immunotherapy. PMBL

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has molecular features that distinguish it from DLBCL and unique clinical features that influence frontline treatment decisions to avoid radiotherapy. Recent comprehensive genomic analyses have advanced our understanding of both DLBCL and PMBL, but in order to improve the cure rate, clinical trials should investigate clinical outcomes within the context of molecular subtypes of DLBCL and focus on high-risk populations.

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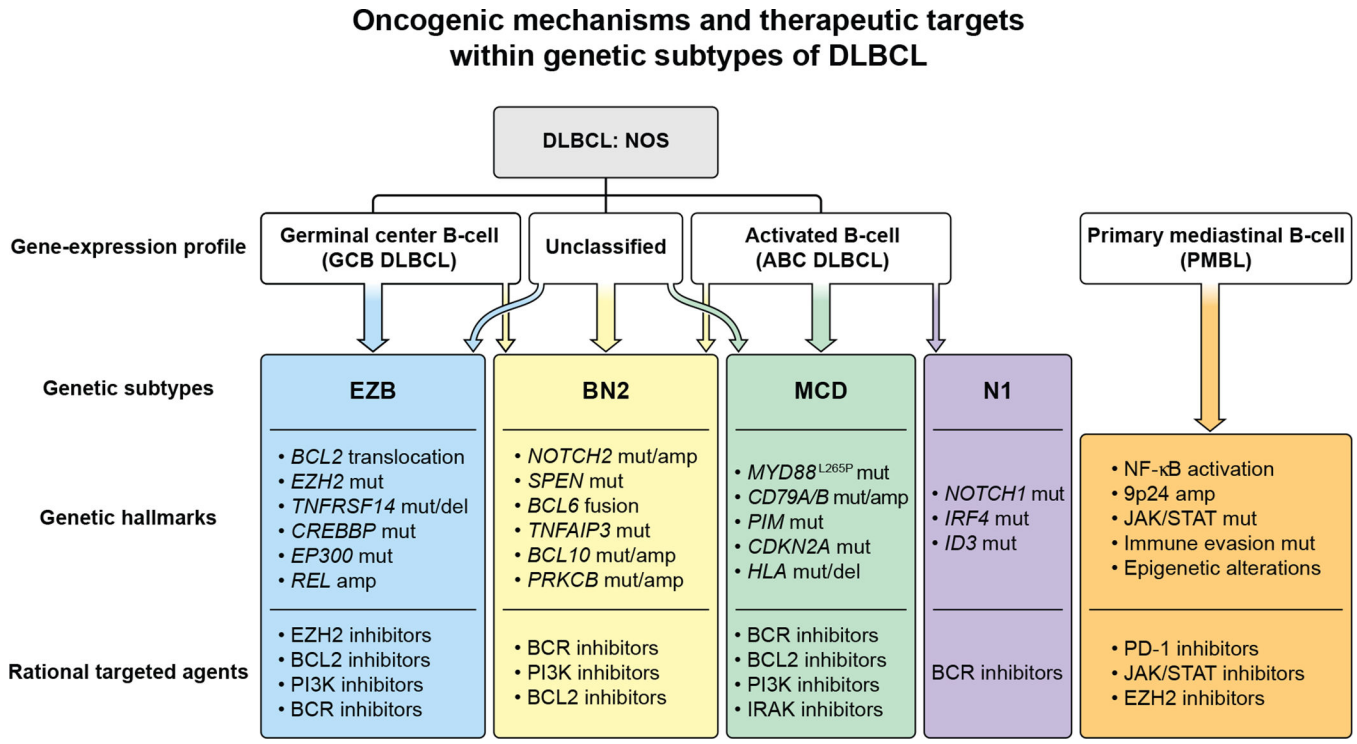

Figure 1.

Oncogenic Mechanisms and Therapeutic Targets within Genetic Subtypes of DLBCL.

Diffuse large B-cell lymphoma can be subdivided by gene-expression profiling into cell-of-origin (COO) phenotypes. The genes overexpressed within these subtypes correspond to the putative developmental stage of the B-cell from which the tumor originated. In DLBCL: NOS, two main phenotypes exist. GCB DLBCL is derived from a B-cell that overexpresses genes associated with the germinal center reaction and ABC DLBCL is derived from a post-germinal center or 'activated' B-cell. The remaining 15–20% of cases of DLBCL: NOS remain unclassified by COO profiling. PMBL is distinct from DLBCL: NOS and derived from the rare post-thymic B-cell with a unique gene expression signature that more closely resembles classical Hodgkin lymphoma than other DLBCL subtypes.

Four genetic subtypes within DLBCL: NOS have been described based on their underlying genetic hallmarks and oncogenic mechanisms. EZB is typically a GCB DLBCL and associated with genetic events including *BCL2* translocations, *EZH2* mutations and *REL* amplifications. Other events include inactivation of *TNFRSF14*, *CREBBP*, and *EP300*. Rational targeted agents in EZB include inhibitors of EZH2 or BCL2 as well as inhibitors of proximal B-cell receptor (BCR) signaling or the phosphatidylinositol 3-kinase (PI3K) signaling pathway. BN2 is found in GCB DLBCL, ABC DLBCL, and unclassified cases. The genetic hallmarks of BN2 include *BCL6* fusions and alterations of the NOTCH pathway. Rational targeted agents in BN2 include inhibitors of BCR or PI3K signaling as well as direct inhibitors of BCL2. MCD is characterized by $MYD88^{L265P}$ and *CD79B* mutations and is almost always ABC DLBCL. Genetic alterations of *HLA-A*, *HLA-B*, or *HLA-C* are seen in MCD and contribute to immune evasion. Rational targets in MCD include inhibitors of Bruton's tyrosine kinase, PI3K, BCL2, and IRAK4. N1 is almost

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exclusively seen in ABC DLBCL and characterized by *NOTCH1* mutations. Rational targeted agents in N1 include inhibitors of BCR signaling.

PMBL is characterized by NF-kB pathway activation and amplification of chromosome 9p24 which encodes for multiple genes that increase signaling via the JAK/STAT pathway including *PDL1*, *PDL2*, and *JAK2*. Further, inhibiting the PD-1 pathway is a rational target in PMBL. Multiple genetic events promoting immune evasion are seen in PMBL including recurrent *CIITA* translocations and recently described mutations in *EZH2* which reduce expression of both MHC class I and MHC class II.

Abbreviations: DLBCL, diffuse large B-cell lymphoma; NOS, not otherwise specified; mut, mutations; del, deletions; amp, amplifications; NF-κB, nuclear factor-κB; JAK/STAT, janus activating kinase/signal transducer and activator of transcriptiong signaling pathway; PI3K, phosphatidylinositol 3-kinase signaling pathway; BCR, B-cell receptor signaling pathway; IRAK4, interleukin-1 receptor-associated kinase 4; PD-1, programmed cell death-1 pathway; Janus Kinase/Signal Transducer and Activator of Transcription

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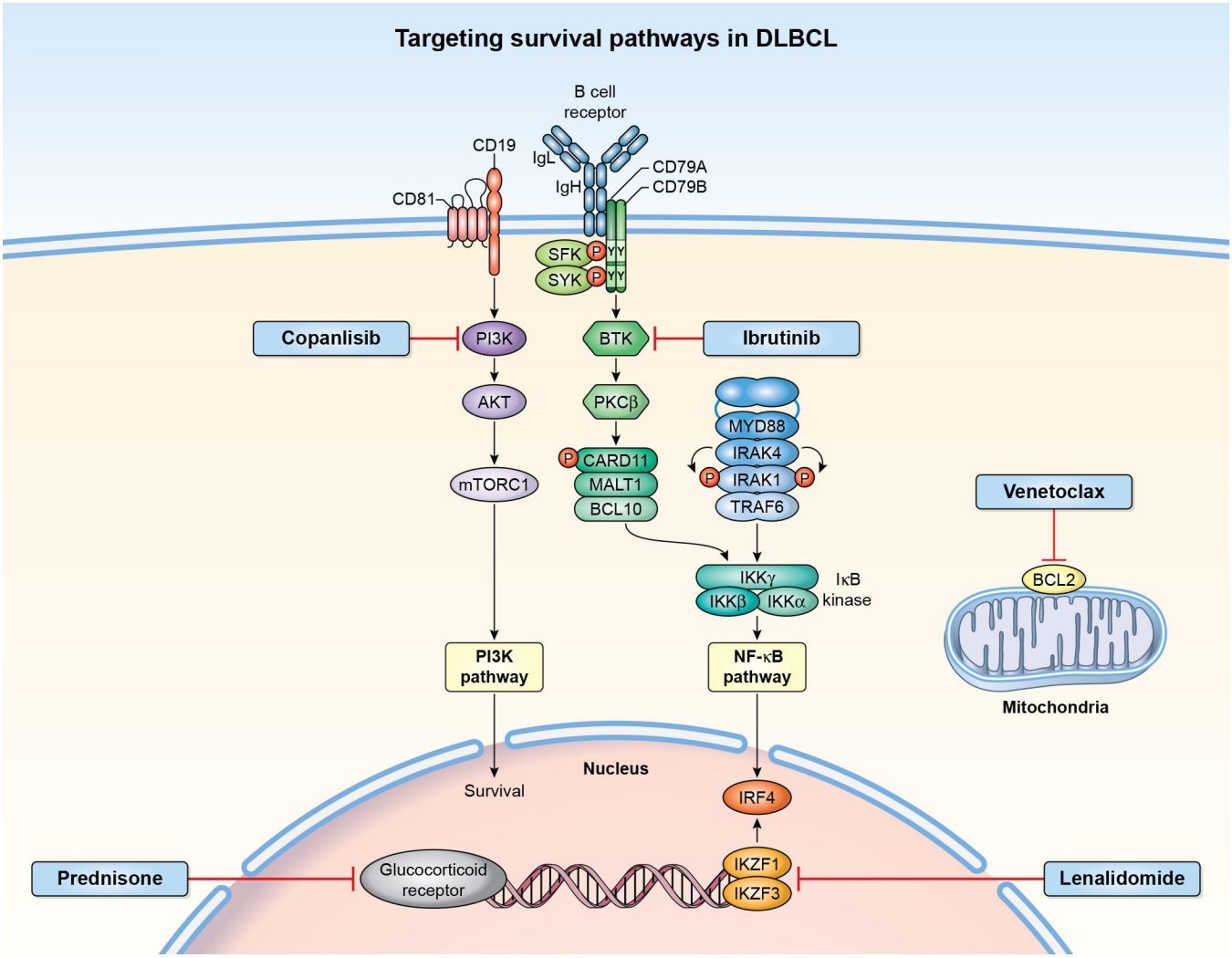

Figure 2.

The key survival pathways implicated in DLBCL with targeted novel agents in clinical development. Copanlisib targets both the α and δ isoforms of phosphatidylinositol 3-kinase (PI3K); an upstream target of the PI3K/Akt/mTOR pathway, which is activated by BCR signaling. Upstream inhibitors of the NF-κB pathway, such as ibrutinib and acalabrutinib, target Bruton's tyrosine kinase (BTK) involved in chronic active BCR signaling. Downstream inhibitors of the NF-κB pathway include lenalidomide which has multiple inhibitory mechanisms including direct targeting of interferon regulatory factor 4 (IRF4) and augmentation of the interferon pathway through degradation of two specific transcription factors, IKZF1 and IKZF3. Venetoclax is an inhibitor of the anti-apoptotic protein BCL-2 and restores the apoptotic ability of tumor cells. Prednisone exerts genotoxic stress in malignant cells through its action on the glucocorticoid receptor and is an essential component of targeted agent combinations.

Abbreviations: DLBCL, diffuse large B-cell lymphoma PI3K, phosphatidylinositol 3-kinase; mTORC1, mammalian target of rapamycin complex 1; SFK, Src-family kinase; SYK, spleen tyrosine kinase; BTK, Bruton's tyrosine kinase; PKCβ, protein kinase C beta; CARD11,

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caspase recruitment domain-containing protein 11; MALT1, mucosa-associated lymphoid tissue lymphoma translocation protein 1; BCL10, B-cell lymphoma/leukaemia 10; MYD88, myeloid differentiation primary response gene 88; IRAK4, interleukin-1 receptor-associated kinase 4; IRAK1, interleukin-1 receptor-associated kinase 1; TRAF6, tumor necrosis factor receptor-associated factor 6; IKK, IKK complex; NF-κB, nuclear factor-κB; IRF4, interferon regulatory factor 4; IKZF1, ikaros family zinc finger protein 1; IKZF3, ikaros family zinc finger protein 3.

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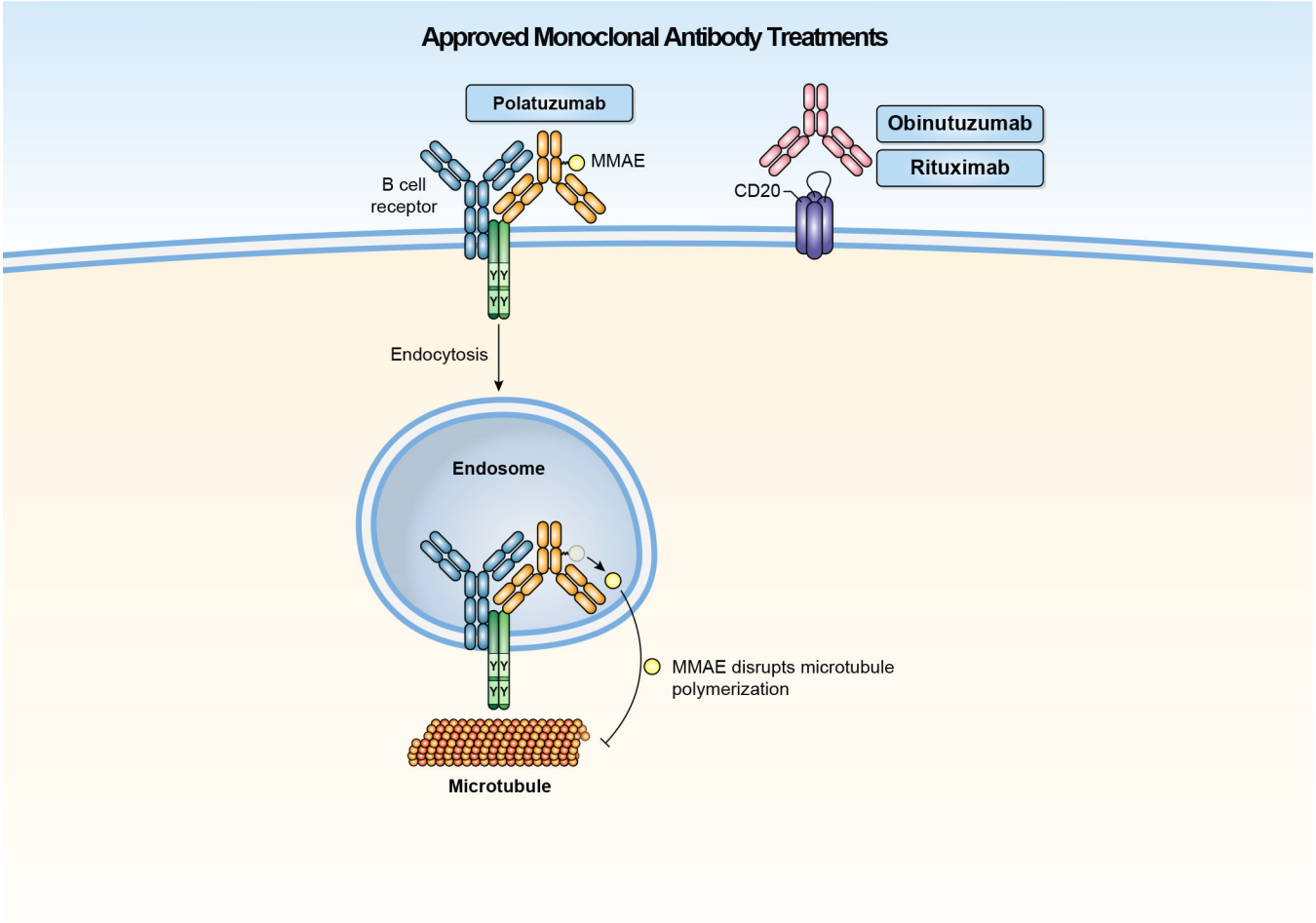

Figure 3.

Targetable surface proteins in DLBCL. The surface expression of CD20 and CD79B are therapeutic targets in all subtypes of DLBCL. CD20 is a surface molecule universally present on DLBCL and the monoclonal antibody rituximab is an essential component of frontline therapy for DLBCL. Obinutuzumab is a glycoengineered, type II anti-CD20 monoclonal antibody targeting CD20 that may improve both direct and antibody-dependent cellular cytotoxicity compared to rituximab and is being studied in novel combinations for relapsed and refractory DLBCL. Polatuzumab Vedotin is an antibody-drug conjugate that includes a monoclonal antibody targeting CD79B conjugated to monomethyl auristatin E (MMAE) via a protein linker. Upon binding to CD79B on the cell surface, MMAE-conjugate enters the cell via endocytosis and is released after exposure to an acidic pH. MMAE can then inhibit microtubule formation.

Abbreviations: DLBCL, diffuse large B-cell lymphoma; MMAE, monomethyl auristatin E

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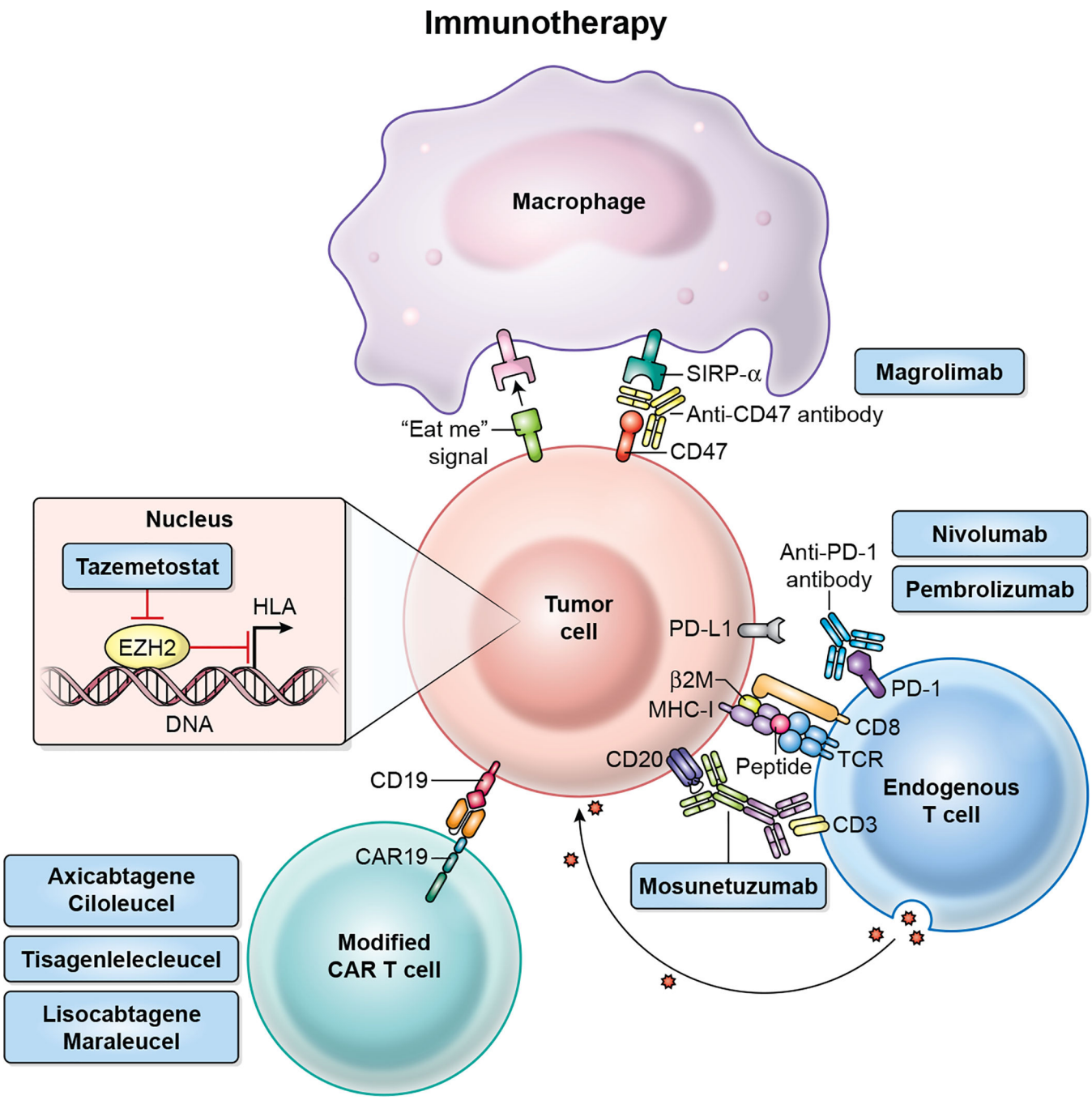

Figure 4.

A variety of novel immunotherapy agents are in development in DLBCL that work through interactions within the tumor microenvironment to promote more effective cytotoxic killing of T-cells or macrophages. Magrolimab is a novel monoclonal antibody targeting CD47 on the surface of tumor cells disrupting the "don't eat me" signal that prevents tumor phagocytosis by macrophages. Nivolumab and pembrolizumab are monoclonal antibodies targeting PD-1 that block the inhibitory signal that tumor cells use to prevent activation of endogeneous T-cells and avoid immune recognition. The PD-1 pathway is particularly

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relevant in PMBL. Mosunetuzumab is a novel bispecific antibody that targets both CD3 on the surface of T-cells as well as CD20 on the surface of malignant B-cells. Bispecific antibodies promote more efficient targeting of B-cells by bringing activated endogenous T-cells in close proximity to tumor cells. Chimeric antigen receptors (CAR) are fusion proteins that include antigen recognition moieties and T-cell signaling domains. The cell surface protein CD19 has emerged as an effective therapeutic target for CAR-T cell therapies. Tazemetostat is an inhibitor of EZH2. EZH2 normally exerts epigenetic control of many oncogenic processes including repression of MHC expression. Inhibitors of EZH2 may therefore enhance various forms of immunotherapy through improved tumor antigen presentation.

Abbreviations: DLBCL, diffuse large B-cell lymphoma; PMBL, primary mediastinal B-cell lymphoma; SIRP-α, signal regulatory protein α; MHC, major histocompatibility complex; HLA, human leukocyte antigen; PD-1, programmed cell death protein 1; PD-L1, programmed death-ligand 1; β2M, beta 2 microglobulin; MHC-I, major histocompatibility complex class I; TCR, T-cell receptor; CAR19, chimeric antigen T-cells targeting CD19; CAR T, chimeric antigen T-cell;

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Table 1.

Recent randomized studies of frontline therapy in diffuse large B-cell lymphoma

Trial Population	Patients (#)	Comparison	Primary outcome	Reference
All DLBCL subtypes, early stage (FLYER)	N=592	R-CHOP × 4 (with 2 additional rituximab) vs. R-CHOP × 6	3-year PFS 94% (95% CI, 91–97) vs 96% (95 CI, 94–99)	Poeschel et al.[57]
All DLBCL subtypes (ALLIANCE)	N=524	DA-EPOCH-R × 6 vs. R-CHOP × 6	2-year PFS 79% (95% CI, 74–84) vs 76% (95 CI, 70–81) HR 0.93 (95% CI 0.68–1.27; *P*=0.65)	Bartlett et al.[64]
All DLBCL subtypes (GOYA)	N=1418	Obinutuzumab + CHOP × 6–8 vs. R-CHOP × 6–8	3-year PFS 70% vs 67% HR=0.92 (95 CI, 0.76–1.11; *P*=0.39)	Vitolo et al.[66]
All DLBCL subtypes (REMoDL-B)	N=1128	Bortezomib + R-CHOP × 6 vs. R-CHOP × 6	30-month PFS 70% (95% CI, 65–75) vs 74% (95 CI, 69–79) HR 0.86 (95% CI 0.65–1.13; *P*=0.28)	Davies et al.[71]
Non-GCB DLBCL (PHOENIX)	N=838	Ibrutinib + R-CHOP × 6 vs. Placebo + R-CHOP × 6	3-year EFS 70% (95% CI, 65–74) vs. 67% (95% CI, 62–72) HR 0.93 (95% CI 0.73–1.20; *P*=0.59)	Younes et al.[75]
ABC DLBCL (ROBUST)	N=570	Lenalidomide + R-CHOP × 6 vs. Placebo + R-CHOP × 6	Median PFS not yet reached – no difference HR 0.85 (95% CI 0.63–1.14; *P*=0.29)	Vitolo et al.[80]
Non-GCB DLBCL, phase 2 (ECOG-ACRIN1412)	N=349	Lenalidomide + R-CHOP × 6 vs. R-CHOP × 6	2-year PFS improved with lenalidomide + R-CHOP HR 0.67 (95% CI 0.44–1.03; *P*(one-sided) = 0.03)	Nowakowski et al.[79]

Abbreviations: DLBCL, diffuse large B-cell lymphoma; R-CHOP, rituximab, cyclophosphamide, doxorubicin, vincristine and prednisone; PFS, progression-free survival; CI, confidence interval; DA-EPOCH-R, dose-adjusted etoposide, vincristine, doxorubicin with cyclophosphamide, prednisone, and rituximab; HR, hazard ratio; non-GCB, non-germinal center B-cell; EFS, event-free survival; ABC, activated B-cell

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