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Review Article

Mechanisms of Immune Evasion and Novel Treatments for Relapsed and Refractory Diffuse Large B-cell Lymphoma

Kamen W. Kossow[1*], Joseph G. Bennett[2] and Marc S. Hoffmann[2]

[1]*Department of Internal Medicine, University of Kansas Health System, Kansas City, KS, USA;* [2]*Department of Hematologic Malignancies and Cellular Therapeutics, University of Kansas Health System, Westwood, KS, USA*

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Abstract

Diffuse large B-cell lymphoma (DLBCL) is the most common subtype of aggressive B-cell non-Hodgkin lymphoma. While a substantial fraction of patients are cured with frontline chemoimmunotherapy, approximately 30% of cases subsequently relapse. DLBCL immune evasion and refractory disease can occur via several mechanisms: downregulation or loss of major histocompatibility complex expression, immune checkpoint activation, tumor microenvironment modulation, and resistance to apoptosis. Addressing these mechanisms of immune evasion in DLBCL has been a focus of ongoing research, leading to the exploration of new therapies. Here, we review the mechanisms of immune evasion and novel immunotherapy treatment strategies for DLBCL.

Introduction

Diffuse large B-cell lymphoma (DLBCL) is a heterogeneous disease with varying biology, clinical manifestations, and responses to treatment.[1] Cases of DLBCL may arise *de novo* or as a transformation event from a more indolent lymphoma such as marginal zone lymphoma or follicular lymphoma.[2] Unfortunately, approximately 30% of patients will not be cured with chemoimmunotherapy.[3] In recent years, the knowledge of disease variability, genetic classification, and immune evasion has increased. Such mechanisms include epigenetic remodeling, inhibition of differentiation, escape from immune surveillance, and alterations in signal transduction pathways, all of which can lead to the development of refractory disease.[3] A greater understanding of this variability allows for improved prognostication and clinical management of DLBCL. Here, we review the current body of literature with regard to immune evasion and discuss novel treatment strategies.

DLBCL has a wide variety of clinicopathologic classifications that were recognized by the 2022 World Health Organization.[4] These distinct diagnostic categories include DLBCL not otherwise specified (NOS), high grade B-cell lymphoma with myelocytomatosis oncogene (MYC) and B-cell lymphoma 2 (BCL-2) rearrangements, T-cell/histocyte-rich large B-cell lymphoma, primary mediastinal large B-cell lymphoma, intravascular large B-cell lymphoma, and primary large B-cell lymphoma of immune-privileged sites, among others.[4] DLBCL can be risk stratified via a variety of methods for subtype differentiation, allowing for more precise prognostication and treatment approaches. Given the heterogeneity of DLBCL, understanding its distinct disease biology and outcomes allows for the development of more targeted approaches for the management of DLBCL. For example, determining the cell-of-origin (COO) should be obtained for all new diagnoses of DLBCL, which can be differentiated into the germinal center B-cell (GCB) type, activated B-cell (ABC) type, and a third unclassifiable type.[5] These distinctions are important at the time of diagnosis, as these subtypes represent different molecular entities and may also respond differently to certain treatments.[5] Generally, ABC-DLBCL is associated with a worse prognosis than GCB-DLBCL.[6] The distinction of COO may influence therapy selection, with the most recent example being the use of Pola-R-CHP (polatuzumab vedotin + rituximab + cyclophosphamide/doxorubicin/prednisone) compared to R-CHOP (rituximab + cyclophosphamide/doxorubicin/vincristine/prednisone) in different COO subtypes, with data indicating improved responses in ABC-DLBCL compared to GCB-DLBCL.[7] Additionally, differentiation by molecular subtype is another possible approach for the individualized management of DLBCL, although its implementation in clinical practice has been challenging, and ongoing trials are being designed to evaluate its application in clinical management.

Several genomic alterations occur in DLBCL, which affect immune status. Over 70% of DLBCL cases harbor genetic alterations

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[*]**Correspondence to:** Kamen W. Kossow, Department of Internal Medicine, University of Kansas Health System, 6745 West 146th Court apartment 35101, Overland Park, KS 66223, USA. ORCID: https://orcid.org/0009-0007-1561-6616. Tel: +1-785-643-1565, E-mail: kkossow@kumc.edu

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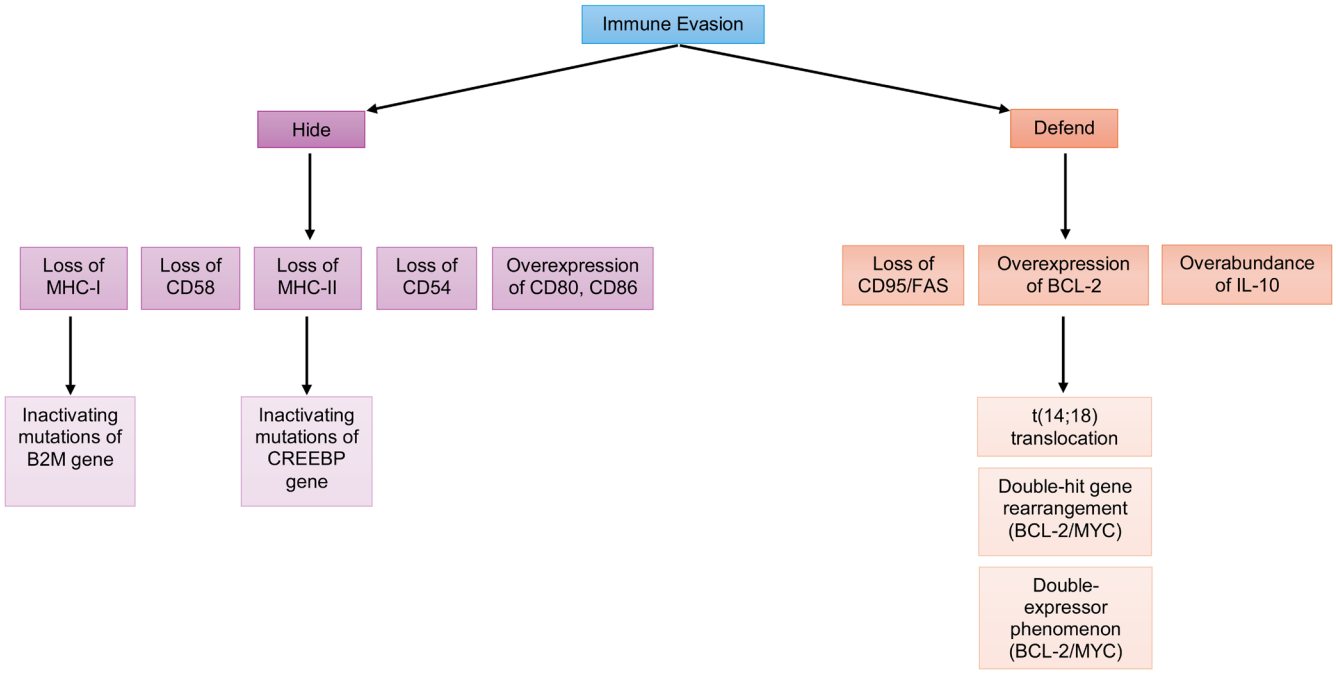

Fig. 1. Mechanisms of immune evasion by lymphoma: "hide" and "defend".[16–19] B2M, beta-2-microglobulin; BCL, B-cell lymphoma; CD, cluster of differentiation; CREBBP, CREB-binding protein; IL, interleukin; MHC, major histocompatibility complex; MYC, myelocytomatosis oncogene.

in genes associated with immune escape.[8] DLBCLs are characterized by somatic hypermutation (SHM) in genes encoding variable regions of the immunoglobulin gene and off target genes such as PIM1.[7] Cases of DLBCL with SHM express higher numbers of IgG neoantigens, leading to increased cell proliferation, increased cancer cell migration, and reduced apoptosis, all resulting in a poor clinical prognosis.[9,10] Another example of alterations affecting immune status is Epstein-Barr virus (EBV) associated DLBCL, which expresses EBV-derived antigen on the tumor cell surface, which have been shown to generate an anti-tumoral response via antigen specific T-cells.[11]

Epigenetic dysregulation in lymphoma leads to the downregulation of tumor suppressor genes, DNA repair proteins, and cell cycle regulators, all of which enhance the ability of tumor cells to evade the immune system.[12] In recent years, the epigenetic changes in DLBCL have been better elicited, with more than 400 loci involving point mutations or recurrent copy numbers in exon sequences primarily in histone modification.[13] Such epigenetic changes in DLBCL have been identified in the CREB-binding protein (CREBBP) and EP300 genes which affect multiple signaling pathways identified as being important for lymphomagenesis.[13] The resulting increase in aberrant DNA methylation associated with epigenetic mutations in CREBBP and EP300 is associated with a poorer prognosis in patients with DLBCL.[12] Patients with DLBCL aged 75 years and older have been found to have increased tumor methylation, indicating the possible role of using therapeutics targeting acetylation/deacetylation mechanisms when considering treatment approaches.[14,15] Given this, the Southwest Oncology Group (SWOG) 1918 phase II/III randomized study is evaluating the incorporation of the oral hypomethylating agent azacitidine with R-miniCHOP in this patient population.[14]

The International Prognostic Index (IPI) and its revisions are the main historical tools for predicting long term survival in aggressive non-Hodgkin lymphomas (NHL) such as DLBCL following treatment with doxorubicin-containing treatment regimens.[15] The IPI incorporates age at diagnosis, Eastern Cooperative Oncology Group performance status, serum lactate dehydrogenase levels, extranodal disease, and clinical stage. In the rituximab era, the original IPI has been validated along with the revised IPI.[15] However, with the improved molecular classification and increased knowledge of immunological escape, additional prognostic models are desperately needed.

Mechanisms of immune evasion

The concept of cancer immunosurveillance was proposed over 60 years ago. Like many other hematologic malignancies, DLBCL can employ various mechanisms to evade immune detection and destruction. Generally, the presence of these immune evasion mechanisms in DLBCL results in a worse prognosis with regard to progression free survival (PFS) and overall survival (OS). Charette and Houot described two mechanisms of lymphoma evasion: hide or defend.[16] Lymphomas and DLBCL can "hide" from immune surveillance via loss or downregulation of major histocompatibility complex 1 (MHC-I), cluster of differentiation 58 (CD58), MHC-II, and/or CD54, as well as via overexpression of CD80 and CD86.[16] Conversely, lymphoma cells can "defend" themselves by loss or downregulation of CD95/FAS, overexpression of BCL-2, or overabundance of interleukin 10 (IL-10) (Figs. 1 and 2).[16–19]

Hide mechanisms

The hide mechanisms occur via loss or downregulation of antigen presenting cells (MHC-I, MHC-II), loss of activation of natural killer (NK) cells (CD58), loss of cell adhesion signaling (CD54), and overexpression of co-stimulatory molecules (CD80, CD86) (Table 1 and Fig. 2).[16,17,20–26]

Loss or downregulation of MHC-I results in the loss of the im-

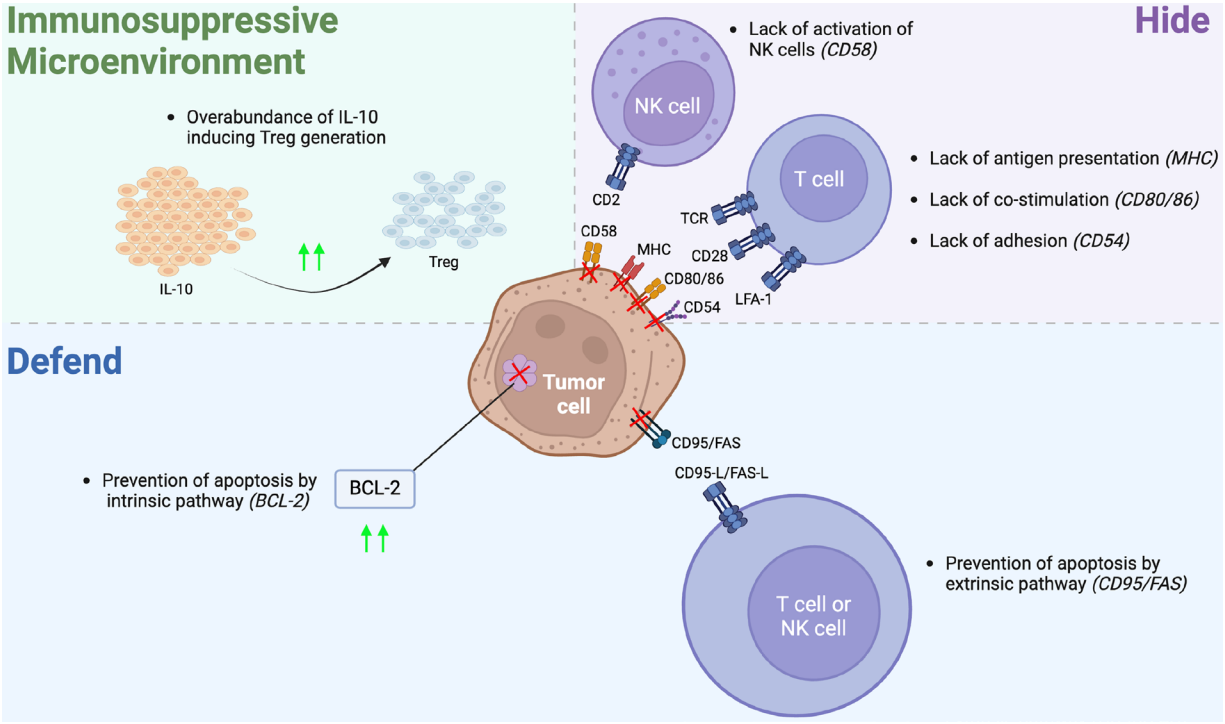

Fig. 2. Mechanisms of immune evasion by lymphoma.[16] Major proposed mechanisms of immune evasion by lymphoma cells and the related molecules involved in each mechanism: "Hide" (MHC-I, MHC-II, CD58, CD54, CD80, CD86), "Defend" (CD95/FAS, BCL-2), "Immunosuppressive Microenvironment" (IL-10, Treg). BCL, B-cell lymphoma; CD, cluster of differentiation; IL, interleukin; MHC, major histocompatibility complex; NK, natural killer; Treg, regulatory T-cells.

munological synapse between CD8+ T-cells and antigen presenting cells, leading to the evasion of immune recognition by tumor cells.[16] In DLBCL, the loss of MHC-I is commonly due to inactivating mutations in the beta-2-microglobulin (B2M) gene, which normally functions to inhibit the human leukocyte antigen 1 (HLA-I) complex for recognition by CD8+ T-cells.[17] Loss of MHC-I occurs in approximately 55–75% of DLBCL cases, while B2M gene mutations occur in 29% of DLBCL cases.[16,17,20] A retrospective

Table 1. Mechanisms of immune resistance: molecules involved in "hide" mechanisms[16]

Molecule	Normal function	Alteration	Result	Prevalence in DLBCL	Prognostication in DLBCL
MHC-I	Antigen presentation to CD8 T-cells	Loss or downregulation	Prevention of antigen presentation; Commonly due to B2M gene mutations	Loss of MHC-I in 55–75%[16]; B2M gene mutation in 29%[17,20]	Worse 5-year PFS and OS with B2M mutation[21]
CD58	Activate NK cells when MHC-I is absent	Loss or downregulation	In conjunction with the absence of MHC-I, escape killing by NK cells	Loss of CD58 in 67%[16,17]	Shortened OS and increased risk of disease progression with loss of CD58[22]
MHC-II	Antigen presentation to CD4 T-cells	Loss or downregulation	Prevention of antigen presentation; Commonly due to CREBBP gene mutations	Loss of MHC-II in 20%[16]; CREBBP gene mutation in 30%[23]	Worse 3-year PFS and OS with CREBBP mutation[24]
CD54	Tumor-to-immune cell adhesion	Loss or downregulation	Diminished interaction between tumor and immune cells	Loss of CD54 in 7%[16]	Worse 2-year OS with loss of CD54[25]
CD80, CD86	Mediate T-cell activation (via CD28) and/or suppression (via CTLA-4)	Overexpression	Inhibition of T-cell activation due to preferential binding to the inhibitory CTLA-4 receptor	CD80 expressed on 81% of cells[26]; CD86 expressed on 90% of cells[26]	Unclear prognostic effects in DLBCL[16]

See Figs. 1 and 2 for illustrations. B2M, beta-2-microglobulin; CD, cluster of differentiation; CREBBP, CREB-binding protein; CTLA, cytotoxic T lymphocyte-associated antigen; DLBCL, diffuse large B-cell lymphoma; MHC, major histocompatibility complex; OS, overall survival; NK, natural killer; PFS, progression free survival.

Table 2. Mechanisms of immune resistance: molecules involved in "defend" mechanisms[16,18,19]

Molecule	Normal function	Alteration	Result	Prevalence in DLBCL	Prognostication in DLBCL
CD95/FAS	Induction of apoptosis	Loss or downregulation	Prevention of apoptosis	Loss of CD95 in 51%*[16]	Worse 5-year OS with loss of CD95[28]
BCL-2	Anti-apoptotic protein regulating the intrinsic apoptosis pathway	Overexpression	Prevention of apoptosis; Commonly due to: t(14;18) translocation; Double-hit gene rearrangement (BCL-2/MYC); Double-expressor phenomenon (BCL-2/MYC)	t(14;18) translocation in 30%[29]; DHL in 5–7%**[19]; DEL in 20–30%[19]	Worse 5-year OS with BCL-2/MYC DHL[30]; Worse 5-year OS and 5-year PFS with DEL[31]
IL-10	Induces Treg generation and stimulates growth of malignant B-cells	Overabundance	Creates tolerogenic microenvironment	Serum elevation of IL-10 in 58%[16]	Worse PFS and OS with elevated serum IL-10[32]

See Figs. 1 and 2 for illustrations. *Extra-nodal DLBCL cases; **65% of DHL cases due to BCL-2/MYC rearrangement. BCL, B-cell lymphoma; CD, cluster of differentiation; DEL, double-expressor lymphoma; DHL, double-hit lymphoma; DLBCL, diffuse large B-cell lymphoma; IL, interleukin; MYC, myelocytomatosis oncogene; OS, overall survival; PFS, progression free survival; Treg, regulatory T-cells.

series found that cases of DLBCL with B2M gene mutations, and subsequent elevations in serum B2M levels, have a significantly worse prognosis compared to cases with normal serum B2M levels (5-year PFS: 41.0% vs 76.1%; OS: 49.2% vs 83.8%).[21]

The loss of MHC-I in DLBCL is associated with the loss or downregulation of CD58. In the absence of MHC-I, CD58 normally functions as a self-missing signal to activate NK cells to eradicate malignant cells.[16] Thus, the loss or downregulation of CD58, in conjunction with the absence of MHC-I, results in tumor cells escaping killing by NK cells. Loss of CD58 occurs in 67% of DLBCL cases.[16,17] A retrospective series found that cases of DLBCL with loss of CD58 have a significantly shorter OS and an increased risk of disease progression compared to cases without loss of CD58.[22]

Similarly, the loss or downregulation of MHC-II results in the loss of the immunological synapse between CD4+ T-cells and antigen presenting cells, leading to the evasion of immune recognition by tumor cells.[16] In DLBCL, the loss of MHC-II is commonly due to inactivating mutations in the CREBBP gene (in the histone acetyltransferase domain), which normally functions to regulate cell growth and differentiation.[16,27] Loss of MHC-II occurs in 20% of DLBCL cases, while CREBBP gene mutations occur in 30% of DLBCL cases.[16,23] A retrospective series found that cases of DLBCL with CREBBP gene mutations have a significantly worse prognosis compared to cases without CREBBP gene mutations (3-year PFS: 52.6% vs 71.3%; OS: 67.8% vs 79.7%).[24] Additionally, loss or downregulation of MHC-II has also been shown to occur via epigenetic alterations in DLBCL, as mentioned previously.[16,27]

CD54, which normally functions in adhesion between immune cells and tumor cells, is commonly lost or downregulated in DLBCL.[16] Loss or downregulation of CD54 results in diminished interaction between immune cells and tumor cells, leading to immune evasion.[16] Loss of CD54 occurs in 7% of DLBCL cases.[16] A retrospective series found that cases of DLBCL with loss of CD54 have a significantly worse prognosis compared to patients with the presence of CD54 (2-year OS: 50.0% vs 77.0%).[25]

CD80 and CD86, members of the B7 co-stimulatory family, normally function to mediate T-cell activation (via CD28) and/or suppression (via cytotoxic T lymphocyte-associated antigen 4 (CTLA-4)).[16] Importantly, CD80 and CD86 bind to the inhibitory receptor CTLA-4 with a much higher affinity than to the activation receptor CD28.[16] Thus, overexpression of CD80 and CD86 preferentially bind to CTLA-4, leading to inhibition of T-cell activation and subsequent immune evasion by lymphoma cells.[16] CD80 is expressed on 81% of DLBCL malignant cells, while CD86 is expressed on 90% of DLBCL malignant cells.[26] Prognostication regarding CD80 and CD86 expression in DLBCL remains unclear, likely due to the dual activity of these co-stimulatory molecules.[16]

Defend mechanisms

The defend mechanisms occur by lymphoma cells becoming resistant to apoptosis (via loss or downregulation of CD95/FAS and overexpression of BCL-2) and by inducing an immunosuppressive microenvironment (via overabundance of IL-10) (see Table 2 and Fig. 2).[16,18,19,28–32]

Cancer cells, including DLBCL cells, can acquire mutations that interfere with normal apoptotic (programmed cell death) pathways. This allows cancer cells to resist cell death induced by the immune system. Apoptosis can occur via the perforin/granzyme pathway with release of cytotoxic granules from NK cells or cytotoxic T-lymphocytes, the extrinsic pathway via CD95/FAS or tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) death receptors, or the intrinsic pathway involving the BCL-2 family of proteins activated by intrinsic signals.[16] The ABC subtype of DLBCL has been found to have high expression of pro and anti-apoptotic proteins that may play a role in the different responses observed between ABC and other subtypes of DLBCL.[33]

CD95/FAS is involved in the tumor necrosis factor (TNF) receptor family and through ligation of CD95L/FASL, it induces apoptosis through its intracellular domain and caspase activation.[16] Loss or downregulation of CD95/FAS in DLBCL results in dysregulation of apoptosis and subsequent evasion of cell death by the immune system. Loss of CD95 occurs in 51% of extranodal DLBCL cases.[16] CD95-positive DLBCL is associated with improved survival and response to R-CHOP (rituximab + cyclophosphamide, doxorubicin, vincristine) therapy.[28,34] A retrospective series by Chatzitolios and colleagues revealed that cases of CD95-negative DLBCL have a significantly worse prognosis compared to CD95-positive cases (5-year OS: 35.0% vs 71.5%).[28]

BCL-2 is an important anti-apoptotic protein involved in the regulation of the intrinsic pathway of mitochondrial apoptosis and is frequently dysregulated in DLBCL.[18] There are three clinically detectable alterations leading to BCL-2 aberrancy in DLBCL: t(14;18) translocation, double-hit genetic rearrangement, and double-expressor phenomenon via immunohistochemistry. The presence of one of these alterations may alter treatment

approaches in DLBCL, with trials underway to evaluate the ideal therapies to utilize. One example is the phase II/III trial evaluating the addition of venetoclax to standard chemotherapy in cases of BCL-2/MYC double-hit and double-expressing lymphomas (#NCT03984448). In DLBCL, gene rearrangements of BCL-2, most frequently a t(14;18) translocation, are associated with a worse prognosis when combined with a MYC rearrangement.[35] The t(14;18) translocation juxtaposes the BCL-2 oncogene and enhancer of heavy chain immunoglobulin, leading to overexpression of the BCL-2 gene.[18] While the t(14;18) translocation occurs more frequently in follicular lymphoma (90% of cases), it is only present in approximately 30% of DLBCL cases.[29] Cases of DLBCL with a t(14;18) translocation may represent histologic transformation from a prior follicular lymphoma or a *de novo* DLBCL subset demonstrating GCB gene expression.[29,36] The MYC proto-oncogene, located on chromosome 8q24, encodes a transcription factor involved in protein synthesis and cellular differentiation.[19] Overexpression of MYC in DLBCL results in cellular proliferation and inhibition of apoptosis (by increasing expression of the tumor suppressor TP53).[19] In DLBCL, the double-hit phenomenon is due to a genetic rearrangement of the BCL-2 (and/or BCL-6) and MYC genes.[19] These malignancies are termed double-hit lymphoma (DHL) and/or high-grade B-cell lymphoma.[19] DHL accounts for 5–7% of all DLBCLs, with a majority (65%) being secondary to a BCL-2/MYC gene rearrangement.[19] Concerning COO classification, a majority (80–90%) of DHL cases occur with the GCB-DLBCL subtype.[19] A retrospective series revealed that cases of DLBCL with a BCL-2/MYC genetic rearrangement are associated with a significantly worse prognosis compared to cases without rearrangement (5-year OS: 33.0% vs 72.0%).[30] Conversely, isolated gene rearrangements of BCL-2, without MYC rearrangement, may not be associated with a worse prognosis highlighting the role that the presence of MYC plays in prognostication.[37,38] Additionally, BCL-2 overexpression with associated MYC overexpression that is not related to chromosomal rearrangements is associated with a poor prognosis in DLBCL cases.[31] The overexpression of BCL-2 and MYC not due to gene rearrangement is defined as the double-expressor phenomenon and cases are termed double-expressor lymphoma (DEL).[19] Cases of DEL account for 20–30% of all DLBCLs, with a majority (66%) of DEL occurring with the ABC-DLBCL subtype.[19,31] A retrospective series revealed that cases of DEL DLBCLs with coexpression of BCL-2 and MYC have a significantly worse prognosis compared to cases without coexpression (5-year OS: 30.0% vs 75.0%; 5-year PFS: 27.0% vs 73.0%).[31] Historically, the expression of BCL-2, in general, has been associated with a worse prognosis when compared to cases of DLBCL without BCL-2 expression, although since the introduction of rituximab as a treatment adjunct for DLBCL, this historically worse prognosis appears to be overcome with the addition of rituximab to standard chemotherapy regimens.[39,40]

In addition to the effects of malignant cells on apoptosis, DLBCL cells also create a tolerogenic microenvironment resulting in resistance to normal immune responses. One important cytokine is IL-10, which stimulates the generation of regulatory T-cells (Tregs) and promotes the growth of malignant B-cells.[16] In DLBCL, an overabundance of IL-10 secretion induces Treg generation, resulting in a tolerogenic microenvironment that limits the effectiveness of cytotoxic T-cells.[16] Elevated IL-10 serum levels occur in 58% of DLBCL cases and have been found to have a significantly worse prognosis with shorter PFS and OS when compared to cases with low levels of serum IL-10.[16,32]

Novel treatments for DLBCL

In recent years, there has been a revolution in the available therapeutics for the treatment of DLBCL. In light of the aforementioned multifactorial immunological mechanisms of resistance, these treatments continue to improve survival and aim to bypass the cellular mechanisms of refractory disease. Some of these treatments include rituximab, chimeric antigen receptor (CAR) T-cell therapy, bispecific antibodies (BsAbs), polatuzumab vedotin, loncastuximab tesirine, tafasitamab/lenalidomide, and bruton tyrosine kinase inhibitors. Clinicians must be aware of these treatments and indications to allow timely referral and administration of these life-saving treatments. Treatment decisions are nuanced, and require careful consideration of prior treatment course, symptomatology, eligibility for novel therapies, performance status, and co-morbidities. When possible, treatments should be tailored to disease characteristics to implement individualized treatment approaches.

Rituximab

The historical example demonstrating that immunotherapy is a powerful tool for DLBCL eradication and cure is rituximab. Rituximab is a monoclonal antibody targeting CD20.[41] CD20 is a membrane protein expressed on normal and neoplastic B-lymphocytes, but it is not present on hematopoietic stem cells.[41] The mechanisms of rituximab-induced cytotoxicity include direct induction of apoptosis, antibody-dependent cellular cytotoxicity (ADCC), and complement-dependent cytotoxicity (CDC).[42] Moreover, rituximab sensitizes NHL cells to chemotherapy (Table 3 and Fig. 3).[41–49]

Rituximab directly induces apoptosis via three major mechanisms. First, rituximab induces the apoptosis of malignant cells by inhibiting the anti-apoptotic cytokine IL-10, which subsequently results in decreased Treg production and decreased B-cell growth.[43] Second, rituximab alters the intrinsic apoptotic pathway by downregulating the production of the anti-apoptotic protein BCL-2, resulting in increased immune system apoptotic activity to target tumor cells.[43] Finally, rituximab alters the extrinsic apoptotic pathway by upregulating Raf-1 kinase inhibitor protein (RKIP), resulting in the downregulation of B-cell lymphoma-extra-large (Bcl-xL) proteins.[44] The downregulation of Bcl-xL results in the sensitization of tumor cells to drug-induced apoptosis.[44] While these mechanisms of apoptosis induction are fundamental to the effectiveness of rituximab, issues with treatment resistance can occur. One mechanism of rituximab resistance associated with apoptosis is diminished CD20 expression on malignant cells leading to cell adherence to stromal cells with subsequent avoidance of apoptosis and enhanced immune evasion by malignant cells.[41,45]

Rituximab's mechanism of action involving ADCC has been studied extensively with regard to its mechanism and role in the development of rituximab antigen escape.[46] Upon infusion of rituximab, these monoclonal antibodies identify and bind to the target CD20 cells.[46] This binding results in the recruitment of NK cells which are subsequently activated and release granules containing perforin and proteases leading to lysis of the target CD20 cell.[46] Rituximab resistance has been shown to occur in the setting of ADCC exhaustion. Bowles and colleagues demonstrated that with the production of rituximab-opsonized B-cells, CD16 on NK cells is severely downregulated resulting in the inhibition of NK cell killing of remaining rituximab-opsonized cells until CD16 can be re-expressed.[45,47] Re-expression of CD16 on NK cells and restoration of cytotoxic activity can take more than 24 hours, allowing tumor cells to proliferate.[45,48] Additionally, blockade of ADCC has been postulated to occur secondary to complement component

Table 3. Rituximab effects on immune resistance in DLBCL and associated issues of treatment resistance[41–44]

Therapy	Therapy target	Immune evasion mechanism's targeted	Mechanism's	Issues of treatment resistance
Rituximab	CD20	Apoptosis	Downregulation of IL-10; Downregulation of BCL-2; Upregulation of RKIP resulting in downregulation of Bcl-xL	Diminished CD20 expression: Decreased expression of CD20 on malignant cells results in adherence to stromal cells and avoidance of apoptosis[41,45]
		ADCC	NK cells are activated and release granules containing perforin and proteases leading to lysis of target CD20 tumor cells	ADCC exhaustion: With production of rituximab-opsonized B-cells, CD16 on NK cells is severely downregulated resulting in inhibition of NK cell killing of remaining rituximab-opsonized cells until CD16 can be re-expressed[45–48]; ADCC blockade: C3 fragments bound to rituximab-opsonized B-cells block access of NK cells[45]
		CDC	Activation of the classical complement pathway via binding of C1 complex to rituximab-opsonized B-cells	CDC exhaustion: Rapid exhaustion of serum complement levels and C3b(i) deposition*[41,45]; Increased complement inhibitory proteins: Tumor cells increase surface expression of complement inhibitor proteins (CD55, CD59)[45]

See Fig. 3 for illustration. *Studied in CLL, not DLBCL. ADCC, antibody-dependent cellular cytotoxicity; BCL, B-cell lymphoma; Bcl-xL, B-cell lymphoma-extra-large; C1, complement component 1; C3, complement component 3; CD, cluster of differentiation; CDC, complement-dependent cytotoxicity; DLBCL, diffuse large B-cell lymphoma; IL, interleukin; NK, natural killer; RKIP, Raf-1 kinase inhibitor protein.

3 (C3) fragments binding to rituximab-opsonized B-cells and blocking the access of NK cells, resulting in another mechanism of rituximab resistance.[45]

In the treatment of DLBCL, rituximab alters CDC via activation of the classical complement pathway upon binding of the complement component 1 (C1) complex to rituximab-opsonized cells, resulting in enhanced immune system eradication of abnormal cancer cells.[41,50] Rituximab resistance has also been shown

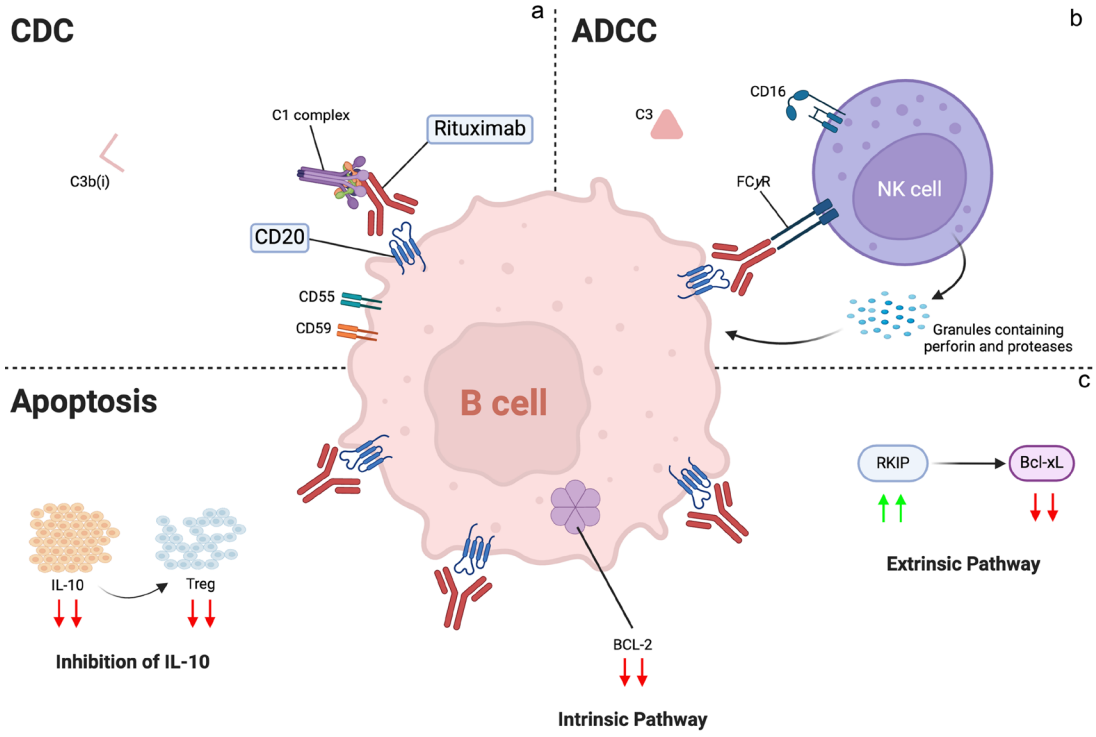

Fig. 3. Mechanisms of rituximab resistance.[41,49] Primary mechanisms of resistance to rituximab in lymphoma and the related molecules involved in each mechanism: Direct induction of apoptosis (diminished CD20 expression on malignant cells leading to cell adherence to stromal cells), ADCC (NK cell, CD16, granzyme and perforin, C3 fragment), CDC (C3b, CD55, CD59). ADCC, antibody-dependent cellular cytotoxicity; Bcl-xL, B-cell lymphoma-extra-large; C1, complement component 1; C3, complement component 3; CD, cluster of differentiation; CDC, complement-dependent cytotoxicity; FcγR, Fc gamma receptor; IL, interleukin; NK, natural killer; RKIP, Raf-1 kinase inhibitor protein.

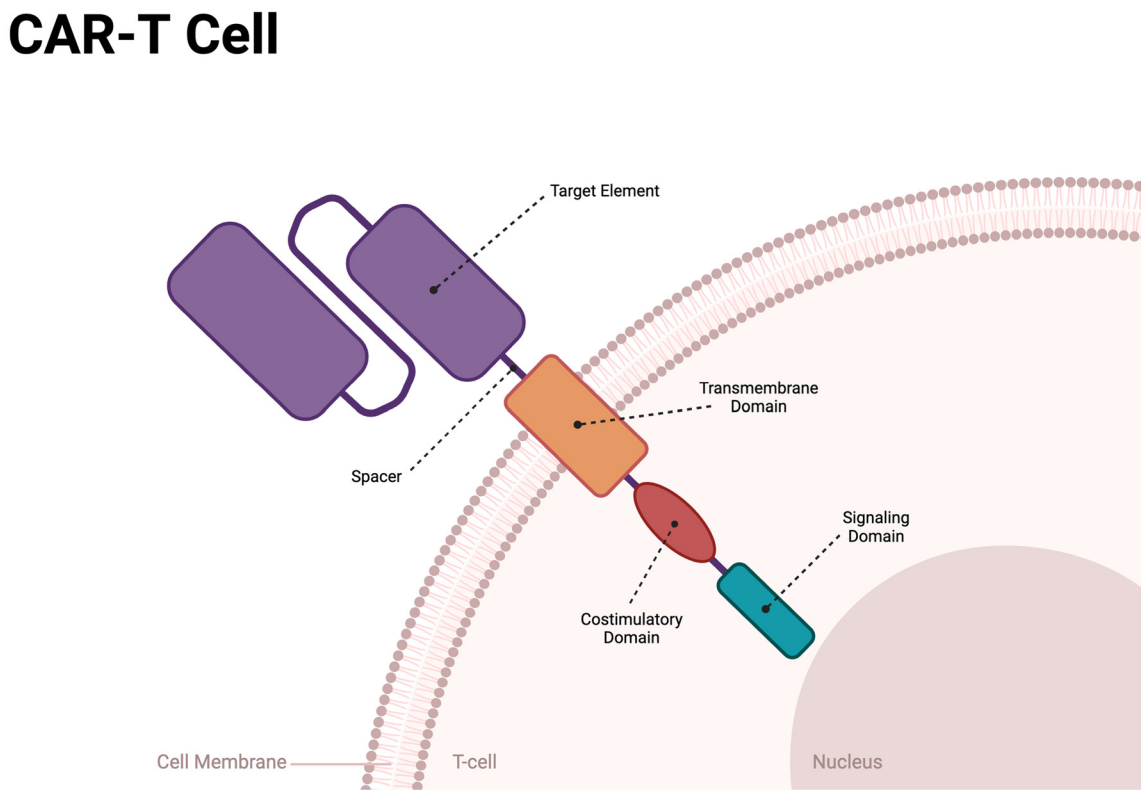

Fig. 4. CAR T-cell construct.[59] CAR-T, chimeric antigen receptor T-cell therapy.

to occur due to CDC exhaustion, although this occurs primarily in malignancies with very high densities of malignant cells such as chronic lymphocytic leukemia (CLL), rather than DLBCL.[41,45] With rituximab infusion in CLL patients, there is rapid exhaustion of serum complement levels and C3b(i) deposition, which theoretically can be applied to rituximab resistance in DLBCL cases.[41,45] Additionally, an increase in complement inhibitory proteins appears to be another possible mechanism of rituximab resistance.[45] This occurs due to tumor cells increasing the surface expression of complement inhibitor proteins such as CD55 and CD59, resulting in the inhibition of normal complement pathway function.[45]

Unfortunately, a fraction of patients with DLBCL will not achieve a cure with initial chemoimmunotherapy, and additional treatment strategies are required for primary refractory disease and cases of relapse.

CAR T-cell therapy

CAR T-cell therapy is a novel immunological treatment that aims to mobilize the immune system against lymphoma cells.[51] After apheresis, T-cells are genetically modified to retain their intracellular signaling domains, but the T-cell receptor is knocked out and replaced with the recognition domain of a monoclonal antibody.[51] As a consequence, these cells retain potent cytotoxic activity but can be directed at any antigen of interest.[52,53] The ideal target for lymphoma treatment is tailoring against malignant cells while sparing normal host cells to limit off-target toxicity. CD19 is one of these surface antigens widely expressed among a variety of B-cell malignancies and is minimally expressed in other tissues.[54] Additionally, the novelty of CAR-T-cell therapy and its efficacy result from a mechanism that is MHC-independent and can bypass the lack of antigen presentation for anti-tumor effects.[16,51] This MHC-independent mechanism is important in the treatment of DLBCL given the frequent loss of MHC-I and MHC-II as mentioned previously. Consequently, CD19-directed CAR-T cells were predicted to have good efficacy against B-cell malignancies without significant off-target effects. During clinical development, this hypothesis proved correct, and CD19-directed CAR-T cells demonstrated potent activity against a variety of B-cell malignancies, including DLBCL, while also having a predictable and manageable safety profile.[53] There are now three Food and Drug Administration (FDA) approved CAR-T-cell constructs for the treatment of relapsed DLBCL: tisagenlecleucel, axicabtagene, and lisocabtagene.[55–57] CAR design includes several structural components, including an antigen binding domain, a hinge region, a transmembrane domain, an intracellular co-stimulatory domain, and a signal transduction domain (Fig. 4).[58,59] The two major processes critical to CAR-T-cell success are expansion and persistence after infusion. Expansion is critical to the development of initial response and activity, and persistence is necessary for response durability with CAR-T-cell therapy.[60] Failure of the CAR-T construct to expand and persist generally results in a lack of treatment efficacy, indicating the importance of evaluating methods to optimize expansion and persistence.[60] Both the modulation of cytokines and the expansion time impact the duration of the anti-tumor effect.[61] Supplementation with numerous different cytokines has been shown to improve expansion and persistence with CAR-T-cell therapy.[60,62] Some examples of utilized cytokines that continue to be studied include IL-2, IL-7, IL-8, IL-9, IL-15, and IL-21.[60,61] Supplementation with IL-15 has been shown to support T-cell proliferation and survival, leading to interest in its role as an adjunct

Table 4. CAR T-Cell Therapy effects on immune resistance in DLBCL and associated issues of treatment resistance[16,51,65]

Therapy	Therapy target	Immune evasion mechanism's targeted	Mechanism's	Issues of treatment resistance
CAR T-cell therapy: Tisagenlecleucel; Axicabtagene; Lisocabtagene	CD19	Loss of MHC-I and MHC-II	CAR T-cells are able to target surface antigens without the need for MHC	T-cell exhaustion: Overexpression of PD-1/PD-L1 and/or CTLA-4 leading to inhibition of T-cell activation[66]
				Antigen (CD19) loss/modulation: Alternative splicing resulting in CD19 isoforms disrupting the target epitope[67]; Alternative splicing resulting in reduced cell surface expression[67]; Interruption in the transport of CD19 to the surface cells[67]

See Figs. 4 and 5 for illustrations. CAR, chimeric antigen receptor; CD, cluster of differentiation; CTLA, cytotoxic T-lymphocyte associated protein; DLBCL, diffuse large B-cell lymphoma; MHC, major histocompatibility complex; PD, programmed death; PD-L, programmed death-ligand.

to CAR-T in the management of lymphoma.[63] In an ongoing phase I/II trial of CD19 CAR-T cells in the treatment of B-cell malignancies (#NCT01865617), a low serum IL-15 concentration after CAR-T-cell infusion was associated with inferior CAR T-cell efficacy.[63] Therefore, trials evaluating the benefits of supplementing CAR-T cells with IL-15 are ongoing. Data regarding the use of supplemental IL-15 with CD19 CAR-T cell therapy in mice has shown increased T-cell and NK cell proliferation, as well as overall enhanced anti-tumor efficacy of human CD19 CAR-T cells compared to mice treated with CAR-T cells alone.[63] The use of IL-15 supplementation to CD19 CAR-T-cell therapy in human subjects with relapsed/refractory DLBCL is currently under investigation in an ongoing phase I clinical trial (#NCT05359211). Additionally, the anti-CD19 CAR-T-cell therapies utilized in DLBCL cases are primarily autologous, and are isolated from the patient's peripheral blood. Manufacturing processes and prolonged wait times for treatment are challenges associated with the use of autologous CAR-T-cell therapy.[64] This has generated interest in the utilization of allogeneic anti-CD19 CAR-T-cell therapy for the treatment of DLBCL. These allogeneic CAR-T-cell therapies, derived from healthy donors, are "off-the-shelf" and do not require apheresis, leading to promise in addressing the aforementioned issues with autologous CAR-T-cell therapy.[64] In relapsed/refractory DLBCL, phase I data on allogeneic anti-CD19 CAR-T-cell therapies (ALLO-501, and ALLO-501A) have shown durable responses compared to those of patients treated with autologous CAR-T-cell therapy.[64] Moreover, findings have shown that the median time from trial enrollment to receiving allogeneic CAR-T-cell therapy was three days, indicating expedited access to therapy.[64] Additionally, the ALPHA2 phase II study of ALLO-501A is ongoing to evaluate its effectiveness in treating DLBCL and follicular lymphoma (#NCT03939026). Continued evaluation of the effects of allogeneic CAR-T-cell therapy on expansion and persistence is crucial for optimizing future therapy.

Despite the high complete response rates observed with CAR-T cells, only 30–40% of eligible patients achieve durable remission.[60] Although enthusiasm for CAR-T cells remains well founded, additional research is needed, with ongoing efforts to evaluate the limitations in efficacy, including factors leading to CAR-T-cell resistance, such as T-cell exhaustion and antigen loss/modulation (Table 4 and Fig. 5).[16,51,65–68] T-cell exhaustion is a dysfunctional state of T-cells that was historically observed in chronic infections and in the presence of tumors.[69] In cancer, T-cell exhaustion occurs due to prolonged antigen exposure and an immunosuppressive tumor microenvironment leading to a loss of effector function and sustained inhibitor receptor expression.[65] CAR T-cell responders have been shown to possess memory-like characteristics, but CAR T-cell non-responders are in a highly exhausted state.[61,70] The overexpression of programmed death 1/programmed death-ligand 1 (PD-1/PD-L1) and CTLA-4 on tumor cells corresponds to CAR-T-cell interactions and has been implicated in T-cell exhaustion.[66] PD-1 is a cell surface receptor which when bound to its ligand, PD-L1, leads to the inhibition of T-cell activation.[71] CAR-T-cell therapy has been shown to induce PD-L1 expression on tumors, leading to PD-1/PD-L1 overexpression and subsequent CAR T-cell exhaustion.[72] CTLA-4 is a negative regulatory protein on T-cells which, when present, leads to the inhibition of T-cell activation.[73] Overexpression of CTLA-4 has also been shown to contribute to CAR T-cell exhaustion and CTLA-4 has been shown to be significantly increased in cases of DLBCL.[65,74] The use of immune checkpoint inhibitors (ICIs) targeting PD-1 (nivolumab, pembrolizumab) and/or CTLA-4 (ipilimumab) has previously been hypothesized to assist in overcoming CAR T-cell exhaustion.[75,76] Despite initial data indicating possible benefits in overcoming T-cell exhaustion, newer data appear to show that this method does not improve CAR-T-cell therapy when used as a salvage strategy after CAR-T-cell therapy failure.[77] Ongoing research to assess the possible benefits of ICI therapy earlier in the course of DLBCL treatment with regard to CAR-T-cell expansion and persistence is vital to determine whether there are benefits in its utilization.

In addition to T-cell exhaustion, another well documented mechanism of treatment resistance with CAR-T-cell therapy is antigen (CD19) loss/modulation.[67] The mechanisms leading to loss of CD19 expression with CAR-T-cell therapy include alternative splicing resulting in disruption of the target epitope of CD19 isoforms, as well as alternative splicing resulting in reduced cell surface expression.[67] Additionally, antigen loss/modulation can occur due to interruption of the transport of CD19 to the cell surface.[67] Tumor heterogeneity plays a role in the persistence of malignant B-cells after CAR-T-cell therapy. In DLBCL, heterogeneous antigen densities of CD19, CD20, and CD22 have been detected in the same patient derived sample.[78] Tumor cells with low or negative antigen expression preceding CAR-T-cell infusion are at risk of antigen escape and secondary clonal expansion.[79] Ongoing efforts to develop CAR-T-cell constructs incorporating multiple antigen targets are being studied with optimism for addressing antigen loss associated with CAR-T-cell therapy.[67] Ongoing studies evaluating these altered CAR-T-cell constructs in B-cell malignancies include the phase I study evaluating CD22 CAR-T cells

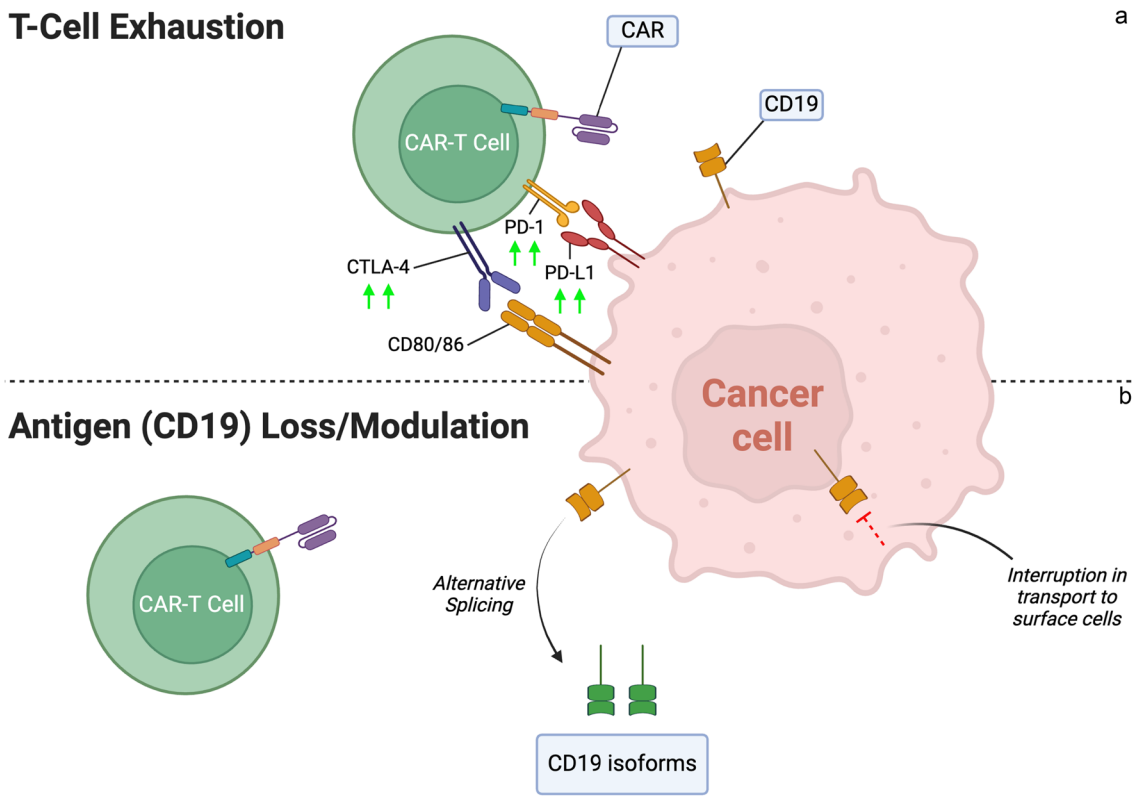

Fig. 5. Mechanisms of CAR-T resistance.[68] Primary mechanisms of resistance to CAR-T in lymphoma and the related molecules involved in each mechanism: T-cell exhaustion (PD-1/PD-L1, CTLA-4), antigen loss/modulation (CD19). CAR, chimeric antigen receptor; CAR-T, chimeric antigen receptor T-cell therapy; PD, programmed death; PD-L, programmed death-ligand; CTLA, cytotoxic T-lymphocyte associated protein; CD, cluster of differentiation.

(#NCT04088890), the phase I/II study evaluating CD19/CD20 CAR-T cells (#NCT03097770), and the phase I study evaluating CD19/CD20/CD22 CAR-T cells (#NCT05418088).

Bispecific antibodies

The BsAbs currently approved for use in DLBCL are glofitamab and epcoritamab. Both of these drugs are "off-the-shelf" BsAbs that activate peripheral and intertumoral endogenous immune cells by co-targeting tumor antigens and NK/T-cells in an FcγR and MHC-independent manner (Fig. 6).[80,81] These activated cells then cause tumor cell death.[80] DLBCL is of particular interest in the BsAb field, as DLBCL cells frequently lack expression of MHC-I and MHC-II molecules.[17,82] Anti-CD20xCD3 BsAbs have shown promising activity in heavily pretreated, high-risk disease groups for DLBCL. Both CD19 and CD20 are surface antigens widely expressed on B cells and are ideal targets for BsAbs in B-cell NHL.[83] Both glofitimab and epcoritamab have been shown to induce responses in cases of refractory DLBCL, as well as cases of CAR-T failure.[84,85] While glofitamab and epcoritamab are approved for use in treating DLBCL, the BsAb mosunetuzumab is approved for use in treating follicular lymphoma. Of note, ongoing phase I/II studies evaluating the use of the combination of mosunetuzumab and the polatuzumab vedotin have shown effectiveness and a favorable safety profile in cases of relapsed/refractory DLBCL.[86,87] Furthermore, this regimen is being evaluated in a phase I/II study (#NCT03677154), specifically in elderly/unfit patients with treatment naïve DLBCL, due to its less toxic adverse effects.

Despite the effectiveness of BsAbs in treating DLBCL, treatment resistance can occur and is commonly due to antigen (CD20) loss/modulation, changes in the tumor microenvironment, and T-cell exhaustion (Table 5).[80,82,83,88,89] As mentioned previously, DLBCL is often initially treated with the anti-CD20 monoclonal antibody, rituximab. After treatment with rituximab, loss of the target antigen (CD20) is known to occur.[80] This loss of CD20 after rituximab treatment leads to antigen loss and subsequent BsAb resistance in the treatment of DLBCL.[80] With CD20xCD3 BsAb treatment in DLBCL, this loss of CD20 has been shown to occur in patients with disease progression and recurrence.[80] Additionally, given that CD3-mediated T-cell activation induced by BsAbs is nonselective, activation of Treg cells can occur, resulting in a tolerogenic microenvironment and subsequent BsAb resistance.[80] Similar to CAR-T-cell resistance, T-cell exhaustion also occurs with BsAb treatment and is another mechanism of treatment resistance. Continuous antigen exposure with BsAb treatment results in the upregulation of inhibitory molecules (PD-1, CTLA-4), and induces a state of T-cell hypo-responsiveness leading to T-cell exhaustion.[88,89] This mechanism of treatment resistance has been reiterated through studies comparing continuous stimulation versus intermittent stimulation of the BsAb blinatumomab, which is approved for use in acute lymphoblastic leukemia.[88] When blinatumomab was disrupted at treatment-free intervals, T-cell functionality was maintained, and transcription reprogramming was induced, thus counteracting T-cell exhaustion.[88] Furthermore, ongoing research evaluating the addition of anti-PD-1 and/or anti-CTLA-4 ICIs to BsAb treatment to assist in overcoming T-cell exhaustion has shown possible benefits.[89]

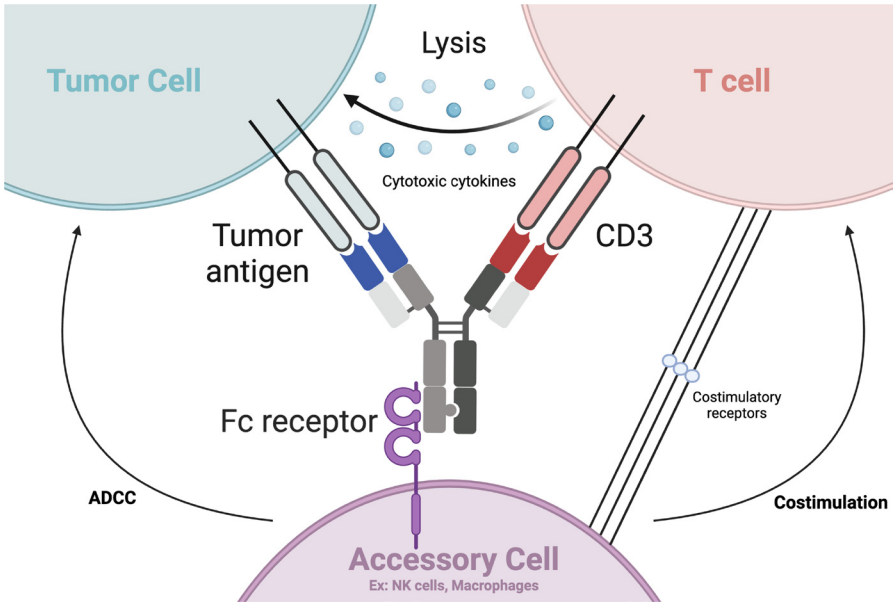

Fig. 6. Bispecific antibody structure.[81] ADCC, antibody-dependent cellular cytotoxicity; CD, cluster of differentiation; NK, natural killer.

Table 5. Bispecific Antibody effects on immune resistance in DLBCL and associated issues of treatment resistance[82,83]

Therapy	Therapy target	Immune evasion mechanism's targeted	Mechanism's	Issues of treatment resistance
BsAbs: Glofitamab; Epcoritamab	CD20xCD3	Loss of MHC-I and MHC-II	BsAbs are able to target surface antigens without the need for MHC	Antigen (CD20) loss/modulation: Loss of CD20 after treatment with rituximab[80]
				Tumor microenvironment: Nonselective T-cell activation leading to activation of Treg cells[80]
				T-cell exhaustion: Continuous antigen exposure upregulates inhibitory molecules (PD-1, CTLA-4) leading to a state of T-cell hypo-responsiveness[88,89]

See Fig. 6 for illustration. BsAbs, bispecific antibodies; CD, cluster of differentiation; CTLA, cytotoxic T-lymphocyte associated protein; MHC, major histocompatibility complex; PD, programmed death; Treg, regulatory T-cells.

Conclusions

The complex interplay between immune dysregulation and the underlying pathophysiology of lymphoma has led to revolutions in the use of immunotherapy for the treatment of DLBCL. The diagnosis of lymphoma represents a failure of the immune system to eradicate abnormal cancer cells in the host. In the era of personalized cancer treatment, immunotherapy represents the opportunity to re-educate the immune system to eradicate lymphoma. These therapies work in a multitude of ways, including the mobilization of the immune system, local delivery of cytotoxic molecules, immunomodulation, and ultimately leading to cell cycle arrest and apoptosis. However, despite the myriad of novel therapeutics available for combating DLBCL, a fraction of patients will still die from their disease or complications of treatment. We must, as a field, continue to explore additional therapeutics, determine optimal treatment combinations, and limit toxicity to improve patient outcomes.

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Conflict of interest

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Author contributions

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