Title

Exploring CD19-targeted Immunotherapy Strategies for human B-cell lymphoma

Abstract

B-cell lymphomas (BCLs) comprise approximately 40 subtypes resulting from mature B-cells' malignant transformation. BCLs are treated differently based on the type and stage of the lymphoma. Multiple therapeutic options exist, including chemotherapy, immunotherapy, radiation therapy, targeted therapy, and stem cell transplantation. Among them, targeted therapy has shown great potential for safer and more effective treatment. Targeted therapies include monoclonal antibodies and nanobodies, CAR-T cell therapies, and Bispecific T-cell engager (BiTE), which operate in diverse ways by targeting a number of molecules including CD79b, CD20, CD30, CD52, and CD19. CD19 is an immunoglobulin superfamily transmembrane glycoprotein of type I which is necessary for setting intrinsic B-cell signaling thresholds by tempering both receptor-dependent and receptor-independent signaling. According to the limitations of conventional therapies and other targets, it seems that CD19 is a proper target for lymphoma. There are several FDA-approved anti-CD19 CAR-T cells such as Axicabtagene Ciloleucel, Tisagenlecleucel, and Lisocabtagene Maraleucel, and Anti-CD19 Monoclonal Antibodies (mABs) such as Loncastuximab Tesirine and Tafasitamab, for which more than a few clinical trials have shown trustworthy results. Blinatumomab is the first FDA-approved antibody produced using BiTE technology which has shown good benefits in B-cell ALL treatment clinical trials. Single-domain antibodies (sdAb) or nanobodies, are the nanoscale VHH fragments of heavy chain-only antibodies (HcAbs) and have been utilized in conjunction with CAR T-cells, yielding promising outcomes. In this review, we aimed to discuss CD19 as an auspicious therapeutic target for lymphoma. Moreover, we talked about different treatment options regarding CD19 targeting, along with the relevant clinical studies, for each of which, the efficacy, safety, and limitations were elaborated.

Keywords: CD-19 Antigen, Molecular Targeted Therapy, Lymphoma , CAR-T cell, Monoclonal Antibody

1. Context

According to the 2017 World Health Organization (WHO) classification over 80 mature lymphoma subtypes are categorized into three main groups: Hodgkin lymphomas (HL), B-cell neoplasms, and NK cell and T-cell neoplasms (1). B-cell lymphomas (BCLs) are a diverse group of over 40 subtypes of neoplasms with diverse physiological and clinical statuses and are caused by the malignant transformation of mature B-cells, most frequently during the germinal center (GC) stage of development. Mantle cell lymphoma (MCL), follicular lymphoma, diffuse large B-cell lymphoma (DLBCL), Burkitt lymphoma, and chronic lymphocytic leukemia are the most prevalent kinds of adult B-cell malignancies. Similar to most cancers, these tumors are generated by specific mutations in tumor suppressor genes (TSGs) and oncogenes (2). According to GLOBOCAN, there were 544,352 cases diagnosed with non-Hodgkin lymphoma (NHL) in 2020, and this number is estimated to grow by 53.1 percent by 2040 (3). NHL is a widespread type of cancer in the United States (US), accounting for approximately 4% of all malignancies. According to the American Cancer Society (ACS), NHL incidence is expected to reach around 80,550 people (44,880 men - 35,670 women), while its death toll is expected to reach 20,180 (11,780 men - 8,400 women) in 2023. The lifetime risk of NHL is around 1:43 and 1:53 in males and females, respectively (4). Based on the information from ACS, BCLs account for approximately 85% of all NHL in the US. The most common type of BCL is DLBCL, which has a prevalence of about 30%, followed by follicular lymphoma with a prevalence of about 20% (4). DLBCL can affect patients of any age; however, it is mostly common among the elderly. The average age at diagnosis is in the mid-60s. It typically begins as a rapidly developing mass in the chest and abdominal deep lymph nodes or the neck and armpit palpable lymph nodes. It can begin in other body organs as well, including the intestines, bones, and even the central nervous system. Although DLBCL is regarded as an aggressive lymphoma, it usually responds effectively to treatment (4).

The average age of patients diagnosed with follicular lymphoma is around 60. It is uncommon in young children. Typically, this lymphoma affects numerous lymph nodes, as well as the bone marrow. Although follicular lymphomas usually respond well to treatment, they remain challenging to cure. When these lymphomas are first discovered, they may not require treatment. Rather than that, treatment may be delayed until the lymphoma begins to cause symptoms. Over time, certain follicular lymphomas can develop into a rapidly developing DLBCL. Although most follicular lymphomas are slow-growing (indolent), some can grow rapidly (4). Treatment of BCLs varies based on the type and stage of the lymphoma.

chemotherapy, immunotherapy, radiation therapy, targeted therapy, and stem cell transplantation are considered as these options for treatment.

Chemotherapy is the first-line therapy for most individuals diagnosed with NHL and can be administered alone or in combination with immunotherapy or radiation therapy. Medications of various classes are frequently used together. CHOP is one of the most often used combinations, which includes Cyclophosphamide, Doxorubicin (also known as Hydroxydaunorubicin), Vincristine (Oncovin), and Prednisone (4). Rituximab is a chimeric anti-CD20 antibody that expands the treatment options in patients with BCLs and is frequently administrated along with chemotherapy (5).

Immunotherapy can either enhance the patient's own immune system or utilize synthetic versions of natural immune system components to kill or hinder the growth of lymphoma cells. There are numerous classes of immunotherapy drugs, including monoclonal antibodies, immune checkpoint inhibitors (ICIs), chimeric antigen receptor T-cells (CAR-T cells), and immunomodulating drugs (4).

Radiation therapy utilizes high-energy rays to destroy cancer cells. It is often used as the main therapy in the early stages since these tumors respond very well to it. In more advanced cases, it is sometimes used besides chemotherapy. Radiation therapy may be used to reduce (palliate) symptoms of lymphoma that have progressed to internal organs including the brain or spinal cord or when a tumor is triggering painful sensations due to nerve compression (4). There are several types of targeted therapies that act in various ways, including monoclonal antibodies, CAR-T cell therapies, Bispecific T-cell engager (BiTE), and nanobodies.These kinds of drugs have a diverse range of targets, including proteasomes, histone deacetylase (HDAC), Bruton's tyrosine kinase (BTK), phosphatidylinositol 3-kinases (PI3Ks), EZH2 (a kind of methyltransferase), CD20, CD19, CD52 (Alemtuzumab), CD30 (Brentuximab vedotin), CD79b (Polatuzumab vedotin) and nuclear export proteins (SINEs) to name a few (4).Monoclonal antibodies directed against the CD20 antigen include Rituximab (Rituxan), Obinutuzumab, Ofatumumab, and Ibritumomab tiuxetan, and each has a distinct mode of action, indicated for a certain type of BCL. Rituximab is frequently combined with chemotherapy to treat some types of NHL, but it may also be administered alone (4). Rituximab demonstrates direct cytotoxic effects via complement and antibody-dependent cell-mediated cytotoxicity, as well as indirect cytotoxic effects via structural alterations, cancer cell sensitization to chemotherapy, and apoptosis (6).

All these CD20-targeting drugs have been proven to possibly reactivate dormant hepatitis B infections, resulting in severe or life-threatening liver disorders. Additionally, they may raise the risk of certain severe infections for several months after their withdrawal. Other minor adverse effects have also been documented concerning these drugs (4). Despite enormous progress in BCL therapy, there remain some limitations, such as low efficacy and high toxicity. Furthermore, there is a need for targets, more specific to BCL, to reach a higher efficacy and lower toxicity.

CD19 is a type I transmembrane glycoprotein member of the immunoglobulin superfamily. This molecule is required for establishing intrinsic B-cell signaling thresholds by influencing both receptor-dependent and receptor-independent signaling. CD19 is crucial for the body's optimal immunological response. It interacts with BCR and other surface molecules to facilitate the recruitment and binding of several downstream protein kinases in both direct and indirect manners. CD19 precedes CD20 in expression in the stages of B-cell differentiation, which is worthy of attention. CD19 is thrice more abundant in mature B-cells than in immature B-cells, with somewhat greater expression in B1 cells than in B2 (typical B) cells. It is a highly accurate surface biomarker for B lymphocytes and is expressed by pre-B-cells until plasma cells are differentiated, and its expression is maintained in aggressive and indolent subtypes of non-Hodgkin lymphoma (7).

Having the limitations of conventional BCL treatments, especially antibodies and multiple therapeutic products engineered against CD20 and the promising results of studies regarding CD19, in this review, our primary objective is to introduce the various types of targeted therapy methods that target CD19 molecules in BCL patients. We will discuss the effectiveness, safety, and limitations of these methods, while also examining relevant clinical studies that are relevant to our research objectives

2. Data Acquisition

A comprehensive literature search was conducted in PubMed to identify relevant studies about CD19-targeted immunotherapy strategies for human B-cell lymphoma. The search strategy utilized a combination of Medical Subject Headings (MeSH) terms and keywords related to CD19 antigen, molecular targeted therapy, cancer, CAR-T cell therapy, BiTE, nanobodies, monoclonal antibodies, and B-cell lymphoma. The search was limited to articles published in English language. Additionally, reference lists of relevant articles and review papers were manually screened to identify additional studies not captured by the initial search strategy.

3. Results

3.1 Anti-CD19 Chimeric Antigen Receptor (CAR) T-cells

Chimeric antigen receptor T (CAR-T) treatment is the branch of cellular immunotherapy that is most quickly evolving and commonly utilized for anticancer therapies. This method, which is relatively new, has greatly changed the field of hematological malignancies. It accounts for over 50% of all cell therapies currently being researched or on the market (8). In this approach, T lymphocytes are extracted from the bloodstream and designed to produce CARs, allowing these modified T lymphocytes to identify and act on cancer cells irrespective of the major histocompatibility complex (MHC). These cells are then reintroduced to the patient after proliferating in vitro to stimulate anticancer immune responses (9). The first generation of CARs had three different parts including the external domain for binding to antigens, the transmembrane domain, and the intracellular part of CD3 for signaling that causes temporary proliferating T-cells and releasing cytokines (10). After that, the second generation of CARs is made by integrating co-stimulatory molecules such as 41-BB or CD28 into the first generation, which helps CAR-T cells survive (11). The third generation is composed by pairing both co-stimulatory molecules together. lately, CAR-T cells have been composed to release cytokines like interleukin-12 (IL-12) for various purposes including improving T-cell survival and enhancing safety and potency by attraction and activation of additional immune cells (Figure 1) (8). There are several pieces of research on CAR-T cells that target CD19 to treat hematologic CD19+ malignancies. Axicabtagene Ciloleucel, Tisagenlecleucel, and Lisocabtagene Maraleucel are FDA-approved and the most well-known of them (Table 1).

3.1.1 Axicabtagene Ciloleucel

Axicabtagene Ciloleucel (Axi-cel) is one of the CAR-T cells that encode anti-CD19 CARs. In October 2017, the FDA granted Axicabtagene Ciloleucel regular approval for the treatment of refractory/relapsed (R/R) large B-cell lymphoma (LBCL) in adult patients who have been administrated at least two lines of systemic therapy (FDA approved 10/18/2017).For composing this CAR-T cell, at first T-cells are concentrated from apheresis peripheral blood, then stimulated with recombinant human interleukin-2 (IL2) and anti-CD3 antibodies before its transduction with a retroviral vector that is incompetent for replication and carrying the anti-CD19 CAR transgene. A murine single-chain variable segment specific for CD19 is coupled to two costimulatory domains generated from human CD3-z and CD28 genes in the CAR protein (12).

In a multicenter, phase two trial, 111 patients with refractory DLBCL, primary mediastinal B-cell lymphoma, or transformed follicular lymphoma were enrolled. Axi-cel was given to 101 of them 82 percent of which displayed an objective response, while 54 percent showed a complete response (CR). Although the majority of patients had CRs during the first month, 23 patients experienced CRs after 15 months. It would have been appropriate to keep track of patients without a CR at the initial evaluation and provide them with a chance to improve. The responses to therapy, even the ones still ongoing, were consistent across significant variables. At baseline, the eight patients with CD19-negative disease had similar response rates to those with CD19-positive disease, suggesting that CD19 detection difficulties rather than actual CD19 negativity were to blame. The findings of this trial suggest that Axi-cel may be safely provided in medical institutions that do transplants, even if they have no prior expertise with CAR T-cell treatment. Although there is a theoretical worry about using immunosuppressive drugs to treat cytokine release syndrome (CRS) or neurologic episodes (NEs), tocilizumab or glucocorticoids did not appear to impair the overall response (OR) in these patients (13).

In another multicenter, phase two trial, of 17 patients with R/R LBCL who have been enrolled, 16 of them have been administered with Axi-cel. 86.7% of the 15 efficacy-evaluable patients had an objective response, 4 (26.7%) patients had a CR, and 9 (60.0%) had a partial response (PR). All 16 patients (100%) showed grade 3 treatment-related adverse events (AEs), the most prevalent of which were neutropenia (81.3%), lymphopenia (81.3%), and thrombocytopenia (62.5%). In 13 (81.3%) patients, CRS occurred (12 cases of grade 1 or 2 and 1 case of grade 4). There were no neurological events observed (14).

ZUMA-1, a single-arm, multicenter, registrational trial at 22 sites in the Israel and USA, had enrolled eligible patients who were at least 18 years old with LBCL confirmed via histology. Between May 19, 2015, and September 15, 2016, this study enrolled 119 patients in phases 1 and 2 of the study, with 108 receiving Axicabtagene Ciloleucel. As of August 11, 2018, 101 patients assessed for phase 2 activity had been followed for a median of 27.1 months, with 84 (83%) having an objective response and 59 (58%) having a CR. The average response time was 11.1 months. The median overall survival was not attained, whereas the median progression-free survival was 5.9 months. In phases 1 and 2, 52 (48%) of the 108 patients who were evaluated for safety suffered significant AEs of grade 3 or above. Twelve patients (11%) had grade 3 or worse CRS, while 35 had grade 3 or worse neurological events (32%) (15).

To decrease toxicity related to treatment, several investigative safety management cohorts were added to ZUMA-1. Mainly, Cohort 6 examined the use of preventive corticosteroids, early

corticosteroids, and tocilizumab intervention to treat CRS and NEs. The findings from cohort 6 suggest that individuals treated with Axi-cel may benefit from preventive corticosteroids, early corticosteroids, and/or Tocilizumab intervention, even in the absence of randomized data. It is promising that no patients who received prophylactic corticosteroids experienced grade 3 or higher CRS, and that the median CRS duration was reduced, with a delay in CRS onset. Additionally, in cohort 6, 68% (n=27/40) of patients did not experience NEs or CRS within the first 72 hours after Axi-cel infusion, which may allow for better corticosteroid use and increase the proportion of patients who can be managed as outpatients. It did not appear that corticosteroid prophylaxis or early interventions with corticosteroids and Tocilizumab had a negative impact on Axi-cel efficacy (16).

3.1.2 Tisagenlecleucel

Tisagenlecleucel is a second-generation anti-CD19 CAR-T cell with the co-stimulatory domain 4-1BB. In May 2018, tisagenlecleucel was approved by the FDA for the treatment of R/R LBCL in adult patients, after at least two courses of systemic therapy (FDA approved 05/01/2018). The main target is CD19+ B-cells. Tisagenlecleucel was tested in phase 2, multicenter, global, pivotal trial, and it showed a great rate of durable response in adult patients with R/R DLBCL (17). Previous studies have indicated the high efficacy with a severe but mostly reversible toxic-effects profile in young adults and children suffering R/R acute lymphoblastic leukemia (ALL) (18). In the Tisagenlecleucel CAR, there is an extracellular antigen-binding domain with the ability of CD19 recognition, as well as 4-1BB costimulatory and CD3-z chain signaling domains inside (18).

A single-group, open-label, multicenter, international phase 2 study enrolled 135 patients with relapsed or refractory DLBCL who were at least 18 years old and had previously undergone two or more courses of treatment, including rituximab and one type of anthracyclines. Tisagenlecleucel was given to 111 patients and the result of the efficacy analysis set indicated an overall response rate of 52 percent among the 93 patients which was the most significant rate among participants. They had three months or longer of follow-up or had stopped participating in the research earlier. In addition, 40% of patients showed a CR whereas just 12% had a PR. In the third month, the OR and CR rates were 38% and 32%, respectively; and at the end of the sixth month, 33% and 29% were reported. In this trial, extensively pretreated adults with R/R DLBCL exhibited a high incidence and durability of response to Tisagenlecleucel treatment (17).

A phase 2, single-cohort, 25-center, global study of Tisagenlecleucel was done on 75 children and young adults with CD19+ R/R B-cell ALL. Within three months, the total remission rate was 81%, and all patients who had responded to therapy were not shown any evidence of minimum residual illness, as determined by flow cytometry. In the sixth month, a 73% event-free rate and 90% overall survival were reported, and at the end of 12 months, they had decreased to 50% and 76%, respectively. Tisagenlecleucel was detectable in the blood for up to 20 months. 73% of patients experienced grade 3 or 4 AEs that were assumed to be associated with Tisagenlecleucel. CRS has registered in around 77% of patients, 48% of whom received tocilizumab. In addition, 40% of patients, showed neurological problems (18).

JULIET (NCT02445248) is a phase 2, single-arm, open-label, multicenter study. Regarding adult patients with the R/R DLBCL who have either relapsed following autologous stem cell transplantation or were ineligible for it. A study has revealed that Tisagenlecleucel has acceptable effectiveness and safety in the Japanese subgroup. Between seventeen patients were enrolled in Japan, and Tisagenlecleucel was administered to 9 of them who had completed more than 3 months of follow-up. The best overall response rate was 77.8%, with five patients (55.6%) in CR and two (22.2%) in PR. CRS developed in six individuals (66.7%), with two patients experiencing grade 3 of CRS (19).

3.1.3 Lisocabtagene Maraleucel

Lisocabtagene maraleucel, also known as liso-cel, is an autologous, CD19-directed CAR-T cell product with a 4-1BB co-stimulatory domain, which is administrated as a sequential infusion of two components containing CD8+ and CD4+ CAR-T cells at equal doses (20). On February 5th, 2021, the FDA approved Liso-cel, marketed under the name Breyanzi®, as a treatment option for adult patients with R/R LBCL after at least two courses of systemic therapy (FDA approved 02/05/2021).

A multicenter study enrolled 344 patients with R/R LBCL including 269 patients who have been administrated with one or more doses of liso-cel. Overall safety and activity of liso-cel were not affected by the dosage level. 100×10^{6} CAR+ T-cells were indicated as the target dosage. In the efficacy-evaluable group, 186 (73%) of the total patients presented an objective response, while 136 (53%) had a CR. The most common grade 3 or more severe AEs were neutropenia (60%), anemia (37%), and thrombocytopenia (27%). In addition, any grade CRS and neurological events have registered in 113 (42%) and 80 (30%) patients, respectively; grade 3 or worse CRS and neurological events were reported in six (2%) and 27 (10%) patients,

respectively. Nine patients (6%) experienced dose-limiting toxicity, including one who died as a result of widespread alveolar injury after receiving 50×10^6 CAR+ T cells (20).

An ongoing, open-label, nonrandomized trial is studying the effects of CAR-T cell therapy using liso-cel on the health-related quality of life (HRQoL) and related symptoms of patients with R/R LBCL. The results show that there is a clinically significant improvement in the quality of life. There were no significant changes in physical performance or pain levels at the 2nd, 12th, and 18th months, but there was a meaningful decrease in fatigue. Furthermore, a higher proportion of patients who responded to the treatment experienced clinically significant improvements in their global health status and QoL compared to non-responders (21).

3.1.4 Challenges and Recent Advances

Despite the enormous advances in cancer therapy and CAR-T cell therapy, there still exist certain limitations. For instance, many intrinsic and extrinsic factors can cause failure in this therapy. Intrinsic factors such as the short persistence of CAR-T cells, and extrinsic factors like the tumor inhibitory microenvironment (22). Also, safety concerns, high cost, the labor-intensive production process, and the long period of its production are other limitations for it (8).The spotlight is now on the Universal CAR-T (UCAR-T) cell treatment, which is expected to improve the current situation. To prevent severe alloimmune rejection caused by MHC mismatch between the donor and the recipient, all CAR-T cell products currently available or in development are autologous, meaning they are produced using T cells from the same patient. UCAR-T cells may also be composed of allogeneic CAR-T cells acquired from healthy donors. UCAR-T cells have different production techniques, safety concerns, and applications while simultaneously having the same killing mechanisms. Also, it has a lower cost and immediate availability (8). Currently, CAR-T cells that utilize nanobody approaches are also in the limelight, which we will discuss later.

3.1.5 Safety and Toxicity

Despite CAR-T cells' clinical efficacy in cancer therapy, their widespread usage is limited by distinct toxicities that are derived from stimulation of tumor-reactive T-cells, which result in a massive release of cytokines that cause cardiovascular, pulmonary, and neurologic side effects. These side effects, known as CRS and/or neurotoxicity (NT), are a leading cause of morbidity and death in certain individuals (23).

A retrospective observational study on all the reports in EU (EudraVigilance, EV) and US (FAERS) databases of adverse drug reactions regarding Axi-cel and Tisagenlecleucel was

performed. Among 1426 reports, the most common adverse reaction was CRS, which was documented in 185 cases for Tisagenlecleucel, 462 cases for Axi-cel in FAERS, and 137 and 498 cases in EudraVigilance, respectively. Both medications and databases had a larger proportion of male patients. This does not imply that male patients are more likely to acquire an Adverse drug reaction (ADR); rather, it is connected to the prevalence of the disease being treated. Males are somewhat more likely than females to develop ALL and DLBCL (24). A study that followed 100 patients from PLAT-02 or PLAT-03 phase 1/2 trials found that the severity of clinical neurotoxicity during EEG recording after CD19-directed CAR-T cell treatment can be accurately reflected by EEG background patterns (25). Although CAR-T cells can cause toxicities, there are ongoing studies aimed at reducing, monitoring, and diagnosing them early.

3.2 Anti-CD19 Monoclonal Antibodies (mABs)

Anti-CD19 Monoclonal Antibodies (mABs) are specifically designed to have enhanced Fc function; after bonding to their receptors these immunoglobulins (Ig) can directly activate signaling pathways for tumor cell death. Besides, by attracting other immune mechanisms they indirectly contribute to complement-dependent cytotoxicity (CDC), antibody-dependent cellular cytotoxicity (ADCC), and antibody-dependent cellular phagocytosis (ADCP) (26).

3.2.1 Complement-dependent cytotoxicity (CDC)

The C1q's interaction as a member of complement proteins and the Fc region of mABs activates membrane attack complex (MAC). MAC then perforates the cellular membrane resulting in cell death following lysis of the targeted cell. Based on the extent of the complement response on tumor cells, cell death can take another route besides MAC activation, by recruiting macrophages to destroy the opsonized targeted cells (26).

3.2.2 Antibody-dependent cellular phagocytosis (ADCP)

The operator cells of ADCP on the other hand express a wide range of FcγR molecule family members, including activating molecules FcγRIIIa, FcγRIIa (CD32A), and FcγRIIIb (CD16b) and inhibitory molecule FcγRIIb (CD32b). Their cumulative effect acts to either induce the ADCP or not. Here macrophages operate mainly by phagocytosis rather than releasing cytotoxic agents (26, 27).

3.2.3 Antibody-dependent cellular cytotoxicity (ADCC)

Natural killer (NK) cells having the most significant role in ADCC are the only cells amongst macrophages and monocytes to express FcγRIIIa exclusively; FcγRIIIa or CD16a is an activating member of FcγR molecule family which upon activation starts a chain of actions that ends up with perforin and granzyme granules being released leading to the elimination of the tumor cell (26, 28).

3.2.4 Loncastuximab Tesirine (ADCT-402)

Loncastuximab Tesirine (also known as Zynlonta® and ADCT-402) received accelerated approval from the FDA on April 23. Loncastuximab Tesirine is an antibody-drug conjugate (ADC) accommodating an anti-CD19 humanized mAB and a pyrrolobenzodiazepine (PBD) dimer toxin acting as a DNA-alkylating factor attached by a cathepsin-cleavable valine-alanine linker. The PBD-linker compound is known as Tesirine (29). The PBD dimer warheads contained in Loncastuximab Tesirine are cytotoxic sequence-selective DNA crosslinking agents. The crosslinks formed by these agents cause minor disturbances in the structure of DNA which hinders the detection system's potential to activate repair mechanisms and grants more favorable biological activity to PBDs in targeted tumor cells. This feature of being nondisturbing to DNA structure is what differentiates PBD dimers from conventionally used chemotherapeutic agents like nitrogen mustard (e.g. cyclophosphamide) and platinum drugs (30)**.**

A phase 1 study was carried out with a 3+3 dose-escalation design in two parts to discover the sufficient dose, during which patients received Loncastuximab Tesirine intravenously once every 21 days as one cycle. Of the 69 patients who used loncastuximab tesirine ≥120 μg/kg, 40.6% (28 patients) reached CR and 18.8% (13 patients) reached PR. All patients were at least 18 years old and had R/R B-cell NHL that was validated histologically, they either could not tolerate conventional therapies, their treatment had failed or they did not have access to other therapy choices (29).

During a phase 2 clinical trial, from the 145 patients after the initial enrollment, 35 patients presented CR and 35 patients also showed PR putting the overall response percentage at 48.3%. Patients in this trial were all at least 18 years old and had R/R DLBCL after receiving two or more multi-agent systemic treatments. They were given 150 μg/kg Loncastuximab Tesirine intravenously once for every three-week period for 2 cycles, and then at the dose of 75 μg/kg for further cycles. Of the most prevalent grade 3 or higher treatment-emergent adverse events (TEAE) were neutropenia (26%) and thrombocytopenia (18%) and serious AEs were observed in 39% of 145 patients (31).

3.2.5 Tafasitamab

Tafasitamab (MOR208 monoclonal antibody or XMAB-5574) is a humanized anti-CD19 monoclonal antibody that is built to have enhanced Fc function, which enables it to have increased ADCP and ADCC. The alterations made in the structure help increase the FcγRIIIa receptor binding, which is expressed by NK cells. As a result, this communication between the Fc domain of monoclonal antibody and FcγRIIIa receptor is enhanced substantially (a 70-254-fold increase, compared to the same interaction but with a normal Fc domain) which is highly needed for the mABs in order for them to be effective (32). There have been studies on the efficacy of Tafasitamab monotherapy; in a study in 2018, Tafasitamab was prescribed for 35 patients aged at least 18 years old with R/R DLBCL for whom one or more regimens including rituximab have been prescribed previously. Tafasitamab was given 12 mg/kg intravenously in 2 stages: an 8-week period followed by a 4-week period if patients had at least reached the stable stage of disease. CR and PR have been reported in 2 and 7 patients respectively. The objective response rate was 26% and 36% among all patients and only assessable patients respectively (33).

Lenalidomide is an immunomodulatory drug that has been granted approval in the management of multiple myeloma initially as there have been studies on its efficacy in a single-agent regiment. The single-agent efficacy of lenalidomide has been the concern of other studies regarding its application in the treatment of R/R indolent lymphoma (34, 35). The mixture of Tafasitamab with lenalidomide received approval in 2020 for the first time for patients with R/R DLBCL (US-FDA, First Approval. Drugs. July 2020). In a study of 80 patients who have been given tafasitamab in combination with lenalidomide, 48 patients presented objective responses; 34 patients had CRs and 14 patients showed PRs which gives the rate of 60% and 43% respectively. Besides 59 out of 80 patients (74%) presented disease control (36). The effects of Tafasitamab have been the focus of other studies concerning other types of B-cell NHL; including a study exploring the potency of Tafasitamab in the therapeutic regimen of R/R B-Precursor Cell ALL which was discontinued due to the low rate of responses to monotherapy with Tafasitamab (37).

3.2.6 DI-B4

DI-B4 is another humanized, anti-CD19 mAB which although having lower CDC features, has potent ADCC. There is an ongoing Phase 1, multi-center clinical trial to calculate the maximum dose of safety and the recommended dose for the next phase of trial. DI-B4 has been given to the patients intravenously each week, for four weeks. Patients in this trial have the following

conditions: R/R CD19 positive indolent B-cell lymphoma, CLL, or Waldenström Macroglobulinaemia (ClinicalTrials.gov Identifier: NCT01805375).

3.2.7 Safety and Toxicity of Anti-CD19 mABs

The most common adverse effects were reported to be IRRs (Infusion- related reactions) in a study with 22 patients, other documented AEs were: fatigue, hypokalemia, pyrexia, constipation, nausea, hyperglycemia, febrile neutropenia, hyperkalemia, dyspnea. The most common hematologic AE and the most common grade ≥3 AE was febrile neutropenia; other hematologic AEs reported in more than two patients were: Anemia, neutrophil count decreased, and platelet count decreased (37). In another study with a treatment regimen of Tafasitamab plus lenalidomide upon cessation of lenalidomide, the occurrence and severity of treatment-emergent AEs were reduced under tafasitamab treatment alone; grade 3 or 4 neutropenia was reported in 6% of 51 patients after lenalidomide cessation. During the co-administration of the two drugs, the most common hematologic AEs were neutropenia, anemia, thrombocytopenia, and leukopenia reported in 5 patients or more. The most common Non-hematological grade 1-2 events reported were diarrhea (32%), rash (27%), and peripheral edema (22%) (38).

3.3 Bispecific T-cell engager (BiTE)

As mentioned before, ALL patients have shown poor prognosis under traditional therapies, hence immunotherapy modalities have developed significantly in recent years. Dual Bispecific T-cell engaging (BiTE) antibodies are newly developed modalities that have shown favorable efficacy, especially in R/R leukemia patients (39). Blinatumomab (BLINCYTO®) is the first class of antibodies constructed by BiTE technology which has gained FDA approval (FDA approved 03/29/2018). The Blinatumomab molecule consists of two arms that bind concurrently to CD3 + cytotoxic T-cells and malignant CD19 + B-cells, leading to the induction of cytotoxic T-cell activity, thus tumor cell lysis (39). Clinical trials of blinatumomab have exhibited promising outcomes in patients with B-cell ALL. For instance In 2015 Topp et all in a phase 2 study consisting of 189 R/R Philadelphia negative ALL patients, After usage of blinatumomab achieved a result of 81 patients (43%, 95% CI 36–50) with either CR or CRh (CR with partial recovery of peripheral blood counts), 63 patients (33%) had a CR and 18 patients (10%) had a CRh (39). In another study in 2017 Kantarjian et all in a phase 3 trial compared the overall survival rate of blinatumomab and traditional care among 405 adult patients with R/R B-cell ALL. Patients treated with blinatumomab achieved a significantly higher overall survival rate of 34% in comparison to the patients who were treated with chemotherapy with an overall survival rate of 16% (40). Also in a phase 2 study in 2018,

Rambaldi et al. demonstrated higher efficacy of blinatumomab over standard of care with an odds ratio of 1.54 (95% CI, 0.61-3.89) in patients with R/R Philadelphia Positive B-cell ALL (41). Viardot et al. in a phase 2 study in 2015, evaluated the effectiveness of this drug in patients with R/R DLBCL. Treatment with blinatumomab resulted in a 43% overall response (CR was 19%) (39). In another phase 2 study in 2018 Gökbuget et al. B-cell ALL adult patients with minimal residual disease achieved a higher relapse-free survival (RFS) (23.6 vs. 5.7 months; P 5 .002) and OS (38.9 vs. 12.5 months; P 5 .002) in minimal residual disease in a result of aforementioned treatment (42). Nevertheless, this drug is known to cause some AEs which restricts its use. Among those the most common and vital AEs are CRS and NT (39). Further investigations on the efficacy and side effects of blinatumomab will hopefully clear the way for extensive utilization of this drug in the future.

3.4 Single-domain Antibodies (Nanobodies)

Single-domain antibodies (sdAb) were first introduced in 1993 when a novel antibody was discovered in the serum of camelids with no light chains by nature, contrary to conventional human antibodies. These heavy chain-only antibodies (HcAbs) were made of merely two heavy chains and a single variable domain (V_HH, ~15kDa) for antigen-binding purposes (43). The nanoscale V_HH fragments of these molecules were then isolated and were observed to be fully capable of interacting with antigens on their own, despite their minuscule size; this explains why they were entitled "nanobodies" (Nbs) in 2003 (44). The 2001 article by Desmyter et al. was one of the earliest studies to discuss the structural superiority of camelid single-domain antibodies over human antibodies. This study revealed that the camelid V_HHs benefitted from a complementarity determining region 3 (CDR3) of greater length than human V_H domains, leading to a higher specificity, affinity, and hydrophilicity (45). This section will elaborate on some of the most pioneering advances in treating different types of B-cell lymphoma with anti-CD19 nanobodies. In 2017, Banihashemi et al. took a great leap in this field by deploying the phage-display antibody technique to acquire anti-CD19 Nbs from the immune Nb library of a one-humped camel. This procedure resulted in a rich library that could be used to target B-cell malignancies as well as B-cell autoimmunity disorders via CD19 molecules on their surface (46). In addition, several studies have associated nanobodies with CAR T-cells, some of which have shown promising outcomes. For instance, in a 2021 study, Wang et al. developed CD19 Nb CAR T-cells, CD20 Nb CAR T-cells, and bispecific Nb CAR T-cells. In vitro incubation of these CAR T-cells with lymphoma tumor cells displayed desirable results, yet further, in vivo studies are required to feel more confident about using nanobody-armed (Anti-CD19 Nbs in our case) CAR T-cells against B-cell malignancies such as lymphomas (47).

Moreover, in a November 2021 study, Zhou et al. generated trispecific CD19xCD20xCD22 Nb CAR T-cells (LCAR-AIO), which exhibited cytotoxicity against B-cell tumors with heterogeneous antigen expression in pre-clinical in vitro and in vivo models. In this study, the simultaneous targeting of three antigens minimized immune escape and thus appeared to be a possible treatment of choice in patients with relapsed B-cell tumors (48).

Finally, anti-CD19 Nbs have been accompanied by liposome-based nanocarriers, which seem utterly fascinating and are yet to be discovered in the coming years. Most recently, Banihashemi et al. prepared liposomal nanocarriers equipped with anti-CD19 V_HHs and packed with anthrax lethal factor (LF). These particles were designed to search for B-cells and deliver the LF upon binding to the target, specifically enough not to harm the normal cells. LF is a mitogen-activated protein kinase (MAPK) pathway inhibitor that acts by blocking MAPK kinases (MKKs) 1, 2, 3, 4, and 6 (49). MAPKs consist of three subgroups all of which are crucial for tumor progression and metastasis: extracellular signal-regulated kinases (ERKs) which contribute to B-cell survival, c-Jun N-terminal kinases (JNKs) and p38-MAPKs which together take part in responses to cellular stress. Inhibiting any of these molecules deprives the B-cells of the support needed for proliferation and thus, results in apoptosis of tumor cells (50). In order to illustrate the diverse array of CD19 targeted therapies available for B-cell lymphoma, Figure 2 showcases the inclusion of Anti-CD19 CAR-T cells, mABs, BiTE molecules, and nanobodies. To conclude, we can see that significant advancements have been achieved in this field, and much more has yet to come; however, more comprehensive clinical studies are required for these methods to gain FDA approval and enter clinical practice.

4. Conclusion

In spite of recent advances in the treatment of hematological malignancies still we require better treatments with higher efficacy and safety. In the field of clinical oncology, various immunotherapeutic techniques and targeted therapies have emerged over the past decade. The number of patients having any of these treatments is growing continuously. CD19 is one of the promising targets for the treatment of B-cell lymphomas. There are several treatment strategies that target CD19 including CAR-T cell therapy, monoclonal antibodies, BiTE, and single-domain antibodies. Anti-CD19 CAR-T cells have shown promising efficacy but also have some limitations and AEs such as CRS and immune effector cell-associated neurotoxicity syndrome (ICANS). Between FDA-approved CAR-T cells, Axicabtagene Ciloleucel showed a higher rate of severe ICANS than Tisagenlecleucel, and Tisagenlecleucel showed a high rate of severe CRS. Also, despite the good efficacy and safety of Lisocabtagene Maraleucel, its real-world

performance needs more validation. Monoclonal antibodies show great potential either taking part in monotherapies like Loncastuximab Tesirine or in combination with Lenalidomide in some studies like Tafasitamab. However monoclonal antibodies need further testing as they undergo development in order to improve their efficacy and decrease their AEs. Blinatumomab, the first class of antibodies constructed by BiTE technology, has shown promising results in patients with B-cell ALL. But it has important AEs such as CRS and neurotoxicity same as CAR-T Cells. Single-domain antibodies can be used in association with CAR-T cells or by themselves which had great progress in pre-clinical studies. As discussed, these studies have shown promising results for these treatments but still, there is a need for more studies for results of the combination of different treatment options and also studies with a higher number of patients with different individual characteristics and various types of B-cell lymphomas. Different approaches to targeting CD19 have shown promising results, using two or more of these approaches might seem reasonable at this time to reach the best efficacies. Some of these drugs have also shown the potential of getting along with other drug classes or cytotoxins. Although CD19 seems like a promising target for BCLs based on what we have seen from them in recent studies, it appears that further trials for the improvement of these medications can be helpful. Meanwhile, it seems like targeting CD19 could bring an opportunity to reinforce the efficacy of other therapeutic regimens.

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Authors' Contributions

Sasan Salehi Nezamabadi conceptualized the review article, conducted the literature review, wrote the main part of the manuscript, and provided critical revisions to the manuscript. Arash Safari Sabet prepared the figures and contributed to the literature review and writing the manuscript. Sana Sadat Peighambardoust and Sadra Behrouzieh contributed to the literature review and writing the manuscript. Seyed Reza Banihashemi provided critical revisions to the manuscript and contributed to writing the manuscript. Saeid Amanpour took on a supervisory role, managed the project administration, and approved the final manuscript. All authors have reviewed and approved the final manuscript.

Ethics

We declare all ethical standards have been respected in preparation of the submitted article.

Conflict of interest

The authors have no conflict of interests to declare that are relevant to the content of this article.

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CAR-T Cell Type	Trade Name	Most Important Disadvantages	Important Adverse Events	Effective Dosage	Company
Axicabtagene Ciloleucel	YESCARTA	- High rate of ICANS - Higher rate of severe ICANS than Tisagenlecleucel[1]	1. CRS 2. ICANS[1] 3. Pyrexia 4. Anemia 5. Hypotension 6. Nausea 7. Neutropenia[3]	$2 * 10^6$ CAR-positive viable T cells/kg of body weight, with a maximum of $2 * 10^8$ CAR-positive viable T cells[2]	Kite Pharma Inc.
Tisagenlecleucel	KYMRIAH	- High rate of severe CRS[1]	1. CRS 2. ICANS[1] 3. Anemia 4. Pyrexia 5. Neutropenia 6. Thrombocytopenia 7. Leukopenia 8. Diarrhea[4]	$0.6\text{-}6 * 10^8$ CAR-positive viable T cells[2]	Novartis Pharmaceuticals Corporation

Lisocabtagene Maraleucel	BREYANZI	- Despite excellent efficacy and safety profiles, its real-world performance needs further validation[1]	1. CRS 2. ICANS[1] 3. Neutropenia 4. Anemia 5. Fatigue 6. Nausea 7. Thrombocytopenia[5]	A single dose containing 50 to 110 * 10^6 CAR-positive viable T cells with a 1:1 ratio of CD4 and CD8 components[2]	Juno Therapeutics, Inc., a Bristol-Myers Squibb Company
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Table 1. Anti-CD19 Chimeric Antigen Receptor (CAR) T-Cells. Immune Effector Cell-Associated Neurotoxicity Syndrome (ICANS), Cytokine Release Syndrome (CRS).

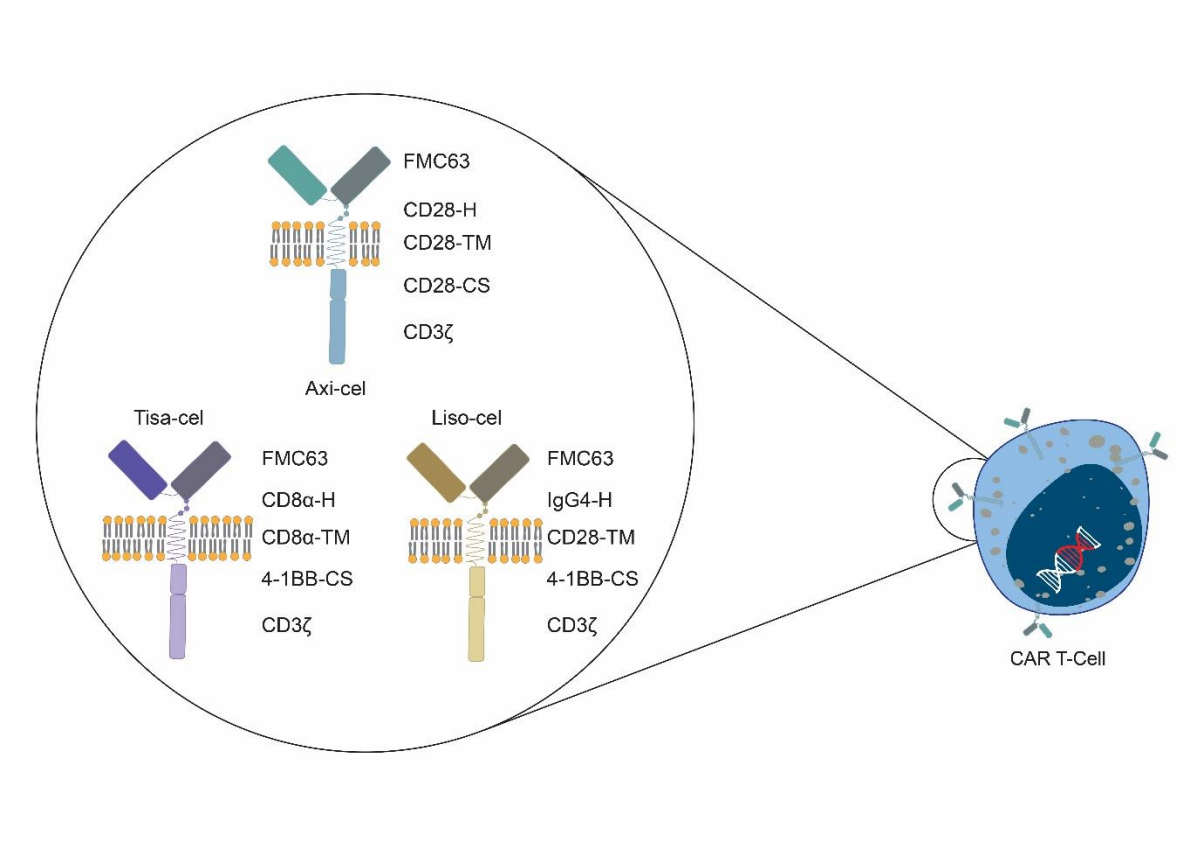

Fig 1. CD19 targeted Chimeric antigen receptor T (CAR-T) cell therapies. One of the Axicabtagene Ciloleucel (Axi-cel), Tisagenlecleucel (Tisa-cel), and Lisocabtagene maraleucel (Liso-cel) can be expressed according to the inserted genes. These three are second-generation CARs; their intracellular part contains CD3ζ and a co-stimulatory domain (CD28 or 4-1BB).

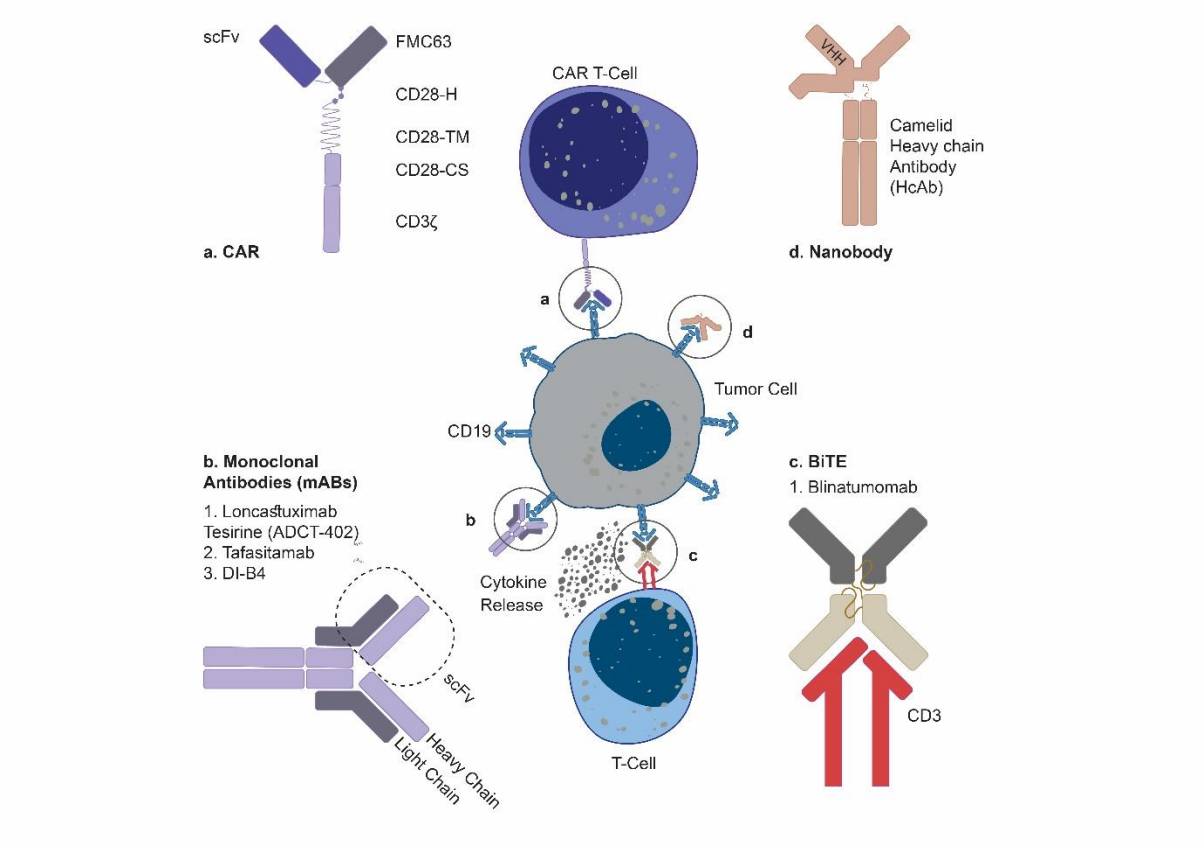

Fig 2. CD19 Targeted Therapies for B-Cell Lymphoma. a. The first generation of chimeric antigen receptors (CARs) with three different parts including the external domain for binding to CD19, the transmembrane domain, and the intracellular part of CD3ζ for signaling promotes transient T-cell proliferation and cytokine release. b. Anti-CD19 Monoclonal Antibodies (mABs) consist of two heavy chains and two light chains. These immunoglobulins (Ig) can directly activate signaling pathways for tumor cell death after binding to CD19. Loncastuximab Tesirine, Tafasitamab, and DI-B4 are three important mABs that target CD19. c. Blinatumomab is a BiTE antibody that has two arms capable of simultaneously attaching to CD3-positive cytotoxic T cells and malignant CD19-positive B cells. This dual binding prompts the activation of cytotoxic T cells, ultimately resulting in the destruction of tumor cells. d. heavy chain antibodies (HcAbs) are made of two heavy chains and a single variable domain (VHH) for antigen-binding purposes. Nanobodies are the nanoscale VHH fragments of HcAbs that are observed to be fully capable of interacting with antigens such as CD19 on their own.