CD19-redirected chimeric antigen receptor (CD19 CAR) T cell in the clinical treatment of human non-Hodgkin's lymphoma and acute lymphoblastic leukemia

Ali R Jazirehi, Jeffrey D Knott, Emily Chiuzzi

Jazirehi AR, Knott JD, Chiuzzi E. CD19-redirected Chimeric Antigen Receptor (CD19 CAR) T Cell in the Clinical Treatment of Human Non-Hodgkin's Lymphoma and Acute Lymphoblastic Leukemia. J Blood Disord Treat. 2019;2(1):7-16.

Response rates for patients with B-cell neoplasms such as Non-Hodgkin's Lymphoma (NHL) and Acute Lymphoblastic Leukemia (ALL) treated with traditional modalities, including chemotherapy, radiation, and bone marrow transplant, are relatively high. However, patients with inherent- or acquired-resistance to first-line treatment options exhibit poor prognosis signifying the need for development of an optimal treatment approach for relapsed/refractory malignancies of B cell origin. Recently, a new form of immunotherapy using genetically engineered chimeric antigen receptor (CAR) T-cells has been developed. CAR T cells trigger apoptosis in tumor targets in an MHC-independent manner upon recognition and ligation to a specific tumor associated antigen (TAA). Typically, engineered CAR T-cells recognize CD19 specifically, a universal B-cell surface marker expressed in many forms of B-cell malignancies. CD19 CAR T cell-redirected immunotherapy is an attractive option for patients with various CD19+ leukemias (e.g., ALL) and relapsed/refractory NHL. In fact, anti-CD19 CAR T-cell therapy has shown remarkable clinical efficacy in the treatment of these patients. Its significant efficacy coupled with limited toxicities makes CD19 CAR T-cell immunotherapy an ideal treatment approach for ALL and NHL. This review summarizes recent developments in the field of CAR T cell therapy with an emphasis on the utilization of various CD19 CAR T cell constructs in the clinical treatment of NHL and ALL.

Key Words: Chimeric antigen receptor; Non-Hodgkin's lymphoma; Immunotherapy; Relapsed/refractory, T cell, leukemia

INTRODUCTION

on-Hodgkin's Lymphoma (NHL) is a collection of lymphocyte neoplasms that arise in the lymphatic system and may spread to all locations of the body. This year an estimated 74,680 people will develop non-Hodgkin's lymphoma with 19,910 expected fatalities [1]. B-cell NHLs make up about 85% of new diagnoses, with diffuse large B-cell lymphoma (DLBCL) and follicular lymphoma (FL) accounting for two thirds of all malignancies [2]. Other major types include mucosal associated lymphoid tissue (MALT) lymphoma (8%), small lymphocytic lymphoma (SLL) (7%), and mantle cell lymphoma (MCL) (6%);T-cell lymphomas represent approximately 10% of NHL diagnoses in the United States [1-3].

NHL ORIGINATES IN GERMINAL CENTERS

Germinal centers (GC) of the lymphatic tissues produce mature, differentiated effector cells using tightly coordinated reactions inside light and dark zones. While this achieves a comprehensive response to pathogens, in NHL this active area of genetic shuffling gives rise to malignant variants of B-cells with unique abilities to escape the immune system [4]. A common theme among NHLs are translocation events in which an exon is relocated under control of a higher expression promoter, generating a phenotype capable of rapid division while lacking checkpoint molecules [4]. For example, follicular lymphoma frequently arises when the t(14;18) (q32;q21) translocation places the anti-apoptotic gene Bcl-2 under control of the robustly expressed immunoglobulin heavy gene locus, IgH, affording the cell evasion of immune checkpoints due to high expression of antiapoptotic Bcl-2 [5]. The differentiation stage associated with germinal center B-cells, in addition to the unique profile of mutations, are key factors in typing NHLs [6]. Common groups of mutations include transcription factor dysregulation (MYC, E2A, NF-κB), deregulation of signaling pathways (MYD88, CARD11, CD79B), immune escape (PD-L1, B2M, CD58, BCL-2, BCL-6), and epigenetic alterations including chromatin and histone arrangement (EZH2, KMT2d, CREBBP) [2,4,5].

TRADITIONAL TREATMENTS FOR NHL

Two important criteria are implemented when selecting treatment for NHL;the grade and the progression of the disease at the time of diagnosis. Intermediate and aggressive (high-grade) lymphomas typically have a higher cure rate with aggressive combination chemotherapy than do indolent (low-grade) lymphomas. The latter are categorized as incurable with standard therapy if diagnosed at an advanced stage [7]. The two most prominent NHL subtypes fall into the indolent or aggressive categories. The first is FL, an indolent lymphoma that represents all indolent lymphomas in terms of its treatment approach which includes combination chemotherapy, interferon (IFN), single agent alkylators, nucleoside analogues, immunotherapy with monoclonal antibodies (mAbs), radiolabeled mAbs, or watchful waiting [8]. The stage at which FL is determines if radiation alone will cure the patient or if more involved approaches such as chemotherapy and/or immunotherapy will be needed. The second, DLBCL, is an aggressive lymphoma who's treatment approach also sets the standard for all lymphomas of its type [9]. The traditional first-line approach for aggressive B-cell NHL was a first-generation combination-chemotherapy treatment, CHOP (cyclophosphamide, doxorubicin, vincristine, and prednisone). In national cooperate group trails of patients with intermediate-grade or high-grade NHL, only around 30% were cured with CHOP alone [10]. The standard of care has since changed to R-CHOP (rituximab-plus-CHOP) for all aggressive forms of NHL. Rituximab, (Rituxan HYCELA™, MabThera®/Rituxan F. Hoffmann-La Roche Ltd) is a chimeric human/murine IgG1 monoclonal antibody [11]. In a study conducted on patients 60-80 years old with DLBCL, 197 patients received eight cycles of CHOP every 3 weeks and 202 patients received eight cycles of R-CHOP every 3 weeks. Results showed a significant increase in complete responses of 63% and 76%, respectively. The toxicities associated with R-CHOP were not significantly higher than those of the standard CHOP chemotherapy, but the event-free and overall survival rates were [12]. With rituximab, lymphoid malignancies such as indolent and aggressive types of NHL and B-cell chronic lymphocytic leukemia (BCC-L) are being treated with a better prognosis compared with conventional chemotherapy. A reduction in hematological adverse effects including correlating infections and neutropenia indicate that elderly patients or those with weak performance status and low degree of tolerability can benefit from its use in when merged with cytotoxic drugs [13]. Patients with FL and other indolent subtypes of NHL have also greatly benefited from immunotherapy rituximab. R-CHOP is currently standard-of-care for aggressive NHL and can be credited with recovery rates of 70%, yet a problem exists with the patients who become resistant or have no response to first-line treatment. These

Department of Biological Sciences, College of Natural and Social Sciences, California State University, Los Angeles (CSULA), Los Angeles, CA 90032, USA

Correspondence: Ali Jazirehi, Department of Biological Sciences, College of Natural and Social Sciences, California State University, Los Angeles (CSULA), Los Angeles, CA 90032, USA. Telephone (323) 343-2050, e-mail Ajazire@calstatela.edu

Received: March 11, 2019, Accepted: May 14, 2019, Published: May 21, 2019,

patients (10-15%) who show no response within a 3-month time frame have a poor chance of recovery and are categorized has having primary refractory disease. Some patients will show an initial response and later relapse (20-25%) resulting in a life expectancy of 3-4 months [14].

Refractory Lymphoma remains the most severe NHL burden

Survival rates for various NHLs with first line therapy vary significantly, but relapsed or refractory disease universally yields a poor survival window. Up to one third of patients with DLBCL will develop relapsed or refractory disease, for which almost all will die [15]. Treatment includes high-dose chemotherapy with allogenic stem cell transplant (ASCT) (2). Yet many patients are unable to undergo ASCT due to age, comorbidities, or refractory disease [16]. In FL, there is a 30% to 40% risk of transplant mortality associated with allogeneic transplantation in patients with relapsed disease [17]. To complicate matters, highly effective first line defenses often give rise to more resilient refractory illness should patients relapse, as the malignancy will express an immune escaped variant with antigen loss phenotype [18]. It is thus necessary to establish more successful treatments with the goal of both higher specificity and lower toxicity to prevent re-emergent malignancy.

Acute lymphoblastic leukemia

Acute lymphoblastic leukemia (ALL) is cancer of immature lymphoid cells and represents the most common form of pediatric malignancy (19). In 2016, there were nearly 6,590 new cases of ALL, with over 1,400 deaths [19]. Children comprise 80% of ALL cases with a peak incidence occurring between 2 and 5 years. Survival in pediatric ALL is now over 90%, however, in adults and the elderly (10% cases) and children with relapsed ALL (up to 15% of patients) it represents a death sentence where survival is measured in months [20]. While some genetic predispositions are correlated with ALL, such as Down syndrome and Bloom syndrome, majority of patients are previously healthy individuals in which malignancy presents spontaneously [21]. Chromosomal aberrations are present in nearly all forms of ALL but alone are not able to propel malignancy [21]. This is further confounded by the weak association of predisposition with environmental triggers [21]. Common genetic abnormalities include rearrangement of mixed-lineage leukemia gene (MLL), a translocation of chromosome 11q23 with over 80 partners that give rise to promotors whose products assist in tumor escape, TP53–retinoblastoma protein tumor-suppressor pathway, chromosomal translocations such as t(12;21) [ETV6-RUNX1], t(1;19) [TCF3-PBX1], and t(9;22) [BCR-ABL1], for which t(9;22) represents the devastating variant known as Philadelphia chromosome [22,23]. Philadelphia chromosome (Ph-positive) ALL is associated more with adults (10-15%) and has a significantly worse outcome with a one-year survival rate of 10% [21].

Additional treatments are required for refractory/relapsed ALL

Prognostic factors with the highest correlation of mortality include: age (less than 1 or older than 19.9 years), Philadelphia or Ph-like ALL, white blood cell count > 30 × 109/L for B-ALL or >100 109/L for T-ALL, and hypodiploidy or complex karyotype [22-24]. Immunotyping and cytogenic analysis are increasingly being used to subtype ALL patients and yield information about prognosis, as well as predict treatment efficacy [24]. For example, tyrosine kinase inhibitors are effective in prolonging 3-year survival rates in ALL patients who bear the Ph-chromosome breakpoint cluster region-proto-oncogene tyrosine-protein kinase (BCR-ABL1) genetic mutation [21]. This is due to the discovery that BCR-ABL1 fusion protein is constitutively active in this genotype [21]. While cure rates are encouraging, approximately 15% of children with ALL will experience relapse, with long term survival rates below 50% [25]. The cause of relapse is not known for individuals in which minimal residual disease (MRD) has been achieved (<0.01%) but is thought to involve emergence of pre-leukemic clones bearing distinct alterations absent in predominant clonal population [26]. Allogeneic stem cell transplantation (ASCT) is used in relapsed or refractory ALL to replace cells damaged by induction therapy and to repopulate progenitor lineages. It remains the only chance at a cure for some variants of aggressive ALL such as Ph-positive ALL in patients over 35 but offers a low rate at long term survival [27].

CD19 chimeric antigen receptor t-cell therapy for the treatment of ALL and NHL

An emerging treatment that has shown dramatic success against relapsing/

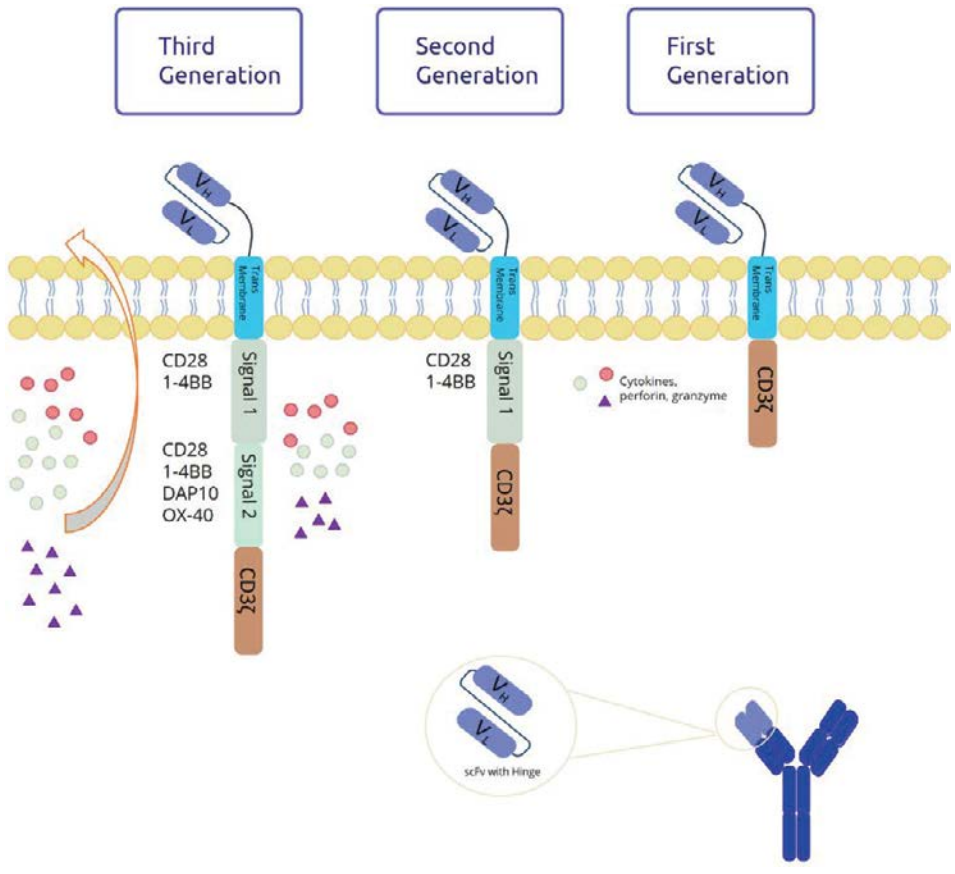

Figure 1) *Generational map of CD19 CAR-T cell development. First generation CARs (right) consisted of only an antigen binding scFv domain with hinge and a cytoplasmic CD3ζ domain, connected with a cell membrane spanning region. Most commonly, the scFv was generated using a monoclonal IgG antibody. Second generation CARs (middle) included addition of costimulatory domain 1-4BB or CD28, resulting in vastly improved cytokine output and robust T-cell proliferation. Third generation CARs (left) combining two costimulatory signals further enhanced proliferation and lytic effector function with increased cytokines and apoptotic potential*

refractory ALL and many NHLs is chimeric antigen receptor T cell (CAR-T) therapy. CAR-T therapy is a potent immunotherapy in which the specificity of a patient's T cell is redirected using chimeric antigen receptors (CARs). Patient's own T-lymphocytes are extracted and transduced to express genetically engineered chimeric antigen receptors (CARs) capable of targeting tumor associated antigens (TAAs) that are present on neoplasms yet absent on other cells [28]. These hybrid T cells are then activated, expanded, and reintroduced to the patient, where upon encountering TAAs in vivo they undergo mitogenic activation and induce apoptosis of target cells [29]. To date there are three generations of CARs (Figure 1). First generation CAR-T cells contained only an epitope binding small chain variable fragment (scFv) constructed from a monoclonal antibody, a flexible immunoglobulin hinge, a lipid bilayer spanning domain, and a signaling domain composed of the CD3ζ T cell receptor base. These cells had detectable anti-tumor activity but rapidly became anergic and struggled with long term persistence [29]. Second generation CARs rectified this shortcoming by including a costimulatory receptor such as CD28, DAP10, OX-40, or 4-1BB in parallel to CD3ζ. These dual signaling receptors aided in persistence and effector functions with significantly improved cytokine output that enhanced proliferation *in vivo* [30]. Third generation constructs received an additional costimulatory domain in series with the first, i.e. CD28 plus 4-1BB, due to observations that choice of costimulatory receptor yielded different effector profiles (30) (Figure 1). For instance, second generation CD28 CARs exhibited robust tumor depletion but struggled to produce sustained expansion, while 4-1BB CARs were detectable in patient's blood for several years following therapy [30].

Components of the CAR-T construct

CAR-T cells recognize antigen in an MHC-independent fashion, enabling a wider range of antigens to be targeted with a single receptor construct as well as circumvention of MHC down-regulation frequently seen in immune escape variants. The antigen specific scFv region is composed of variable light and variable heavy monoclonal antibody chains of which CD19 has shown the most success [31]. A hinge region connects the scFv and membrane anchoring domains and imparts flexibility to the receptor. Most commonly these include IgG1-4 variants [32]. Investigators have reported that choice of hinge influences CAR activity by modulating crosslinking with endogenous TCRs [32]. For instance, Guest and colleagues found CD19 scFv CARs benefited from addition of a CH2CH3 hinge whereas carcinoembryonic antigen (CEA) CARs performed better without incorporation of any hinge [33]. The membrane-spanning domain influences crosslinking flexibility as well as stability of the CAR within the membrane [32]. It is well documented that costimulatory domains (CD28, DAP10, OX-40, or 4-1BB) in addition to CD3ζ benefit activation and in vivo persistence of CAR-T cells, but due to the homogenous nature of clinical trials as well as the cost of preparing each CAR-T treatment, it remains to be seen if CD28 or 4-1BB in combination with CD28/DAP10/OX-40/4-1BB provide greater benefit when compared against a similar patient cohort using an alternate third generation costimulatory sequence [34,35]. The most common construct includes second generation CD28 and CD3ζ such as in the FDA-approved axicabtagene ciloleucel, or 4-1BB with CD3ζ as seen in tisagenlecleucel.

Patient preconditioning relies on lymphodepleting chemotherapy

Patient conditioning using chemotherapy to reduce cancer burden prior to infusion of CAR-T cells is frequently performed during a 1-2 week period while autologous cells are prepared [36]. Most commonly cyclophosphamide or cyclophosphamide and fludarabine are administered, with recent trials more frequently using only high-dose cyclophosphamide at concentrations of $1g/m^2$ to $3g/m^2$ [37] although pentostatin has also been used with success [38]. Researchers at the Memorial Sloan-Kettering Cancer Center (MSKCC) have shown in vivo T-cell persistence and peripheral blood tumor load are inversely correlated, suggesting larger target cell numbers cause clearance of CAR T-cells [39]. In this same study at MSKCC, investigators compared both conditioned and unconditioned patient cohorts using 1.5 g/m^2, 3 g/m^2, or no cyclophosphamide pretreatment among 8 CLL patient cohorts, allowing a direct comparison to be made. The study found conditioning correlated with higher response rates and longer overall survival despite lower number of CAR T-cells transferred in the conditioned group [39]. Additionally, a study conducted at the Baylor College of Medicine in which no conditioning was performed showed poorer T-cell persistence compared to other 2nd generation CAR-T therapy trials [40]. To date, the MSKCC study is the only one allowing a direct comparison of preconditioning routes to be made, but preclinical animal models suggest preconditioning overall aids in response [41]. Future trials with the aim of homogenizing preconditioning treatment are needed to further evaluate whether the efficacy of preconditioning chemotherapies against cancer cells themselves is responsible for greater CAR-T response or if their lymphoablative effect is responsible. Most likely it is a combination of the two.

Preparation of CAR samples for infusion to patients

To generate each CAR-T sample for transfusion to patients, lymphocytes are first separated from patient blood using leukapheresis and density gradients. Cells are then enriched or depleted using antibody adsorbed paramagnetic beads to generate the desired ratio of CD4+/CD8+ population [42]. Investigators have reported high proportions of CD8+ lymphocytes correlate with poorer in vivo proliferation due to decreased IL-2 output [43]. Further, CD4+ cells derived from naive T cells were more potent cytokine producers versus central memory or effector memory subtypes, while CD8+ cells from central memory subsets offered enhanced persistence [43].

Once the desired CD4+/CD8+ ratio is achieved, the cells will be activated using αCD3/αCD28 antibodies with or without IL-2, IL-7, and IL-15 [33]. There is high variability in cytokine selection and concentration between protocols with some results correlating exhaustion of T cells with increasing IL-2 concentrations [33]. Delivery of the CAR gene segment is performed using either lentiviral or retroviral transduction, with upwards of 90% of cells successfully adopting the CAR phenotype, but they are sometimes as low as 4% [33]. Recently, electroporation of vectors carrying transposase/transposons have shown transfection efficiency similar to viral methods, with the benefit of significantly reduced cost [35]. CAR expressing cells are then purified to >99% and expanded in flasks, static culture bags, or more recently bioreactors until a desired cell concentration is reached (discussed below under Clinical Trials) [44]. Patients are then infused with the purified product in a hospital setting to monitor for cytokine release syndrome, which occurs in nearly all patients.

CD19-redirected CARs and toxicities

The most promising CAR-T therapies are those using CD19 reactive scFv domains. CD19 is a 95kDa surface antigen expressed in all B-cell populations present in pre-B cells and is maintained through maturity. CD19 CARs are thus able to recognize nearly all B-cell malignancies with less risk of widespread off-target cytotoxicity, and do not have to compete with solid tumors. The most common toxicity is cytokine release syndrome (CRS), with as high as 39% of patients developing severe CRS (sCRS) of grade 3 or higher [45]. CRS is characterized as endothelial activation resulting in hypotension, inflammation, and fever in the presence of elevated serum IL-6 and INFγ. Patients with higher cancer burden typically experience more severe CRS as one would expect. The robust T-cell activation responsible for CRS may often be controlled using corticosteroids such as dexamethasone or methylprednisone with or without the anti-IL-6 antibody tocilizumab [46]. Lymphodepleting preconditioning is performed in most current trials and generally confers a better response rate, however treatment with fludarabine and cyclophosphamide is a predictor of sCRS and CRES [47].

The second most common toxicity is neurotoxicity, termed CAR-T-cell-related encephalopathy syndrome (CRES). In current trials using the FDA approved CD19 CAR T-cell treatments Yescarta and Kymriah, up to 57% of patients develop CRES ≥ grade 3 [48]. CRES is thought to occur after endothelial activation causes a breakdown of the blood brain barrier in which cytokines IL-6, INFγ, and TNFα invade the CSF, although a smoking gun has not been identified [49]. The same treatment modalities for CRS are used in treating CRES, including in-hospital monitoring with tocilizumab with or without steroids [49]. Most CRES is self-limiting within 7-28 days of onset, however CRES ≥ grade 4 does not respond to anti-IL-6 and/or steroid treatments as well as sCRS [49].

Augmenting CAR T-cell therapy and limitations

While most patients do not develop life threatening toxicities, the severity in patients with runaway CRS, CRES, or both demonstrates the need for prophylactics. Researchers at University of Texas MD Anderson Cancer Center created a CRES grading algorithm to reduce time between symptom onset and treatment intervention during high grade CRES [50]. Further, because sCRS and CRES are the largest predictors for TRM, predictive biomarkers are being explored to better instruct researchers which patients may be at higher risk for these toxicities. A study of 133 patients treated with CD19 CAR T cells found CRS ≥ grade 3 correlated with high serum von Willebrand Factor (VWF) and angiopoietin-2 (Ang-2), two proteins released during endothelial activation [47]. Importantly, they found elevated preexisting levels of these biomarkers significantly predicted risk of developing sCRS [47]. Investigators also analyzed infused cell concentrations with toxicity to determine a dosing window that maximizes therapeutic

benefit before toxicity risk increases. However, they found that while infusion of CAR T-cells leading to peak peripheral CD8+ CAR T-cells < 10 per µl and CD4+ CAR T-cells <5 per µl correlated with lower incidence of grade ≥2 CRS, it also correlated with reduced efficacy in NHL patients, giving evidence a narrow window exists to achieve low CRS and high therapy [47]. Another study found elevated serum IL-6 and IFN concentrations on day 1 after CAR T-cell infusion were predictors of severe CRS among NHL and B-ALL patients, and that decreased TGF- correlated with risk of neurotoxicity [51].

As CAR T-cell therapy is further adopted, it's possible these proteins may be read as prophylaxis toward indicating patients at greater risk of toxicity. Some investigators have utilized HSCT in patients after receiving CAR T therapy as a means to prolong remission, although there does not seem to be significance between overall survival among patients who received HSCT and those who have not [46,52]. One study assessing HSCT following CD19 or CD22 CAR therapy in B-ALL patients suggests MRD is a more effective indicator to whether HSCT is successful in prolonging relapse free survival. Out of 25 patients who achieved MDR-negative status, relapse within 24 months was seen in 13.5% of patients who underwent HSCT, while those who did not undergo HSCT saw an 80% relapse rate [50].

Another technique altogether may include augmenting the cytokine profile of CAR T cells. Because CAR T cell persistence and disease burden are inversely correlated, lymphodepletion is commonly undertaken, which has significant toxicity risks and excludes low-performing patients. Researchers at the Manchester Cancer Research Centre recently developed a mouse model that utilizes first and second generation CD19 CARs engineered to express IL-12 in vivo [52]. Using a lymphoreplete model, they were successful eradicating B-cell lymphoma without the need for lymphodepletion while still seeing a long-term survival rate of 25% [52].

The largest limitation encumbering CAR-T cell therapy adoption is the lack of uniform protocols that exist, as well as high cost [39,42,44]. Due to the high cost of labor- intensive treatments, lack of uniformity in steps including cell number per infusion, transducing vector used, final T-cell population phenotype extending beyond CD4+/CD8+ decisions [43], as well as preconditioning agent used [37], the reproducibility of results and side effects between trials remain challenges.

Summary of clinical trials

CD19 CAR-T Therapy in NHL: CAR T-cell therapy has had a recent exciting breakthrough, with two CAR T-cell products that have received FDA approval: Axicabtagene ciloleucel (Yescarta) for DLBCL and tisagenlecleucel (Kymriah) for adult DLBCL subtypes and pediatric ALL. Clinical trials using these two agents have shown remarkable efficacy, while increasing the homogeneity of treatment, resulting in more predictable toxicities and reliable post administration proliferation. In a trial conducted at the University of Pennsylvania, investigators treated a total of 14 patients with DLBCL and 14 patients with follicular lymphoma (FL) using tisagenlecleucel [53]. A high percent of both groups had refractory disease, 86% of DLBCL and 57% of FL patients. Complete response (CR) was seen in 6 of 14 DLBCL patients (43%) and 10 of 14 FL patients (71%). At a median follow-up of 28.6 months, 57% of all patients remained progression-free (95% CI, 36 to 73), including 70% of patients in the FL group and 43% of DLBCL patients. cytokine release syndrome (CRS) and neurotoxicity (NT) remained the most common adverse events, though grade 3 or higher CRS was low at 18%. The ZUMA-1 trial by Kite evaluated axicabtagene ciloleucel in 7 patients with refractory DLBCL who had no remaining chemotherapy treatment options and found CR in 4 patients (57%) and an overall response (OR) in 5 patients (71%), with 3 patients (43%) remaining disease free at 12 months [54]. In a 2014 study by Khondoker et al. 8 of 13 patients evaluated with assorted advanced B-cell malignancies achieved CR and 4 had partial remissions [55]. One patient experienced SD (lymphoma). Acute NT were present in some patients but resolved within three weeks after infusion. One patient died 16 days after the infusion due to an unknown cause.

CD19 CAR-T Therapy in ALL: In two trials of 16 and 30 patients with relapsed or r/r B-cell ALL (n=16 was comprised only of adults with a median age of 50, n=30 comprised 26 children/young adults and 4 adults >22 years), both achieved CR with rates ≥ 90% [56,57]. In the 30-patient study CRS was experienced by 100% of the patients with 27% being severe, however the investigators noted C-reactive protein (CRP) functioned as a reliable indicator for CRS severity and allowed successful abatement with tocilizumab [56]. A total of 53 adults with B-ALL received Kymriah at the Memorial Sloan Kettering Cancer Center [58]. CR was seen in 44 patients (83%). Median overall survival was 12.9 months, and patients with low disease activity (predicted by blasts of <5%) saw a median survival of 20.6 months [58]. A predictor of adverse events such as CRS and NT include marrow blasts >5% [58]. Another trial investigating the efficacy of CTL-019 in 75 children and young adults with relapsed/refractory (r/r) B-ALL found an overall survival at 6 months of 90%, and 12 months survival of 76% [59]. CRS occurred in 77% of patients, of which 48% required administration of tocilizumab [59]. CTL-019 was shown to be effective in another recent trial with a similar patient cohort of 59 r/r B-ALL patients aged 1.5-24 years who received 4-1BB CTL-019 infusions as a single agent treatment. 55 of 59 patients (93%) experienced complete remission with a 79% overall survival at 12 months [60-65]. Recent CD19 clinical trials in NHL and ALL treatment are summarized below in Table 1.

TABLE 1

Summary of CD19 CAR-T cell clinical trials in ALL and NHL patients

Summary of trial	Drug/ treatment	Infused cell amount (per kg)	Over-all response rate	Complete remission	Disease free progression	Notable toxicities	Reference
n=30 (children/ adults) r/r ALL	CTL019 (Kymriah)	Range: 0.76 × 106 to 20.6 × 106	OS=78%	90%	Sustained remissions at 6-month event-free survival=67%, Predicted probability of persistence with CTL019=68%, Probability of relapse-free B-cell aplasia=73%	Severe: CRS=27%, Any grade: CRS=100%, NT=43%, B-cell aplasia in patients with a response=100%	56
n=28 DLBCL or r/r FL	CTL019 (Kymriah)	Range: 3.08 × 106 to 8.87 × 106	64%	FL=71% , DLBCL=43%	Sustained remissions of patients with response at 28.6-month (median) follow-up: FL=89%, DLBCL=86%	Severe: CRS=18%, NT=11% Any grade: CRS=57%, NT=39%	53
n=93 adults r/r DLBCL	Tisagenlecleucel (Kymriah)	Range: 0.1 × 108 to 6.0 × 108	52%	(Complete response) 40%	12-month post first response: relapse-free survival=65%, CR relapse-free survival=79%	Severe: CRS=22%, Neurologic events=12%, Cytopenias (>28 days)=32% Any grade: CRS=58%, Neurologic events=21%, Cytopenias=44%	61
n=101 adults r/r DLBCL, PMBCL or (transformed) FL	Axicabtagene ciloleucel (Yescarta)	2×10^6	ORR=83%	58%	Progression-free survival median=5.9 months OS median not reached, estimated at 50.5 % for 24 months.	Severe: CRS=11%, neurologic events=32% Any grade: neutropenia=16%, anemia=94%, thrombocytopenia=34%	62
n=20 adults r/r CLL or ALL	CART-19/ CTL019 (Kymriah)	Minimum dose: 1.5×10^7 (Average dose: 5 x109)	CL=42.9% ,ALL=83.3%	CLL=21.4% , ALL=83.4%		Severe: CRS (CLL)=42.86%, CRS (ALL)=83.33%	63

n=7 adults refractory DLBCL	KTE-C19 (Yescarta)	2 x 10^6	71%	57%	CR at 12+ months=43%	Severe: CRS=14%, NT=57% Any grade: CRS=86%, NT=86%	54
n=53 adults relapsed B-cell ALL	Autologous 19-28z+ CAR T-cells	1 x 10^6 or 3 x 10^6		83%	29-month (median) follow-up: event-free survival=6.1-months (median) OS=12.9-months (median)	Severe: CRS=26%, NT=42% Any grade: CRS=85%, NT=44%	58
n=59 children/ adults r/r/ ALL	CTL019 (Kymriah)	1 x 10^7 to 1 X 10^8	OS at 12-month (median) follow-up=79%	93% (1-month post infusion)	12-month (median) follow-up: CR =58%	Severe: CRS=27% ,B cell aplasia (continuing CR patients)=71% Any grade: CRS=88%	60
n=75 pediatric/ young adult (r/r B-cell ALL)	Tisagenlecleucel (Kymriah)	Range: 0.2 × 106 to 5.4 × 106		3-month overall remission rate=81% CR=60% Incomplete hematologic recovery + CR=21%	6-month follow-up: Patients with a response having relapse-free survival=80%, OS=90% 12-month follow-up: Patients with a response having relapse-free survival=59%, OS=76%	Severe: CRS=46%, Neurologic event=13%, Infection=24% Any grade: CRS=77% Neurologic event=40% Infection=43% B-cell aplasia in patients with a response=100%	59
n=63 children / young adult (r/r ALL)	Tisagenlecleucel (Kymriah)	One flat dose for patients based on weight: >50 kg infused with 0.1-2.5 x 10^8 or ≤50 kg infused with 0.2-5 x 10^6	83%	63%	At a 4.8 month median follow-up, continuation of response not reached.	Severe: CRS=49%, NT=18% Any grade: CRS=79%, NT=65%	64
n=21 children / young adults (r/r B-cell ALL)	autologous CD19-CAR T-cells	Either 1×10^6 or 3×10^6. If an insufficient number of cells were generated for the required dose, the entire CAR T-cell product was infused.	median follow up (10 months) overall survival=51.6%	66.70%		Any grade: CRS=76%, NT=29%	65
n=30 adults (r/r B-cell ALL)	autologous CD19-CAR T-cells	2 × 10^5, 2 × 10^6, or 2 × 10^7		86%		Any grade: CRS=83% Only severe incidences of NT were reported at 50%	46

REFERENCES

1. American Cancer Society. Key Statistics for Non-Hodgkin Lymphoma.
2. Armitage JO, Gascoyne RD, Lunning MA, et al. Non-Hodgkin lymphoma. Lancet. 2017;390: 298-310.
3. Cancer Network Home of the Journal Oncology. Non-Hodgkin Lymphoma.
4. Basso K, Dalla-Favera R. Germinal centers and B cell Lymphomagenesis. Nat Rev Immunol. 2015;15: 172-84.
5. Kridel R, Sehn LH, Gascoyne RD. Pathogenesis of follicular lymphoma. J Clin Invest 2012;122: 3424-31.
6. Challa-Malladi M, Lieu YK, Califano O, et al. Combined genetic inactivation of b2-microglobulin and CD58 reveals frequent escape from immune recognition in diffuse large B cell lymphoma. Cancer Cell. 2011;20: 728-40.
7. Smith MR. Non-Hodgkin's lymphoma. Curr Probl Cancer. 1996;20: 76-7.
8. Tan BR, Bartlett NL. Treatment advances in non-Hodgkin's lymphoma. Expert Opin Pharmacother. 2000;1: 451-66.
9. Jazirehi AR, Bonavida B. Cellular and molecular signal transduction pathways modulated by rituximab (rituxan, anti-CD20 mAb) in non-Hodgkin's lymphoma: Implications in chemosensitization and therapeutic intervention. Oncogene. 2005;24: 2121-43.
10. Fisher RI, Gaynor ER, Dahlberg S, et al. Comparison of a standard regimen (CHOP) with three intensive chemotherapy regimens for advanced non-Hodgkin's lymphoma. N Engl J Med. 1993;328: 1002-6.
11. Cartron G, Watier H, Golay J, Solal-Celigny P. From the bench to the bedside: ways to improve rituximab efficacy. Blood. 2004;104: 2635-42.
12. Coiffier B, Lepage E, Brière J, et al. CHOP chemotherapy plus rituximab compared with CHOP alone in elderly patients with diffuse large-B-cell lymphoma. N Engl J Med 2002;346: 235-42.
13. Plosker GL, Figgitt DP. Rituximab: a review of its use in non-Hodgkin's lymphoma and chronic lymphocytic leukemia. Drugs. 2003;63: 803-43.
14. Onea AS, Jazirehi AR. CD19 chimeric antigen receptor (CD19 CAR)-redirected adoptive T-cell immunotherapy for the treatment of relapsed or refractory B-cell Non-Hodgkin's Lymphomas. Am J Cancer Res. 2016;6: 403-24.
15. Teras LR, DeSantis CE, Cerhan JR, et al. 2016 US lymphoid malignancy statistics by World Health Organization subtypes. Ca Cancer J Clin. 2016;66: 443-59.
16. Friedberg JW. Relapsed/refractory diffuse large B-cell lymphoma. Hematology Am Soc Hematol Educ Program 2011;2011: 498-505.
17. Khouri IF. Allogeneic stem cell transplantation in follicular lymphoma. Best Pract Res Clin Haematol. 2011;24: 271-7.
18. Gisselbrecht C, Glass B, Mounier N, et al. Salvage regimens with autologous transplantation for relapsed large B-cell lymphoma in the rituximab era. J Clin Oncol. 2010;28: 4184-90.
19. American Cancer Society. Key Statistics for Acute Lymphocytic Leukemia (ALL) 2018.
20. Hunger SP, Lu X, Devidas M, et al. Improved survival for children and adolescents with acute lymphoblastic leukemia between 1990 and 2005: a report from the children's oncology group. J Clin Oncol. 2012;30: 1663-9.
21. Terwilliger T, Abdul-Hay M. Acute lymphoblastic leukemia: A comprehensive review and 2017 update. Blood Cancer J. 2017;7: e577.
22. Mullighan CG, Collins-Underwood JR, Phillips LA, et al. Rearrangement

of CRLF2 in B-progenitor-and Down syndrome-associated acute lymphoblastic leukemia. Nat Genet. 2009;41: 1243-6.

23. Zhang J, Mullighan CG, Harvey RC, et al. Key pathways are frequently mutated in high-risk childhood acute lymphoblastic leukemia: a report from the Children's Oncology Group. Blood. 2011;118: 3080-7.
24. Inaba H, Greaves M, Mullighan CG. Acute lymphoblastic leukemia. Lancet 2013;381: 1943-55.
25. Ceppi F, Duval M, Lecler JM, et al. Improvement of the outcome of relapsed or refractory acute lymphoblastic leukemia in children using a risk-based treatment strategy. PLoS One. 2016;11: e0160310.
26. Ma X, Edmonson M, Yergeau D, et al. Rise and fall of subclones from diagnosis to relapse in pediatric B-acute lymphoblastic leukemia. Nat Commun 2015;6: 6604.
27. Alvarnas JC, Brown PA, Aoun P, et al. Acute lymphoblastic leukemia, version 2.2015. J Natl Compr Canc Netw. 2015;13: 1240-79.
28. Jackson HJ, Rafiq S, Brentjens RJ. Driving CAR-T cells forward. Nat Rev Clin Oncol. 2016;13: 370-83.
29. Hartmann J, Schüßler‐Lenz M, Bondanza A, et al. Clinical development of CAR-T cells-challenges and opportunities in translating innovative treatment concepts. EMBO Mol Med. 2017;9: 1183-97.
30. van der Stegen SJ, Hamieh M, Sadelain M. The pharmacology of second-generation chimeric antigen receptors. Nat Rev Drug Discov. 2015;14: 499-509.
31. Sommermeyer D, Hill T, Shamah S, et al. Fully human CD19-specific chimeric antigen receptors for T-cell therapy. Leukemia. 2017;31: 2191-9.
32. Dotti G, Gottschalk S, Savoldo B, et al. Design and development of therapies using chimeric antigen receptor-expressing T cells. Immunol Rev. 2014;257: 107-26.
33. Guest RD, Hawkins RE, Kirillova N, et al. The role of extracellular spacer regions in the optimal design of chimeric immune receptors: evaluation of four different scFvs and antigens. J Immunother. 2005;28: 203-11.
34. Makita S, Yoshimura K, Tobinai K. Clinical development of anti‐CD19 chimeric antigen receptor T‐cell therapy for B‐cell non‐Hodgkin lymphoma. Cancer Sci. 2017;108: 1109-18.
35. Singh H, Figliola MJ, Dawson MJ, et al. Manufacture of clinical-grade CD19-specific T cells stably expressing chimeric antigen receptor using Sleeping Beauty system and artificial antigen presenting cells. PLoS One. 2013;8: e64138.
36. Zhao Z, Chen Y, Francisco NM, et al. The application of CAR-T cell therapy in hematological malignancies: advantages and challenges. Acta Pharm Sin B. 2018;8: 539-51.
37. Davila ML, Brentjens R, Wang X, et al. How do CARs work? Early insights from recent clinical studies targeting CD19. Oncoimmunology. 2012;1: 1577-83.
38. Porter DL, Levine BL, Kalos M, et al. Chimeric antigen receptor–modified T cells in chronic lymphoid leukemia. N Engl J Med. 2012;365: 725-33.
39. Brentjens RJ, Rivière I, Park JH, et al. Safety and persistence of adoptively transferred autologous CD19-targeted T cells in patients with relapsed or chemotherapy refractory B-cell leukemias. Blood. 2011;118: 4817-28.
40. Savoldo B, Ramos CA, Liu E, et al. CD28 costimulation improves expansion and persistence of chimeric antigen receptor-modified T cells in lymphoma patients. J Clin Invest. 2011;121: 1822-6.
41. Pegram HJ, Lee JC, Hayman EG, et al. Tumor-targeted T cells modified to secrete IL-12 eradicate systemic tumors without need for prior conditioning. Blood. 2012;119: 4133-41.
42. Vormittag P, Gunn R, Ghorashian S, et al. A guide to manufacturing CAR-T cell therapies. Curr Opin Biotechnol. 2018;2018: 164-81.
43. Sommermeyer D, Hudecek M, Kosasih PL, et al. Chimeric antigen receptor-modified T cells derived from defined CD8+ and CD4+ subsets confer superior antitumor reactivity in vivo. Leukemia. 2016;30: 492-500.
44. Sadeghi A, Pauler L, Annerén C, et al. Large-scale bioreactor expansion of tumor-infiltrating lymphocytes. J Immunol Methods. 2011;364: 94-100.
45. Jin Z, Xiang R, Kai Qing K, et al. The severe cytokine release syndrome in phase I trials of CD19-CAR-T cell therapy: a systematic review. Ann Hematol. 2018;97: 1327–35.
46. Turtle CJ, Hanafi L, Berger C, et al. CD19 CAR-T cells of defined CD4+:CD8+ composition in adult B cell ALL patients. J Clin Invest. 2016;126: 2123-38.
47. Hay KA, Hanafi L, Li D, et al. Kinetics and biomarkers of severe cytokine release syndrome after CD19 chimeric antigen receptor–modified T-cell therapy. Blood. 2017;130: 2295-306.
48. Locke FL, Neelapu SS, Bartlett NL, et al. Phase 1 results of ZUMA-1: a multicenter study of KTE-C19 anti-CD19 CAR T cell therapy in refractory aggressive lymphoma. Mol Ther. 2017;25: 285-95.
49. Gust J, Hay KA, Hanafi L, et al. Endothelial activation and blood-brain barrier disruption in neurotoxicity after adoptive immunotherapy with CD19 CAR-T cells. Cancer Discov. 2017;7: 1401-19.
50. Boyaidzis MM, Dhodapkar MV, Brentjens RJ, et al. Chimeric antigen receptor (CAR) T therapies for the treatment of hematologic malignancies: clinical perspective and significance. J Immunother Cancer. 2018;6: 137.
51. Turtle CJ, Hanafi L, Berger, et al. Immunotherapy of non-Hodgkin's lymphoma with a defined ratio of CD8+ and CD4+ CD19-specific chimeric antigen receptor-modified T cells. Sci Transl Med 2016;8: 355a116.
52. Kueberuwa G, Kalaitsidou M, Cheadle E, et al. CD19 CAR T Cells Expressing IL-12 Eradicate Lymphoma in Fully Lymphoreplete Mice through Induction of Host Immunity. Mol Ther Oncolytics 2018;8: 41-51.
53. Schuster SJ, Svodoba J, Chong EA, et al. Chimeric antigen receptor T cells in refractory B-cell lymphomas. N Engl J Med. 2017;377: 2545-54.
54. Locke FL, Neelapu SS, Bartlett NL, et al. Phase 1 results of ZUMA-1: a multicenter study of KTE-C19 anti-CD19 CAR T cell therapy in refractory aggressive lymphoma. Mol Ther. 2017;25: 285-95.
55. Kochenderfer JN, Dudley ME, Kassim SH, et al. Chemotherapy refractory diffuse large B-cell lymphoma and indolent B-cell malignancies can be effectively treated with autologous T cells expressing an anti-CD19 chimeric antigen receptor. J Clin Oncol. 2015;33: 540-9.
56. Maude SL, Frey N, Shaw PA, et al. Chimeric antigen receptor T cells for sustained remissions in leukemia. N Engl J Med. 2014;371: 1507-17.
57. Davila ML, Riviere I, Wang X, et al. Efficacy and toxicity management of 19-28z CAR cell therapy in B cell acute lymphoblastic leukemia. Sci Transl Med. 2014;6: 224ra25.
58. Park JH, Rivière I, Gonen M, et al. Long-term follow-up of CD19 CAR therapy in acute lymphoblastic leukemia. N Engl J Med. 2018;378: 449-59.
59. Maude SL, Laetsch TW, Buechner J, et al. Tisagenlecleucel in children and young adults with B-cell lymphoblastic leukemia. N Engl J Med. 2018;378: 439-48.
60. Maude SL, Teachey DT, Rheingold SR, et al. Sustained remissions with CD19-specific chimeric antigen receptor (CAR)-modified T cells in children with relapsed/refractory ALL. J Clin Oncol 2016;34: 3011-1.
61. Schuster SJ, Bishop MR, Tam CS, et al. Tisagenlecleucel in adult relapsed or refractory diffuse large B-cell lymphoma. N Engl J Med. 2019;380: 45-56.
62. Neelapu SS, Locke FL, Bartlett NL, et al. Axicabtagene ciloleucel CAR T-cell therapy in refractory large B-cell lymphoma. N Engl J Med. 2017;377: 2531-44.
63. NIH U.S. National Library of Medicine ClinicalTrials.gov. CART19 to Treat B-Cell Leukemia or Lymphoma That Are Resistant or Refractory to Chemotherapy 2019.

64. Lee DW, Kochenderfer JN, Stetler-Stevenson M, et al. T cells expressing CD19 chimeric antigen receptors for acute lymphoblastic leukemia in children and young adults: a phase 1 dose-escalation trial. Lancet. 2015;385: 517-28.

65. O'Leary MC, Lu X, Huang Y, et al. FDA Approval Summary: Tisagenlecleucel for Treatment of Patients with Relapsed or Refractory B-cell Precursor Acute Lymphoblastic Leukemia. Clin Cancer Res. 2019;25: 1142-6.