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Review Series

Cellular therapy for hematologic malignancies is a rapidly evolving field, with new iterations of novel constructs being developed at a rapid pace. Since the initial reports of chimeric antigen receptor T cell (CAR T cell)success in CD19⁺ B cell malignancies, multiple clinical trials of CAR T cell therapy directed to CD19 have led to the approval of this therapy by the FDA and the European Medicines Agency for specific indications. Despite strikingly similar efficacy, investigators at multiple centers participating in these studies have observed the nuances of each CAR T cell product, including variability in manufacturing, availability, and toxicity profiles. Here we review state-of-the-art clinical data on CD19-directed CAR T cell therapies in B cell hematologic malignancies, advances made in understanding and modeling associated toxicities, and several exciting advances and creative solutions for overcoming challenges with this therapeutic modality.

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State of the art in CAR T cell therapy for CD19+ B cell malignancies

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Cellular therapy for hematologic malignancies is a rapidly evolving field, with new iterations of novel constructs being developed at a rapid pace. Since the initial reports of chimeric antigen receptor T cell (CAR T cell) success in CD19+ B cell malignancies, multiple clinical trials of CAR T cell therapy directed to CD19 have led to the approval of this therapy by the FDA and the European Medicines Agency for specific indications. Despite strikingly similar efficacy, investigators at multiple centers participating in these studies have observed the nuances of each CAR T cell product, including variability in manufacturing, availability, and toxicity profiles. Here we review state-of-the-art clinical data on CD19-directed CAR T cell therapies in B cell hematologic malignancies, advances made in understanding and modeling associated toxicities, and several exciting advances and creative solutions for overcoming challenges with this therapeutic modality.

Introduction

The chimeric antigen receptor (CAR) constructs that would eventually become tisagenlecleucel (tisa-cel) and axicabtagene ciloleucel (axi-cel) were first reported in 2009. These second-generation, CD19-specific CAR T cell constructs were composed of a single-chain variable fragment (scFv) derived from the murine anti-CD19 clone FMC63 and fused to a transmembrane domain, and the endodomains of a T cell costimulatory receptor (4-1BB in tisa-cel and CD28 in axi-cel) and CD3ζ (1, 2). Both constructs were tested in vitro and in xenograft mouse models, and academic investigators soon scaled up these processes to treat patients with B cell malignancies in phase I clinical trials (reviewed in ref. 3). Although these constructs target the same epitope of CD19, seemingly minor differences in the constructs, manufacturing processes, and final cell products generated significant variability in clinical toxicity and CAR T cell kinetics in patients. Remarkably, these CD19-directed T cell products induced complete responses in patients with previously refractory or multiply relapsed B cell malignancies of different origins, including diffuse large B cell lymphoma (DLBCL), chronic lymphocytic leukemia (CLL), and B cell acute lymphoblastic leukemia (B-ALL) (4–6). Since these initial reports, three pivotal studies led to FDA and European Medicines Agency approvals for the CD19-specific CAR T cell products tisa-cel and axi-cel (7–9). Class-specific toxicities include cytokine release syndrome (CRS) and immune effector cell–associated neurotoxicity syndrome (ICANS). CRS spans a spectrum of severity, from a mild flu-like syndrome with high fevers, fatigue, and myalgias to multi–organ system failure requiring intensive supportive care such as, intubation, vasopressors, and hemodialysis (10). CAR T cell–related ICANS, previously referred to as neurotoxicity- or CAR-related encephalopathy syndrome, is a protean clinical syndrome characterized by confusion, obtundation, seizures, visual/auditory hallucinations, amnesia, expressive aphasia, and—in rare cases—potentially lethal cerebral edema (10, 11).

Toxicities associated with CAR T cells

The unique toxicity profile of CAR T cell therapies targeting CD19, namely CRS and ICANS, began to emerge in the first clinical trials (4, 5, 12, 13). A combination of clinical acumen and correlative immunologic studies identified the mechanisms of toxicity and led to current management strategies.

Cytokine release syndrome. CRS is a potentially life-threatening systemic inflammatory response triggered by release of proinflammatory cytokines such as IL-1, IL-2, IL-6, TNF-α, IFN-γ, GM-CSF, MCP-1, and MIP-1β; the frequency and severity of CRS correlate with antigen-dependent T cell activation and expansion (13–15). CRS has variable time to onset and can begin within the first 24 hours after CAR T cell infusion; delayed CRS has also been observed (16, 17). Initial symptoms of CRS include fever and tachycardia, and can progress to hypotension, hypoxia, and signs of end-organ dysfunction; CRS is managed primarily with the anti–IL-6 receptor monoclonal tocilizumab (18–21). CRS differs from a similar syndrome, cytokine storm, a steroid-responsive, antigen-independent immune activation mediated by cytokines such as TNF-α (22, 23). CRS is a clinical syndrome mediated by antigen-specific T cell activation and expansion, with strong interactions with innate immune compartments mediated by the IL-6 signaling pathway. Initial attempts to manage CRS with high-dose steroids and TNF-α blockade failed, leading to the first successful use of tocilizumab in refractory CRS (13, 18). No other agents have been approved to date for managing CAR T cell–associated CRS, but several investigators have prescribed third-line agents in the setting of

tocilizumab- and corticosteroid-refractory CRS; these include siltuximab (anti–IL-6 antibodies), anakinra (to block IL-1), and chemotherapy (T cell lytic agents, such as cyclophosphamide).

Patient- and treatment-specific factors associated with severe CRS include the use of lymphodepletion chemotherapy, higher CAR T cell dose, disease burden, and elevated baseline inflammatory markers (24). Prior CAR T cell studies that did not use lymphodepleting chemotherapy (LDC) reported lower toxicity as well as decreased efficacy compared with regimens containing it. While the effects of LDC are likely multifactorial, its use is hypothesized to increase levels of homeostatic cytokines such as IL-7 and IL-15, while also depleting inhibitory Treg populations, thereby enhancing CAR T cell expansion (25, 26).

ICANS and fludarabine-related neurotoxicity. ICANS has been observed with both the CD19-directed bispecific T cell engager (blinatumomab) and CD19-specific CAR T cells, and has been observed with other, non-CD19⁺ constructs (27). ICANS is commonly associated with, and temporally follows, CRS, and clinical laboratory findings of severe ICANS often overlap with severe CRS, including elevated C-reactive protein, ferritin, and cytopenias, suggesting a mechanistic link between CRS and ICANS (28). Initially there were concerns that CNS disease may predispose to ICANS, but recent work has suggested that the mere presence of CNS disease may not lead to increased toxicity (29). Unlike CRS, ICANS is primarily managed with high-dose steroids. Tocilizumab does not ameliorate ICANS, probably because it does not efficiently cross the blood-brain barrier (BBB); worse, tocilizumab also transiently increases circulating levels of IL-6 (20, 30), which may potentiate the underlying inflammatory cascade. Although MRI and electroencephalography are commonly performed during acute ICANS, little is known about the implications of various findings associated with this syndrome, such as focal areas of edema on imaging, or diffuse or frontal background slowing in θ and δ frequency ranges on electroencephalography (31). Given that severe ICANS is often associated with decreased overall survival (OS), further studies are warranted (31).

Correlative studies in samples of patients with ICANS have revealed endothelial activation through the angiopoietin (ANG)/TIE2 axis, as well as overall disruption of the BBB, and suggest these underlying mechanisms for CAR-related ICANS (28). While ANG1 is constitutively produced by vascular (including brain) pericytes and platelets, ANG2 is stored in Weibel-Palade bodies and is released upon endothelial cell (EC) activation in the setting of inflammatory insults. Displacement of ANG1 by ANG2 causes increased EC activation and microvascular permeability (28). Patients with severe ICANS also exhibit elevated levels of inflammatory cytokines and protein and T cell infiltrates within the CNS, suggesting increased BBB permeability. These observations suggest that early EC activation initiates a self-perpetuating cycle of increased BBB permeability, cytokine transit, vascular pericyte stress, additional EC activation, and further increases in BBB permeability (28). More recent nonhuman primate models using a CD20-specific 4-1BB–based CAR successfully recapitulated both CRS and ICANS in rhesus macaques (32). After infusion of truncated EGFR–tagged (EGFRt-tagged) CD20-specific CARs into non–tumor-bearing primates, without LDC, robust CAR T cell expansion analogous to observations in

human patients was seen. These monkeys experienced B cell aplasia as well as fever, tremor, lethargy, and ataxia, all consistent with CRS/ICANS. Supporting correlative markers included elevated serum cytokines, disproportionately high cerebrospinal fluid levels of IL-6, IL-2, GM-CSF, and VEGF, and significant accumulation of both CAR⁺ and CAR⁻ T cells consistent with pan–T cell encephalitis (32).

With the increasing use of fludarabine-containing regimens, clinicians should be cautious of fludarabine dosing and fluctuations in creatinine clearance, given the rising incidence of late-onset fludarabine-associated neurotoxicity (33). Initial studies of fludarabine reported neurologic symptoms 20–250 days after drug exposure that manifested as worsening visual disturbances, peripheral neuropathy, dementia, ataxia, weakness, coma, and death (34). Patients with fludarabine toxicities had findings consistent with white matter demyelination, necrosis, enlarged astrocytes and oligodendrocytes, and white matter changes evident upon MRI and autopsy (35). Elimination of fludarabine's active metabolite, F-araATP, depends on adequate renal function, rendering patients with abnormal renal function particularly susceptible to toxicities (36). Increased BBB permeability in CRS/ICANS may further predispose patients to fludarabine-related neurotoxicity (37).

MAS/HLH and the CRS spectrum. Macrophage activation syndrome/hemophagocytic lymphohistiocytosis (MAS/HLH) is a clinical syndrome associated with high fevers, hepatosplenomegaly, liver/renal dysfunction, coagulopathy, cytopenias, hyperferritinemia, and hypertriglyceridemia, with evidence of hemophagocytosis often noted upon bone marrow biopsy (38). Interestingly, these characteristics commonly occur in patients experiencing severe CRS, with considerable overlap of cytokine profiles including elevated levels of IFN-γ, IL-6, and IL2RA (39, 40). Although rare, fatal cases of MAS/HLH have been observed in CAR T cell–related CRS/ICANS. Unfortunately, standard NOD–SCID–γ chain receptor–knockout (NSG) mouse models failed to predict or recapitulate CRS and ICANS, partly because of the NOD genetic background, which reduces function of the innate immune system (41). Work by Giavridis et al. (42) and Norelli et al. (43) used SCID-beige and humanized-SGM3 mouse models, respectively, which partly recapitulated much of the clinical syndrome of CRS and ICANS (Figure 1). These data demonstrated that although T cells are the primary source of both IFN-γ and GM-CSF, myeloid cells are the primary producers of inflammatory factors such as IL-6, IL-1, and inducible NOS, which play key roles in the pathogenesis of CRS (42, 43). As excessive macrophage activation appears to be a shared characteristic in both severe CRS and ICANS, targeting the MAS pathway may ultimately yield therapeutic benefits for both clinical syndromes. Additional studies have demonstrated GM-CSF's potential role in CRS (44), and clinical trials targeting multiple aspects of the MAS pathway are likely to open in the coming year.

Grading and difficulty of cross-trial comparisons. Early clinical trials using CAR T cell therapy lacked a unified grading system for CRS and ICANS. Although early use of a modified Common Terminology for Criteria for Adverse Events (CTCAE v4.03) was attempted, it became clear that standard CTCAE grading was insufficient to truly capture CAR T cell–related toxicities. Thus, a published consensus among early investigators incorporated clin-
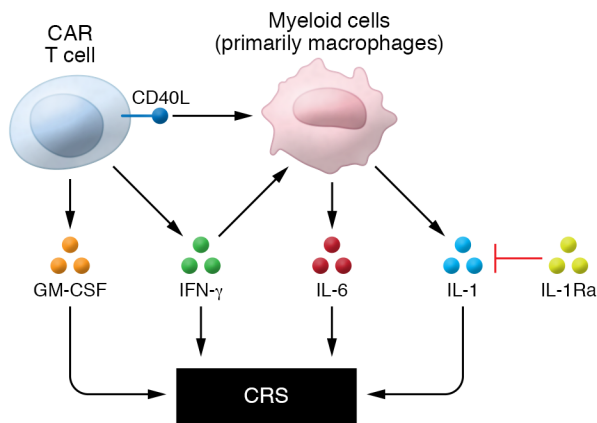
Figure 1. Summary of mechanisms discovered in two animal models designed to recapitulate cytokine release syndrome.

ical factors such as hypoxia and hypotension in combination with organ-specific CTCAE grading (16). In parallel, investigators at the University of Pennsylvania developed a CRS grading scale for their CD19-directed CAR T cell trials focusing on clinically relevant factors and interventions, including need for hospitalization and varying degrees of organ-specific toxicities (45). Unlike Lee et al. (16), the Penn criteria defined grade 3 CRS as any organ dysfunction requiring hospitalization, including grade 4 liver function abnormalities, grade 3 creatinine, coagulopathy requiring blood products, hypotension treated with multiple fluid boluses or low-dose pressors, and hypoxia requiring supplemental oxygen, leading to more severe grading (10, 17). Separately, investigators at Memorial Sloan Kettering Cancer Center developed criteria using duration of intervention for hypoxia and/or hypotension, while investigators at MD Anderson Cancer Center developed the CAR-TOX criteria, modifying Lee et al. (16) slightly by including organ toxicities in the definition of grade 1 CRS (46, 47).

In an attempt to harmonize these various approaches, an expert group comprising academic centers and industry partners convened at a 2018 meeting supported by the American Society for Transplantation and Cellular Therapy. These new criteria defined CRS as the presence of fever with/without hypotension and/or hypoxia. Grading depends on the degree of vasopressor and respiratory support, with clear delineations based on the type of intervention. End-organ toxicities were removed from the process of grading severity, as such toxicities were felt to be managed symptomatically in accordance with standard medical practices. ICANS was defined as "a disorder characterized by a pathologic process involving the central nervous system following any immune therapy that results in the activation or engagement of endogenous or infused T cells and/or other immune effector cells," with the consideration that this system could be applied to adverse effects of immune effector cell–engaging therapies beyond CAR T cells. Additionally, a scoring algorithm was devised to include an element for assessing receptive aphasia, which is now termed the Immune Effector Cell–Associated Encephalopathy (ICE) score (10). Adopting these consensus criteria in future studies and in clinical practice will enable cross-study comparisons of new and existing immune effector cell therapies.

Summary of current FDA-approved indications for CD19-directed CAR T cell therapy

There are two CD19-directed CAR T cell products approved and one nearing approval for various indications that originate from B cell malignancies: tisagenlecleucel (tisa-cel) for pediatric B-ALL and large cell lymphoma, axicabtagene ciloleucel (axi-cel) for large cell lymphoma, and, nearing approval, lisocabtagene maraleucel (liso-cel) for large cell lymphoma. The results of each registration trial are summarized in Table 1 and described in more detail below.

Tisa-cel for B-ALL. The FDA approved the first CAR T cell therapy in August 2017 for relapsed/refractory pediatric and young adult B-ALL up to 25 years of age based on the phase II, global ELIANA study (9). In this trial, 75 patients were infused following LDC (fludarabine 30 mg/m^2 i.v. for 4 days, cyclophosphamide 500 mg/m^2 i.v. for 2 days). The overall complete remission rate within 3 months was 81%; all patients in remission were negative for minimal residual disease (MRD) as scored by flow cytometry. Impressively, the event-free and OS rates of these patients were 50% and 76%, respectively, at 12 months, with a median follow-up of 13.1 months. Forty-six percent of patients experienced grade 3+ CRS, based on the grading criteria developed at the University of Pennsylvania (45), while only 13% experienced grade 3+ neurotoxicity. In this cohort, the median number of prior therapies was 3 (range 1–8), with 61% of patients having relapsed after a prior allogeneic stem cell transplant (SCT). Responses were equivalent regardless of prior SCT, presence of high-risk mutations such BCR-ABL1, MLL, hypoploidy, or BCR-ABL1–like gene signatures. Because there was no correlation between cell dose and CAR T cell expansion, as measured by mean area under the curve from day 0 to day 28 (AUC$_{0-28d}$) and maximum transgene level (C$_{max}$), a wide target cell dose of 0.2×10^6 to 5.0×10^6 CAR$^+$ viable T cells was allowed for commercial tisa-cel in B-ALL. The median persistence of CAR T cells was 168 days in responding patients. Most importantly, there was no difference in OS with or without censoring at the time of allogeneic SCT, with only 8 of 75 patients undergoing later allogeneic SCT after CAR T cell therapy (9).

In subsequent analyses of this trial, patients with B cell recovery within 6 months of infusion experienced earlier loss of CAR T cell persistence, as determined via transgene copy number (48). Furthermore, no relationships were detected among clinical response, safety, expansion, and CD4/CD8 ratio of infused product (48). Longer-term follow-up demonstrated similarly impressive response rates, with relapse-free and OS rates at 18 months of 66% (95% CI, 52%–77%) and 70% (95% CI, 58%–79%), respectively (49). Of the responders, 19 patients relapsed; 14 of 19 patients exhibited loss of CD19 in their tumor despite ongoing B cell aplasia, a phenomenon previously attributed to hemizygous deletions spanning the *CD19* locus and de novo frameshift and missense mutations in extracellular portions of *CD19* as well as CAR transduction of leukemic blasts leading to epitope masking (50, 51). As cellular immune responses against the murine portions of the anti-CD19 scFv (FMC63) have been demonstrated (52), subsequent generations of CD19$^+$ CAR T cells may utilize humanized versions of the anti-CD19 scFv. Preliminary data for such humanized anti-CD19 CAR T cell therapy (CTL119) have demonstrated efficacy in relapsed/refractory disease after failure of murine-derived (FMC63) anti-CD19 CAR T cells (53, 54).

Table 1. Summary of efficacy and toxicity from advanced CD19-directed CAR T cell therapies

	Tisagenlecleucel (95)	Axicabtagene ciloleucel (56)	Lisocabtagene maraleucel (69)
Construct	Anti-CD19–4-1BB–CD3ζ	Anti-CD19–CD28–CD3ζ	Anti-CD19–4-1BB–CD3ζ
Follow-up, months	24	27.1	12
Median prior therapies	3	3	3
Overall response	54% ORR, 40% CR	83% ORR, 58% CR	73% ORR, 53% CR
Median OS, months	10.3, not reached for patients in CR	Not reached	Not reached
PFS at 2 years, %			
All patients	Not reported	39	Not reported
In CR	78	72	Not reported
Grade 3–4 AEs	23% CRS, 11% NT	12% CRS, 31% NT	2% CRS, 10% NT
CRS grading scale used	Penn	Lee	Lee
Treatment locale	Inpatient or outpatient	Inpatient only	Inpatient or outpatient
Approval status	FDA-approved for pediatric ALL and adult R/R DLBCL	FDA-approved for adult R/R DLBCL and PMBL	Not yet approved

AEs, adverse events; CR, complete response; NT, neurotoxicity; ORR, overall response rate; OS, overall survival; PFS, progression-free survival; PMBL, primary mediastinal B cell lymphoma; R/R, relapsed/refractory.

Axi-cel for DLBCL. In October 2017, axi-cel was the second CAR T cell product to be approved in the United States. The approval was based on a single-arm, phase II, multicenter registration trial (ZUMA-1) for relapsed or refractory large B cell lymphoma after two or more lines of systemic therapy, including DLBCL not otherwise specified, primary mediastinal large B cell lymphoma, high-grade B cell lymphoma, and DLBCL arising from follicular lymphoma (55). Of 101 assessable patients who received axi-cel after LDC (fludarabine 30 mg/m² i.v. and cyclophosphamide 500 mg/m² i.v. for 3 days), 83% had an objective response, including a complete response rate of 58%. At the time of data cutoff, 39% of patients had ongoing responses with a median follow-up of 27.1 months. Interestingly, 39% of patients who had either a partial response or stable disease at 1 month eventually converted to a complete response by 6 months (56). Of the study population, 39% had complete responses, and importantly, in this group, the median duration of response had not been reached after 2 years. Notably, in this disease, some consider 2 years of complete remission from chemotherapy as representing cure (57) and no longer requiring routine follow-up imaging. CD19 positivity was not required for eligibility, as the frequency of preexisting CD19⁻ disease was considered negligible. Retrospective analysis of CD19 expression by immunohistochemistry did not predict response, likely owing to decreased sensitivity of immunohistochemistry compared with CAR scFv recognition; however, a subset of patients who relapsed did exhibit CD19⁻ disease only at relapse (58). Responders demonstrated higher CAR T cell expansion (AUC$_{0-28d}$ and C$_{max}$) than nonresponders, and although very low levels of axi-cel could be detected in responders at day +180, only three patients with ongoing complete remissions had detectable CAR T cells at 24 months (55). The role of expansion and persistence thus seems to differ from that seen with tisa-cel; this may be related to their different costimulation domains. Regarding

toxicity, 11% of patients experienced grade 3+ CRS (per Lee et al., ref. 16), while 32% experienced grade 3+ neurotoxicity. Two treatment-related deaths from HLH and cardiac arrest were reported (56).

Given these impressive data, two groups independently sought to describe the off-protocol, commercial "real-world" experience of treatment with axi-cel in patients who were eligible based on the prescribing label, but who may or may not have met the stricter eligibility requirements of the registration trial. At the 2018 American Society of Hematology (ASH) Annual Meeting, Nastoupil et al. (59) and Jacobson et al. (60) reported on a collective 269 patients who received axi-cel in October 2017. These groups reported objective response rates of 79% and 71%, respectively, and complete response rates of 50% and 44% respectively, similar to rates reported by the pivotal ZUMA-1 study (61). Interestingly, when Nastoupil et al. and Jacobson et al. examined patient populations based on ZUMA-1 eligibility criteria, only 51% and 40% met ZUMA-1 eligibility, respectively, with most ineligibility linked to performance status, renal dysfunction, and cardiac dysfunction (59, 60). Unlike tisa-cel, axi-cel is prescribed using fixed weight-based dosing of 2×10^6 CAR⁺ T cells/kg, with a maximum dose of 2×10^8 CAR⁺ T cells. Correlative studies from the ZUMA-1 study indicated that the CD4/CD8 ratio of infused product was not predictive of response; however, more recent work (62) demonstrated that preinfusion polyfunctionality of the T cell compartment was associated with improved clinical outcomes, albeit at the expense of increased toxicity. CD19 loss and downregulation of CD19 and other B cell antigens, as well as PD-L1 upregulation, may underlie possible tumor resistance and/or relapse (60, 63, 64).

Tisa-cel for DLBCL. The international, phase II, JULIET study (8) led to the May 2018 approval of tisa-cel for adult patients with relapsed or refractory large B cell lymphoma after two or more lines of systemic therapy, including DLBCL not otherwise specified, high-grade B cell lymphoma, and DLBCL arising from follicular lymphoma. Ninety-three patients infused with tisa-cel after LDC (fludarabine 25 mg/m² i.v. and cyclophosphamide 250 mg/m² i.v. for 3 days) were evaluable for efficacy, with a median time from infusion to data cutoff of 14 months (8). The best objective response rate was 52% (95% CI, 41%–62%), with 40% of all patients achieving a complete response. As in the ZUMA-1 study (61), responses occurred across all subgroups regardless of molecular subtype, prior transplant, and double/triple-hit disease (8). The median duration of response for the 35% of patients who had ongoing responses at the data cutoff, including 34% with ongoing complete responses, had not been reached at 19.3 months of follow-up (65), again indicating the potential curative nature of CAR T cell therapy. Although CD19 positivity was required per proto-

col, retrospective analysis of CD19 expression levels did not indicate that expression level correlated with response. Unlike with axi-cel, similar in vivo expansion (C_{max} and AUC_{0-28d}) was observed in responders and nonresponders and persistent CAR transgene levels were observed for up to 2 years in most patients with durable responses. Like in ELIANA, activity was seen across a wide dose range, leading to the FDA-approved dosage of 0.6×10^8 to 6.0×10^8 CAR+ T cells. Twenty-two percent of patients experienced grade 3+ CRS (according to the Penn Criteria [ref. 45]), while 12% experienced grade 3+ neurotoxicity. No deaths were attributed to CAR T cell treatment, CRS, or neurotoxicity. Unlike the ZUMA-1 study (61), JULIET allowed for bridging therapy between leukapheresis and CAR T cell infusion (8). Thus, a subset of 7 patients achieved a complete response to salvage chemotherapy; this response aligned with response rates predicted by an international, multicohort, retrospective, non-Hodgkin lymphoma (NHL) research study (SCHOLAR-1) (66), and proceeded with tisa-cel infusion. Despite absence of radiographic disease, tisa-cel expanded in all 7 patients, and transgene levels were detectable for more than 2 years, with 5 of 7 patients remaining disease-free at >12 months (8, 67); these data indicate that a large burden of antigen is not necessary for the CAR T cells to expand and persist in patients. Additional data supported tisa-cel's use in isolated, secondary CNS disease, again suggesting that active systemic disease may not be required for tisa-cel expansion and activity (29), demonstrating that CAR T cell therapy may penetrate disease compartments that are considered sanctuaries from standard chemotherapy and antibody therapy.

Liso-cel for DLBCL. Another promising CD19-specific construct in development is lisocabtagene maraleucel (liso-cel), previously known as JCAR017. Like axi-cel and tisa-cel, liso-cel is a second-generation CD19-specific CAR T cell construct using the FMC63-derived scFv. Although it shares a 4-1BB costimulatory domain with tisa-cel, liso-cel's structure uses a modified IgG4 hinge and transmembrane domain rather than CD8 hinge and transmembrane domains. Its predefined manufacturing process of 1:1 CD4+/CD8+ CAR T cells is based on preclinical work suggesting synergistic enhancement of antitumor activity by administration of a defined ratio of CD4+ to CD8+ cells in a xenograft model of Burkitt lymphoma (68). Data presented at ASH's 2019 annual meeting indicate that when liso-cel was administered across three dose levels in 268 patients, the objective response rate was 73%, with a best overall complete response rate of 53%. The median progression-free survival of all patients was 6.8 months, with a median OS of 19.9 months. CRS (graded according to Lee et al., ref. 16) occurred in 42% of patients, and 2% exhibited grade 3+ severe CRS. Neurotoxicity was also detected in about 30% of patients, with 10% having grade 3+ or higher (69).

Moving beyond currently approved indications

Earlier lines of therapy or combination with SCT. There is substantial motivation to move CAR T cell therapy earlier in the patient treatment course, rather than focusing on end-stage disease. Relapsed/refractory lymphoma is currently defined as chemotherapy-refractory disease following two lines of therapy or relapsed following autologous SCT. Data from the phase III ORCHARRD trial (70), the largest second-line trial using rituximab- and anthracy-cline-based first-line regimens, indicated 2-year progression-free survival and OS of 24%–26% and 38%–41%, respectively; only 33%–37% of patients completed autologous SCT, highlighting a substantial need in this patient subset. Studies are under way to investigate axi-cel, tisa-cel, and liso-cel in earlier lines of therapy for aggressive lymphomas, including for first-line DLBCL (ZUMA-12/axi-cel, NCT03761056, ClinicalTrials.gov) and second-line DLBCL versus standard-of-care autologous SCT (ZUMA-7/axi-cel, NCT03761056; Belinda/tisa-cel, NCT03570892; and TRANSFORM/liso-cel, NCT03575351). Additional studies are evaluating the use of CAR T cells in earlier-line therapy after first relapse or MRD+ pediatric and adult leukemia (NCT03628053).

Various groups have attempted to incorporate CAR T cell therapy after autologous SCT; in all instances, CAR T cell infusion followed preconditioning chemotherapy and stem cell infusion. The initial studies used first-generation (CD3ζ only) and second-generation (CD28ζ) constructs introduced into T cells with differing viral and transposon-based transduction methods (71). These studies included patients with various B cell NHL (B-NHL) subtypes with either active disease or undetectable disease by imaging, and reported 1-year progression-free survival of 75% (71, 72). Sauter et al. (73) recently reported a larger series of poor-risk, chemosensitive relapsed and refractory B-NHL patients who underwent CAR T cell therapy after SCT. Of the 15 patients, 67% experienced severe neurotoxicity, with a 2-year progression-free survival of 30%. Interestingly, the proportion of naive-like (CD45RA+CCR7+) CAR T cell populations in the infused product was inversely correlated with progression-free survival (73).

CD19-directed CAR T cell therapy beyond adult high-grade lymphomas. Although current approvals of CAR T cell therapies only encompass pediatric/young adult B-ALL and adult DLBCL, success was originally achieved in indolent lymphomas and adult B-ALL, but these indications have yet to obtain FDA approval (4–6). Axi-cel is currently being studied in multiple disease settings, including follicular and marginal-zone lymphomas (ZUMA-5/NCT03105336), mantle cell lymphoma (MCL) (ZUMA-2/NCT02601313) (74), chronic lymphocytic leukemia (CLL) (ZUMA-8/NCT03624036), and adult (ZUMA-3/NCT02614066) and pediatric/young adult ALL (ZUMA-4/NCT02625480). Recently presented data from ZUMA-2 ($n = 28$), KTE-X19 in MCL, appeared promising with an overall response rate of 86%, complete response rate of 57%, and ongoing responses in 83% of patients at 12 months. Tisa-cel is also under investigation for pediatric NHL in a multicenter phase II protocol (BIANCA, NCT03610724), for follicular lymphoma in a multicenter phase II study (ELARA, NCT03568461), for relapsed/refractory adult B-ALL in a randomized, phase III study comparing CAR T cell therapy with blinatumomab or inotuzumab (Oberon/NCT03628053), and for primary CNS lymphoma in a pilot single-center study (NCT04134117). Although liso-cel has yet to obtain FDA approval, additional studies involving CLL/small lymphocytic leukemia as well as MCL are ongoing (NCT03331198, NCT02631044). Other studies are examining the role of combinatorial therapies to improve on already impressive CAR T response rates. Axi-cel is currently being studied in combination with PD-L1 blockade (ZUMA-6, atezolizumab, NCT02926833), 4-1BB agonistic antibodies (ZUMA-11, utomilumab, NCT03704298), and rituximab and/or lenalido-
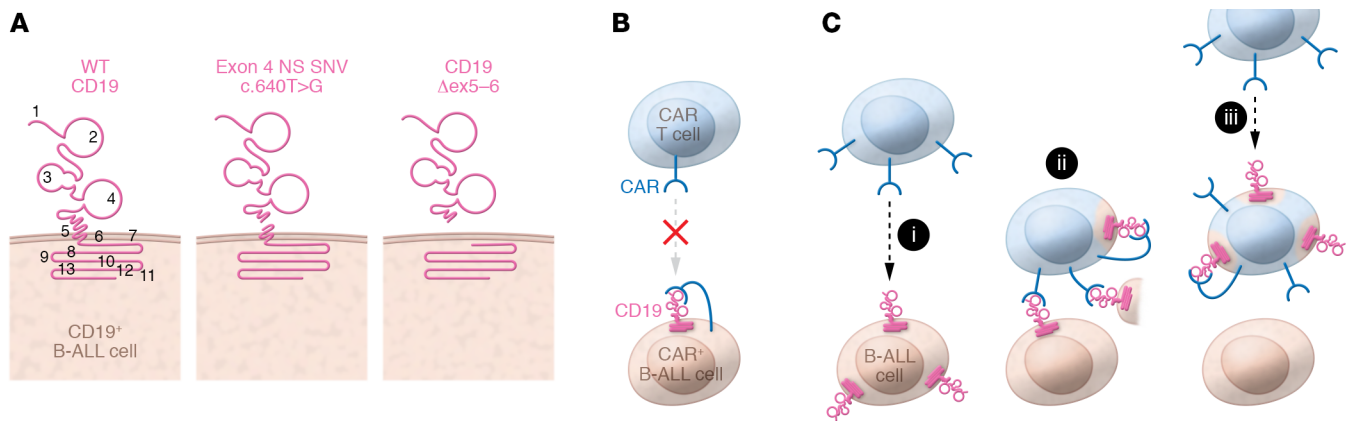
Figure 2. Examples of mechanisms underlying CD19⁻ loss in B cell malignancies. (**A**) Wild-type CD19 and observed frameshift mutations that result in lack of surface expression of the CD19 molecule in B cell leukemia. (**B**) CAR-transduced B cells with self-binding ("masking") of CD19 by the CAR expressed on the cell surface of leukemic B cells. (**C**) Trogocytosis, in which CAR T cells "rip off" the CD19 molecules from the leukemic blasts by membrane transfer, resulting in lowered expression of CD19 by the leukemic blasts. In (i), CAR T encounters B-ALL cell; in (ii), CAR T has trogocytosed bits of membrane-bound CD19 from the B-ALL cell onto the CAR T cell, and some of the CD19 on the CAR T cell is masked; in (iii), the CAR T cell with membrane-bound CD19 may become susceptible to lysis by another CAR T cell.

mide (ZUMA-14, NCT04002401). Early results demonstrated that combining atezolizumab with axi-cel resulted in a more than 20-fold higher CAR T cell expansion without significant increases in CAR T cell–related toxicities (75). Tisa-cel is being evaluated in combination with ibrutinib (NCT03876028) and with the PD-1 inhibitor pembrolizumab (Portia, NCT03630159). Finally, liso-cel is being studied in combination with the PD-L1 inhibitor durvalumab and the novel cereblon-modulating agent CC-122 (PLATFORM, NCT03310619).

Mechanisms of tumor resistance

Despite dramatic treatment responses in a large subset of patients with otherwise refractory disease, upwards of 50% of patients eventually relapse after CD19-directed CAR T cell therapy. Further research is therefore warranted to determine mechanisms of disease resistance and to evaluate potential approaches to improve overall response rates and response durability. The factors underlying disease resistance likely fall into three categories: poor T cell fitness/expansion, loss of the target antigen, and, most recently, tumor mutations that lead to resistance to CAR T cell–mediated killing (76).

Poor T cell fitness or expansion. Porter et al. (45) first described the role of CAR T cell expansion and persistence as correlating with treatment responses. Among patients who achieved complete response, median transgene expansion was more than 73,000 copies/μg (assayed via PCR), while nonresponders displayed minimal expansion, with a median of 420 copies/μg; peak transgene copy number was correlated with response. In the same patient series, CAR T cells persisted long-term, as assessed via flow cytometry and quantitative PCR, and all responding patients had ongoing B cell aplasia, suggesting functional persistence of their CD19-directed CAR (45). Fraietta et al. (77) later established that CAR T cells from patients who achieved complete response were enriched for the expression of memory-related genes, including IL-6/STAT3 signatures, whereas nonresponders upregulated expression programs involved in effector differentiation and exhaustion. These findings were further validated by the report that remission was associated with an elevated frequency of CD8⁺CD27⁺CD45RO⁻ memory-like T cells before CAR T cell manufacturing (77). Prior work had demonstrated that pretreatment with ibrutinib, a BTK/ITK inhibitor, improved expansion of CD19-directed
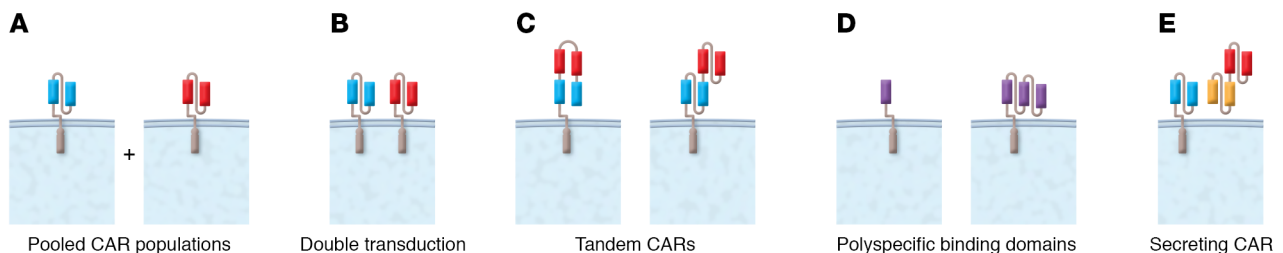

Figure 3. Summary of strategies for targeting multiple antigens with CAR T cells. (**A**) Pooled CAR populations: Two cell populations are transduced with two different CARs, each with a single specificity (red and blue); these two cell products are then pooled or coinfused into the patient. (**B**) Double transduction: One cell population is cotransduced with two CARs, each with a single specificity (red and blue). (**C**) Tandem CARs: Two different binding specificities (red and blue) are molecularly linked in tandem and fused to a single transmembrane/signaling domain. The specificities can be combined in different formats to achieve productive binding of each. (**D**) Polyspecific binders: A cross-reactive CAR (purple) binds with two different specificities. These can be linked together in tandem to form multimeric binding domains. (**E**) Secreting CARs: The CAR has one specificity (blue) and a second binder is secreted from the CAR (red). This can also take the form of a T cell engager (yellow) if the secreted form has a bispecific format.

CAR T cells in association with deceased expression of the immune checkpoint PD-1 (78). Additional work is under way using coculture with PI3K inhibitors during CAR T cell manufacturing to enrich for a memory-like phenotype and improved functional persistence of CAR T cells (79). Taken together, these data suggest that T cell fitness drives CAR T cell function, and that T cell fitness is amenable to improvement with small-molecule drugs.

Given that patients with hematologic malignancy may not recover T cell fitness, because of either disease or prior therapies, CAR T cell approaches using allogeneic CAR T cells are under development. With allogeneic T cell products, the endogenous T cell receptor needs to be removed to prevent graft-versus-host disease in the recipient. To this end, gene-editing approaches using TALENs or CRISPR/Cas must be used to knock out the T cell receptor, which can be done in one or two steps with gene transfer of the CAR transgene. Early clinical investigations of the use of allogeneic CAR T cell therapy for relapsed/refractory B-ALL (UCART19) have reported overall complete response rates of 88%, with 86% of responses having no MRD detectable by flow cytometry or quantitative PCR. However, although initial responses are promising, subsequent host-mediated rejection of these allogeneic products may limit efficacy or have other unforeseen consequences (80).

Loss of the target antigen. To date, multiple mechanisms of antigen loss have been reported in patients who relapse after CAR T cell therapy, underscoring the strong selection pressure exerted by this therapy. Frameshift mutations leading to CD19 transmembrane domain loss were detected in a subset of previously CD19+ patients with CD19− disease relapse after CAR T cell therapy (Figure 2 and ref. 81). In addition to harboring genomic alterations, a series of CD19− B-ALL relapses resulted from splice variants, with loss of the exon encoding the epitope targeted by FMC63 (50) or loss of the anchoring transmembrane domain (Figure 2A). In immature B cell malignancies, Gardner et al. reported a conversion of acute lymphoblastic to acute myeloid leukemia in a patient with mixed-lineage leukemia; this genetically identical — but phenotypically myeloid — relapse was impervious to CD19 targeting (82). "Loss" of CD19 antigen expression has also been observed in the context of antigen masking due to CAR transduction into leukemic blasts (51) (Figure 2B). CAR-transduced blasts effectively mask the target epitope from external CAR T cell killing through self-binding of CD19 on the cell surface (51). Although retrospective analysis identified several patients with CAR-transduced B cells in their preinfusion products, only one had CD19− relapse of disease, indicating that this event is quite rare. Finally, in in vitro and animal models, CARs have been shown to induce reversible antigen loss through trogocytosis, in which the target antigen is transferred to CAR T cells during establishment of an immune synapse (Figure 2C). This transfer of target antigen led to a decrease in target density on tumor cells as well as an increase in fratricide and subsequent T cell exhaustion (83); this mechanism of relapse has not been definitively observed in patients.

Analogous to historical data with antimicrobial therapy, where serial targeting of a single pathway can lead to resistance, approaches to target multiple surface antigens simultaneously are under way with the hypothesis that these are more likely to avoid antigen−

relapse (Figure 3). Although dual targeting with two separately transduced CAR T cell populations is possible (Figure 3A), transduction of a single cell population with pooled vectors (Figure 3B) or a bicistronic vector expressing dual-antigen specificity (Figure 3C) had higher efficacy in animal models of antigen− relapse (84, 85). Based on early data from a phase I study of a bispecific CD19/CD20 second-generation CAR construct, the objective response rate was 91%, with an 82% complete response rate at the recommended phase II dose and no relapses to date, although follow-up is limited (86). Other approaches using multiple scFvs attached to a single intracellular signaling domain allow for a reduced transgene size while providing multi-antigen specificity; for example, such tandem CARs targeting CD19/CD22, CD19/CD37, and CD19/CD79b are currently under investigation (87–89). Although scFv has been the primary binding moiety employed in CAR T cell constructs to date, alternative binders such as camelids, adaptor proteins, and modified ligands/receptors are also under clinical development. Naturally occurring binders may achieve the same dual-antigen targeting without extensive modification to scFv orientations and with reduced immunogenicity (Figure 3D). This approach was validated in a recent study of IL13Ra2 for glioblastoma, and similar approaches include using a proliferation-inducing ligand (APRIL) as well as a trimeric APRIL (TriPRIL) variant engineered to improve binding to its natural receptors, BCMA and TACI (90–92). Finally, approaches using CAR T cells as a platform to recruit a broader immune infiltrate are promising (93), as T cells can be engineered to secrete immune checkpoint–blocking scFvs or bispecific T cell engagers (Figure 3E) to allow local immune targeting within the tumor microenvironment of an otherwise broadly-expressed antigen without systemic toxicity (94).

Concluding remarks

The field of CAR T cell therapy is growing rapidly. We undertook this Review with the thought of covering all of CAR T cell therapy for hematologic malignancies, but then found that there were enormous progress and data to cover even within the limited scope of B cell malignancies: from variations in CAR molecular design; to clinical observations describing efficacy with multiple CD19-directed CAR T cell products, their unpredicted but relatively similar toxicities, and how systems had to be devised to measure and quantify and report them; to correlations and mechanisms discovered in the clinic and then modeled in vitro and in animals to discover and test pathways and druggable targets. Not only is CAR T cell therapy transformative in its potential to treat cancer with a single infusion, but its complex, cellular nature and its role in directing other cell-cell interactions, whether between CAR T cell and tumor cell or endothelial cell or macrophage, lend themselves to deep scientific probing and manipulation of its functions.

Acknowledgments

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