Review

Molecular Cancer Therapeutics

What CAR Will Win the CD19 Race?

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Abstract

Adoptive transfer of T cells engineered with synthetic receptors is emerging as a new pillar in the treatment of cancer. The adoptive cell therapy furthest along in clinical development is the engineering of T cells to express chimeric antigen receptors (CAR) against the CD19 antigen. Several platforms have shown remarkable activity in patients with relapsed or refractory B-cell malignancies. In 2017, the FDA approved the first CAR T cell products tisagenlecleucel (Kymriah, Novartis) and axicabtagene ciloleucel (Yescarta, Gilead), and others are expected to follow shortly. Despite their activity, CAR T cell approaches have limitations that will need to be addressed, including excessive toxicity, relapses mediated via antigen escape, difficulties overcoming the suppressive tumor microenvironment, high manufacturing costs and retail prices, and patient access, among others. The CAR T cell product that better addresses those challenges will obtain a critical competitive advantage.

Introduction

Chimeric antigen receptors (CAR) are synthetic fusion proteins that contain extracellular antigen recognition motifs and intracellular signaling domains (1). Manufacturing CAR T cells involves the collection of autologous peripheral blood mononuclear cells, isolation of T cells, transduction of a transgene encoding for the CAR, and expansion to reach a critical number of T cells, and formulation of the clinical-grade therapeutic product (2). CD19-targeted CAR T cell approaches have proven remarkably efficacious for the treatment of patients with relapsed and/or refractory B-cell malignancies. Most clinical programs utilize either gammaretroviral or lentiviral transduction platforms to genetically engineer T cells to express CAR constructs encompassing a binding moiety, typically a single-chain variable fragment (scFv) derived from either the FMC63 or the SJ25C1 murine monoclonal antibodies, a spacer or hinge, a transmembrane domain, and either the 4-1BB or the CD28 costimulatory molecules, and the ζ-chain of the CD3 complex (Fig. 1; ref. 1). The election of costimulatory domain remains the most important structural difference between currently available second-generation CD19-directed CAR T cells. CD28 is a member of the immunoglobulin superfamily of costimulatory and inhibitory receptors and 4-1BB is a member of the TNF receptor superfamily and both induce similar levels of tyrosine phosphorylation, enhance TCR signaling that increases cytokine production, proliferation, and cytolytic activity (1). Importantly, 4-1BB costimulatory domains appear to ameliorate T-cell exhaustion and promote long-term persistence compared with CD28 costimulatory domains (3).

This article discusses the three drug maker platforms further along in clinical development. Tisagenlecleucel was initially developed at the University of Pennsylvania and subsequently licensed to Novartis in 2012 for development and commercialization. Similarly, axi-cel was initially developed at the Surgery Branch in NCI's Center for Cancer Research and later licensed to Kite Pharma. Finally, Juno Therapeutics CD19 programs derive from their relationship with Memorial Sloan Kettering Cancer Center (MSKCC) and the Fred Hutchinson Cancer Research Center (FHCRC) and Seattle Children's Hospital. Overall response rates (ORR) for patients with relapsed/refractory non-Hodgkin lymphoma (NHL) or acute lymphoblastic leukemia (ALL) receiving CD19-directed CAR T cell therapy have ranged between 50% and 90%. However, the clinical development of these therapeutics remains challenging due to morbidity and mortality risks mainly due to cytokine release syndrome (CRS) and neurotoxicity, also known as CAR T cell–related encephalopathy syndrome (CRES; ref. 4). Nevertheless, on August 30, 2017, the FDA made tisagenlecleucel (CTL019, Kymriah, Novartis) the first gene therapy available in the United States. Tisagenlecleucel, a CD19-directed CAR T cell therapy, is approved for the treatment of patients up to 25 years of age with B-cell precursor ALL that is refractory or in second or later relapse (5). Subsequently, axicabtagene ciloleucel (KTE-C19, axi-cel, Yescarta, Gilead) was approved for the treatment of adult patients with relapsed or refractory large B-cell lymphoma after two or more lines of systemic therapy (October 17, 2017; ref. 6) and tisagenlecleucel for the treatment of patients with relapsed/refractory diffuse large B-cell lymphoma (DLBCL) ineligible for or relapsing after autologous stem cell transplantation (SCT; May 2, 2018; ref. 7). In addition to the regulatory validation, the CAR T business model has been validated by the acquisition of Kite Pharma by Gilead for $11.9B and shortly thereafter by that of Juno Therapeutics by Celgene for $9B. Despite the uncertainties surrounding these novel therapeutics, the investments made by Gilead, Celgene, and Novartis have positioned all three CAR T cell pioneers in a race to dominate the B-cell cancer therapy field. But, which one is most likely to emerge victorious?

Efficacy

The efficacy of several CD19-directed CAR T cell therapy platforms is being tested in NHL, ALL, chronic lymphocytic leukemia (CLL) and other CD19-positive B-cell malignancies. All data to

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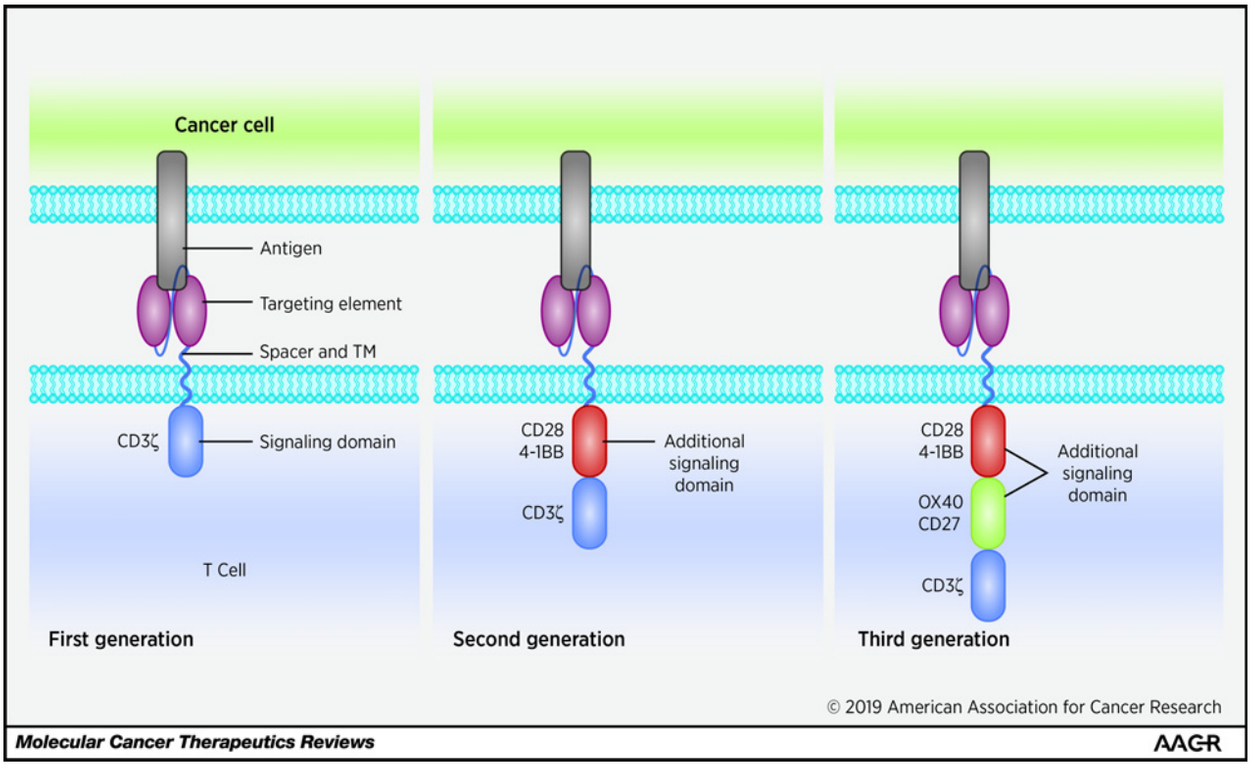

Figure 1.
Structure of CARs. First-generation CARs included a tumor antigen binder, typically an scFv tethered to the ζ-chain of the CD3 complex via a linker and a transmembrane (TM) domain. Second-generation constructs result from adding a costimulatory motif such as CD28 (axi-cel) or 4-1BB (tisagenlecleucel) to a first-generation CAR scaffold. Third-generation CARs include more than one costimulatory domain.

date have been generated in single-arm uncontrolled studies mainly involving patients with ALL or DLBCL.

ALL

CAR T cell therapy in ALL has consistently rendered high response rates (Table 1). The Children's Hospital of Philadelphia reported a complete response (CR) rate of 90% in 30 patients (25 pediatric and 5 adults) treated with tisagenlecleucel, including 15 who had previously failed SCT (8). Of note, 24% of patients evaluated did not receive tisagenlecleucel due to failure of their T cells to meet predefined criteria for *in vitro* proliferation (9). Durable remissions up to 24 months correlated with persistent CAR T cells. The 6-month probability of CAR T cell persistence and overall survival (OS) were similar at 68% and 78%, respectively. CRS was universal, being severe in 27% of patients, frequently linked to high tumor burden (8). A follow-up analysis of this study reported a CR rate of 93%, with several CRs sustained over 3 years after infusion. However, 21 (36%) patients relapsed, of whom 14 had CD19-negative disease (10). These results provided the rationale for the pivotal ELIANA clinical trial, in which 75 pediatric patients (median age, 11 years) received tisagenlecleucel at 25 centers in North America, Europe, Japan, and Australia (11). Patients had failed a median of 3 prior therapies (range, 1–8). The ORR within 3 months was 81% (61 responders). CR was achieved by 60% of patients and 21% achieved CR with incomplete blood count recovery (CRi). Flow cytometry failed to detect minimal residual disease (MRD) in 95% of responders by day 28. Median response duration was not reached, and the relapse-free survival (RFS) and event-free survival (EFS) at 12 months were 59% and 50%, respectively. Most relapses were CD19 negative. With a median follow-up of 13.1 months, the OS at 12 months was 76% and the median duration of tisagenlecleucel persistence was 168 days (range, 20–617 days; ref. 11).

The National Cancer Institute treated 45 children and young adults with axi-cel (containing a CD28 costimulatory domain), reporting a CR rate of 60% (12). At the time of the analysis, all patients remained alive, and 88.9% remained disease-free (range, 5–28 months). However, the vast majority of responding patients in CR underwent allogeneic SCT, likely due to the limited persistence of the CAR T cells, which did not exceed 68 days in any patient (13).

The MSKCC group has reported on 53 adult patients with relapsed/refractory ALL who received the CD28-containing JCAR015 CAR in a phase I trial (14). The CR rate was 83%, and the MRD-negative CR rate was 67%. The median EFS and OS were 6.1 and 12.9 months, respectively, both of them being longer among patients with MRD-negative CR. In fact, all 9 patients with an MRD-positive CR relapsed with CD19-positive blasts, but only 16 relapses were reported among the 32 patients who achieved MRD-negative CR, 4 of them being CD19 negative. Of the 44 patients who achieved CR, 26 (59%) did not receive any further therapy, of whom 17 relapsed and died. Of the 18 patients who received subsequent therapy post JCAR015 therapy, 17 received an allogeneic SCT (after a median time of 74 days after CAR T cell

Table 1. Selected CAR T cell clinical trials in relapsed/refractory ALL

Group/study	CAR T cell	Population	CR rate (%)	Median RFS (months)	Median OS (months)	CRS	CRES
UPenn/CHOP	Tisagenlecleucel	Pediatric ($n = 25$) and adults ($n = 5$)	90	NR	NR	100% (27% severe)	17% severe
ELIANA (global, multicenter)	Tisagenlecleucel	Pediatric ($n = 75$)	81	NR (59% at 12 months)	NR (76% at 12 months)	77% (47% severe)	40% (13% severe)
MSKCC	JCAR015	Adults ($n = 53$)	83	NR (median EFS 6.1)	12.9	85% (26% severe)	43% (42% severe)
ROCKET	JCAR015	Adults ($n = 38$)	59	4.4	8.1	22% severe	56% severe
NCI	axi-cel	Pediatric and young adults ($n = 21$)	67	NR	NR	76% (28% severe)	43% (5% severe)
FHCRC	liso-cel	Adults ($n = 30$)	100	NR	NR	83% (23% severe)	50% (50% severe)

Abbreviations: CR, complete response; RFS, relapse-free survival; CRS, cytokine release syndrome; CRES, CAR T cell–related encephalopathy syndrome; NR, not reported.

infusion), of whom 6 relapsed, 6 died, and 5 are still alive in CR. Patients with a low baseline disease burden (<5% bone marrow blasts) had improved outcomes, with a median EFS of 10.6 months and a median OS of 20.1 months, compared with those with higher baseline disease burden. The median persistence of JCAR015 was short at 14 days (range, 7–138), which likely guided the decision to proceed to allogeneic STC in one third of responders (14). The ROCKET trial explored the safety and efficacy of JCAR015 in adult patients with relapsed/refractory $CD19^+$ B-ALL (15). Lymphodepletion consisted of either fludarabine 25 mg/m^2 × 3 days and cyclophosphamide 30–60 mg/kg × 1 day or cyclophosphamide 1–3 g/m^2. Median age was 39 years (range, 19–69 years), and the median number of prior therapies was 2 (range, 1–7), including 37% of patients who had undergone prior SCT and 50% who had received blinatumomab. Thirty-two patients were evaluable for response, but only 8 of them received fludarabine and cyclophosphamide as lymphodepletion. The ORR was 52%, and the median OS and RFS were 7.3 and 4.4 months, respectively. Nine (64%) of 14 responders were alive at last follow-up (15).

Thirty adult patients with relapsed/refractory ALL were treated at FHCRC with lisocabtagene maraleucel (liso-cel, JCAR017, Juno Therapeutics, Celgene), given at a defined 1:1 CD4:CD8 ratio. An MRD-negative CR was achieved by 93% of patients. The addition of fludarabine to the lymphodepleting regimen improved liso-cel persistence and RFS (16).

NHL

CD19-directed CAR T cell therapy has shown activity in heavily pretreated patients with CD19-positive NHL (Table 2). Fifteen patients with advanced B-cell malignancies (9 DLBCL, 2 indolent NHL, and 4 CLL) were treated at the National Cancer Institute (NCI), with a single dose of axi-cel after cyclophosphamide and fludarabine (17). The ORR was 80% (DLBCL 67%, indolent NHL 100%, CLL 100%). Three of four CRs in DLBCL were ongoing, and their duration ranged from 9 to 22 months. CAR T cells peaked within 15 days after infusion and remained detectable for up to 75 days (17). In the phase II ZUMA-1 trial, 111 patients with refractory DLBCL, primary mediastinal B-cell lymphoma (PMBCL) or transformed follicular lymphoma received axi-cel (2×10^6 cells/kg) following cyclophosphamide and fludarabine (18). The leukopheresis product was shipped fresh and T cells underwent a 6-day serum-free manufacturing process involving retroviral transduction in a closed system without the use of CD3/CD28 beads (19). The median age was 58 years (range, 23–76) and 69% of patients had received at least three prior therapies, including 21% of patients who had progressed after autologous SCT. The best ORR was 82% and the best CR was 58%. Persisting axi-cel was detected in 71% of patients remaining in response at 1 year. Durable responses were observed in patients with and without detectable persisting axi-cel (20). Responses were consistent across key clinical factors (e.g., cell-of-origin subtype, international prognostic index, use of tocilizumab) and across key biological covariates (e.g., CD4:CD8 ratio, CD19 expression). After a median follow-up of 15.4 months, 42% of patients had a sustained response (40% in CR), and the OS rate at 18 months was 52% (18).

Twenty-eight adult patients with heavily pretreated, relapsed, or refractory NHL (14 DLBCL and 14 follicular lymphoma) received tisagenlecleucel at the University of Pennsylvania (21). The ORR was 64% with a CR rate of 43% in DLBCL. Importantly,

Table 2. Multicenter CAR T cell clinical trials in relapsed/refractory aggressive NHL

Multicenter trial	SCHOLAR-1	ZUMA-1	JULIET	TRANSCEND NHL-001	
Agent	Standard CIT	Axi-cel	Tisagenlecleucel	Liso-cel	
CAR T cell dose	NA	2×10^6/kg	Median 3.1×10^8 (range, 0.1–6)	DL1: 5×10^7 DL2: 1×10^8	
Lymphodepletion	NA	Flu 30 mg/m^2 and Cy 500 mg/m^2 on days −5, −4, and −3	Flu 25 mg/m^2 and Cy 250 mg/m^2/day for 3 days or Bendamustine 90 mg/m^2/day for 2 days	Flu 30 mg/m^2 and Cy 300 mg/m^2 for 3 days	
Median follow-up (months)	NA	15.4	5.6	6	
No. of patients	636	108	81	65[a]	88[a]
Median age		58	56	61	
Indication	Refractory DLBCL	Refractory DLBCL, FL, PBMCL	r/r DLBCL	r/r DLBCL NOS or TFL (Core group)	r/r DLBCL NOS, TFL, PMBCL (full group)
Efficacy					
Best ORR (%)	26	82	53	80	74
Best CR (%)	7	58	40	55	52
3-mo ORR (%)	NR	39	38	65	53
3-mo CR (%)	NR	33	32	54	44
6-mo ORR (%)	NR	NR	37	47	35
6-mo CR (%)	NR	NR	30	42	31
Ongoing CR (%)	NR	40	30	42	
Median DOR (months)	NR	11.1	NR	9.2	5
Median OS (months)	6.3	NR	NR	NR	NR
Safety					
All grades CRS	NA	93	58	36	35
Grade 3+ CRS (%)	NA	13	23	1	1
All grades CRES	NA	64	21	21	19
Grade 3+ CRES (%)	NA	28	12	15	12

Abbreviations: Flu, fludarabine; Cy, cyclophosphamide; CIT, chemoimmunotherapy; No., number; DLBCL, diffuse large B-cell lymphoma; TFL, transformed follicular lymphoma, NOS, not otherwise specified; PMBCL, primary mediastinal B-cell lymphoma; ORR, overall response rate; CR, complete response; DOR, duration of response; CRS, cytokine release syndrome; CRES, CAR T cell–related encephalopathy syndrome; NR, not reported; NA, not applicable.

[a]Evaluable patients.

deep responses were more frequently observed among patients with follicular lymphoma with an CR rate of 71%. At a median follow-up of 29.3 months, all patients in CR by 6 months regardless of their NHL histology remained in CR. Among 16 patients who had a CR, 14 had detectable tisagenlecleucel DNA 6 to 24 months after infusion. At a median follow-up of 28.6 months, 86% of patients with DLBCL responders and 89% of follicular lymphoma responders sustained their responses (21). In the pivotal JULIET study, tisagenlecleucel was given to adult patients with relapsed/refractory DLBCL who had failed at least two prior lines of therapy and were not eligible for autologous SCT at 27 sites in 10 countries (22). Ninety-nine of the 147 enrolled patients were infused. Median patient age was 56 years, and the median number of prior therapies was 3 (47% of patients had failed autologous SCT). Lymphodepletion was administered to 93% of patients (73% fludarabine plus cyclophosphamide and 19% bendamustine). The median tisagenlecleucel dose was 3.1×10^8 (range, $0.1–6 \times 10^8$). The primary analysis focused on the 81 patients who received tisagenlecleucel manufactured at the U.S. site and were followed for at least 3 months or discontinued therapy earlier. Their ORR was 53.1% (CR, 39.5%). The CR and PR rates were 32% and 6% at 3 months and 30% and 7% at 6 months. Importantly, 95% of patients achieving a CR at 3 months remained in CR at 6 months. Tisagenlecleucel was detectable in responders for up to 367 days. Neither the median duration of response nor the median OS were reached. The 6-month RFS and OS were 73.5% and 64.5%, respectively.

Liso-cel was tested in patients with relapsed/refractory NHL in the TRANSCEND NHL-001 trial (23). The study included two different data sets. The DLBLC CORE ($n = 67$) data set included high-grade B-cell lymphoma (double/triple hit), DLBCL NOS (not otherwise specified) *de novo* or transformed from follicular lymphoma, all of them with an ECOG 0–1 and no prior allogeneic SCT. These patients met inclusion into a planned pivotal cohort. The DLBCL FULL data set ($n = 91$) included the CORE data set as well as patients with other NHL subtypes such as transformed CLL, marginal zone lymphoma, PMBCL, and grade 3b follicular lymphoma. Liso-cell was given as a single dose at one of two dose levels (DL1, 5×10^7 cells; DL2, 1×10^8 cells; ref. 23). In the FULL data set, the best ORR was 74%, and the best CR rate was 52%. The 3-month and 6-month CR rates were 44% and 31%, respectively. In the CORE data set, the best ORR and CR rates were 80% and 55%, but when only patients treated at DL2 (selected for the ongoing pivotal trial) were considered ($n = 27$), those rates were 81% and 63%, respectively. The 3-month and 6-month CR rates in the latter group were 68% and 50%. Across doses, 80% of patients in the CORE data set with CR at 3 months remained in CR at 6 months, and 92% of responders at month 6 continued in response as of the data cutoff date.

Safety

CAR T-cell therapies directed against the CD19 antigen have been associated with distinct adverse events, particularly CRS, CRES, and B-cell aplasia. The severity and frequency of these toxicities varies depending on the tumor type and on the CAR T cell platform.

CRS manifests when large numbers of CAR T cells become activated, proliferate, and release large amounts of inflammatory cytokines such as IFNγ, IL6, and TNFα. Clinically, CRS is characterized by fever, capillary leak, coagulated, and in severe cases end-organ failure (24). CRES usually develops after the onset of CRS and sometimes after CRS has resolved. Although not completely understood, CRES is associated with high systemic cytokine levels, endothelian activation, blood–CSF barrier disruption, and myeloid cell accumulation or activation. The clinical manifestations of CRES vary and may include seizures, tremors, delirium, expressive aphasia, myoclonus, and/or obtundation (25). Most cases of CRS or CRES are reversible and most cases of CRS quickly reverse after administration of tocilizumab, which is an anti–IL-6R monoclonal antibody approved by the FDA for the management of this complication. It is important to note that different trials have used different grading and management guidelines (e.g., different tocilizumab dosing) to manage CRS and CRES (26) and that the incidence and severity of such CAR-related complications are influenced by multiple factors such as indication, CAR T cell dosing, tumor, and/or antigen burden.

ALL

In the multicenter pediatric ELIANA trial, 24% of patients received tisagenlecleucel in an outpatient setting but CRS was reported in 77% of patients, requiring ICU admission for a median of 7 days in 47% of them (11). Thirty (40%) patients experienced CRES being grade 3 in 10 (13%). Four grade 3 CRES events were unresolved in 3 patients at the time of discontinuation. Severe neutropenia unresolved by day 28 was reported in 40 (53%) patients and 18 of them had grade 3/4 infections. All responders experienced B-cell aplasia and most received immunoglobulin replacement (11). Tisagenlecleucel-induced tocilizumab-refractory CRS was involved in the death of 3 patients with adult ALL, although all deaths occurred in the context of concomitant systemic infections (27).

In the MSKCC phase I trial in adult patients with relapsed/refractory ALL, CRS occurred in 85% of patients receiving JCAR015, a CD28-containing CAR, being severe in 14 (26%) of 53 patients, which caused the death of 1 of them (14). Tocilizumab was administered to 19 patients. Twenty-two (39%) patients experienced severe CRES. Patients with ≥5% bone marrow blasts were associated with a higher risk of severe CRS (41% vs. 5%) and CRES (59% vs. 14%) compared with those with low preinfusion disease burden. Peak CAR T cell expansion was significantly higher in patients with severe neurotoxic effects (14). Five fatal CRES events occurred in the adult ALL ROCKET trial investigating the activity of JCAR015 (28). Severe CRS or severe CRES occurred in 22% and 56% of patients, respectively (15). The study was placed on clinical hold by the FDA after 3 patients died. The hold was lifted after fludarabine was removed from the lymphodepleting regimen but 2 more patients died after experiencing cerebral edema, and Juno Therapeutics elected to halt the development of JCAR015. All fatalities were characterized by rapid (within 7 days) JCAR015 expansion with high levels of $CD8^+$ cells and IL2 and TNFα. CAR-induced T-cell activation results in endothelial cell activation in the central nervous system, which promotes capillary leak, disseminated intravascular coagulation, and breakdown of the blood–brain barrier (29), which was observed in post-mortem examination (28). Factors associated with an increased risk of neurotoxicity include ALL diagnosis, high $CD19^+$ bone marrow cells, high CAR T cell dose, CRS, and preexisting neurologic comorbidities (29). Patients with evidence of endothelial activation prior to the administration of lymphodepletion may be at an increased

CRES risk. Liso-cel therapy in adult ALL has also been associated with significant toxicity as 23% and 50% of patients experienced severe CRS or CRES (16).

NHL

In the University of Pennsylvania trial of tisagenlecleucel in patients with aggressive NHL, severe CRS or CRES occurred in 18% and 11% patients, respectively. One patient with follicular lymphoma died due to encephalitis several months after infusion (30). Notably, sustained reappearance of B-cells was observed in 8 of 16 patients who achieved a CR, coupled with an improvement in immunoglobulin levels in a significant number of patients starting 6 months after tisagenlecleucel infusion (21). In the JULIET study (22), CRS occurred in 58% of patients, which was grade 3/4 in 23% of patients according to the University of Pennsylvania CRS grading scale. Tocilizumab was administered to 15% of patients for CRS management, and glucocorticoids were received by 11% of patients. Grade 3/4 CRES was reported in 12% of patients, whereas cytopenias lasting longer than 28 days or infections occurred in 27% and 20% of patients, respectively. Three patients died within 30 days after infusion due to disease progression (22).

Axi-cel therapy was associated with frequent grade $\geq$3 toxicities including fever (80%), hypotension (27%), and CRES (40%) in the NIH trial (17), whereas in the ZUMA-1 trial, the most common grade $\geq$3 adverse events were neutropenia (78%), anemia (43%), and thrombocytopenia (38%). CRS was reported in 93% of patients, being grade $\geq$3 in 13%. Neurotoxicity occurred in 64% of patients, and it was grade $\geq$3 in 28% of them (21% encephalopathy; ref. 18). Peak expansion and area under the curve were associated with grade $\geq$3 CRES but not with CRS. Interleukin-6, -10, -15, and -2Rα and granzyme B were significantly associated with grade $\geq$3 CRS and neurotoxicity. Tocilizumab was administered to 43% of patients and glucocorticoids to 27% of patients for the management of CRS and/or CRES, and its administration did not affect efficacy. Forty-four percent of patients died due to disease progression ($n = 37$), adverse events ($n = 3$, 2 due to CRS and 1 due to pulmonary embolism), or other causes post progression ($n = 4$; ref. 18). The CD4:CD8 ratio of the axi-cel product did not affect efficacy or safety. By contrast, the TRANSCEND NHL-001 study provided liso-cel at a fixed 1:1 CD4:CD8 ratio in an attempt to gain more control over the expansion of the product post infusion and to minimize toxicity (31). The most frequent severe adverse events were cytopenias. In the FULL data set, CRS occurred in 35% of patients but was grade 3/4 in only 1% of patients, whereas grade 3/4 CRES occurred in 19% of patients (grade 3/4 in 12%). The odds ratio of these complications was 8-fold higher in patients with higher baseline tumor burden, LDH levels, and levels of inflammatory markers (32). In the CORE data set, the grade 3/4 CRS and neurotoxicity were 1% and 15%, respectively, but only 0% and 7% among those treated at DL2, and no deaths were due to any of these toxicities. Based on this encouraging safety profile, outpatient administration of liso-cel is currently under investigation (33).

Limitations and Potential for Improvement of Current CD19-Targeted CARs

The limitations of CAR T cell therapy vary according to a number of factors, including tumor type, patient population, lymphodepleting regimen, and CAR T cell design.

A variety of lymphodepleting regimens containing cyclophosphamide, either alone or in combination with other chemotherapeutic agents, have been used to facilitate the engrafting of the adoptive engineered T-cell therapies. The role and optimal agents of lymphodepleting regimens are still unknown, but growing evidence indicates that intensification of cyclophosphamide-based lymphodepleting regimens with the addition of fludarabine results in enhanced CAR T cell expansion and persistence. Clinically, the latter translates into an increased but still manageable toxicity profile and, more importantly, into improved response rates and survival in both ALL and NHL (16, 31). The underlying mechanisms by which fludarabine promotes such beneficial effects may include an increased availability of homeostatic cytokines through the suppression of regulatory T cells, favorably altering the tumor microenvironment, and/or reducing anti-CAR immunogenicity (16, 31).

Differences in costimulatory molecules appear to determine CAR T cell functionality, with 4-1BB generally preferentially inducing central memory cells and CD28 preferentially inducing effector memory differentiation (1). These differences affect persistence (favored by 4-1BB) and expansion (favored by CD28; ref. 1), which in turn may affect clinical outcomes. Generally speaking, the challenge in ALL has been the prevention of relapse and excessive toxicity, whereas in aggressive NHL, it has been the achievement of CR. In the ELIANA study in pediatric ALL, the ORR was 81%, but the RFS at 12 months was 59%, with no evidence of relapse among responders after 9 months after infusion (11). Of note, the vast majority of relapses were CD19 negative, indicative of antigen escape to tisagenlecleucel. Similarly, the ORR in the MSKCC trial in adult ALL was 83%, but relapses were universal among patients who failed to achieve an MRD-negative CR, and they occurred in 50% of patients who achieved an MRD-negative CR. Notably, this CD28-based CAR was associated with a CD19-negative relapse rate of only 16%. EFS curves plateaued after 20 months after infusion, at which point the EFS rate was below 20% (14). Although 4-1BB CAR T cells appear to be associated with longer persistence and improved EFS compared with CD28 CAR T cells, marked differences in eligibility criteria (e.g., patient age) and lack of randomized studies preclude a balanced comparison. However, available data appear to suggest that clinical efficacy and safety can be markedly improved by enrolling younger patients with lower tumor burden. Further research is warranted to prove that CAR T cell therapy can be safely administered to adult patients with relapsed/refractory ALL.

In aggressive NHL, the ORR reported with tisagenlecleucel, axi-cel, and liso-cel has ranged from 50% to 80% and durable CR rates between 30% and 42%. Clearly, this level of efficacy markedly improves upon that observed with currently available options for relapsed/refractory DLBCL (34). This has been recently supported by the retrospective SCHOLAR-1 study, which reported an ORR of 26% and a CR rate of 7% with standard therapies among patients with refractory aggressive NHL (34). However, the use of different costimulatory molecules (CD28 vs. 4-1BB), differences in patient populations (relapsed/refractory vs. only refractory), the allowance (or not) of preinfusion bridging therapy, the use of a fixed CD4:CD8 ratio in TRANSCEND NHL-001, and differences in follow-up duration make difficult a comparison across studies evaluating the efficacy of CAR T cell therapies in aggressive DLBCL. Those limitations notwithstanding, tisagenlecleucel, axi-cel, and liso-cel appear to induce similar response rates. CD19-negative relapse post CAR T cell therapy is a serious problem, particularly

among patients with ALL. CAR T cells targeting other B-cell antigens such as CD22 are being developed and appear to be efficacious in patients relapsing after CD19-directed CAR T cell therapy (35). CAR T cell therapies engineered to simultaneously target more than one B-cell antigen (e.g., CD19 and CD22; ref. 36) are currently being tested in clinical trials in an attempt to prevent CD19 antigen escape.

Regarding safety, multivariable analysis of baseline characteristics of 133 patients with B-cell malignancies, including aggressive NHL, treated with liso-cel identified high marrow tumor burden, lymphodepletion using cyclophosphamide and fludarabine, higher CAR T cell dose, thrombocytopenia before lymphodepletion, and manufacturing of CAR T cells without $CD8^+$ central memory T-cell selection as independent CRS predictors (37). Examination of these baseline variables as well as baseline levels of markers of inflammation (e.g., macrophage-derived cytokines such as IL6 and IL10; ref. 38) should be investigated prospectively as predictors of CRS and CRES severity and to guide preemptive anticytokine therapy. Severe CRS and/or CRES remain associated with a high risk of morbidity and, in some cases, mortality, such as that reported in adult ALL trials either with JCAR015 (ROCKET trial; ref. 15) or with tisagenlecleucel (27). For that reason, the administration of CAR T cell therapies must be restricted to centers with experience in the management of such adverse events. CAR T cell therapies targeting antigens expressed outside of the B-cell lineage will require careful development to prevent on-target/off-tumor toxicity (39).

Given that the administration of tocilizumab does not appear to limit the cytotoxic potential of CAR T cells (18), earlier institution of tocilizumab therapy merits investigation. In a safety expansion cohort of the ZUMA-1 study, patients received prophylactic treatment with 750 mg of levetiracetam twice a day on day 0 and 8 mg/kg of tocilizumab on day 2 after axi-cel infusion (40). Early tocilizumab use appeared to reduce the risk of CRS but not CRES, which is in keeping with the marginal effect observed with this agent in the management of CRES. Higher tumor burden and higher baseline markers of inflammation and/or endothelial activation consistently increase the risk of severe CRS, which highlights the criticality of stringent patient selection and appropriate tumor debulking prior to CAR T cell infusion (32, 41). The lack of randomized trials, differences in patient populations, and the use of different toxicity grading systems prevent discerning safety differences across platforms. However, tisagenlecleucel appears to be associated with a higher risk of severe CRS but lower risk of severe CRES than axi-cel, with the latter requiring in-hospital administration and admission for at least 7 days (6). Both agents are available only under Risk Evaluation and Mitigation Strategy programs. Liso-cel has been associated with a risk of severe CRS as low as 1% but similar CR rates as tisagenlecleucel or axi-cel. That favorable toxicity profile may provide a competitive advantage in aggressive NHL provided such preliminary results are confirmed in ongoing pivotal trials.

Remaining Challenges

Although CAR T cell therapies are clearly poised to become staples in the treatment algorithms of some of the most aggressive hematologic malignancies, it is worth noting that only a few hundred patients have been treated in uncontrolled studies thus far, and that follow-up is still short, which prevents drawing conclusions regarding long-term toxicities, response duration, and the curative potential of these therapies. These are important considerations given the high price associated with both tisagenlecleucel ($475,000) and axi-cel ($373,000). Data reported by the University of Pennsylvania on more than 500 patient-years of follow-up on a trial of a retrovirally transduced CAR T cell against HIV revealed no evidence of vector-induced cell immortalization or persistent clonal expansion (42), suggesting a negligible risk of long-term toxicity. Furthermore, long-term follow-up of patients treated in the University of Pennsylvania's initial pilot study in patients with high-risk relapsed/refractory CLL showed that 27% of patients achieved a CR, and that all of them remain ongoing, in some cases beyond 4 years after infusion, suggesting that some patients can be cured after a single CAR T cell infusion (43).

The addressable cancer patient population is very limited for both tisagenlecleucel (relapsed/refractory pediatric ALL or DLBCL) and axi-cel (relapsed/refractory DLBCL). Short-term, this situation will remain unchanged as the most likely next approval will be liso-cel, also in relapsed/refractory DLBCL. Looming large over CD19-targeted CAR T cell therapies is the uncertainty as to their competitiveness against standard therapies when used in earlier lines of therapy. Randomized controlled studies will be required to demonstrate whether CAR T cell therapy could displace allogeneic SCT as standard therapy for pediatric patients with ALL progressing after first-line therapy or whether they could improve on the efficacy of autologous SCT in patients with relapsed DLBCL.

Deaths due to cerebral edema post infusion have only been reported in clinical trials of CARs incorporating the CD28 costimulatory domain. CD28 costimulation has been associated with faster CAR T cell expansion compared with the expansion of CAR T cells fueled by 4-1BB costimulation such as tisagenlecleucel or liso-cel. Nonetheless, CRES also afflicts patients treated with CARs that incorporate 4-1BB costimulation as well as patients treated with bispecific anti-CD3/CD19 T-cell engagers (i.e., blinatumumab; ref. 29). Improvements in CAR T cell design (44) or the use of novel engineered T-cell platforms associated with lower cytokine release (45) warrant clinical investigation. A better understanding of the pathophysiology of CRS and CRES is needed to better manage these toxicities.

Conclusions

Ultimately, the success of CAR T cell therapies will depend not only on their clinical efficacy and safety but also on the implementation of the necessary strategies to facilitate their access to all patients who need them. Insurance companies currently make decisions regarding coverage for CAR T cell therapies on a case-by-case basis, whereas Medicare is yet to create a code for these therapies. Currently, hospitals face the tough predicament of purchasing and delivering the therapy at risk or else keep patients waiting until the insurer guarantees reimbursement. Solving access and reimbursement issues is critical because it is estimated that the cost of these therapies once the hospital and physicians' fees are accounted for widely exceeds the half a million dollars mark. Given that the number of patients with relapsed/refractory DLBCL eligible for CAR T cell therapy in the United States alone is 7500, the total expenditures would surpass $3B (46). Although these issues are sorted out, the increasing competition in the field of adoptive T-cell therapies will stimulate the development of more

effective and affordable products that will facilitate patient access to CAR T cell therapies, which may be curative in some hematologic malignancies.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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