CAR T Cells

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Keywords

Non-Hodgkin lymphoma · Immunotherapy · Adoptive cell therapy · Chimeric antigen receptor (CAR) T cell · Cytokine release syndrome (CRS) · Toxicity · Cellular therapy · CAR-T cell related encephalopathy syndrome · Neurotoxicity · Axicabtagene ciloleucel · Tisagenlecleucel · Lisocabtagene maraleucel · Brexucabtagene autoleucel

1 Introduction

In 1891, Dr. William B. Coley, an American surgeon, made a compelling observation that immune system can be triggered to shrink tumors. The quest to exploit the power of immunotherapy however was forestalled by an era of chemotherapy that ensued. During World War II, the accidental sinking of a US naval ship led to a group of sailors developing pancytopenia due to poisoning from mustard gas (nitrogen mustard). The observation prompted wide-scale screening of these chemical compounds with cytotoxic potential; further clinical trials led to the first Food and Drug Administration (FDA) approval of a chemotherapy drug, nitrogen mustard. The immunotherapy field took further impetus, not until the last two decades, due to our deeper understanding of the immune system and the cellular and molecular pathways leading to tumor development. Two groundbreaking therapies which have shown great promise in this field involve "taking the breaks off" and "pushing the pedal" of the immune system. These therapies, namely, immune checkpoint inhibitors and adoptive cell therapy, respectively, have been successful in a variety of malignancies, while the former mostly in solid tumors and the latter in hematological malignancies.

Adoptive cell therapy includes both genetically engineered TCR (T-cell receptor) therapy and CAR (chimeric antigen receptor) T-cell therapy. The former requires antigen presentation by innate T cells, while the latter has receptors transduced in T cells which offers antigen-presenting cell (APC) independent effector T-cell function and antigenic specificity.

Adoptive T-Cell Therapy Adoptive T-cell therapy such as allogeneic hematopoietic cell transplantation and donor lymphocyte infusion (DLI) has been clinically utilized for greater than three decades. Although an immune therapy, they use T cells in the crudest of forms, with varying degree of success, and have become the treatment of choice for many relapsed refractory hemato-

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logical cancers due to lack of more effective or less toxic options. However due to its nonselective nature (HLA disparity) and off-tumor toxicity, allogeneic transplantation comes with significant treatment-related morbidity and mortality, both acute and long-term.

TCR and CAR T-cell therapies emerged to mitigate this nonspecific alloreactivity and bypass immune tolerance and enhanced effector function. Antigen recognition by the αβ moieties on T-cell receptor surface is cardinal for TCR therapy and binds both intracellular and/or extracellular peptides in a major histocompatibility complex (pMHC)-dependent presentation by antigen-presenting cells. The αβTCR activation requires concerted effects of receptors CD4 and CD8. TCR lacks an intrinsic intracellular signaling moeity and, thus, once activated triggers its binding to CD3 complex and through a complex mechanism, yet to be elucidated, leads to an optimal cytotoxic anticancer T-cell activity.

Transfection of T cells with virally inserted chimeric antigen receptors not only retains the extracellular antigen specificity but also is able to function in an MHC and co-receptor-independent manner. The technology was pioneered by Dr. Gideon Gross and Dr. Zelig Eshhar 30 years ago [26]. Dr. Carl H. June and Dr. Bruce Levine furthered the CAR therapeutic strategy from bench to bedside by treating patients with relapsed acute lymphoblastic leukemia. Its unparalleled therapeutic efficacy in this devastating disease led the way to an explosion of CAR T-cell therapies in clinical trials. A brief summary of CAR T-cell evolution is shown in Fig. 1. In this chapter, we will review the various aspects of CAR T-cell and their efficacy, toxicity, and management in different tumors presented in recent clinical trials and its future potential.

2 Chimeric Antigen Receptor Structure and Function

The simplest level of CAR structure consists of an extracellular domain, hinge, transmembrane domain, and an intracellular signaling domain (Fig. 2). The CAR T-cell ectodomain recognizes the extracellular tumor antigen and initiates downstream signal transduction, which channels through the hinge, transmembrane, and costimulatory domains leading to a complex cascade of CAR T-cell activation, transcription factor expression, cell proliferation, survival, and cytokine release resulting in cytotoxic activities.

Ectodomain or Extracellular Domain (ECD) The extracellular target-binding site in a CAR structure is the single most important factor that serves as a lock and key for target antigen specificity. The ECD is directed against a well-documented target on the cancer's cell surface, which can be a carbohydrate, protein, or glycolipid structures. An ECD against an appropriate tumor-associated antigen (TAA) is the most crucial component of a CAR T cell (Table 1). Selection of the target TAA is essential and ideally will be universally expressed on the targeted cancer cells, infrequently lost in refractory disease, and not expressed on nonessential normal tissue. The most commonly used ectodomain is derived from the single-chain variable fragment (scFv) of a tumor antigen-reactive murine monoclonal antibody. The scFv is formed by a light chain and heavy chain (which in general are antigen-binding regions of a B-cell monoclonal antibody), connected by a flexible peptide linker which enhances the affinity of the CAR to target antigens. The scFvs (Fig. 1) are synthesized from one of the various expression strategies either from murine or humanized antibodies. The scFv obviates the need for tumor antigen processing and MHC class restriction to lock the target, unlike TCR gene therapy which requires peptide procession and major HLA restriction. The ECD is connected to intracellular domains by an extracellular hinge region and a transmembrane (TM) region.

Hinge (Spacer) This is generally derived from the constant Fc portion of IgG subclass immunoglobulins (such as IgG1 and IgG4) and IgD or CD8 domains and connects the antigen recognition part, scFV, with the transmembrane domain.

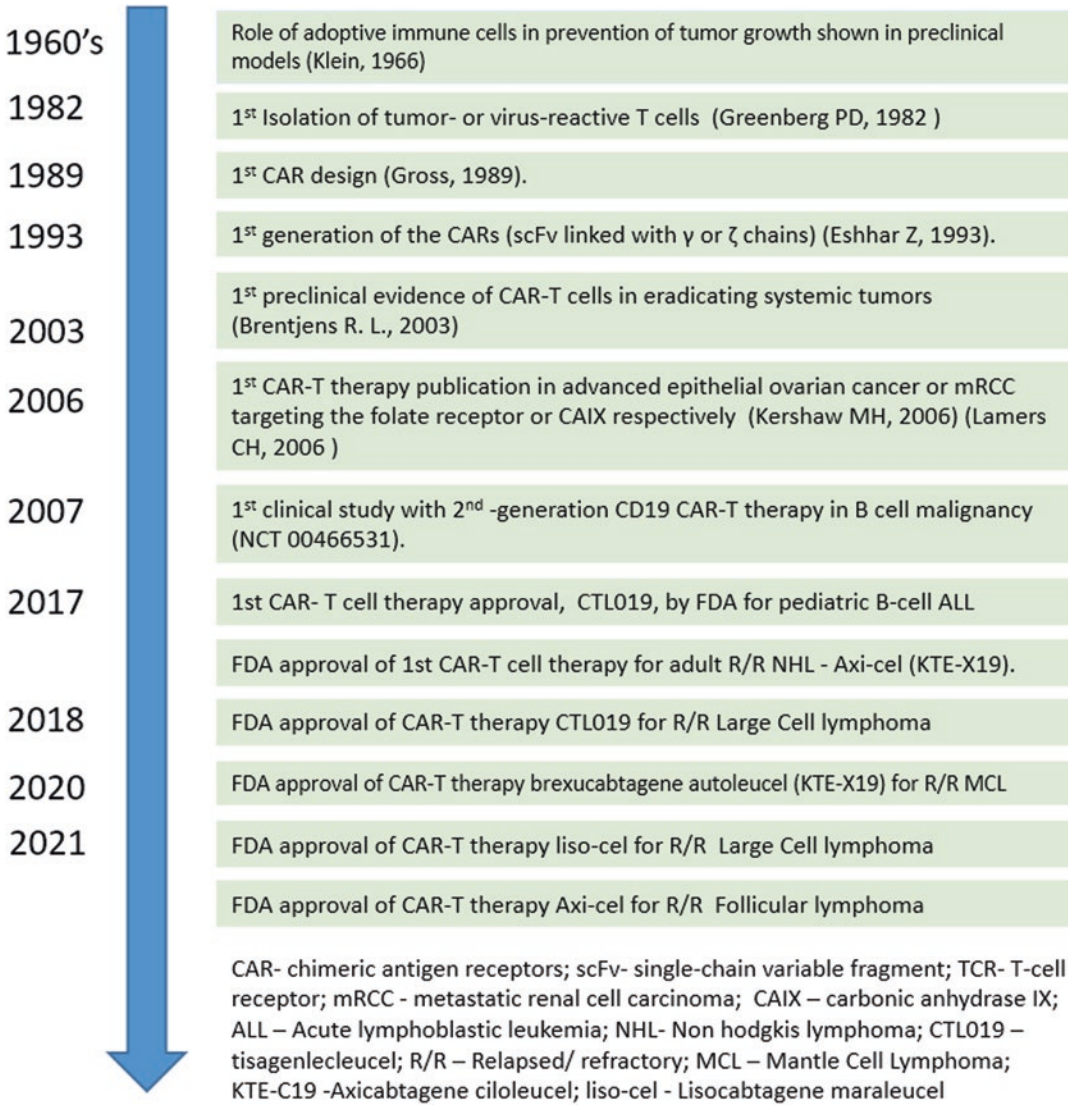

Fig. 1 Timeline of progress in the development of CAR T-cell therapies

The hinge, though inconspicuous in the overall structure, has a significant impact on the overall function and cytokine signature during T-cell expansion [3]. Though the length of the hinge region affects the flexibility of the scFv, it can increase Fc vulnerability for interaction with off-target FcR receptors and has the potential to nullify CAR efficacy by unintentional CAR and/or innate immune response activation. Research is underway to improve CAR T-cell persistence and antitumor efficacy by improved hinge structure through point mutations which can optimize the aforementioned interactions [31].

Transmembrane Domain Between the hinge and the signaling endodomains lies the transmembrane domain. This forms an integral part of the CAR structure and spans across the cell membrane and functions as signal gateway to the intracellular compartment. This is usually derived from CD3-ζ, CD4, CD8, or CD28 molecules.

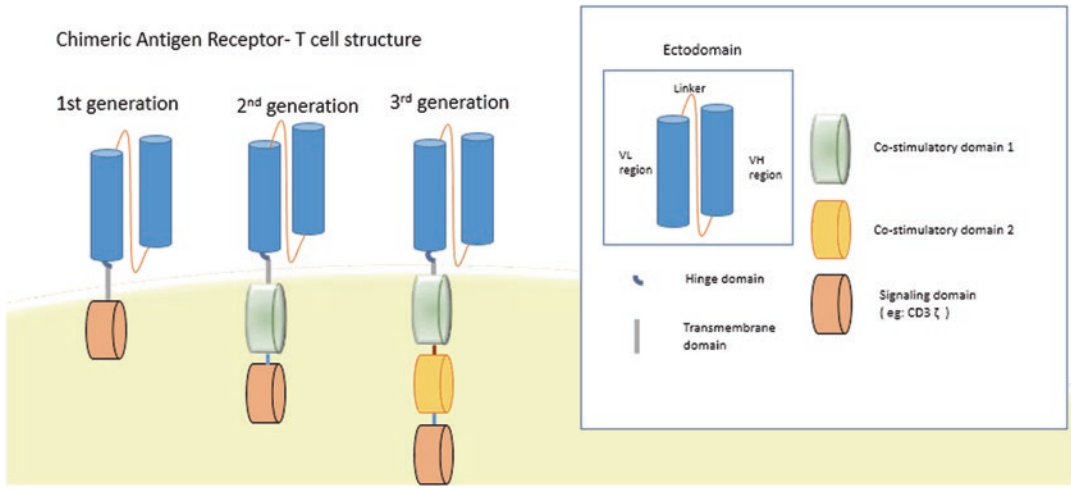

Fig. 2 Structure of first-, second, and third-generation chimeric antigen receptor

Intracellular Domain The first-generation CAR design consisted of only Fcγ (the γ-chain from FcεRI) or CD3ζ (ζ-chain of the TcR complex) intracellular domain. Thus, the modified T-cell activation was dependent on exogenous IL-2, which although was shown to have impressive tumor killing in preclinical model, the effect could not be translated in vivo due to poor T-cell expansion, less stability, and anti- tumor activity due to absent interaction with the TCR and costimulatory receptors. Subsequently, costimulatory domains were added to the CAR constructs to create the second (CD28 or 4-1BB)- and the third generation (combinations of CD28, ICOS, OX40/CD134 and 4-1BB/CD137)-CARs. The addition is shown to be more therapeutically effective due to enhanced persistence, less differentiation, less exhaustion, prolific expansion, cytotoxicity, memory, and efficacy over the first generation.

More novel designs of CARs are under development. Bivalent CARs, targeting two distinct TAA in the same CAR molecule, are generated by coupling two different single-chain fragment variable. Tandem CARs (Tan CARs) generated through co-transduction, generating a pool of T cells containing two or more CAR T cells, appear to be successful in preclinical models and theoretically develop synergistic responses due to multiple targets and reduced likelihood of antigen-loss relapses [28, 60]. The fourth-generation CARs which have functional modification in addition to its structural change, the so-called TRUCKs (T-cells redirected for universal cytokine-mediated killing), use T cells as vehicles to produce and release a tumoricidal cytokines inside the targeted tumor tissue. This causes direct killing and also a second wave of immune recruitment [14]. To deliver the pleotropic effects of CAR T cells in a controlled manner, preclinical tests are ongoing with the so called smart T cells which are furnished with one of the different technologies including a presence of suicide gene, switchable dual-antigen receptors, or synthetic control devices (using inducible caspase 9 (iCasp9), Synthetic Notch (synNotch) receptors.) [79].

3 Manufacturing and Treatment

Building autologous CAR T cells requires a series of well-organized steps (Fig. 3). The process starts with the collection and enrichment of CD3+ lymphocytes through the process of leukapharesis. The principle of leukapharesis is same as that for peripheral blood stem cell (PBSC) collection in hematopoietic stem cell

Table 1 TAA that are actively investigated in clinical trials

Cancer type	TAA
Colorectal carcinoma	CEA EGP-40
Liver	CEA GPC3
Breast cancer	CEA Mesothelin ROR1 erb-B 2,3,4
CNS tumors	EGFRvIII EphA2 (glioblastoma) EGFR GD2 (neuroblastoma) CD171 (neuroblastoma) IL13-Rα2 (glioblastoma) Her-2/ ErbB2 (medulloblastoma)
Lung cancer	EGFR GPC3 Mesothelin (mesothelioma) ROR1
Renal	VEGFR-II CAIX CD70
Gynecological cancers	FR-α MUC1 MUC16 FBP (ovarian) CD44v7/8 (cervical cancer) CD70 (ovarian cancer)
Mesothelioma	FAP
Prostate	PSMA PSCA
Pancreatic cancer	Mesothelin CD70 CD24 FAP HER2 Prostate stem cell antigen MUC1
Hematological	CD19, CD20 and CD22, CD38, κ-light chain (NHL) CD30 (Hodgkin's lymphoma) CD33 (AML) BCMA, NY-ESO-1, NKG2D ligands, SLAMF7 (CS1),CD138 (syndecan-1) (myeloma)

CEA, carcinoembryonic antigen; EGP-40, colon cancer-associated Ag; GPC3,Glypican 3; ROR-1, receptor tyrosine-kinase like orphan receptor 1; CD, cluster of differentiation; EGFRvIII, epidermal growth factor receptor vIII; ErbB, erythroblastosis oncogene B; EPHA2, EPH receptor A2; FAP, fibroblast activation protein alpha; GPC3, glypican 3; GD2, gangliocide; HER2, human epidermal growth factor receptor 2; VEGFR, vascular endothelial growth factor receptor; iCas9, inducible caspase-9 (safety switch); IL13Rα2, Interleukin-13 receptor subunit alpha-2; CA IX, carbonic anhydrase IX; FR-α, folate receptor alpha; MUC1, mucin 1, cell surface associated; FBP, folate-binding protein; FAP, fibroblast activation protein; BCMA, B-cell maturation antigen; NY-ESO-1, New York esophageal squamous cell carcinoma 1; NKG2D, natural killer group 2 member D; SLAM7, self-ligand receptor of the signaling lymphocytic activation molecule

transplant. The collection process in CAR T-cell patients however presents unique challenges. Apart from the target cells for collection being small, mature lymphocytes (in contrast to stem cell collection which targets large, immature CD34+ stem cells), potential CAR T recipients often have active disease, cytopenias, and poor T-cell function due to multiple prior therapies. Factors that have shown to adversely impact T-cell collection include older age, pre-collection thrombocytopenia, multiple prior cancer treatments, non-mobilized lymphocytes, presence of circulating blasts, and natural killer cells [5, 6, 72]. The success has shown to be influenced by the nature of the T cells collected (naïve or early memory phenotype elicit a greater antitumor potential) [23, 33]. A minimum absolute peripheral blood lymphocyte count greater than 100–200 cells/mL is expected to result in successful T-cell collection [52, 65].

Leukapheresis This is the process of filtering blood from the donor for the purpose of T-cell collection, originally pioneered by Freireich and colleagues. Leukapheresis, usually well tolerated and safe, is an outpatient procedure involving the placing a dependable venous access (central or peripheral), removing blood and filtering the peripheral blood mononuclear cells [70]. The remainder of the blood is returned to the circulation. In CAR T-cell patients, adverse events are reported in <15% during apheresis and can manifest as hypotension requiring fluid bolus, agitation, vomiting, fevers, and procedure-related pain. Severe side effects in the form of syncope, citrate toxicity, and vascular injuries are uncommon, described to occur in less than 0.5% in incidence [5, 6, 11].

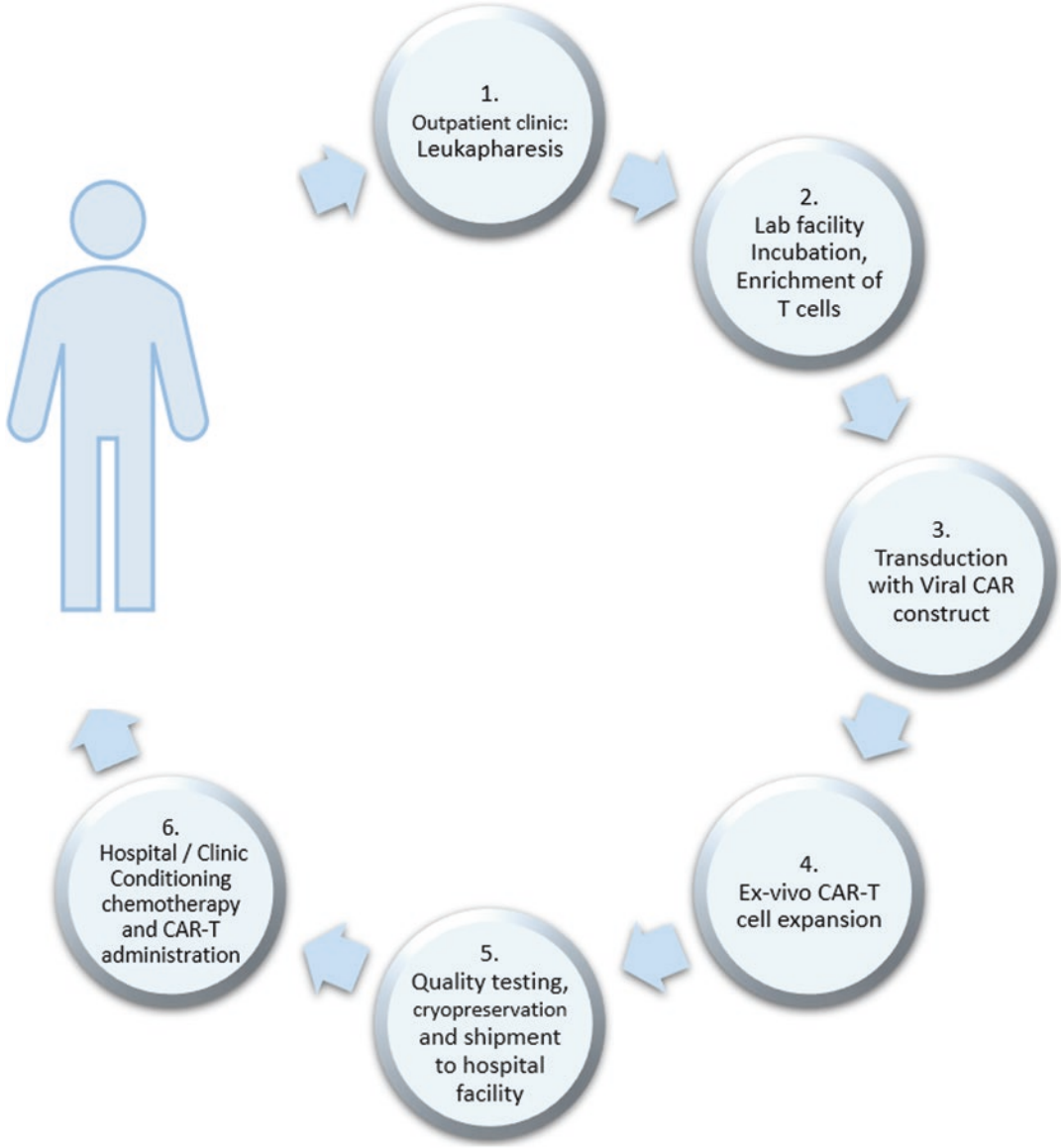

Fig. 3 Simplified version of manufacturing process of autologous CAR T cell therapy

FDA-approved instruments are available to perform extraction of T cells from the blood that is withdrawn, which involves elutriation, a technique which relies on the application of centrifugal force to the continuous or semicontinuous flow of anticoagulated whole blood. This results in the separation of cell layers based on its density. The mononuclear cell layer (both monocytes and lymphocytes) is sandwiched between the dense polymorphonuclear cell/red blood cell (RBC) layers and the less dense platelets. The is followed by purification of the T cell from other blood cells by a complex process of washing and antibody-bead conjugate selection [64]. The extracted apheresis product is shipped to the lab, either as a fresh or frozen product depending on the planned manufacturing procedure, where T cells are incubated and genetically modified with a viral vector encoding the CAR and expanded. There are three major types of stable gene expres-

sion vectors used for clinical applications: gamma retroviral vectors, lentivirus vectors, and the transposon/transposase system. Lentivirus vectors have a safer integration site profile than gamma retroviral vectors and hence commonly used in clinical practice for generating CAR T-cell therapies. Other methods of gene transfer are currently being investigated. Viral transduction is followed by the expansion of modified T cells before the cells are cryopreserved. The cryopreserved cells are transferred back to the hospital center for administration.

Conditioning Chemotherapy Conditioning chemotherapy is a part of most of the CAR T-cell protocols and has shown to improve outcomes. The most utilized regimen is fludarabine and cyclophosphamide, but other regimes such as bendamustine have also been utlilized. The impact of the conditioning chemotherapy on the cancer to cause an objective tumor response in patients with chemotherapy resistant cancers is hypothesized to be very low as majority of patients enrolled in these studies have highly refractory and heavily pretreated disease [8, 16, 34, 55, 73, 75]. The conditioning helps to create a less competitive environment for the adoptive transferred T cells by promoting host lymphocyte depletion, more supportive cytokine milieu, decreased immunosuppressive cells such as regulatory T cells, and myeloid-derived suppressor cells [32, 78].

CAR T-Cell Infusion Once the cryopreserved product is received by the treating center and the patient deemed ready for infusion, the staff thaws the cells at the bedside, confirms the patient's identification, and infuses the cells via gravity over approximately 30 minutes. Though the infusion of CAR T cells is generally safe, the ensuing toxicity of the treatment varies by the type of product, dose, disease burden, and patient characteristics. Hence, the site of administration of CAR T-cell infusion can be both inpatient and outpatient. Given the toxicities of the currently approved products (axicabtagene ciloleucel and tisagenlecleucel) which require early identification and specific medical interventions, including transfer to intensive care for successful outcome, these are often administered in the inpatient setting although acute infusion reactions are rare. Patients are often premedicated with antipyretics and antihistamines. Systemic steroids including hydrocortisone are generally avoided due to concerns about lymphotoxicity and arrested expansion. After the CAR T-cells are infused, patients require close monitoring while they are at risk for the development of cytokine release syndrome (CRS) and immune effector cell-associated neurotoxicity syndrome (ICANS).

The side effect profile of the currently approved CAR T precludes wide-scale application in the outpatient setting. In ZUMA-1 trial, patients could be discharged at day 7 post treatment in the absence of any sign of CRS or ICANS, while in ELIANA and JULIET trial, patients could be discharged same day [48, 52, 69]. Patients are also instructed to have a caregiver present 24 hours a day and stay locally within 2 hours for at least 4 weeks following CAR T-cell infusion that allows prompt access to hospital that is equipped to manage CAR T-cell toxicities. A portion of patients with tisagenlecleucel and lisocabtagene have been infused as outpatients; however this requires intensive monitoring, education of staff, and coordination of care. In TRANSCEND NHL 001 study, out of the 269 patients who received at least 1dose of liso-cel, 25 patients were treated in the outpatient setting, and approximately a third of these patients did not require any further hospitalization. For patients who required hospitalization, the median time from liso-cel infusion to hospitalization was 5 days (range 3–22) [2].

4 CAR T-Cell Therapy in Different Cancer Types

4.1 Hematological Malignancies

Diffuse Large B-Cell Lymphoma (DLBCL) Patients with chemotherapy-refractory DLBCL have a dire prognosis, with no

curative treatment options available until recently [15, 19]. The majority of second-line patients are not eligible for hematopoietic stem cell transplant due to chemotherapy-refractory disease, age, and/or comorbidities. The international, multi-cohort retrospective non-Hodgkin's lymphoma research (SCHOLAR-1) study retrospectively evaluated outcomes in patients with refractory DLBCL. Refractory was defined as progressive disease or stable disease as best response at any point during chemotherapy (after four cycles of first-line or two cycles of later-line therapy) or relapsed within 12 months of autologous stem cell transplantation. The objective response rate noted in this group was a dismal 26% (with CR at 7%) to the next line of therapy, and the median overall survival was 6.3 months. Only 27% of patients were alive at 2 years. Outcomes were consistently poor across all patient subgroups.

The clinical efficacy of CAR T-cell therapy in this refractory group of patients in pivotal CAR T-cell trials is gratifying with impressive response rates and sustained durability. There are three CAR T-cell products that are FDA approved as of 2021, tisagenlecleucel (CTL019, Kymriah), axicabtagene ciloleucel (axi-cel, KTE-19, Yescarta), and lisocabtagene maraleucel (Lis-cel, Breyanzi). Tisagenlecleucel was approved for the treatment of pediatric relapsed and/or refractory B-cell precursor acute lymphoblastic leukemia, and on August 30, 2017, the same product was further approved in relapsed or refractory large B-cell lymphoma. Axicabtagene was approved for use in relapsed or refractory large B-cell lymphoma including primary mediastinal large B-cell lymphoma, in October 18, 2017 [52]. Liso-cel is the most recent CAR T to receive approval for DLBCL. On February 5, 2021, the FDA approved this treatment for adult patients with non-Hodgkin's lymphoma after two or more lines of systemic therapy, including DLBCL not otherwise specified (NOS) (including transformed DLBCL), high-grade B-cell lymphoma, primary mediastinal large B-cell lymphoma, and follicular lymphoma grade 3B.

Axicabtagene Ciloleucel The CAR T-cell construct (CD28 costimulatory domain) is derived from the initial NCI-designed CAR construct. The same CAR vector construct was further used in the pivotal ZUMA 1 trial, which included patients with refractory diffuse large B-cell lymphoma, primary mediastinal B-cell lymphoma, or transformed follicular lymphoma (TFL).

Patients achieved an objective response rate (ORR) of 83%, with a complete response (CR) rate of 58%, and 42% of the patients continued to have a response, with 40% continuing to have a CR with a median follow-up of 27 months [45]. The molecular subgroups of DLBCL did not have an impact on the response rate; ORR was 88% (CR 57%) and 76% (CR 59%) in germinal center B cell and activated B-cell DLBCL subgroups, respectively [38, 52]. Median PFS for the whole group was 5.9 months. In a recent real-world analysis of axi-cel in the standard of care setting (n = 295), the safety and efficacy in patients with relapsed/refractory LBCL was comparable to the registrational ZUMA-1 trial [51].

Tisagenlecleucel The 4-1BB costimulation domain used in this product is known to be associated with longer persistence of CAR T cells and less T-cell exhaustion. Schuster et al. reported a 57% CR rate in pilot study of 28 patients with refractory B-cell lymphomas treated with this construct (CTL019). Among refractory DLBCL, CR rate was 43%. This included three double-hit lymphoma patients (one histologic transformation) all who had complete responses. The JULIET study was built upon the aforementioned study and included relapsed/refractory DLBCL and transformed follicular lymphoma, with ORR of 52% with 40% achieving CR and 14% achieving PR. At 6 months from infusion, the ORR was 37% with a CR rate of 30%. The median duration of response was not reached with 26 months of median follow-up [68, 69].

Lisocabtagene Maraleucel TRANSCEND NHL 001, a large multicenter trial, which started

as a phase I first-in-human study of JCAR017, used a defined composition of CD19-directed CAR T cell (equal ratio of CD4+ and CD8+ CAR T cells) and used 4-1BB costimulatory domain. In this trial, which has the largest cohort of patients for any CAR T study to date in large-cell lymphoma, 344 patients underwent leukapheresis for manufacture of liso-cel, of whom 269 patients received at least 1 dose of liso-cel. The trial reported an ORR of 74% for the entire patient population, with CR rate of 53%. The estimated duration of response rate at 1 year was 55% for the total population and 65% among those who achieved a complete response. Median progression-free survival was 6.8 months.

The core group, which had patients with high-grade B-cell lymphoma (double/triple hit), DLBCL-NOS de novo or TFL (treated with 5×10^7 cells in a single dose) had an overall response rate of 76% and a CR rate of 47%. In comparison, those treated with higher dose (1×10^8 cells in a single dose) had an overall response rate of 80% and a CR rate of 63%. Among 16 double/triple hit patients, best ORR was 81%, and 3-month CR rate was 60%. In those who relapsed within 12 months of a stem cell transplant, the ORR was 85% [1, 2]. (Table 2)

Mantle cell lymphoma: Eight patients with mantle cell lymphoma (MCL) (four of them receiving Cy/Flu conditioning) were included in the study at Fred Hutchinson Cancer Research Center, with no CRs reported and only two PRs in the cohort of MCL [73]. The phase I TRANSCEND study included patients with MCL; however results reported were primarily for patients with relapsed large B-cell lymphomas. In the NCI trial (NCT00924326), which included 22 patients with relapsed/refractory advanced-stage lymphoma, there was 1 one patient with MCL who experienced a CR and had ongoing response +17 months [38]. Given the promising results from NCI trial, the CD19-targeted CAR T-cell product KTE-X19 (Tecartus; brexucabtagene autoleucel) was investigated in patients with relapsed/refractory MCL in the ZUMA-2 trial (NCT02601313). In an intention-to-treat analysis of the 74 patients, the responses were unprecedented in this highly aggressive disease cohort, which included MCL with Ki67 > 30% (82%), Tp53 mutated (17%), blastoid/pleomorphic (31%), with an ORR of 85% (CR 59%). At a median follow-up of 12.3 months, 57% of the 60 patients in the primary efficacy analysis were in remission. At 12 months, the estimated progression-free survival and overall survival were 61% and 83%, respectively. This study led to the first and only CAR T-cell therapy approval in MCL to date.

Indolent Lymphoma An indolent B-cell lymphoma can have ominous clinical features, either manifesting as early relapse after therapy or by transformation histologically to DLBCL or high FLIPI scores (as in high-risk follicular lymphoma). These features have been consistently associated with poor outcomes. Relapse of follicular lymphoma (FL) after first-line treatment with R-CHOP within 2 years defines a unique category of patients at substantially high risk of death from lymphoma.

The first patient report of CAR T therapy in lymphoma was on a phase I trial at the NCI with a second-generation CD19-targeted CAR T (CD28 costimulatory domain) where a patient with advanced relapsed/refractory FL received lymphocyte-depleting regimen with cyclophosphamide and fludarabine. The day after the last fludarabine dose, the patient received $1x10^8$ anti-CD19 CAR Ts intravenously, followed by $3x10^8$ anti-CD19 CAR Ts the next day. After the second CAR T infusion, the patient received 720,000 IU/kg IL-2 intravenously every 8 hours, for a total of eight doses. The patient achieved a PR for 32 weeks after anti-CD19 CAR T therapy. A follow-up trial from the NCI group was conducted in patients with FL or marginal zone lymphoma (MZL). In this trial, patients (four FL and one MZL) were treated with a single infusion of CAR-transduced T cells. IL-2 was also administered intravenously 3 hours after the CAR T infusion at a dose of 720,000 IU/kg every 8 hours; doses of CAR Ts ranged from $0.3x10^7$ to $3.0x10^7$ CAR Ts/kg bodyweight. Results from this trial showed that three of four patients with FL

Table 2 Summary of the three anti-CD19 CAR T-cell therapy in aggressive B-cell NHLs
Abbreviations: *DLBCL-NOS* **Diffuse Large B cell Lymphoma – not otherwise specified,** *FL* **Follicular Lymphoma,** *SCT* **Stem cell transplant,** *HGBCL* **High grade B cell lymphoma**

Study	ZUMA-1	JULIET	TRANSCEND
Co-stimulatory domain	CD-28	4-1BB	4-1BB
Median Age (Range), years	58 (23-76)	56 (22-76)	60 (20-82)
Evaluable Patients	101	93	269
Response	ORR: 83% CR: 58%	ORR: 52% CR: 40% PR: 12%	ORR: 74% in FULL dataset CR: 52% in FULL dataset
Lymphoma Subtype and FDA approval	DLBCL- NOS Transformed FL HGBCL PMBCL	DLBCL- NOS Transformed FL HGBCL	DLBCL- NOS Transformed FL HGBCL PMBCL FL grade 3B
Double Hit Lymphoma	5 patients	27%	13%
Relapse Post Auto SCT	21%	49%	33%
Bridging Therapy	Not Permitted	92% received	59% received.

achieved PR, with a follow-up between 8 and 17 months, and the one patient with MZL achieved PR, with a follow-up of 12 months [34].

The NCI trial included two patients with FL who both achieved CR; however one patient developed myelodysplastic syndrome requiring treatment after a remission lasting of 19 months. The second patient has an ongoing CR 11+ months at the time of report [38].

Building up on the success in aggressive B-cell lymphoma, ZUMA-5 trial enrolled patient in a phase II, multicenter, single-arm study of axi-cel for R/R indolent advance stage NHL, including FL and MZL. 146 patients (124 FL; 22 MZL) received axi-cel. With a median follow-up of 17.5 months, the ORR was 92% among efficacy-evaluable patients with 76% CR rate. In patients with FL (n = 84), the ORR was 94% (80% CR rate); in those with MZL (n = 20), the ORR was 85% (60% CR rate). This study led to FDA's first CAR T therapy approval in FL, and current indication includes treatment of adult patients with relapsed or refractory FL after two or more lines of systemic therapy.

Refractory FL (14 patients) who relapsed within 24 months of initial diagnosis and/or remained refractory to least 2 lines of therapy were treated in the University of Pennsylvania trial using CTL019 [68]. At the time of the most updated report, 3-month ORR and CR rates were reported as 79% and 50%, respectively. The results looked very promising for this high-risk group of patients, defined by prior multiple therapies (median number 5) and relapsed post-autologous/ allogeneic, with a median progression-free survival (PFS) that was not reached. 70% of patients were disease-free after a median follow-up of 29 months. It remains unclear if responding patients will have sustained durable responses, and/or potential cure, or if the disease will eventually relapse as happens with many indolent lymphoma therapies. ELARA is a phase II study evaluating the efficacy and safety of tisagenlecleucel in patients with heavily pre-treated relapsed/refractory FL. In the early interim analysis of the 52 evaluable patients who received tisagenlecleucel (median follow-up, 6.5 months), the ORR was 83% and with CRR was 65%. The treatment was overall very tolerable, and in patients with best response of CR, the responses appear durable [20]. Turtle et al. published their experience with the use of 1:1 ratio CD4/CD8 CAR T in 8 patients with FL even of 8 patients with FL achieving complete remission

(CR; 88%) after CAR T cells. The median time to CR was 29 days (range, 27–42), and all who achieved CR remained in remission (median follow-up, 24 months; range, 5–37). One patient received additional therapy (allogeneic HCT) while still in CR. One patient with stable disease at first restaging received radiation 2.3 months after CAR T cells and has not progressed 36 months after CAR T-cell infusion. The study demonstrated a high rate of durable CR in high-risk FL patients treated with CD19 CAR T cells, comparable to that reported in another study where CRs were only seen in the cohort that received fludarabine/cyclophosphamide conditioning chemotherapy with none in the cyclophosphamide alone conditioning arm (0/2 at 0%) [29, 73].

In CLL, CAR T cells have produced responses ranging from 57 to 74%, with CRs lower in comparison to DLBCLs and range from 21 to 29% [55]. In patients who attained a CR, responses were deep (with minimal residual disease negative) and very durable suggesting the potential of cure in these patients with advanced CLL. There was evidence of long-term persistence of CTL019 cells as detected by flow cytometry or quantitative polymerase chain reaction [37, 57, 58]. The group at the NCI also reported the data on 20 patients treated with allogeneic anti-CD19 CAR T cells in patients with different B-cell malignancies who progressed after allogeneic hematopoietic stem cell transplantation (alloHSCT). T cells obtained from each recipient's alloHSCT donor source were used for the engineered T-cell production. In this study, five patients had CLL with one patient achieving complete response and one with partial response. A durable CR (> 30 months) was reported in a patient with chronic lymphocytic leukemia. There was no new reported graft versus host disease (GVHD) related to the allogeneic CAR T-cell infusion. This clinical benefit was seen in patients even despite prior DLI failure showing the potential superiority of the engineered T cells [9]. Based on preclinical models suggesting synergy, a clinical trial is evaluating anti-CD19 CAR T cells combined with the BTK inhibitor ibrutinib, which to date has achieved an almost 90% minimal residual disease (MRD) negative marrow CR was observed in patients with high-risk, TP53 positive relapsed CLL. Though this is a small study with short follow-up, it shows that a combinatorial approach would enhance the potency of CAR T-cells [25]. Several studies are currently ongoing to prove this concept on a wider population cohort [21, 22].

Hodgkin's and T-Cell Lymphoma In HL, the treatment decision regarding a combined modality approach and duration of chemotherapy is mainly based on the stage and presence of poor prognostic features. Despite the high cure rates, relapses occur in approximately 10% to 15% of patients with localized Hodgkin's disease and approximately a third of those with advanced-stage disease. Around 10% to 15% of patients will have refractory disease to first-line therapy. With the advent of ASCT, anti-CD30 antibody, and checkpoint inhibitors, a major proportion of these patients are salvageable. The patients who fail these therapies comprise the major unmet need in Hodgkin's lymphoma. The immunosuppressive tumor environment and the relative paucity of the malignant RS cells make it challenging to seek an appropriate target to be explored in the CAR T-cell platform. In addition, despite the B-cell origin of the lymphoma, CD19 is generally absent in RS cells. The two main targets that are currently explored are CD123 (expressed in RS cells and other immune cells in tumor microenvironment) and CD30 antigen (expressed in RS and some activated T cells in the tumor microenvironment). In T-cell lymphomas, targeting CD30 with CAR T-cells does appear to be an attractive therapeutic option; however this TAA is not universal and thus has been tested mostly in anaplastic large-cell lymphoma (ALCL).

A phase I, dose-escalation study using CAR T cells targeting CD30 included patients with relapsed/refractory CD30+ Epstein-Barr-virus negative HL (n = 7) or ALCL (n = 2). Three dose levels (DL) were investigated; two patients received 2 x 10^7 CAR+ cells/m2 (DL1), two patients received 1 x 10^8 CAR+ cells/m2 (DL2), and five patients received 2 x 10^8 CAR+ cells/m2

(DL3). The responses reported to date include two out of seven complete responses (CR), three out of seven stable disease (SD), and two progressive disease (PD) in patients with relapsed/refractory HL. Of two patients with ALCL, one had a CR that persisted 9 months after the fourth infusion of CD30. The modest response from anti-CD30 CAR T cells was likely due to two main reasons, one due to the heavy microenvironmental T-cell suppressive infiltrate in Hodgkin's lymphoma and second, which was common to these trials, was the absence of conditioning therapy. Currently two parallel phase I/II trials (NCT02690545 and NCT02917083/RELY30) at two independent centers involving patients with relapsed or refractory HL are ongoing. In the study, a total of 41 patients with R/R HL received autologous CD30 CAR Ts after lymphodepletion with either bendamustine alone, bendamustine, and fludarabine or cyclophosphamide and fludarabine. All patients had received at least 2 prior lines of therapy (and as many as 23) and a median of 7 prior therapies. Of the 37 evaluable patients, 34 received fludarabine conditioning. Two of those patients had attained complete remission prior to CAR T-cell infusion and were not included in the efficacy analysis. The treatment led to objective responses in 23 of 32 (72%) patients, consisting of 19 complete responses and 4 partial responses. Three additional patients had stable disease. 1-year PFS and OS for all evaluable patients were 36% and 94%, respectively [63].

Acute Lymphoblastic Leukemia Acute lymphoblastic leukemia (ALL) is the most common cancer in children and adolescents in the United States with an annual incidence of over 3000 cases [77], with 10-year overall survival reaching almost 80% [77]. Achieving a CR in relapsed patients occurs in about a third of patients [18, 54]. The prognosis is grim for patients with primary refractory disease, and relapse post allogeneic hematopoietic stem cell transplantation (HSCT) results in a median overall survival of 3–6 months.

CAR T cells have shown to be very promising in these groups of patient with induction of remission rates as high as 70–90% seen across multiple trials with different CAR T-cell constructs (scFv and costimulatory domains) and in heavily pretreated with prior CD19 targeted therapies (e.g., blinatumomab) or SCT. Remission is also seen in Philadelphia chromosome-positive (Ph+) disease and in down syndrome-associated ALL [40, 43].

Tisagenlecleucel is the only FDA-approved autologous CD19-targeted CAR T-cell product for treatment of R/R B-cell ALL in patients under 25 years old. The multicenter international ELIANA trial that led to its approval reported an ORR rate of 81%. Majority of patients in the study were not bridged to transplant. The rates of event-free survival and overall survival were 73% and 90%, respectively, at 6 months and 50% and 76% at 12 months. The median duration of remission was not reached. Tisagenlecleucel has been found to have an ongoing persistence of at least 20 months at the time of the report.

In the NCI trial, in ALL patients treated with CD19 CAR T cell with a CD28 costimulatory domain, three quarters of MRD-negative responders proceeded to HSCT. Relapse rate was significantly higher in subjects who did not have a HSCT after CAR therapy (6/7; 85.7%) compared to those who did (2/21; 9.5%) ($p = 0.0001$) [43].

In the ZUMA-3 phase I study, KTE-X19 (same construct of axi-cel) is being evaluated in adult patients with R/R ALL. The interim analysis reported showed encouraging efficacy with manageable safety. The CR rate was noted to be 68%, and all patients were MRD negative. The phase II portion of the study is ongoing (NCT02614066).

It is challenging to draw definitive conclusions from these studies and many open question currently remain; once MRD negative status is achieved, whether to consolidate with HSCT, especially for transplant-naïve patients, or is CD19-CAR T a better bridging therapy than other novel therapies (e.g., blinatumomab) if an MRD negative status can be achieved prior to HSCT.

Multiple Myeloma Patients with relapsed and refractory multiple myeloma (RRMM) who progress on immunomodulatory agents, proteasome inhibitors, and anti-CD38 antibodies have dismal outcomes and have a high unmet need for novel therapies including CAR T. BCMA (CD269), a tumor necrosis family receptor superfamily member (TNFRSF17.4), which is unique to the mature B-cell lineage cells including post germinal center B cells, plasmablasts, and normal plasma cells, is currently the main target being tested in CAR T-cell trials in myeloma. Though there are no FDA approvals, there are a few strong contenders in the race. In the first-in-human clinical trial of BCMA-specific CAR T-cell therapy conducted at the NCI (CD28 costimulatory domain), ORR as high as 81% was obtained with some patients achieving a stringent CR and minimal residual disease (MRD) undetectable disease in bone marrow [4, 10]. Bluebird Bio's bb2121 cell therapy product (4-1BB costimulatory domain), currently marketed as idecabtagene vicleucel (ide-cel), has further set the benchmark in multiple myeloma in the large phase II study (KarMMa) study. The study included patients with triple-class-exposed relapsed/refractory myeloma. The median number of prior therapies was 6 (range, 3–16), and 94% had previously undergone at least one autologous hematopoietic stem cell transplant. The phase II results of this study showed an ORR, CR, and median duration of response of 73%, 33%, and 10.7 months, respectively, across the target dose levels of 150–450 × 10^6 CAR+ T cells and 82%, 39%, and 11.3 months at the highest target dose of 450 × 10^6 CAR+ T cells, a response independent of the degree of BCMA expression. A multicenter, randomized, open-label, phase III study, KarMMa-3 is currently open to evaluate the role of ide-cel as an earlier line of treatment [62, 50].

Nanjing Legend Biotech in China recently reported long-term follow-up results from LCAR-B38M CAR T-cell trial, LEGEND-2 study (NCT03090659) (using 4-1BB costimulatory domain), a clinical trial featuring a CAR T therapy with 2 BCMA-targeting single-domain antibodies designed to confer avidity. Patients on this trial had fewer lines of prior therapy and achieved an ORR of 88% with CR in 74% of patients, with overall favorable safety profile. At 18 months, the PFS rate was 50% for all pts. and 71% for MRD negative-negative patients with CR [76, 80]. The same construct under the name ciltacabtagene autoleucel (cilta-cel; JNJ-4528) is currently undergoing a phase I/II study in patients with RRMM who have received at least three prior lines of therapy or are double class refractory to a proteasome inhibitor and an immunomodulatory drug.

Overall response rate per independent review committee (primary endpoint) was 95% with a stringent CR rate of 56%. Of 52 MRD-evaluable patients, 94% were MRD-negative at 10^5. The 6-month PFS and OS rates were 87% and 94%, respectively.

Other BCMA CAR T trials with different products are currently ongoing with data preliminary at this point [56]. BCMA CAR Ts hold great promise with high efficacy and mild and manageable cytokine release syndrome. Other targets being explored in myeloma are listed in Table 1.

Solid Tumors CAR T cells for solid cancers have not yet been able to reproduce the success of their hematological counterparts. Solid tumors present a more complex array of surface proteins, and trials so far have shown an inefficient homing of CAR T cells to tumor locations. Apart from the low persistence after infusion, the ability of T cells to survive through the immunosuppressive microenvironment in solid tumors (T_{reg} cells, MDSCs, TAMs, tumor-associated neutrophils, and immature DCs) has been equally challenging. There are several ongoing trials worldwide, with different targets under investigation (Table 1).

Toxicity and Management The unique and major toxicities of CAR T treatment include cytokine release syndrome (CRS) and neurotoxicity most recently coined as immune effector cell-associated neurotoxicity syndrome (ICANS). CRS and ICANS are completely reversible in

most instances and early recognition is paramount. Less common side effects include B-cell aplasia, hemophagocytic lymphohistiocytosis (HLH)/macrophage activation syndrome (MAS), anaphylaxis, and tumor lysis syndrome (TLS).

CRS, an inflammatory syndrome observed not just solely with CAR T but also with other immune effector cell therapies, involves a constellation of symptoms that range in severity from mild to being fatal. Symptoms tend to occur early with CD28 costimulatory domain CARs than in those treated with 4-1BB costimulatory domain CARs. The median time to onset was 2 days (range, 1 to 12 days) in axi-cel and 3 days (range, 1–51) in tisagenlecleucel. Symptoms include fever, rigors, hypotension, tachycardia, hypoxia, capillary leak, in severe cases cardiac dysfunction, respiratory failure, renal failure, hepatic failure, and disseminated intravascular coagulation. T-cell and tumor cell interaction releases massive amount of cytokines such as interferon-γ (IFN-γ), tumor-necrosis factor α, and interleukins (IL-6, IL-8, IL-10, IL-15, IFN-g, and MCP-1). This leads to monocytes and macrophage activation which further trigger a pro-inflammatory cascade of cytokines and unrestrained progression of CRS. There also exists a deregulated endothelium (due to increased Ang2:Ang1 ratio and VWF) which plays a role in triggering concurrent ICANS. The incidence of CRS was reported in 93% of patients (grade ≥ 3 in 13%) in ZUMA-1 (axi-cel), 58% of patients (grade ≥ 3 in 22%) in JULIET trial (tisagenlecleucel), and 37% of patients (grade ≥ 3 in 2%) in TRANSCEND NHL 001 trial (liso-cel). Factors that predict severe CRS, included high tumor burden, high bone marrow involvement, high baseline inflammatory state, rising IL6, baseline thrombocytopenia, and therapy-related factors such as the use of high-intensity lymphodepletion with cyclophosphamide and fludarabine, higher CAR T-cell dose, and type of costimulatory domain (e.g., CD28 > 4-1BB) [2, 52, 69].

There is considerable difference and overlap in the management of these toxicities across grades, clinical trials, and different institutions. The American Society for Blood and Marrow Transplantation (ASBMT) recently came up with a consensus grading system for CRS and neurotoxicity associated with effector cell therapies for use across clinical trials and for approved therapies [44]. Organ toxicity associated with CRS is graded according to CTCAE v5.0. Most patients have a compromised immune system or have ongoing neutropenia, and the symptoms mimic sepsis syndrome; clinical management needs a concerted effort from the CAR T specialist and infectious disease team. Sepsis guidelines should be followed with blood cultures, imaging, and empiric broad-spectrum antibiotics.

Early CRS with grade 1 can be managed with supportive measures including antipyretics, antiemetics, intravenous fluids, and empiric antibiotics as appropriate. Grade 2 is defined in the presence of fever (≥38.0 °C) with hypotension not requiring vasopressors and/or hypoxia requiring use of oxygen delivered by low-flow nasal cannula (≤6 L/minute) or blow-by. In addition to fluid bolus, IL6 blocking agents (tocilizumab or siltuximab) should be considered if deterioration to require vasopressors or to grade 3 CRS. Shifting patient for more intensive care in critical care unit should be considered in these scenarios. Dexamethasone is reserved if hypotension persists despite IL6 blockade or fluid boluses or if there is high risk for severe CRS (high tumor burden). Grade 3 is defined as fever (≥38.0 °C) with hypotension requiring one vasopressor with or without vasopressin and/or hypoxia requiring high-flow nasal cannula (>6 L/minute), facemask, nonrebreather mask, or Venturi mask not attributable to any other cause [44]. IL6 blocking agents should be used immediately if not used before and should be managed in critical care unit. Steroids (dexamethasone preferred over methylprednisolone due to better central nervous system penetration) are often needed in cases of refractory to IL-6 blockade. Dexamethasone is dosed 10 to 20 mg every 6 hours for grade 3 and up to methylprednisolone 1000 mg/day for grade 4. If clinical improvement is noticed, consider keeping the duration of steroids as minimum with short taper due to the theoretical possibility of abrogating T-cell efficacy. The median time to

CRS parameter	Grade 1	Grade 2	Grade 3	Grade 4
Fever	Temperature ≥ 38 °C	Temperature ≥ 38 °C	Temperature ≥ 38 °C	Temperature ≥ 38 °C
		With		
Hypotension	None	Not requiring vasopressors	Requiring a vasopressor with or without vasopressin	Requiring multiple vasopressors (excluding vasopressin)
		And/or[†]		
Hypoxia	None	Requiring low-flow nasal cannula[‡] or blow-by	Requiring high-flow nasal cannula[‡], facemask, nonrebreather mask, or venturi mask	Requiring positive pressure (e.g., CPAP, BiPAP, intubation, and mechanical ventilation)
Management	• Antipyretics • Antiemetics • IV fluid • Sepsis work-up • Growth factors and antibiotics if neutropenic	Conservative measures as in grade 1 IL-6 blockade +/− corticosteroids Supplemental oxygen as needed	Transfer to intensive care unit Conservative measures as in grade 1 Vasopressors for hypotension + corticosteroids Supplemental oxygen as needed	Transfer to intensive care unit Conservative measures as in grade 1 Vasopressors for hypotension +corticosteroids Supplemental oxygen as needed

CRS resolution ranges from 7 days (axicabtagene ciloleucel) to 8 days (tisagenlecleucel).

Refractory cases of CRS are rare and are associated with high mortality. Other agents being used and considered investigational include anti-TNFα (etanercept), IL-1R inhibitor (anakinra), T-cell depleting alemtuzumab and ATG, cyclophosphamide, ibrutinib, and GM-CSF inhibition.

ASTCT CRS consensus grading and management

ASBMT guidelines for ICANS

Anti-IL6	Tocilizumab	Siltuximab
Origin	Humanized monoclonal antibody	Human-murine IGκ chimeric monoclonal antibody
Target	IL-6 receptor antagonist	Binds to soluble IL-6
FDA	Approved in August, 2017 for the management of severe CRS	Off label use
Dose and frequency	Minimum interval of 8 hours to a maximum total of 4 tocilizumab doses 4–8 mg/kg (max 800 mg)	One dose in 3 weeks 11 mg/kg IV

ICANS, a unique neurotoxicity syndrome, is the second most-common adverse event that can occur concurrently with or after resolution of CRS or in the absence of CRS. The incidence in clinical trials was reported in 64% (grade ≥ 3 in 32%) of patients in ZUMA-1(axicel), 39% (grade ≥ 3 in 12%) of patients in JULIET trial (Tisagenlecleucel), and 19% (grade ≥ 3 in 12%) of patients in TRANSCEND NHL 001 (liso-cel) trial. Though there is similarity in the pathophysiology to CRS, the exact mechanism is still elusive. Severity seems to correlate with high tumor burden and a more severe CRS [27, 67]. An analysis showed higher levels of cytokines, which are usually associated with a systemic inflammation (i.e., IL-6, IL-10, and IFN-γ), in patients who develop severe ICANS indicating a correlation between systemic inflammation and ICANS. Some of the earliest signs can be subtle and can often be missed during routine assessment. This includes diminished attention, impaired handwriting which can deteriorate quickly to language disturbance, confusion, disorientation, agitation, aphasia, somnolence, and tremors. More severe cases of ICANS are associated with motor weakness, seizures, incontinence, mental obtundation, increased

intracranial pressure, papilledema, and cerebral edema.

Manifestation of CRES can be biphasic; the first phase occurs concurrently with CRS (more common), and a second phase after CRS resolves or in the absence of CRS. The management involves a multidisciplinary approach, close hemodynamic monitoring, aggressive medical and supportive care, and use of specific drugs with IL6 blocking agents: tocilizumab, siltuximab, or steroids [53]. Though IL-6 blockade can reverse CRES during the first phase, it is found to be suboptimal by itself during second phase, likely due to decreased blood-brain barrier (BBB) permeability in the absence of an inflammatory phase. Corticosteroids should be considered as a first-line treatment during this second phase. Similar to CRS, ASBMT guidelines for ICANS were proposed to harmonize the neurological toxicity grading and utilize the assessment of five neurological domains (Table 3). A 10-point immune effector cell-associated encephalopathy (ICE) score is assessed across this five domains, which includes elements for assessing orientation, naming, command-following, writing, and attention. Other neurological domains assessed for ICANS grading include level of consciousness, seizures, motor weakness, and raised intracranial pressure/cerebral edema.

ASBMT guidelines for ICANS

ICE:
• Orientation: Orientation to year, month, city, hospital – 4 points
• Naming: Ability to name three objects (e.g., point to clock, pen, button) – 3 points
• Following commands: Ability to follow simple commands (e.g., "Show me 2 fingers" or "close your eyes and stick out your tongue") – 1 point
• Writing: Ability to write a standard sentence (e.g., "Our national bird is the bald eagle") – 1 point
• Attention: Ability to count backwards from 100 by 10 – 1 point

ASBMT ICANS grade	Defining features of grade	Management
Grade 1	• ICE score 7–9 and/or depressed level of consciousness but awakens spontaneously • No seizures, motor weakness, or raised ICP/ cerebral edema	• Aspiration precautions and IV hydration • Seizure prophylaxis with levetiracetam • EEG • Imaging of brain • Consider tocilizumab if there is concurrent CRS
Grade 2	• ICE score 3–6 and/or depressed level of consciousness but awakens to voice • No seizures, motor weakness, or raised ICP/ cerebral edema	• Supportive care as in grade 1 • Consider dexamethasone or its equivalent of methylprednisolone
Grade 3	• ICE score 0–2 and/or depressed level of consciousness but awakens to tactile stimulus • Any clinical seizure focal or generalized that resolves rapidly, or nonconvulsive seizures on EEG that resolve with intervention • No motor weakness • Focal/local edema on neuroimaging	• Supportive care as in grade 1 • Dexamethasone 10–20 mg IV q 6 hours or its equivalent of methylprednisolone • Control seizures with benzodiazepines (for short-term control) and levetiracetam +/− phenobarbital and/or lacosamide • High-dose methylprednisolone 1000 mg/day for focal/ local edema

ASBMT ICANS grade	Defining features of grade	Management
Grade 4	• ICE score 0 and patient is unarousable or requires vigorous or repetitive tactile stimuli to arouse or stupor or coma • Life-threatening prolonged seizure (>5 min); or repetitive clinical or electrical seizures without return to baseline in between • Deep focal motor weakness such as hemiparesis or paraparesis • Diffuse cerebral edema on neuroimaging; decerebrate or decorticate posturing; or cranial nerve VI palsy; or papilledema; or Cushing's triad	• Supportive care as in grade 1 • High-dose methylprednisolone 1000 mg/day • Control seizures with benzodiazepines (for short-term control) and levetiracetam +/− phenobarbital and/or lacosamide • Imaging of spine for focal motor weakness • Lower ICP by hyperventilation, hyperosmolar therapy with mannitol/ hypertonic saline, and/ or neurosurgery consultation for ventriculoperitoneal shunt in patients with cerebral edema

Abbreviations: ASBMT: American Society for Bone Marrow Transplant; CRS, cytokine release syndrome; EEG: electroencephalogram; ICANS, immune effector cell-associated neurotoxicity syndrome; ICE: immune effector cell-associated encephalopathy; ICP: intracranial pressure; IV, intravenous

Hemophagocytic lymphohistiocytosis (HLH)/ macrophage activation syndrome (MAS) is an uncommon event (1% incidence with CAR- T therapies) characterized by extreme immune activation, cytokine release, lymphohistiocytic tissue infiltration, multiorgan failure, and even death if not recognized early. HLH can mimic events of T-cell therapy such as fevers, cytopenias, hyperferritinemia, and elevated C- reactive protein (CRP) and rarely can have overt presentation with rapid splenomegaly, or evidence of hemophagocytosis. Traditional diagnostic criteria of HLH are unreliable due to symptom overlap with CAR T adverse events. Clinical expertise and judgment on a case-by-case basis is paramount, and in majority of cases, HLH/MAS is managed in same way as for CRS and resolves with CRS resolution [44]

B-cell aplasia is an on-target off-tumor effect of CAR T cell and uncommonly can persist for years in patients, leading to hypogammaglobulinemia [47, 61, 66]. Hypogammaglobulinemia can occur as early as 9 weeks after CAR T-cell infusion, and immunoglobulin replacement has shown to lower the risk of infections in such cases [34, 35, 47, 57]. GVHD is a concern with Allo HSCT CAR T products; however the risk has been fairly low in early clinical trials mostly due to the dampening of the natural alloreactivity from the CAR T generation process [9, 12, 36]. Other toxicities rarely associated with CAR T-cell therapy include pneumonitis, fatal infections, anaphylaxis, and tumor lysis syndrome. Due to the potential risk of insertional mutagenesis with CAR T generation and with use of conditioning chemotherapy, the long-term adverse events with this therapy are currently unclear and would need to be careful calibration in the future years to assess the overall safety.

5 Resistance Pathways

Prognosis of patients after failure of CAR T is poor. The resistance of the tumor and the cause of T-cell failure is an area of active research; some potential mechanisms include loss of target, genetic reprogramming, and T-cell exhaustion. In the international trial which included young adults and pediatric patients with acute lymphoblastic leukemia, around third of the relapses were with CD19-negative variants [39, 47]. The same phenomenon was also observed in two of the patients treated in the NCI trial for children and young adults with refractory B-cell malignancies with CD19-CAR T cells [42]. There are several mechanisms postulated for this escape mechanism including alternative splicing, CD19 gene deletion, or mutation. The loss of target has also been shown in treatment with other immunotherapeutic agents including rituximab leading to CD20-negative relapses. A phenomenon called trogocytosis or shaving has been used to explain

this mechanism with monoclonal antibodies, where the receptor drug complex is removed by the receptor monocytes and macrophages expressing Fcγ which can bind the drug bound to the CD receptor of the cell. This leads to drug clearance and also leads to selection of target-negative tumor cells. It could also be the presence of a sub-detection level presence of a CD19-negative clone [71, 74]. Selection pressure, with genetic reprogramming and lineage switch, has been demonstrated as another uncommon mechanism of relapse. Multiple groups have shown the emergence of relapses with a myeloid phenotype and loss of expression of B lymphoid lineage antigens, in ALL patients treated with anti CD19 CAR T [24, 30]. T-cell exhaustion, a fundamental phenomenon seen with T cells, was first described in chronic viral infections in mice, exposed to chronic recurrent or repetitive antigens. This was subsequently reported in human chronic viral infections and cancer [7, 49]. This would incapacitate T-cell functionality, proliferative potency, and cytokine release with subsequent limitation of lytic capability. Consequent to this, there is upregulation of multiple inhibitory receptors/immune checkpoints (PD1 and PDL-1) that bind to their ligands expressed by tumor cells and antigen-presenting cells in the tumor microenvironment (TME) [13]. It is been established that the absence of costimulatory domain can pave the way to tumor resistance and the presence of costimulatory domain protects against PD-1 upregulation and other mediators of resistance in tumor microenvironment. CD19 CAR T cells incorporating the 4-1BB costimulatory domain were shown to be more persistent than those incorporating CD28 in clinical trials showing clues regarding the role of costimulation domain. 4-1BB costimulation has shown to abrogate the persistent exhaustion induced by CAR signaling [17, 46]. Trials are underway using different combinatorial approach of using costimulation domains in CAR T-cell.

Despite these early interpretations, our knowledge of the resistance phenomenon in CAR T is still in infancy, and clear understanding of these pathways is critical to build up on the early success of CAR T.

6 Future Directions

CAR T holds great promise in the treatment of hematological and solid malignancies. It is clear that the scope of this engineered T-cell product is something beyond the scope of our current understanding. Future trials are currently underway to identify and optimize CAR structure (including multispecific CAR T cells; tandem CARs or Tan CARs) and reduce the toxicity of treatment by using suicide switch technology (caspase 9 (iCasp9) and Synthetic Notch (synNotch) receptors. Allogeneic off-the-shelf CAR T-cell therapy is underway with minimal GVHD and reduced wait times, can meet the high demand of relapsing patients, and avoids the use of heavily pretreated autologous T cells. CAR T cells with dissociated signaling domains and switch receptors, which have the potential to combat tumor antigen resistance, with improved efficacy and durability of response, are underway [14, 41, 59]. As we learn more on the technology that allows heightened efficacy, safety, proliferation, expansion, and inflammatory cell recruitment, there would be more customizable CAR designs and therapies to tailor to a personalized approach for our patients.

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