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REVIEW

A Review of Chimeric Antigen Receptor T-Cell Therapy for Myeloma and Lymphoma

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Abstract: Collectively, hematological malignancies account for the fourth most common malignancy. Myeloma and lymphoma are the most common types of hematological malignancies. Unfortunately, the management of refractory myeloma and lymphoma remains challenging. The discovery of new immunological therapies, namely chimeric antigen receptors T cells (CAR-T), outlined unprecedented B cell malignancies results. In this context, the CAR-T-based approach has led to the proliferation of many clinical studies. In this review, we will deal with the CAR-T structure, and we will summarize the primary clinical studies assessing the risks and benefits of CAR-T cell therapy. We will also deal with the adverse events and management of cytokine release syndromes/immune effector cell-associated neurotoxicity syndrome (ICANS). Subsequently, we will review potential future improvements to overcome refractoriness and improve expansion while decreasing CAR-T's off-target effects. The advances in the CAR-T platform represent a step forward with promising unlimited future possibilities that made it a paradigm-shifting for the management of B cell malignancies.

Keywords: multiple myeloma, relapsed, refractory, treatment, chimeric antigen receptor, T cells, cytokine release syndrome, lymphoma, leukemia

Introduction

Hematological malignancies are collectively the fourth most common of all cancers in the United States.[1] Despite the major leaps forward in treatment options, relapsed and refractory disease remains a challenge. Progress for hematological malignancies has been exceptionally rapid due to improvements in treatment protocols, including the development of targeted therapies. For example, the multiple myeloma (MM) 5-year relative survival rate increased from 25% in the 1970s to 56% in 2012, and close to 75% in US-academic centers.[1,2] However, it is estimated that every 9 minutes, someone in the US dies from a hematological malignancy.[1] When chemotherapeutic options fail, novel immunologic approaches are needed.

During initial tumor progression, tumor cells escape immune recognition and become less immunogenic in a process termed "cancer immune editing."[3] The role of immunotherapy in stimulating the immune response against cancer cells might represent the future of hematological malignancy treatments. A new treatment concept for genetically engineered T cell immunotherapy is now available. This so-called chimeric antigen receptor CAR-T cell therapy leads to considerable overall response rates, even in highly pretreated and refractory hematological malignancies. Hence, the US Food and Drug Administration (FDA) granted the first breakthrough

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designation for a CD19-CAR-T cell therapy for patients with relapsed/refractory NHL in 2017.[4] Also, another approval was granted for patients 25 or younger with relapsed/refractory B-cell acute lymphoblastic leukemia (B-ALL). Others followed this approval for relapsed refractory mantle cell lymphoma in 2020 and diffuse large B-cell lymphoma in 2021.[5,6] More approvals are likely to follow 2021.

CAR-T consists of genetically modified cells either through transfection (DNA plasmid inclusion) or transduction (using viral vector), introducing a new antigen on the T-cell surface to enable cancer cell detection. Since its introduction, multiple methods for CAR-T production have developed. Transduction methods may use either a lentivirus or gamma-retro virus as vectors for genetic modification, whereas the transfection method could transfer the new genes to a T-cell without using a virus vector. Examples of transfection methods include Sleeping Beauty® or piggyBac™ methods.[7,8]

The end product is a new T-cell powered by a specific antibody directed against a selected antigen. One advantage of these CAR-T activities is that they do not depend on antigen HLA presentation. The CAR molecule consists of three parts: 1) an extracellular domain containing a single-chain fragment variable directed against a specifically targeted antigen and an antigen recognition site connected with a linker. The extracellular domain is then attached with a hinge to 2) a transmembrane domain, part of CD3, CD8, CD28 or FcεRI, which is then connected to 3) an intracellular domain, consisting of the intracytoplasmic activating domain (CD28, CD27, CD134, CDB7, or CD3ζ) with or without a second costimulatory factor (CD28, or 4-1BB). Figure 1.

CAR-Ts could be autologous or off-the-shelf allogenic, depending on the source. Autologous CAR-T is more frequently used because it has a simpler structure, and its clinical development started earlier. However, allogenic CAR-T is a more convenient off-the-shelf option.[9,10] Due to GVHD concerns, allogenic CAR-Ts are usually supplemented with a suicidal gene or death receptor-like CD20, protease/protease inhibitor system, synthetic notch receptors, or a small molecule gated zeta chain associated protein kinase 70 (ZAP70) suicidal switch.[9,10]

CAR-T Engineering Issues

Several factors can influence CAR-T's overall outcomes and can be divided into CAR-T manufacturing factors and clinically related factors. First, CAR-T cell quality is assessed by color, presence of transgenes, number and percentage of T-cells and viable T-cells, CD4:CD8 ratio, the extent of expression of CAR on the cell surface, cytokine production, presence of bacterial endotoxins, the risk of insertional oncogenesis, presence of residual magnetic beads, and sterility. Secondly, clinical factors include immune-dependent cancer antigen selection, preferably cancer-specific antigen; CAR-T persistence in the patient; and the associated toxicity profile with cytokine release syndrome (CRS) and immune effector cell-associated neurotoxicity syndrome (ICANS). While CAR-T's goal is to direct the new T-cells against cancers, it should be noted that the off-target effect should always be considered. For example, persistent CAR-T against SLAM-F7 or Kappa light chain in multiple myeloma, theoretically, could lead to prolonged immune suppression, which in turn might offset the benefit of myeloma control. Similarly, B-cell aplasia is a concern for CD19 CAR-T treatment.[11,12]

Table 1 summarizes the currently available/proposed targets for myeloma and lymphoma with a list of potential off-target expressions. CD19 is the primary target for lymphoma clinical trials, whereas B-cell maturation antigen (BCMA) is the main target for myeloma.[13] CD19 is a transmembrane protein expressed on the surface of normal and neoplastic B cells that modulates intracellular signaling pathways, including the B cell receptor signaling pathway that is dysregulated in many B-NHL types.[14] BCMA supports survival and promotes cell growth and chemotherapy resistance.[15] Hence as expected, expression of BCMA increases with progression from MGUS to active myeloma and is associated with worse outcomes.[15] Many other targets could be exploited as alternative options for CAR-T in MM and lymphoma treatment. (Table 1).

Data from clinical trials are accumulating about each of those different targets. However, herein we will summarize the most clinically relevant results.

CAR-T Cells in Clinical Trials for Lymphoma

CAR-T Cells for Diffuse Large B Cell Non-Hodgkin's Lymphoma

Outcomes for patients with relapsed/refractory aggressive B cell non-Hodgkin lymphoma (B-NHL) are poor. The efficacy of salvage chemotherapy regimens for refractory diffuse large B cell lymphoma (DLBCL) is dismal, with response rates ranging from 20–31% and complete

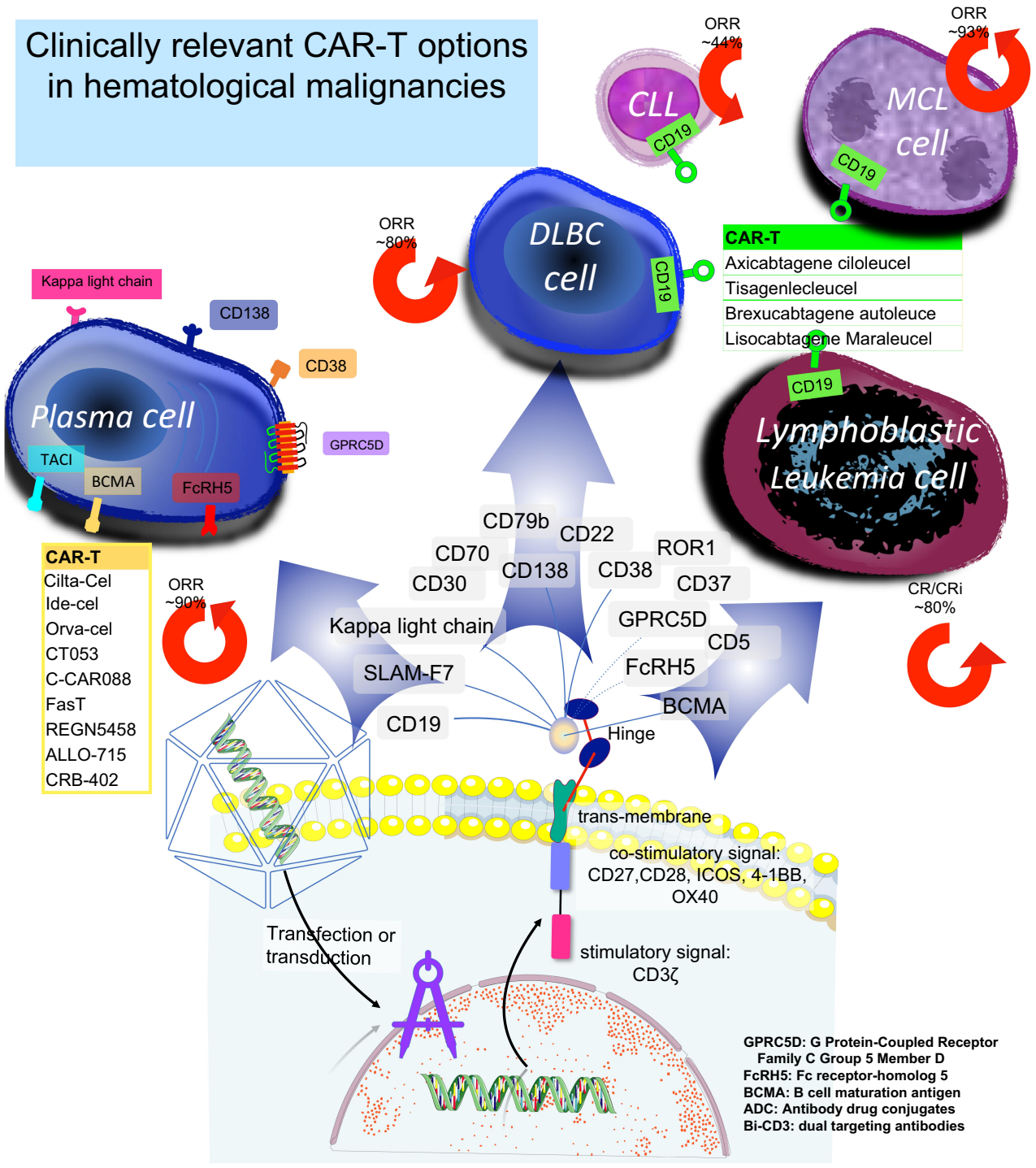


Figure 1 CAR-T cell use in hematological malignancies.

response rates 2–15%.[16] Overall survival for patients with relapsed/refractory DLBCL is estimated to be 6.3 months after salvage therapy initiation, with only 20% of patients alive at two years.[16] Similarly, the overall survival of mantle cell lymphoma (MCL) patients who have failed front-line therapy and BTK inhibition is 2.9 months, only slightly improved to 5.8 months for patients fit for additional therapy.[17] Patients with indolent B cell non-Hodgkin lymphoma enjoy a longer survival, but relapse is inevitable Responses and overall life expectancy are shorter with

Table 1 CAR Antigens Being Examined in Hematological Malignancies

Target	Indication	On Tumor Target	Off-Tumor	Refs
BCMA	Multiple myeloma BCMA+ B cell lymphomas and leukemias	Near universal expression by MM cells in most patients	Plasmacytoid, DCs	[15,68,69]
SLAMF7	Multiple myeloma	Expression in 95% of MM	Plasma cells, NK cells, NK-like T cells, CD8+ T cells	[29,70]
CD38	Multiple myeloma	Most MM 80–100%	Early B-cells, NK cells, activated T-cells, basophils, monocytes, hematopoietic progenitors, DCs, cardiac and smooth muscle cells, cornea, gut, pancreas	[71–73]
CD138	Multiple myeloma	Most MM cells in most patients 90–100%	Epithelial cells, pre-B-cells	[74]
CD56	Multiple myeloma	Strong expression in 70–90% MM	Muscle cells, neurons, NK cells, NK-like T-cells	[75–77]
CD74	Multiple myeloma	95% of plasma cells in >50% of patients	DCs, B-cells, DC, activated-T-cells, monocytes, macrophages	[35,78]
CD40	Multiple myeloma	Variable expression most MM 70–100%	Plasma cells, DCs, APCs	[36,79]
Kappa Light Chain	Multiple myeloma FL, MZL, MCL	Expression in 35% of the MM	Clonogenic MM precursors, mature B-cells	[80,81]
Lewis Antigen	Multiple myeloma	Expression in 52% of MM	Epithelial cells and granulocytes	[82]
NY-ESO -1/LAGE-1	Multiple myeloma	Expression in about 34% of the HLA-A2 positive MM.	Restricted expression to germ cells and malignant tissues	[83,84]
CD19	Multiple myeloma B-NHL, CLL, B-ALL	Expressed only on 5% of MM cells Nearly universal expression in B-NHL	Clonogenic MM precursors, pan-B-cell marker	[80,85]
CD229	Multiple myeloma	Expressed in all plasma cell dyscrasias, especially with plasma cells showing the CD56+ aberrant phenotype.	Expressed on T and NK cells.	[86,87]
NKG2D	Multiple myeloma	NKG2D upregulated in response to DNA damage, infection with certain pathogens, and importantly, malignancies like MM.	Present on NK cells, invariant NKT cells, γδ T-cells, CD8 T-cells, and a small fraction of CD4 T-cells.	[88]
APRIL	Multiple myeloma	A proliferation-inducing ligand (APRIL) is members of the tumor necrosis factor (TNF) family. APRIL stimulates BCMA	B lymphocytes, fibroblasts.	[89,90]
GPRC5D	Multiple myeloma	A member of the G protein-coupled receptor family	Hair follicles	[91]
FcRH5	Multiple myeloma	A member of the immunoglobulin receptor superfamily and the Fc-receptor like family.	Epstein-Barr virus- transformed lymphocytes, spleen, and the terminal ileum of the small intestine	[92]
CD79b	B-NHL, B-ALL	Expressed on the surface of 2/3 of B-NHL	B lymphocytes	[93,94]
CD20	B-NHL	Expressed by 90% of B-NHL and 40% of B-ALL	Pan-B cell marker, follicular dendritic cells	[95]

(*Continued*)

Table 1 (Continued).

Target	Indication	On Tumor Target	Off-Tumor	Refs
CD22	B-NHL, B-ALL, CLL	Expressed by most B-NHL, B-ALL and CLL	Epithelioid histiocytes, B lymphocytes	[62,96]
ROR1	MCL, CLL, B-ALL	Highly expressed in CLL, but less than 10% expression in B-ALL	B-lymphocyte precursors	[97,98]
CD30	Hodgkin lymphoma, B-NHL, T-NHL	Highly expressed in ALCL and classical Hodgkin lymphoma; variable expression in other PTCL and B-NHL	Granulocytes, plasma cells, activated B, T ad NK cells, monocytes	[37]
CD70	B-NHL, AML, MM, T-NHL	Highly expressed in DLBCL, FL, LPL and Hodgkin lymphoma; CLL and AML	Activated B and T cells, thymic stromal cells, NK cells, dendritic cells; Aberrantly overexpressed by multiple solid tumors	[99]
CD7	NK/T cell lymphoma, T-ALL, AML	Highly expressed in T-ALL and most NK cell lymphomas; Expressed in a subset of myeloid malignancies	T lymphocytes, NK cells, thymocytes; variably expressed in monocytes, early myeloid cells, pre-B cells	[100,101]
CD4	PTCL, NOS, AITL, ALCL, CTCL	Expressed in many post-thymic T cell lymphomas	T helper cells, thymocytes, granulocytes, macrophages, DC	[102]
CD5	T-ALL, PTCL, CTCL	Uniformly expressed on T-ALL; Variable expression in PTCL and CTCL	Expressed on virtually all peripheral T lymphocytes, thymocytes;	[66,103]
TCR (TRBC1)	PTCL, NOS, AITL, ALCL	>95% of PTCL homogeneously express either TRBC1 or TRBC2	T lymphocytes	[104,105]
CD37	B-NHL, CLL, PTCL, CTCL, T-PLL	Highly expressed in both B-NHL and T-NHL	Expressed on non-neoplastic B and T cells	[102]

Abbreviations: BCMA, B-cell maturation antigen; DC, dendritic cells; B-ALL, B cell acute lymphoblastic leukemia; CLL, chronic lymphocytic leukemia; B-NHL, B cell non-Hodgkin lymphoma; NK, Natural Killer; APC, antigen presenting cell; MM, multiple myeloma; ROR1, receptor tyrosine kinase-like orphan receptor 1; PTCL; NOS, peripheral T cell lymphoma; not otherwise specified; AITL, angioimmunoblastic T cell lymphoma; ALCL, anaplastic large cell lymphoma; CTCL, cutaneous T cell lymphoma; T-PLL, T-cell prolymphocytic leukemia; T-NHL, T cell non-Hodgkin lymphoma; FL, follicular lymphoma; MCL, mantle cell lymphoma; MZL, marginal zone lymphoma.

each subsequent line of therapy.[18] Although CAR-T therapy is still early in development, early results promise to improve these outcomes considerably.

CAR-T cells targeting the CD19 antigen on B-NHL cells' surface are furthest in clinical development, with FDA-approved anti-CD19 CAR-T constructs for DLBCL and MCL and approvals for additional constructs and indications anticipated within the next 1–2 years.

Axicabtagene ciloleucel was the first anti-CD19 CAR-T approved for lymphoma in the United States.[4] In the landmark Phase 2 ZUMA-1 study, 101 patients with DLBCL refractory to chemotherapy or relapsed after autologous stem cell transplant were treated with a single dose of axicabtagene ciloleucel, which elicited an 82% objective response rate and 54% complete response rate.[19] At a median follow-up of 15.4 months, 42% of patients had a continued response, with an unprecedented 40% still in complete response.[19] The estimated 2-year progression-free survival of patients who were in CR at three months was 72%. The latest long-term follow-up from the ZUMA-1 study (median follow-up 39.1 months) was presented at the ASH annual meeting in 2020 and reported durable responses with 47% 3 years overall survival.[20] Tisagenlecleucel is a second-generation anti-CD19 CAR-T construct that is commercially available for use in patients with relapsed/refractory DLBCL following at least two lines of prior therapy. In the phase 2 JULIET study, 93 relapsed/refractory DLBCL patients received tisagenlecleucel infusion. At a median follow-up of 14 months, the overall response rate was 52%, with the majority of patients achieving a complete response, and the 12-month relapse-free survival was 79% in patients who achieved a complete response.[21] In the TRANSCEND NHL 001 study, a third CD19-directed CAR-T therapy, lisocabtagene maraleucel, enrolled patients with a median of

three previous lines of systemic treatment, and 67% had chemotherapy-refractory disease, 3% had secondary CNS involvement, 33% of patients had a previous autologous stem cell transplant, and 3% had a previous allogenic stem cell transplant.[22] TRANSCEND NHL 001 demonstrated 73% ORR and 53% CR rate and has just received an approval for commercial use soon.[5,22] Importantly, these unprecedented response rates have been confirmed in so-called "real world" or standard of care analyses,[23–25] and older patients have also been shown to derive benefit from CAR-T administration with manageable side effects.[26] Importantly, the safety analyses highlighted some differences in side effect profiles of these constructs. The JULIET study used a different grading system for CRS. However, the risk of grade ≥3 cytokine release syndrome was notably lower with lisocabtagene maraleucel (2% vs 13% and 22% with axicabtagene ciloleucel and tisagenlecleucel, respectively), and the risk of grade ≥3 neurotoxicity was higher with axicabtagene ciloleucel (28% vs 10–12% with the other two constructs).[19,21,22]

The current FDA indications for anti-CD19 CAR-T therapy in lymphoma require a failure of two lines of therapy. With more experience in the management of expected side effects of CAR-T therapy and the development of safer constructs, multiple studies are now investigating the incorporation of anti-CD19 CAR-T therapy earlier for chemo-refractory aggressive B-NHL. At a median follow-up of 3.5 months, all nine transplant-ineligible patients with DLBCL in the first relapse treated with lisocabtagene maraleucel in the PILOT study achieved an objective response with no CRS or neurologic events reported.[27] Importantly, five of those patients were successfully treated in the outpatient setting.[27] In the planned interim analysis of the ZUMA-12 trial, 15 patients with double-or triple-hit lymphoma who had FDG avid disease on an interim PET after 2 cycles of induction chemotherapy were treated with axicabtagene ciloleucel. Of those 15 patients with more than three months of follow-up after CAR-T infusion, 80% achieved a CR, with the majority achieving durable response.[28,29] Axicabtagene ciloleucel is being evaluated in a Phase 3 study randomizing DLBCL patients who fail first-line therapy to standard salvage chemotherapy + autologous stem cell transplant versus axicabtagene ciloleucel (ZUMA-7, NCT03391466).

CAR-T Cells for Mantle Cell Lymphoma

In 2020, brexucabtagene autoleucel was approved for use in relapsed/refractory mantle cell lymphoma.[6] The ZUMA-2 study included patients relapsed after or refractory to at least two lines of therapy, including anti-CD20 therapy, anthracycline or bendamustine, and BTK inhibition.[30] Of the 60 patients included in the efficacy analysis, 67% achieved a complete response, and 57% maintained their response at a median follow-up of 12.3 months.[30] CRS was nearly universal (91%) and occurred early with a median time to onset of 2 days, but grade ≥3 CRS occurred in only 15%, and there were no fatal CRS events. Neurotoxicity was also frequent (31% grade ≥3 neurologic events) but was fully reversible in the majority of patients (complete resolution in 86% of patients, the median time to resolution 12 days).[30] The preliminary results of lisocabtagene maraleucel in relapsed/refractory mantle cell lymphoma suggest similar response rates and enhanced safety. Fifty-nine percent of relapsed/refractory MCL patients achieved CR after lisocabtagene maraleucel, including 57% of patients with blastoid variant MCL. CRS was seen in 50% of the cases, but only a single grade ≥3 CRS event occurred, and less than 10% experienced grade ≥3 neurologic events.[31]

CAR-T Cells for Indolent Lymphoma

CD19-directed CAR-T therapy has also demonstrated promising results in indolent B-NHL. The ORR for patients with indolent B-NHL (predominantly follicular lymphoma) treated with axicabtagene ciloleucel was 92%, with a CR rate of 75%.[32] Tisagenlecleucel has also demonstrated impressive preliminary overall and complete response rates (83% and 65%, respectively) in relapsed/refractory follicular lymphoma, with the median duration of response not reached.[33] Multiple novel CD19 CAR-T constructs are still under investigation in B-NHL.

Due to concerns about the risk of neurotoxicity, in the registration studies for the currently approved anti-CD19 CAR-T therapies, patients with central nervous system involvement were excluded. However, "real world" experience in patients with DLBCL and secondary CNS involvement has shown no significant difference in the safety of axicabtagene ciloleucel or tisagenlecleucel.[33–36] Future studies are explicitly targeting patients with CNS involvement. One such proposed study will investigate a novel CD19 CAR-T construct in patients with relapsed primary CNS lymphoma and is planned to incorporate both intravenous and direct intraventricular administration of CD19 CAR-T cells (NCT04443829).

CAR-T Cells for Hodgkin Lymphoma and T-Cell Lymphoma

The superior efficacy of anti-CD19 CAR-T therapy and broad applicability in patients with aggressive B-NHL after multiple lines of therapy has led to trials evaluating alternative targets that may expand access to patients with Hodgkin lymphoma and T cell non-Hodgkin lymphoma. CD30 is a tumor necrosis factor receptor that is over-expressed in Hodgkin lymphoma and some T-NHL subtypes.[37] Anti-CD30 therapy has proven successful in these lymphoma types, which has led to the exploration of anti-CD30 CAR-T therapy in patients with relapsed/refractory disease. In a study of anti-CD30 CAR-T therapy in patients with multiple relapsed Hodgkin lymphoma who had seen up to seven lines of prior therapy, including anti-CD30 therapy with brentuximab vedotin, response rates were promising (ORR 72%, CR 59%) and durable.[38] This construct has also been tested in a limited number of CD30 + anaplastic large cell lymphoma, a T-NHL subtype, with mixed results.[39]

Multiple Myeloma CAR-T Cells in Clinical Trials

After the approval of several new drugs in the last decade, the multiple myeloma management landscape has substantially changed. Treatment options for multiple myeloma have substantially improved over time, and therapeutic options include agents such as proteasome inhibitors (PIs), immunomodulatory drugs (IMiDs), monoclonal antibodies (MoAbs), antibody-drug conjugates, nuclear export inhibitors, and stem cell transplantation. However, overall survival for patients with IMiDs, PIs, and MoAbs-refractory disease remains around six months.[40] Hence, new treatments with a novel mechanism of action are needed. Harnessing the immune system's ability to overcome refractoriness to conventional drugs can be achieved with monoclonal antibodies, antibody-drug conjugates, T-cell engagers, and chimeric T-cell based therapies.

Multiple myeloma CAR-T cells mostly targeted BCMA (also known as CD269 and TNFRSF17) in early clinical results.[41] BCMA is a 20 kilodalton, type III membrane protein that is part of the tumor necrosis receptor superfamily. Initial clinical trials chose BCMA as the target because it is predominantly expressed in B-lineage cells and plays a critical role in B cell maturation and subsequent differentiation into plasma cells with a relatively higher expression on malignant plasma cells.[41] Prior to delivering CAR-Ts, almost all clinical trials used the same conditioning chemotherapy of fludarabine 30 mg/m^2 and cyclophosphamide 300 mg/m^2 on days −5, −4, and −3.

Currently, three major ongoing clinical trials pathways are exploring BCMA CAR-T. First, Idecabtagene Vicleucel (ide-cel) CAR-T cells for patients with relapsed/refractory multiple myeloma (RRMM).[42] Ide-cel was tested in the KarMMa clinical trials; after lymphodepletion chemotherapy, it was given with a dose-escalation fashion then dose-expansion phase. The expansion phase enrolled patients who had received ≥3 prior lines of therapy. The overall response rate for the expansion dose, 150–450 x10^{-6} CAR-T, was 76%, including 39% patients with CR and 30 patients (out of 37 patients tested) achieving MRD negative status to the level 10^{-4} by next gene sequencing. Those results are unprecedented for patients with highly refractory multiple myeloma. Notably, the duration of response in that group of patients was around 11.3 months, and the median progression-free survival was around 12.1 months. Safety signal was significant for cytokine release syndrome (CRS) higher than or equal to grade 3 in about 7% of patients. The CRS incidence correlated with CAR-T dose. Out of 128 patients enrolled in the trial, 107 patients experienced CRS, while only seven patients had a CRS grade ≥3. One patient had grade 5 CRS.[43] Most patients required at least one dose of tocilizumab for CRS management. Twenty-seven percent of patients reported neurologic toxicity, which is mostly of grade 1/2. However, one patient had grade 3 neurotoxicities and one patient had grade 4 neurotoxicities.

Building on those results, it was felt that enriching CAR-T cells with memory T-cells to increase the persistence of CAR-T cells will help with more durable responses. The bb21217 CAR-T cells are produced by culturing Ide-cel with a PI3K inhibitor.[44] Clinically, bb21217 is undergoing a Phase 1 multicenter study for MM patients who received ≥3 prior regimens, including PI and IMiD agents. Investigators published initial results on 44 patients. The CR rate was 18%. CRS developed in 67% of patients, including one patient with grade 5 CRS. More importantly, higher expression of CD127, a long-lasting memory T-cell marker, was positively correlated with duration of response (DOR), while multiple markers were associated with differentiated T-cells and exhaustion (eg EOMES+, TBET+) correlated negatively with DOR.[45]

Secondly, Orvacabtagene-Autoleucel (orva-cel), also previously known as JCARH125, is equipped with

a fully human binder.[46] Orva-cell was evaluated in the EVOLVE phase 1 study and showed promising results with CAR-T dosing between 50 and 150 x 10^6 cells without dose-limiting toxicities.[47] Recently, another 51 patients were added to the EVOLVE dose escalation with CAR-T dose between 300–600 x 10^6 cells after lymphodepleting chemotherapy. Orva-cel yielded a 91% ORR with 39% CR in a highly refractory population. However, it is too early to report on the final overall survival or median PFS.[46] Based on those results, another ongoing trial, NCT04394650, is testing the next generation manufacturing platform designed to deliver a CAR T-cell product with less-differentiated composition and reduce turnaround time (Nex-T^{TM}). Nex-T CAR-T will likely replace orva-cel.

Third, Ciltacabtagene Autoleucel (Cilta-Cel),[48] also known as LCAR-B38M or JNJ-4528, is genetically engineered to contain a 4-1BB costimulatory domain and two binding sites that attach to BCMA to confer avidity. The overall response rate was 94.8% (95% CI 88.4–98.3), with a stringent CR rate of 55.7% (95% CI 45.2–65.8), VGPR of 32.0% (95% CI 22.9–42.2), and partial response rate of 7.2% (95% CI 3.0–14.3). Also, out of 52 patients with evaluable minimal residual disease (MRD), 94.2% were MRD negative 10^{-5} by next gene sequencing. The 6-month PFS and OS rates were 87.4% and 93.8%, respectively. Unfortunately, eight patients died during the trial due to toxicities. Table (3) summerizes pivotal CAR-T trials.

CAR-T Related Toxicities

Cytokine Release Syndrome (CRS)

CRS is a systemic inflammatory response observed after adoptive T-cell therapy. This condition results from upregulation of CD25 and CD69, secretion of cytokines IL-6, IL-10 and IFN γ, the proliferation of immune cells, and production of granzyme and perforin.[49] This toxicity is non-antigen specific, and it is related to high immune activation and, in some patients, CRS-related clinical and laboratory findings are like macrophage activation syndrome/hemophagocytic lymphohistiocytosis (MAS/HLH).

Clinically, CRS presents with cardiac (tachycardia and arrhythmias), gastrointestinal (nausea and vomiting), laboratory (coagulation, renal and hepatic), neurological, respiratory, skin, vascular (hypotension), and constitutional (fever, rigors, headaches, malaise, fatigue arthralgia) symptoms. However, after ruling out infection, fever, hypotension, and hypoxia are the mainstay of CRS clinical manifestations.[50]

Initial reports speculated that CRS is essential for clinical response against cancer; however, it is clear now that tumor burden is also related to the increased incidence of CRS,[51] and the absence of CRS does not preclude tumor response.

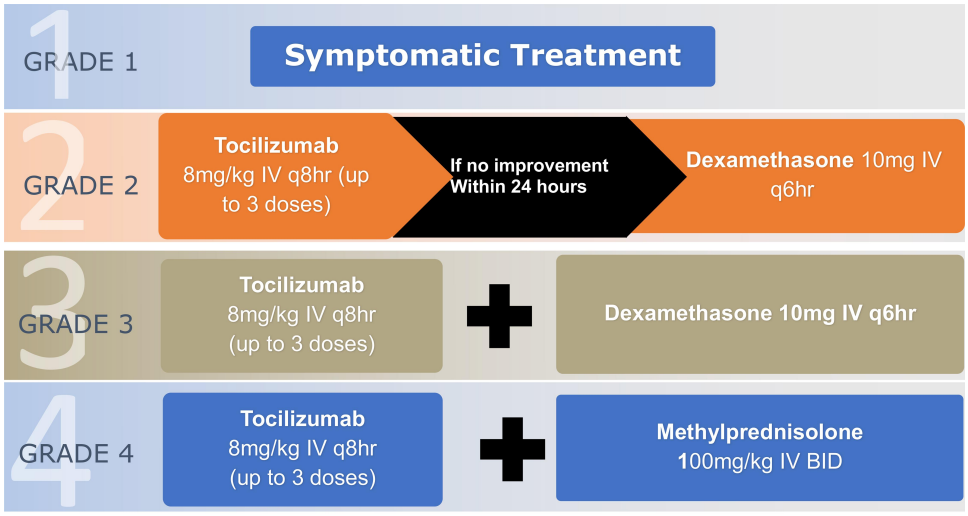
CRS is graded by different methods with minor difference.[50,52,53] In general, CRS severity depends on hypotension and hypoxia. Earlier, Lee et al[53] published criteria for grading CRS, which was later modified to the ASTCT criteria.[50] However, CRS-induced end-organ damage remains a part of the CTCAE (currently v. 5.0) grading system.[54]

While grade 1 CRS is defined as the presence of constitutional symptoms with or without fever, mild hypotension and mild hypoxia are the mainstays of grade 2 CRS. However, hypotension that requires vasopressors or hypoxia that requires a low-flow nasal cannula (6 L/minute) is considered grade 3 CRS. The use of multiple vasopressors (excluding vasopressin) or hypoxia requiring positive pressure defines grade 4 CRS.[54]

Management of CRS syndrome is summarized in Figure 2. The management of CRS grade ≥ 2 requires tocilizumab. At least two doses of tocilizumab must be available before infusion of CAR-T cells. In severe or refractory cases, siltuximab, anakinra, and cyclophosphamide might be helpful. Figure 2.

Neurotoxicity: Immune Effector Cell-Associated Toxicity (ICANS)

The earliest manifestations of ICANS are tremor, dysgraphia, mild difficulty with expressive speech (especially in naming objects), impaired attention, apraxia, and mild lethargy. Seizures are possible, hence the use of levetiracetam prophylaxis in most CAR-T protocols.[55] In general, the diagnosis is made by clinical symptoms. Except with seizures, electroencephalography (EEG) or brain imaging findings are non-specific in ICANs.[56] However, MRI brain might be helpful to role out while the underlying mechanisms. The increased blood-brain barrier permeability does not shield the CSF from high serum cytokine concentrations; also, the presence of CAR-T cells in CSF might play a role in producing local cytokines.[57] Nonetheless, the exact mechanism behind neurotoxicity is not fully elucidated. While severe neurotoxicity is more common with severe CRS, ICANS could also happen without CRS. Early severe CRS (within 36 hours) with elevated IL-6 and monocyte chemoattractant protein-1 (MCP) might indicate a higher risk for developing ICANS.[56]



	Management of Neurotoxicity	
	Concurrent CRS	**No Concurrent CRS**
Grade 1	Symptomatic treatment	Symptomatic treatment
Grade 2	**Tocilizumab** 8mg/kg IV; repeat q8hr (up to 3 doses) *If no improvement within 24 hrs administer* ***Dexamethasone*** *10mg IV q6hr*	**Dexamethasone** 10mg IV q6hr
Grade 3	**Tocilizumab** 8mg/kg IV; repeat q8hr (up to 3 doses) + **Dexamethasone** 10mg IV q6hr	**Dexamethasone** 10mg IV q6hr
Grade 4	**Tocilizumab** 8mg/kg IV; repeat q8hr (up to 3 doses) + **Methylprednisolone** 1000mg/kg IV per day with first dose of **tocilizumab**	**Methylprednisolone** 1000 mg/kg IV per day for 3 days
seizure prophylaxis (eg, levetiracetam)		

Figure 2 Management to CRS/neurotoxicity.

The CAR-T-cell-therapy-associated toxicity 10-point neurological assessment (CARTOX-10) is one of the most commonly used scores because it is easy to use clinically.[55] In the CARTOX-10, one point is assigned for each of the following tasks that are performed correctly: orientation to year, month, city, hospital, and President/Prime Minister of the country of residence (total of 5 points); name three objects (maximum of 3 points); write a standard sentence (1 point). Normal cognitive function is defined by an overall score of 10. A score between 7–9 defines Grade-one (mild impairment); the grade-two score is 3–6; while a score of 0–2, stage 1–2 papilloedema, CSF opening pressure <20 mmHg or partial/non-convulsive seizure on EEG that is responding to benzodiazepine defines grade-three (severe impairment). Grade four is obtundation, stage three to five papilloedema, CSF opening pressure ≥20 mmHg, cerebral edema, or generalized seizure/unresponsive non-convulsive status epilepticus. Figure 2 includes a summary of ICANS treatment.

Challenges and Future Directions

Challenges with Clinical Efficacy

Despite the success rates, some patients relapse after CAR-T therapy. Also, in myeloma, anti-BCMA CAR-Ts have limited clinical efficacy with a median progression-free survival < 12 months. In addition, in lymphoma, not all patients achieve a durable response with anti-CD19 CAR-Ts. This limitation is due to multiple factors:

Table 2 Directed CAR-T Studies in Lymphoma

Challenge: Limited Efficacy of CD19 CAR-Ts in B-NHL		
Strategies Under Investigation	**Selected Clinical Trials**	**Refs**
Tandem/Multiplexed CAR-T therapy to reduce CD19 escape	NCT04260932, NCT04697290, NCT03881761, NCT04723914, NCT03870945, NCT03271515, NCT04486872, NCT04215016, NCT04007029, NCT04553393 (CD19/CD20 CAR-T) NCT04715271, NCT04539444, NCT03593109, NCT04648475, NCT04649983, NCT04204161, NCT03287817, NCT04412174, NCT04626908, NCT04029038 (CD19/CD22 CAR-T) NCT04603872, NCT04162353 (CD19/BCMA CAR-T) NCT04429438 (multiple targets)	
Adjunctive therapy to improve efficacy of CD19	NCT04381741, NCT04163302, NCT04539444, NCT02706405 (PD1 inhibition) NCT04484012, NCT04257578 (Acalabrutinib) NCT04234061 (ibrutinib) NCT04697940, NCT04553393 (Decitabine) NCT03310619 (multiple combinations)	[50] [51]
Modified CD19 CAR constructs	NCT03929107, NCT04381741 (IL7 x CCL19 expression) NCT04213469 (PD1 knockout) NCT03790891, NCT03497533, NCT03720496, NCT03910842 (PDL1 inhibition and T activation domains) NCT04037566 (HPK1 edited) NCT04450069 (adaptable CAR-T construct)	[52]
Consolidative allogeneic HSCT	NCT03366350, NCT03110640	
Challenge: Undefined Treatments for B-NHL Post-CD19 CAR-T Relapse		
Strategies Under Investigation	**Selected Clinical Trials**	**Refs**
Retreatment with CD19 CAR-T	NCT04419909	
Treatment with alternatively targeted CAR-Ts	NCT04036019, NCT04316624 (CD20 CAR-T)	
Other immunotherapies/ immunomodulatory therapies	NCT04703686, NCT02290951 (CD20 x CD3 bispecific Ab) NCT02650999 (pembrolizumab) NCT02926833 (atezolizumab) NCT04205409 (nivolumab) NCT04136756 (NKTR-255) NCT03648372 (TAK-981) NCT04074330 (TAK-981 + rituximab)	
Radiation therapy to residual sites to re-prime response	NCT04601831, NCT04473937	
Challenge: Limited Access to CAR-T Therapy		
Strategies Under Investigation	**Selected Clinical Trials**	**Refs**
Outpatient administration of CAR-T Allogeneic CAR-T therapy	NCT03744676, NCT01853631, NCT03233854 NCT03939026, NCT04416984, NCT04637763, NCT03166878, NCT04264039, NCT03666000, NCT04026100, NCT03229876, NCT04035434, NCT04629729 (CD19) NCT03398967 (CD19 + CD20/CD22) NCT04030195 (CD20) NCT04264078, NCT04620655 (CD7) NCT04502446 (CD70) NCT04288726 (CD30) NCT03881774 (cord blood derived CAR-T) NCT01430390 (EBV-CTL CAR-T)	
Shorten manufacturing time	NCT04638270 (CD19) NCT04303247 (CD19 + CD22)	

(*Continued*)

Table 2 (Continued).

New indications	NCT04443829, NCT04532203, NCT4608487 (PCNSL)	
Earlier employment of CAR-T therapy	NCT04531046, NCT03570892, NCT03483103	
Alternative targets in:		
Hodgkin lymphoma	NCT03383965, NCT02917083, NCT04268706, NCT04653649, NCT04526834, NCT03049449, NCT03602157, NCT04008394, NCT02663297, NCT04083495, NCT02690545 (CD30)	
Peripheral T cell lymphoma	NCT04004637, NCT04033302, NCT04599556, NCT03690011, NCT04480788 (CD7) NCT04594135, NCT03081910 (CD5) NCT03590574 (TRBC1) NCT04219319, NCT04162340,	
Cutaneous T cell lymphoma	NCT04712864, NCT03829540 (CD4)	
B-cell non-Hodgkin lymphoma	NCT04169932, NCT03664635, NCT04176913, NCT03277729 (CD20) NCT04163575, NCT04571138, NCT04007978, NCT03262298, NCT02315612 (CD22) NCT04609241 (CD79b) NCT04662294 (CD70) NCT02954445 (BCMA) NCT04223765 (kappa light chain) NCT02706392 (ROR1) NCT04136275 (CD37) NCT04427449 (CD44v6)	
Challenge: Safety Concerns with CAR-T Therapy		
Strategies Under Investigation	**Selected Clinical Trials**	**Refs**
Targeted studies in vulnerable populations	NCT04661020 (elderly) NCT04088864, NCT04610125, NCT03373071, NCT03448393, NCT03241940 (pediatrics)	
Adjunctive therapies to prevent CRS/ neurotoxicity	NCT04359784, NCT04432506, NCT04148430, NCT04150913, NCT04205838 (anakinra) NCT04603872 (dasatinib) NCT03954106 (defibrotide) NCT04514029 (IT dexamethasone + simvastatin) NCT04314843 (lenzilumab)	[106]
Inducible "safety switch"	NCT03696784 (Caspase 9)	

1. Intrinsic factors due to CAR-T exhaustion or senescence leading to target-positive relapses. A potential strategy to overcome this would be using naïve and stem/central memory CAR-T because it has a better proliferation ability to overcome this exhaustion.[58]
2. Tumor-related factors relating to target loss; for example, biallelic loss of BCMA might play a role against reapplying the same CAR-T treatment upon progression.[59] One future option here is to include more than one target in CAR-T design. Another option is to target the ligand of the receptor; for example, in myeloma, a proliferation-inducing ligand (APRIL) is a ligand of BCMA and Transmembrane activator and CAML interactor (TACI); therefore, by targeting APRIL, we could exploit the benefits of BCMA and avoid tumor escaping mechanism.[60]

Clinical trials are underway, incorporating CAR-T with multiple targets to improve efficacy and reduce antigenic escape. Both sequential and combinatorial CAR-T therapy are being investigated. One example in lymphoma is the dose-finding study of MB-CAR-T2019.1, the tandem CD19 and CD20 targeted CAR-T product was well tolerated and had promising response rates in lymphoma patients.[61,62] CD22, CD30, CD38,

Table 3 BCMA Directed CAR-T Studies in Myeloma

	Idecabtagene Vicleucel (Ide-Cel) /bb2121	**bb21217**	**Orvacabtagene-Autoleucel (Orva-Cel)**	**Ciltacabtagene Autoleucel (Cilta-Cel) /LCAR-B38M**	**P- BCMA-101**
Sponsor	BMS	BMS	BMS	Jansen/China	Poseida Therapeutics
Study	KARMMA Study CRB-401[13,42,43]	CRB-402[44]	EVOLVE Ph 1/2 Trial[46]	CARTITUDE-1[48]/ LEGEND	PRIME[107]
Design	Lentiviral vector 4–1BB	Ide-cel cultured with PI3Ki to enrich memory like T cells	Fully human (CD28/41BB). 1:1 CD4:CD8 ratio	BCMA-targeting with two single chain binding sites	PiggyBac® transposon-based system
Population (n)	128 patients	24 escalation and 22 in expansion	51 pts dose escalation	97 pts (29 in Phase1, 68 in Phase2)	43 pts
Median Number of Prior lines	6 lines	6 lines	6 lines		7
CAR-T dose (cell/kg)	150–450 $x10^6$	150–450 x 10^6	300–600 $x10^6$	Target dose of 0.75×10^6	0.75–15 x 10^6
Refractory to IMiD and PI	98%	–	–	–	100%
Refractory to IMiD, PI, and CD38 MoAb	84%	57%	92% exposed	87.6% 41.2% penta-refractory	93%
Previous ASCT	94%	–	–	–	58%
ORR	73%	55%	91%	94.8%	57%
CR≥	33%	18%	39%	55.7%	
PFS/DFS/DOR	10.7 months	11.9 months	–	NR	–
OS	19.4 months		–	NR	–
Median time to CRS	1 day	3 days		7 days	–
Grade 3/4 CRS	6%	2 pts (1 death)	1 pt	4.1%	1 pt
Neurotoxicity ≥grade 3	3%	3 pts	2 pts	10.3%	1 pt
Reference	NEJM/ASH2020 NCT02658929	ASH2020 NCT03274219	ASCO2020 NCT03430011	ASH2020 NCT03548207	ASH2020 NCT03288493
Next generation	Bb21217	–	NEX-T	–	Nano-plasmid

and CD70 directed CARs are additional targets under investigation in conjunction with CD19 CAR-Ts in B-NHL (Tables 1 and 2).[63,64] It remains to be seen if these multi-targeted CAR-T constructs will produce more remissions.

3. Tumor-microenvironment related factors. Some patients relapse with target+ cells and CAR-T circulating. This type of relapse suggests that CAR-T cell persistence and antigen presence are not sufficient to exert immunity. Perhaps immune suppression in the tumor microenvironment may have a role here.

Finally, the best timing of CAR-T administration in earlier lines of treatment remains questionable.

Challenges with Target Selection

Targeting T cell antigens for peripheral and cutaneous T cell lymphoma has proven challenging. T cell antigens

are frequently downregulated or lost during T cell lymphomagenesis,[65] limiting CAR-T's applicability in T cell non-Hodgkin lymphoma. Furthermore, expression of the target antigen on the CAR-T cell surface results in fratricide, with blunted expansion and CAR-T cells' reduced viability. Finally, targeting markers expressed by normal T cells results in profound immunosuppression and risk of infection, in contrast to B-cell aplasia seen with CD19 CAR-T, resulting in hypogammaglobulinemia. Nevertheless, T cell antigen targets such as CD4, CD5, CD7, CD37, and TRBC1 (Table 1) are in clinical development for lymphoma patients. Other promising targets such as NKG2D, APRIL, GPRC5D, and FcRH5 are following. (Table 1) Additional modification of the CAR-T cells to down-regulate the target antigen expression has been employed to counteract fratricide.[66] Incorporation of a "kill switch" may reduce the risk of T-cell aplasia.

The Future of CAR-T Manufacturing

Currently approved anti-CD19 CAR-T therapies are autologous products, which may require up to four weeks for manufacturing. For patients whose disease progresses rapidly, this constraint may deprive a portion of patients of this potentially life-saving therapy. Induced pluripotent stem cells were transduced with target-specific or BCMA chimeric antigen receptors to generate CAR-Ts that demonstrated effective target-specific cell killing in preclinical studies.[67] This technology is being developed to generate a renewable source of allogenic CAR-T products that may significantly reduce the time from patient identification to CAR-T infusion.[10] Off-the-shelf, allogenic CAR-Ts from various sources and targeting various tumor-specific antigens are currently in clinical development (Table 2). In addition to the typical side effects of autologous CAR-Ts, allogenic CAR-Ts have additional potential but not insurmountable complications, including graft versus host disease and graft rejection. Similarly, derived allogenic CAR-NK (natural killer) cells are also under investigation.

Conclusion

Mounting evidence indicates that immune therapy will be the next revolution in hematological malignancies care. CAR-Ts' technology is a platform with vast opportunities to develop a plethora of different manufacturing techniques and structures. Many questions remain unanswered relating to the target selection and best timing in treatment lines to employ CAR-T therapy. However, this treatment is a launchpad for unlimited possibilities in the future.

Disclosure

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