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Review

Emerging trends in clinical allogeneic CAR cell therapy

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SUMMARY

There has been significant progress in the clinical development of allogeneic off-the-shelf chimeric antigen receptor (CAR)-engineered cell therapies for the treatment of cancer and autoimmune diseases. Unlike autologous CAR cell therapies, allogeneic approaches overcome challenges such as high costs, labor-intensive manufacturing, and stringent patient selection. This makes allogeneic therapies a more universally applicable option for a diverse patient population. In this review, we examine recent clinical advancements in allogeneic CAR cell therapies, including CAR-T cell therapy derived from healthy donor peripheral blood mononuclear cells, as well as CAR-NK cell therapy from cord blood or induced pluripotent stem cells. We provide an overview of their genetic engineering strategies, clinical designs, and outcomes, highlighting their promising efficacy and safety. Additionally, we summarize key preclinical developments, address key challenges, and explore future directions to provide insights into emerging trends in the field.

INTRODUCTION

Adoptive cell therapy has significantly transformed cancer immunotherapy, particularly through the use of chimeric antigen receptor (CAR)-engineered T (CAR-T) cell therapy. This treatment modality has demonstrated remarkable efficacy in managing hematologic malignancies in clinical settings.[1–5] The successful outcomes from US Food and Drug Administration-approved CAR-T therapies targeting $CD19^+$ B cell malignancies and $BCMA^+$ multiple myeloma (MM) offer renewed hope for patients with various cancer types, as well as for those affected by other conditions, including autoimmune disorders, viral infections, and cardiovascular diseases.[5–8] However, the current autologous approach for generating CAR-T cell products presents several challenges, including high costs, extended manufacturing timelines, and limited accessibility for patients. This is especially critical for individuals with progressive disease or those who have undergone prior treatments, as they may lack sufficient or functional T cells for CAR-T production (Figure 1).[9–11] Consequently, there is a burgeoning interest in developing allogeneic CAR-T cell products, which promise greater accessibility and affordability for a broader patient population.

Apart from conventional CAR-T cells, other CAR-engineered immune cell types, including natural killer (NK), invariant natural killer T (NKT), gamma delta T (γδ T) cells, and macrophages are under investigation (Figure 1).[12–18] Among these, CAR-NK and CAR-NKT cell therapies have been tested in clinical trials, demonstrating promising outcomes.[19–21] Several sources can be used to generate allogeneic CAR cells, including healthy donor peripheral blood mononuclear cells (PBMCs), cord bloods, and stem cells such as induced pluripotent stem cells (iPSCs) and hematopoietic stem and progenitor cells (HSPCs) (Figure 1).[22–27] Tailored genetic engineering strategies and culture methods have been developed for each cell type and source. Two major concerns for allogeneic CAR cell therapies are graft-versus-host disease (GvHD) and host-mediated allorejection.[11,28]

GvHD is the primary safety concern in allogeneic CAR cell therapy, primarily mediated by donor CAR-T cells. These T cells can identify the host's major histocompatibility complex (MHC) molecules as foreign, leading to an immune response. Clinically, GvHD can range from mild to severe, presenting with symptoms such as skin rashes, diarrhea, liver dysfunction, and respiratory issues.[29,30] Severe GvHD can greatly affect patient quality of life and may necessitate aggressive immunosuppressive treatment. Strategies to mitigate GvHD involve careful matching of donor and recipient MHC profiles, employing genetic engineering techniques to remove endogenous T cell

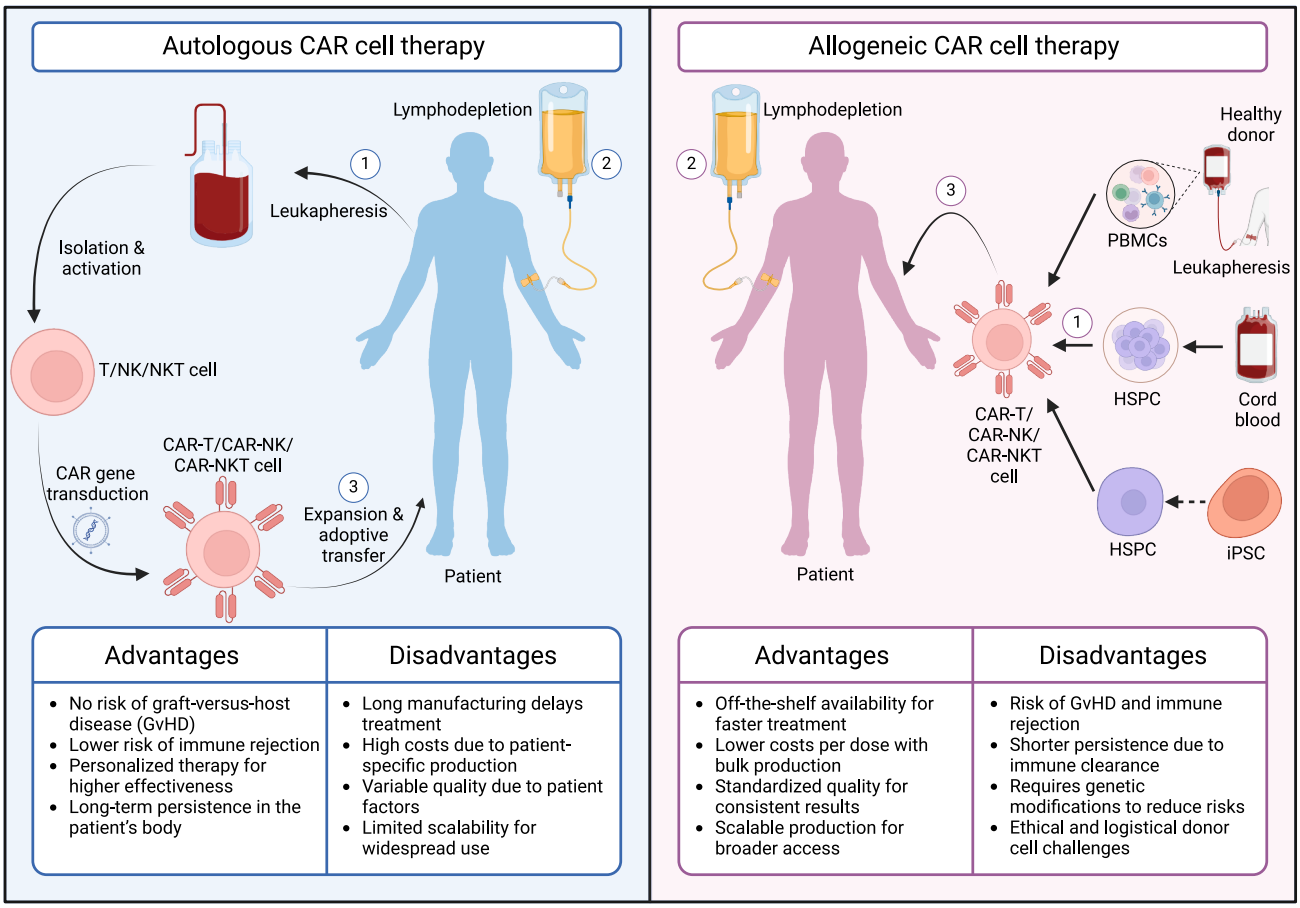

Figure 1. Comparison of autologous and allogeneic CAR cell therapies

The left panel depicts autologous CAR cell therapy, in which T, NK, or NKT cells are collected from the patient through leukapheresis, subsequently activated, genetically engineered to express a CAR, expanded, and reinfused into the same patient after undergoing lymphodepletion. Conversely, the right panel illustrates allogeneic CAR cell therapy, where immune cells are sourced from a healthy donor via leukapheresis or derived from hematopoietic stem and progenitor cells (HSPCs), including those obtained from cord blood or differentiated from induced pluripotent stem cells (iPSCs). These cells are then engineered to express a CAR, expanded, and administered to the patient after lymphodepletion. The advantages and disadvantages of each therapeutic approach are also discussed. PBMC, peripheral blood mononuclear cell.

receptors (TCRs), and administering prophylactic immunosuppressive agents.[13,31–33] While GvHD poses significant risks, the graft-versus-leukemia (GvL) effect is beneficial as it reflects the graft's ability to target and eliminate residual cancer cells.[34] Therefore, balancing the management of GvHD while preserving GvL activity is crucial in allogeneic CAR cell therapy.

Host cell-mediated allorejection presents a primary efficacy concern in allogeneic CAR cell therapy. The immune response is mainly driven by host T and NK cells, which identify the donor CAR cells as foreign due to MHC molecule differences.[11,35,36] This recognition initiates a series of immune reactions aimed at eliminating the infused T cells. Allorejection can significantly reduce the therapeutic effectiveness of allogeneic CAR-T cells.[11] If the host immune system successfully rejects these cells, the intended benefits, such as tumor targeting and elimination, may not be achieved, potentially leading to treatment failure as the CAR-T cells cannot persist and function effectively. Strategies to mitigate allorejection include meticulous matching of donor and recipient MHC profiles, employing genetic engineering techniques to ablate MHC class I and MHC class II molecules while overexpressing NK inhibitory ligands such as human leukocyte antigen E (HLA-E), and the administration of immunosuppressive agents.[11,24,25,37]

With advancements in new technologies and increased biological understanding of allogeneic CAR cells, numerous cell products have entered clinical trials for the treatment of blood cancers and autoimmune diseases. In this review, we analyze recent clinical developments in allogeneic CAR cell therapies, providing a comprehensive overview of their genetic engineering strategies, clinical designs, and outcomes, while emphasizing their promising efficacy and safety profiles. Furthermore, we summarize encouraging preclinical advancements in other allogeneic CAR therapies, particularly focusing on CAR-engineered NKT cells and γδ T cells. We also address the challenges these therapies encounter and explore future directions, aiming to highlight emerging trends in the field.

CLINICAL TRIALS EVALUATING ALLOGENEIC CAR CELL THERAPY

An increasing number of clinical trials are currently being conducted to evaluate allogeneic CAR cell therapy for the treatment of cancers, as well as other diseases, including autoimmune disorders and viral infections (Figure 2). The earliest clinical trial in this area began in 2009 and, by 2024, over 50 new trials have been registered, with this number continuing to rise each year. Given that this field is still in its early stages, the majority of these trials are phase 1 studies. A significant portion of the current clinical trials is focused on investigating allogeneic CAR-T cell therapy, followed by CAR-NK cells, CAR-γδ T cells, and other cell types. Among these trials, CD19-targeting CAR-T cell products are predominantly utilized to treat B cell malignancies. Additionally, various CAR targets are actively being developed to address other hematological cancers, such as acute myeloid leukemia (AML) and non-Hodgkin lymphoma (NHL), as well as solid tumors including hepatocellular carcinoma, lung cancer, and ovarian cancer. Besides cancer, allogeneic CAR cell therapy has also been investigated for the treatment of lupus nephritis, systemic lupus erythematosus, severe aplastic anemia, and COVID-19 (Figure 2). In autoimmune diseases, CAR-T cells have been primarily designed to target autoreactive B cells, with strategies focusing on CD19 and BCMA.[8,38] For COVID-19, a universal off-the-shelf NKG2D-ACE2 CAR-NK cell therapy has been developed, targeting the spike protein of SARS-CoV-2 and NKG2D ligands expressed on the surface of infected cells, and this therapy has progressed to the clinical trial (NCT04324996). Here, we summarize the key clinical trials involving allogeneic CAR cell therapy, including their clinical designs and outcomes (Table 1).

Allogeneic donor-derived, TCR-ablated CAR-T cell therapy for the treatment of B cell malignancies and MM

Healthy donor-derived T cells have been widely utilized in allogeneic CAR cell therapy. These cells have undergone genome editing to mitigate GvHD and have been employed in several clinical trials targeting $CD19^+$ B cell malignancies and $BCMA^+$ MM (Figure 3A).

The first CAR-T cell product tested in clinical trials was UCART19, a genome-edited, donor-derived allogeneic anti-CD19 CAR-T cell therapy designed for the treatment of relapsed or refractory B cell acute lymphoblastic leukemia (ALL).[39,40] For manufacturing, healthy donor PBMCs were activated with anti-CD3 and anti-CD28 ExpAct beads, followed by transduction with a third-generation self-inactivating lentivirus encoding the CD19-targeting CAR. These cells then underwent multiplex gene editing via electroporation with transcription activator-like effector nuclease (TALEN) mRNA targeting the *TRAC* and *CD52* genes.[39,40] The mRNA encoding TALENs was utilized to knock out genes responsible for the TCR α constant chain and CD52. This dual approach aimed to minimize the risk of GvHD by reducing the population of TCR αβ-positive T cells and to conferred resistance to the anti-CD52 monoclonal antibody alemtuzumab.[45]

In total, 21 patients, including 7 pediatric and 14 adult individuals, were enrolled in a multicenter, phase 1 clinical trial to evaluate the safety and antileukemic activity of UCART19 (NCT02808442 and NCT02746952). All patients underwent lymphodepletion with fludarabine and cyclophosphamide, with or without alemtuzumab. Pediatric patients received UCART19 at doses ranging from 1.1 to 2.3×10^6 cells per kg, while adults received doses of 6×10^6 cells, 6 to 8×10^7 cells, or 1.8 to 2.4×10^8 cells in a dose escalation study.[40]

In terms of safety outcomes from the clinical trial, cytokine release syndrome (CRS) was the most prevalent adverse event, affecting 19 patients (91%). Among these, 3 patients (14%) experienced severe grade 3 to 4 CRS. Additional adverse effects noted included grade 1 to 2 neurotoxicity in 8 patients (38%), grade 1 acute skin GvHD in 2 patients (10%), and grade 4 prolonged cytopenia in 6 patients (32%). Two treatment-related fatalities were documented: one caused by neutropenic sepsis in a patient presenting with concurrent CRS, and the other resulting from pulmonary hemorrhage in a patient with persistent cytopenia.[40] Regarding efficacy outcomes, 14 out of 21 patients (67%) achieved either a complete response (CR) or a CR with incomplete hematological recovery within 28 days after infusion.[40]

Overall, the treatment yielded manageable side effects alongside high CR rates, comparable with those seen with autologous CAR-T cell therapies.[40,46,47] UCART19 therapy demonstrated minimal incidence of GvHD, and no detrimental expansion of unedited TCR αβ-positive T cells from the infused product was detected. Nevertheless, the studies underscored several challenges, including the limited persistence of UCART19 and the complications arising from intensive lymphodepletion necessary to prevent host-mediated rejection. These complications included cytopenias and a heightened risk of viral infections.[40]

Another important observation from this trial involved four cancer patients who underwent lymphodepletion with fludarabine and cyclophosphamide, omitting alemtuzumab at the investigator's discretion due to concerns about the associated risk of viral infections. Following the use of alemtuzumab, five patients (24%) experienced grade 3 or higher viral infections, including cytomegalovirus, adenovirus, human metapneumovirus, and BK virus.[40] In contrast, in the four patients at high risk for viral infections who did not receive alemtuzumab, no expansion of UCART19 was observed.[40] This raises important considerations regarding the utilization of CD52 knockout and alemtuzumab strategies, suggesting that further evaluation of their safety and efficacy in clinical trials is warranted.

Another clinical trial is underway using BCMA-targeting allogeneic CAR-T cells, known as ALLO-715, to treat patients with relapsed or refractory MM (NCT04093596).[41] The manufacturing process for ALLO-715 is similar to that of UCART19, utilizing a lentivector encoding a second-generation anti-BCMA CAR. In total, 43 patients underwent lymphodepletion using fludarabine, cyclophosphamide, and alemtuzumab before receiving ALLO-715.[41] Regarding safety, grade ≥3 adverse events were reported in 38 patients (88.0%). CRS was observed in 24 patients (55.8%), with one instance classified as grade ≥3 (2.3%). Neurotoxicity was recorded in 6 patients (14%), with no grade ≥3 events reported. Infections occurred in 23 patients (53.5%), with 10 cases (23.3%) classified as grade ≥3. In terms of efficacy, 24 patients (55.8%) demonstrated a therapeutic response.[41] Among those

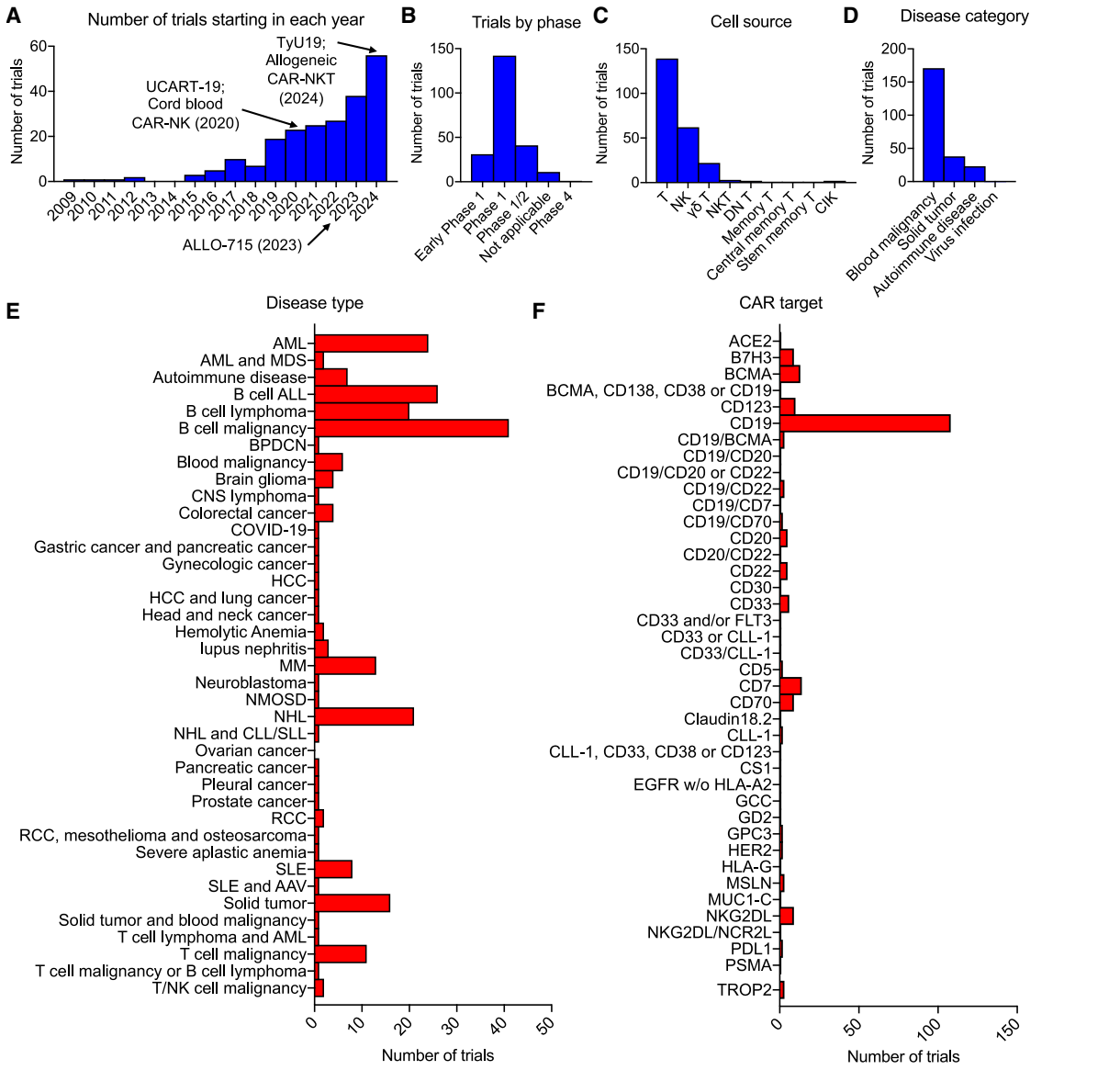

Figure 2. Landscape of clinical trials evaluating allogeneic CAR cell therapy

Distribution of clinical trials by initiated year (A), phase (B), cell source (C), disease category (D), disease type (E), and CAR target (F). Key breakthroughs and their respective reporting years are indicated in (A). DN T, double-negative T cell; CIK, cytokine-induced killer cell; AML, acute myeloid leukemia; MDS, myelodysplastic syndrome; ALL, acute lymphoblastic leukemia; BPDCN, blastic plasmacytoid DC neoplasm; DC, dendritic cell; CNS, central nervous system; HCC, hepatocellular carcinoma; MM, multiple myeloma; NMOSD, neuromyelitis optica spectrum disease; NHL, non-Hodgkin lymphoma; CLL, chronic lymphocytic leukemia; SLL, small lymphocytic lymphoma; RCC, renal cell carcinoma; SLE, systemic lupus erythematosus; AAV, anti-neutrophil cytoplasmic antibody (ANCA)-associated vasculitis; ACE2, angiotensin converting enzyme 2; BCMA, B cell maturation antigen; Claudin18.2, claudin-18 isoform 2; CLL-1, C-type lectin-like molecule-1; EGFR, epidermal growth factor receptor; HLA-A2, human leukocyte antigen A2; GCC, guanylyl cyclase C; GD2, disialoganglioside GD2; GPC3, glypican-3; HER2, human epidermal growth factor receptor 2; HLA-G, human leukocyte antigen G; MSLN, mesothelin; NKG2DL, NKG2D ligand; NCR2L, NCR2/NKp44 ligand; PDL1, programmed death-ligand 1; PSMA, prostate-specific membrane antigen; TROP2, trophoblast antigen 2.

treated with 320×10^6 CAR-T cells, 17 patients (70.8%) showed a response, including 11 patients (45.8%) achieving a very good partial response or better, and 6 patients (25%) achieving either a CR or stringent CR.[41] Overall, this first-in-human phase 1 trial involving heavily pretreated MM patients demonstrates the feasibility and acceptable safety of ALLO-715, along with preliminary evidence of its anti-myeloma efficacy.

Several other clinical trials have evaluated allogeneic donor-derived, TCR-ablated CAR-T cell therapies, utilizing TALENs or CRISPR-Cas9 to disrupt the endogenous TCR while targeting a diverse range of antigens.[48,49] Examples also include CD22-targeting UCART22, CD19/CD20 dual-targeting CTA101, and CD7-targeting WU-CART007, all of which have shown clinical promise.[50–52] Importantly, none of these trials reported cases

Table 1. Manufacturing techniques and clinical outcomes in allogeneic CAR cell clinical trials

Therapeutic cell types	Disease indications	Manufacturing strategy	Preconditioning strategy	Dosing regimen and strategy	Clinical results	ClinicalTrials.gov ID and reference
Allogeneic CD19-targeting CAR-T cells (UCART19)	B cell acute lymphoblastic leukemia (ALL) (7 child patients)	healthy donor PBMCs were stimulated using anti-CD3 and anti-CD28 ExpAct beads, followed by transduction with a lentiviral vector encoding a CD19 CAR. The cells were then subjected to multiplex gene editing through the electroporation of TALEN mRNA targeting the *TRAC* and *CD52* genes	6 patients received lymphodepletion with • fludarabine (150 mg/m^2) • cyclophosphamide (120 mg/kg) • alemtuzumab (1 mg/kg) 1 patient received lymphodepletion with • fludarabine (150 mg/m^2) • cyclophosphamide (120 mg/kg)	1.1–2.3 × 10^6 cells per kg of body weight	safety: 19 patients had CRS, 3 had grade 3–4 CRS, 8 had grade 1–2 neurotoxicity, 2 had grade 1 skin GvHD, 6 had grade 4 prolonged cytopenia, and 2 treatment-related deaths occurred efficacy: 14 patients had a complete response (CR) or CR with incomplete hematological recovery 28 days after infusion	NCT02808442[39,40]
	B cell ALL (14 adult patients)		11 patients received lymphodepletion with • fludarabine (90 mg/m^2) • cyclophosphamide (1,500 mg/m^2) • alemtuzumab (1 mg/kg or 40 mg) 3 patients received lymphodepletion with • fludarabine (90 mg/m^2) • cyclophosphamide (1,500 mg/m^2)	3 dose levels: 6 × 10^6 cells, 6–8 × 10^6 cells, or 1.8–2.4 × 10^8 cells		NCT02746952[40]
Allogeneic BCMA-targeting CAR-T cells (ALLO-715)	multiple myeloma (MM) (43 patients)	ALLO-715 was manufactured by Allogene from healthy donor PBMCs. Activated T cells were transduced with a lentiviral vector encoding a BCMA CAR, followed by multiplex gene editing using TALEN to knock out the *TRAC* and *CD52* genes	patients received lymphodepletion with: • fludarabine (90 mg/m^2) • cyclophosphamide (900 mg/m^2) • alemtuzumab (3-day dose of 30, 60, or 90 mg)	4 dose levels: 40 × 10^6 cells, 160 × 10^6 cells, 320 × 10^6 cells, or 480 × 10^6 cells	safety: 38 patients had grade ≥3 adverse events, 24 had CRS with 1 having ≥3 CRS, 6 had neurotoxicity, 23 had infections with 10 having ≥3 infections efficacy: 24 patients had a response among patients treated with 320 × 10^6 cells, 17 had a response including 11 with very good partial response or better and 6 with a CR/stringent CR	NCT04093596[41]

(*Continued on next page*)

Table 1. *Continued*

Therapeutic cell types	Disease indications	Manufacturing strategy	Preconditioning strategy	Dosing regimen and strategy	Clinical results	ClinicalTrials.gov ID and reference
Allogeneic CD19-targeting CAR-T cells (TyU19)	severe myositis (1 patient) and systemic sclerosis (2 patients)	T cells were isolated from healthy donor PBMCs, transduced with lentivector encoding CD19 CAR, and then were knocked out for HLA-A, HLA-B, CIITA, TRAC, and PD-1 by electroporation-based CRISPR-Cas9 gene editing	patients received lymphodepletion with ● fludarabine (25 mg/m^2) ● cyclophosphamide (300 mg/m^2)	1 × 10^6 cells per kg of body weight	safety: no CRS or serious adverse events efficacy: the infused CAR-T cells persisted for over 3 months, achieving complete B cell depletion within 2 weeks of treatment	NCT05859997[42]
Allogeneic cord-blood-derived CD19-targeting CAR-NK cells	relapsed or refractory NHL (6 patients) and chronic lymphocytic leukemia (CLL; 5 patients)	NK cells were isolated from cord blood unit, stimulated with K562 feeder cells, and transduced with retrovector encoding CD19 CAR, IL-15, and inducible caspase-9	patients received lymphodepletion with ● fludarabine (30 mg/m^2) ● cyclophosphamide (300 mg/m^2)	3 dose levels: 1 × 10^5 cells, 1 × 10^6 cells, or 1 × 10^7 cells per kg of body weight	safety: no CRS neurotoxicity, GvHD, or increased inflammatory cytokines efficacy: 8 patients had a response; of these patients, 7 had a complete remission, and 1 had remission of the Richter's transformation component but had persistent CLL	NCT03056339[16,19]
Allogeneic iPSC-derived CD19-targeting CAR-NK cells	B cell lymphoma (86 patients; 18 patients received regimen A that was CAR-NK monotherapy, and 68 patients received regimen B that was CAR-NK + rituximab)	CAR-NK cells were generating using an iPSC *ex vivo* culture method, expressing a CD19 CAR, IL-15, and high-affinity non-cleavable CD16 Fc receptor[43,44]	patients received lymphodepletion with ● fludarabine (30 mg/m^2) ● cyclophosphamide (500 mg/m^2)	dose escalation commencing at 3 × 10^7 cells as a single dose on day 1 and done independently for individual regimens	safety: no DLT occurred in regimen A, while one DLT in regimen B. CRS was reported in 1 patient in regimen A and in 9 patients in regimen B, but no grade 3 or higher CRS, neurotoxicity, or GvHD of any grade were reported efficacy: for regimen B, in patients with follicular lymphoma, the OR and CR rates were 100% and 85%, respectively; in those with large B cell lymphomas, the OR and CR rates were 38% and 25%, respectively	NCT04245722[21]
Allogeneic CD19-targeting CAR-NKT cells	relapsed or refractory B cell non-Hodgkin lymphoma (NHL) (7 patients) and relapsed B cell ALL (2 patients)	NKT cells were isolated from healthy donor PBMCs, transduced with an anti-CD19 CAR, IL-15, and shRNAs targeting *B2M* and *CD74*	patients received lymphodepletion with ● fludarabine (regimen not reported) ● cyclophosphamide (regimen not reported)	3 dose levels: 10 × 10^6 cells, 30 × 10^6 cells, or 300 × 10^6 cells	safety: no early adverse events except grade 1 CRS in one patient efficacy: of the 7 NHL patients, 3 had an initial partial response and 2 evolved into a CR; 1 ALL patient achieved a CR with incomplete hematologic recovery	NCT00840853[20]

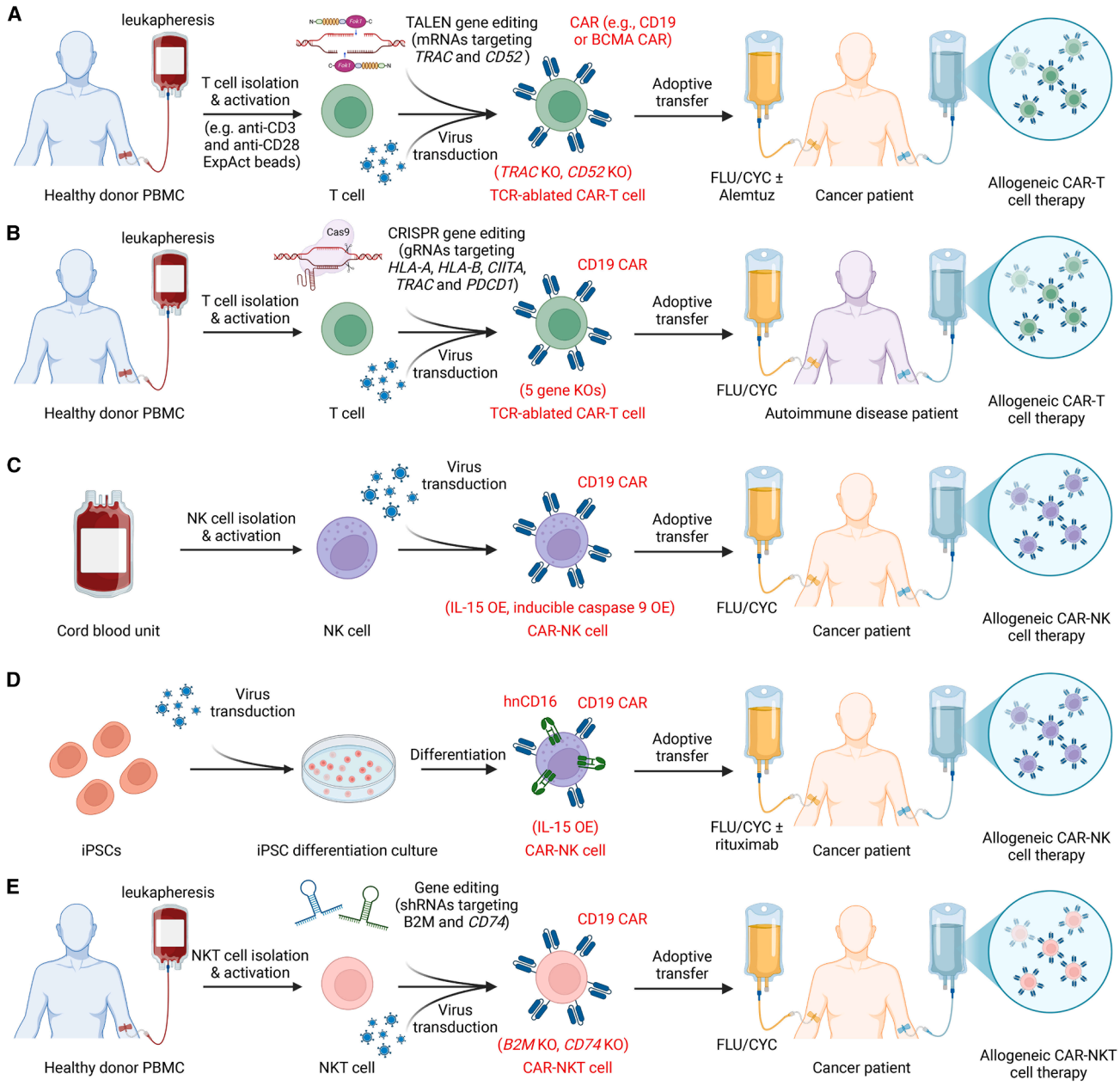

Figure 3. Examples of clinical designs in allogeneic CAR cell therapy

Five categories of allogeneic CAR cell therapies have been successfully implemented in clinical trials, demonstrating promising anti-tumor efficacy and safety. These include healthy donor PBMC-derived TCR-ablated CAR-T cell therapy for cancer (A) and autoimmune diseases (B), cord-blood-derived CAR-NK cell therapy for cancer (C), iPSC-engineered CAR-NK cell therapy for cancer (D), and healthy donor PBMC-derived CAR-NKT cell therapy for cancer (E). PBMC, peripheral blood mononuclear cell; TALEN, transcription activator-like effector nuclease; KO, knockout; OE, overexpression; FLU, fludarabine; CYC, cyclophosphamide; Alemtuz, alemtuzumab; shRNA, short hairpin RNA.

of GvHD. The incidence of grade 3 or higher CRS varied, with no cases observed in CTX130, 16.7% in CTA101, and 31% in WU-CART007.[50–52] Immune effector cell-associated neurotoxicity syndrome (ICANS) was observed in no more than 10% of patients across all trials.[50–52] In terms of efficacy, UCART22 achieved response rates of 67% at dose level 2 (DL2) and 50% at DL3.[50] CTA101 demonstrated that 60% of patients achieved CR or CR with incomplete hematologic recovery (CRi), with sustained minimal residual disease (MRD) negativity.[51] WU-CART007 reported an objective response rate of 43% at DL2, with CR/CRi patients maintaining MRD negativity and a median response duration of 86 days[52]

Additionally, a trial evaluating CD70-targeting CTX130, which incorporates TRAC CAR knockin rather than simple TCR

knockout, demonstrated a favorable safety profile with no reported GvHD, dose-limiting toxicities (DLTs), grade 3+ CRS, or ICANS.[53] This trial also reported disease control in 81.3% of patients.[53] Overall, these findings underscore the clinical potential of allogeneic donor-derived CAR-T cell therapy, highlighting both its promising efficacy and favorable safety profile in cancer treatment.

Allogeneic donor-derived, multiplex genome-edited, TCR-ablated CAR-T cell therapy for the treatment of severe myositis and systemic sclerosis

The application of CAR-T cell therapy for treating autoimmune diseases is emerging as a novel and promising trend.[38] Recent clinical trials have demonstrated sustained and significant elimination of autoreactive B cells through autologous CD19- and/or BCMA-targeting CAR-T cells, leading to effective control of autoimmune conditions with minimal safety concerns.[54–57] A notable clinical trial utilized an allogeneic CD19-targeting CAR-T cell product known as TyU19 to treat various autoimmune diseases, including severe myositis and systemic sclerosis (Figure 3B).

This CAR-T cell therapy involved multiplex gene editing to ablate five specific molecules via electroporation-based CRISPR-Cas9 gene editing: HLA-A, HLA-B, CIITA, TRAC, and PD-1.[42] The *TRAC* gene was knocked out to disrupt TCR expression, thereby reducing the risk of GvHD. HLA-A, HLA-B, and CIITA were targeted to eliminate HLA-I and HLA-II molecules, minimizing the likelihood of host T cell-mediated allorejection. Additionally, PD-1 knockout was performed to alleviate immune checkpoint inhibition. Notably, while multiple gene knockouts were achieved, the CAR transduction process demonstrated sufficient efficacy, yielding a CAR-positive cell percentage of 29.79%, with TRAC, HLA-A, HLA-B, and CIITA knockout rates of 99.80%, 77.49%, 91.01%, and 84.81%, respectively.[42] Importantly, the high gene editing efficiency did not compromise CAR-T cell yields, with each batch producing over 100 patient doses.

The trial recruited a total of three patients: one with severe immune-mediated necrotizing myopathy and two with diffuse cutaneous systemic sclerosis. Patients underwent a standard lymphodepleting regimen, which included 25 mg/day/m^2 of fludarabine from day −5 to day −3, along with 300 mg/day/m^2 of cyclophosphamide on days −5 and −4. Each patient subsequently received an intravenous infusion of 1×10^6 CAR-positive TyU19 cells/kg.[42]

The TyU19 therapy demonstrated an excellent safety profile. Post-infusion, none of the three patients exhibited symptoms indicative of CRS or GvHD, and vital signs remained stable throughout the monitoring period.[42] In terms of efficacy, the infused cells persisted for over 3 months, achieving complete B cell depletion within 2 weeks of treatment. During a 6-month follow-up, deep remission was observed, as demonstrated by significant improvements in clinical response index scores for both conditions, alongside documented reductions in inflammation and fibrosis.[42] Specifically, TyU19 effectively alleviated severe skeletal muscle damage in the patient with refractory immune-mediated necrotizing myopathy, and reversed extensive fibrotic damage in critical organs of both patients with diffuse cutaneous systemic sclerosis.[42]

In summary, this clinical trial underscores the potential of allogeneic off-the-shelf CAR-T cell therapy in improving the management of severe refractory autoimmune diseases. Unlike conventional management strategies that rely on ongoing immune suppression, the responses observed in all three patients persisted even after the normalization of lymphocyte levels. Although the sample size was limited, this study clearly demonstrates the feasibility and safety of employing allogeneic CAR-T cells in treating autoimmune diseases. Given that autoimmune conditions typically do not pose an immediate threat to life as cancer does, the safety criteria for cell therapy in these patients should be more stringent.[38] While only a single dose of 1×10^6/kg CAR-T cell product was administered to each patient, with minimal side effects reported, higher doses or multiple infusions could be safely explored in future studies. There remains significant potential for advancing allogeneic CAR-T cell therapy in the treatment of autoimmune disorders.

Allogeneic cord-blood-derived CAR-NK cell therapy for the treatment of B cell malignancies

Innate immune cells, such as NK cells and macrophages, possess significant anti-tumor capabilities and do not induce GvHD. These cells have been extensively utilized in allogeneic cell therapy. However, compared with CAR-T cells, CAR-NK cells exhibit limitations, including restricted expansion and persistence *in vivo*. This necessitates additional genetic engineering, such as incorporating IL-15, to enhance their functional performance within the host.[15,16,58,59] Furthermore, CAR-NK cells are sensitive to cryopreservation, making careful storage and timely administration into patients essential. Notably, NK cells derived from allogeneic sources, including cord blood or iPSCs, can be safely administered without requiring full HLA matching.[60] Allogeneic NK cells have demonstrated a strong safety profile after infusion for adoptive immunotherapy in cancer patients.[61,62]

A recent clinical phase 1 and 2 trial investigated the use of allogeneic CD19-targeting CAR-NK cells to treat patients with relapsed or refractory NHL or chronic lymphocytic leukemia (CLL) (NCT03056339; Figure 3C).[16,19] In this study, CAR-NK cells were generated from HLA-mismatched cord blood. Briefly, the cord blood unit was thawed, and NK cells were purified and cultured in the presence of engineered K562 feeder cells and IL-2. On day 6, these NK cells were transduced using a retroviral vector expressing genes encoding the CD19 CAR, IL-15, and inducible caspase-9 (iC9) as a safety switch. The efficiency of CAR transduction for the infused CAR-NK cell product was approximately 49.0%.[19]

The CAR-NK cells were administered in a single infusion at one of three doses (1×10^5, 1×10^6, or 1×10^7 CAR-NK cells per kg of body weight) after lymphodepleting chemotherapy with fludarabine (30 mg/m^2) and cyclophosphamide (300 mg/m^2), administered daily for 3 consecutive days.[19]

The safety profile of the allogeneic CAR-NK cells was impressive. The infusion of CAR-NK cells did not lead to the development of CRS, neurotoxicity, or GvHD, and there was no observed increase in inflammatory cytokines, including IL-6, above baseline levels.[19] Regarding efficacy, of the 11 treated

patients, 8 (73%) exhibited a clinical response; among these, 7 patients (4 with lymphoma and 3 with CLL) achieved complete remission, while 1 patient experienced remission of the Richter's transformation component but had persistent CLL. Notably, responses were rapid, manifesting within 30 days post-infusion across all dosage levels. The infused CAR-NK cells expanded and maintained low levels of persistence for at least 12 months[19]

As the first-in-human trial of allogeneic CAR-NK cell therapy, this clinical study highlights the promising safety results associated with CAR-NK cell products, even with IL-15 engineering. The absence of serious toxicity during the trial indicated that the caspase-9 safety switch (activated by rimiducid) was not required for eliminating the CAR-NK cells. However, it should be noted that, as no patients received cryopreserved and thawed CAR-NK cells, and still demonstrated anti-tumor responses,[19] it poses a unique challenge. Consequently, optimal arrangements between hospitals and manufacturing facilities are needed to ensure that freshly cultured cells are timely and properly administered to cancer patients. While this requirement may limit the true off-the-shelf potential of CAR-NK cell therapy, strategic advancements should be considered to facilitate their accessibility and utility in clinical settings.

Allogeneic iPSC-derived CAR-NK cell therapy for the treatment of B cell malignancies

In addition to cord-blood-derived CAR-NK cells, which primarily involve the activation and transduction of human endogenous NK cells, a recent clinical trial has reported on the use of iPSC-engineered $CD19^+$ CAR-NK cell therapy, specifically FT596, for the treatment of patients with B cell malignancies (NCT04245722; Figure 3D).[21] This innovative approach combines genetic engineering with *ex vivo* stem cell differentiation technology, facilitating the generation of CAR-NK cells with high yield and potent anti-tumor efficacy.[43,44]

FT596 incorporates three anti-tumor modalities: a CD19 CAR, a high-affinity, non-cleavable CD16 Fc receptor, and an IL-15/IL-15 receptor fusion. In this clinical trial, FT596 was evaluated both as monotherapy (regimen A) and in combination with rituximab (an anti-CD20 monoclonal antibody) (regimen B).[21] The latter regimen benefits from CD16-mediated antibody-dependent cellular cytotoxicity, providing dual tumor-antigen recognition that efficiently mitigates tumor antigen escape.[44,58]

In the trial, a total of 86 patients with B cell lymphoma received FT596; 18 patients were treated with FT596 monotherapy, while 68 patients received the combination with rituximab. Each treatment cycle consisted of conditioning chemotherapy with cyclophosphamide (500 mg/m^2) and fludarabine (30 mg/m^2) administered intravenously on days −5 to −3, followed by FT596 at various doses and schedules, with or without a single dose of rituximab (375 mg/m^2) intravenously on day −4.[21]

The safety profile of FT596 was notably positive, with no significant differences in adverse events between regimen A and regimen B. No DLTs occurred in regimen A, while one DLT with prolonged grade 4 thrombocytopenia was observed in regimen B. CRS was reported in 1 (6%) of 18 patients in regimen A (maximum grade 1) and in 9 (13%) of 68 patients in regimen B, but no grade 3 or higher CRS, neurotoxicity, or GvHD of any grade were reported.[21] Importantly, 39 patients received a second cycle of FT596 after the first cycle, with no increase in overall adverse events or grade 3 or higher toxicities compared with the first cycle. This includes no increase in grade 3 or higher neutropenia, thrombocytopenia, or instances of infection, suggesting that the safety profile of FT596 is promising.[21]

Given that FT596 incorporates the high-affinity CD16 component, which potentially enhances therapeutic activity when administered alongside monoclonal antibodies, the evaluation of activity was particularly focused on patients receiving regimen B.[21] In patients with follicular lymphoma, the objective response (OR) rate was 100%, with an 85% CR rate. In those with large B cell lymphomas, the OR rate was 38%, and the CR rate was 25%. When excluding *de novo* diffuse large B cell lymphoma, the OR and CR rates in this subgroup were notably higher at 82% and 64%, respectively.[21] However, because this report did not include data from regimen A, it is challenging to ascertain the anti-tumor efficacy of FT596 on its own. The overall enhanced efficacy observed in the combination regimen may be attributed to the administration of rituximab, which also possesses intrinsic anti-tumor activity. Therefore, careful and comprehensive evaluation of the allogeneic CAR-NK cell products is essential.

Overall, this clinical trial demonstrates high safety and encouraging preliminary efficacy in patients who had undergone extensive prior treatments, including hematopoietic stem cell transplantation and autologous CAR-T cell therapy.[21] iPSCs represent an unlimited source for generating allogeneic CAR-NK cells and other cell types. This technology allows for the uniform production of multiple doses of a given iPSC-derived cell product from a well-characterized, clonal master cell bank with defined transgene copy numbers and integration sites. Furthermore, these products can be cryopreserved to maintain a sustainable inventory. The uniformity of transgene expression ensures consistent treatment products with predictable clinical characteristics across patients.[25,26,63,64] Consequently, these iPSC-derived products enable the administration of multiple doses of therapeutic cells to cancer patients, potentially enhancing anti-tumor efficacy outcomes. The success of the FT596 clinical trial indicates the feasibility of employing iPSC technology to develop CAR-engineered therapeutic cells for cancer treatment, including iPSC-derived CAR-T cells, CAR-NKT cells, and CAR macrophages.[27,64–67] Importantly, the efficient gene-editing capabilities of iPSCs allow for the simultaneous modification of multiple genes, and this approach can optimize both the anti-tumor efficacy and safety profiles of iPSC-derived cells, ultimately leading to improved clinical outcomes.

Allogeneic donor-derived CAR-NKT cell therapy for the treatment of B cell malignancies

NKT TCRs specifically recognize the non-polymorphic MHC molecule CD1d, which significantly reduces the risk of inducing GvHD.[68–72] Consequently, both NKT and CAR-NKT cells are more suitable for use in allogeneic settings, offering a higher safety profile compared with conventional T cells and CAR-T cells. Although the development of CAR-NKT cell therapy has progressed more slowly than that of CAR-T cell therapy, several clinical trials have demonstrated the safety and

efficacy of CAR-NKT cells, including trials involving autologous GD2-targeting CAR-NKT cells to treat pediatric patients with neuroblastoma.[73,74]

Recently, a clinical trial reported the use of allogeneic CD19-targeting CAR-NKT cell therapy for patients with relapsed or refractory B cell malignancies (NCT00840853) (Figure 3E). These NKT cells were isolated from the leukapheresis product of a single HLA-unmatched healthy donor. The CAR-NKT cells were supposed to co-express a CD19-targeting CAR, soluable IL-15, and short hairpin RNAs (shRNAs) targeting *B2M* and *CD74* to downregulate HLA-I and HLA-II molecules, respectively.[20]

Patients were enrolled across three dose levels: 1×10^7 (DL1), 3×10^7 (DL2), and 1×10^8 (DL3) CAR-NKT cells/m^2, administered after lymphodepleting conditioning with cyclophosphamide and fludarabine.[20] Seven patients with relapsed/refractory B cell NHL (cohort A) were enrolled at all three dose levels, while two patients with relapsed B cell ALL (cohort B) were enrolled at DL1.

Notably, there were no early adverse events attributable to the allogeneic CAR-NKT cell product, aside from grade 1 CRS observed in one patient. Among the seven NHL patients (six with diffuse large B cell lymphoma and one with follicular lymphoma), three exhibited an initial partial response, with two cases evolving into a CR. One patient with ALL achieved a CR along with full hematologic recovery, showing no evidence of leukemia at the 4-week follow-up.[20]

In conclusion, initial results from the first-in-human clinical evaluation of allogeneic CAR-NKT cells suggest that these cells are well tolerated and can mediate ORs in patients with relapsed or refractory NHL and ALL, even at low doses.[20] Although the full results have yet to be reported and the outcomes of allorejection and shRNA knockdown efficacy remain to be evaluated, these findings indicate the feasibility of allogeneic CAR-NKT cell therapy for treating hematological cancers. Future research directions will be essential to explore the potential of allogeneic CAR-NKT cell therapies in addressing solid tumors and autoimmune diseases.[75,76]

GENETIC ENGINEERING STRATEGIES IN CLINICAL TRIALS

Innovations in CAR designs

CAR design has undergone significant evolution, transitioning from first-generation constructs to second- and third-generation CARs that incorporate co-stimulatory domains to enhance signaling and improve therapeutic efficacy. Despite these advancements, several key challenges persist, including antigen escape, on-target off-tumor toxicity, and CAR-T cell exhaustion. To address these limitations, next-generation CAR constructs are being developed to improve specificity, durability, and safety. Among the most promising strategies under clinical investigation are bispecific CARs, logic-gated CARs, and the synthetic TCR and antigen receptor (STAR) platform.[77–79] These innovations collectively represent a paradigm shift in CAR-based immunotherapy, with the potential to enhance therapeutic outcomes while minimizing toxicities.

One of the primary limitations of conventional CAR-T cell therapy is tumor antigen escape, a phenomenon in which malignant cells downregulate or alter target antigens to evade immune detection.[80,81] To mitigate this issue, bispecific CARs have been engineered to recognize and engage two distinct tumor-associated antigens, reducing the likelihood of immune evasion and enhancing therapeutic durability. Several ongoing clinical trials are evaluating bispecific CAR constructs in hematologic malignancies. Trials such as NCT04499573 and NCT03463928 investigate allogeneic CD19/CD22 CAR-T therapies in relapsed or refractory leukemia, while NCT03824964 assesses a CD19/CD22 CAR-NK therapy for B cell lymphoma. Additionally, NCT05507827 explores CD19/CD22 CAR-T cells combined with regulatory T cell-enriched hematopoietic stem cell transplants to mitigate GvHD. Beyond CD19/CD22, other bispecific CAR constructs targeting CD19/CD20 (NCT06014762), CD20/CD22 (NCT05607420), CD33/CLL-1 (NCT05987696), and CD19/CD70 (NCT05667155, NCT05842707) are being explored in clinical trials.

While CAR-T cell therapies have achieved significant success in hematologic malignancies, their application in solid tumors has been hampered by challenges such as on-target off-tumor toxicity. To improve tumor specificity and minimize adverse effects, logic-gated CARs have been developed, incorporating sophisticated activation mechanisms that restrict CAR activity to tumor-specific environments. One such approach is the Tmod system, which employs a dual-receptor logic gate to enhance selectivity.[82] The NCT06682793 trial is evaluating this system in EGFR-positive solid tumors exhibiting HLA-A*02 loss of heterozygosity. The Tmod platform integrates an activator CAR that recognizes EGFR and a blocker receptor that detects HLA-A*02 expression, ensuring selective CAR activation in tumor cells lacking HLA-A*02 while sparing normal cells that retain it. This strategy represents a significant advancement in the application of CAR-T therapy to solid tumors, offering improved tumor selectivity and reduced toxicity. Similarly, the SENTI-202 trial (NCT06325748) investigates a logic-gated CAR-NK cell therapy for AML and myelodysplastic syndromes. SENTI-202 employs a TME-dependent activation mechanism, ensuring that CAR-NK cells are only activated in response to tumor-specific antigen combinations, thereby reducing systemic toxicity and expanding the potential of CAR-NK therapy beyond hematologic malignancies.[83]

Another major limitation of CAR-T therapies is their susceptibility to functional exhaustion, which is partly attributed to intrinsic defects in CAR signaling. To address this issue, the synthetic STAR platform has been developed. Unlike conventional CAR constructs, STAR integrates an antigen-recognition domain derived from an antibody with constant regions from the TCR, allowing it to engage endogenous CD3 signaling pathways.[84] This design prevents tonic signaling in the absence of antigen, thereby reducing exhaustion and promoting long-term persistence. Upon antigen engagement, STAR mediates robust and physiologically relevant TCR-like signaling, leading to enhanced proliferation and reduced dysfunction compared with traditional CAR-T cells. In preclinical models of solid tumors, STAR-T cells have demonstrated superior efficacy compared with both BBζCAR-T and 28ζCAR-T cells, with a favorable toxicity profile.[84] Currently, the STAR-T platform, which utilizes CRISPR-Cas9-mediated disruption of *TRAC* to integrate an

anti-CD19-STAR construct, is under clinical evaluation in the NCT05631912 trial for patients with relapsed or refractory B cell NHL.

The ongoing advancements in CAR engineering are reshaping the clinical landscape of both autologous and allogeneic CAR cell therapy, paving the way for more effective and safer immunotherapies. Next-generation CAR designs, including bispecific CARs, logic-gated CARs, and STAR constructs, offer promising strategies to overcome antigen escape, improve tumor specificity, and mitigate exhaustion in allogeneic cell therapy. While these innovations hold great potential for enhancing clinical outcomes in both hematologic and solid malignancies, further clinical validation is necessary to confirm their efficacy and safety.

Additional gene engineering strategies

The integration of gene editing technologies has significantly advanced allogeneic CAR-T and CAR-NK therapies by enhancing their safety, persistence, and efficacy. Various strategies, including CRISPR-Cas9 and TALEN, have been implemented to improve immune evasion, introduce safety mechanisms, and enhance tumor-targeting capabilities. These approaches have revolutionized adoptive cell therapy, expanding its clinical applicability across hematologic and solid malignancies.

Cytokine engineering has emerged as a critical strategy for enhancing the efficacy of CAR-T and CAR-NK cell therapies by modulating their survival, proliferation, and functional properties. A prominent approach involves the co-expression of IL-15, which has been shown to enhance the persistence and cytotoxic potential of engineered immune cells in several clinical trials evaluating allogeneic CAR-NK and CAR-NKT cell products (NCT04245722, NCT06342986, NCT04623944, NCT05182073, NCT06733935, and NCT03774654). Beyond IL-15, IL-10 has been incorporated into CAR constructs to enhance the persistence and functionality of cord-blood-derived NK cells, with its therapeutic efficacy currently being investigated in refractory or relapsed B cell NHL (NCT06707259). Collectively, these studies underscore the importance of cytokine engineering in advancing next-generation CAR-based immunotherapies.

Gene editing technologies are widely used to enhance the safety and efficacy of allogeneic CAR-T therapies by reducing GvHD and immune rejection. Key strategies include knocking out endogenous TCRs to minimize GvHD, as seen in the NCT05350787 trial with ThisCART19A, where TCR$\alpha\beta$/CD3 complex retention in the endoplasmic reticulum prevents surface expression and reduces alloreactivity.[85] Similarly, NCT04093596 evaluated ALLO-715, using CRISPR-Cas9 to disrupt TCR and HLA, minimizing GvHD and immune rejection.[41] In NCT05336409, iPSC-derived CAR-NK cells targeting CD19 had *B2M* and *CIITA* knockouts to reduce T cell-mediated rejection, with HLA-E introduced to counter NK cell clearance.[16,19] TALEN-based genome editing has also been utilized in UCART19, where *TRAC* and *CD52* gene knockouts enhance persistence and reduce alloreactivity in patients with B-ALL.[39,40] Additionally, CRISPR-Cas9-mediated CAR knockin into the TRAC locus is being investigated in several trials (NCT06680037, NCT05757700, NCT03666000, NCT04629729) to ensure consistent CAR expression and reduce alloreactivity.[86] The ANCHOR trial (NCT00840853) explores CAR-NKT cells with shRNA targeting B2M and CD74 to enhance immune evasion and prolong persistence.[20] Collectively, these advancements highlight the pivotal role of gene editing in optimizing allogeneic CAR-based immunotherapies, offering innovative approaches to improve safety, durability, and therapeutic efficacy.

Inducible suicide switches have also been integrated into CAR constructs to enable controlled elimination of engineered immune cells in cases of severe toxicity. The iC9 system, the most widely used, allows for pharmacologically triggered depletion of CAR-T or CAR-NK cells, reducing risks such as CRS and neurotoxicity. In the NCT03056339 clinical trial, CAR-NK cells engineered with anti-CD19 CAR, IL-15, and iC9 have demonstrated enhanced safety through this approach.[16,19] Another safety mechanism involves a truncated epidermal growth factor receptor (tEGFR) switch, incorporated into an IL-15-expressing, iPSC-derived CAR-NK cell product for treating relapsed or refractory CD19-positive B cell malignancies (NCT05336409).[87] This tEGFR variant, which includes a cetuximab-binding epitope, enables selective depletion of infused cells when needed, providing an additional safeguard in CAR-based therapies.

Another gene engineering strategy involves the knockout of CD38 in donor-derived CAR-NK cells to prevent antibody-mediated fratricide. This fratricide occurs when anti-CD38 monoclonal antibodies, such as daratumumab or isatuximab, bind to CD38 on NK cells, triggering Fc receptor-mediated cytotoxicity and leading to NK cell depletion.[88] By eliminating CD38 expression, this approach enhances NK cell survival and functionality, thereby improving their therapeutic efficacy (NCT06342986 and NCT05182073). Additionally, bispecific T cell engager (BiTE) technology has been integrated into gene-edited CAR platforms to strengthen anti-tumor responses. In the ongoing clinical trial NCT06150885, Vδ2 $\gamma\delta$ T cells are engineered via mRNA electroporation to express a nanobody-based HLA-G-targeting CAR while simultaneously secreting a PD-L1/CD3ε BiTE construct. This design recruits bystander T cells by bridging PD-L1 on tumor cells with CD3ε on T cells, amplifying the immune response. Furthermore, PD-1 knockout has emerged as a key strategy to enhance the persistence and cytotoxic function of allogeneic CAR-T cells by overcoming tumor-induced exhaustion. The phase 1 clinical trial NCT04637763 incorporated CRISPR-Cas9-mediated PD-1 knockout, demonstrating a manageable safety profile and promising efficacy for NHL patients.[89]

The continued integration of gene editing and cytokine engineering into CAR-T and CAR-NK therapies represents a major advancement in immunotherapy, offering unprecedented control over safety, persistence, and efficacy. As these innovations move through clinical trials, they hold the potential to overcome key limitations of adoptive cell therapy, paving the way for broader clinical applicability. With ongoing refinements and emerging technologies, gene-engineered CAR-based therapies are poised to reshape the landscape of cancer treatment and beyond.

PRECONDITIONING STRATEGIES IN CLINICAL TRIALS

Lymphodepletion is a key component in the success of CAR-based cell therapies, significantly improving their therapeutic

outcomes.[90] This process typically involves the administration of chemotherapy or lymphodepleting agents before the infusion of CAR-engineered cells. By reducing the patient's lymphocyte levels, lymphodepletion facilitates a more favorable environment for the engraftment and expansion of the infused therapeutic cells.

In all clinical trials of allogeneic CAR-T, CAR-NK, and CAR-NKT cell therapies with reported results, patients underwent at least a standard preconditioning regimen before receiving CAR-engineered cell products, similar to the preconditioning used in approved autologous CAR-T therapies (Table 1).[6,19,21,42,90,91] This typical lymphodepletion regimen consisted of fludarabine and cyclophosphamide (Flu/Cy), typically administered intravenously from day −5 to day −3 before the infusion of therapeutic cells. The only exception was UCART19 therapy, where lymphodepletion lasted for 7 days before UCART19 infusion on day 0.[40] The dosage of these lymphodepletion agents varied depending on the trial. For example, in TyU19 therapy, Flu was given at 25 mg/day/m^2 and Cy at 300 mg/day/m^2, while in FT596 therapy, Flu was administered at 30 mg/day/m^2 and Cy at 500 mg/day/m^2.[21,42]

In addition to the standard lymphodepletion regimen, some trials incorporated additional treatments to mitigate adverse events and/or enhance efficacy outcomes. For instance, in the UCART19 trial, 81% of patients received the CD52 monoclonal antibody alemtuzumab, which targets T cells and malignant cells, as part of the preconditioning regimen.[40,92] The remaining 19% of patients were excluded from alemtuzumab treatment due to concerns about viral infection risks, as per the investigator's discretion.[40] In cases with high tumor burden, cytoreduction was also allowed before initiating lymphodepletion.[40] Similarly, in the ALLO-715 trial, all 43 patients received anti-CD52 monoclonal antibody ALLO-647 over a 3-day period as part of the preconditioning regimen.[41] In the case of cord-blood-derived CAR-NK therapy (NCT03056339), patients also received Mesna before and after the Cy dose from day −5 to day −3 to prevent bladder toxicity induced by cyclophosphamide.[16,19] This approach to preconditioning was tailored to optimize both the safety and efficacy of the CAR-engineered cell therapies.

DOSING REGIMENS AND STRATEGIES IN CLINICAL TRIALS

Dosage size

The dosage regimen of allogeneic cell therapies in clinical trials varies across different products, influenced by study design, patient population, and disease indication (Table 1). Dosage can be determined based on either body weight or a fixed cell number. In the UCART19 trial for pediatric B-ALL, therapeutic cells were infused at 1.1–2.3 × 10^6 cells/kg, whereas in the TyU19 trial for severe myositis and systemic sclerosis, the infusion dose was 1 × 10^6 cells/kg.[39,40,42] Similarly, dose escalation was employed in a clinical trial using allogeneic cord-blood-derived CD19-targeting CAR-NK cells, with doses of 1 × 10^5, 1 × 10^6, or 1 × 10^7 cells/kg.[16,19] Among these trials, UCART19 was associated with grade ≥3 CRS and prolonged cytopenia, whereas the other therapies did not report severe adverse events.[39,40] For fixed-dose regimens, the lowest dose was 6 × 10^6 cells in UCART19 treatment for B-ALL, while the highest dose was 480 × 10^6 cells in the ALLO-715 trial for MM, where grade ≥3 adverse events occurred in 88% of patients.[39,40,57] Notably, two cases demonstrated high-dose administration without severe toxicity. In one case, allogeneic iPSC-derived CD19-targeting CAR-NK cells were infused at an initial dose of 3 × 10^7 cells, with no grade ≥3 CRS, neurotoxicity, or GvHD observed.[21] Therapeutic efficacy was demonstrated by a 100% OR rate in follicular lymphoma and a 38% OR rate in B cell lymphoma. In another case, CAR-NKT cells were administered at 300 × 10^6 cells, with only grade 1 CRS reported, achieving an OR rate of 43%.[20] These findings highlight the complex relationship between dosage, cell type, and safety profile in allogeneic cell therapies. While T cell-based products such as UCART19 and ALLO-715 show dose-dependent toxicity, iPSC-NK- and NKT-based therapies demonstrate a favorable safety profile even at high doses. These observations imply the potential of alternative immune cell platforms in mitigating severe adverse events while maintaining therapeutic efficacy, informing future strategies for optimizing dosing regimens in allogeneic cell therapy.

Dosing schedules

Clinical trials for allogeneic CAR-based cell therapies typically follow a standardized dosing regimen, beginning with a pre-infusion lymphodepletion phase, followed by a single infusion of therapeutic cells, with or without additional regimen treatment according to the study design. This section focuses on the pre-infusion schedule, as infusion protocols remain largely uniform across trials. The conditioning regimen commonly includes fludarabine and cyclophosphamide, both of which play critical roles in creating an optimal environment for infused cells. Fludarabine depletes lymphocytes by inhibiting DNA synthesis, thereby impairing immune cell proliferation. Cyclophosphamide induces broad myeloablation, effectively suppressing residual T cells and mitigating anti-CAR immune responses.[93] The dose of fludarabine varies between 25 and 150 mg/m^2, whereas cyclophosphamide dosing ranges from 300 to 1,500 mg/m^2, with some studies using weight-based dosing, such as 120 mg/kg for pediatric patients.[16,19,39,40,42] While fludarabine and cyclophosphamide are widely employed for pre-conditioning, additional lymphodepletion strategies may be required in specific settings. In trials involving CD52 knockout allogeneic CAR-T cells, an anti-CD52 monoclonal antibody alemtuzumab is incorporated to further deplete host T and NK cells and minimize GvHD.[45] For instance, in the UCART19 trial, alemtuzumab was co-infused with fludarabine and cyclophosphamide, while the ALLO-715 trial administered alemtuzumab over 3 days at doses of 39, 60, or 90 mg.[39–41] In contrast, trials involving CAR-NK cells typically omit alemtuzumab while maintaining robust efficacy and a favorable safety profile. As detailed in previous sections, CAR-NK cells have demonstrated effective tumor control while reducing toxicity risks, including GvHD and severe CRS. These findings suggest that allogeneic CAR-NK therapies may require less aggressive lymphodepletion, potentially enhancing their clinical applicability, especially in settings where immune suppression-related complications need to be minimized.

While infusion protocols remain largely uniform across trials, the exploration of multiple dosing strategies is an area of active research. Emerging strategies involve administering multiple infusions of CAR-T cells to sustain therapeutic pressure on tumors, particularly in cases where a single infusion may be insufficient. This method is under investigation to determine its efficacy in enhancing overall treatment outcomes.[94] Incorporating multi-dosing strategies into allogeneic CAR-based cell therapy protocols necessitates careful consideration of factors such as dosing intervals, cumulative cell doses, and patient-specific characteristics. Ongoing clinical trials continue to evaluate the safety and efficacy of these approaches, aiming to refine treatment paradigms and improve patient outcomes.

PRECLINICAL DEVELOPMENT OF ALLOGENEIC CAR CELL THERAPY

Building on the progress observed in clinical studies of allogeneic CAR-T, CAR-NK, and CAR-NKT therapies, preclinical research continues to expand the scope of allogeneic cell therapy by exploring novel CAR-engineered immune cell types and optimizing manufacturing strategies. While allogeneic CAR-T cell therapies face challenges such as GvHD and immune rejection, alternative cell sources, including CAR-NKT, CAR-γδ T, and CAR-mucosal-associated invariant T (MAIT) cells, offer distinct advantages due to their natural resistance to GvHD and unique tumor-targeting mechanisms.[75] These unconventional T cell subsets are being extensively studied to address both hematologic malignancies and solid tumors, with a focus on enhancing persistence, safety, and therapeutic efficacy. Preclinical efforts also aim to improve the scalability and consistency of allogeneic cell production through advanced genetic engineering and innovative cell sourcing strategies, such as the use of HSPCs and iPSCs.

Based on the promising safety and efficacy of donor-derived CAR-NKT cells in clinical trials for B cell malignancies, ongoing preclinical studies are exploring strategies to overcome a major limitation: the low abundance of NKT cells in human peripheral blood, which typically constitute only 0.001%–1% of total blood cells.[95–97] This scarcity presents a significant challenge for generating consistent and sufficient numbers of allogeneic NKT cells suitable for CAR engineering. To address this, alternative cell sources such as HSPCs from cord blood and iPSCs are being investigated. For instance, Li et al. developed universal CAR-NKT (^{U}CAR-NKT) cells by engineering HSPCs to express an invariant NKT TCR while ablating HLA genes to prevent allorejection.[23,24] These cells were differentiated *ex vivo* using a clinically guided culture method,[22,98] yielding high-purity and functionally robust ^{U}CAR-NKT cells.[23,24] Preclinical studies demonstrated that these cells effectively targeted both hematologic malignancies and solid tumors through multiple mechanisms, including cytotoxicity against tumor cells and selective depletion of immunosuppressive tumor-associated macrophages (TAMs) and myeloid-derived suppressor cells.[23,24] Similarly, Urakami et al. successfully engineered HER2-targeting CAR-NKT cells derived from iPSCs under feeder-free conditions while maintaining stable CAR expression.[99] *In vitro*, these cells exhibited strong cytotoxicity against HER2-positive cancer cell lines.[99] In a xenograft model using SK-OV-3 ovarian cancer cells, HER2 CAR-NKT cell treatment significantly prolonged survival compared with control or non-CAR NKT cell groups.[99]

CAR-γδ T cells are gaining significant attention in both preclinical and clinical studies due to their innate ability to recognize infected or malignant cells without MHC restriction. This unique property enables their broader and safer application in allogeneic settings. A study by Nishimoto et al. successfully demonstrated large-scale manufacturing of anti-CD20 CAR-γδ T cells from healthy donor-derived Vδ1 T cells.[100] These cells were expanded using an agonist anti-Vδ1 monoclonal antibody and showed potent tumor-killing activity *in vitro*, along with robust cytokine production.[100] *In vivo*, anti-CD20 CAR-Vδ1 T cells effectively suppressed tumor growth in B cell lymphoma xenografts.[100] In a more recent approach, Huang et al. engineered Vδ2 γδ T cells expressing a nanobody-based HLA-G CAR (Nb-CAR) with a secreted PD-L1/CD3ε BiTE.[101] Preclinical studies revealed that these Nb-CAR.BiTE γδ T cells efficiently eliminated PD-L1 and HLA-G-positive solid tumors *in vitro* and in xenograft models, suggesting a promising strategy for overcoming tumor immune evasion.[101]

While CAR-MAIT cell therapy has not yet entered clinical trials, preclinical studies highlight its feasibility and strong anti-tumor activity. One study successfully generated mesothelin-targeting CAR-MAIT cells by activating MAIT cells with the agonist 5-OP-RU, followed by lentiviral CAR transduction.[102,103] These engineered CAR-MAIT cells showed strong cytotoxicity against mesothelin-expressing ovarian cancer cells *in vitro* and demonstrated an additional mechanism of targeting TAMs through MR1/TCR interactions.[103] This dual-targeting capability suggests that CAR-MAIT cells can simultaneously attack tumor cells and modulate the immunosuppressive TME.[103] Although *in vivo* data evaluating the anti-tumor efficacy of CAR-MAIT cells are currently limited, these findings suggest that CAR-MAIT cells hold significant promise for the treatment of solid tumors, particularly those originating in mucosal tissues.

Collectively, these preclinical studies demonstrate the expanding potential of allogeneic CAR-engineered cell therapies. The development of novel CAR-NKT, CAR-γδ T, and CAR-MAIT cells provides promising strategies for overcoming the limitations of allogeneic conventional CAR-T therapies. Continued research into optimizing cell sources, improving manufacturing scalability, and refining tumor-targeting mechanisms will be crucial for translating these findings into clinical applications.

CURRENT CHALLENGES AND LIMITATIONS

Allogeneic CAR-engineered cell therapies offer distinct advantages over autologous approaches in the clinical setting, including immediate availability and large-scale production capabilities. However, their clinical translation faces several challenges, particularly related to immune compatibility, persistence, manufacturing scalability, and regulatory hurdles. Addressing these limitations is essential to advancing the clinical success of allogeneic CAR cell therapies.

GvHD induced by allogeneic CAR cells

GvHD remains a major safety concern in allogeneic CAR-T cell therapy, as donor-derived T cells can recognize host tissues

as foreign, triggering immune-mediated toxicity. In clinical trials, strategies such as TCR knockout, HLA matching, and the use of NK cells or unconventional T cells with a low risk of GvHD have been employed to mitigate this risk, as detailed in previous sections. Despite these advancements, balancing GvHD suppression with the preservation of GvL effects remains a clinical challenge, as excessive immune editing may compromise anti-tumor function.

To enhance the GvL effect while maintaining safety in allogeneic CAR cell therapy, several strategies have been developed. One approach involves selecting tumor-associated antigens highly expressed on leukemic cells but absent on normal tissues, such as CD19, CD22, and BCMA, to minimize off-target toxicity.[104] Additionally, engineering CARs with reduced affinity for target antigens helps decrease the risk of attacking healthy cells with low antigen expression.[105,106] Modulating cytokine signaling, such as through IL-15 or IL-7/IL-21 engineering, enhances CAR cell persistence while mitigating exhaustion and preventing excessive inflammation.[107] Furthermore, safety mechanisms have been incorporated to allow controlled elimination of CAR cells in the case of toxicity, including the iC9 suicide gene, which induces apoptosis upon activation,[108–110] and rituximab-based depletion strategies (e.g., CD20 or EGFRt expression) that enable selective CAR cell removal if necessary.[111,112] These strategies collectively improve the therapeutic efficacy of allogeneic CAR cell therapy while maintaining safety.

Immune rejection and persistence of CAR cells

Host immune surveillance poses another major obstacle to the durability and effectiveness of allogeneic CAR cells. The recipient's immune system often recognizes the infused cells as foreign, leading to allorejection and reduced persistence of CAR-T cells. This immune response involves both cellular mechanisms, such as T cell- and NK cell-mediated rejection, and humoral mechanisms, including the production of alloantibodies. In addition to the strategies discussed in previous sections, such as the deletion of HLA-I and HLA-II molecules on allogeneic CAR cells, other approaches include engineering CAR-T cells to express bacterial immunoevasins that degrade cytotoxic immunoglobulins, or to overexpress Fc receptors such as CD64, which interfere with antibody-mediated clearance.[11] However, these interventions must be carefully balanced to maintain immune functionality and avoid excessive immunosuppression.

Manufacturing and scalability challenges

The transition of allogeneic CAR cell therapy from small-scale clinical trials to widespread clinical application presents significant manufacturing and logistical challenges. Large-scale expansion of allogeneic CAR cells requires bioreactor systems that introduce shear stress, fluctuations in gas exchange, and non-physiological culture conditions, all of which can compromise cell quality and viability. Additionally, donor-to-donor variability remains a concern, as variations in cell source can impact therapy consistency. While master cell banks derived from iPSCs offer a theoretically unlimited and standardized supply of allogeneic CAR cells, their establishment requires extensive time and validation.[113] Meanwhile, although lentiviral vectors provide stable transgene expression, their high cost and lengthy production process drive interest in alternatives such as electroporation and lipid nanoparticles, which may enable more scalable and cost-effective manufacturing. Overcoming these challenges is critical for translating allogeneic CAR therapies into clinical practice.

The manufacturing challenges associated with allogeneic CAR cell therapies are further compounded by the distinct biological characteristics of different cell types. In the case of CAR-T cells, mitigating the risk of GvHD necessitates the use of gene-editing technologies, such as TCR knockout strategies, to prevent alloreactivity.[114] However, these modifications add complexity to the production process, requiring stringent validation to ensure both safety and efficacy. Additionally, donor selection and matching remain critical considerations, as variability in donor-derived T cells can influence therapy consistency and effectiveness.[114] Conversely, CAR-NK cells present an alternative with a lower risk of GvHD, yet their manufacturing introduces unique hurdles. One key limitation is their relatively short persistence *in vivo*, necessitating the development of strategies to prolong survival and enhance cytotoxic potential.[115] Moreover, the intrinsic signaling pathways of NK cells require further optimization to ensure efficient CAR expression and function.[116] Cryopreservation remains another significant challenge, as post-thaw viability and cytotoxicity can be substantially reduced, limiting the feasibility of off-the-shelf applications. Addressing these cell-type-specific manufacturing constraints is critical for advancing the clinical translation and large-scale deployment of allogeneic CAR-based therapies.

Regulatory hurdles and patient access issues

Despite the promise of allogeneic CAR cell therapies in reducing costs compared with autologous approaches, the establishment of large-scale manufacturing infrastructure requires substantial initial investment. Regulatory complexities surrounding the standardization, testing, and distribution of these therapies further complicate their clinical implementation. Ensuring consistent product quality while complying with evolving regulatory frameworks remains a major challenge, particularly given the relative novelty of allogeneic therapies. Limited clinical data compared with autologous CAR-T therapies also contribute to regulatory uncertainty, slowing approval timelines and restricting patient access.[117]

CONCLUSIONS

Current clinical outcomes of allogeneic CAR cell therapy are promising. Compared with FDA-approved autologous CAR-T cell therapies, allogeneic CAR cell therapies have yet to achieve comparable clinical efficacy. For instance, the first FDA-approved CAR-T cell therapy, Kymriah (tisagenlecleucel), was approved in 2017 based on a clinical trial involving 63 patients with relapsed or refractory B cell precursor ALL, demonstrating an overall remission rate (ORR) of 83% within 3 months of treatment.[118] Similarly, the first FDA-approved BCMA-targeting CAR-T therapy, Abecma (idecabtagene vicleucel, ide-cel), received approval in 2021 based on a trial in 100 patients with relapsed and refractory MM, showing an ORR of 72%, including a stringent CR rate of 28%.[119] While allogeneic CAR cell

therapies hold promise, they currently fall short of these efficacy benchmarks (Table 1). Advancements in cell engineering, persistence-enhancing strategies, and immune evasion mechanisms are needed to improve their durability and mitigate allorejection, thereby enhancing their therapeutic potential.

Nevertheless, compared with the autologous CAR cell therapies, allogeneic CAR cell therapies significantly reduce both costs and manufacturing complexities. The acquisition cost of CAR-T cell therapy ranges from $373,000 to $475,000 per infusion, not including additional procedures or facility fees.[120] Moreover, these therapies typically require administration in an inpatient setting, involving the infusion of modified T cells and subsequent disease status monitoring, which incurs additional expenses of approximately $79,466 to $85,267.[121,122] In contrast, allogeneic CAR cell therapy presents a more cost-effective alternative. For instance, HSPC-derived allogeneic CAR-NKT cells generated using a clinically guided culture method can yield substantial quantities from a single cord blood donor containing approximately 5×10^6 $CD34^+$ HSPCs. This process enables researchers to produce up to 10^{12} CAR-NKT cells, sufficient for 1,000 to 10,000 doses for cancer patients, effectively lowering the cost of CAR cell therapy to about $5,000 per patient.[22,23] While the efficacy of allogeneic CAR cell therapy has not yet reached the levels of autologous CAR-T cell therapy, its low cost and availability of ready-to-use cell products allow for multiple doses for patients, conferring significant potential benefits.

A critical evaluation of allogeneic CAR cell therapy must consider the trade-offs between persistence, safety, and therapeutic efficacy. While strategies to enhance persistence, such as the incorporation of cytokine support (e.g., IL-7 and IL-15) or genetic modifications to reduce immunogenicity, can improve long-term efficacy, these approaches may also introduce safety concerns, such as uncontrolled expansion or increased risks of CRS and GvHD.[9,11,17] On the other hand, stringent safety measures, including fail-safe switches or reduced activation potential, may compromise therapeutic efficacy by limiting the expansion and persistence of allogeneic CAR cells *in vivo*. Striking the right balance requires ongoing optimization of cell engineering strategies, immune evasion mechanisms, and dosing regimens to maximize clinical benefits while minimizing risks. Future studies should aim to refine these parameters to ensure that allogeneic CAR cell therapy achieves both durable responses and an acceptable safety profile in diverse patient populations.

Building on the current preclinical and clinical experiences with allogeneic CAR cell therapies, several other future directions can be pursued. First, the exploration of combination therapies is essential. Various strategies have been developed to enhance autologous CAR-T cell therapy, including chemotherapy, oncolytic viruses, immune checkpoint blockade, and genetic modifications involving cytokines and chemokines.[123–126] These strategies can be readily adapted for use with allogeneic CAR cell therapies.

Second, the development of diverse therapeutic cell types and sources necessitates the selection of specific cell products tailored to distinct disease indications. For instance, given the unique pharmacokinetics and pharmacodynamics of allogeneic CAR-NKT cells, characterized by their trafficking patterns, persistence, expansion kinetics, and functional activity *in vivo*, these cells have the ability to migrate into the bone marrow.[22] Utilizing these cells to target bone marrow-resident tumors, such as MM and myeloid malignancies, is particularly relevant. Additionally, various allogeneic CAR cell products should be systematically compared in terms of safety, efficacy, and manufacturing feasibility to identify the most promising candidates for clinical application.

Third, allogeneic CAR cell therapy can facilitate personalized medicine approaches. By integrating specific antigen and marker detection associated with the patient's tumor, allogeneic CAR cells can be selected and engineered to express either a single CAR or multiple CARs. This enables the concurrent delivery of various allogeneic CAR cell products, either simultaneously or sequentially, allowing for efficient targeting of tumor cells with specific and personalized antigen recognition. Ultimately, this strategy aims to achieve optimal anti-tumor efficacy while mitigating the potential for tumor antigen escape.

Overall, the transition from autologous to allogeneic CAR cell therapies aims not only to address the limitations associated with autologous methods but also to provide a standardized and scalable solution to meet the pressing needs of cancer patients.[127] This evolution in therapeutic approaches has the potential to significantly enhance the accessibility and effectiveness of cancer treatments, ultimately improving patient outcomes across a range of diseases. Progress in allogeneic CAR cell therapy will require collaborative efforts among scientists, clinicians, and biotechnology companies to drive innovation and ensure successful implementation.

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DECLARATION OF INTERESTS

L.Y. is a scientific advisor to AlzChem and Amberstone Biosciences and a cofounder, stockholder, and advisory board member of Appia Bio. None of the declared companies contributed to this study.

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