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REVIEW

# Expanding the frontier of CAR therapy: comparative insights into CAR-T, CAR-NK, CAR-M, and CAR-DC approaches

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## Abstract

Chimeric antigen receptor (CAR) therapies have demonstrated remarkable clinical efficacy in hematological malignancies, validating their therapeutic potential. However, challenges such as therapeutic resistance and limited accessibility hinder their broader application. To overcome these limitations, alternative CAR-based cell therapies, including CAR-Natural Killer (CAR-NK), CAR-macrophage (CAR-M), and CAR-dendritic cell (CAR-DC) therapies, have been proposed. Compared with CAR-T, CAR-NK cells have a higher safety profile in terms of cytokine release syndrome (CRS) and neurotoxicity, while being naturally cytotoxic, making them a promising option. Despite these advantages, CAR-NK therapy is limited by issues such as insufficient tissue infiltration and low transduction efficiency. CAR-M cells, with their potent infiltration capabilities and ability to function as antigen-presenting cells, also hold promise but face challenges related to suboptimal viral transduction efficiency. CAR-DCs are emerging as a highly promising approach and are currently undergoing active investigation. This review summarizes the profiles, current clinical trials, and comparative advantages and limitations of CAR-T, CAR-NK, CAR-M, and CAR-DC therapies. Finally, we discuss the key challenges to be addressed and the future prospects of these evolving CAR-based cell therapies.

**Keywords** CAR-T · CAR-M · CAR-NK · CAR-DC · Cellular immunotherapy · Hematological malignancies

## Introduction

Hematologic cancers are among the most common types of malignancies, and recent advancements in treatment have significantly improved patient outcomes. Traditional therapies have been complemented by emerging approaches, such as CAR T-cell therapy and immune checkpoint inhibitors, which are reshaping the landscape of treatment. Among these, CAR T-cell therapy has demonstrated impressive results, particularly in the treatment of hematologic malignancies, and is increasingly being utilized for early-stage intervention, offering new hope for patients.

CARs are synthetic modular proteins that guide the reactivity of immune cells towards a specific target. This adaptable platform has shown significant clinical efficacy in treating hematological malignancies, and its potential for broader application is driving rapid technological advancements and substantial investments from both academia and the biopharmaceutical sector. In 1989, G. Gross and colleagues engineered the first CAR structure and incorporated it into T cells [1]. To date, CAR-T cells have developed to the fifth generation and are widely applied in hematological

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malignancies (Figs. 1 and 2); however, they continue to encounter substantial challenges. Due to the distinct characteristics of hematological malignancies, NK cells and M cells have garnered considerable interest. However, new challenges have emerged, prompting a shift in focus from direct cytotoxicity to antigen-presenting cells, such as dendritic cells (DCs). As a result, CAR-based immune cell therapies, including CAR-NK, CAR-M, and CAR-DC have emerged. In this article, we will review the four predominant CAR immune cell therapies, detailing their benefits, drawbacks, associated clinical trials, and potential solutions for these drawbacks.

## CAR-T

CAR-T cell therapy involves the genetic modification of T cells to express CARs, which enable the targeted recognition of tumor cells, the activation of immune responses, and the destruction of malignant cells. CAR design is a critical part of CAR-T cell therapy (Fig. 1). CARs require a higher affinity range than T cell receptors (TCRs), which means that CAR-mediated cytotoxicity relies on higher densities of cell surface antigens. The advantage of CAR is their ability to regulate the density of antigens, provide greater signal intensity, and compensate for the functional shortcomings of T cell exhaustion [2].

CAR-T cell therapy has a wide range of clinical applications, especially in the treatment of acute lymphoblastic leukemia, non-Hodgkin lymphoma and multiple myeloma, and its efficacy has also been widely recognized [3]. CAR-T research started earlier, and numerous clinical trials are currently in progress. Early- phase CAR-T clinical trials focused on hematologic malignancies, with the main targets being CD19 and BCMA. Among them, CD19-targeting CAR-T has shown significantly better outcomes in B-cell Lymphoma compared to other targets, while BCMA-targeting CAR-T has proven more effective in the treatment of multiple myeloma. As clinical research advances, autologous CAR-T therapies targeting a single antigen can no longer fully address the demands of clinical treatment, leading to an increasing focus on both CAR-T and allogeneic CAR-T therapies targeting multiple antigens [4]. Additionally, several new targets, such as GPC-3 and BAFFR. (NCT05620706) (NCT05370430) have emerged as promising candidates for CAR-T-based therapies.

## Confronting problems

The successful application of CAR-T cell therapy in B-ALL and lymphoma demonstrates its significant potential in anti-tumor therapy. However, several challenges remain, including immunosuppressive tumor microenvironment of CAR-T cells, toxicity of CAR-T cells. These are also barriers to the application of CAR-T treatment (Fig. 3).

### Challenges related to the exhaustion and expansion of CAR-T cells

The efficacy of CAR-T cell therapy is closely related to the persistence of CAR-T cells in the human body, and the exhaustion and proliferation of CAR-T cells are significant factors influencing their longevity [5]. CAR-T cell exhaustion refers to a dysfunctional state characterized by the loss of antigen-specific T cells due to sustained antigen stimulation, excessive signaling from costimulatory domains of the CAR [6]. In vitro studies have shown that the upregulation of PD-1, Lag3, Tim3, TIGIT and CTLA-4 is a

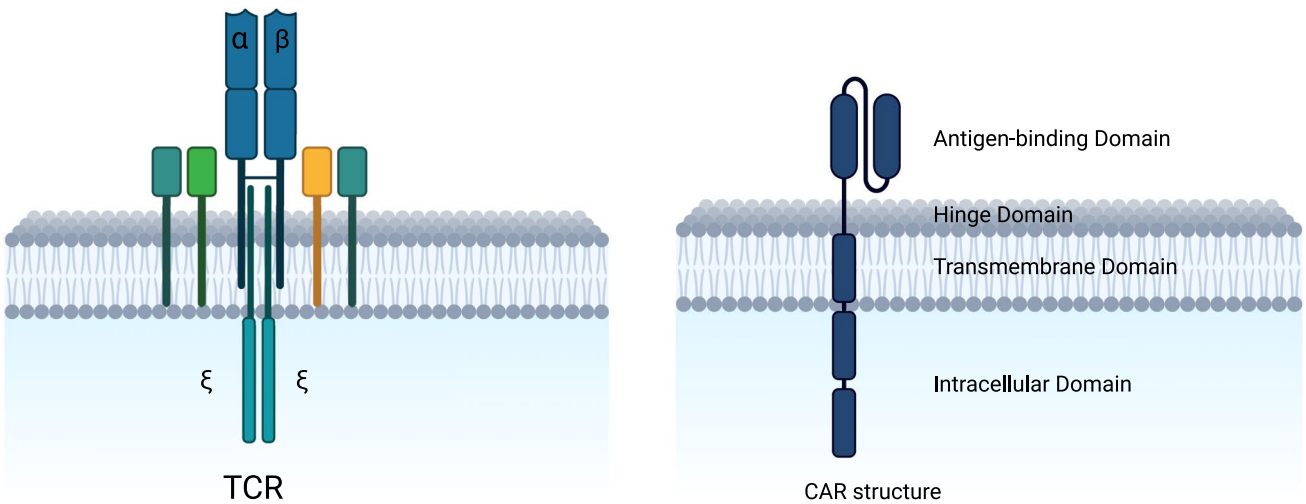


**Fig. 1** Comparison of TCR structure and CAR structure. The basic CAR structure mainly includes four parts: the extracellular target antigen-binding domain, the hinge region, the transmembrane-binding domain, and the intracellular signaling domain

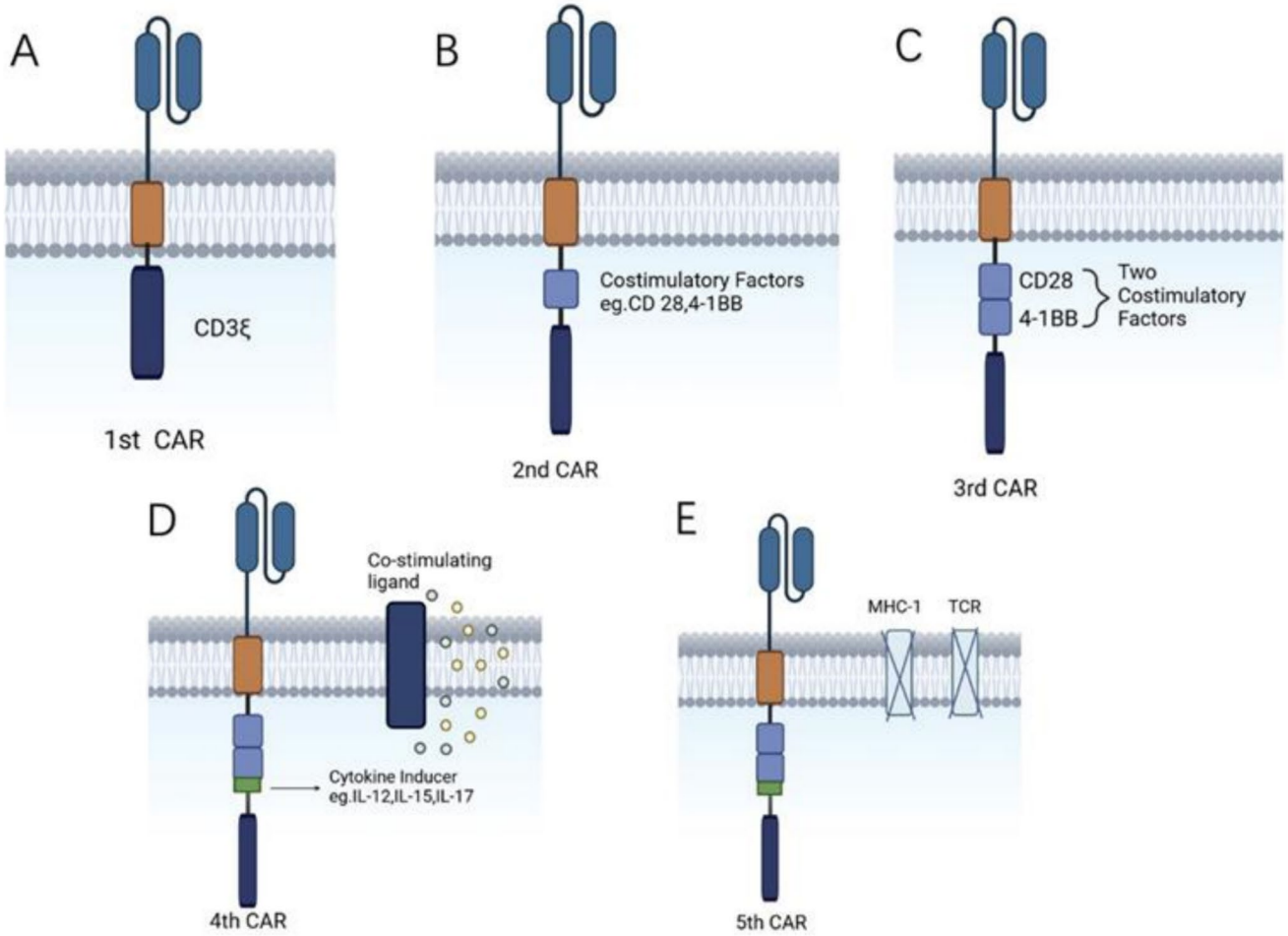


**Fig. 2** Structure diagram of the 1st~5th generation CAR. **a** First-generation: A chimeric TCR fragment independent of MHC is constructed by replacing the light and heavy chains of the antibody with a single strand of the variable region (ScFv). **b** Second generation: Costimulatory factors such as CD28, 4-1BB, ICOS, and OX40 are added to the first generation. **c** Third generation: The combination of two co-stimulators can simultaneously enhance the continuous expansion and anti-tumor ability of CAR-T cells, such as the synergistic stimulation of CD28 and 4-1BB can optimize CAR-T cell function, such as persistence and resistance to exhaustion. **d** Fourth generation: pro-inflammatory cytokines are introduced, including IL-12, IL-15, IL-17, IL-18, etc. **e** Fifth generation: Able to recognize a wider range of antigens

marker of T cell exhaustion and the main cause of loss of anti-tumor function [7, 8]. In addition, cytokines, transcription factors, and epigenetic modifications play an important role in the development of CAR-T cell exhaustion. IL-5 has been shown to reduce mTORC1 activity, thereby enhancing proliferative potential and reducing apoptosis [9]; IL-7 contributes to the prolonged persistence of CAR-T cells in vivo by activating pathways such as STAT5 [10]. Transcription factors, such as TOX [11], NR4A [12], and modulation of c-Jun expression [13], have been found to improve the resistance of CAR-T cells to exhaustion. As for epigenetics, DNA methylation is a key epigenetic mechanism for establishing stable gene silencing programs [14]. T cells undergo de novo DNA methylation during and after the peak of the anti-tumor response, resulting in terminal differentiation associated with exhaustion. These epigenetic changes contribute to the formation of a precursor-exhausted T cell state [15] (Fig. 4).

To address CAR-T cell exhaustion and expansion, the first is to enhance the proliferation and differentiation activity of CAR-T cells. Some studies have shown that oncolytic viruses can be combined to enhance the transport and anti-tumor activity of CAR-T cells in tumors through synergistic effect [16, 17]; Additionally, the medium used during the expansion phase of CAR-T cells can affect their performance in humans, [18] therefore, it is possible to optimize the medium to enhance the proliferation and differentiation ability of CAR-T cells [19]. Another approach is to add cytokines that promote the production of undifferentiated CAR-T cells, such as interleukin mixtures, which can affect the memory, proliferation, and differentiation capabilities of CAR-T cells, thereby improving their persistence in the human body [20]. The second is to target the process of

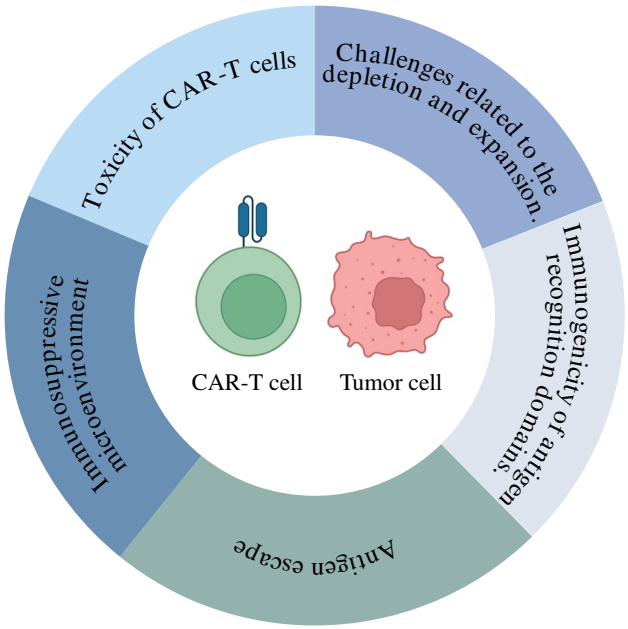


**Fig. 3** Issues facing CAR-T cell therapy. Current issues faced by CAR-T cell therapies include CAR-T cells exhaustion, immunogenicity issues of antigen-binding domains, antigen escape, immunosuppressive microenvironment and toxicity of CAR-T cells

CAR-T cell exhaustion, first of all, by targeting the intrinsic pathway of T cells to overcome exhaustion, for example, the persistent expression of PD-1 is related to CAR-T dysfunction, so PD-1 can be internally blocked to improve CAR-T function [21]. Secondly, the interaction between CAR and antigen can be restricted or disrupted through CAR design, and the persistent antigen stimulation can be attenuated, such as by inducing a "short rest" phase to restore the effector function of exhausted T cells [22]. In addition, deletion of DNMT3A in CAR T cells has been shown to block methylation of multiple key genes that regulate human T cell differentiation, prevent CAR-T cell exhaustion and enhance antitumor activity [23].

## Immunogenicity of antigen recognition domains

At present, most CAR-T cell therapies use murine-derived CARs, which are recognized as foreign by the human immune system by the human immune system. This recognition contributes to the immunogenicity of antigen recognition domains such as single-chain variable fragments (scFvs), and represents a major factor in CAR-T cell immunogenicity and subsequent exhaustion [24]. To address this challenge, the researchers have explored the use of nanobodies and antibody humanization techniques to reduce the immunogenic potential of CAR constructs.

The nanobody, is derived from the variable domain of heavy-chain antibody (VHH). They were first discovered in dromedaries [25] by Hamers-Castermans et al., and were subsequently widely found in camelidae [26] and sharks [27]. However, scFv often exhibit compromised stability and antigen affinity, and is prone to aggregation

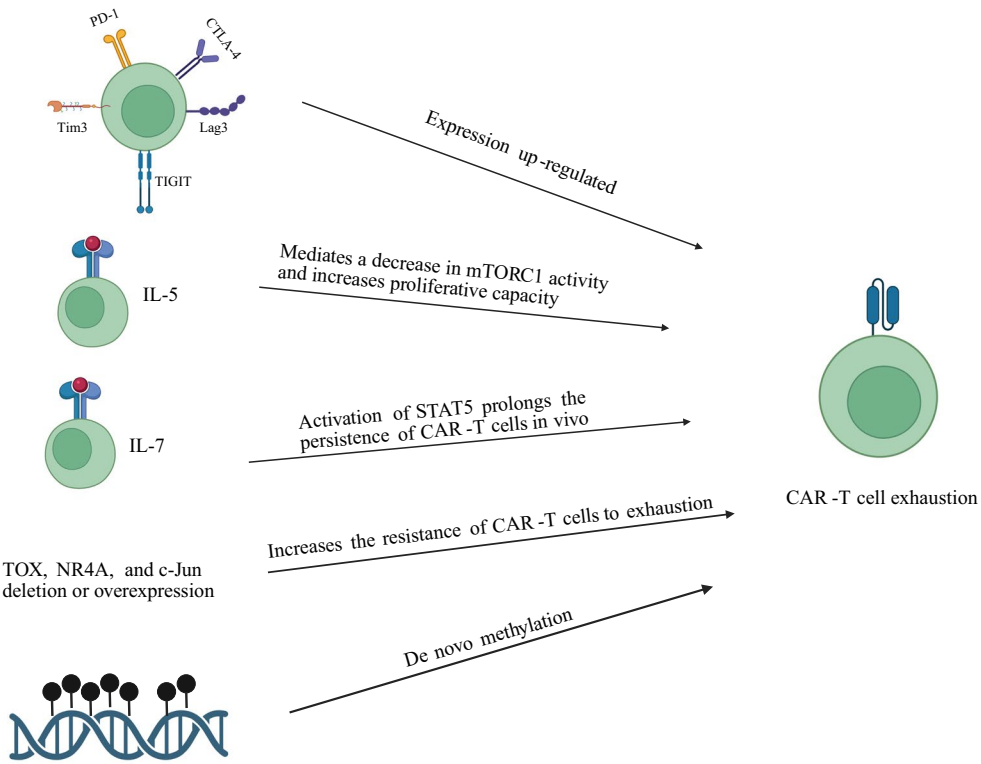


**Fig. 4** Mechanisms of CAR-T cell exhaustion. **a** In vitro studies have shown that the up-regulation of PD-1, Lag3, Tim3, TIGIT and CTLA-4 is a marker of T cell exhaustion and the main cause of loss of anti-tumor function. **b** IL-5 has been shown to reduce mTORC1 activity, thereby enhancing proliferative potential and reducing apoptosis; IL-7 contributes to the prolonged persistence of CAR-T cells in vivo by activating pathways such as STAT5. **c** Transcription factors, such as TOX,11 NR4A, and modulation of c-Jun expression, have been found to improve the resistance of CAR-T cells to exhaustion. **d** T cells undergo de novo DNA methylation during and after the peak of the anti-tumor response, resulting in terminal differentiation associated with exhaustion

or misfolding. These limitations may be due to structural issues, such as the low folding stability of the variable light (VL) domain and the exposure of hydrophobic residues at the interface between the variable heavy (VH) and VL domains [28]. Nanobodies, which completely lack both VL and constant domains [29], are conformationally stable and maintain comparable binding capacity, specificity, and solubility to scFv [26]. In addition, they are less prone to aggregation and misfolding, and their high sequence similarity to human VH gene family III, make them less immunogenic and more suitable for clinical application [25]. Moreover, nanobodies typically require only minor sequence modifications for humanization [30]. In a preclinical trial evaluating dual variable heavy-chain domain of VHH (dVHH) NS7CAR-Ts, the constructs demonstrated high specificity for CD7, enhanced proliferative capacity, and reduced cross-reactivity with other proteins compared to scFv-based CD7-targeted CAR-T therapy. Clinically, a favorable safety profile was observed, during the clinical trial phase, with 80% of patients experiencing only mild CRS, similar to that reported with scFv-based NS7CAR-T treatment [31].

Both CARs derived from murine sources and nanobodies from the Camelidae family are non-human in origin. Non-human antibodies can elicit human immune responses, potentially compromising therapeutic efficacy. To address this issue, the researchers have employed antibody humanization techniques. Common strategies include complementary determining regions (CDR), grafting based on framework regions (FWR) homology, germline humanization, and the use of IgG-derived sequences, with the choice of method depending on the specific application. Humanization techniques enables the optimization of antibody properties immunogenicity, specificity, and affinity [32]. Researchers have also developed a universal humanized nanobody scaffold, which retain structural stability while acquiring antigen specificity and affinity through CDR transplantation [30]. Studies have shown that human-derived CAR-T exhibit improved long-term efficacy in treating relapsed/refractory B-ALL than murine CAR-T cell therapy, with a CR rate of 75% in the human-derived group, which is better than that in the murine group [33, 34]. It is worth mentioning that humanized CAR-T can be used as salvage therapy after relapse or failure after murine CD19 CAR-T therapy [35, 36].

### Antigen escape

Antigen evasion is one of the common methods for tumors to escape immune surveillance. Clinical evidence suggests that some patients who get CAR-T cell therapy for acute B-lymphoblastic leukemia will have a recurrence of CD19-negative leukemia, especially those with long-term CAR-T cells [37]. CD19 negativity leads to the failure of CD19-targeted CAR-T cells, a phenomenon known as antigen escape. Studies have found that antigen escape in tumor cells is associated with the expression of several CD19 splice variants, such as Δexon-2 and Δexon-5/6, which lack the transmembrane domain of CD19 and consequently evade recognition by CAR-T cells [37]. CAR-induced immune pressure promotes the selective expression of these splice variants, enabling tumor cells to evade CAR-T cell surveillance [38]. Another clinically observed mechanism of antigen escape is lineage switching, which is frequently seen in patients with mixed lineage leukemia (MLL) who develop acute myeloid leukemia (AML) following CAR-T cell therapy [39]. This phenomenon may be related to KMT2A rearrangement [40]. In the face of antigen escape, one strategy is to simultaneously target multiple antigens on the surface of the target cell. Numerous clinical trials have investigated CAR-T cells targeting two antigens concurrently (e.g. NCT05438368, NCT05437341, NCT05437328), which can preserve therapeutic efficacy even if one antigen undergoes escape. Additionally, CAR molecule can be engineered to recognize multiple antigens simultaneously, i.e., "tandem CARs" [37]. Studies have shown that Tandem CAR T cells targeting both HER2 and IL13Rα2 exhibit enhanced anti-tumor activity and therapeutic potency compared to single-molecule CAR [41]. Another strategy involves improving CAR-T cell responsiveness to targets with low antigen density. Studies have shown that the activity of CARs is influenced by antigen density, and additional immunoreceptor tyrosine-based activation motifs (ITAM) into CARs can improve the recognition of tumor cells with low antigen density [42]. In addition to that the reason for the weakened response of CD19 CAR-T cells to resistant low antigen density tumors is inadequate CAR signaling. Enhancing CAR signaling intensity can improve the effectiveness of CD19 CAR-T cells against such tumors [42]. For example, CAR-T cell therapy combined with drugs that increase the expression of target antigens, in addition to directly demethylating lymphoma cells to inhibit tumor growth, decitabine (DAC) can also upregulate CD19 expression on lymphoma cells [43]; bryostatin 1 can up-regulate the expression of CD22 to enhance the effect of CD22 CAR-T or prevent low expression of CD22 on the surface of tumor cells [44]. Alternatively, the density threshold of the CAR can be lowered by changing the affinity of the CAR to recognize antigens, especially to enhance the affinity of ScFv for its target [37].

### Immunosuppressive microenvironment

The immunosuppressive microenvironment (TME) refers to the internal environment in which tumors are located, which is mainly composed of tumor cells, stromal cells

(such as cancer-associated fibroblasts), immune cells and their secreted factors, vascular endothelial cells, and extracellular matrix (ECM), is a complex integrated system. These immunosuppressive components closely interact with malignant cells facilitating their survival and immune escape [45, 46]. Simultaneously, they impair the anti-tumor efficacy of CAR-T cells, leading to CAR-T cell exhaustion [47].

At present, no breakthrough has been made in overcoming the barrier that TME hinder the CAR-T therapy; however, several promising strategies remain under exploration. The first is to target immunosuppressive cells in TME. Key immunosuppressive components include regulatory T cells (Tregs), tumor-associated macrophages (TAMs), and myeloid-derived suppressor cells (MDSCs) [48–50]. For Tregs, knockout of CD74 has been shown to specifically reduce their infiltration within the TME, thereby promoting the infiltration and accumulation of CAR-T cells in tumors [51]. It has been shown that blocking the signaling of CD47/SIRPα and CD24 SIGLEC-10 can effectively promote the phagocytosis of TAMs against tumor cells [52]. The microenvironment can also be modulated by developing armored CAR-T cells secreting immunostimulatory cytokines, such as interleukin IL-12, IL-18, or IL-15-armed CAR-T cells [53]. The second is the strategy of modulating metabolic dysregulation, using metabolic pathways to promote the formation of memory T cells or the reversal of effector function by nutritionally restricted TME, which has shown promising improvements in CAR-T cell tumor control in clinical trials [54]. Morgane Boulch et al. proposed a method to deepen the interaction between T cells and the microenvironment and use the cytokines that interact with them by studying the different roles of CAR4 and CAR8 T cells in TME [55].

### Toxicity of CAR-T cells

CAR-T cell therapy may lead to an overactivation of the immune response, which results in CRS. CAR-T cells are rapidly activated upon binding to their target antigen, leading to the secretion of large amounts of granzyme B, perforin, IFN-γ, and TNF-α. Granzyme B activation of cells is associated with widespread expression of Gasdermin E (GSDME), which induces pyroptosis and promotes the release of damage-associated molecular patterns (DAMPs) [56–58]. DAMPs are recognized by pattern recognition receptors (PRRs) and trigger macrophage activation [57]. Current studies have identified L-6, IL-10, and IFN-γ as key cytokines involved in CAR-T cell-associated CRS, with IL-6 being the central mediator [59, 60]. IL-6 and its downstream effectors play an important role in the development of clinical symptoms during CRS. Elevated IL-6 levels can induce a coagulation cascade leading to disseminated intravascular coagulation and myocardial dysfunction [61]. In addition, the colocalization of macrophages and CAR-T cells involves CD40L–CD40 interactions [62], which directly induces IL-6 production [63]. This suggests the existence of a potential CAR-T cell–macrophage regulatory axis [64].

To prevent and treat CRS, therapeutic strategies targeting cytokines such as interleukin IL-1 and IL-6, which are implicated in CRS pathogenesis, can be employed. For example, IL-1 is an important cytokine involved in CRS, so the IL-1 receptor antagonist Anakinra can be used to treat CRS to some extent [65]. Similarly, CRS is predominantly mediated by IL-6, IL-6 receptor antagonists have shown efficacy in mitigating this condition [66]. Studies have shown that the severity of CRS is positively correlated with tumor burden [67], therefore, traditional radiotherapy and chemotherapy can be used before CAR-T cell infusion to reduce tumor burden and prevent CRS.

## CAR-NK

The various challenges encountered by CAR-T cells have prompted increasing interest in the use of other immune cells for CAR therapy, particularly natural killer (NK) cells. NK cells have no MHC restriction and can kill tumor cells non-specifically, exhibiting strong anti-tumor activity. CAR-NK cells have some significant advantages over CAR-T cells, such as a better safety profile, especially in terms of CRS and neurotoxicity [68]. CAR-NK cells can not only possess the inherent ability to kill tumor cells but can also be engineered to recognize a broad range of tumor cell types through CARs. This is especially advantageous as NK cell receptors can recognize ligands present on a variety of tumor cells, thereby positioning CAR-NK cell therapy as a promising approach with substantial therapeutic potential [69].

CAR-NK cells are structurally similar to those of CAR-T cells, but typically use costimulatory factors that are more suitable for NK cells. For example, the upregulation of CD244 can enhance the signal-enhancing ability of NK cells and its natural toxicity to tumor cells [70]. Additionally, DAP12 and DAP10 are involved in NK cell signaling, and as a result, CAR-NK cells can be engineered to incorporate these signaling molecules [71, 72]. Studies have shown that DAP12-based CAR-NK cells perform better than CD3-based CAR-NK cells, which are similar to CAR-T [71].

## Clinical trails

Currently, CAR-NK is primarily being explored in clinical studies targeting hematological malignancies, including B-lymphocytic leukemia and multiple myeloma. But most of them remain in the experimental stage and the results have not yet been published (Table 1). The most common target n these studies is CD19, which is mainly used for B-cell lymphoma; The second most common target is BCMA, which is mainly used for the treatment of multiple myeloma. Among them, there is also CD19/CD70 Bispecific for B-cell Non-Hodgkin Lymphoma (NCT0566715) (NCT05842707); BCMA/GPRC5D Bispecific for Multiple Myeloma (NCT06594211); and CD33/CLL1 Bispecific for Acute Myeloid Leukemia (NCT05215015). For example, in a phase 1 dose-escalation trial of 4-1BB co-stimulatory CD19-specific CAR-NK cell therapy for R/R large B-cell lymphoma, repeated administration of CAR-NK cells in 8 patients. The objective response rate (ORR) was 62.5% and the complete response (CR) of 50%. Grade 3 febrile neutropenia was observed in 37.5% of patients, and grade 3 thrombocytopenia occurred in 50% of patients. Importantly, no cases of long-term or delayed cytopenia were reported. Studies have also shown that high-dose, repeated dosing was necessary to obtain better anti-tumor efficacy [73]. Additionally, a Phase 1 clinical trial evaluating the safety and efficacy of CD33-targeted CAR-NK cell therapy in patients with R/R AML showed that CD33 CAR-NK had a more favorable safety profile compared to CAR-T and helped mitigate side effects related to limited NK cell proliferation. But the durability needs to be further improved. The minimal residual disease–negative complete response (MRD-CR) was 60%. Furthermore, 70% of patients developed fever within 2 days of the first round of CAR-NK cell infusion, and no AEs above grade 3 were observed beyond hematological toxicity [74].

## Advantage

Compared with CAR-T cells, CAR-NK can kill tumors offer a dual mechanism of tumor cell killing, and can effectively kill some tumor cells that do not express the specific antigens recognized by the CAR [68]. CAR-NK cells retain their inherent cytotoxicity, enabling them to kill tumor cells through natural mechanisms [75]. At the same time, it can also exert the role of ADCC through CD16 to kill tumor

**Table 1** Clinical trial of CAR-NK cell therapy in recent years

| Tumor type | Specific target | Source of NK-cells | Phase | NCT number | Result |
|---|---|---|---|---|---|
| T/ NK Cell Hematologic Malignancies | CD7 | CD7 UCAR-T cells | Early Phase 1 | NCT04264078 | No result posted |
| Acute Lymphoblastic Leukemia | CD19 | allogenic CD19-CAR-NK cells | Early Phase 1 | NCT05739227 | No result posted |
| Acute Lymphocytic Leukemia in Relapse | CD19 | Anti-CD19 CAR NK Cells | Phase 1/Phase 2 | NCT06631040 | No result posted |
| Acute Myeloid Leukemia | CD33/CLL1 | IBR733-T01 | Early Phase 1 | NCT05215015 | No result posted |
| B-cell Non Hodgkin Lymphoma | CD19 | anti-CD19 CAR-NK | Phase 1 | NCT04887012 | No result posted |
| B-cell Non Hodgkin Lymphoma | CD19 | anti-CD19 CAR-NK | Phase 1 | NCT05472558 | CR:50% |
| B-cell Non Hodgkin Lymphoma | CD19/CD70 | CB dualCAR-NK19/70 | Phase 1 | NCT05667155 | Ongoing |
| Multiple Myeloma | BCMA | AsclepiusTCG02 | Phase 1/Phase 2 | NCT03940833 | Ongoing |
| Multiple Myeloma | BCMA | BCMA CAR-NK Cells | Early Phase 1 | NCT05008536 | No result posted |
| Multiple Myeloma | BCMA/ GPRC5D | ACT-001 CAR-NK cell | Not Applicable | NCT06594211 | Ongoing |
| Multiple Myeloma | NKG2D | NKG2D Chimeric Antigen Receptor NK Cell Injection | Early Phase 1 | NCT06379451 | Ongoing |
| Multiple Myeloma/Plasma Cell Leukemia | BCMA | Human BCMA targeted CAR-NK cells injection | Early Phase 1 | NCT06045091 | Ongoing |
| Refractory B-Cell Lymphoma | CD19 | Anti-CD19 CAR NK Cells | Early Phase 1 | NCT03690310 | No result posted |
| Refractory B-Cell Lymphoma | CD22 | Anti-CD22 CAR NK Cells | Early Phase 1 | NCT03692767 | No result posted |
| Refractory or Relapsed B-cell Non-Hodgkin Lymphoma | CD19/CD70 | Cord Blood-derived | Phase 1/Phase 2 | NCT05842707 | Ongoing |
| Refractory/ Relapsed Acute Myeloid Leukemia | CD123 | CD123-CAR-NK cells | Early Phase 1 | NCT05574608 | No result posted |
| Refractory/ Relapsed Acute Myeloid Leukemia | NKG2D | NKG2D CAR-NK | Not Applicable | NCT05734898 | No result posted |
| Refractory/ Relapsed Non- Hodgkin Lymphoma | CD19 | CAR-NK-CD19 Cells | Phase 1 | NCT05410041 | No result posted |
| Relapsed/ Refractory Acute Myeloid Leukemia | CD33 | anti-CD33 CAR NK cells | Phase 1 | NCT05008575 | CR:60% |
| Relapsed/ Refractory Diffuse Large B Cell Lymphoma | CD19 | anti-CD19 CAR NK cells | Early Phase 1 | NCT05673447 | No result posted |

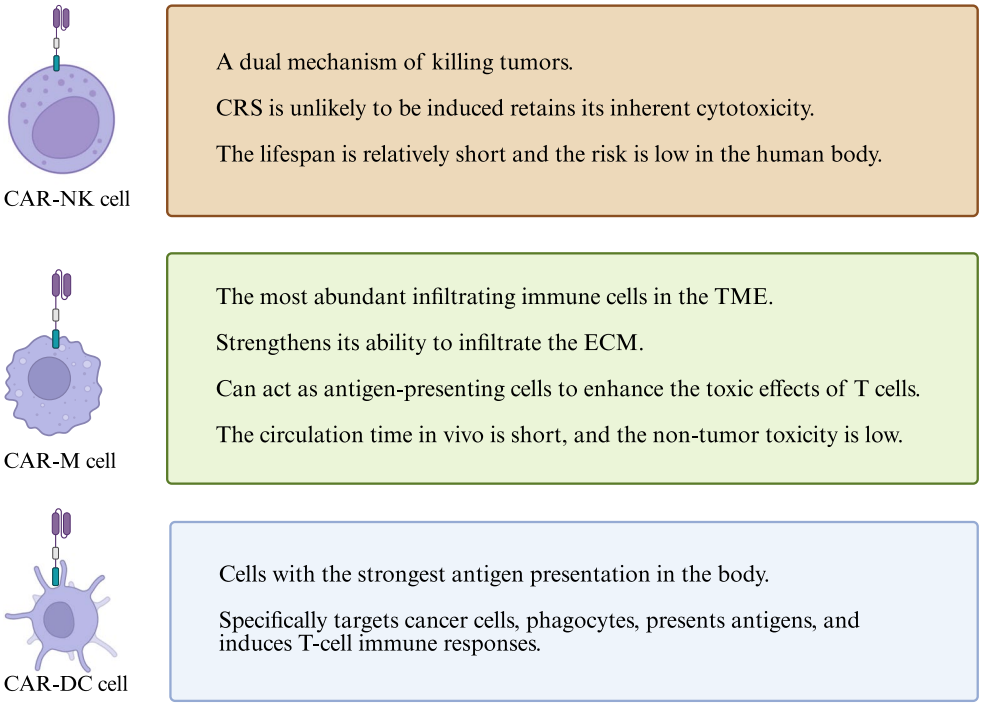


**Fig. 5** Comparison of the advantages of CAR-NK, CAR-M and CAR-DC

cells [76]. CAR-NK cells have a shorter lifespan in the human body and they are also associated with a lower risk of damaging healthy tissues [77]. Furthermore, CAR-NK cells are less likely to induce CRS because of the different secretion of cytokines. NK cells mainly secrete IFN-γ [78]; CAR-T cells secrete IL-1a, IL-1Ra, IL-2, IL-2Ra, IL-6, TNF-α, MCP-1, IL-8, IL-10, and IL-15, all of which are cytokines that are highly correlated with CRS [68] (Fig. 5).

## Confronting problems

"The relatively short lifespan of CAR-NK cells contributes to their safety within the human body, reducing the likelihood of damage to normal tissues. However, at the same time, the limited persistence of CAR-NK also restricts the efficacy of CAR-NK cell therapy to a certain extent. In order to enhance the efficacy of CAR-NK cells in vivo, external cytokine assistance is required. But it also comes with side effects [79], such as inhibition of regulatory T cells [80].

### Low tumor targeting efficiency

Low tumor targeting efficiency has been a challenge for CAR cell therapy. The tumor microenvironment significantly contributes to the low targeting efficiency of CAR-NK cells in tumor tissues. First, the immunosuppressive signal in TME inhibits NK cell clearance on tumors cells. In addition to tumors and stromal cells, various immune cells in TME, such as TAMs, regulatory T cells, and neutrophils—release immunosuppressive substances that hinder the antitumor effect of NK cells [81]. Secondly, the poor availability of nutrients in TME further impedes the ability of NK cells to clear tumors. This is because the exhaustion of oxygen and nutrients caused by explosive tumor growth leads to the formation of a hypoxic and nutrient-deficient TME, impairing NK cell's regular functioning [81]. In addition to inhibiting NK cell clearance, TME also influences the interaction of checkpoint molecules with CAR-NK cells [82]. Current strategies primarily involve gene editing to remove checkpoint components from NK cells. Studies have shown that deletion of checkpoint components can effectively enhance the anti-tumor activity of CAR-NK cells [83, 84].

### Lentiviral transduction efficiency is low

Lentiviral transduction is widely used methods [69] for gene modification and delivery in CAR cell therapy. However, the inherent resistance of NK cells to virus infection presents a challenge in achieving high effectiveness of lentiviral transduction in CAR-NK cell therapy. Chemicals are currently used to improve transduction efficiency, such as protamine sulfate or polymers that can be used to remove the charge on the cell membrane to enhance transduction efficiency [85]. In hematopoietic stem cells and progenitor cells, cyclosporine A and rapamycin are employed to overcome lentiviral restriction barriers [86, 87].

## CAR-M

Macrophages are specialized phagocytic and antigen-presenting cells. They are classified into two types: M1 and M2. M1 macrophages exert pro-inflammatory effects in TME and play a major role in anti-tumor, whereas M2 macrophages promote tumor metastasis, survival and growth [88]. Inducing M2 macrophages to polarize into M1 macrophages could potentially reverse the tumor microenvironment. Therefore, in addition to CAR-T and CAR-NK, CAR-M has emerged, with the potential to address the difficulties of CAR-T and CAR-NK.

Due to the different functions of macrophages, the construction of CAR in CAR-M is slightly different from that of CAR-T and CAR-NK. The CAR construct in CAR-M is mainly composed of scFv, CD8 hinge and transmembrane domains targeting the corresponding molecules, and CD3ζ intracellular domains [89, 90]. For macrophage function, Morrissey and colleagues introduced different cytoplasmic domains. Among them, the CAR composed of Megf10 and FcRy domains showed efficacy in phagocytosis antigenic markers, while the Bai1 and MerTK domains failed to induce phagocytic activity in macrophages [91]. In addition, the researchers also tried to use the inflammatory signaling domain, hoping to have a pro-inflammatory effect, which can induce the transformation of M2 macrophages into M1 macrophages [92]. For example, the integration of the intracellular signaling domains of TLR4 and TLR2 into the CAR framework (the latest grouping in Endnote) can promote the production of CD86, MHC-II, and TNF-α by macrophages, and accelerate tumor regression, which are characteristic of M1 macrophage production [93]. As for the CD3ξ intracellular domain, it was mainly used in the construction of CAR in CAR-T cells in the past, but now it is also widely used in the construction of CAR in CAR-M cells due to its excellent signal transduction ability [94]. CAR-M CAR has progressed to the second generation, which is constructed from induced pluripotent stem cells and has an intracellular domain that binds CD3ζ and TLR4. Furthermore, it has been demonstrated to possess the capacity to augment the elimination of tumor cell and regulate the tumor environment [95].

### Advantage

Macrophages are the most abundant infiltrating immune cells in the TME. The physical barrier formed by the ECM surrounding the tumor is the most important factor affecting the infiltration of immune cells. The ECM is composed of highly organized fibrous molecules, glycoproteins and other macromolecules. Its synthesis and degradation are mainly regulated by matrix metalloproteinases (MMPs) and metalloproteinase tissue inhibitors (TIMP), MMP can degrade ECM, and TIMP is an important enzyme family that inhibits the activity of MMP [96]. Macrophages are the main source of MMP, which enhances their ability to infiltrate the ECM than T cells and NK cells. This confers an advantages of CAR-M cell therapy. Taking advantage of this, Zhang et al. designed a CAR-M consisting of an extracellular scFv region targeting HER2 and intracellular activated CD147, which can trigger downstream signaling pathways with the activated CD147 domain of HER2-positive target cells, thereby promoting the production of MMP and even further promoting the infiltration of T cells, while the phagocytosis and inflammatory cytokine secretion of CAR-M cells are not affected [97].

In addition to their phagocytic function, macrophages can also act as antigen-presenting cells. Furthermore, CAR-M can also act as an antigen-presenting cell to enhance the toxic effect of T cells. Compared with CAR-T cells, CAR-M cells exhibit a shorter circulation time in vivo and lower non-tumor toxicity, making them less likely to induce CRS [98]. IL-6 is a cytokine that may trigger CRS, while CAR-M cells produce IL-6, and interestingly, two different results emerged: mice treated with CAR-147 macrophages had lower levels of IL-6 than in the control group, while CAR-iMac cells showed elevated IL-6 production [99]. The specific causes need further research (Fig. 5).

### Confronting problems

As immune cells, macrophages also face the problem of low viral transduction efficiency. At present, adenoviral vectors [100] and Vpx-modified lentiviruses [101] are mainly used for transduction, which can overcome the problem of low efficiency of macrophage transduction CAR to a certain extent. CAR-M cells also face expansion problems. Macrophages do not proliferate after injection in vitro or in vitro, so the number of CAR-M cells in the patient's body is limited. This limitation may impact the efficacy of the therapy [99]. Furthermore, the migration characteristics of macrophages in vivo will also affect the efficacy. When CAR-M cells are injected in vitro, they initially tend to traverse the lungs before predominantly residing in the liver. Additionally, the limited quantity of CAR-M cells further constrains their effectiveness [102].

### Clinical trails

At present, only a limited number of clinical studies on CAR-M have been conducted, with seven identified, six of which focus on solid tumors. The remaining one is for the hematologic tumor Relapsed/Refractory TCL, which is divided into two parts. The first phase evaluates the

safety, tolerability and appropriate dose of the CAR-M drug MT-101 targeting CD5; The second phase assesses the safety, tolerability, and efficacy of MT-101 based on comparative experiments in the first phase (NCT05138458). The clinical trial is ongoing and is expected to be completed by October 2025. There are few clinical studies of CAR-M in hematological malignancies, and additional trials are necessary to further investigate the anti-tumor activity, therapeutic efficacy and safety of CAR-M.

## CAR-DC

DC cells are the strongest antigen-presenting cells in the body, and most of the DCs in the human body are in a non-mature state. These cells possess a high migration capacity and antigen phagocytosis ability, enabling them to effectively absorb, process, and present antigens. Following antigen uptake, these cells undergo differentiation into mature DCs. During maturation, DCs can migrate from antigen-exposed peripheral tissues into secondary lymphoid organs, make contact with T cells, and elicit an immune response, including induction of specific cytotoxic T lymphocytes (CTLs).

In vitro DC cells are primarily derived from autologous monocytes to generate DCs. CAR-loaded DCs are capable of specifically targeting cancer cells, phagocytosis cells, presenting antigens and inducing T cell immune responses. It is currently believed to have the potential to target a wide range of cancers; however, no relevant research has been published at present (Fig. 5).

Clinical trials of CAR-DCs are still in the experimental stage. There is one for hematologic malignancies: the combination of CAR-T and CAR-DC targeting CD19 in relapsed and refractory B lymphoma (NCT05585996).

## Conclusion

This review provides an overview of current CAR cell therapies, encompassing their design, ongoing clinical trials, applications, and strategic considerations in hematological malignancies. Additionally, a comparative analysis of CAR-M, CAR-NK, and CAR-T therapies highlighted their respective advantages and challenges in clinical applications. Although CAR-T cell therapy is widely used in hematological malignancies, it still faces many problems, such as antigen escape, depletion and expansion of cells themselves, etc. In contrast, CAR-NK and CAR-M therapies exhibit promising potential: CAR-NK offers improved safety and natural cytotoxicity, while CAR-M excels in tumor microenvironment infiltration and antigen presentation, similar to CAR-NK but with enhanced toxicity. However, both CAR-NK and CAR-M face obstacles such as homing efficiency and viral transduction issues.

Looking forward, CAR-DC therapy holds theoretical promise due to dendritic cells' antigen-presenting capabilities, although its efficacy requires further validation in experimental studies. As CAR cell therapy continues to advance rapidly, leveraging technological innovations across disciplines holds the key to overcoming current challenges and enhancing therapeutic outcomes for cancer patients.

In conclusion, CAR cell therapy represents a burgeoning field, from the pioneering CAR-T to the recent developments in CAR-NK, CAR-M, and CAR-DC therapies. Continued interdisciplinary efforts are poised to surmount existing barriers, promising significant advancements in the treatment of hematologic tumors and beyond.

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No datasets were generated or analysed during the current study.

## Declarations

**Competing Interests** The authors declare no competing interests.

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