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Review

# Advances in cell therapy: progress and challenges in hematological and solid tumors

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**Cell-based therapies harness the endogenous ability of the immune system to fight cancer and have shown promising results in the treatment of hematological malignancies. However, their clinical application beyond B cell malignancies is hampered by numerous hurdles, ranging from relapsed disease to a hostile tumor microenvironment (TME). Recent advances in cell engineering and TME modulation may expand the applicability of these therapies to a wider range of cancers, creating new treatment possibilities. Breakthroughs in advanced gene editing and sophisticated cell engineering, have also provided promising solutions to longstanding challenges. In this review, we examine the challenges and future directions of the most prominent cell-based therapies, including chimeric antigen receptor (CAR)-T cells, tumor-infiltrating lymphocytes (TILs), and natural killer (NK) cells, and emerging modalities. We provide a comprehensive analysis of emerging cell types and combination strategies translated into clinical trials, offering insights into the next generation of cell-based cancer treatments**

## Highlights

Cell engineering advancements, including base editing and dual antigen targeting, improve safety and efficacy across cancer types.

Novel approaches, such as *in vivo* chimeric antigen receptor (CAR)-T cell generation and CAR-macrophages aim to overcome current therapeutic limitations.

Allogeneic strategies and induced pluripotent stem cell-derived immune cells offer potential for off-the-shelf treatments, improving accessibility.

Metabolic and genetic reprogramming enhance cell function in hostile tumor environments, potentially expanding efficacy to solid tumors.

Combination strategies, particularly with checkpoint inhibitors, address challenges posed by the immunosuppressive tumor microenvironment.

Ongoing clinical trials of novel cell therapeutics show promising results across various cancer types, paving the way for future translational efforts.

Future directions incorporate advanced gene editing, synthetic biology, and artificial intelligence-driven approaches to create more targeted and controllable immune effector cells.

## Adoptive cell therapies: revolutionizing cancer treatment

**Adoptive cell therapies** (see Glossary; Table 1) are innovative approaches for the treatment of cancer that harness the power of the immune system to target malignancies. These therapies involve the use of living cells, such as T cells, NK cells, and macrophages, which are engineered or selected to recognize and eliminate cancer cells. While allogeneic hematopoietic stem cell transplantation (allo-HSCT; Box 1) remains the best-known and most established cell therapy, newer modalities, such as **CAR**-T cell therapy ,have shown remarkable efficacy in treating certain hematological cancers [1,2]. Recent clinical successes, particularly in refractory leukemias and lymphomas, have catapulted cell-based therapies to the forefront of cancer research. Despite revolutionizing the treatment landscape for hematological malignancies, such as B cell cancers, extending the therapeutic reach of these therapies to solid tumors has proven challenging. Key obstacles include relapsed disease, complex manufacturing processes, and the immunosuppressive **TME** [3]. Furthermore, limited cell persistence, poor tumor infiltration in solid tumors, and limited accessibility due to complex production processes hinder the broader application of these promising therapies [4].

Addressing these challenges necessitates a multifaceted approach. Current efforts focus on enhancing cell persistence for prolonged therapeutic effects, improving tumor infiltration in solid tumors, and developing 'off-the-shelf' allogeneic products to increase accessibility and reduce manufacturing complexities [5].

In this review, we examine the challenges and recent developments in adoptive cell therapies, focusing on strategies to enhance cell persistence, improve tumor infiltration, and modulate

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the immunosuppressive TME. By analyzing the current state of clinical research and emerging trends, including an overview of the complex landscape of cell therapy clinical trials (Table 2), this comprehensive exploration offers insights into the future directions of these therapies. Reflecting the intense research activity in this field, we seek to illuminate the path forward for this revolutionary approach to cancer treatment, highlighting its potential to transform therapeutic strategies across a wider spectrum of malignancies.

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Table 1. Current landscape of immunotherapy: overview of therapy types and their clinical development[a]

| Therapy type | Key features | Clinical status | Major challenges | Notable targets/approaches |
|---|---|---|---|---|
| CAR-T cells | Gene transduction equips T cells with antigen-specific CARs [5] | FDA approved for several B cell malignancies; multiple clinical trials for solid tumors | Manufacturing complexity; CRS; ICANS; ICAHT, IEC-HS<br>Secondary malignancies, antigen selection<br>CAR design<br>T cell fitness | CD19, BCMA, GPRC5D, CD45 (universal CAR-T using CRISPR base editing), ADGRE2 and CLEC12A, CD7, CD30 (hematological)<br>Dual targeting (e.g., CD19/CD22)<br>HER2, PSCA, Claudin 18.2, IL13Rα2, EGFR variant III and wild-type EGFR (solid tumors) |
| TCR engineered T cells | Genetically modifies T cells to target specific antigens, including intracellular ones | Clinical trials for various cancers | Potential off-target effects<br>HLA restriction<br>Manageable toxicities | TAAs (overexpressed in cancer cells) and tumor-specific antigens (unique to cancer cells) [44]<br>Protocol: lymphodepletion, TCR-T infusion, IL-2 administration [99] |
| TILs | Naturally occurring T cells isolated from tumors<br>Expanded *ex vivo* [100] | FDA approved (lifileucel) for melanoma<br>Clinical trials for various solid tumors, including NSCLC, breast, ovarian, and cervical cancers [101,102] | Insufficient numbers<br>Unfavorable TME<br>*Ex vivo* expansion challenges | Neoantigen-specific TILs<br>Combined with checkpoint inhibitors |
| NK cells | Recognize stressed/abnormal cells without prior sensitization<br>Support adaptive responses [36] | Clinical trials (Phase 1/2) for hematological cancers | Limited persistence *in vivo*<br>Challenges to large-scale production | Targeting MHC-I-deficient tumors [103]<br>Allogeneic use of iPSC-derived NK cells (no GvHD)<br>CAR-NK cells<br>Cytokine-activated NK cells<br>No ICANS<br>Mild CRS |
| DCs | Professional antigen-presenting cells<br>Stimulate T cell responses [51] | FDA approved (sipuleucel-T) for prostate cancer<br>Multiple clinical trials | Limited efficacy as monotherapy<br>Manufacturing complexity | *In situ* DC vaccines<br>Combination with checkpoint inhibitors<br>Targeting specific DC subsets |
| CAR-M | Engineered macrophages<br>Express CARs<br>Enhance phagocytosis and antigen presentation [104,105] | Early clinical trials (Phase 1) | Limited efficacy as monotherapy | HER2-targeting CAR-M (CT-0508)<br>Combination with PD-1 inhibitors |
| iPSC-derived immune effector cells | Derived from iPSCs<br>Potential for 'off-the-shelf' products | Preclinical and early clinical trials | Safety concerns<br>Scalable manufacturing challenges<br>Limited persistence/immune rejection | iPSC-derived CAR-T cells<br>iPSC-derived NK cells |
| MSCs | Dual-natured immune modulators<br>Potential delivery vehicles for CAR-T therapy<br>MSC-derived exosomes with similar capabilities [60] | Preclinical studies and early clinical trials | Variable outcomes in modulating CAR-related products<br>Paradoxical role in tumor promotion | Enhancing CAR-T cell function<br>Oncolytic properties [59]<br>Exosome-based delivery |

[a]Abbreviations: CAR-M, chimeric antigen receptor macrophages; CRS, cytokine release syndrome; DCs, dendritic cells; FDA, US Food and Drug Administration; GvHD, graft-versus-host disease; ICAHT, immune effector cell-associated hematological toxicity; ICANS, immune effector cell-associated neurotoxicity syndrome; IEC-HS, immune effector cell-associated hemophagocytic lymphohistiocytosis-like syndrome; iPSC, induced pluripotent stem cell; MHC, major histocompatibility complex; MSCs, mesenchymal stem cells; NSCLC, nonsmall cell lung cancer; PSCA, prostate stem cell antigen; TAAs, tumor-associated antigens; TCR, T cell receptor.

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Box 1. Allogenic stem cell transplantation

Allo-HSCT remains a crucial treatment for various hematological malignancies, especially acute myeloid leukemia (AML). Since its introduction by E. Donnall Thomas in 1957, allo-HSCT has evolved significantly, often representing the only curative option for patients at high risk for AML [84]. The primary challenge in allo-HSCT is maintaining an optimal balance between effective immune reconstitution, minimal GvHD, and sustained disease control [85]. Recent advancements have focused on addressing this challenge through various strategies. Conditioning regimens have evolved from traditional myeloablative approaches to include reduced-intensity (RIC) and non-myeloablative (NMA) options. These less intense regimens have expanded allo-HSCT eligibility to older patients and those with more comorbidities, relying more on the **graft-versus-leukemia (GvL)** effect for disease control [84]. Novel therapies for steroid-refractory GvHD have emerged, including ruxolitinib (a JAK1/2 inhibitor) for both acute and chronic GvHD, and belumosudil (a ROCK2 inhibitor) for chronic GvHD. These treatments offer new mechanisms of action in GvHD management [86,87]. Balancing GvHD and GvL effects remains a central challenge. Ongoing research focuses on strategies to separate these effects, including selective T cell depletion, targeted therapies against specific alloreactive T cell subsets, and manipulation of **regulatory T cells (Tregs)** to promote tolerance. Future directions in allo-HSCT research aim to refine personalized transplant approaches, integrating factors such as disease biology, donor characteristics, and individual patient risk profiles. As we gain more insight into the complex immunological mechanisms of allo-HSCT, we expect to develop increasingly precise therapies that can effectively distinguish between GvHD and GvL effects. These advancements hold promise for improving the efficacy and safety of allo-HSCT, potentially expanding its role in treating hematological disorders and offering better outcomes for patients.

## Challenges and recent progress in prominent cell-based therapies

### CAR-T therapies

CAR-T cell therapy genetically modifies T cells to express CARs, enabling them to recognize and attack specific cancer antigens. These engineered T cells bind to target cells, proliferate, and destroy them through cytokine release and direct cytotoxicity. Development focuses on antigen selection, CAR design, gene transfer, and T cell optimization (Box 2). CAR-T therapies have shown exceptional efficacy in treating hematologic malignancies, achieving high overall and complete response rates in conditions such as relapsed/refractory B cell lymphomas, multiple myeloma, and follicular lymphoma [1,2].

However, despite these successes, significant safety concerns remain. CAR-T therapies are frequently associated with adverse events, such as **cytokine release syndrome (CRS)** and neurotoxicity, commonly known as **immune effector cell-associated neurotoxicity syndrome (ICANS)** [6], which is mediated by microglia [7] (Figure 1).

Further complicating CAR-T therapy is the risk of **graft-versus-host disease (GvHD)** and host-versus-graft rejection in cases where allogeneic cells are used (Figure 1) [8]. Additionally, rare cases of postinfusion T cell lymphomas raise concerns about vector integration potentially leading to malignant transformation, although these instances remain uncommon [9].

Anti-B cell CAR-T therapy can lead to B cell aplasia and hypogammaglobulinemia, potentially increasing infection risk, particularly during the first month post treatment (Figure 1); consequently, some patients may require immunoglobulin replacement therapy, with the decision based on factors such as the severity of hypogammaglobulinemia, presence of recurrent infections, and individual clinical assessment [10,11] .

Hematological toxicities represent another significant challenge, particularly immune effector cell-associated hematological toxicity (ICAHT), which affects all hematopoietic lineages. Prolonged neutropenia increases the risk of potentially lethal infections. In rare cases, hyperinflammatory responses can also trigger immune effector cell-associated hemophagocytic lymphohistiocytosis-like syndrome (IEC-HS), leading to pancytopenia. Moreover, the US Food and Drug Administration (FDA) has issued warnings regarding the potential risk of secondary malignancies, particularly myeloid neoplasms [9].

## Glossary

**Adoptive cell therapy:** a cutting-edge approach in immunotherapy where immune cells, typically T cells or NK cells, are extracted from a patient or donor, engineered or expanded *ex vivo* to boost their anti-cancer efficacy, and subsequently reinfused into the patient to target and eradicate malignant cells.
**Chimeric antigen receptor (CAR):** innovative engineered protein that integrates an antigen-recognition domain with intracellular signaling components, enabling immune cells , such as T cells, NK cells, or macrophages, to identify and engage specific antigens present on cancer cells.
**Cytokine release syndrome (CRS):** critical immune response characterized by the excessive and rapid release of cytokines into the bloodstream, leading to severe systemic inflammation and potentially life-threatening complications.
**Exosomes:** diminutive extracellular vesicles released by cells, which transport proteins, lipids, and RNA, and have a pivotal role in intercellular communication and various physiological processes.
**Graft-versus-host disease (GvHD):** occurs when donor immune cells attack the recipient's tissues after an allogeneic HSC or organ transplant, leading to significant and often severe complications.
**Graft-versus-leukemia (GvL):** describes a beneficial immune response in which donor-derived immune cells target and destroy residual leukemia cells in the recipient following an allogeneic HSC transplant, enhancing therapeutic outcomes.
**Immune checkpoint inhibitors (ICIs):** novel class of therapeutics that block proteins enabling tumors to evade immune surveillance, thus enhancing the capacity of the immune system to recognize and target cancer cells.
**Immune effector cell-associated neurotoxicity syndrome (ICANS):** potential side effect of CAR-T therapy, characterized by neurological symptoms including confusion and altered mental status. It is caused by activation of the immune system in response to the therapy.
**Major histocompatibility complex (MHC) molecules:** essential cell surface proteins with a crucial role in the immune response by presenting peptide antigens to T cells, with MHC class I molecules interacting with CD8+ T cells and MHC class II molecules with CD4+ T cells.

The loss of target antigens undermines the efficacy of CAR-T therapy [12], prompting researchers to explore diverse strategies to overcome this obstacle. Compounding this issue is **trogocytosis**, a process in which target antigens are transferred to T cells (Figure 1). This phenomenon reduces antigen density on tumor cells, diminishes CAR-T activity, and can lead to **T cell exhaustion** and fratricide, further complicating treatment outcomes [13].

In solid tumors, CAR-T efficacy remains challenging, primarily due to the immunosuppressive TME and tumor heterogeneity. The heterogeneous nature of cancer cells in solid tumors can lead to incomplete responses or relapses, because some cancer cells may not express the targeted antigen. Furthermore, solid TMEs can hinder CAR-T persistence [4].To combat this, short-lived CAR-T cells are being explored to maintain functional effector pools and combat exhaustion [4]. While multiple infusions could enhance efficacy, this approach faces hurdles in autologous settings due to high costs and production complexities. Allogeneic 'off-the-shelf' products offer a potential solution but introduce risks such as GvHD. To mitigate these risks, allogeneic products require gene editing to knock out the **T cell receptor (TCR)**, preventing them from being alloreactive and causing GvHD [14]. However, this modification comes with its own challenges. While it reduces the risk of GvHD, these edited cells might have limited persistence because they could be rejected by the immune system of the host. Moreover, multiple infusions may increase therapy-associated toxicities [14]. To further improve efficacy in solid tumors, researchers have developed a novel CAR construct that incorporates cytokine signaling capabilities, specifically a truncated IL-2 receptor β-chain domain and a STAT3-binding motif, in addition to the traditional TCR signaling and co-stimulatory domains. This innovative approach, which mimics the third signal of T cell activation, has demonstrated superior *in vivo* persistence and enhanced antitumor effects in both liquid and solid tumor models compared with conventional CAR designs [15].

On-target off-tumor toxicity remains a significant challenge in CAR-T therapy, affecting both hematological and solid tumor treatments. This phenomenon occurs when CAR-T cells attack healthy tissues expressing the targeted antigen, leading to potentially severe side effects (Figure 1). While this issue is present in hematologic malignancies, it becomes particularly problematic in solid tumor treatments due to the broader expression of target antigens across various tissues. For instance, HER2-targeted CAR-T cells have shown promise in treating certain cancers, but have also caused severe pulmonary toxicity due to low-level HER2 expression in lung tissue. To address this challenge, innovative strategies are being developed to enhance the specificity of CAR-T cells. A recent study presented a novel dual CAR NOT gate system incorporating an inhibitory CAR (iCAR) recognizing HLA-A02 alongside an activating CAR targeting HER2. This approach aims to effectively treat tumors with HLA-A02 loss of heterozygosity while sparing normal tissues expressing both antigens [16]. Another approach to enhance CAR-T specificity involves modifying the affinity of the CAR-binding domain. For instance, a study on CD229-targeted CAR-T cells for multiple myeloma demonstrated that a single amino acid substitution in the CAR-binding domain, combined with c-Jun overexpression, maintained antitumor efficacy while significantly reducing toxicity against healthy lymphocytes expressing lower levels of CD229 [17].

Researchers have developed a range of innovative approaches to enhance the efficacy, safety, and applicability of CAR-T therapy across various cancer types (Figure 1). Significant progress in novel target selection and CAR design has been made, aimed at addressing issues such as antigen loss, toxicity, limited persistence, and efficacy. Significant progress has emerged in novel target selection and CAR design, specifically aimed at overcoming the obstacles encountered with earlier iterations of the treatment.

**Monocyte-derived dendritic cells (MoDCs):** specialized subset of DCs that originate from monocytes and are integral to antigen presentation and activation of immune responses.
**Neoantigen:** novel antigens arising from tumor-specific mutations that can elicit an immune response, making them prime targets for personalized cancer immunotherapy.
**NK cell engagers:** therapeutic agents designed to boost the activity of NK cells, directing them to effectively target and destroy cancer cells through enhanced immune engagement.
**Oncolytic viruses:** genetically engineered or naturally occurring viruses that selectively infect, replicate within, and destroy cancer cells while sparing healthy tissue, potentially stimulating the immune system to recognize and attack tumors.
**Optogenetics:** use of light to control genetically modified cells.
**Regulatory T cells (Tregs):** subset of T cells with a critical role in modulating immune responses, maintaining self-tolerance, and preventing autoimmune diseases.
**Synthetic biology:** the design and construction of new biological parts and systems to enhance cellular functions, such as improved tumor recognition or cytokine production.
**T cell exhaustion:** describes a state of dysfunction in T cells resulting from chronic antigen exposure, characterized by diminished effector functions, sustained expression of inhibitory receptors, and altered transcriptional profiles.
**T cell receptor (TCR):** molecule on the surface of T cells that recognizes and binds to specific antigens presented by MHC molecules, thereby initiating and orchestrating the immune response.
**Trogocytosis:** biological process in which one cell transfers membrane fragments and associated proteins to another cell upon cell–cell contact. This phenomenon occurs between various types of immune cell and can have a role in immune responses and cell signaling.
**Tumor-associated antigens (TAAs):** abnormal or overexpressed proteins present on cancer cell surfaces that can serve as targets for immune system attack or biomarkers for cancer diagnosis and treatment.
**Tumor-associated macrophages:** immune cells found within the TME that can either support or inhibit tumor growth, often displaying functions

For instance, a combined CAR targeting adhesion G protein-coupled receptor E2 (ADGRE2) and a chimeric receptor targeting C-type lectin domain family 12 member A (CLEC12A) has been developed to selectively target acute myeloid leukemia (AML) cells (Figure 1). ADGRE2 is a surface receptor overexpressed on AML cells, while CLEC12A is a myeloid lineage antigen expressed on most AML blasts. This dual-targeting approach aims to enhance specificity for leukemia cells while sparing normal hematopoietic stem cells (HSCs) and progenitor cells during AML treatment [18]. Another promising target is CD7, a pan-T cell antigen highly expressed in acute T cell leukemia (T-ALL) and some peripheral T cell lymphomas. However, its expression can lead to fratricide among CAR-T cells. This challenge can be addressed through strategies such as genomic disruption of *CD7* [19], which prevents fratricide and supports CAR-T cell expansion and efficacy, or by leveraging naturally occurring CD7-T cells to generate CD7-CAR ($CD7^{-}CARCD7^{-}$) T cells [20]. Additionally, pharmacological inhibition of key CAR/CD3ζ signaling kinases with agents such as ibrutinib and dasatinib can also prevent fratricide and enhance CAR-T cell function [21].

One of the major engineering advancements in CAR-T therapies includes the development of universal CAR-T cell products. A universal CAR-T cell therapy targeting the pan-leukocyte marker CD45 has been created using CRISPR base editing of HSCs, introducing a protective mutation to prevent recognition of healthy cells while maintaining essential CD45 functions and hematopoiesis [22].

More sophisticated CAR-T cell technologies have been developed. One innovative approach is the Uni-Vect system, a novel lentiviral vector that combines constitutive CAR expression with antigen-induced production of accessory molecules (Figure 1). This platform enables activation-dependent expression of IL-12 for enhanced efficacy, an IL-6 antibody to mitigate CRS, and transcription factors to modulate T cell biology [23]. Complementing this, researchers are designing CARs with logic gates for improved specificity and exploration of combination therapies. For instance, CAR-T cells are being paired with **oncolytic viruses** to enhance tumor infiltration [24]. Additionally, modulating T cell extrinsic pathways, such as inhibiting TGF-β [25], can help preserve T cell function in the immunosuppressive TME. Moreover controlling CAR expression on the surface of T cells through targeted knockin into a certain locus or through inducible or switchable CAR systems allows for more precise regulation of T cell activity, potentially mitigating exhaustion by preventing overstimulation [26,27].

A novel approach to overcome cardiac fibrosis involves generating transient antifibrotic CAR-T cells *in vivo* by delivering modified mRNA in T cell-targeted lipid nanoparticles [28]. This represents an innovative application of CAR-T technology that could result in an off-the-shelf vaccine-like product to generate CAR-T cells *in vivo* in patients with cancer.

Beyond engineering the CAR-T cells themselves, researchers are also exploring how the patient's own biology can impact treatment efficacy. An emerging factor influencing the success of immunotherapies, including CAR-T therapy, is the gut microbiome. A favorable microbiome enhances immune responses, while an unfavorable one may impede treatment. A study on anti-CD19 CAR-T therapy for B cell malignancies found that recent antibiotic exposure correlated with worse survival and increased neurotoxicity. Stool analysis revealed specific bacterial taxa, particularly *Clostridia* spp, associated with better responses. This research highlights microbiome modulation as a potential strategy to improve outcomes for various immunotherapies, including CAR-T [29]. By manipulating the microbiome, it may be possible to enhance the efficacy of these treatments while reducing their associated toxicity [29].

Despite these promising developments, current CAR-T approaches face limitations due to their reliance on autologous T cells, which are both difficult and expensive to manufacture. There are

distinct from macrophages in healthy tissues.

**Tumor microenvironment (TME):** encompasses the intricate network of non-cancerous cells, signaling molecules, and extracellular matrix that surrounds and interacts with a tumor, significantly influencing its growth and progression.

Table 2. Examples of ongoing clinical studies for cell-based therapies[a]

| Cancer type | Intervention | Phase | No. of patients | Outcome | Clinical trial | Refs |
|---|---|---|---|---|---|---|
| CAR-T cells | | | | | | |
| Multiple melanoma | GRPC5D-targeted CAR-T cells | 1 | 17 | ORR 71% | NCT04555551 | [64] |
| B cell malignancies | 19(T2)28z1xx CAR-T cells | 1 | 30[b] | ORR 82% (entire cohort)<br>ORR 88% (lowest dose cohort ($25\times10^6$) CAR-T cells) | NCT04464200 | [66] |
| CD30+ Hodgkin lymphoma and CD30+ non-Hodgkin lymphoma | CD30.CAR-T cells | 1/2 | 40[b]<br>60[b] | ORR 72%, CR 59% in patients with active disease (31) | NCT02690545<br>NCT02917083 | [65] |
| B cell acute lymphoblastic leukemia | AUTO3, dual CD19/CD22 CAR-T cells | 1/2 | 23[b] | RR 13/15<br>(CR or CR with incomplete BM recovery 1 month after treatment) | NCT03289455 | [12] |
| | Recombinant polymer-conjugated IL15 receptor agonist + dual CAR19/22 | 1 | 56[b] | Measurable residual disease negative remission 8/9 | NCT03233854 | [67] |
| Gastrointestinal tract malignancies | Claudin 18.2 CAR-T cells | 1 | 134[b] | ORR 48,6% | NCT03874897 | [68] |
| Glioblastoma | CART-EGFR-IL13Rα2 | 1 | 18[b] | Objective radiographic response criteria not met | NCT05168423 | [70] |
| Prostate cancer | PSCA-directed CAR-T cells | 1 | 14[b] | 4 of 14 participants with PSA declines >30% | NCT03873805 | [71] |
| Sarcoma | HER2-specific CAR-T cells | 1 | 36[b] | Clinical benefit in 50% | NCT00902044 | [72] |
| TILs | | | | | | |
| NSCLC, melanoma, squamous cell carcinoma of head and neck | Lifileucel autologous TILs | 2 | 178[b] | 21.4% | NCT03645928 | [32] |
| Soft tissue sarcoma | TILs + LTX-315 | 2 | 6[b] | OR criteria not met, BOR SD 3/4 | NCT03725605 | [73] |
| DC vaccines | | | | | | |
| Pleuramesothelioma | DC vaccine + PD1 inhibitor + standard-of-care chemotherapy | 1/2 | 15[b] | Recruiting | NCT05765084 | [82] |
| Glioblastoma | DC vaccine + TLR inhibitor | 2 | 24 | PFS 8.1 months; OS 26.6 months | NCT01204684 | [83] |
| CAR-macrophages | | | | | | |
| Solid tumors overexpressing HER2 | Anti-HER2 CAR-macrophages | 1 | 48[b] | 4/14 stable disease | NCT04660929 | [74] |
| CAR-monocytes | | | | | | |
| Solid tumors overexpressing HER2 | Anti-HER2 CAR-monocytes | 1 | 6 | N/A | NCT06254807 | |
| NK cells | | | | | | |
| CD19+ B cell malignancies | Anti-CD19 CAR-NK cells derived from cord blood | 1/2 | 49[b] | day + 30 and day+ 100<br>OR 48.6% (entire cohort)<br>CAR-NK derived from optimal CBUs: d + 30<br>OR 75% versus non-optimal CBU 28.6% | NCT03056339 | [40] |
| AML relapse after allo-HSCT | Cytokine-induced memory-like NK cells + DLIs | 1/2 | 110 | CR 50% | NCT03068819 | [76] |
| | Cytokine-induced memory-like NK cells | 2 | 60[b] | Composite CR 87% | NCT02782546 | [77] |
| TCR therapy | | | | | | |
| Refractory solid cancers | Patient-specific neoTCR | 1 | 21[b] | 5/16 stable disease | NCT03970382 | [78] |

[a]Abbreviations: allo-HSCT, allogeneic hematopoietic stem cell transplantation; AML, acute myeloid leukemia; BM, bone marrow; BOR, best objective response; CBU, cord blood units; CR, complete remission; DC, dendritic cell; DLI, donor lymphocyte infusion; NK, natural killer; NSCLC, nonsmall cell lung cancer; OR, overall response; ORR, overall response rate; OS, overall survival; PFS, progression-free survival; PSA, prostate-specific antigen; PSCA, prostate stem cell antigen; SD, standard deviation; TCR, T cell receptor.
[b]Patient number updated according to ClinicalTrials.gov after publication.

### Box 2. Evolution of CAR-T cell generations and next-generation innovations

First-generation CARs, developed by Eshhar in 1997, involved T cells equipped with a single-chain variable fragment (scFv) linked to CD3ζ to bypass major histocompatibility complex (MHC) restrictions. However, their therapeutic effectiveness was limited due to T cell exhaustion from the lack of co-stimulatory signals [5].

Second-generation CARs were an improvement with an additional 4-1BB co-stimulatory domain, enhancing T cell persistence. Second-generation CD19 CAR-T therapies, now commercially available, have shown long-term effectiveness, with cells remaining active in patients for up to 10 years [88].

Third-generation CARs, featuring two co-stimulatory domains (e.g., CD28/4-1BB), showed improved persistence but did not significantly outperform second-generation CARs in terms of their antitumor activity for blood cancers [89]. However, they might be more beneficial for treating solid tumors with complex TMEs [90].

Next-generation CARs address current limitations, such as tumor localization, accurate tumor cell recognition, long-term proliferation, resistance to the TME, safety, and creation of off-the-shelf products. Advances in synthetic biology are driving these innovative designs, collectively referred to as 'next-generation CARs'. These innovations include, for example:

Logic gates:

- 'OR': next-gen CAR-T cells activate any of several target antigens, enhancing flexibility but needing careful safety measures [91].
- 'AND': they require two antigens for activation, improving targeting accuracy and reducing damage to normal tissues [92].
- 'AND-NOT': activation occurs with one antigen but is inhibited by another, adding specificity to avoid nontumor cells [93].
- Adaptor dependency: these CAR-T cells are precisely controlled by external ligands or adaptors, allowing versatile targeting of multiple tumor-associated antigens [94].

Pharmacological switches:

- On-switch: activation of CAR-T cells can be controlled by administering specific drugs [21].
- Off-switch: deactivation or destruction of CAR-T cells via drugs to manage side effects and improve safety [95].

Secretion: engineered to secrete molecules, such as bispecific T cell engagers (BiTEs) or cytokines, these CAR-T cells show improved persistence, specificity, and overall antitumor activity [96].

TME response: these CAR-T cells are designed to activate in response to the TME, reducing off-target effects and improving precision [97].

To extend the fitness of CAR-T cells, synthetic knock-in sequences have been identified using CRISP/Cas9 screens [98].

also approaches attempting to develop potent allogeneic T cells that are not rejected by the recipient's immune system with the aim to provide easily accessible off-the-shelf therapies. For example, a receptor (alloimmune defense receptor ADR) was engineered that mediates deletion of activated host T and NK cells by transiently upregulating co-stimulatory receptors upon activation, thereby preventing rejection of allogeneic T cells (Figure 1) [30].

## Tumor-infiltrating lymphocytes

TILs have similarly shown considerable promise, particularly in the treatment of solid tumors, such as melanoma. Research is increasingly focusing on **neoantigen**-specific TILs, which have demonstrated superior efficacy in achieving complete and durable tumor regression compared with unselected TILs [31]. Recently, the FDA approved lifileucel, a one-time autologous TIL therapy, marking a milestone in the use of TILs for solid cancers [32]. Although response rates in non-melanoma solid tumors remain lower, durable complete responses have been observed, supporting further research into this therapeutic avenue [3]. A key factor influencing TIL efficacy is their metabolic state, with metabolic stressors, such as glucose deprivation and hypoxia, in the TME impairing their function and contributing to T cell exhaustion (Figure 1) [33]. Research into improving T cell metabolism, including strategies to maintain mitochondrial fitness, is

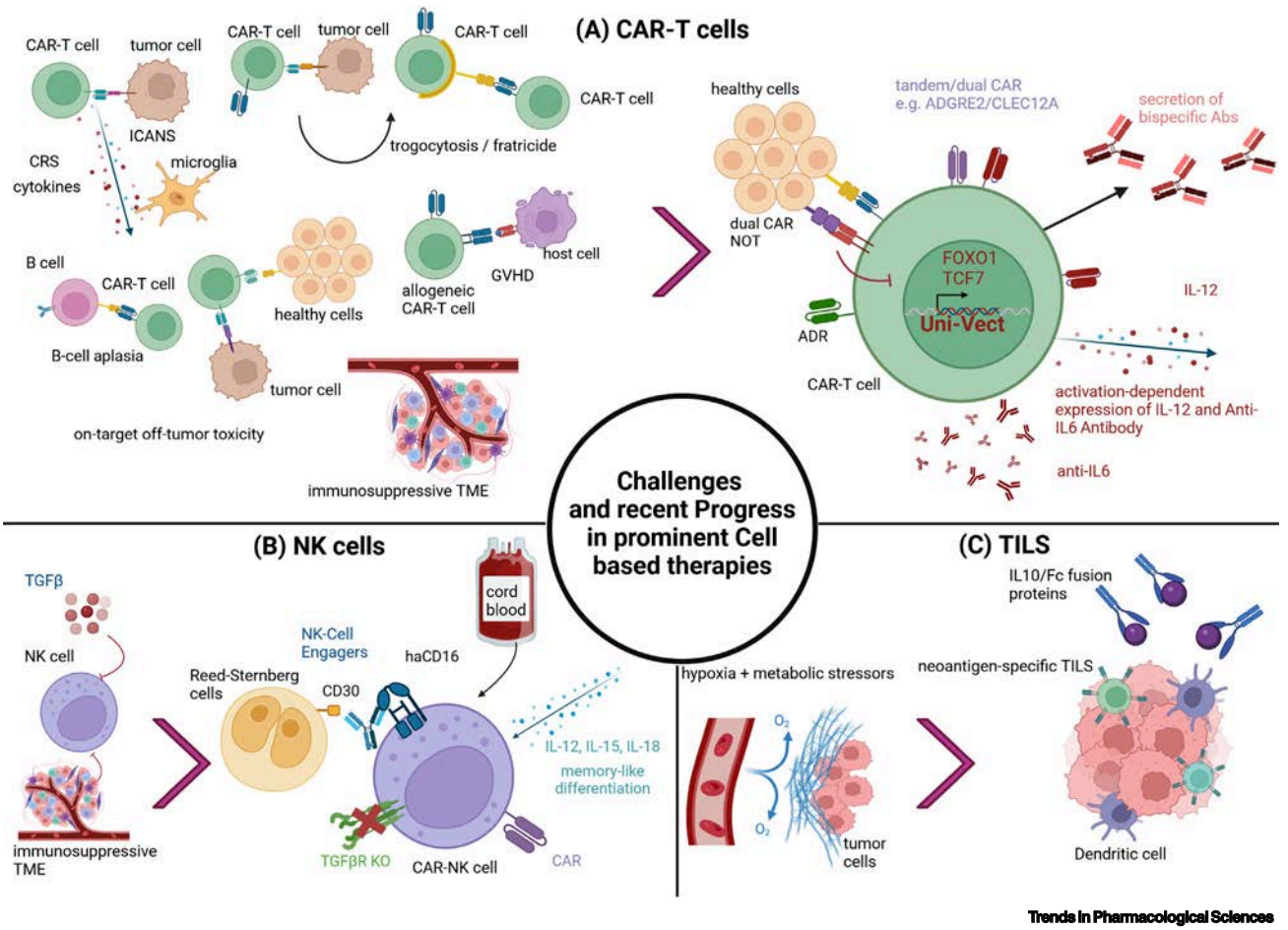



Figure 1. Comprehensive overview of current cell-based cancer immunotherapies. (A) Chimeric antigen receptor (CAR)-T cells. The panel illustrates key challenges (left) and recent progress (right) in CAR-T cell therapy. Challenges include: cytokine release syndrome (CRS) and immune effector cell-associated neurotoxicity syndrome (ICANS) depicted by cytokine release from activated CAR-T cells leading to inflammatory responses and microglia activation in the brain; on-target off-tumor toxicity, shown by CAR-T cells attacking healthy cells expressing the same antigen, such as B cell aplasia in anti-CD19 therapy; trogocytosis, where target antigens are transferred from tumor cells to CAR-T cells, which can lead to fratricide (i.e.. CAR-T cells attacking each other); immunosuppressive tumor microenvironment (TME); and graft-versus-host disease (GvHD) risk in allogeneic CAR-T cells depicted by mismatched CAR-T cells causing immune reactions against the host tissues. Recent progress includes: dual CAR systems for enhanced specificity, such as the NOT gate system with inhibitory and activating CARs, and ADGRE2/CLEC12A targeting; the Uni-Vect system for controlled accessory molecule expression (e.g., IL-12, anti-IL6 antibodies); secretion of bispecific antibodies (Abs) that engage both the tumor antigen and CD3 on T cells, enhancing the recruitment and activation of other immune cells to the tumor site; and universal CAR-T approaches, such as the alloimmune defense receptor (ADR) for 'off-the-shelf' products. (B) Natural killer (NK) cells: challenges (left) include the immunosuppressive TME (represented by TGFβ) and short persistence of NK cells in patients. Advances (right) show NK cell engagers directing cells to tumors, *ex vivo* cytokine stimulation (IL-12, IL-15, and IL-18) of cord blood-derived NK cells to enhance function and provide a memory-like phenotype; TGFβ receptor knockout (TGFBR KO) for TME resistance; and high-affinity CD16 (haCD16) for enhanced antibody-dependent cellular cytotoxicity. Cord blood is illustrated as a source for NK cells, addressing the challenge of limited persistence. (C) tumor-infiltrating lymphocytes (TILs): challenges (left) include T cell exhaustion in the TME and effects of hypoxia and metabolic stressors. Recent progress (right) shows neoantigen-specific TILs for targeted therapy and IL-10/Fc fusion proteins restoring exhausted CD8+ TIL function. Pink right-facing arrowheads indicate progression from challenges to advancements. Figure created using BioRender (biorender.com).

ongoing. Notably, an IL-10/Fc fusion protein was shown to restore the function of exhausted CD8+ TILs, leading to improved tumor eradication and more durable responses when combined with adoptive T cell transfer or immune checkpoint blockade therapies [34]. However, the manufacturing process for T cell-based therapies remains a significant hurdle, because it is expensive, complex, and time-consuming, requiring specialized facilities that limit its widespread accessibility [35].

### Natural killer cell therapy

Complementing T cell-based approaches, innate immune cell therapies are emerging as powerful tools in cancer immunotherapy. Unlike T cells, NK cells recognize and target a variety of stressed or abnormal cells without prior sensitization, including metastatic and **major histocompatibility complex (MHC)**-I-deficient tumor cells [36]. NK cell therapy is advancing with the development of **NK cell engagers**, which direct NK cells specifically to tumors (Figure 1) [37]. *Ex vivo* cytokine stimulation methods are being used to enhance NK cell function and provide a memory-like phenotype, including feeder cell methods using membrane-bound IL-15 and soluble IL-12, IL-15, and IL-18 [38].

To overcome limitations in the TME, researchers are exploring the knockout of TGFβ receptors to resist TGFβ-mediated suppression and engineering cells to express high-affinity CD16a (haCD16a) receptors or IL-2 for improved tolerance to the immunosuppressive TME and enhanced antibody-dependent cellular cytotoxicity (ADCC) (Figure 1) [25,39]. Another limitation is the relatively short persistence of NK cells in the patient, which can reduce their long-term therapeutic efficacy and may require repeated infusions [38]. Additionally, the therapeutic potential of NK cells is heavily influenced by their source, with functional heterogeneity affecting clinical outcomes. Recent research on cord blood-derived NK cells has highlighted this issue, demonstrating that NK cells from optimal cord blood units (CBUs) exhibited greater functionality and are enriched in effector-related genes compared with NK cells from suboptimal CBUs [40]. The need for conditioning regimens in allogeneic NK cell therapies remains a topic of debate, with further clinical studies required to clarify their necessity.

Future research aims to enhance NK cell trafficking, metabolic profile, and effector capacity [36,40,41]. Developing scalable manufacturing processes and maintaining cell viability post cryopreservation are crucial areas of focus. Questions persist regarding the optimal duration of NK cell persistence in patients and its impact on clinical efficacy [36,40]. Besides CAR-NK approaches, new principles are emerging, such as the combination of a bispecific CD30/CD16 antibody with cytokine-activated cord blood and blood-derived NK cells [42].

## Emerging cancer cell-based therapies

### T cell receptor therapy

TCR therapy genetically modifies a patient's T cells to express receptors targeting specific cancer antigens: Unlike CAR-T therapy, TCR-T therapy can recognize intracellular antigens presented by MHC molecules, broadening its potential targets (Figure 2) [43]. This approach focuses on **tumor-associated antigens (TAAs)**, which are overexpressed in cancer cells, and tumor-specific antigens, which are unique to cancer cells [44]. Recent clinical trials have shown promising results for TCR-T therapeutics in both hematological malignancies and solid tumors. Notably, TCR-T therapy demonstrated better outcomes in solid tumors compared with CAR-T therapy [45]. This superior performance may be attributed to the ability of TCRs to recognize a wider range of antigens, their higher sensitivity to low antigen concentrations, and better *in vivo* functionality compared with CAR-T cells [46]. However, TCR therapy, while promising, faces several technical challenges. For instance, manufacturing TCR-T products is complex, time-consuming, and requires specialized facilities. To overcome these technical limitations, off-the-shelf allogeneic products are being evaluated, even though these products may increase the risk for GvHD. Strategies to mitigate this include using specific T cell subsets or gene-edited T cells [35,43]. Future perspectives include developing more efficient manufacturing processes, enhancing T cell persistence and function in immunosuppressive environments, and combining TCR-T with other immunotherapies to improve outcomes across a broader range of cancers.

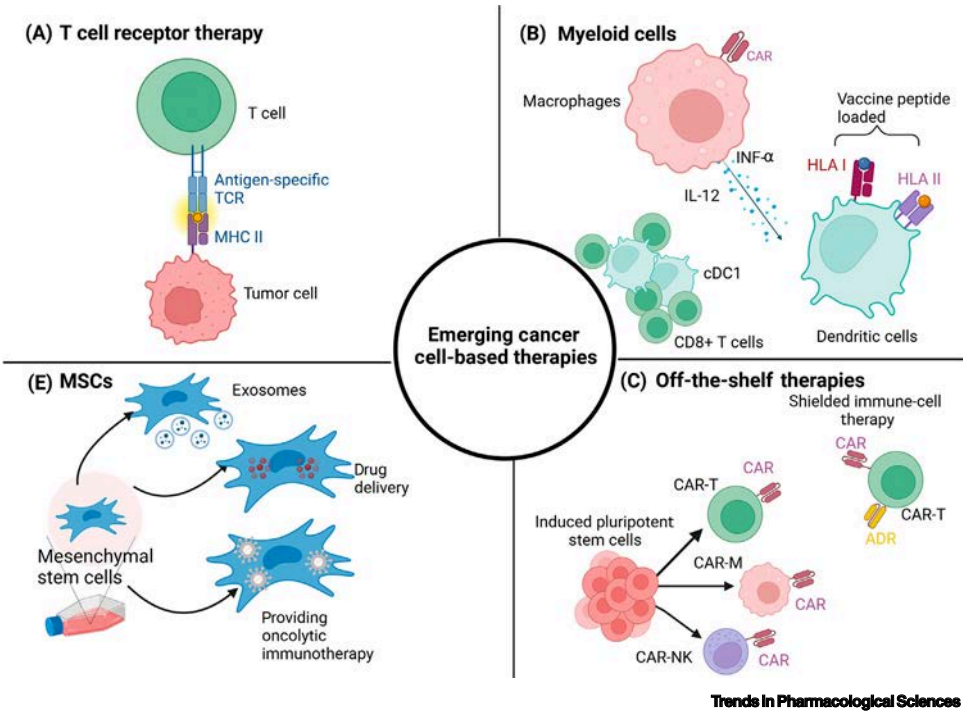



Figure 2. Emerging principles of cell-based cancer therapies. (A) T cell receptor (TCR) therapy: genetically engineered T cells expressing antigen-specific TCRs target intracellular cancer antigens presented by major histocompatibility (MHC) molecules, expanding their therapeutic potential in solid tumors. (B) Myeloid cell therapies, including macrophage-based and dendritic cell (DC) approaches. Chimeric antigen receptor (CAR)-macrophages are engineered to enhance phagocytosis and reshape the tumor microenvironment (TME), while advanced DC strategies involve *in situ* DC vaccines and converting tumor cells into conventional (c)DC1-like cells to activate immune responses against antigens. (C) Off-the-shelf therapies: induced pluripotent stem cells (iPSCs) provide a versatile source for generating ready-to-use CAR-T cells, CAR-macrophages, and CAR-natural killer (NK) cells, facilitating large-scale production. The incorporation of an alloimmune defense receptor (ADR) helps shield these therapeutic cells from immune rejection by the host, increasing their persistence and effectiveness in the TME. (D) Mesenchymal stem cells (MSCs): explored for delivering therapeutic agents, such as TNF-related apoptosis-inducing ligand (TRAIL) and oncolytic viruses, and providing oncolytic immunotherapy, leveraging their ability to release exosomes and influence the TME. Figure created using BioRender (biorender.com).

## Dendritic cell therapies

Cell-programming approaches represent another frontier in cancer immunotherapy, offering unique ways to harness the power of the immune system. In dendritic cell (DC) therapy, researchers are exploring innovative strategies to enhance DC-based vaccines, focusing on improving antigen delivery and optimizing DC maturation. These approaches include developing *in situ* or *in vivo* DC vaccines through direct TAA delivery using DC-targeting immunoliposomes, genetic vectors, or TAAs fused to monoclonal antibodies targeting DC surface receptors [47]. Additionally, efforts are being made to exploit specific naturally available DC subsets [47]. The WT1 transcription factor has emerged as a major target in clinical trials [48], along with other important antigens, such as prostate acidic phosphatase (PAP), human telomerase reverse transcriptase (hTERT), and survivin [47].

Recently, a method to reprogram tumor cells into antigen-presenting conventional DCs (cDCs), specifically cDC1-like cells, *in vivo* using the transcription factors PU.1, IRF8, and BATF3 was reported. cDCs are a subset of DCs with a crucial role in initiating and directing T cell responses (Figure 2). The cDC1 subtype is particularly effective at cross-presenting antigens to CD8+ T cells and producing cytokines that promote antitumor immunity. By harnessing the unique

T cell recruitment and activation properties of cDC1s, this approach aims to enhance immune responses and overcome limitations of current cancer immunotherapies [49,50].

However, DC vaccines alone have demonstrated limited efficacy, prompting the recommendation to combine them with other treatments, such as T cell therapies. Challenges to DC therapy include overcoming the immunosuppressive TME and addressing technical hurdles to using **monocyte-derived DCs (MoDCs)** [51]. Other limitations include the complexity and cost of production, variability in patient responses, and difficulty in identifying the most effective antigens for specific cancer types [51].

### CAR-macrophages

A novel approach in macrophage-based therapy involves engineering macrophages with CARs, creating CAR-macrophages (CAR-M) (Figure 2). These cells can effectively target tumor antigens, enhance phagocytosis, and modulate the TME. Notably, the chimeric Ad5F35 serotype of the Ad5 vector is used for CAR-M generation due to its high transduction efficiency in macrophages, which are otherwise challenging to genetically modify. When macrophages are transduced with the Ad5F35 vector, they adopt a potent M1-like phenotype, enhancing their tumoricidal capabilities. Additionally, CAR-M cells promote epitope spreading through antigen processing and presentation, further stimulating tumor antigen-specific T cell responses and amplifying the antitumor immune response [52,53]. For macrophage-based therapies, future directions include targeting pathways that regulate macrophage polarization [53], using induced pluripotent stem cells (iPSCs) as an alternative cell source, and developing new gene delivery methods [54]. Moreover, innovative experimental strategies have demonstrated marked potential to exploit the monocyte–macrophage transition to selectively introduce exogenous cytokines into the immunosuppressive TME in a spatially and temporally constrained manner, thereby avoiding toxicity associated with systemic cytokine therapy. HSCs have been engineered to produce inflammatory cytokines, such as IFN-α or IL-12, under transcriptional and miRNA-mediated control, thereby acting as a natural shuttle into the TME (Figure 2). Upon entering the tumor, monocytes differentiate toward **tumor-associated macrophages**, which induces the secretion of inflammatory cytokines, an approach that has shown promising results in breaking the highly suppressive TME in preclinical models of cold tumors, such as glioblastoma [55,56].

### Induced pluripotent stem cells

iPSCs are being explored as an unlimited source for generating various immune cells for cancer immunotherapy, including T cells, NK cells, and macrophages (Figure 2). This technology has been previously combined with the CAR platform and has progressed from the preclinical setting to clinical trials. [57]. A promising example is using T cell-derived iPSCs (TiPS) to generate 'off-the-shelf' CAR-T cells. Traditional autologous CAR-T cell production is time-consuming and expensive, whereas TiPS offer scalability. However, early TCR or CAR expression in TiPS can result in cells with an innate-like phenotype. By disabling the TCR and carefully timing CAR expression, researchers generated TCR-deficient CD8αβ+ CAR-T cells with functionality similar to that of peripheral blood CAR-T cells. These iPSC-derived CAR-T cells showed effective tumor control in a mouse leukemia model without causing GvHD [58]. Future research aims to optimize differentiation protocols, improve safety through non-integrating reprogramming methods [57], and enhance cell trafficking and persistence in solid tumors.

### Mesenchymal stem cells

Mesenchymal stem cells (MSCs) are versatile, self-renewing cells that can be harvested from various sources, including adipose tissue, umbilical cord, amniotic fluid, and bone marrow [54]. The role of MSCs in cancer development and progression appears to be context dependent

and remains largely enigmatic to date. In cancer immunotherapy, MSCs show promising results in enhancing CAR-T cell function and providing oncolytic immunotherapy [59], potentially amplifying antitumor effects. MSC-derived **exosomes** may have similar capabilities [60]. However, research outcomes on the ability of MSCs to modulate CAR-related products have been variable. Paradoxically, MSCs have also been implicated in promoting tumor growth and metastasis in certain contexts [61]. Currently, MSCs are being explored as delivery vehicles for therapeutic agents, such as TNF-related apoptosis-inducing ligand (TRAIL) [62] and oncolytic viruses [63] (Figure 2). Future studies should focus on clarifying the role of MSCs in tumor growth, optimizing their engineering, and exploring combination therapies.

While these emerging trends and future directions paint an exciting picture of the potential for cell-based cancer therapies, it is crucial to understand how current innovations are being translated into clinical practice. The journey from laboratory breakthroughs to patient bedsides is complex and multifaceted, involving rigorous testing and evaluation through clinical trials. These trials not only assess the safety and efficacy of new therapies, but also provide valuable insights that shape the future direction of research. As we transition from exploring future possibilities to examining the current clinical landscape, we gain a clearer picture of the challenges and opportunities that lie ahead in bringing these innovative therapies to patients.

## The complex landscape of cell therapy clinical trials

The clinical landscape for cell therapies in cancer treatment is dynamic and rapidly evolving, with numerous clinical trials (Table 1) underway testing various approaches across different cancer types. It is crucial to understand how current innovations are being translated into clinical practice to gain a clearer picture of the challenges and opportunities that lie ahead in bringing these innovative cell therapies to patients.

For CAR-T therapy, trials are exploring its efficacy in a range of hematological malignancies and solid tumors, aiming to address current limitations and expand the applicability of the therapy. In hematological diseases, new targets are being explored. For example, CAR-T cells recognizing the G-protein-coupled receptor, class C, group 5, member D (GPRC5D) have shown efficacy in preclinical multiple myeloma models, including activity in a BCMA antigen escape mode. A Phase 1 trial targeting GPRC5D in multiple myeloma recently reported promising results [64]. In a study of CD30-directed CAR-T cell therapy for patients with heavily pretreated Hodgkin lymphoma, the treatment demonstrated a high overall response rate (ORR) of 72% (59% complete responses; CRs) with manageable toxicity, suggesting its potential as an effective option for relapsed or refractory disease beyond traditional B cell malignancies [65].

Moreover, improved CAR designs are also being evaluated in the clinic to improve the efficacy of CAR-T cell therapies. A CD19-targeting CAR comprising a calibrated signaling module, termed 1XX, demonstrated extended persistence and tumor clearance at lower doses compared with conventional CARs in preclinical studies. This was confirmed in a Phase 1 study, in which patients receiving a low dose of $25\times10^6$ CAR-T cells achieved an 88% ORR and 75% CR with low rates of severe CRS and ICANS, potentially giving rise to novel therapeutic modalities and targets for this disease [66]. Currently, dual antigen-targeting CAR-T cells are being evaluated to address the challenge of antigen loss, a major cause of treatment failure in single-target therapies. For example, one trial evaluated AUTO3, a dual-targeted CAR-T cell therapy simultaneously targeting CD19 and CD22, in pediatric and young adult patients with relapsed or refractory B-ALL. This approach showed a favorable safety profile and high initial remission rate (86%), addressing the challenge of antigen loss [12]. The second trial combined a bispecific CAR targeting CD19 and CD22 (CAR19-22) with NKTR-255, a novel recombinant polymer-conjugated IL-15 receptor

agonist, in adults with relapsed/refractory B-ALL. This combination demonstrated safety, high remission rates (89%), and improved 12-month progression-free survival compared with historical controls [67].

In solid tumors, targets such as Claudin 18.2 are emerging, with interim results showing promise in digestive system cancers [68]. In glioblastoma treatment, promising results have been shown with CARv3-TEAM-E T cells, which target both EGFR variant III and wild-type EGFR through secretion of a T cell-engaging antibody molecule [69]. Another approach involves intrathecal delivery of bivalent CAR-T cells targeting both EGFR and IL13Rα2 [70]. Prostate stem cell antigen (PSCA)-targeted CAR-T cell therapy for metastatic castration-resistant prostate cancer induced prostate-specific antigen (PSA) declines and radiographic improvements in some patients despite limited persistence [71], while HER2-targeted treatment for advanced soft tissue sarcoma achieved clinical benefit in 50% of patients with observable CAR-T cell expansion, both demonstrating manageable safety profiles and offering insights into tumor immune dynamics [72].

TIL therapy is being extensively studied in clinical trials for solid cancers. In nonsmall cell lung cancer, lifileucel showed an objective response rate of 21.4% in a Phase 2 study [32]. In soft tissue sarcoma, TIL therapy combined with oncolytic peptides showed modest efficacy [73]. While not a major breakthrough, these initial results offer a glimmer of hope and warrant further investigation in this difficult-to-treat cancer entity.

CAR-M therapy is still in its early clinical stages, with CT-0508, an autologous HER2-targeting CAR-M therapy generated from a leukapheresis product, receiving Fast Track designation from the FDA. CAR-M therapy, while promising, has shown limited efficacy in early clinical trials. CT-0508 manufacturing was deemed feasible for all enrolled patients and demonstrated favorable safety and tolerability with no dose-related toxicities. However, suboptimal therapeutic outcomes were observed with CAR-M monotherapy, as well as with combination treatment with PD-1 inhibition in a substudy of this trial. To potentially enhance efficacy, Carisma Therapeutics, the company that developed CT-0525, initiated a clinical trial investigating the potential of CAR-monocyte therapy as means of reducing manufacturing time and increasing cell dosing, tumor infiltration, persistence, and potentially clinical outcomes [74].

NK cell therapies are also advancing, with Phase 1 clinical trials for universal off-the-shelf iPSC-derived NK cell products underway. A groundbreaking Phase 1/2 trial has already shown remarkable promise. In this study, patients with relapsed or refractory CD19-positive cancers received infusions of HLA-mismatched anti-CD19 CAR-NK cells derived from cord blood. The results were striking: an ORR of 73%, offering new hope to patients who had exhausted other treatment options [75]. However, innovations do not stop there. Researchers are also exploring novel approaches for patients who have undergone allogeneic hematopoietic stem cell transplantation (allo-HSCT) for AML. These strategies involve harnessing the power of donor memory-like (ML) NK cells, supercharged *ex vivo* with IL-12 and IL-15. This approach aims to boost the anti-cancer efficacy of NK cells, potentially reducing the risk of relapse in patients with AML post transplant [76,77].

TCR-T therapy clinical trials have shown promising results in both blood cancers and solid tumors. In one Phase 1 trial using nonviral precision TCR replacement, 31.25% of patients with refractory solid cancer achieved at least stable disease [78].

Combination therapies are emerging as a key trend, particularly the integration of cell therapies with **immune checkpoint inhibitors (ICIs)** [79]. This approach shows promise in overcoming resistance to single-agent therapies and improving overall treatment outcomes. Cell-based

therapies, such as CAR-T cells and DC-targeted approaches, including mRNA vaccines (e.g., melanoma FixVac) [80], have demonstrated potential in treating various cancers by stimulating antigen-specific immune responses. However, their efficacy can be limited by immunosuppressive mechanisms in the TME. To address this challenge, combining cell-based therapies with checkpoint blockade (e.g., PD-1/PD-L1 inhibitors) has become a promising strategy to enhance antitumor immunity, potentially converting 'cold' tumors into 'hot' ones with a strong immune infiltrate, as shown for the anti-PD1 plus MDM2-inhibitor combination for melanoma [81]. Ongoing clinical trials are exploring the combination of DC vaccines with ICIs, such as a Phase 1/2 trial testing a WT1 DC vaccination in combination with PD-L1 inhibition and conventional chemotherapy [82], and another approach combining autologous tumor lysate-pulsed DC vaccination with TLR agonists in malignant gliomas [83]. These combinatorial approaches aim to leverage the strengths of both cell-based therapies and checkpoint inhibition to create more robust and durable antitumor responses.

## Outstanding questions

How can we enhance cell therapy efficacy in solid tumors?

What strategies can mitigate severe side effects, such as CRS?

Can we develop truly 'off-the-shelf' allogeneic therapies without rejection risks?

How do we optimize manufacturing processes for wider accessibility?

What combination strategies will yield the most durable responses?

## Concluding remarks and future perspectives

Cell-based cancer therapies are showing promising results in clinical trials, yet expanding their application beyond B cell malignancies remains a key hurdle. Significant challenges remain in enhancing efficacy for solid tumors, mitigating side effects, developing true 'off-the-shelf' therapies, optimizing manufacturing, and identifying optimal combination strategies (see Outstanding questions). Recent advancements in cell engineering, including base editing and dual-antigen targeting, are improving safety and efficacy across cancer types. Novel approaches, such as *in vivo* CAR-T generation and CAR-macrophages, aim to overcome current limitations, particularly in solid tumors. Allogeneic strategies and iPSC-derived immune cells offer potential for improving accessibility. However, these advanced therapies require careful patient stratification, and there is a lack of reliable biomarkers for patient selection and assessing target engagement. Combination strategies, particularly with checkpoint inhibitors, are being explored to address challenges posed by the immunosuppressive TME. Looking ahead, advanced gene editing, **synthetic biology**, **optogenetics**, and artificial intelligence (AI)-driven approaches may create more targeted and controllable immune effector cells. Synthetic biology could enable the creation of 'smart' therapeutic cells with enhanced antitumor capabilities, while optogenetics offers the potential for precise, real-time control of cell behavior *in vivo*. While promising, these approaches present new challenges in terms of safety, ethics, and regulation. Thus, realizing the potential of cell-based cancer therapies for a range of malignancies requires overcoming significant challenges in their development, delivery, and personalization.

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### Declaration of interests

R.Z. received honoraria from Novartis, MNK, Incyte, Medac, Neovii and VectivBio outside the submitted work. The remaining authors declare no conflict of interest related to this study.

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