Emerging Frontiers in Cancer Immunotherapy: Recent Advances in CAR-NK Cell and CAR-Macrophage Therapies

David Drew

Department of Natural Science and Mathematics, University of Houston, Texas, USA
Corresponding author: dddrew@uh.edu

Abstract:

Chimeric antigen receptor (CAR) cell therapies have revolutionized the field of cancer treatment with the breakthrough successes of CAR-T cell therapy in hematological malignancies. However, CAR-T cell efficacy in solid tumors has been limited by challenges such as the immunosuppressive tumor microenvironment (TME) and safety concerns like cytokine release syndrome (CRS) and graft-versus-host disease (GvHD). This review focuses on the latest advancements in CAR-NK cell and CAR-macrophage therapies, which offer potential solutions to these limitations. CAR-NK cells exhibit natural cytotoxicity and MHC-independent tumor targeting, providing a safer profile with lower risks of GvHD and CRS compared to CAR-T cells. Innovations in CAR-NK cell therapy includes structural optimizations, gene editing with CRISPR-Cas9, and the development of multispecific CARs, all contributing to enhanced anti-tumor efficacy, especially in solid tumors. Meanwhile, CAR-macrophages are effective in remodeling the TME through phagocytosis and immune activation, addressing the challenges of solid tumor infiltration. This review compares the mechanisms of action, clinical applications, and safety profiles of CAR-NK cells and CAR-macrophages, highlighting the synergistic potential of these therapies. Although clinical research on both therapies is still in its early stages, the promising preclinical and early clinical trial results underscore their potential as alternative immunotherapies for refractory cancers. Future research will focus on overcoming challenges in production scalability, improving in vivo persistence, and personalizing CAR therapies through precision medicine approaches. These two therapies will likely play pivotal roles in the next generation of cancer immunotherapies.

Keywords: CAR-NK cells; CAR-macrophages; cancer immunotherapy; tumor microenvironment (TME).

1. Introduction

Over the last decade, chimeric antigen receptor (CAR)-based cell therapies have emerged as a leading approach in cancer immunotherapy [1-5]. The initial successes of CAR-T cell therapy, particularly in preclinical research and clinical trials, demonstrated potent anti-tumor effects and remarkable improvements in response and remission rates compared to traditional treatments for various hematological malignancies [2, 4, 5]. However, these successes have not been replicated in solid tumors, and clinical trials for CAR-T cells targeting solid tumors have only recently been approved [2, 4, 5].

The challenges in applying CAR-T cell immunotherapy to solid tumors are numerous. They include limited trafficking and infiltration of T cells into the tumor, difficulties in in vivo expansion and persistence, the immunosuppressive tumor microenvironment (TME), tumor resistance and escape mechanisms, as well as safety problems including graft-versus-host disease (GvHD) and cytokine release syndrome (CRS) [4, 5] . Recent studies exploring the application of CAR technology to natural killer (NK) cells and macrophages have shown promising results, although clinical research in these areas is still in its early stages [1-4]. Each type of immune cell—NK cells and macrophages—plays a unique role in the immune system and presents distinct advantages and limitations with regards to CAR-based therapy. NK cells, as cytotoxic innate immune system lymphocytes, are capable of MHC-independent targeting of tumor cells and possess a superior safety profile compared to CAR-T cells, with minimal risk of GvHD and CRS. This is due to their different targeting and cytokine signaling mechanisms and their low persistence in vivo [2-4]. NK cells utilize multiple recognition pathways, balancing signals from activating receptors—which bind to stress-induced ligands and non-classical MHC class I molecules—and inhibitory receptors, which recognize classical MHC class I molecules [2, 3]. Additionally, NK cells recognize the Fc fragment of antibodies and engage in antibody-dependent cellular cytotoxicity [2-4]. The downregulation of MHC molecules is a common antigen escape mechanism in tumors, which is countered by the MHC-independent recognition pathways of NK cells [2, 3]. However, like T cells, NK cells are not

abundant among tumor-infiltrating lymphocytes (TILs) in the TME and struggle to resist the immunosuppressive environment of solid tumors [2-4].

Macrophages, another critical component of the innate immune system, are phagocytic cells that target dead and dying cells and are key players in antigen presentation and recruitment of both the innate and adaptive immune system. Tumor-associated macrophages (TAMs) are often of the immunosuppressive M2 phenotype, which is crucial for maintaining the immunosuppressive TME, and they constitute the majority of tumor-infiltrating immune cells [2-4]. However, macrophages exhibit high phenotypic plasticity and can also adopt the pro-inflammatory M1 phenotype, which plays an immunostimulatory role through the secretion of inflammatory cytokines [1, 4]. Macrophages support cancer immunity through the phagocytosis of cells undergoing immunogenic cell death and through antibody-dependent cellular phagocytosis, which facilitates the recruitment of TILs via antigen presentation [1, 4]. While macrophages share some safety concerns with T cells, such as CRS, they have an advantage in tumor infiltration due to their ability to recognize the hypoxic environment of the TME directly [1, 4].

The basic design principles of CAR technology are consistent across NK cells, macrophages, and T cells. A typical CAR consists of an extracellular recognition domain—typically the light and heavy chain variable regions of a tumor antigen antibody—fused with the intracellular CD3ζ signaling domain, along with costimulatory domains like CD28 and 4-1BB (CD137) [1-5]. While the majority of CARs share this TCR-derived structure, NK cells have their own non-TCR signaling receptors, and some studies have explored CAR constructs that utilize the intracellular components of activating NK cell receptors to enhance cytotoxicity [2-4]. CAR-T cell therapy has the most extensive body of preclinical research and the largest number of clinical trials, particularly in the context of liquid cancers. There are currently five FDA-approved CAR-T cell treatments targeting relapsed B cell malignancies, including lymphomas, B cell leukemias, and multiple myelomas [2, 4, 5]. In contrast, CAR NK cells and CAR macrophages remain primarily of preclinical interest, with a growing body of literature supporting their efficacy, but less than a hundred clinical trials registered on ClinicalTrials.gov for both types of treatments [2, 4, 5]. Among these, only one clinical trial by Liu et al. has reported results—a phase I trial involving allogeneic cord blood-derived anti-CD19 IL-15 armored CAR-NK cells targeting relapsed and refractory CD19+ malignancies (NCT03056339) [2-4].

This review will explore the latest advances in CAR-NK and CAR-macrophage therapies, comparing their efficacy, advantages, and limitations with each other and with CAR-T cell therapy. Future perspectives and challenges in CAR cancer immunotherapy will be discussed, with the aim of identifying unmet therapeutic needs and formulating effective strategies to address these challenges, thereby paving the way from bench to clinic for these novel therapies.

2. Latest Advances in CAR NK Cells

2.1 Structural Improvements of CAR NK Cells

Various studies have explored structural optimization and innovative designs for CAR constructs in CAR-NK cells. Many of these advancements have been derived from CAR-T cells, particularly third- and fourth-generation constructs, which incorporate cytokine co-expression and additional co-stimulatory domains [2-4]. Specific optimizations for NK cell signaling pathways have also been investigated, such as employing NK cell-activating receptors and costimulatory domains [2-4]. Research into bispecific and multispecific CAR-NK cells offers significant potential for treating refractory cancers, relapse in patients, and improving efficacy in solid tumors [2-4].

Next-generation CAR constructs, particularly third- and fourth-generation CAR-T designs, include co-expression of cytokines, inhibitory components, and logic gates, which have notably enhanced the CAR-T cell therapy's efficacy and safety [2, 3]. These strategies are being utilized in CAR-NK cell therapy, leading to improved anti-tumor action, especially in CD19+ hematological malignancies like acute lymphoblastic leukemia (ALL) and multiple myeloma (MM) [6-12]. There have also been some reports of success in solid tumors, such as glioblastoma, lung cancer and stomach cancer [13-18]. New designs incorporating inhibitory domains from killer cell immunoglobulin-like receptors (KIRs) aim to prevent effector cell fratricide resulting from trogocytosis, where CAR-NK cells acquire tumor antigens from target cells [19]. Additionally, inhibitory signals like PD-1 can be inverted by integrating the extracellular PD-1 receptor into the CAR construct [18]. Programmable logic-gated CARs have also been adapted to CAR-NK cells, with one example being MyD88, an activator regulated by the small molecule rimiducid [20]. Despite the generally favorable safety profile of CAR-NK cells, inducible caspase-9 (iC9) switches activated by small molecules have been introduced as a safety mechanism [9, 20].

Furthermore, the incorporation of extracellular NKG2D receptor domains and NK cell-specific costimulatory domains, such as 2B4 and DNAM1, is hypothesized to enhance NK cell proliferation and persistence by engag-

ing NK-specific activating pathways [11]. This has been shown to increase anti-tumor efficacy in both tumor xenograft models of solid tumors and liquid cancers. CAR constructs can also harness antibody-dependent cellular cytotoxicity (ADCC) by integrating the extracellular domain of the CD16 (FcγRIII) receptor [21]. Bispecific and tandem CAR designs have garnered significant interest, with examples such as CD19 and mesothelin tandem CARs for gastric cancer and bispecific GD2-NKG2D CARs for glioblastoma, which have demonstrated enhanced anti-tumor efficacy and cytotoxicity in vivo [17, 18]. The use of these designs holds promise for improving solid tumor targeting and leveraging NK-specific antitumor mechanisms like NKG2D receptors and ADCC.

2.2 Gene Editing in CAR NK Cells

The precision and versatility of CRISPR-Cas9 gene editing have played a crucial role in advancing CAR-NK cell therapy. One study demonstrated that CRISPR-Cas9 gene knockout of CD38 in CD38 CAR-NK cells reduced effector cell fratricide, enabling an effective anti-tumor response against acute myeloid leukemia (AML) by targeting the CD38 tumor-associated antigen (TAA), which is also expressed in NK cells at significant levels [11]. Besides introducing CAR constructs, accessory co-stimulatory molecules, switches, and other elements—each requiring genetic engineering—other gene-editing applications are essential for CAR-NK therapy. Co-expression of the CXCR1 chemokine receptor with an NKG2D CAR has been reported to increase tumor infiltration and anti-tumor effects in ovarian tumor xenografts [21]. To minimize potential in vivo side effects, the CAR and CXCR1 were delivered transiently in mRNA form via electroporation, while other transduction methods, such as viral vectors, allowed integration of the desired transgene into the NK cell genome [2, 3, 21]. As the understanding of NK cells in cancer biology becomes more refined, gene editing will play an increasingly prominent role in refining CAR-NK cell therapy through rational engineering strategies.

2.3 Application of CAR NK Cells in Different Tumors

Preclinical research on CAR-NK cells, similar to CAR-T cells, has primarily achieved successes in hematological malignancies. In addition to widely studied antigens like CD19, CAR constructs targeting antigens such as CD123 in AML and FMS-like tyrosine kinase 3 (FLT3) have shown promise [12, 22]. Tumor-specific antigens have also been used, such as NPM1-mutant neoepitope CAR-NK constructs targeting NPM1-mutated AML [10]. Recently, efforts have expanded to address solid tumors with CAR-NK cells. Studies targeting solid tumors with HER2, EGFR, and tissue factor (TF)-specific CARs have shown promising results in vivo using tumor xenograft models [14, 23, 24]. Other studies targeting solid tumors include c-Met CAR-NK cells for hepatocellular carcinoma and prostate stem cell antigen (PSCA) CAR-NK cells for pancreatic cancer, all demonstrating therapeutic efficacy [25].

While these preclinical results support further exploration of CAR-NK cells in solid tumors, most ongoing clinical trials remain focused on hematological malignancies, and no CAR-NK cell therapies have been FDA-approved. To date, only three phase I/II clinical trials involving CAR-NK therapy are registered with the ClinicalTrials.gov database, and just one has published results (NCT03056339). Liu et al. conducted a phase I trial using allogeneic cord blood-derived anti-CD19 IL-15-armored CAR-NK cells to target relapsed and refractory CD19+ malignancies, including ALL [26]. The trial showed a 48.6% overall response (OR) rate at day 30 and day 100 endpoints, with a 37.8% complete remission (CR) rate at one year. Patients maintained CR with a 70% probability over one year, and importantly, no significant CRS or neurotoxicity was observed [26]. These results provide strong justification for continuing efforts to translate CAR-NK therapy into clinical practice.

2.4 Production and Clinical Application of CAR NK Cells

One of the major challenges in CAR-NK cell therapy is the search for an "off-the-shelf" CAR-NK cell source for clinical use. Unlike allogeneic CAR-T cell therapy, which carries risks such as GvHD, allogeneic CAR-NK cell therapy offers safety and enhances NK cell anti-tumor effects, making it feasible to develop a universal NK cell source [2-4]. The NK-92 cell line, frequently used in preclinical studies, must be irradiated due to its lymphoma origins, preventing proliferation in vivo, which makes it unsuitable for therapeutic use [2,-4]. Current manufacturing efforts face hurdles such as low transduction efficiency and high production costs.

Various strategies have been used to improve transduction rates while maintaining NK cell viability, including viral and other methods such as optimized viral vectors (BaEV-LVs) and retroviral vectors [24, 27, 28]. Non-viral methods include the piggyBac transposon system, direct electroporation of CAR plasmids, and charge-altering releasable transporters (CARTs) [29-31]. These methods enable larger CAR constructs and co-expression of additional genes. Another challenge in manufacturing is the need for extensive NK cell expansion. Feeder cells, paired with stimulatory cytokines, are typically used, but the cost of obtaining and culturing feeder cells is prohibitive for large-scale production [2-4, 12]. Alternative approaches,

such as feeder-free systems and induced pluripotent stem cells (iPSCs), show promise in producing high yields of CAR-NK cells while maintaining viability and persistence [2-4, 32, 33].

2.5 Safety and Persistence of CAR NK Cells

The favorable safety profile of CAR-NK cells has been widely documented in preclinical studies, with no significant adverse effects except in cases involving IL-15 co-expression, where mild CRS was observed [2-4 9]. The MHC-independent recognition pathways and limited cytokine release of NK cells allow for allogeneic transplantation without the risk of GvHD or CRS, major concerns in CAR-T cell therapy [2-4]. However, the primary challenge in CAR-NK cell therapy remains low persistence and limited response to treatment [2-4]. Efforts to improve CAR-NK cell resistance to immunosuppressive mechanisms, particularly in solid tumors, continue to be a focus of research.

3. Latest Advances in CAR Macrophages

3.1 Development and Design of CAR Macrophages

CAR-macrophages share structural similarities with CAR-NK cells, consisting of a fused extracellular recognition and intracellular signaling domain, and co-stimulatory molecules [1, 4]. However, macrophage-specific intracellular domains activate unique functions not found in cytotoxic immune cells [1, 4]. For instance, the FcRγ signaling domain is essential for phagocytosis, while the CD147 domain promotes the secretion of matrix metalloproteinases (MMPs), facilitating tumor infiltration by degrading the extracellular matrix (ECM) [1, 4, 34]. Unlike CAR-NK cells, CAR-macrophages excel at infiltrating solid tumors and remodeling the tumor microenvironment (TME) [1, 4, 34]. Furthermore, CAR-macrophages can be reprogrammed from the M2 to M1 phenotype, increasing their anti-tumor efficacy [1, 4, 34]. Efforts to optimize CAR-macrophages are focused on overcoming their inability to proliferate in vivo and addressing challenges such as liver migration and macrophage checkpoints like CD47-SIRPα, which inhibit phagocytosis [1, 4, 34].

3.2 Role of CAR Macrophages in Tumor Microenvironment

CAR-macrophages play a vital role in shaping the TME. Upon activation, they are readily repolarized into the M1 phenotype, which resists immunosuppression and secretes pro-inflammatory cytokines [1, 4, 34]. These cytokines stimulate nearby immune cells and enhance phagocytosis and antigen presentation [1, 4, 34]. Studies have demonstrated that CAR-macrophages improve tumor clearance in vitro, reduce tumor burden, and extend survival in tumor xenograft mouse models [1, 4, 34]. Furthermore, a synergistic effect has been observed between CAR-T cells and CAR-macrophages, enhancing the combined anti-tumor response through cytokine signaling [1, 4, 34].

3.3 Preclinical and Clinical Studies of CAR Macrophages

Preclinical research on CAR-macrophages has been limited by the inability of differentiated macrophages to proliferate, complicating large-scale manufacturing [34]. One promising approach involves in vivo transfection of macrophages with CAR transgenes. For example, a study using a nanoporter-hydrogel system achieved transfection of CD133 CAR constructs into microglia within the tumor resection cavity, successfully managing relapse in post-operative glioblastoma (GBM) models [35]. Other methods have included using lipid nanoparticles to deliver CAR constructs, demonstrating potential for in vitro CAR expression in macrophages [36]. Ex vivo fabrication of CAR macrophages from iPSCs and hematopoietic stem cells (HSCs), which can readily expand, be modified with a CAR construct, and then differentiated into CAR-macrophages, is another promising approach [37, 38]. Gene editing technologies have been applied to improve the efficacy of CAR-macrophages, particularly in enhancing the pro-inflammatory M1 phenotype [39].

4. Comparative Analysis and Discussion of CAR NK Cells and CAR Macrophages

4.1 Comparison of Biological Characteristics and Mechanisms of Action

CAR-NK cells and CAR-macrophages have unique immunological properties that grant them different advantages in anti-tumor immunity. CAR-NK cells benefit from distinct recognition pathways that detect stress-induced ligands, downregulation of classical MHC molecules, and presentation of non-classical MHC molecules, which are often found in cancer cells [2-4]. This makes CAR-NK cells especially effective in targeting treatment-resistant and refractory cancer cells, as they exploit immune escape mechanisms used by tumors [2-4]. However, CAR-NK cells are challenged by poor in vivo persistence, poor expansion, and difficulties infiltrating solid tumors [2-4]. Once inside, they are susceptible to exhaustion and immunosuppression, similar to other tumor-infiltrating immune cells [2-4]. In contrast, CAR-macrophages lack direct cytotoxicity but are highly effective at reshaping the TME and engaging the broader immune system [1, 4, 34].

Macrophages naturally infiltrate solid tumors, where they make up the majority of tumor-resident immune cells, typically in the immunosuppressive M2 phenotype [1, 4, 34]. However, their phenotypic plasticity allows them to be polarized into pro-inflammatory M1 macrophages, which help relieve suppression of surrounding immune cells and induce an endogenous immune response [1, 4, 34]. This capability is enhanced by macrophage-mediated phagocytosis and tumor antigen presentation following the immunogenic cell death of cancer cells [1, 4, 34]. Additionally, both CAR-NK cells and CAR-macrophages benefit from antibody-directed responses against tumors, leveraging NK cell cytotoxicity and macrophage phagocytosis [1-4, 34].

4.2 Comparison of Clinical Applications and Efficacy

Both CAR-NK cells and CAR-macrophages show great potential in clinical applications, but there is a more extensive body of clinical research on CAR-NK cells. There is at least one completed clinical trial with published results for CAR-NK cells, while CAR-macrophages have only one active phase I clinical trial targeting HER2-positive solid tumors, according to ClinicalTrials.gov (NCT04660929) [26]. The clinical trial for CAR-NK cells reported impressive outcomes, with a 48.6% overall response (OR) rate and a 37.8% complete remission (CR) rate after one year [26, 40]. CAR-NK cells also exhibit an excellent safety profile, showing minimal risk of toxicities and side effects like GvHD, CRS and neurotoxicity, while maintaining their cytotoxic effects on tumors [2-4]. In contrast, CAR-macrophages are prone to CRS due to the secretion of pro-inflammatory cytokines, which poses a significant safety concern for patients [34]. CRS symptoms, such as fever, joint pain, and fatigue, must be monitored, and treatment with steroids (e.g., dexamethasone and methylprednisolone) and tocilizumab (an FDA-approved cytokine antibody) can mitigate these side effects [41]. In some preclinical CAR constructs, suicide switches like inducible caspase-9 (iC9) have been incorporated, allowing treatment cessation in cases of severe toxicity [9, 20].

4.3 Comparison of Safety and Side Effects

As CAR-NK and CAR-macrophage therapies evolve, next-generation CAR designs will likely adopt features from CAR-T cell research, including dual, tandem, and multispecific CAR constructs [1, 5]. These designs help overcome antigen escape mechanisms and improve specificity for solid tumors [1, 5]. For example, programmable CAR constructs like the synNotch circuit combine AND/NOT gates to integrate signals from multiple antigens, managing the risk of side effects [1, 5]. Synthetic cytokine circuits are also employed to ensure that cytokines interact only with the CAR-modified cells, reducing the risk of CRS and neurotoxicity [1, 5]. Gene editing technologies, such as CRISPR, are expected to enhance the efficacy of CAR therapies as well. By silencing antigens expressed in both cancer and immune cells, such as CD38 in AML, researchers can prevent effector cell fratricide and maintain treatment efficacy [1, 5, 11]. As understanding of immune checkpoints and NK/macrophage signaling grows, gene knockout strategies will also be used to disrupt inhibitory pathways, improving CAR cell persistence and activity in the TME.

5. Future Directions and Research Challenges

5.1 Technological Developments and Innovations, Challenges in Clinical Application

Both CAR-NK and CAR-macrophage therapies face significant hurdles in clinical application and manufacturing. One emerging trend in CAR-NK research is the pursuit of a universal CAR-NK cell source, which would significantly reduce production costs and time [2-4]. Cell lines like NK-92 have been optimized for high expansion and viability in laboratory settings, but their clinical utility is limited by poor in vivo persistence and proliferation [2-4]. CAR-macrophages face even greater challenges due to their inability to proliferate once fully differentiated [1, 4, 34]. Early studies on in vivo gene editing of macrophages were complex, costly, and yielded low numbers of tumor-infiltrating CAR-macrophages [34]. A promising solution for both therapies involves using iPSCs and HSCs, which can be expanded in the lab before differentiation into CAR-NK or CAR-macrophages [1-4, 34-36]. This approach allows for a streamlined, GMP-compliant fabrication process, although optimal timing for CAR introduction and stem cell expansion remains an area of active research. As clinical trials increase, CAR-NK cell therapy will likely expand beyond hematological malignancies to include solid tumors, while CAR-macrophages will be studied beyond HER2-positive cancers. Advances in CAR construct design, safety features, and manufacturing processes will make these therapies accessible to a wider patient population, addressing an urgent unmet need for effective cancer immunotherapies.

5.2 Personalized Medicine and Precision Therapy

Some early efforts have been made toward personalized CAR-NK and CAR-macrophage therapies. One study used clinical trial, transcriptomic, and proteomic data

to identify potential CAR targets, creating a database of over 100 targets and 100,000 target pairs for designing patient-specific therapies and improving patient matching with existing CAR treatments [42]. Another study developed organoids derived from patient cancer cells to model healthy colorectal tissue and tumor tissue, utilizing a luciferase-based viability assay to visualize CAR-NK-92 toxicity across different targets [43]. These models provide a valuable tool for optimizing antigen selection and treatment efficacy before clinical application. As machine learning algorithms, genomic datasets, and patient-derived tumor models improve, the potential for personalized CAR cell therapy will grow, offering tailored treatments based on patient-specific factors. This will be especially important as CAR-NK and CAR-macrophage therapies become established as viable alternatives to CAR-T cell therapies.

6. Conclusion

CAR-NK cell and CAR-macrophage therapies represent significant advancements in cancer immunotherapy and is a solution to the limitations faced by CAR-T cell therapy, particularly in targeting solid tumors. While CAR-T cell therapy has demonstrated notable successes in hematological malignancies, it struggles with the complexities of the TME, limited trafficking, and high risks of CRS and GvHD [1, 5]. In contrast, CAR-NK cells provide a safer alternative with minimal risk of CRS and GvHD due to their innate immune properties and MHC-independent mechanisms [2-4]. Similarly, CAR-macrophages bring unique advantages by remodeling the TME and enhancing anti-tumor immunity through phagocytosis, antigen presentation, and polarization from immunosuppressive M2 macrophages to pro-inflammatory M1 phenotypes [1, 4, 34]. In terms of structural and technological improvements, both CAR-NK cells and CAR-macrophages are benefiting from advancements initially developed for CAR-T cells [1, 5]. Third- and fourth-generation CAR constructs with co-stimulatory molecules, cytokine co-expression, and logic gates, have been adapted to improve the effect and safety of CAR-NK cells [1-5]. These innovations, alongside gene editing technologies, are enabling more precise design of CAR cells to improve tumor targeting, persistence, and resistance to TME immunosuppression. The versatility of CAR designs, such as bispecific and tandem CARs, is further increasing their effectiveness, especially in refractory cancers and solid tumors.

CAR-macrophages, while still in the early stages of clinical research, show promise in overcoming the challenge of solid tumor infiltration which they are naturally suited to. Their ability to reshape the TME by releasing pro-inflammatory cytokines and promoting antigen presentation sets them apart from other CAR-based therapies [1, 4, 34]. However, their clinical application is limited by their inability to proliferate in vivo and the complexity of their manufacturing process [1, 4, 34]. As iPSCs and HSCs emerge as viable platforms for scalable production, the use of these stem cell sources could revolutionize the large-scale manufacturing of CAR-NK cells and CAR-macrophages, reducing costs and manufacturing time.

Although clinical studies on CAR-NK and CAR-macrophages is still lacking compared to CAR-T cells, early results are promising. Studies, such as the phase I trial of cord blood-derived anti-CD19 IL-15-armored CAR-NK cells, have demonstrated durable responses in patients with minimal toxicity [26]. CAR-macrophage therapy is also advancing, with ongoing clinical trials targeting HER2-positive solid tumors showing potential in reshaping the TME and boosting immune responses [40]. Looking forward, future research will likely focus on overcoming the remaining challenges in CAR-NK and CAR-macrophage therapy, such as improving in vivo persistence, expanding the range of targetable tumors, and further optimizing CAR constructs for both efficacy and safety. The integration of programmable CAR designs, synthetic cytokine circuits, and gene editing will enable more precise and personalized therapies, aligning with the emerging field of precision medicine. Personalized approaches, leveraging genomic, transcriptomic, and proteomic data, along with patient-derived tumor models, will play a critical role in optimizing CAR therapies to meet individual patient needs.

In conclusion, CAR-NK cells and CAR-macrophages have immense potential to fill unmet therapeutic gaps left by CAR-T cell therapy, especially for patients suffering from treatment-resistant cancers or those ineligible for CAR-T therapy. With continued technological innovations, the development of personalized therapies, and further clinical trials, CAR-NK and CAR-macrophage therapies could become essential components of the future landscape of cancer immunotherapy.

References

[1] Chen, Y., Yu, Z., Tan, X., Jiang, H., Xu, Z., Fang, Y., & Tu, J. (2021). CAR-macrophage: A new immunotherapy candidate against solid tumors. BIOMEDICINE & PHARMACOTHERAPY, 139, Article 111605. https://doi.org/10.1016/j.biopha.2021.111605

[2] Wendel, P., Reindl, L., Bexte, T., Künnemeyer, L., Särchen, V., Albinger, N., & Ullrich, E. (2021). Arming Immune Cells for Battle: A Brief Journey through the Advancements of T and NK Cell Immunotherapy. CANCERS, 13, Article 1481. https://doi.

org/10.3390/cancers13061481
[3] Khawar, M., & Sun, H. (2021). CAR-NK Cells: From Natural Basis to Design for Kill. FRONTIERS IN IMMUNOLOGY, 12, Article 707542. https://doi.org/10.3389/fimmu.2021.707542
[4] Pan, K., Farrukh, H., Chittepu, V., Xu, H., Pan, C., & Zhu, Z. (2022). CAR race to cancer immunotherapy: from CAR T, CAR NK to CAR macrophage therapy. JOURNAL OF EXPERIMENTAL & CLINICAL CANCER RESEARCH, 41, Article 119. https://doi.org/10.1186/s13046-022-02327-z
[5] Sterner, R., & Sterner, R. (2021). CAR-T cell therapy: current limitations and potential strategies. BLOOD CANCER JOURNAL, 11, Article 69. https://doi.org/10.1038/s41408-021-00459-7
[6] Xiao, L., Cen, D., Gan, H., Sung, Y., Huang, N., Xiong, H.,& Xu, X. (2019). Adoptive Transfer of NKG2D CAR mRNA-Engineered Natural Killer Cells in Colorectal Cancer Patients. MOLECULAR THERAPY, 27, 1114-1125. https://doi.org/10.1016/j.ymthe.2019.03.011
[7] Leivas, A., Valeri, A., Córdoba, L., García-Ortiz, A., Ortiz, A., Sánchez-Vega, L.,& Martínez-López, J. (2021). NKG2D-CAR-transduced natural killer cells efficiently target multiple myeloma. BLOOD CANCER JOURNAL, 11, Article 146. https://doi.org/10.1038/s41408-021-00537-w
[8] Cichocki, F., Bjordahl, R., Goodridge, J., Mahmood, S., Gaidarova, S., Abujarour, R.,& Miller, J. (2022). Quadruple gene-engineered natural killer cells enable multi-antigen targeting for durable antitumor activity against multiple myeloma. NATURE COMMUNICATIONS, 13, Article 7341. https://doi.org/10.1038/s41467-022-35127-2
[9] Silvestre RN, Eitler J, de Azevedo JTC, Tirapelle MC, Fantacini DMC, de Souza LEB, Swiech K, Covas DT, Calado RT, Montero PO, Malmegrim KCR, Figueiredo ML, Tonn T and Picanc¸o-Castro V (2023) Engineering NK-CAR.19 cells with the IL15/IL-15Ra complex improved proliferation and anti-tumor effect in vivo. Front. Immunol. 14:1226518. doi: 10.3389/fimmu.2023.1226518
[10] Dong, H., Ham, J., Hu, G., Xie, G., Vergara, J., Liang, Y.,& Romee, R. (2022b). Memory-like NK cells armed with a neoepitope-specific CAR exhibit potent activity against NPM1 mutated acute myeloid leukemia. PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF THE UNITED STATES OF AMERICA, 119
[11] Gurney, M., Stikvoort, A., Nolan, E., Kirkham-McCarthy, L., Khoruzhenko, S., Shivakumar, R.,& O'Dwyer, M. (2022). CD38 knockout natural killer cells expressing an affinity optimized CD38 chimeric antigen receptor successfully target acute myeloid leukemia with reduced effector cell fratricide. HAEMATOLOGICA, 107, 437-445. https://doi.org/10.3324/haematol.2020.271908.
[12] Caruso, S., De Angelis, B., Del Bufalo, F. et al. Safe and effective off-the-shelf immunotherapy based on CAR.CD123-NK cells for the treatment of acute myeloid leukaemia. J Hematol Oncol 15, 163 (2022). https://doi.org/10.1186/s13045-022-01376-3
[13] Huang, Y., Zeng, J., Liu, T., Xu, Q., Song, X., & Zeng, J. (2020). DNAM1 and 2B4 Costimulatory Domains Enhance the Cytotoxicity of Anti-GPC3 Chimeric Antigen Receptor-Modified Natural Killer Cells Against Hepatocellular Cancer Cells in vitro. CANCER MANAGEMENT AND RESEARCH, 12, 3247-3255. https://doi.org/10.2147/CMAR.S253565
[14] Han, J., Chu, J., Chan, W., Zhang, J., Wang, Y., Cohen, J.,& Yu, J. (2015). CAR-Engineered NK Cells Targeting Wild-Type EGFR and EGFRvIII Enhance Killing of Glioblastoma and Patient-Derived Glioblastoma Stem Cells. SCIENTIFIC REPORTS, 5, Article 11483. https://doi.org/10.1038/srep11483
[15] Zhang, Y., Zhang, C., He, M., Xing, W., Hou, R., & Zhang, H. (2024). Co-expression of IL-21-Enhanced NKG2D CAR-NK cell therapy for lung cancer. BMC CANCER, 24, Article 119. https://doi.org/10.1186/s12885-023-11806-1
[16] Wang, J., Toregrosa-Allen, S., Elzey, B., Utturkar, S., Lanman, N., Bernal-Crespo, V.,& Matosevic, S. (2021). Multispecific targeting of glioblastoma with tumor microenvironment-responsive multifunctional engineered NK cells. PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF THE UNITED STATES OF AMERICA, 118, Article e2107507118. https://doi.org/10.1073/pnas.2107507118
[17] Cao, B., Liu, M., Huang, J., Zhou, J., Li, J., Lian, H.,& Zhu, K. (2021). Development of mesothelin-specific CAR NK-92 cells for the treatment of gastric cancer. INTERNATIONAL JOURNAL OF BIOLOGICAL SCIENCES, 17, 3850-3861. https://doi.org/10.7150/ijbs.64630
[18] Lu, C., Guo, C., Chen, H., Zhang, H., Zhi, L., Lv, T., & Zhu, W. (2020). A novel chimeric PD1-NKG2D-41BB receptor enhances antitumor activity of NK92 cells against human lung cancer H1299 cells by triggering pyroptosis. MOLECULAR IMMUNOLOGY, 122, 200-206. https://doi.org/10.1016/j.molimm.2020.04.016
[19] Li, Y., Basar, R., Wang, G., Liu, E., Moyes, J., Li, L., & Rezvani, K. (2022). KIR-based inhibitory CARs overcome CAR-NK cell trogocytosis-mediated fratricide and tumor escape. NATURE MEDICINE, 28. https://doi.org/10.1038/s41591-022-02003-x
[20] Wang, X., Jasinski, D., Medina, J., Spencer, D., Foster, A., & Bayle, J. (2020). Inducible MyD88/CD40 synergizes with IL-15 to enhance antitumor efficacy of CAR-NK cells. BLOOD ADVANCES, 4, 1950-1964. https://doi.org/10.1182/bloodadvances.2020001510
[21] Ng, Y., Tay, J., & Wang, S. (2020). CXCR1 Expression to Improve Anti-Cancer Efficacy of Intravenously Injected CAR-NK Cells in Mice with Peritoneal Xenografts. MOLECULAR THERAPY-ONCOLYTICS, 16, 75-85. https://doi.org/10.1016/j.omto.2019.12.006
[22] Oelsner, S., Waldmann, A., Billmeier, A., Roder, J., Lindner, A., Ullrich, E., & Wels, W. (2019). Genetically engineered

CAR NK cells display selective cytotoxicity against FLT3-positive B-ALL and inhibit in vivo leukemia growth. INTERNATIONAL JOURNAL OF CANCER, 145, 1935-1945. https://doi.org/10.1002/ijc.32269

[23] Hu, Z. (2020). Tissue factor as a new target for CAR-NK cell immunotherapy of triple-negative breast cancer. SCIENTIFIC REPORTS, 10, Article 2815. https://doi.org/10.1038/s41598-020-59736-3

[24] Strecker, M., Wlotzka, K., Strassheimer, F., Roller, B., Ludmirski, G., König, S., & Burger, M. (2022). AAV-mediated gene transfer of a checkpoint inhibitor in combination with HER2-targeted CAR-NK cells as experimental therapy for glioblastoma. ONCOIMMUNOLOGY, 11, Article 2127508. https://doi.org/10.1080/2162402X.2022.2127508

[25] Liu, B., Liu, Z., Zhou, M., Lin, J., Chen, X., Li, Z., & Liu, T. (2019). Development of c-MET-specific chimeric antigen receptor-engineered natural killer cells with cytotoxic effects on human liver cancer HepG2 cells. MOLECULAR MEDICINE REPORTS, 20, 2823-2831. https://doi.org/10.3892/mmr.2019.10529

[26] Marin, D., Li, Y., Basar, R., Rafei, H., Daher, M., Dou, J.,& Rezvani, K. (2024). Safety, efficacy and determinants of response of allogeneic CD19-specific CAR-NK cells in CD19⁺ B cell tumors: a phase 1/2 trial. NATURE MEDICINE. https://doi.org/10.1038/s41591-023-02785-8

[27] Colamartino, A., Lemieux, W., Bifsha, P., Nicoletti, S., Chakravarti, N., Sanz, J.,& Haddad, E. (2019). Efficient and Robust NK-Cell Transduction With Baboon Envelope Pseudotyped Lentivector. FRONTIERS IN IMMUNOLOGY, 10, Article 2873. https://doi.org/10.3389/fimmu.2019.02873

[28] Müller, S., Bexte, T., Gebel, V., Kalensee, F., Stolzenberg, E., Hartmann, J.,& Ullrich, E. (2020). High Cytotoxic Efficiency of Lentivirally and Alpharetrovirally Engineered CD19-Specific Chimeric Antigen Receptor Natural Killer Cells Against Acute Lymphoblastic Leukemia. FRONTIERS IN IMMUNOLOGY, 10, Article 3123. https://doi.org/10.3389/fimmu.2019.03123

[29] Du, Z., Ng, Y., Zha, S., & Wang, S. (2021). piggyBac system to co-express NKG2D CAR and IL-15 to augment the in vivo persistence and anti-AML activity of human peripheral blood NK cells. MOLECULAR THERAPY-METHODS & CLINICAL DEVELOPMENT, 23, 582-596. https://doi.org/10.1016/j.omtm.2021.10.014

[30] Ingegnere, T., Mariotti, F., Pelosi, A., Quintarelli, C., De Angelis, B., Tumino, N.,& Moretta, L. (2019). Human CAR NK Cells: A New Non-viral Method Allowing High Efficient Transfection and Strong Tumor Cell Killing. FRONTIERS IN IMMUNOLOGY, 10, Article 957. https://doi.org/10.3389/fimmu.2019.00957

[31] Wilk, A., Weidenbacher, N., Vergara, R., Haabeth, O., Levy, R., Waymouth, R.,& Blish, C. (2020). Charge-altering releasable transporters enable phenotypic manipulation of natural killer cells for cancer immunotherapy. BLOOD ADVANCES, 4, 4244-4255. https://doi.org/10.1182/bloodadvances.2020002355

[32] Yang, Y., Badeti, S., Tseng, H., Ma, M., Liu, T., Jiang, J.,& Liu, D. (2020). Superior Expansion and Cytotoxicity of Human Primary NK and CAR-NK Cells from Various Sources via Enriched Metabolic Pathways. MOLECULAR THERAPY-METHODS & CLINICAL DEVELOPMENT, 18, 428-445. https://doi.org/10.1016/j.omtm.2020.06.014

[33] Ueda, T., Kumagai, A., Iriguchi, S., Yasui, Y., Miyasaka, T., Nakagoshi, K.,& Kaneko, S. (2020). Non-clinical efficacy, safety and stable clinical cell processing of induced pluripotent stem cell-derived anti-glypican-3 chimeric antigen receptor-expressing natural killer/innate lymphoid cells. CANCER SCIENCE, 111, 1478-1490. https://doi.org/10.1111/cas.14374

[34] Klichinsky, M., Ruella, M., Shestova, O., Lu, X., Best, A., Zeeman, M.,& Gill, S. (2020). Human chimeric antigen receptor macrophages for cancer immunotherapy. NATURE BIOTECHNOLOGY, 38, 947-+. https://doi.org/10.1038/s41587-020-0462-y

[35] Chen, C., Jing, W., Chen, Y., Wang, G., Abdalla, M., Gao, L.,& Jiang, X. (2022). Intracavity generation of glioma stem cell-specific CAR macrophages primes locoregional immunity for postoperative glioblastoma therapy. SCIENCE TRANSLATIONAL MEDICINE, 14, Article eabn1128. https://doi.org/10.1126/scitranslmed.abn1128

[36] Ye, Z., Chen, J., Zhao, X., Li, Y., Harmon, J., Huang, C.,& Xu, Q. (2022). In Vitro Engineering Chimeric Antigen Receptor Macrophages and T Cells by Lipid Nanoparticle-Mediated mRNA Delivery. ACS BIOMATERIALS SCIENCE & ENGINEERING, 8, 722-733. https://doi.org/10.1021/acsbiomaterials.1c01532

[37] Zhang, J., Webster, S., Duffin, B., Bernstein, M., Steill, J., Swanson, S.,& Slukvin, I. (2023). Generation of anti-GD2 CAR macrophages from human pluripotent stem cells for cancer immunotherapies. STEM CELL REPORTS, 18. https://doi.org/10.1016/j.stemcr.2022.12.012

[38] Paasch, D., Meyer, J., Stamopoulou, A., Lenz, D., Kuehle, J., Kloos, D.,& Moritz, T. (2022). Ex Vivo Generation of CAR Macrophages from Hematopoietic Stem and Progenitor Cells for Use in Cancer Therapy. CELLS, 11, Article 994. https://doi.org/10.3390/cells11060994

[39] Wang, X., Su, S., Zhu, Y., Cheng, X., Cheng, C., Chen, L.,& Zhang, J. (2023). Metabolic Reprogramming via ACOD1 depletion enhances function of human induced pluripotent stem cell-derived CAR-macrophages in solid tumors. NATURE COMMUNICATIONS, 14, Article 5778. https://doi.org/10.1038/s41467-023-41470-9

[40] National Library of Medicine (U.S.). (2021, February -). CAR-macrophages for the Treatment of HER2 Overexpressing Solid Tumors. Identifier NCT04660929. https://www.clinicaltrials.gov/study/NCT04660929

[41] Ayuketan, A., Jäger U. Management of Cytokine Release Syndrome (CRS) and HLH (2022). The EBMT/EHA CAR-T

Cell Handbook. Springer, Chapter 26. https://www.ncbi.nlm.nih.gov/books/NBK584171/ doi: 10.1007/978-3-030-94353-0_26

[42] MacKay, M., Afshinnekoo, E., Rub, J., Hassan, C., Khunte, M., Baskaran, N.,& Mason, C. (2020). The therapeutic landscape for cells engineered with chimeric antigen receptors. NATURE BIOTECHNOLOGY, 38, 233-+. https://doi.org/10.1038/s41587-019-0329-2

[43] Schnalzger, T., Groot, M., Zhang, C., Mosa, M., Michels, B., Röder, J., & Farin, H. (2019). 3D model for CAR-mediated cytotoxicity using patient-derived colorectal cancer organoids. EMBO JOURNAL, 38, Article e100928. https://doi.org/10.15252/embj.2018100928