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Beyond CAR-T: The rise of CAR-NK cell therapy in asthma immunotherapy

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Abstract

Asthma poses a major public health burden. While existing asthma drugs manage symptoms for many, some patients remain resistant. The lack of a cure, especially for severe asthma, compels exploration of novel therapies. Cancer immunotherapy successes with CAR-T cells suggest its potential for asthma treatment. Researchers are exploring various approaches for allergic diseases including membrane-bound IgE, IL-5, PD-L2, and CTLA-4 for asthma, and Dectin-1 for fungal asthma. NK cells offer several advantages over T cells for CAR-based immunotherapy. They offer key benefits: (1) HLA compatibility, meaning they can be used in a wider range of patients without the need for matching tissue types. (2) Minimal side effects (CRS and GVHD) due to their limited persistence and cytokine profile. (3) Scalability for "off-the-shelf" production from various sources. Several strategies have been introduced that highlight the superiority and challenges of CAR-NK cell therapy for asthma treatment including IL-10, IFN-γ, ADCC, perforin-granzyme, FASL, KIR, NCRs (NKP46), DAP, DNAM-1, TGF-β, TNF-α, CCL, NKG2A, TF, and EGFR. Furthermore, we advocate for incorporating AI for CAR design optimization and CRISPR-Cas9 gene editing technology for precise gene manipulation to generate highly effective CAR constructs. This review will delve into the evolution and production of CAR designs, explore pre-clinical and clinical studies of CAR-based therapies in asthma, analyze strategies to optimize CAR-NK cell function, conduct a comparative analysis of CAR-T and CAR-NK cell therapy with their respective challenges, and finally present established novel CAR designs with promising potential for asthma treatment.

Introduction

Asthma, a chronic lung disease affecting over 300 million people globally [1], remains a significant healthcare burden. Despite advancements in treatment, severe asthma can lead to frequent flare-ups and life-threatening complications [2, 3]. Current therapies like corticosteroids offer some relief but may not fully control the underlying inflammation or prevent exacerbations [4]. Although several FDA-approved mAb therapies, such as omalizumab and dupilumab, offer some clinical benefit in severe atopic asthma, these treatments necessitate frequent administration due to their short half-life. Furthermore, they primarily manage symptoms by reducing exacerbation frequency and severity, rather than providing a definitive cure [5].

The involvement of NK cells in asthma remains complex and not fully understood. While traditionally known for their antiviral function [6], NK cells might play a dual role in allergic asthma, either promoting or suppressing the disease [7]. Their activation is influenced by a balance of inhibitory and stimulatory signals from various

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receptors [8–10]. These receptors recognize molecules like MHC-I, cytokines, and microbial components. Human NK cells can be categorized into two subsets based on CD56 expression [11]. Studies have observed changes in NK cell phenotype and function in asthma patients. For example, patients with severe asthma exhibit increased expression of CD69 and NKG2D on their NK cells [12, 13]. Research using mouse models further highlights the complexity of NK cells' role in asthma. Some studies suggest their involvement in allergic inflammation [14], while others indicate minimal or even a resolving effect [4, 15]. Furthermore, NK cells have been shown to produce various immune mediators like IFN-γ and TNF-α upon IgE stimulation, and they can even attack IgE-coated target cells [16]. This suggests a potential contribution of NK cells to IgE-mediated allergic responses.

Recent successes in cancer immunotherapy utilizing CAR-based therapies against tumor-specific neoantigens (e.g., HSP70) suggest the potential application of CAR technology for targeted immunotherapies in asthma. CAR technology offers an approach to engineer immune cells for antigen-specific recognition [17]. Several researchers have explored the potential of CAR-T cell therapy for allergic diseases like asthma [18–22]. One approach focuses on eliminating B cells that produce IgE, a key antibody in allergic reactions. Studies are investigating CARs that bind to membrane-bound IgE on B cells, offering the potential for long-term suppression with a single treatment [18, 19]. Another approach targets eosinophils, white blood cells involved in allergic inflammation. Engineered CAR-T cells can eliminate these cells and potentially suppress asthmatic symptoms [20, 21]. Additionally, research is ongoing to manipulate Tregs with CARs to suppress allergic airway inflammation [22]. While no current clinical trials test CAR therapy for asthma, these studies suggest a promising future direction for treating allergic diseases. Although CAR-T cell therapy has succeeded in various diseases, limitations like adverse effects, cytotoxicity, and T-cell exhaustion (terminal differentiation) highlight the need for alternative cell types [23–25].

NK cells offer several advantages over T cells for CAR-based immunotherapy in the context of asthma. Unlike T cells requiring antigen presentation, NK cells utilize innate receptors for activation. This allows CAR-NK cells to retain cytotoxic function even if the target molecule changes, potentially overcoming a limitation of CAR-T cell therapy [26]. Additionally, NK cells have a shorter lifespan in vivo, potentially reducing the risk of severe side effects associated with CAR-T cells, such as CRS and GVHD [27].

While the core structure of the CAR construct might be similar in CAR-NK and CAR-T cells, NK cells offer additional functionalities. They utilize co-stimulatory molecules like NKG2D and CD244 (2B4) to amplify their cytotoxic activity and cytokine production [27]. Furthermore, NK cells possess a broader recognition repertoire beyond the specifically targeted antigen through receptors like NCRs, NKG2D, and DNAM-1 [28, 29]. This broader recognition potential translates to a more robust and potentially more effective targeting strategy in the context of asthma.

Although research on CAR cell therapy for asthma is in its early stages, the inherent advantages of NK cells, such as readily available cell sources and potentially lower risk of complications compared to CAR-T cells, suggest a promising future direction. To comprehensively explore this potential, this review will delve into several key areas. First, we'll examine the evolution and production of CAR designs. Next, we'll critically assess existing preclinical and clinical studies of CAR-based therapies in asthma to understand current progress and identify areas for further research. Additionally, we'll explore strategies to optimize CAR-NK cell function, focusing on ways to modulate their natural abilities for enhanced efficacy and safety in asthma treatment. Furthermore, a comparative analysis of CAR-T and CAR-NK cell therapy will be conducted, highlighting their respective advantages and limitations in the context of asthma. Finally, we'll discuss established and novel CAR designs with promising potential for treating severe asthma. By comprehensively addressing these objectives, this review aims to provide a valuable resource for researchers and clinicians seeking to understand the potential of CAR-NK cell therapy as a novel and promising approach to managing severe asthma.

Evolution of CAR design: From first generation to enhanced functionalities

While CAR-T cell therapy is currently a leading focus in adoptive immunotherapy with success in treating leukemia and lymphomas, CAR-NK cell therapy is emerging as a promising new area with growing preclinical research [30]. The foundation for ACT was established in 1982 by Rosenberg et al., who developed an in vitro culture system capable of activating and expanding autologous PB lymphocytes. The system included NK cells, T cells, NKT cells, and monocytes from PB, with the addition of IL-2. These cultured lymphocytes exhibited enhanced killing abilities compared to NK cells and cytotoxic T lymphocytes, earning them the name lymphokine-activated killer cells [31]. The rapid development of gene engineering in the 1980s introduced novel methods in the field of ACT. The pioneering work of Yoshihisa Kuwana in 1987 described the first CAR, a fusion protein combining the antigen-binding domain of an antibody with the signaling domain of a T cell receptor [32]. This concept was further refined by Arthur et al. in 1991 with the creation of a CAR

incorporating the CD3ζ intracellular signaling domain. This iteration marked the final form of the first-generation CAR, characterized by a scFv of immunoglobulin and CD3ζ signaling domains [33]. While First-generation CARs demonstrate antigen recognition, their clinical efficacy is diminished by the inadequate activation and proliferation of the T cells expressing these CARs [34]. In the early 2000s, the significance of co-stimulators was acknowledged and incorporated into CARs, resulting in the second generation. The positive outcomes of clinical trials using second-generation CAR-T targeting CD19 and CD20 in hematopoietic malignancies led to the first FDA approval of CAR-T (Tisagenlecleucel, Kymriah, and Novartis) in 2017 [35–38]. To enhance its effectiveness, multiple domains from co-stimulators (CD28, CD27, OX40, 4-1BB, etc.) were inserted into CARs. The ongoing pursuit of improved clinical efficacy has driven the exploration of further functionalities within CAR design. Immunomodulatory factors such as cytokines, chemokines, growth factors, IFN, and TNF were added to construct the fourth generation of CARs [39, 40]. The newest generation of CAR technology, known as the fifth generation, introduces an additional co-stimulatory domain to activate novel signaling pathways within CAR cells. This innovative design integrates a co-stimulatory molecule with a fragment of a cytokine receptor, such as the IL2Rβ chain, which then interacts with STAT proteins and CD27 molecules inside the cell. This enhanced structure allows for antigen-specific activation of the JAK/STAT pathway, promoting cell proliferation and preventing premature exhaustion) of the CAR cells [41].

The function of CAR therapy is indeed determined by the structure of the CAR molecule, which acts as a bridge between the immune system and target cells [42]. This section will delve into the specific influence of each component of the CAR molecule on its functionality (Fig. 1).

Extracellular target-binding domain

This domain, typically incorporating a scFv comprising a heavy chain and a light chain connected through a linker, a nanobody, or a ligand specific for the target antigen, dictates the targeting specificity of CAR cells. Beyond specificity, the binding affinity between the target-binding domain and its cognate antigen significantly influences CAR performance. Deviations from optimal binding affinity, either excessively high or low, can compromise therapeutic efficacy [43]. Consequently, additional factors such as charge density of the target antigen [44], epitope location [45], and overall target antigen density on the target cell surface [46] must also be

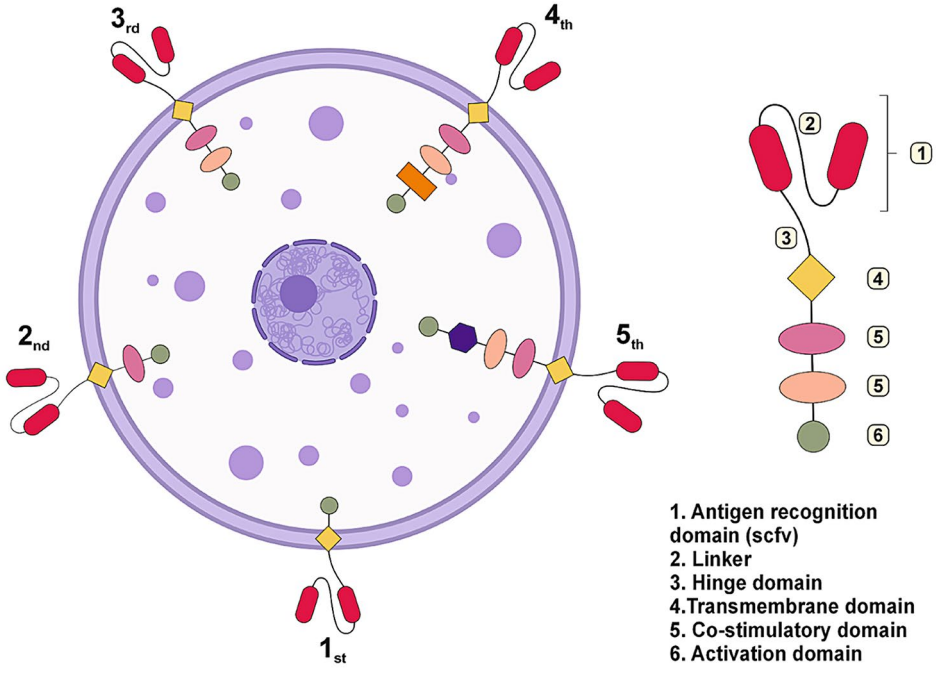

Fig. 1 Unveiling the Emergence and Development of CAR NK Cells: A Classification into Five Generations: The figure depicts a sequential representation of CAR development, categorized by their structural characteristics and costimulatory signaling molecules. 1st generation: The first-generation design, characterized by a simple ScFv-CD3ζ configuration, omits co-stimulatory signals, thereby compromising therapeutic efficacy. 2nd and 3rd generation: The inclusion of one or two costimulatory molecules has been shown to augment NK cell activation and persistence. 4th generation: This approach utilizes transgenic integration to achieve cytokine secretion, specifically IL-12, fostering a multifaceted immune response against targeted cells. 5th generation: The integration of a costimulatory signaling molecule within the cytokine receptor domain of a CAR enhances the coordinated cytolytic activity of CAR- NK cells

meticulously considered during CAR design for optimal therapeutic outcomes.

Spacer or hinge domain

The hinge domain, situated extracellularly, acts as a crucial linker between the target-binding domain and the transmembrane domain of the CAR molecule. Emerging evidence suggests that the hinge domain also influences CAR function. The spatial positioning of the target antigen relative to the plasma membrane can be influenced by the hinge length, consequently affecting the binding efficiency of the CAR molecule. Therefore, for optimal design, CAR development should incorporate hinge lengths tailored to the specific target antigen for enhanced therapeutic efficacy [47].

Transmembrane domain

While the primary function of the transmembrane domain is to bind the CAR molecule to the cell membrane, recent research suggests it exerts a more multifaceted influence. This domain can impact the expression level and stability of the CAR itself, modulate the formation of the immunological synapse, and potentially be involved in the dimerization of endogenous signaling molecules [48, 49]. The transmembrane domain frequently utilized in NK cells is sourced from CD3ζ, CD8, or CD28. However, alternative transmembrane segments like NKG2D, 2B4, and DNAM-1 have also been employed for this purpose [50].

Intracellular signal domain

This part integrates both signaling and costimulatory domains to activate NK or T cells. The signaling domain, typically CD3ζ or FcεRIγ, harbors ITAMs that mimic the TCR signaling function and initiate the activation cascade within the cell. Additionally, Costimulatory domains, frequently derived from the CD28 family (CD28, ICOS) or the TNFR family (4-1BB, OX40, CD27), synergize with costimulatory molecules to amplify intracellular activation signals [51, 52].

CAR-NK therapy uses similar engineered receptors (CARs) as CAR-T cell therapy. When genetically modified with a CAR, NK cells gain targeted killing ability alongside their natural cytotoxicity [53]. Researchers are enhancing NK cell killing by replacing components of the T cell CAR (CD3z, CD28, and/or CD137) with signaling molecules naturally found on NK cells, such as 2B4, NKG2D, DAP10, or DAP12. This modification led to a boost in NK cell activation and their ability to destroy cells [54]. To enhance the design of CAR for NK cells, Li et al. conducted a study comparing a single CAR-T cell design with nine different CAR-NK cell designs. These CAR-NK cells featured four distinct transmembrane domains and various intracellular signaling domains, all aimed at targeting the same mesothelin antigen. The research showed that CAR-NK cells equipped with the NKG2D transmembrane domain, 2B4 co-stimulatory domain, and CD3ζ signaling domain displayed potent and specific cytotoxicity against the target antigen [50].

Cell sources and production of CAR-NK

Similar to the well-established workflow for autologous CAR-T therapy, other CAR-engineered immune cells such as CAR-NK, CAR-macrophage, and CAR-CIK typically follow a standardized process. This includes:

1. Leukapheresis to isolate and enrich patient cells.
2. Activation and expansion of the cells.
3. Genetically modifying the cells with a CAR using viral (such as Lentivirus, adeno-associated virus, and γ-retrovirus) or nonviral (such as e transposon systems, CRISPR/Cas9 systems and mRNA electroporation platforms) methods.
4. Expanding the CAR cells outside the body (ex vivo).
5. Formulating and cryopreserving the final cell product.
6. Administering lymphodepleting treatment followed by patient reinfusion of the CAR cells [55–57].

Manufacturing CAR cell therapy in the traditional way has several hurdles. The process itself is lengthy, taking up to 22 days and requiring specialized labs, which significantly inflates the cost and potentially delays treatment for patients in urgent need [58]. Furthermore, Isolating cells can be tricky, with a risk of contamination from other blood cells. Additionally, not all these cells readily accept the CAR modification, leading to inconsistencies [59]. The extended time these cells spend growing outside the body (ex vivo) and the processes of freezing and transport can further diminish their effectiveness. Moreover, CAR cell therapy may not be suitable for everyone. Patients who received stem cell transplants may have an altered immune system that reduces the treatment's efficacy [60, 61]. New developments offer a promising solution to the challenges of CAR cell therapy. Using CAR products derived from donors who are either fully compatible (HLA-identical) or partially compatible (HLA-haplotype matched) with the patient offers a way to overcome these issues [62].

Several cell sources can be used to generate NK cells for CAR-NK therapy, including PBMCs, hPSCs, iPSCs, UCB, and the NK line cell line which are compared in Table 1. PBMCs are a traditional source of NK cells for CAR-NK therapy. These NK cells are typically mature (CD56dimCD16bright) and exhibit consistent characteristics across individuals [63]. They also express high levels of activating receptors (NKG2D, NKp44, NKp46) that enhance their ability [64] However, there are some

Table 1 Comparation between different sources of NK cells for CAR NK cell production

Sources	Advantage	Disadvantage	References
PB	1. Strong killing potential 2. express high levels of activating receptors 3. Mature phenotype 4. High safety and low toxicity	1. Donor dependent 2. Heterogenous cell population 3. Limited availability of NK cells 4. Low gene transduction efficiency 5. Potential for GvHD with allogeneic cells 6. Limited lifespan 7. Decreased viability and function after cryopreservation 8. Lengthy and complex collection and storage	[63–68]
UCB	1. Abundant source and better proliferation capacity 2. Easier collection and storage 3. Reduced risk of GVHD 4. Lower immunogenicity 5. higher proportion of NK cells (15–30%) 6. higher proportion of memory cells 7. higher purity (92.3%) of CAR-NK cells	1. Heterogenous cell population 2. immature phenotype 3. lower levels of activating receptors and adhesion molecules 4. higher level of the inhibitory receptor NKG2A 5. diminished cytotoxic function in vitro 6. risk of incomplete differentiation and oncogenicity 7. Numerically few and therefore requires ex vivo expansion	[30, 69, 70, 74–78, 214]
hPSCs and iPSCs	1. Homogeneous population 2. unlimited source and high proliferation capacity 3. stronger cytotoxicity 4. more efficient at expressing CAR structures 5. amenable to genetic engineering 6. potency to production of "off-the-shelf" CAR-NK cell 7. May exhibit epigenetic memory 8. No need for eradiation before injection 9. High concentration and purity of NK cells (> 90%)	1. Complicated production process 2. Higher Immunogenicity 3. Risk of oncogenicity 4. Potential for amplified genetic variations 5. low ADCC due to low CD16 expression 6. heterogeneity among different iPSC lines presents	[69, 89, 90, 93, 95–99]
NK cell line	1. homogeneity population 2. lesser GVHD risk 3. lack specific inhibitory receptors KIRs 4. amenable to genetic engineering 5. unlimited source and high proliferation capacity 6. lower levels of inhibitory receptors such as NKG2A 7. potency to production of "off-the-shelf" CAR-NK cell 8. less affected by the freezing and thawing process	1. need for eradiation before injection 2. Deficiency of ADCC due to lack of CD16 expression 3. Limited survival in vivo potential carrier of genetic abnormalities	[79–84]

key limitations associated with using PB-NK cells. Firstly, the limited availability of NK cells in PB (around 10–15%) presents a challenge for collection and ex vivo expansion, potentially hindering large-scale production. Secondly, PB-NK cells exist in various stages of maturity, resulting in a diverse range of receptor expression profiles. This inherent heterogeneity makes it difficult to guarantee the standardization and stability of the final CAR-NK cell product, which is crucial for consistent therapeutic outcomes [65]. In addition, The precise eradication of T cells is essential to minimize the impact of GVHD [66]. Furthermore, incorporating the CAR gene into PB-NK cells is less efficient compared to other cell sources. Prolonged expansion of PB-NK in the lab (in vitro) can shorten telomeres, which are structures on chromosomes that influence cell lifespan. Finally, freezing PB-NK cells (cryopreservation) further reduces their viability and effectiveness [67, 68].

Another source of NK cells is UCB. UCB is a valuable source of stem cells found in the umbilical cord and placenta after childbirth. These stem cells are unique in their ability to differentiate into various cell types, including blood cells, nerve cells, and immune cells [69]. UCB is rich not only in HSCs but also in immune cells like T cells and NK cells [70]. This makes it a promising source material for developing CAR-T and CAR-NK cell therapies [71, 72]. Compared to adult PB, immune cells in UCB are younger and less developed. They have a greater ability to multiply and a higher proportion of NK cells (15–30%) [73].In addition, this immaturity translates to a specific profile: lower CD16 expression, higher CD56 bright expression, NKG2A positivity, and lower KIR expression [74]. Studies suggest that CAR cells derived from UCB have a higher proportion of memory cells compared to those from adult blood. This could lead to longer persistence in the body and a more sustained immune response, potentially resulting in a more durable therapeutic effect [75, 76]. Additionally, UCB-derived transplants are less likely to cause GVHD, reducing the risk of this serious side effect [77]. Nevertheless, UCB-NK cells exhibit diminished cytotoxic function in vitro due to reduced levels of adhesion molecules and activating receptors, coupled with heightened expression of the inhibitory receptor NKG2A. Moreover, there is a

possibility that they may be at risk of incomplete differentiation, restricted lethality, and tumorigenicity [78].

While NK cells can be obtained from PB or UCB, there's another option. Scientists can also use mature NK cell lines that have undergone modifications to bypass replicative senescence, a natural cellular process that limits cell division [79]. These lines, like NK-92, NKL, HANK1, IMC-1, SNK-6, and SNT-8, can be easily expanded in large quantities to provide enough cells for treatments. Clonal NK cell lines offer advantages for research due to their rapid growth, homogeneity of product, and lack of expression of inhibitory receptors especially inhibitory KIRs and NKG2A [80, 81]. Additionally, the inherent absence of T cells in NK-92 cell lines eliminates the risk of GVHD following infusion [82]. Moreover, The NK-92 cell line exhibits remarkable cryopreservation tolerance, demonstrating high post-thaw viability and functional retention [83]. However, they are genetically abnormal and require radiation before use, limiting their survival in patients to just 48 h. In addition, they lack a key molecule (CD16) needed for ADCC [80]. It's important to note that only NK-92 cells are currently approved for use in clinical trials, making them a valuable source for manufacturing CAR-NK therapies [84].

Unlike traditional immunotherapies that require personalized and time-consuming engineering of immune cells for each patient, "off-the-shelf" CAR-NK cells offer a game-changer. These pre-engineered cells are readily available for immediate use, eliminating the lengthy and expensive customization process. This approach boasts several advantages: faster treatment initiation, overcoming challenges associated with obtaining high-quality cells from individual patients, and a standardized manufacturing process that ensures consistent and predictable treatment effectiveness [85]. Allogenic UCAR cell therapy offers a promising alternative for patients with insufficient immune cells. Unlike autologous CAR cells derived from the patient themselves, UCARs are engineered from healthy donor immune cells. However, to prevent GVHD due to mismatched immune systems, UCARs require additional gene editing. While convenient as an off-the-shelf product, there are limitations. Extensive gene editing raises the risk of mutations and requires advanced technical expertise. Additionally, clinical trials have shown limited persistence and expansion of allogeneic UCAR cells within patients. This challenge could be overcome by finding ways to reduce the recipient's immune rejection response or by minimizing the immunogenicity of the infused cells [86]. Therefore, Both autologous and allogeneic NK cell transfers struggle with low numbers of infused cells, requiring optimization protocols to increase cell population size for clinical use [87].

Another source, hPSCs, encompassing both human hiPSCs and hESCs, possess the unique capabilities of self-renewal and differentiation into various cell types [88]. iPSCs offer another promising approach for off-the-shelf CAR-NK cell therapy which facilitates the ex vivo expansion of NK cells by over 1,000-fold, achieving a final cell product with a purity exceeding 90% [89]. These cells, created by reprogramming adult cells, have the potential to become an unlimited source of CAR therapy due to their ability to multiply endlessly [90]. Originally developed by Takahashi using Sox2, Oct3/4, c-Myc, and Klf4 [91], iPSCs share similar properties with embryonic stem cells, including indefinite self-renewal and the ability to differentiate into various cell types. This technology offers a convenient way to obtain pluripotent stem cells without using embryos [92]. The process of generating iPSC-CAR-NK cells involves modifying the iPSCs in their undifferentiated state before guiding them to become mature and homogenous populations of $CD45^{+}CD56^{+}$ NK cells through a two-step differentiation process. First, they are stimulated by vascular endothelial growth factor, stem cell factor, and bone morphogenic protein 4 to differentiate into HSCs. Then, these stem cells are further differentiated into NK cells using stem cell factor, FLT3L, IL-3, IL-7, and IL-15 [65, 93]. Finally, the differentiated iPSC-CAR-NK cells are expanded in large numbers for clinical use by co-culturing with artificial APC (K562 and aAPC cells expressing mbIL15/mbIL21+41BBL) [94]. Studies show that Phenotypically, the iPSC-derived NK cells express specific markers, including NKG2D, CD56, CD16, NKG2A, NKG2C, and KIR which are also found on natural NK cells and may even exhibit stronger cytotoxicity effects than primary NK cells [95, 96]. Additionally, they appear to be more efficient at expressing CAR structures, making them a promising avenue for developing off-the-shelf CAR-NK cell therapy [93]. Despite the potential of iPSC-derived CAR-NK cells, several crucial issues necessitate cautious evaluation before widespread clinical application. First, heterogeneity among different iPSC lines presents a challenge. Variations in differentiation capacity can lead to inconsistencies in the final cell product. Second, the robust proliferative capacity and pluripotency of iPSCs raise safety concerns. Genetic variations within these cells have the potential to be amplified during differentiation and expansion, potentially compromising the stability and safety of the cell therapy product and even increasing the risk of oncogenicity. Furthermore, iPSCs may exhibit residual DNA methylation patterns reflecting their original somatic cell source. This phenomenon, termed "epigenetic memory," could potentially influence their differentiation trajectory, favoring the development of cell lineages distinct from those of the donor cells. Finally, the immunogenicity of CAR-NK cells derived from iPSCs is a concern. These cells may be recognized and eliminated by the recipient's immune system, potentially reducing their therapeutic

efficacy [69, 97, 98]. While amenable to genetic engineering for enhanced function, iPSC-derived NK cells exhibit a diminished ADCC response due to low expression of the critical Fc receptor CD16. This limitation can be potentially addressed through the integration of a high-affinity CD16 variant, restoring the efficacy of the ADCC mechanism [99].

When CARS comes to NK cells produced from the differentiation of stem cells, there are two methods to consider. One option is to introduce CARs to the stem cells first and then differentiate them into CAR-expressing NK cells. The other approach involves introducing CARs into the already differentiated NK cells, following a process similar to that seen in primary NK cells or NK cell lines. The challenge lies in the fact that NK cells are diverse and resistant to direct transduction, transfection, or nucleofection in vitro. This difficulty could lead to high costs associated with obtaining CAR-NK cells. NK cells obtained from stem cell differentiation, on the other hand, are more uniform and exhibit superior transduction efficiency. Nonetheless, it is crucial to verify whether the integrated CAR has any impact on NK cell differentiation, a consideration that does not arise when engineering NK cells directly [100].

A new approach for off-the-shelf CAR therapy involves generating the CAR molecules directly inside a patient's body. This approach uses specially designed vectors to deliver the CAR gene precisely to specific immune cells. Ideal vectors for this purpose must be skilled delivery agents. They need to be highly specific, targeting only the desired immune cells and avoiding unintended changes elsewhere in the body. Additionally, they must efficiently deliver the CAR gene and promote either long-term or short-term expression of the protein depending on the treatment plan. Finally, practicality and safety are critical considerations. The vector production process should be readily scalable for large-scale production, and the final formulation must be stable for long-term storage as an off-the-shelf treatment, with affordability ensuring broad patient access. Notably, safety remains a key concern, and the vectors themselves must possess a well-established safety profile [69, 101].

CAR-NK cell engineering utilizes various gene transduction methods, each with its own advantages and limitations. Retroviral and lentiviral vectors offer efficient delivery but raise concerns about genotoxicity and reduced NK cell viability. Electroporation and liposomal transfection provide a faster and less variable approach, but the introduced genes are short-lived. DNA transposons offer a promising alternative with stable integration, low immunogenicity, and affordability, but face hurdles related to low efficiency and potential cell death during the DNA delivery process (Fig. 2) [102, 103].

Since CAR-T cells are living cell therapy, strict quality checks are essential throughout production to ensure a safe and effective final product. This involves controlling the production materials (specifically cells and gene editing vectors), ongoing monitoring and testing during production, final product release testing, validating the entire manufacturing process, and performing stability studies. These studies assess factors like CAR expression levels, lymphocyte types present, cell purity, cell viability, and ratios, effectiveness in lab tests (in vitro potency), and contamination control (including sterility testing, mycoplasma checks, checks for replication-competent viruses, rapid detection of microbes, endotoxin testing, and others.) [59, 104–106].

Preclinical and clinical study on CAR therapy in asthma

Current asthma treatments, while effective for many, have limitations. Despite managing symptoms in a majority of patients, some remain resistant to medications like anti-inflammatory drugs and bronchodilators [22]. Additionally, long-term use of inhaled corticosteroids can lead to undesirable side effects such as high blood pressure and osteoporosis [107]. This lack of a definitive cure, particularly for severe asthma, necessitates the exploration of alternative treatment avenues.

IgE-mediated allergic asthma, affecting over half of all allergic asthmatics, poses a significant health burden with uncontrolled symptoms despite available medications. A survey among asthmatic patients showed that 75% of them prefer non-drug treatments for their asthma. Therefore, a treatment method that has a longer and more stable effect is desirable. In recent years, the use of cell therapy based on CAR therapy has had many successful results in the field of cancer and other diseases. It seems that the use of this therapeutic method in the treatment of asthma and AHR can be welcomed by everyone [18].

The presence of mIgE enables T Cells to target and eliminate cells involved in IgE production, including germinal center B cells, plasmablasts, plasma cells, and memory B cells [108, 109]. Achieving high specificity for FcεRIα-based CARs requires meeting four key criteria. First, precise mIgE binding strength for T cell activation and target cell elimination [110]. Second, efficient discrimination against healthy FcεRI-expressing cells to avoid side effects [111, 112]. Third, distinction between FcεRI-bound and FcεRII-bound IgE for specificity and to prevent non-specific immune activation, and Finally, high-affinity binding to membrane-anchored mIgE while minimizing competition from circulating IgE [113, 114]. To address this, a study using low-affinity FcεRIα-based CARs was conducted, demonstrating their ability to induce selective T cell responses against cells expressing transmembrane IgE but not free IgE or FcεRI/

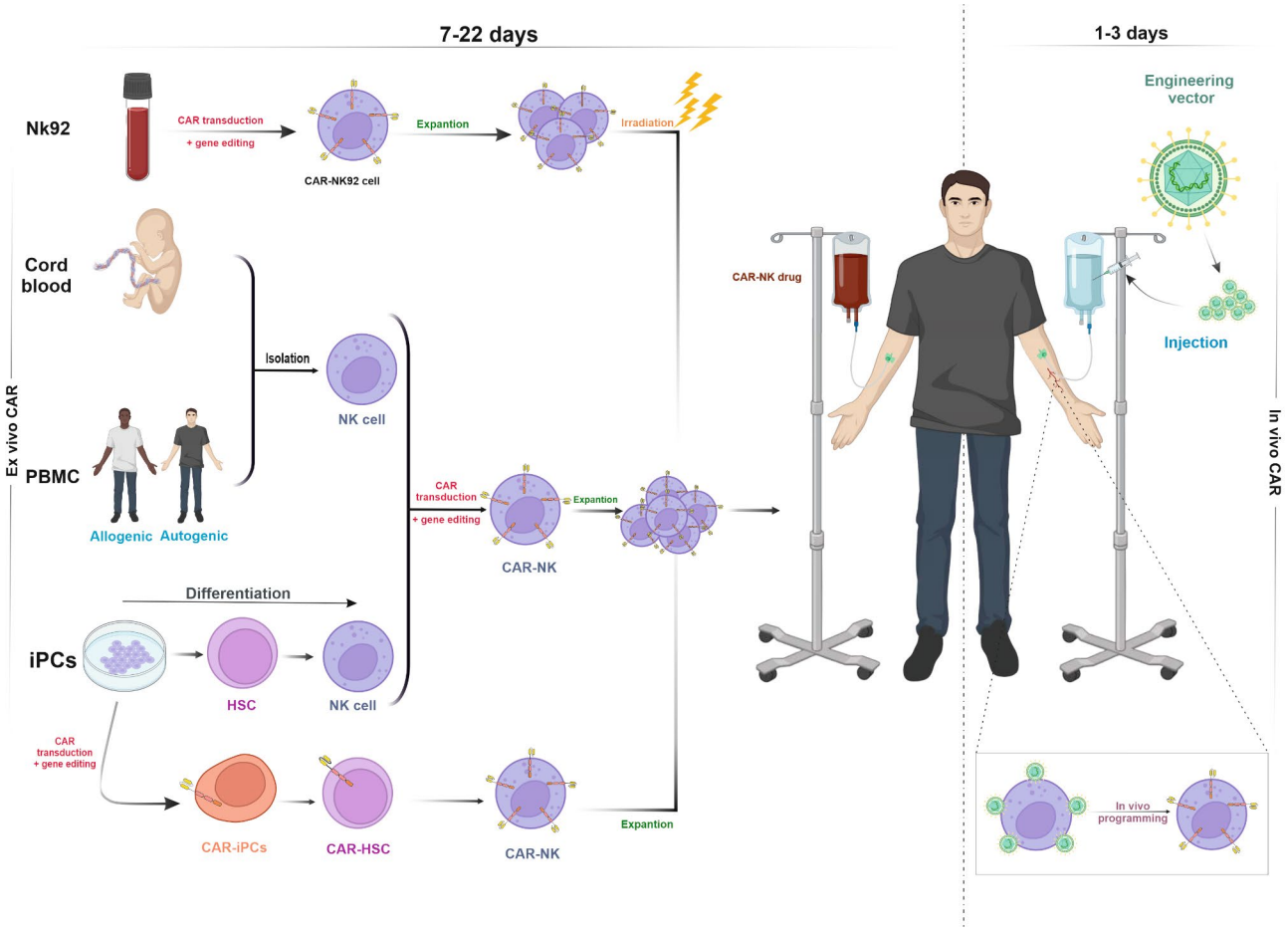

Fig. 2 CAR-NK cells production: This figure summarizes the diverse approaches to CAR-NK cell production. The traditional ex vivo method involves isolating NK cells from sources like peripheral blood (PBMCs), umbilical cord blood (UCB), or NK cell lines, followed by activation, gene modification with CAR vectors, expansion, cryopreservation, and administration. While readily available, PBMCs have limitations in NK cell quantity and function. UCB offers younger and more proliferative NK cells, but concerns remain about their in vitro activity and differentiation. Cell lines provide rapid growth but have limitations related to genetic abnormalities and pre-treatment needs. Additionally, human pluripotent stem cells (hPSCs) including induced pluripotent stem cells (iPSCs) are emerging as a promising source due to their self-renewal and potential for generating large numbers of homogenous and highly cytotoxic CAR-NK cells. The figure also highlights a novel in vivo approach that delivers CAR genes directly into a patient's body, bypassing the need for ex vivo manipulation. This comprehensive overview emphasizes the importance of cell source selection and the potential of in vivo gene delivery for future advancements in CAR-NK cell therapy

FcεRII-bound IgE. These CARs possess the specificity and potency required for targeting IgE-expressing B cells in ACT for asthma and allergic diseases. Adejuwon [18] constructed FcεRIα-based CAR-T cells of both the 2nd and 3rd generation. These CARs were then introduced into primary human CD8+T cells via lentiviral transduction, paving the way for in vitro assessment of their longevity and ability to selectively destroy IgE-expressing B cells. Second generation CARs effectively eliminated target cells.

Driven by the goal of developing a single-dose therapeutic approach for IgE suppression in allergic disease, Christie et al. constructed CARs targeting the EMPD of mIgE. This specific binding domain exhibits exclusive expression on all IgE-producing B cell populations, potentially enabling long-lasting IgE suppression with a single administration. EMPD's exclusive expression on IgE+B cells allows CARs to target and eliminate them while avoiding competition from circulating free IgE due to EMPD's absence on secreted forms. One particular CAR, 2E3E10, exhibited remarkable effectiveness against both U266 and Daudi cells expressing mIgE, triggering both potent cytotoxicity and IFN-γ production. This was particularly noteworthy given the relatively low expression level of mIgE on U266 cells. The results of this study demonstrate the feasibility of using EMPD-specific CARs to reprogram T cells to target and eliminate mIgE-expressing B cells, suggesting a potential therapeutic strategy for allergic diseases such as asthma [19].

SEA, a significant phenotype of refractory asthma,, is driven by eosinophilic inflammation [21]. Targeting eosinophils and related cytokines(IL-4,IL-5 and IL-13)

shows promise, but long-term use limits effectiveness and affordability [115]. A recent article by Jin et al. developed a promising approach for asthma cure using long-lasting CAR-T cells targeting IL-5Rα + eosinophils [20]. IL-5Rα is highly expressed on the surface of mature eosinophils and progenitors. By binding IL-5Rα to IL-5, it causes the release of various cytokines and intensification of inflammation [116]. These CAR-T cells, dubbed Immortal-like and Functional IL-5 CAR-T cells, demonstrate remarkable persistence and efficacy in suppressing asthma symptoms. They engineered IL-5 CAR-T to produce an IL-4 mutein, a modified version of IL-4 that effectively blocks the signaling of both IL-4 and IL-13. In multiple models of asthma, a single administration of IL-5 CAR-T cells in immunocompetent mice, without any prior conditioning, effectively eliminated pathological eosinophils and blocked the actions of IL-4/IL-13. This resulted in long-lasting suppression of type 2 inflammation and asthmatic symptoms. In line with this study, Chen et al. developed a CCAR-T utilizing IL-5-CD28-CD3ζ receptor. In this work, an IL-5-based CCAR configuration was created that does not rely on scFv for binding to the target. This system harnesses T cells to selectively eliminate eosinophils. IL-5 CCAR-T cells effectively neutralized eosinophils in vitro and mouse asthma models (the HDM and OVA-stimulated. Additionally, these IL-5-anchored CCAR-T cells had a lasting impact on reducing eosinophils, and IL-5 levels, and preventing airway inflammation for three months, surpassing the typical active therapeutic period of single mAb-injections which is four weeks [21].

CD4 + Tregs balance allergic airway inflammation [117]. Skuljec et al. used CARs to redirect Tregs to lung epithelium in asthmatic mice. CAR-Tregs accumulated in lungs and lymph nodes, reducing AHR and eosinophils. They also inhibited IL-5 production, mucus production, and Th2 cytokine levels, while increasing TGF-β1 and IL-10 expression. In comparison to unmodified Treg cells, CAR-modified Tregs demonstrated remarkable superiority in managing asthma, highlighting the importance of specific Treg cell activation in the affected organ [22].

According to www.clinicaltrials.gov accessed on 28 April 2024, there are currently no ongoing clinical trials in asthma to test the effectiveness of CAR-based therapy—preclinical studies on the applications of CARs in Asthma are summarized in Table 2.

Fungal asthma, a specific type of asthma, is primarily caused by AF. This fungus triggers the release of IL-25 and IL-33 cytokines from respiratory epithelial cells through the action of various proteases. These cytokines, in turn, stimulate eosinophils and contribute to AHR, leading to inflammation in the airways [118, 119]. Dectin-1, a type C lectin receptor on immune cells like lymphocytes, macrophages, and bronchial epithelial cells, strengthens immune responses by recognizing beta-glucans on the surface of AF [120, 121]. Dectin-1 CAR T cells show promise for fungal airway inflammation treatment. This strategy has the potential to be more targeted and have fewer side effects compared to traditional drug therapies. . Seif et al.'s engineered CD8 + Af-CAR-T cells with dectin-1 protected mice from lung damage, preserved alveolar structure and reduced necrosis around the bronchi, suggesting promise for future asthma therapies [122]. Kumarasan et al. developed a Dectin-CAR that specifically recognizes laminarin, a component of AF, and restricts fungal growth. This CAR also significantly enhances IFN-γ production by over 6-fold [123]. Targeting Dectin-1 with CAR-NK cells holds promise for treating asthma, but needs more optimization, human trials, and safety evaluation.

NK cell, subtypes, activation, and function mechanisms

Unique among innate lymphoid cells, NK cells play a vital role in immunoregulation and cytotoxicity within the immune system [17]. NK cells develop from HSCs in the bone marrow through a series of differentiation steps [124]. NK cells present in the blood, lymph nodes, and tissues and classified in two main subpopulations based on the levels of CD56 and CD16: CD56brightCD16- and CD56dimCD16+. These cells lack CD3 expression [125].

CD56brightCD16^{-} NK cells are a distinct subset of NK cells found predominantly in secondary lymphoid organs. Though lacking expression of KIRs, they exhibit

Table 2 Summary of preclinical studies on the applications of CARs in Asthma

Author/Year	Cell Type	CAR Generation	Target	Outcome
Adejuwon [18]	Human CD8 + T cells	Second and Third Generation (CD28)	IgE	Second-generation CARs are highly effective at eliminating cells expressing mIgE
Christie et al. [19]	Human T cells	Second Generation (CD28)	IgE	CAR 2E3E10 exhibited robust cytotoxicity and IFNγ production against U266 and Daudi cells expressing mIgE
Jin et al. [20]	Murine CD8 + T cells		IL-5Rα+	Engineered IL-5 CAR-T target and eliminated IL-5Rα + eosinophils and and blocked the actions of IL-4/IL-13
Chen et al. [21]	Murine CD3^{+} T cells		IL-5	IL-5-anchored CCAR-T cells selectively eliminated eosinophils
Skuljec et al. [22]	Murin Tregs cells		Tregs	CAR-modified Treg cells inhibited excessive mucus production in the lungs and prevented a surge in allergen-specific IgE and Th2 cytokine levels

potent immunomodulatory functions by producing a diverse array of cytokines, including IFN-γ, IL-10, and IL-2. In contrast, CD56dimCD16+NK is highly cytotoxic and induces ADCC. They exhibit abundant expression of perforin and granzyme B, cytotoxic molecules crucial for their cytolytic function. Furthermore, KIRs expressed on NK cells play a central role in self- and non-self-recognition, contributing to their precise targeting of infected or transformed cells. This group comprises 90% of circulating NK cells and is also found in the spleen and lungs. Compared to other tissues, the lungs harbor a high proportion of NK cells, particularly the CD56dimCD16+subset which circulate between the lungs and blood [126, 127]. CD56dimCD16+NK cells are typically associated with rapid responses in acute inflammation, and CD56brightCD16- NK cells can contribute to immune regulation in chronic settings. Additionally, CD56dimCD16+NK cells demonstrate an increased capacity to produce cytokines when encountering target cells. Despite significant progress in characterizing NK cell subsets, the precise boundaries between them, are still being elucidated [128, 129].

NK cells act as early responders in the immune system, playing a critical role in defending against viral infections and fighting against cancer. NK cells are a key part of the immune system's defense against cancer, particularly hematopoietic tumors. They can eliminate abnormal cells without prior sensitization, unlike other immune cells. Unlike T cell-based therapies, which have shown limited success, NK cells offer a promising alternative due to their inherent ability to directly kill cancer cells through various mechanisms and promote anti-tumor immune response [130]. They have a similar function to CD8+T cells. They augment the immune system by stimulating other immune cells [131, 132]. NK cells constantly integrate signals from numerous surface receptors (Fig. 3). These receptors act as a finely tuned switchboard, sending both inhibitory and activating signals that determine the cell's response. This dynamic interplay allows NK cells to rapidly adapt and respond to environmental

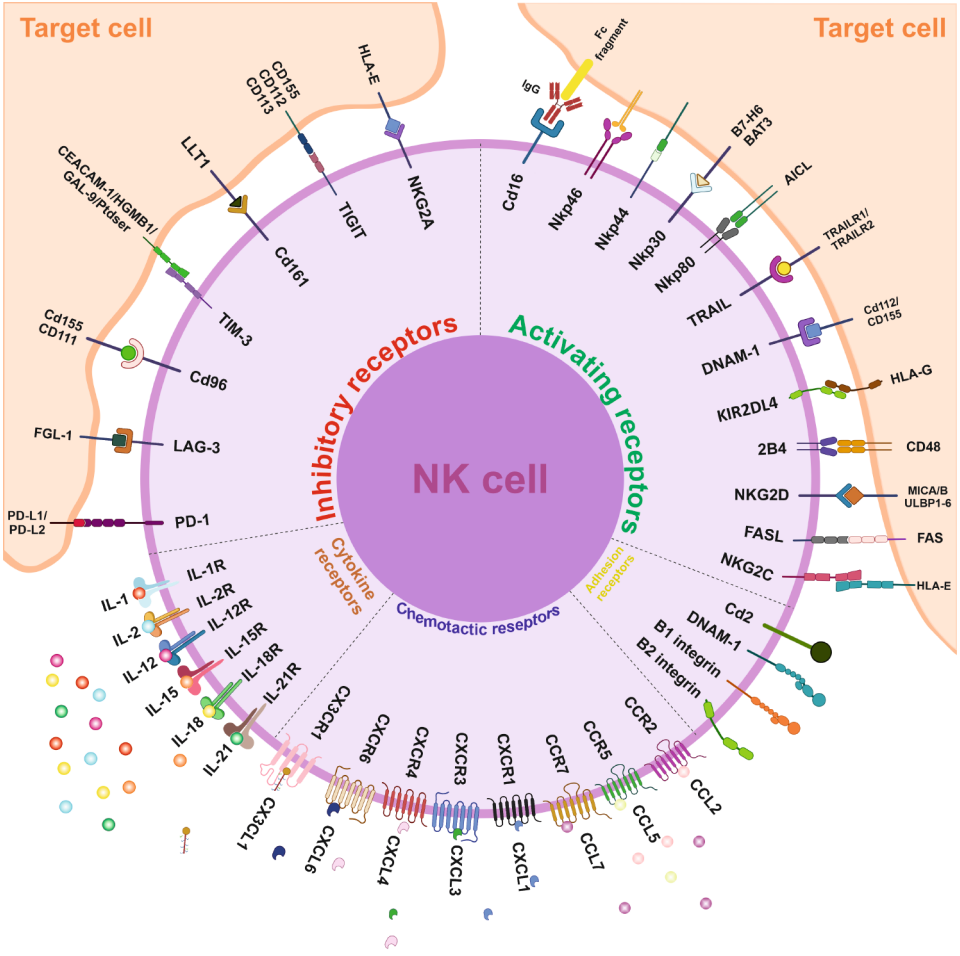

Fig. 3 NK cells receptors: NK cells rely on a combination of activating and inhibitory receptors to determine their actions. Additionally, receptors specific to interleukins (signaling molecules) allow NK cells to respond to interleukins' effects. Finally, receptors for chemotactic and adhesive factors guide NK cell movement and attachment, further influencing their activities

changes [133]. Most of the inhibitory receptors on NK cells recognize MHC class I molecules. These include two main groups: KIRs with inhibitory motifs and the CD94-NKG2A heterodimer. PD-1, TIGIT, LAG-3, and CD96 are other inhibitory receptors [134, 135]. Many activating receptors exist for NK cells which NCRs (NKp46, NKp44, NKp30, etc.) and CD16 can be mentioned in this list [10]. MICA and MICB, molecules related to MHC class I proteins, activate NK cells through the NKG2D receptor which is one of several factors influencing their activity. These interactions play a crucial role in recognizing and eliminating inflammated cells. MICA and MICB are present on the surface of virus-infected and stressed cells as well as in soluble forms [136]. Furthermore, the NKG2D receptor initiates NK cell activation utilizing the adapter molecule DAP10 [137]. Conversely, upon binding to the appropriate ligands in NK cells, NCRs enlist adapter molecules DAP12, CD3ζ, or FcεRIγ which carry the ITAM. These adapter molecules trigger the commencement of activation signaling cascades. CD16 is unique in its ability to trigger some responses in resting NK cells without additional signals. However, it primarily activates them in collaboration with other receptors. The receptor DNAM-1, a member of the Immunoglobulin superfamily, has the capability to stimulate NK cells through its interaction with CD112 [137]. Furthermore, IL-15, IL-12, IFN type 1, and DCs are considered very strong NK cell activators [138].

NK cells produce appropriate immune responses through producing chemokines such as chemokine CCL 3, CCL 5, and CXCL 8 and cytokines such as TNF-α, IFN-γ, GM-CSF, IL-5, 13, 10 or interacting with cells such as macrophages, T cells, B cells, granulocytes, and DCs [126, 139–141]. IFN-γ is the main cytokine of NK cells, which is produced through stimulation of cytokines such as IL-12 and IL-18 and plays a significant role in treating asthma [142].

Recent studies clarify that Th2-polarizing DCs and immature DC cells are killed by NK cells through helper molecule DNAX 1 (DNAM-1) and NKp30. As a result, the number of DCs involved in Th2 immune responses is reduced [143]. Furthermore, the combined action of both NK cell subsets exerts its cytotoxicity on DCs by way of direct contact with eosinophils and leads to their apoptosis through a different pathway from caspases and degranulation of NK cells [12, 144, 145]. Additionally, In the presence of lipoxin A4, NK cells dial down the production of superoxide anions, key players in inflammatory processes [145].

In the lung, NK cells can trigger apoptosis in neutrophils, promoting the resolution of acute inflammation. This process is mediated by the caspase pathway, involving the Fas and NKp46 receptors on neutrophils and NK cells [146]. While, neutrophils play an important role in the maturation of NK cells [147].

NK cells activate plasmacytoid DCs through NKp46, exerting anti-inflammatory functions. In mice, lung plasmacytoid DCs suppress T-cell division and effector T-cell generation induced by myeloid DCs, thereby regulating lung inflammation [126].

Research suggests decreased activity of lung NK cells compared to their counterparts in PB. This adaptation might serve a crucial purpose. Given the lung's constant exposure to allergens, the subdued NK cell response could help prevent excessive inflammation and tissue damage [148].

While the number of IL-4 producer -NK cells increases in allergic asthma the number of NK cells producing IFN-γ decreases. In contrast, in neutrophilic asthma, the level of IFN-γ and granzyme B secreted from NK cells is increased [126]. Moreover, during severe asthma, the ratio of NK cells to CD4+T cells within the lungs is reduced. NK cell numbers in the bloodstream are also lower. In addition, findings reveal the impaired ability of NK cells to trigger eosinophil cell death in patients with severe asthma [12, 13]. The research discussed above, unveils a critical dysfunction in asthma–impaired NK cells. These immune cells are not only reduced in number but also show abnormal reactions to allergens, potentially hindering the fight against asthma. The reduced NK cell activity highlights the potential of drug or cell therapies to improve asthmatic patients' immune response by increasing NK cell populations and enhancing their abilities.

Unveiling the difference: A comparative analysis of Treg and NK cell

NK cells and Treg cells play crucial roles in immune responses to allergens, interacting with APCs to orchestrate the response. While NK cells make up a significant 5–20% of circulating lymphocytes, Treg cells constitute less than 5% [149].

NK cells originate from lymphoid progenitor cells within the bone marrow and mature in secondary lymphoid organs. Subsequently, they populate diverse tissues such as PB, lung, liver, skin, bone marrow, and kidney. In contrast, Treg cells expand in the thymus and PB and express TCR [150, 151]. Compared to Tregs, the human lung harbors a significantly higher amount of NK cells, which is one of the body's primary reservoirs for this critical immune cell population [152].

NK cells comprise two main groups differentiated by CD56 expression: CD56dim and CD56bright. The dominant population, CD56dim NK cells, circulate within the blood and exhibit robust cytotoxic activity, directly eliminating infected or tumor cells. In contrast, CD56bright NK cells, residing primarily in lymphoid tissues,

contribute to immune defense by secreting cytokines and chemokines. According to the direct killing function of NK cells, in inflammatory or infectious reactions, all these cells perform killing while only some T cells act as cytolytic [153, 154]. Treg cells, as a subset of CD4+T cells, have high expression of the IL-2 receptor α chain (CD25) [155]. CD4+CD25+Tregs can be further classified into two main subsets based on their origin: thymus-derived Tregs and peripherally-derived Tregs. Studies demonstrated that Treg cells derived from the periphery, display the ability to regulate the immune response to foreign antigens [156]. Additionally, NK cells differ from Treg cells in the absence of CD3 [157].

While both NK cells and T cells utilize MHCs on APCs for recognition, NK cells exhibit greater flexibility. This flexibility stems from their ability to recognize receptors directly on target cells through non-MHC-dependent mechanisms [140, 158]. Unlike Treg cells, which recognize antigens with rearranged receptors in a clonal form, NK cells express random combinations of active and inhibitory receptors to recognize antigens by integrating them [151]. In contrast to Tregs, which primarily utilize the αβTCR and TLRs for recognition, NK cells possess a diverse cluster of activating (e.g., NKp44, NKp30, NKp46) and inhibitory receptors (e.g., NKG2A, KIR3DL1, KIR2DL1, KIR3DL2) [155].

In general, NK cells have a broader range of potential targets through the recognition of diverse antigens. However, their recognition lacks the precision and specificity achieved by Treg cells. Compared to T cells, whose population can double within 10 h upon activation, NK cells exhibit slower proliferation, requiring approximately 1.5 days to achieve the same feat [159, 160]. NK cells generally have a shorter lifespan and rely on immediate responses. On the contrary, T cells can form specialized memory cells which persist for long-term immune protection [160, 161].

Both cells utilize perforin granzymes to induce target cell death. Additionally, they express apoptotic ligands such as FASL. However, unlike phagocytic cells, neither NK cells nor Treg cells digest debris from eliminated cells [155]. Distinct from T cells, NK cells can kill by natural mechanisms and ADCC [162].

NK cells just like Treg cells can produce anti-inflammatory cytokines including IL-10 and IFN-γ [152]. Studies have shown that Treg cells suppress immune response and promote tolerance to foreign antigens by secreting IL-10 and TGF-β [163]. Among the types of NK cells, this function is performed by CD56dimCD16+, which secretes fewer cytokines than T cells [129, 164]. Low cytokine secretion can counteract the harmful effects of TGF-β in asthmatic patients.

In addition, unlike T cells, these cells are easy to transduce [88]. As the last comparison, GVHD is rarely reported in treatment with NK cells. This may be partially explained by the reduced proliferative and cytokine secretion capacity of NK cells compared to T cells. A study by Ruggeri et al. using mouse models confirms this finding, demonstrating that NK cells can eliminate APCs, thereby exerting a protective effect against GVHD [165] (Table 3). However, other factors such as differences in target cell recognition and immune regulation require further investigation [166].

Table 3 A comparative analysis of Treg and NK cell

Feature	NK Cells	Treg Cells
Source	PB, BM, UCB, cell lines, HSPCs, hESCs, iPSCs	PB, Thymus, UCB
Frequency in peripheral blood	5–20%	< 5% of circulating T cells
phenotype	CD3- CD4+ CD56 + CD16+	CD3 + FOXP3 + CD4 + CD127low CD25 + CD16+
Antigen Recognition	MHC-dependent and independent mechanisms	Antigen-specific (TCR)
Receptors	NKRs	αβTCR TLR
Pro inflammatory cytokines	Yes	No
Apoptosis-inducing ligands (TRAIL, FasL, …)	Yes	Yes
Perforin/granzymes	Yes	Yes
ADCC	Yes	No
Phagocytosis	No	No
GVHD Risk	Rare	High

The strength and challenges of NK cells as immunotherapy candidates

In the previous chapter, we discussed certain significant characteristics of NK cells. In this section, we will delve into the details of utilizing these features in CAR-NK, as well as the challenges and prospects that lie ahead.

NCR

NCRs are one of the activating receptors on the surface of NK cells which induce NK cells to kill targets and produce cytokines [167]. The NCR family comprises NKp30 (CD337), NKp44(CD336), and NKp46 (CD335), are determined by the genetic sequences NCR3, NCR2, and NCR1, respectively [168]. A comparison of the different NCRs comprises is presented in Table 4 NK cells with high NCR surface density exhibit stronger cytotoxicity against target cells [169].

NKp30 plays a multifaceted role in the immune response. Encoded by the NCR3 gene, this single-unit transmembrane protein with an immunoglobulin-like domain exists in three forms (a, b, and c) due to

Table 4 A comparative analysis of the various NCR components

Feature	Nkp30	Nkp44	Nkp46
Gene	NCR3	Not specified	NCR1
Structure	Single transmembrane protein with one immunoglobulin-like domain (a, b, c isoforms)	Single transmembrane protein with one immunoglobulin-like domain	Single transmembrane protein with two immunoglobulin-like domains
Expression	Present on resting and activated NK cells	Induced only after NK cell activation	Present on resting and activated NK cells
Function	Targets infected or cancerous cells - Regulates cytokine production (promotes Th1 cytokines and inhibits IL-10)	Targets infected or cancerous cells	Targets infected or cancerous cells
Adaptor molecule	CD3ζ and FcRγ	DAP-12	CD3ζ and FcRγ
Signaling strength	Potentially weaker due to fewer ITAMs in adaptor complex	Potentially weaker due to fewer ITAMs in adaptor complex	Potentially stronger due to more ITAMs in adaptor complex
Disease association	Decreased expression in severe asthma patients	May regulate allergic responses	May play a role in resolving inflammation
CAR-T cell therapy	Promising candidate to enhance cytotoxicity	May be effective despite lacking specific signaling domains	Antigen-binding domain used for target recognition

alternative splicing, each with distinct functions [170]. Found on both resting and activated NK cells, NKp30 goes beyond just targeting infected or cancerous cells [171]. It influences the immune response by regulating cytokine production. NKp30a and NKp30b promote the release of immunostimulatory Th1 cytokines like IFN-γ and TNF-α, while NKp30c acts as an immunoregulator by inducing IL-10 production [172–174].

NKp44, another member of the NCR family, shares a similar structure with a single external immunoglobulin-like domain but differs in its expression pattern [168]. Unlike NKp30 and NKp46 which are present on both resting and activated NK cells, NKp44 expression is typically induced only after activation [171]. This controlled expression helps maintain a balanced immune response.

While NKp30 and NKp44 are single-unit transmembrane proteins with one extracellular domain, NKp46 is unique with two such domains [168]. Interestingly, NKp46 is present on both resting and activated NK cells, allowing for quicker responses [171]. To trigger NK cell activation, NCRs rely on adaptor molecules containing an ITAM signaling. NKp46 and NKp30 partner with CD3ζ and FcRγ, while NKp44 utilizes DAP-12 for signal transmission [175]. Importantly, NKp46 and NKp30 have the potential to deliver stronger activating signals due to their adaptor complexes containing more ITAMs compared to NKp44 [176].

NK cells in severe asthma show lower NCR expression, particularly NKp30 and NKp46 [177]. Linking NCRs to weaker anti-inflammatory function. NCR1 may regulate allergic responses by reducing airway inflammation [178]. Additionally, Studies suggest NCRs might play a role in resolving acute inflammation. Human NK cells can induce neutrophil apoptosis through the NKp46 and Fas pathway in a laboratory setting [146]. This mechanism could potentially help reduce asthma. Elhaik Goldman et al. link NCR1 to reduced allergic lung inflammation [179]. NCR1-deficient mice showed worse symptoms, suggesting it dampens allergies by controlling granulocyte infiltration into the airways and IgE production.NCRs' potent immune activation potential makes them promising for CAR-T therapy. Studies by Divanji et al. showed enhanced cancer cell cytotoxicity with CAR-T cells expressing NKp30, NKp44, NKp46 [131]. Interestingly, subsequent research by the same group revealed that even though NKp44 and NKp46 CARs lack specific signaling domains, they can still interact with and activate their natural adaptor molecules within NK cells [180]. Kasahara et al. explored incorporating NKp44's antigen-binding domain into CAR design for broad target recognition in CAR-T cells [181]. This highlights NCRs' potential in CAR therapy, but further research is needed for CAR-NK applications in diseases like asthma.

Dap 10, Dap 12, and NKG2D

NKG2D is a critical activating receptor in NK cells which has a short cytoplasmic tail that lacks the ability to initiate intracellular signaling [182, 183]. NKG2D, located on the cell membrane, requires adaptor proteins for stability and signal transduction [184]. These adaptor proteins, such as Dap10 and Dap12, trigger distinct signaling pathways. DAP10 utilizes a YxxM motif to activate PI3K [185, 186], whereas DAP12 employs an ITAM motif to recruit and activate ZAP70-Syk, ultimately leading to cytokine release and cell killing [187, 188].

As some studies show promising effects of Dap10 and Dap 12 such as improved persistence, cytokine secretion, and cytotoxicity in CAR-T therapy [189–191], some others show their encouraging effects in CAR-NK therapy [183, 192]. Xiao et al. conducted a study on NK cells using a novel CAR with NKG2D and DAP12 components. The study demonstrated that NK cells with DAP12-containing NKG2D-CAR had higher cytotoxicity and IFN-γ release compared to NK cells with

CD3ζ-containing NKG2D-CAR [183]. The incorporation of DAP12 signaling in CAR designs has been associated with a triad of adverse consequences: NK cell exhaustion, hyperactivation, and CRS. These limitations necessitate the investigation of alternative co-stimulatory domains, such as DAP10, for safer and more effective CAR-NK cell therapies [193]. NKG2D-DAP10 increases NK cytotoxicity according to Wilton et al.'s research [192]. In addition, pent et al. employed DAP10 and DAP12 in the development of CAR-NK cells, utilizing them as the principal intracellular domains for the transmission of signals from NK stimulatory receptors. As a result, DAP10,12 CAR-NK cells may exhibit increased lifespan, enhanced cytotoxic, and suppress allergic responses through IFN-γ production.

IL-10

NK cells are a source of IL-10, but this anti-inflammatory cytokine is also generated by various immune cell types such as TH1, TH2, TH17, Teff cells, FoxP3−and FoxP3+Treg cells, B cells, and myeloid cell subsets [194, 195]. IL-10 exerts its effects through interaction with a specific receptor complex composed of IL-10Rα and IL-10Rβ subunits. Ligand binding triggers the Jak/STAT pathway, with Jak1 and Tyk2 kinases phosphorylating STAT3 [196, 197]. Although STAT3 is essential for this process, research suggests that STATE1 and STATE5 also contribute to IL-10 signaling [198]. Nuclear translocation of phosphorylated STAT3 subsequently induces the expression of anti-inflammatory genes, including SOCS-3 [199]. SOCS-3 acts as a negative regulator, inhibiting the production of pro-inflammatory cytokines and antigen presentation by downregulating key signaling pathways like NF-κB and MAPKs [200]. Additionally, IL-10 can activate the p38 MAPK pathway, leading to the induction of HO-1, a protein with further anti-inflammatory properties. In conclusion, IL-10 employs a multifaceted signaling network involving the Jak/STAT pathway and HO-1 induction to suppress inflammation and maintain immune homeostasis [199].

IL-10 is a key anti-inflammatory cytokine in asthma, reducing lung damage and disease severity [201, 202]. TH cell-derived IL-10 plays a crucial role in establishing tolerance to allergens and resolving allergic inflammation, with impaired production linked to severe asthma [203, 204]. Studies show IL-10 limits TH2 cells, reduces eosinophilic inflammation, and mitigates AHR. Genetic variations in IL-10 expression are linked to asthma risk and severity [195]. IL-10 also exerts a regulatory influence on Th2 lymphocyte production of IL-4 and IL-5, thereby modulating IgE-mediated mast cell activation. Animal studies further corroborate these anti-inflammatory effects [205].

Beyond TH cells, recent research highlights the immunomodulatory role of IL-10 from other immune cell populations. Treg Foxp3 cells contribute to suppressing allergic lung inflammation through IL-10 production [206]. Additionally, IL-10-producing ILC2s promote epithelial barrier integrity by suppressing pro-inflammatory mediators like IL-6 and IL-8, thereby mitigating tissue damage during allergic reactions [207]. Myelomonocytic cells exposed to IL-10 upregulate IL-1 receptor antagonists, leading to diminished eosinophil recruitment and reduced AHR [208]. However, despite these compelling findings, a comprehensive understanding of the precise mechanisms by which IL-10 regulates the diverse phenotypes of asthma remains elusive [202].

Building on the promise of CAR-T cell therapy, researchers are exploring the potential of incorporating IL-10 to enhance their efficacy and broaden their application. Mohseni et al. engineered HLA-A2 CAR-Tregs to express IL-10, boosting their suppressive ability [198]. This finding aligns with Huang et al.'s work, where CAR-T cells engineered for IL-10 secretion exhibited improved proliferation and effector function [209]. Expanding on this concept, Chen et al. pioneered the use of IL-10R as a therapeutic target in AML by engineering CAR-T cells to recognize AML cells expressing IL-10R as the antigen-binding domain. The study observed an increase in Treg cells and strongly supported the therapeutic potential of incorporating IL-10 into CAR-T cell therapy [210]. Overall, in asthma, CAR-NK cells could offer a novel anti-inflammatory approach by leveraging their ability to produce IL-10 (Fig. 4).

KIRs

KIRs contribute to the regulation of NK cell activity. These highly polymorphic molecules are expressed on the surface of CD56dimCD16+NK cells and transduce signals that modulate cytotoxic function [126, 127, 211]. Two types of KIR are inhibitory and activating receptors. Inhibitory and activating KIRs interact with HLA-I molecules to regulate NK cell responses [212].

Inhibitory KIRs expressed by NK cells suppress cytotoxicity through binding of NK cells and MHC-1 on healthy cells [213]. One of the mechanisms to enhance the cytotoxic activity of CAR-NK is silencing inhibitory KIRs. KIR-blocking antibodies are one of the approaches to silence inhibitory KIRs [214].

Unlike most KIR receptors with clear-cut inhibitory or activating functions, KIR2DL4 presents a unique case. While it possesses structural features associated with both types, only its activating function has been definitively established [215, 216]. KIR2DL4 (CD158d), expressed on the surface of NK cells recognizes HLA-G as its ligand [215, 217]. HLA-G polymorphisms are associated with increased susceptibility to AHR and asthma

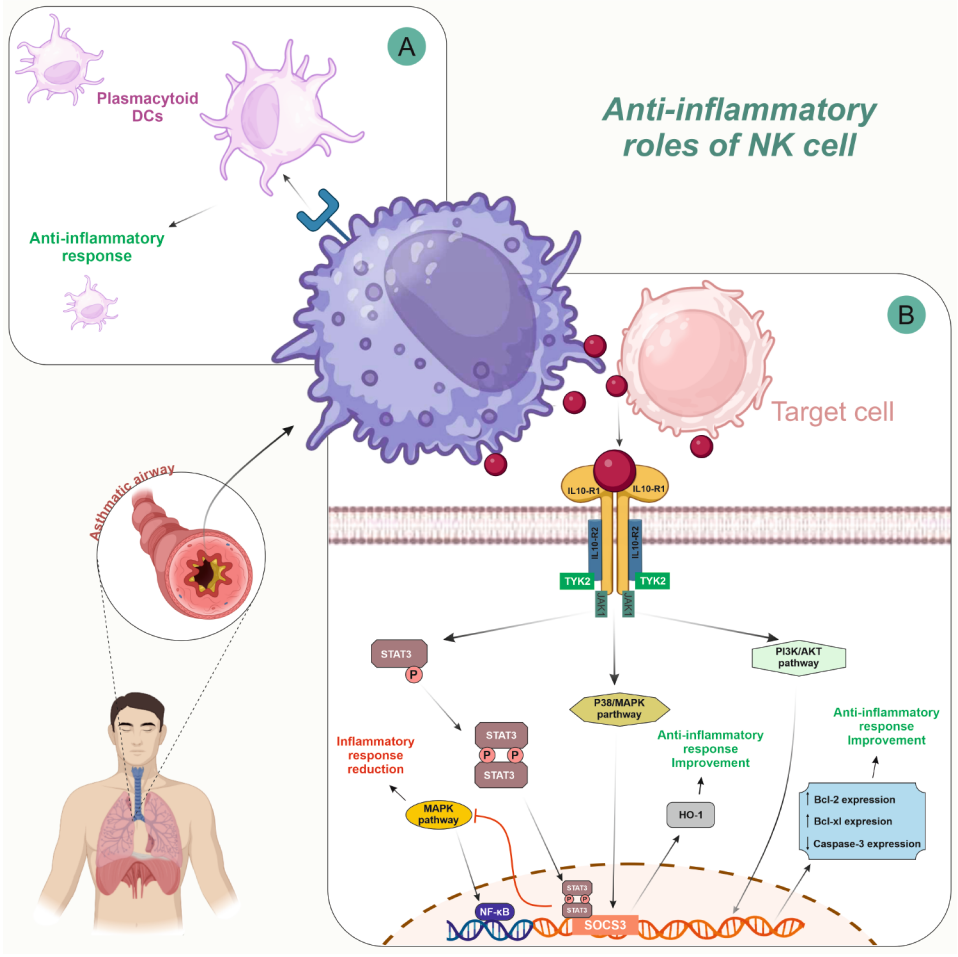

Fig. 4 NK Cells Promote Healing (Anti-inflammatory Actions): NK cells have surprising anti-inflammatory capabilities through Nkp46 receptor and IL-10. **(A)** By Nkp46 receptor They can connect with plasmacytoid dendritic cells, triggering responses that dampen inflammation. **(B)** Via IL-10 production, NK cells can suppress inflammation in three ways. STAT3 pathway reduces inflammatory molecule production (Nf-kB gene expression) through inhibition of MAPK pathway of NfkB. p38/MAPK pathway helps increase the production of HO-1, a molecule with anti-inflammatory properties. PI3K/AKT pathway reduce pro-inflammatory signals and caspase-3 to induce anti-inflammatory responses

development [218]. Interestingly, research suggests HLA-G expression can increase in various tissues during inflammation [219]. This includes the bronchial epithelium (lining) of asthmatics' lungs [218, 220]. Recent research suggests a more nuanced understanding of HLA-G function. This raises the intriguing possibility of an analogous interaction between NK cells and HLA-G in the lung microenvironment, potentially influencing asthma development.

KIR2DL4 exhibits a unique genetic polymorphism. Two common variants exist within the transmembrane region, with the 9 A-TM allele containing a single nucleotide deletion in exon 6. This deletion results in a defective receptor incapable of inducing IFN-γ secretion upon ligand binding [216]. IFN-γ variations may impact asthma risk. Firstly by affecting Th1/Th2 balance, as previously reviewed by Szabo et al. [221], and secondly, through NK cell-derived IFN-γ in tolerogenic DC generation [222]. This suggests a more complex role for KIR2DL4 in allergy.

When KIR2DL4 binds to HLA-G, the ITIM motif within KIR2DL4 recruits phosphatases like SHP-1 and SHP-2, leading to the subsequent inhibition of CD16/FcγRIIIa signaling in human NK cells [223]. This CD16 inhibition aligns with the self-missing hypothesis, as CD16 is involved in ADCC. However, KIR2DL4 activation can also trigger the release of pro-inflammatory cytokines like TNF-α, IL-1α, IL-1β, IL-6, and IL-8 from NK cells, highlighting its potential role in promoting inflammatory responses [224, 225]. This activating signal appears to be mediated by FcεRIγ and is independent of the ITIM motif [224, 226, 227]. Soluble HLA-G induces similar cytokine secretion, possibly via endosomal KIR2DL4 binding and a DNA-dependent protein kinase subunit, AKT, and NF-κB [225, 228].

Activating KIRs interact non-covalently with ITAM-containing DAP-12. When the activating KIR binds to DAP-12, it triggers a chain reaction within the NK cell. This reaction involves the phosphorylation of various molecules downstream of the initial interaction, ultimately leading to NK cell activation [229, 230]. Researchers are exploring further enhancements to CAR-NK cells by leveraging activating KIRs. These approaches include stimulating them using mAb or specialized molecules called NK Cell Engagers [231]. However, a major hurdle lies in the high degree of similarity (97–98%) between the external regions of activating and inhibitory KIRs [230]. Designing protein tools for specific targeting of activating KIRs is challenging. A promising strategy uses peptide-MHC complexes recognized by activating KIR2DS2 [232]. Studies have shown that introducing these complexes through a DNA vaccine approach can be effective in activating CAR-NK cells in mice engineered to express specific KIRs [233]. Interestingly, allelic variations in KIR2DS2 itself don't affect its ability to recognize specific peptide-MHC complexes [232]. This suggests a single complex can activate NK cells regardless of the KIR2DS2 isoform they express. Since all isoforms respond, targeting specific isoforms for therapy offers no additional benefit.

In conclusion, KIRs, particularly KIR2DL4, play a complex and multifaceted role in regulating NK cell activity and potentially influencing asthma development. Silencing inhibitory KIRs may enhance CAR-NK cells, but KIR2DL4-HLA-G interaction is complex. Activating KIRs, like KIR2DS2, hold promise for improved CAR-NK therapy.Future investigations in humans are necessary to determine the effectiveness of this approach and its potential impact on improving CAR-NK cell-based treatments for asthma and other diseases.

NKG2A

NKG2A (CD159a) plays a critical role in regulating the activity of NK cells, acting as an inhibitory immune checkpoint receptor. It recognizes HLA-class I and E molecules on healthy cells, preventing NK cells from attacking them and thereby preventing autoimmune reactions [234]. This recognition system allows NK cells to distinguish "self" from "non-self" cells within the body [235]. NKG2A overexpression leads to NK cell exhaustion (dysfunction) [235, 236]. Blocking NKG2A might restore NK cell activity [237]. High NKG2A suppresses NK cells. This effect is likely mediated by the repression of STAT1, NF-κB, and p38 MAPK signaling pathways, which are thought to be key mechanisms in NK cell activation [238].

NKG2A has a multifaceted role in regulating NK cell function. CD56bright NK cells, characterized by low KIR expression but high NKG2A levels, exhibit diminished cytotoxicity and IFN-γ production compared to CD56dim NK cells with high KIR expression and lower NKG2A [4, 239]. Furthermore, NKG2A may be involved in various immune-mediated diseases, including autoimmune diseases, inflammatory conditions, parasitic infections, and transplant rejection [240, 241].

NKG2A blockade enhances NK cell function. Studies demonstrate that blocking NKG2A with antibodies like Monalizumab can restore cytotoxicity, IFN-γ production, and ADCC in NK cells isolated from patients [242]. This finding is further supported by gene editing approaches that eliminate NKG2A, confirming its role as an inhibitory checkpoint [243]. However, the impact of NKG2A blockade appears to be context-dependent. While Grote et al. observed limited effects on CD276-CAR NK-92 cell cytotoxicity against melanoma upon NKG2A knockout or blocking it with an antibody [244], a significant increase in cytotoxicity was seen against AML cells when combined with knockouts of CBL-B and TIGIT. This suggests the potential benefit of targeting multiple inhibitory pathways for optimal efficacy [245]. Additionally, studies employing CRISPR/Cas9 to disrupt NKG2A demonstrated enhanced cytotoxic activity of CD33-CAR-NK cells [246]. Besides CRISPR-mediated CARs, Combining allogeneic CAR-NK therapy with anti-NKG2A antibodies has also been proposed to overcome NKG2A's inhibitory role [247]. The potential for translating these findings to asthma treatment warrants further investigation, particularly considering the potential benefits of enhanced NK cell cytotoxicity and IFN-γ production.

TGF-β

TGF-β is a small signaling protein with a surprisingly large impact on human health which exists in three isoforms (TGF-β1, TGF-β2, and TGF-β3) [248]. Among immune cells, NK cells stand out alongside eosinophils and epithelial cells as significant producers of TGF-β, comparable to monocytes [249]. It acts as an immunosuppressive cytokine, dampening T, B, and NK cell activity to maintain immune balance [250]. Beyond the immune system, TGF-β arranges cellular processes by attracting repair cells (macrophages and fibroblasts), stimulating the formation of structural tissue support (fibronectin), and even influencing ASM cell growth [248]. Research further reveals TGF-β's impact on essential biological processes, regulating cell proliferation, differentiation, and death, as well as influencing the extracellular matrix and stem cell fate, ultimately affecting development, tissue repair, and immune responses across multiple organs [251]. Excessive TGF-β activity can suppress the immune system, hindering target cell elimination and potentially worsening asthma, a disease marked by immune dysregulation [252].

TGF-β signaling, via Smad protein activation, regulates gene expression. A specific Smad3 gene variation is linked to asthma susceptibility [253, 254]. TGF-β plays a complex and multifaceted role in asthma [255]. TGF-β exhibits pro-fibrotic properties, promoting the growth of ASM cells and the deposition of extracellular matrix by fibroblasts [251]. This excessive tissue remodeling ultimately leads to structural changes that constrict the airways in asthma [248]. Biopsies and BAL fluid from asthmatic patients reveal elevated levels of TGF-β, further supporting its involvement in the disease [256]. TGF-β contributes to airway remodeling in asthma by promoting EMT, a process where airway epithelial cells transform into scar-forming fibroblasts [256]. This suggests a potential dysfunction in epithelial repair mechanisms in asthmatic patients [257, 258]. TGF-β secreted by eosinophils induces airway constriction and AHR, as well as promoting goblet cell metaplasia, excessive mucus production, tissue injury, and structural alterations in the airways [259]. In contrast, some studies suggest TGF-β might also have anti-inflammatory properties. This is relevant to asthma, where a weak immune response with low IL-10 and TGF-β production is linked to severe cases [252]. The combined action of TGF-β and IL-10 enables the regulation of pro-inflammatory cells and the mitigation of exaggerated inflammatory reactions [260]. In stark contrast to NK cells, the release of IL-10 and TGF-β by Treg cells in humans plays a pivotal role in effectively dampening airway inflammation [261]. Moreover, excessive TGF-β production by NK cells in asthma might contribute to the overall disease process [260].

In terms of considering the interaction of NK cells with TGF-β, it has been demonstrated to suppress the cytotoxic responses of NK cells. Findings were observed in a study involving human NK cells, where the introduction of TGF-β to IL-2/15-stimulated NK cells led to a decrease in the expression of IFN-γ, granzyme B. In research conducted by Trotta et al., it was found that TGF-β suppresses NK cell activation induced by CD16 binding. The authors proposed that the inhibitory impact on NK-cell ADCC responses is facilitated by Smad3, which acts downstream of TGF-β signaling through its receptor. These effects were reversed upon the inhibition or knockdown of the TGF-β receptor [262].

The potential therapeutic implications of these inhibitory findings are intriguing. Panek et al.'s research using experimental models demonstrates that blocking specific pathways downstream of TGF-β signaling can effectively reduce airway fibrosis [248]. Additionally, research on SARS-CoV-2 infection suggests that inhibiting TGF-β might restore NK cell function, potentially offering new avenues for treatment [263]. Moreover, Co-administration of TGF-β antagonists with NK cells has demonstrated the ability to maintain their cytotoxicity and sustain the expression levels of activating NK receptors such as NKG2D and CD16. The utilization of Fresolimumab, an antibody that neutralizes TGF-β, and galunisertib, an inhibitor targeting TGF-βRI, has been highlighted for their safety and tolerability profiles in human subjects [82].

In a strategically separated investigation, CRISPR/Cas9 disrupts TGF-βR2 in NK cells [264], enhancing their resilience against target cells and resistance to TGF-β-mediated immunosuppression, leading to stronger anti-tumor effects in models of AML and glioblastoma [265].

In a comparable approach, CAR-NK cells were engineered to overexpress a dnTGF-βR, which exhibits a strong affinity for TGF-β without initiating subsequent signaling cascades. This alteration effectively alleviates the inhibitory impacts of TGF-β, consequently enhancing NK cell cytotoxicity and significantly augmenting the release of perforin, granzyme, and IFN-γ [266, 267].

These approaches are also employed in CAR-T cells to obstruct the impact of TGF-β by integrating the CAR with the dnTGF-βRII which acts as a decoy receptor for the cytokine, thereby inhibiting the initiation of its downstream signaling pathway [268]. CAR-T cells use dnTGF-βRII to block TGF-β, enhancing their antitumor effect [155, 269]. Given the advantages of developing TGF-β resistant CAR-T cells, a phase 1 clinical trial was carried out to evaluate the efficiency of CAR-T cell therapy modified with dnTGF-βRII in cancer [270].

In summary, researches suggest a complex role for TGF-β in asthma. Its presence seems to be dose and time-dependent, potentially contributing to airway fibrosis and cough. Unfortunately, TGF-β suppresses NK cells. CAR studies suggest removing TGF-β may benefit asthma. Future research could engineer CAR cells to target TGF-β or block its production in asthmatic cells, offering a new treatment approach (Fig. 5).

CCLs

CCLs such as CCL3 (MIP-1α), CCL4 (MIP-1), and CCL5 termed RANTES have a role in the pathogenesis of asthma by inducing type 2 inflammation [271]. CCL3 is expressed highly by macrophages, NK cells, neutrophils, and other immune cells in inflammatory conditions [272]. CCL3 could potentially function as a significant genetic modulator for T-lymphocytes, macrophages, and chemo-attractants responsible for mononuclear cells [273]. Conversely, increased expression of CCR5, a CCL3 receptor, can stimulate T cells to produce more IL-2, potentially mitigating asthma symptoms [274].

CCL4 is produced by a wide range of cells including monocytes, NK cells, neutrophils, T and B lymphocytes, as well as epithelial and endothelial cells lining tissues, and fibroblasts within connective tissue [275, 276]. CCL4 acts against eosinophils after releasing from an activated

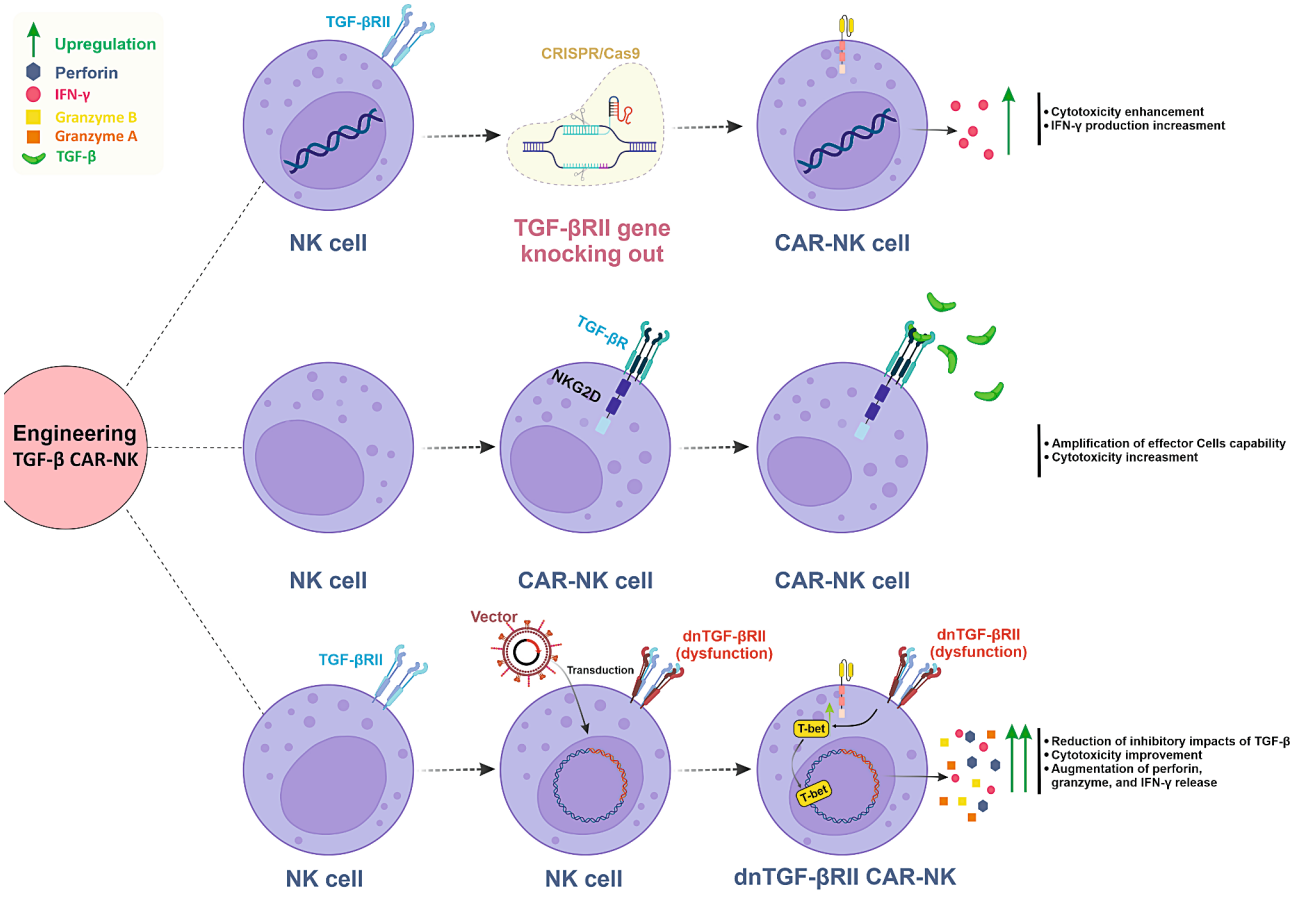

Fig. 5 TGF-β CAR NK cells: methods for engineering NK cells to overcome the suppressive effects of TGF-β. These engineered cells are known as TGF-β CAR NK cells. One method uses CRISPR/Cas9 technology to delete the gene responsible for making the TGF-β receptor on the NK cell. Without this receptor, the TGF-β molecule can't bind and suppress the NK cell's activity. As a result, these engineered NK cells produce more interferon gamma and become more cytotoxic. Another method builds a new receptor molecule for the NK cell. It uses the ectodomain of the TGF-β receptor, but combines it with the endo domain of NKG2D. This allows the engineered NK cell to recognize target cells but bypasses the TGF-β suppression. This approach improves NK cell function and reduces the inhibitory effects of TGF-β. dnTGF-β CAR-NK cells lack both the functional receptor and the ability to respond to TGF-β signals. Consequently, the TGF-β molecule has no suppressive effect, and these engineered NK cells show increased production of interferon gamma, perforin, and granzymes

form of them, especially in inflammatory conditions of airways [277]. On the other hand, some studies demonstrated that CCL4 can exacerbate asthma and AHR [271]. Immune cells such as monocytes, NK cells, and T cells are attracted to the inflamed area by binding CCL4 to its receptors CCR5 and CCR8 [278]. A study by Capelli et al. found significant differences in CCL4 levels between the BAL of chronic bronchitis patients and control subjects [279]. Additionally, Dupilumab treatment reduced CCL4 expression in nasal polyps, potentially mitigating excessive inflammation [280].

CCL5 is a potent chemoattractant for monocytes, T helper cells, DC cells, eosinophils, and NK cells at the site of inflammation. CCL5 exhibits a higher binding affinity for CCR1 and CCR5 compared to CCR3. Th1 cells usually have CCR1 and CCR5 on their surface, while Th2 cells mostly express CCR3 [281, 282]. Elevated concentrations of CCL5 have been documented in individuals diagnosed with asthma [283]. The use of antibodies to specifically target CCL5 in a murine model of allergic airway disease has revealed the capacity to hinder inflammation within the airways [284]. Additionally, Allard et al.'s findings demonstrate that CCL5 activates monocytes in the exacerbation of rhinovirus-induced asthma [285]. As a result, Reducing CCL2, CXCL8, and CXCL10 in CAR-NK cells via gene editing might be beneficial due to their inflammatory effects.

ADCC and CD16

NK cells utilize a variety of effector mechanisms in order to eradicate target cells. Of particular importance is the involvement of CD16A (FcγRIIIA), a low-affinity Fc receptor expressed on their surface. CD16A specifically recognizes the Fc region of antibodies pre-bound to target cells, initiating ADCC. ADCC is a mechanism by which antibodies induce the elimination of target cells

through non-phagocytic means [286]. ADCC can be mediated by several human antibody classes: IgG, IgA, and IgE [287]. This process empowers NK cells to function as potent cytotoxic effectors against cells targeted for destruction by antibodies [167, 288].

ADCC represents a critical mechanism for NK cells to eliminate virally infected cells. Furthermore, in vitro ADCC capacity has been linked to susceptibility to asthma following respiratory infections [289]. As prominent ADCC effectors, NK cells utilize three primary mechanisms to eliminate target cells: cytotoxic granule exocytosis, Fas ligand signaling, and release of perforins and granzymes. The release of perforin and granzymes from granules represents the principal and best-characterized mechanism employed in ADCC [290, 291]. While various myeloid lineage cells can mediate ADCC, NK cells appear to be the key effectors in vivo. The clinical effectiveness of many targeted mAb therapies has been shown to be dependent on NK cell activity [292]. This highlights the critical role of NK cells and their high expression of activating CD16A in ADCC, making them a target for optimizing antibody interactions in the context of immunotherapy.

Screening for ADCC activity could identify patients needing alternative immunotherapy or interventions to boost their ADCC [293]. CAR-NK cells co-expressing CD16A and CD64 (FcγRI) show promise, demonstrating enhanced and sustained ADCC [294] (Fig. 6).

Perforin and granzyme

NK cells eliminate stressed, transformed, or infected cells, including damaged epithelial cells found in asthmatic lungs by releasing cytolytic granules that consist of perforin and granzymes [295, 296]. These granules work in a coordinated fashion: perforin creates pores in the target cell membrane, while granzymes infiltrate the cell and trigger apoptosis through multiple pathways. Granzyme B can achieve this in two ways: by activating a special suicide program (caspase-3 cleavage) or through other methods not depending on this program [297, 298]. Notably, research suggests a link between impaired NK cell cytotoxicity and the development of severe asthma, highlighting the importance of this process in maintaining lung health [13].

Perforin-deficient mice develop asthma-like symptoms (Mathias et al.), suggesting its critical role in preventing the disease [299].

Benralizumab, a therapeutic agent for severe eosinophilic asthma, exerts its multifaceted effects through binding to their IL-5Rα, blocking survival signals and leveraging NK cells to eliminate them [300]. This activation triggers ADCC, prompting NK cells to release perforin and granzyme, ultimately inducing apoptosis in both eosinophils and basophils [252, 300, 301]. Recent in vitro investigations provide additional insights into this mechanism, elucidating the essential function of an eosinophil-NK cell synapse in the induction of eosinophil apoptosis by benralizumab. In a significant breakthrough, Dagher et al. revealed that benralizumab can eliminate eosinophils through macrophages via two distinct pathways: directly engulfing eosinophils in a process called ADCP and stimulating macrophages to produce TNF, a molecule known to trigger cell death. Eosinophils upexpress TNFR1 during apoptosis. Interestingly, the presence of NK cells further amplified TNFR1-mediated apoptosis by releasing another signaling molecule, IFN-γ [302].

NK cells targeting eosinophils show promise for eosinophilic asthma treatment, offering a potential alternative to IL-5 biologics with fewer side effects [13]. Further research on enhancing NK cell function is warranted (Fig. 6).

Fas/FasL

Fas (CD95, APO-1), a type I transmembrane protein belonging to the TNFR superfamily, harbors a cytoplasmic death domain. Its ligand, FasL, a 40 kDa protein appertaining to the TNF superfamily, triggers apoptosis. Fas-FasL pathway triggers cell death in activated immune cells, maintaining immune balance by eliminating potentially harmful lymphocytes and preventing tissue damage [303, 304].

While NK cells express a diverse array of death ligands, only FasL and TRAIL have been demonstrably implicated in direct target cell cytotoxicity during NK-mediated killing in humans and mice [305, 306]. Upon ligand binding, Fas triggers distinct signaling pathways categorized as apoptotic and non-apoptotic. Mechanistically, in an apoptotic way, Fas engagement by FasL recruits FADD, which subsequently binds procaspase-8. This interaction leads to the formation of the DISC and activation of caspase-8. Activated caspase-8 then cleaves and activates downstream caspases, such as caspase-3 and caspase-7, ultimately culminating in apoptosis [307, 308]. The activation of nonapoptotic pathways by Th2 cells using Fas-mediated mechanisms might be involved in the progression of pulmonary inflammation. The regulation of Fas signaling within Th2 cells is crucial in the inhibition of type 2 inflammatory responses. Distinguishing Th2 cell Fas signaling is challenging. Fas-FasL antagonists might harm other immune cells. More specific therapies require further research [309].

FasL inhibition resulted in enhanced T cell activation, with a dose-dependent and time-dependent increase in IL-5, IL-9, GM-CSF production, and airway eosinophilia. Paradoxically, activated T cells suppressed eosinophilic airway inflammation and allergic asthma, likely through a subsequent reduction in cytokine production [309, 310]. FasL restricts the proliferation and expansion of

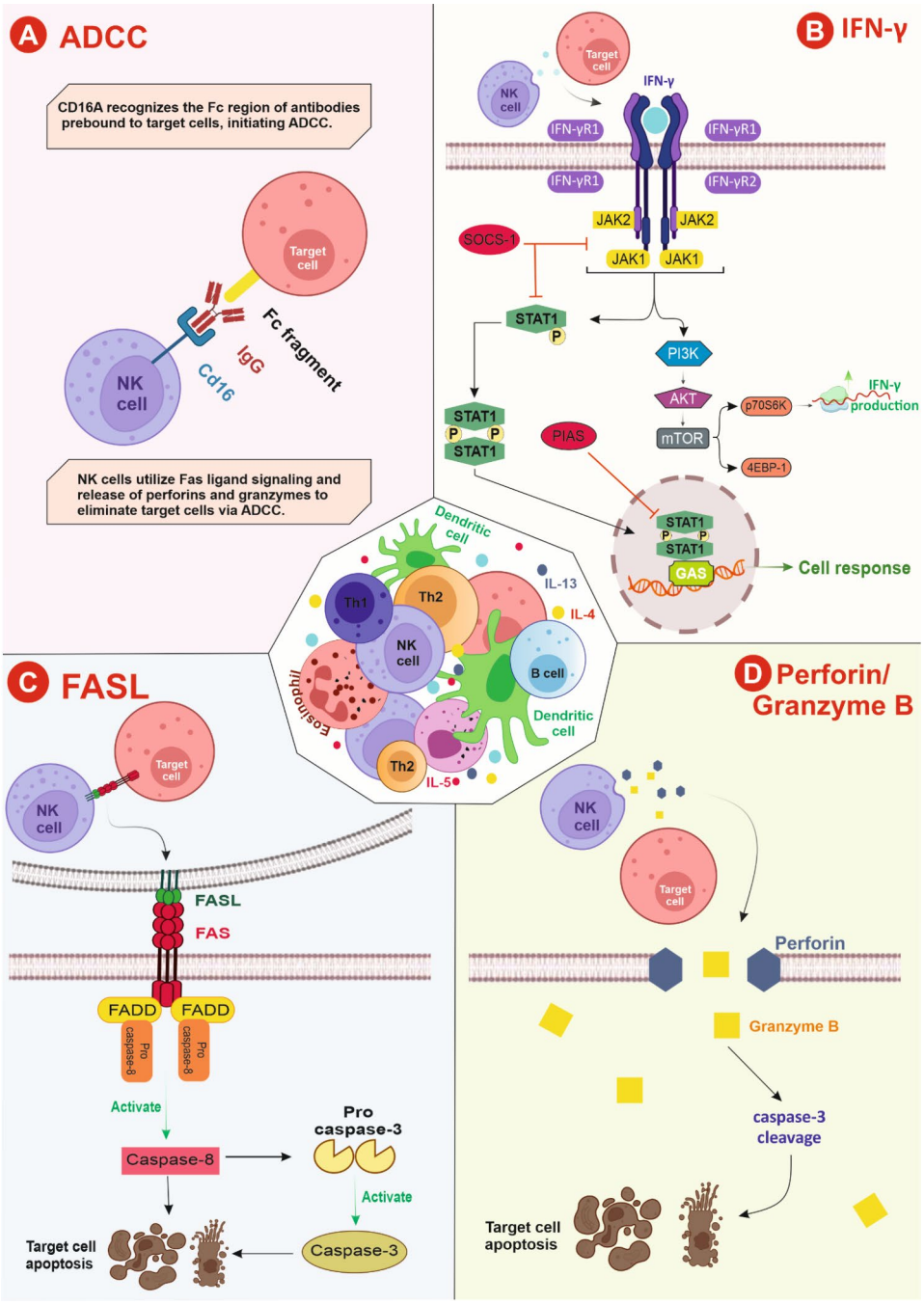

Fig. 6 NK cell's cytotoxicity: The image describes how NK cells fight against target cells within the inflamed airways of an asthmatic patients. **(A)** NK cells utilizes CD16A to recognize Fc region of antibodies pre-bound to target cells, initiating ADCC. ADCC induce the elimination of target cells through FAS ligand signaling and release of perforin/granzymes. **(B)** NK cells release IFN-γ, which is a signaling molecule. It binds to receptors on target cells, activating a protein named STAT1.Then, Two STAT1 molecules combine and bind to DNA, promoting the production of proteins crucial for fighting against allergens by specific gene known as GAS. Also, IFN-γ can activate additional pathways involving proteins such as mTOR. This loop ultimately leads to increased IFN-γ production, further amplifying the immune response. **(C)** NK cells contain specialized granules filled with perforin and granzyme B. Perforin creates pores in the target cell's membrane. Granzyme B enters the target cell through the pores, initiating apoptosis. **(D)** FasL binds to Fas receptors on target cells. This Binding triggers recruitment of FADD, which activates procaspase-8 into caspase-8. Caspase-8 directly cleaves target proteins, inducing apoptosis. Notably, caspase-8 can also activate caspase-3, another cell death executioner to robust more apoptosis response

antigen-specific T cells, consequently diminishing the production of Th2 cytokines. [311]

NK cells may play a role in asthma by regulating neutrophil activity in the lungs [146]. Asthmatic patients show airway neutrophilia, suggesting disrupted communication between these cells [312]. NK cells from asthmatic patients also exhibit abnormal function [313].

CAR-NK cells targeting the Fas-FasL pathway offer promise for new asthma treatments. Studies show engineering CAR-NK cells with a modified Fas receptor lacking the death domain (ΔFAS) improves their persistence [314]. CAR-NK cells targeting FasL offer a new approach for asthma by inducing Fas-mediated apoptosis in immune cells, potentially reducing inflammation Building on the success of CAR cell therapies that exploit the Fas/FasL pathway to enhance cytotoxicity [100, 315] (Fig. 6).

IFN-γ

NK cells and NKT cells are crucial producers of IFN-γ during the innate immune response. In contrast, CD8+and CD4+T cells become significant sources of IFN-γ during the adaptive immune response [199]. The secreted IFN-γ binds to its receptor IFNGR on cells, regulating the immune response. IFN-γ can also induce APCs to release IL-12, reactivating IFN-γ production. This forms a positive feedback loop in inflamed settings and can reduce asthmatic inflammation [316]. T-bet, a transcription factor from the T-box family (TBX21 gene) promotes IFN-γ production in Th1 cells and NK cells, but not Th2 cells [316]. Introducing T-bet into Th2 cells via transduction turns them into IFN-γ-producing Th1 cells [317]. This process could be a promising approach to mitigating asthma by potentially suppressing the release of excessive Th2 cytokines.

IFN-γ has several signaling pathways. In the Canonical pathway, IFN-γ locks onto its receptor, which triggers a domino effect. Two proteins, JAK1 and JAK2, get activated and modify a specific site on another protein known as STAT1. This activated STAT1 pairs up with another STAT1, and together they move to the nucleus. There, they bind to their specific gene (GAS). As a result, GAS increases the production of proteins that fight infection [318]. Meanwhile, SOCS hinders JAK and STAT activation. In addition, PIAS restrains gene transcription induced by IFN-γ [319]. During a non-canonical pathway, it activates a series of proteins including STAT1, PI3K, and Akt. This activation eventually leads to a protein named mTOR playing a role in the IFN response. Additionally, mTOR works with p70S6 kinase to increase the production of IFN-γ [320, 321].

Th1 IFN-γ suppresses asthma by inhibiting Th2 cells [322]. Production of IFN-γ by NK cells has the potential to induce the polarization of CD4+T-cells towards a Th1 phenotype [323]. In addition, IFN-γ inhibits mucus, chitinases, and eosinophilia through the airway epithelium independently of Th2 cell activation [324], and might favor allergic inhibition by reducing IgE production [325]. Patients diagnosed with allergic asthma demonstrate elevated levels of IL-4-producing NK cells and reduced levels of IFN-γ-producing NK cells circulating in the bloodstream [326]. Both the IFN-γ+NK cells and the concentration of IFN-γ exhibited a notable decrease in individuals with severe asthma in comparison to those in good health. This finding indicates that NK cells derived from severe asthma patients may possess an inherent deficiency that results in diminished levels of IFN-γ expression and secretion [4]. Therefore, enhancing the ability of NK cells to produce IFN-γ holds promise as a therapeutic strategy for alleviating the symptoms of asthma [323]. Numerous studies support IFN-γ's inhibitory role in asthma pathogenesis. In a study by Renzi et al., infants with lower levels of IFN-γ in their PB experienced more severe asthma symptoms. Additionally, the ratio of IFN-γ to IL-4 was lower in infants requiring medication for their symptoms [327, 328]. Another research shows that decreased levels of IFN-γ+CD4+cells and lower concentrations of IFN-γ were found in individuals diagnosed with allergic asthma [329]. Additionally, Ex vivo studies suggest impaired IFN-γ responses in asthma patients compared to healthy individuals during viral infection [330]. In contrast, the transcriptomic analysis showed that TNF-α and IFN-γ exposure affects gene expressions in ASM, reducing corticosteroid sensitivity and potentially worsening pediatric asthma symptoms [194]. NK cells' cytotoxic properties via IFN-γ elimination of target cells in asthma suggest potential for enhanced efficacy through CAR therapy [331, 332] (Fig. 6).

DNAM-1

DNAM-1 (CD226), a transmembrane glycoprotein of the immunoglobulin superfamily, plays a pivotal role in immune regulation [333, 334]. In NK cells, DNAM-1 functions as a key activating receptor. Deficiency in DNAM-1 impairs essential NK cell processes like proliferation, activation, and effector functions [335–337]. It works alongside other activating receptors like NKG2D and NCRs to trigger cell killing by binding specific ligands [335]. DNAM-1 signals activation through its interaction with two ligands, PVR (CD155) and Nectin-2 (CD112) [338]. Notably, DNAM-1 functions as a co-stimulatory receptor, harboring an ITAM-like motif that amplifies signaling pathways initiated by ITAMs within immune cells [339].

Intriguingly, the immunoregulatory role of DNAM-1 extends beyond NK cell activation and might hold relevance in asthma and AHR. Studies suggest that DNAM-1

signaling promotes the activation, proliferation, and differentiation of Th1 and Th17 cells which are considered to be protective in asthma [336]. Additionally, DNAM-1 in CD4+T cells promotes IFN-γ production while its deficiency favors the production of pro-inflammatory IL-4 and IL-13 cytokines [340, 341]. Similarly, DNAM-1+CD8+T cells exhibit enhanced production of IFN-γ and granzyme B, further supporting their potential role in reducing inflammation [342].

DNAM-1's versatility makes it a target for CAR-NK engineering. Studies show engineered NK cells with activated DNAM-1 exhibit direct cytotoxicity [343], supporting the potential of CAR-NK for targeting tumor antigens [344, 345]. CAR-NK-92 cells engineered with a DNAM-1 intracellular domain exhibit enhanced cytotoxicity compared to CAR-NK cells incorporating a CD28 costimulatory domain. CAR-NK-92 cells, which possess both the DNAM-1 and 2B4 costimulatory domains, demonstrated the most elevated cytolytic activity [346]. Adoptive transfer of NK cells with increased DNAM-1 levels can be very helpful in the treatment of asthma, considering that these cells play an important role in the production of cytokines and intensification of the overreaction of the airways.

DNAM-1 CAR-NK cells may outcompete inhibitory receptors like TIGIT and CD96 for binding to PVR and Nectin-2 ligands, potentially leading to enhanced activation and improved cytotoxicity. Therefore, incorporating DNAM-1 into CAR-NK presents a promising avenue for alleviating asthma symptoms, further investigation is necessary to elucidate potential complications and definitively assess its efficacy.

TNF-α

TNF-α, encoded by a gene on chromosome 6, is a key cytokine implicated in inflammatory processes [347]. Structurally, this homotrimeric protein of 157 amino acids is primarily produced by activated macrophages, T-lymphocytes, and NK cells [348]. TNF-α can augment NK cell cytotoxicity [349], and its pleiotropic effects on various biological processes make its influence on immune regulation in asthma multifaceted [350]. Notably, research suggests TNF-α can modulate both pro-inflammatory and anti-inflammatory mechanisms [351]. TNF-α exists in both soluble (sTNF-α) and transmembrane (tmTNF-α) forms. Initially synthesized as tmTNF-α, it requires cleavage by TNF-α-converting enzyme for release as the active sTNF-α [352]. This cytokine exerts its effects by binding to two distinct TNF receptors, TNFR1 and TNFR2 [350]. The interaction of TNF-α with these receptors, particularly TNFR1, triggers signaling cascades that mediate diverse biological processes [348]. Notably, TNFR1 signaling is primarily linked to pro-inflammatory and apoptotic pathways, while TNFR2 signaling is crucial for mononuclear cell activation and proliferation [353, 354].

Emerging evidence suggests a multifaceted role for TNF-α in asthma pathogenesis. The airways of individuals with neutrophilic and severe asthma exhibit elevated TNF-α levels [355]. TNF-α promotes the production of Th2 cytokines (IL-4, IL-5, IL-13), which further enhance ASM contraction [356, 357]. Furthermore, TNF-α, IFN-γ, and IL-1β may increase CD38 expression (cyclic ADP ribose hydrolase) in ASMs, potentially leading to increased intracellular Ca2+signaling and AHR induction [358]. TNF-α also synergizes with IL-17 cytokines to recruit neutrophils, contributing to neutrophilic inflammation [115, 359]. TNFR1-mediated hyperactivation of the NF-κB pathway is associated with upregulation of pro-inflammatory genes and airway inflammation in asthma patients [360]. Conversely, TNFR2 signaling demonstrates an anti-inflammatory effect by suppressing Th2 and Th17 cell differentiation and decreasing cytokine levels [361, 362]. In contrast to the prevailing view, certain research indicates that TNF-α binding to TNFR2 can enhance T cell activation, proliferation, and survival during airway inflammation [363]. Notably, TNF-α appears to exert these effects primarily on CD8+Teff cells [364–372]. These seemingly contradictory findings highlight the complex interplay between TNF-α and its receptors in asthma. TNFR1 drives inflammation, while TNFR2's therapeutic potential requires further exploration.

CAR-NK cells engineered to target TNF-α may offer therapeutic potential for asthma. These cells could eliminate mucus-producing goblet cells or deplete inflammatory immune cells, potentially improving symptoms [348, 373–385]. However, the precise role of TNF-α in asthma and CAR-NK therapy warrants further investigation.

CAR-NK vs. CAR-T: Unveiling potential, challenges, and solutions

NK cells offer several advantages over T cells for CAR-based immunotherapy. Unlike T cells, which require antigen presentation for activation, NK cells are activated through their innate receptors. This allows CAR-NK cells to retain their cytotoxic function even if the target loses or downregulates its antigen, Additionally, NK cell-based therapies are associated with a lower risk of severe side effects compared to CAR-T cell therapy. This is because NK cells have a limited lifespan in the body, reducing the chance of complications like CRS, ICANS, and GVHD [27]. These advantages position CAR-NK cells as a promising next-generation cellular therapy. A detailed description of the comparison of CAR-T vs. CAR-NK is discussed below (Table 5).

Table 5 Comparison of CAR-T vs. CAR-NK

Feature	CAR-T	CAR-NK
Source of Immune Cells	Autologous T lymphocytes	Allogeneic NK cells (peripheral blood, cord blood, cell lines)
Activation Mechanism	MHC-dependent, requires antigen presentation	MHC-independent, innate immune response
Availability	Personalized, requires T cell collection	Off-the-shelf, readily available from various sources
Manufacturing Complexity	High, requires complex autologous expansion	Lower, simplifies manufacturing with allogeneic approach
Manufacturing Cost	High due to personalized production	Lower potential cost due to scalability of allogeneic approach
Delivery Vector	Primarily lentiviral vectors (VSV-G pseudotyped)	BaEV lentiviral vectors, exploring non-viral methods
CAR Structure (Costimulation)	T cell receptor (TCR) primary signaling, costimulatory domains (CD28, 4-1BB, ICOS, OX-40) for enhanced activation	Distinct co-stimulatory molecules (NKG2D/CD244)
Antigen Recognition	Primarily MHC-dependent (single-chain antibody)	Multifaceted recognition (scFv, NCRs, NKG2D, DNAM-1)
Persistence Strategies	Engineered expression of IL-15 shows promise	Short-term IL-12/15/18 exposure for memory-like NK cells
Persistence Necessity	May vary depending on disease (short-term vs. long-term)	Likely disease-specific, potentially less critical than for CAR-T in some cases
Expansion	Prone to exhaustion with prolonged culture, requiring optimization of cytokine cocktails (IL-2, IL-15, etc.) and potentially shorter durations.	Limited proliferation due to telomere shortening, but enhanced by specific cytokine combinations (IL-21, feeder cells) or single agents (IL-15).
Antigen Loss	Highly susceptible, necessitating multi-pronged approaches (dual-targeted CARs, sequential therapy) with ongoing challenges.	Potentially less impacted due to intrinsic cytotoxicity (NCRs, death receptor ligands) and independent target recognition via NKG2D and NDAM1 signaling.
Cytotoxicity Profile	Potent cytokine storm upon activation (TNF-α, IL-1β, IL-2, IL-6)	Less inflammatory cytokine profile (GM-CSF)
CRS/ICANS risk	High risk	Lower risk
GVHD	High risk	Lower risk

Source of immune cells

A critical advantage of CAR-NK cells over CAR-T cells lies in the source of allogeneic immune effector cells. CAR-T cell therapy necessitates the utilization of autologous T lymphocytes, rendering patients who have undergone prior lymphodepletion therapies ineligible due to insufficient T cell counts. This limitation can significantly delay, or even preclude, treatment initiation. Studies have demonstrated that a substantial proportion of patients fail to meet the minimum T cell collection threshold for CAR-T cell manufacturing [386].

CAR-NK cells offer a compelling alternative in this regard. Unlike T cells, NK cells exhibit reduced alloreactivity and minimal risk of GVHD due to their MHC-independent activation pathways. This characteristic enables the development of off-the-shelf CAR NK cell products. Allogeneic NK cells can be sourced from diverse origins, including PB [63], UCB [75], hESCs, iPSCs [93], or cell lines like NK-92 [80].

Manufacture and cost

While CAR-T has revolutionized asthma treatment [18–21, 387], access remains limited due to high costs and complex, personalized manufacturing [388]. Recent research explores cost reduction through distributed, automated CAR-T manufacturing, demonstrating significant savings compared to centralized processes [389–391]. An alternative approach is the development of off-the-shelf CAR-T therapies. These therapies involve the large-scale production of genetically modified cells at a central location, enabling them to be used allogeneically for multiple patients [392].

In contrast to CAR-T cells, CAR-NK cells exhibit a naturally diminished propensity to induce GVHD, enabling their allogeneic application without the need for complex gene editing interventions [393]. Similar to CAR-T cells, cytokines can independently induce NK cell proliferation to levels sufficient for clinical application [394, 395]. While feeder cells significantly augment this expansion, their complete removal from cultures remains challenging, posing potential safety risks [396–399]. To address this, researchers are exploring alternative feeder-free methods. One promising approach utilizes dissolvable, polymer-based microspheres that gradually release growth factors and nutrients, thereby facilitating NK cell expansion [400].

Delivery approach

Delivering CAR constructs to T and NK cells utilizes distinct approaches due to their inherent biological differences. CAR-T cell manufacturing traditionally relies on viral vectors, such as retrovirus, adeno-associated virus vectors, and especially lentivirus, particularly VSV-G pseudotyped lentiviral vectors, to achieve efficient and sustained CAR expression [102]. However, NK cells present a challenge due to their low VSV-G receptor expression, hindering lentiviral transduction [401]. A promising solution lies in the Baboon

envelope pseudotyped lentiviral vector, which significantly enhances NK cell transduction and even facilitates the delivery of CRISPR components for potential gene editing [402]. While other lentiviral envelope options like gibbon ape leukemia virus envelope and feline endogenous retrovirus envelope protein (RD114-TR) exist, BaEV currently appears to be the most effective vector for NK cell transduction [102, 403, 404]. Safety concerns regarding insertional mutagenesis associated with some viral vectors require further investigation in the context of CAR-T therapy [405], although long-term studies with CAR-T therapy haven't reported such malignancies [288].

Beyond viral vectors, research explores non-viral delivery for CAR-T/NK cells due to limitations of viral vectors such as immunogenicity, cost [102, 406]. These methods (Sleeping Beauty, PiggyBac transposons) offer a safer integration profile but lower efficiency [102]. Alternatively, mRNA CAR constructs with electroporation or lipid nanoparticles achieve high, but transient, CAR expression [407]. DNA nano vectors represent another promising strategy, enabling non-integrating CAR expression with minimal immunogenicity and genotoxicity [408]. CRISPR-Cas9 unlocks targeted CAR-NK engineering, including CAR integration and inhibitory gene deletion [409]. Novel delivery methods like biomaterial vehicles and microrobots explore ex vivo-independent approaches [410, 411]. The field is rapidly evolving, with BaEV lentiviral vectors showing promise for CAR-NK, while non-viral and innovative delivery methods hold potential for safer and more versatile CAR-T and CAR-NK therapies.

CAR Structure

CAR-NK and CAR-T cells share a fundamental CAR structure with three key domains [412]. However, a key distinction lies in their co-stimulatory molecules. CAR-T cells typically utilize CD28 or 4-1BB, while CAR-NK cells leverage NKG2D and 2B4 [27].

Persistent

The field of ACT faces a crucial challenge: ensuring the long-term persistence of infused immune effector cells. The conventional view suggests that NK cells have a lower potential for long-term persistence compared to T cells [413]. This limited lifespan is believed to contribute to the modest clinical success observed in NK cell-based therapies [414, 415]. Consequently, CAR-NK cell development has primarily focused on enhancing cytotoxic function, with less emphasis placed on promoting persistence [183, 416].

However, research offers a promising approach to improving CAR-NK cell persistence. Engineering NK cells to express immunostimulatory cytokines like IL-15 has demonstrated enhanced survival [417, 418]. Moreover, short-term exposure to a combination of IL-12, IL-15, and IL-18 can generate memory-like NK cells exhibiting improved persistence, potentially leading to more efficacious CAR-NK cell therapy [419, 420]. In contrast, CAR-T cell development has successfully incorporated costimulatory domains into their design, enriching memory-like CAR-T cells and leading to enhanced persistence and durable treatment responses in both preclinical models and clinical trials [421]. Furthermore, engineering CAR-T cells to express immunostimulatory cytokines such as IL-7, IL-12, IL-15, IL-18, IL-21, and IL-23, creating so-called "armored CARs," has shown promise in improving persistence and is currently being evaluated in ongoing clinical trials [422, 423].

Inducible promoters offer a promising strategy to enhance the functionality, safety, and specificity of CAR cells. These promoters are designed to activate only upon a specific trigger, such as recognition of an antigen, metabolite, drug, or a particular cell signaling pathway [424]. NFAT promoters control CAR-T cell transgene expression [425], while NF-κB promoters in CAR-NK cells trigger IL-12 secretion upon activation, boosting cytotoxicity and monocyte recruitment [426].

Pre-conditioning regimens using lymphodepletion is a crucial factor influencing the persistence of infused T cells in CAR-T therapy, and this approach may hold similar importance for CAR-NK therapy [427]. Typically, a fludarabine/cyclophosphamide combination is used prior to CAR-NK infusion to minimize host rejection of the infused NK cells [428]. Studies in unmodified NK cell therapy suggest that high-dose lymphodepletion promotes in vivo NK cell expansion and persistence, highlighting its potential role in enhancing CAR-NK cell therapy efficacy [64]. CAR-T therapy shows promise with selective lymphodepletion using CD52 antibodies and knockout CAR-T cells [429]. Whether such an approach can be applied to CAR-NK therapy remains to be explored.

Expansion

For CAR-T cells, prolonged ex vivo expansion can be detrimental, leading to a state of exhaustion that negatively impacts their memory phenotype, longevity, and proliferative capacity upon reintroduction [430, 431]. Achieving sufficient numbers of CAR-T cells for therapy hinges on the ex vivo expansion duration. Traditional protocols cultivate T cells for 9–14 days, but recent trends suggest potential benefits of shortening this period. Studies indicate that a shorter ex vivo culture may limit excessive T-cell differentiation, potentially enhancing efficacy [432]. However, cytokines like IL-2, commonly used for expansion, can induce exhaustion and decrease persistence. Conversely, expansion with IL15 or a combination of IL-2, IL-4, and TGF-β resulted in less differentiated

CAR-T [433–435]. Similarly, studies suggest that incorporating IL-4, IL-7, and IL-21 promotes stemness and inhibits exhaustion [436].

A crucial stage in CAR-NK cell production is expanding the transduced cells. However, limited NK cell proliferation capacity hinders achieving sufficient numbers. Telomere shortening restricts their proliferative potential, but incorporating IL-21 into the culture medium can address this [437]. Co-culturing NK cells with feeder cells expressing IL-15, 4-1BB ligand, and MICA also enhances proliferation and viability [438]. While cytokines optimize ex vivo expansion, specific combinations like IL-2, 15, and 18 with anti-CD3 and CD52 beads expedite expansion [27, 439]. Notably, IL-15 alone, without IL-2, enhances cytotoxicity and expansion without activating regulatory T cells [27, 395]. IL-21 also promotes NK cell proliferation, particularly in synergy with feeder cells [440].

Antigen loss

A critical challenge shared by both CAR-T and CAR-NK cell therapies is antigen loss, which can lead to treatment failure [441]. Several strategies have been explored to address antigen loss in CAR-T therapy, including the development of dual-targeted CAR T cells and sequential administration of CAR-T cell products targeting distinct antigens [442–447]. However, these approaches are not without limitations, as documented cases of relapse due to antigen loss in patients receiving dual-targeted CAR-T cell therapy are highlighted [442–444]. Preclinical models have shown promise for additional approaches, including multi-antigen targeting using CARs incorporating ankyrin repeat domains, and engineering CAR-T cells to modulate the endogenous immune system. These approaches all hold promise for overcoming antigen loss and limiting relapse in CAR-T therapy [448–450].

CAR-NK cells may possess a potential advantage in overcoming antigen loss compared to CAR-T cells. Their intrinsic cytotoxic arsenal, mediated by natural cytotoxicity NCR and death receptor ligands, offers a built-in resistance mechanism against CAR target antigen loss [214, 451]. Moreover, intracellular signaling of NK cells (NKG2D and DNAM-1) can contribute to the identification of the target cell independent of CAR, antigen-loss does not affect the efficacy of CAR-modified NK cells [452, 453]. Li et al. [454] explored the use of an antigen-specific inhibitory KIR-based receptor that effectively inhibited aCAR-mediated trogocytosis and the associated immunomodulatory consequences. This approach successfully preserved the ability of NK cells expressing the same antigen to exert key effector functions against target cells [454]. Beyond inhibitory KIR antagonism, cytokine treatment strategies are explored to reduce inhibitory KIR levels on NK cells, thereby enhancing CAR-NK cell activity against HLA-A/B/C+targets. Studies indicate superior KIR downregulation with IL-12, IL-15, and IL-18 compared to IL-15 alone [455].

Cytotoxicity

A key area of research is optimizing the cytotoxicity of CAR-T and CAR-NK cells. One strategy involves engineering costimulatory domains into the CAR construct to enhance the activating intracellular signaling of the modified cells. While the TCR serves as the primary signaling receptor for T cell cytotoxicity [411], various transmembrane costimulatory molecules, such as CD28, 4-1BB, ICOS, and OX-40, can amplify downstream signaling pathways when incorporated within the costimulatory domain of the CAR. Each of these costimulatory molecules influences distinct signaling cascades that impact CAR-T cell cytotoxicity, proliferation, and persistence [456, 457]. In contrast, NK cells utilize a complex network of activating and inhibitory receptors that recruit specific downstream signaling mediators. Fine-tuning these signaling cues determines the balance between NK cell activation and inhibition [411]. Studies suggest that tailoring CAR engineering specifically for NK cells by incorporating NK-specific signaling domains, such as 2B4 and DAP10/DAP12, within the costimulatory domain, can enhance CAR-NK cell efficacy and IFN-γ secretion compared to CAR constructs designed for T cells [457, 458].

Side effects

Despite the remarkable response rates achieved with CAR-T cell therapy, a significant drawback lies in the potential for severe side effects [459]. These complications are primarily attributed to the potent cytokine storm triggered upon CAR-T cell activation [460, 461]. CRS and ICANS are prominent examples, manifesting as fever, multi-organ failure, and neurological symptoms [461, 462]. Management of these cytokine-mediated toxicities often necessitates corticosteroids and, in severe cases, anti-cytokine antibodies [463]. This cytotoxicity profile stands in stark contrast to that observed with CAR-NK cells. Early clinical trials with CAR-NK cells have not reported CRS or neurotoxicity, potentially due to a distinct cytokine release pattern upon activation [27]. CAR-T cell activation triggers the release of a pro-inflammatory cytokine storm, including TNF-α, IL-1β, IL-2, and IL-6 [462]. Conversely, CAR-NK cells appear to secrete a less inflammatory cytokine profile, with GM-CSF being a notable example [452]. This suggests that CAR-NK cells might offer a potentially safer cytotoxicity profile compared to CAR-T cells, with a reduced risk of severe side effects. Further research is warranted to definitively compare the direct cytotoxic potential of these cell types, but the emerging evidence suggests a

promising avenue for CAR-NK cell therapy with potentially improved tolerability.

Improving CAR-NK therapy safety and efficacy

Use of immune checkpoints like PD-L2 and CTLA-4

The immune checkpoint molecule PD-L2, which is a ligand for PD-1, plays a role in regulating inflammation in asthma. Studies suggest that a deficiency in PD-L2 leads to increased activity of immune cells such as eosinophils and lymphocytes, potentially contributing to excessive response to allergens in asthmatic patients [464, 465]. Studies by Akbari et al. suggest that PD-L2 deficiency may worsen AHR, inflammation, and IL-4 secretion [466]. PD-L2 on CAR-T cells represents a promising strategy for future asthma treatment. Its well-established immunosuppressive properties suggest it could effectively dampen the inflammatory response characteristic of asthma.

Liu et al. investigated CTLA-4 as a checkpoint in CAR macrophages targeting M1 microglia, implicated in neuroinflammation. Engineered CAR-macrophages with the CTLA-4 showed promise in reducing M1 microglia activity [467]. A separate study suggests that CAR-T cells engineered with CTLA-4 in their cytoplasmic tail lead to a significant reduction in CRS, a common complication of CAR cell therapy. Zhou et al. suggest CTLA-4 incorporation in CAR-T cells promotes self-tolerance and potentially reduces trogocytosis, a process limiting CAR-T durability. This strategy could enhance CAR cells lifespan and efficacy [468]. Therefore, the usage of CTLA-4 in CAR therapy can be very useful in various aspects.

AI and ML in CAR cell therapy

Despite the remarkable achievements of CAR cell therapy, several challenges remain. These include optimizing CAR design and cell products, enhancing the time-consuming, and expensive manufacturing processes, improving response rates and remission durability, and minimizing toxicity [469, 470]. ML and AI offer exciting solutions to these challenges and are revolutionizing the field of medical research [471].

AI holds immense promise for CAR T-cell therapy, particularly in tackling the persistent challenge of target identification. Multi-omics approaches, encompassing a wide range of biological data including genomics, epigenomics, transcriptomics, T cell receptor repertoire profiling, proteomics, metabolomics, and microbiomics, generate vast datasets. AI algorithms can effectively explore these datasets to identify promising targets for CAR cell design [470, 472, 473]. Furthermore, AI facilitates the characterization of CAR cell phenotype. By employing multifaceted ML algorithms, researchers can analyze extensive datasets of the functional and structural characteristics of CAR cells, leading to a deeper understanding of their therapeutic potential [474].

To address manufacturing obstacles, the development of an automated AI-facilitated CAR cell manufacturing platform is underway. AI can analyze the platform's accumulated data, providing valuable insights and driving decisions for process improvement [469, 475]. Beyond manufacturing, AI offers the potential to enhance CAR cell function in several ways. First, AI can analyze vast datasets generated from advanced techniques like scRNA-seq and TCR-seq to identify crucial genetic markers associated with treatment response. ML algorithms can then leverage these markers to predict individual patient responses, paving the way for personalized treatment strategies [476, 477]. Second, AI has the potential to improve CAR cell activation [478] and enhance gene editing methodologies for CAR cell design [479].

Moreover, AI can identify and manage adverse events like CRS [480–482]. AI-powered methods can analyze cytokine profiles and leverage existing clinical data to rapidly diagnose CRS, significantly aiding clinicians [480]. AI can also address the challenge of differentiating CAR cells from other blood cells. One study achieved a remarkable 99.63% accuracy in CAR cell identification using an AI model trained on microscopy images [483].

Developing robust AI-driven CAR cell prediction models necessitates the utilization of extensive omics and clinical datasets. The establishment of comprehensive multi-omics datasets is critical for the successful implementation of AI approaches [484]. Data limitations arise from evolving clinical practices (e.g., CAR dosage adjustments) [485], hindering AI integration in CAR cell therapy. Careful consideration and robust data collection strategies are crucial for successful integration.

Novel CAR Designs

The field of CAR engineering is actively exploring modifications to existing strategies. This relentless research and investigation aims to overcome limitations and develop a more selective and potent approach to CAR cell therapy [486]. Having reviewed the established CAR designs, we will now turn our attention to some promising new directions in CAR design (Fig. 7).

Bispecific and dual car

To improve the efficacy of CAR cell therapy, researchers are exploring dual or multi-targeting functionalities within the CAR design. This can take two main approaches: First, multi-epitope targeting, where the CAR recognizes multiple distinct sites (epitopes) on the same target antigen, potentially strengthening the binding and enhancing cytotoxicity activity. Second, bispecific or multi-specific CARs can be engineered to recognize entirely different antigens on the target cell surface. This

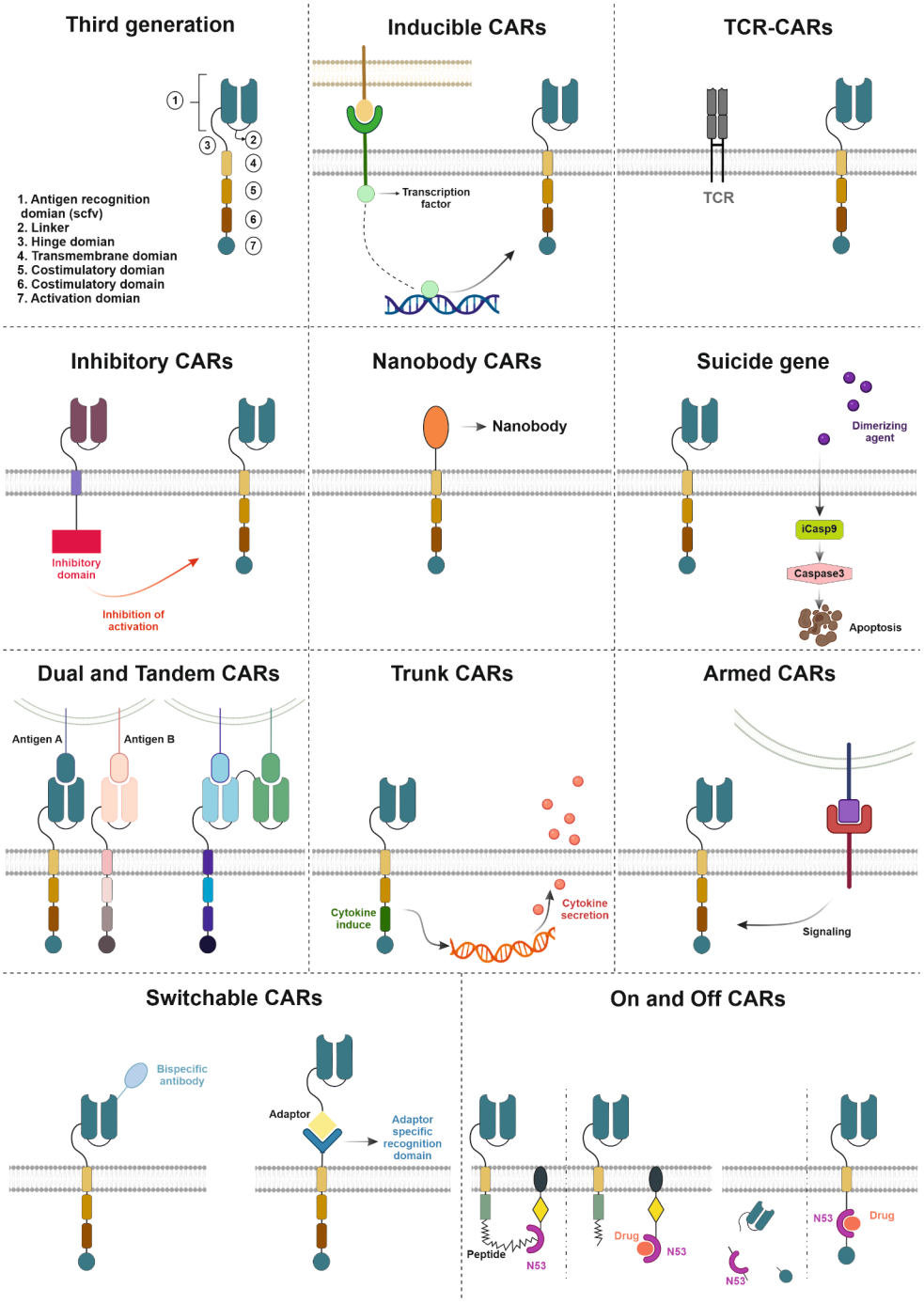

Fig. 7 Novel CAR design: This figure explores novel CAR designs beyond established approaches. Researchers are investigating strategies to improve efficacy, safety, and target recognition. These include bispecific/dual CARs for multi-antigen targeting, TRUCK CARs for targeted cytokine release, armored CARs for co-expressing beneficial proteins, and nanobody/FHVH CARs for reduced immunogenicity. Suicide genes provide a safety measure for rapid cell elimination, while inducible and switchable CARs offer precise control over activation. Inhibitory-CARs address NK cell fratricide, and TCR-CARs broaden target recognition. Finally, "ON and OFF" CARs utilize drugs to regulate activity precisely. This comprehensive overview highlights the dynamic field of CAR design, constantly innovating to enhance therapeutic potential

strategy aims to improve antigen recognition and prevent evasion [486].

TRUCK CAR

The TRUCK approach represents a novel strategy in CAR cell therapy. This approach involves engineering CAR cells to secrete specific transgenic cytokines upon CAR engagement with target antigens. Examples of such cytokines include IL-7 [487], IL-12 [488], IL-15 [489], IL-18 [332], IL-23 [490], and IL-33 [491]. This targeted cytokine release at the antigen site offers a two-pronged benefit: first, it can provide an auto-stimulatory effect, enhancing the activity of the CAR cells themselves. Second, it can activate other immune cell types, leading to a more robust anti-asthma response [492].

Armored CAR

Armored CAR cells are designed to co-express different proteins with the CAR, for example, antibodies or their fragments [493]. The multifaceted nature of armored CAR NK cells, with their ability to produce a wide range of cytokines, proteins, and enzymes, holds significant promise for the development of next-generation CAR NK cells with improved therapeutic efficacy [214].

Nanobody and FHVH CAR

To enhance CAR cell therapy, researchers are exploring alternative targeting domains with reduced immunogenicity and improved expression within cells. These alternatives move beyond the conventional scFv by utilizing shorter extracellular fragments. One promising approach involves single-domain variable heavy-chain antibodies derived from camelid antibodies (also known as nanobodies) [494]. Alternatively, a FHVH can be employed, minimizing the risk of an immune response against the targeting domain in patients [495].

Suicide gene

While NK cells generally exhibit a safer profile compared to T cells in cell-based therapies, additional safety measures can be crucial for emergency situations [496]. One strategy for achieving rapid termination of such therapies involves incorporating a suicide gene into the therapeutic transgene. In the context of NK cells, researchers have explored the iCasp9 safety switch for this purpose. This system utilizes a modified caspase-9 molecule fused to the human FK506 binding protein. This system allows for the selective elimination (apoptosis) of effector cells by introducing a small-molecule drug. CAR cells engineered with iCasp9 have demonstrated effectiveness in controlling cytotoxicity under unfavorable conditions [27, 75, 497].

Inducible CAR

The ability to control gene expression with precise timing and magnitude is crucial in various biological applications. One approach involves inducible expression systems, where gene expression can be triggered by specific cellular signals and transcription factors. Examples include synthetic Notch signaling [498, 499] STAT5, AP-1, and NFκB [500]. These systems offer researchers a powerful tool to manipulate gene expression in a tightly regulated manner. Alternatively, hypoxia-inducible factor-1α can be utilized to restrict gene expression to specific cellular environments with low oxygen levels [501]. This approach provides a strategy for targeted gene activation in response to distinct physiological conditions.

Switchable CAR

sCAR represents a novel approach to enhance the precision and safety of CAR cell therapy. Unlike conventional CAR-T cells that directly target antigens, sCAR cells lack this recognition domain. Instead, they rely on a specific "switch" molecule to control their activation. Two main strategies have emerged in sCAR design. The first utilizes an antibody-based switch where the sCAR recognizes the Fab fragment of an antigen-specific antibody pre-bound to the cell, offering precise control over activation and targeting [502–504]. Alternatively, an adaptor protein switch can be employed, such as zipFv, which combines a scFv with a leucine zipper fragment. This adaptor protein bridges the gap between the sCAR and the antigen-specific antibody, enabling controlled activation of sCAR cells. This innovative approach allows for a more refined control over CAR cell activity, potentially improving the safety and efficacy of CAR cell therapy [505].

Inhibitory-CAR

To address the challenge of NK cell fratricide mediated by trogocytosis, a novel strategy utilizes iCARs targeting NK-cell-specific inhibitory receptors. These iCARs essentially deliver a "don't kill me" signal to NK cells. The co-expression of antigen-targeting activating CARs alongside these self-recognizing iCARs has demonstrated efficacy in preventing trogocytosis-mediated NK fratricide and enhancing the overall activity of CAR-NK cells [454].

TCR-CAR

TCR-CAR-NK cells represent a novel approach to engineering NK cells for target recognition. Unlike conventional NK cells, which recognize targets independently of MHC-I molecules, TCR-CAR-NK cells leverage TCRs to broaden their targeting capabilities. TCRs are specialized surface molecules that enable T cells to recognize specific antigen fragments presented by MHC-I

molecules on target cells. By engineering NK cells with TCRs, researchers aim to equip them with the ability to recognize targets based on their specific surface protein expression (proteome) [214]. Studies have demonstrated the feasibility of this approach. Walseng et al. successfully transduced NK-92 cells with a TCR-CAR, enabling these engineered cells to react against MHC-I-positive target cells [506]. In essence, the integration of TCRs with CAR-NK cells offers a promising strategy for enhancing the target recognition potential of NK cells.

On and off CAR

ON and OFF-controllable CAR signaling systems utilize established drugs to regulate CAR activity precisely. Multiple strategies exist: lenalidomide can trigger CAR degradation for swift termination [507], dasatinib can induce gradual downregulation potentially mitigating T cell exhaustion [508], and researchers are exploring drug-induced proteolytic cleavage for rapid CAR inactivation [509, 510]. Drug-regulatable expression systems offer precise control over gene expression but face limitations like unintended "leakiness" in the off state, reduced expression compared to always-on systems, and a limited range between on and off states. Researchers are constantly innovating to address these challenges. A recent development, signal neutralization by an inhibitable protease platform, demonstrates significant improvements – it achieves a tight shut-off state, a wider range of expression control, and even greater potency compared to traditional, always-on systems in various pre-clinical models. This advancement highlights the ongoing efforts to refine drug-regulatable expression systems for broader applications [509].

Logic gate CAR

To improve the specificity of a targeting system, researchers are exploring the use of biological circuits with Boolean logic (Fig. 8). These circuits demand the engagement of multiple specific targets in a defined order for activation. An AND-gate design requires two or more targets for activation, achievable by separating signaling and costimulatory components into distinct entities [511].

Both receptors in the AND-gate design must bind their specific ligands to trigger a full-fledged cellular response against the target. An alternative approach to achieve AND-gate logic involves sequential detection of multiple targets, as exemplified by synthetic Notch or synthetic intramembrane proteolysis receptor systems [499, 512, 513]. These designs utilize two gene expression cassettes. The first cassette constitutively expresses a synthetic receptor containing a transcription factor. Upon ligand (target A) binding, this receptor undergoes cleavage, releasing the transcription factor. The freed transcription factor then translocates to the nucleus and drives the inducible expression of a conventional targeting system (e.g., CAR) specific for a second target (antigen B). This sequential activation strategy ensures that only when both targets are present can a full response be initiated. However, a potential limitation of AND-gate designs is the risk of off-target effects. If healthy tissue expresses the target recognized by the inducible system (e.g., CAR) and is co-localized with the primary target cells, off-target activation can still occur [498].

Researchers are exploring AND-NOT-gate logic as an alternative strategy to enhance target specificity [514, 515]. Unlike AND-gate systems, AND-NOT gates require the presence of a specific target molecule (antigen A) and the simultaneous absence of another target (antigen B) to trigger a response. This is achieved by co-expressing two distinct signaling modules: one that activates upon binding antigen A and another that inhibits upon binding antigen B. The inhibitory module effectively overrides the activation signal if antigen B is present, preventing an unwanted response. An example of this approach involves co-expressing an activation module targeting a specific molecule with an inhibition module targeting a common cellular marker (e.g., HLA-A2). This AND-NOT-gate strategy offers the potential for highly selective targeting of cells lacking the inhibitory marker, thereby increasing targeting specificity while mitigating the risk of off-target activation [515, 516].

While AND- and AND-NOT-gated systems offer promising advantages in terms of target specificity, these designs also face noteworthy challenges. First, these circuits necessitate the co-expression of multiple transgenes. The resulting increase in the genetic payload size can significantly hinder the efficiency of transgene integration within target cells [517]. Second, early attempts at constructing these computational circuits using synthetic biology, such as the synthetic Notch system, often relied on non-human components like viral transcription factors. The potential immunogenicity of these foreign components presents a significant hurdle for translating such designs into clinical applications. Finally, these systems rely on the presence or absence of multiple specific targets in a defined combination. This increased complexity raises the probability of antigen escape, as the target entity needs only to modify the expression pattern of one of the multiple targets to evade detection [518].

Achieving OR-gate logic in target recognition can be accomplished through various strategies. A straightforward approach involves the co-infusion of distinct targeting systems (e.g., CAR cell products) against multiple antigens, essentially creating a 'cocktail treatment.' Clinical trials have demonstrated the efficacy of this approach, with studies showing improved survival rates in patients receiving combined targeting of EGFR and CD133 or CD19 and CD20. However, this method increases the

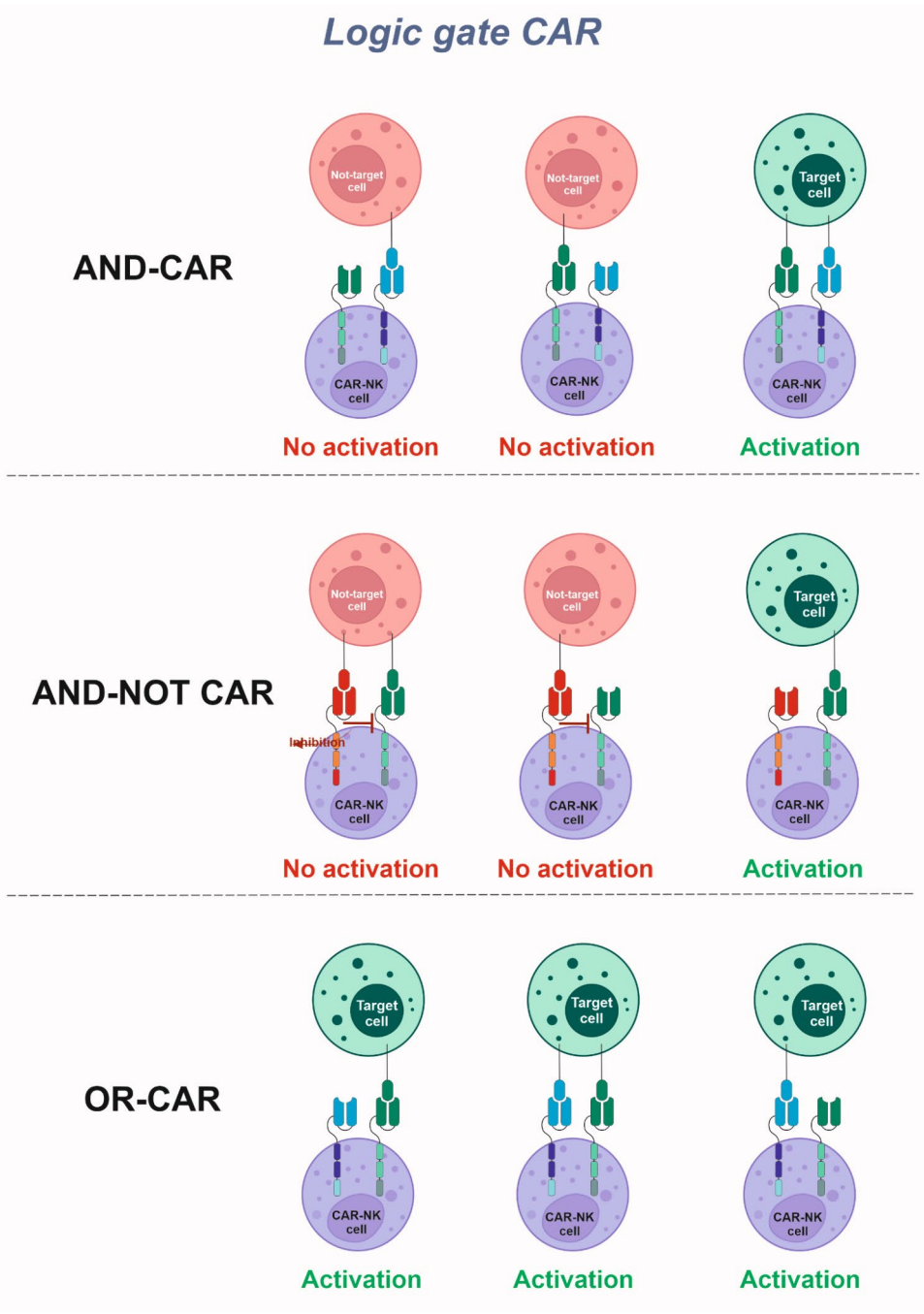

Fig. 8 Logic gate CAR: This figure explores the concept of Boolean logic circuits for enhanced target specificity in CAR-NK cell therapy. AND-gate and AND-NOT-gate designs require engagement of multiple targets for activation, offering high specificity but with challenges like complex transgene integration, potential immunogenicity, and vulnerability to antigen escape. The simpler OR-gate logic can be achieved through co-infusion of distinct CAR products or tandem targeting systems within a single construct. Overall, this figure highlights the ongoing advancements in CAR design towards achieving precise and safe targeting strategies

workload for researchers and necessitates further investigation into potential toxicity associated with administering multiple targeting agents [519, 520].

Another strategy employs tandem targeting systems, which incorporate multiple antigen recognition domains (e.g., scFvs) within a single construct. Activation can occur upon binding to any of the targeted antigens. Tandem systems targeting CD19/CD20 and CD19/CD22 have shown promising results in pre-clinical models [521, 522]. Furthermore, designs incorporating even more targets, such as CD19/CD20/CD22, have demonstrated therapeutic potential against antigen-heterogeneous

targets. However, with an increasing number of targets, safety considerations become paramount [523, 524].

A more versatile approach is exemplified by the split, universal, and programmable (SUPRA) CAR system developed by Wong et al. This system allows for the creation of various logic gates within CAR cells. The SUPRA CAR design splits the CAR into two components: a membrane-bound zipCAR and a freely circulating zipFv. The zipFv can bind to the zipCAR via complementary leucine zippers, forming a complete functional CAR. By utilizing different zipCAR/zipFv combinations, the SUPRA system can rapidly reprogram CAR cells to achieve different logic gates. For instance, the simultaneous addition of two zipFvs creates an OR-gate, enabling CAR cells to potentially target multiple antigens. However, the limited in vivo half-life of the zipFv currently restricts the broader application of the SUPRA CAR system [42, 505].

Conclusion and future perspective

While CAR-T cell therapy has shown remarkable progress, challenges such as GVHD, complex manufacturing, and the potential for severe side effects remain. CAR-NK cells offer a compelling alternative approach to address these limitations. Their "off-the-shelf" nature, potentially lower cost, and reduced risk of GVHD and CRS position them as a next-generation cellular immunotherapy.

In this review, we aimed to suggest potential strategies to enhance CAR-NK efficacy for the management of severe asthma and establish promising CAR design. NK can express IL-10, IFN-γ, perforin-granzyme, FASL, KIR, DAP, DNAM-1, TGF-β, TNF-α, CCL, NKG2A, TF, and EGFR. We discussed that for enhancing CAR-NK efficacy in asthma, we can modulate IL-10, IFN-γ, ADCC, perforin-granzyme, FASL, and KIR and decrease TGF-β, TNF-α, CCL, NKG2A, TF, and EGFR using gene-editing.

NK cells are a powerful weapon in the immune system's arsenal, wielding an array of tools to combat threats. These tools include cytokine production (such as IL-10 and IFN-γ) to modulate immune responses, ADCC to eliminate target cells, and direct target cell lysis through the perforin-granzyme system. Additionally, NK cells express FasL to trigger apoptosis in target cells, while receptors like KIRs, DAP, and DNAM-1 control their activation. NK cells also produce cytokines like TGF-β and TNF-α, influencing immune suppression and inflammation, respectively. They even release chemokines (CCL) to recruit other immune cells and express NKG2A, a receptor that can inhibit their activation. Finally, transcription factors and the EGFR further regulate their functions. By strategically modulating these diverse NK cell functions using gene editing, CAR-NK can be a promising approach to managing severe asthma.

The field of CAR cell therapy is undergoing a revolution with a focus on designing more precise and effective therapies. AI is revolutionizing CAR cell therapy by addressing key hurdles. AI can pinpoint promising targets for CAR design by analyzing massive biological datasets. It can also delve into CAR cell characteristics to optimize their function. Additionally, AI is being used to automate manufacturing, potentially making this therapy more accessible. AI can further personalize treatments by identifying genetic markers for response, and improve cell function by enhancing activation or reducing side effects. However, successful AI integration relies on robust data collection strategies to keep pace with the evolving field of CAR cell therapy.

Researchers are exploring various avenues to achieve an efficient therapy. One approach involves engineering CARs to recognize multiple targets simultaneously (bispecific CAR) or to secrete cytokines upon target engagement (TRUCK CAR). Additionally, alternative targeting domains with reduced immunogenicity (nanobodies) and safety mechanisms like suicide genes are being investigated. Furthermore, researchers are developing innovative methods for controlling CAR activity, including inducible CARs and switchable CARs. To enhance target specificity, complex logic gates mimicking Boolean operations (AND, AND-NOT, OR) are being explored. These advancements offer promising possibilities for the future of CAR cell therapy, paving the way for safer and more targeted treatments.

Abbreviations

ACT	Adoptive cellular therapy
ADCC	Antibody-dependent cellular cytotoxicity
ADCP	Antibody-dependent cellular phagocytosis
ADP	Adenosine diphosphate
AF	Aspergillus fumigatus
AHR	Airway hyper-reactivity/airway hyperresponsiveness
AI	Artificial intelligence
Akt	Ak strain transforming
AML	Acute myelogenous leukemia
APC	Antigen-presenting cell
ASM	Airway smooth muscle
BaEV	Baboon Endogenous Retrovirus
BALF	Bronchoalveolar lavage fluid
cAMP	Cyclic antimicrobial peptides
CAR	Chimeric antigen receptor
CCAR-T	Cytokine-anchored chimeric antigen receptor T
CCL	(C-C motif) ligand
CCL	CC chemokine ligand
CCR	CC chemokine receptor
COPD	Chronic obstructive pulmonary disease
CRISPR	Clustered regularly interspaced short palindromic repeats
CRS	Cytokine Release Syndrome
CTL	Cytotoxic T lymphocyte
CTLA-4	Cytotoxic T-lymphocyte-associated antigen 4
CXCL	Chemokine (C-X-C motif) ligand
DAP	Diaminopimelic acid
DC	Dendritic cell
DISC	Death-inducing signaling complex
DNA	Deoxyribonucleic acid
DNAM-1	DNAX accessory molecule-1
dnTGF-βR	Dominant-negative TGF-β receptor
EGF	Epidermal growth factor
EGFR	Epidermal growth factor receptor
EGFR	Epidermal growth factor receptor

EMPD	Extracellular membrane-proximal domain
EMT	Epithelial-mesenchymal transformation
EPX	Eosinophil peroxidase
FADD	Fas-associated death domain protein
FASL	Fas ligand
FcεR	The high-affinity IgE receptor
FDA	Food and Drug Administration
FHVH	Fully human heavy-chain-only variable domain
FLT3L	Fms-like tyrosine kinase 3 ligand FoxP3:nuclear transcription factor Forkhead box P3
GM-CSF	Granulocyte-macrophage colony-stimulating factor
GPC3	Glypican-3
GVHD	Graft versus host disease
HDM	House Dust Mite
hESC	Human embryonic stem cell
hiPSC	Human induced pluripotent stem cell
HSCs	Hematopoietic stem cells
HLA	Human leukocyte antigens
HO-1	Heme oxygenase-1
hPSC	Human pluripotent stem cell
ICANs	Cell-Associated Neurotoxicity Syndrome
iCARs	Inhibitory chimeric antigen receptors
iCasp9	Inducible caspase-9
IFN-γ	Interferon-gamma
IL	Interleukin
ILC	Innate lymphoid cell
iPSC	Induced pluripotent stem cells
ITAM	Immunoreceptor tyrosine-based activation motif
ITT	Immunoglobulin tail tyrosine
Jak/STAT	Janus kinase/signal transducers and activators of transcription
KIR	Killer-cell Immunoglobulin-like Receptor
LAG-3	Lymphocyte activation gene-3
mAb	Monoclonal antibodies
MAPK	Mitogen-activated protein kinase
MHC	Major histocompatibility complex
MICA and B	Major histocompatibility complex class I chain-A and B
mIgE	Transmembrane IgE
MIP-1	Macrophage inflammatory protein-1
ML	Machine learning
mTOR	The mammalian target of rapamycin
NCR	Natural cytotoxicity receptor
NFAT	Nuclear factor of activated T-cells
NF-κB	Nuclear factor kappa B
NK	Natural killer
NKG2D	Natural killer group 2D
NSCLC	Non-Small Cell Lung Cancer
OVA	Ovalbumin
PB	Peripheral blood
PBMC	Peripheral blood mononuclear cells
PD	Programmed cell death protein
PD-1	Death protein 1
PI3K	Phosphatidylinositol 3-kinase
PVR	Poliovirus receptor
PVRL2	Poliovirus receptor-related 2
RANTES	Regulated upon activation normal T cell expressed and secreted
SARS-CoV-2	Severe acute respiratory syndrome coronavirus 2
ScFv	Single-chain variable fragment
SEA	Severe eosinophilic asthma
SOCS-3	Suppressor of cytokine signaling 3
SUPRA-CAR	Split universal and programmable chimeric antigen receptor
T reg	Regulatory T cells
T-bet	T-box transcription factor TBX21
TCR	T-cell receptor
TEFF	Effector T cell
TF	Tissue factor
TGF-β	Transforming growth factor-beta
TGF-βR	Transforming growth factor-beta receptor
Th2	T-helper cell type 2
TIGIT	T cell immunoreceptor with immunoglobulin and ITIM domain
TKI	Tyrosine kinase inhibitor
TLR	Toll-like receptor
TNBC	Triple-negative breast cancer
TNF	Tumor necrosis factor
TNFR	Tumor necrosis factor receptor
TNF-α	Tumor necrosis factor-alpha
TRUCK	T cells Redirected for antigen-unrestricted Cytokine-initiated Killing
TSLP	Thymic stromal lymphopoietin
Tyk2	Tyrosine kinase 2
UCAR	Universal chimeric antigen receptor
UCB	Umbilical cord blood
VSV-G	Vesicular-stomatitis-virus-G protein
ZAP70-Syk	Zeta chain of T cell receptor-associated protein kinase 70-Spleen tyrosine kinase

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