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REVIEW ARTICLE

Emerging Innate Immune Cells in Cancer Immunotherapy: Promises and Challenges

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Abstract

Immune checkpoint inhibitor (ICI)-based therapy has made an unprecedented impact on survival benefit for a subset of cancer patients; however, only a subset of cancer patients is benefiting from ICI therapy if all cancer types are considered. With the advanced understanding of interactions of immune effector cell types and tumors, cell-based therapies are emerging as alternatives to patients who could not benefit from ICI therapy. Pioneering work of chimeric antigen receptor T (CAR-T) therapy for hematological malignancies has brought encouragement to a broad range of development for cellular-based cancer immunotherapy, both innate immune cell-based therapies and T-cell-based therapies. Innate immune cells are important cell types due to their rapid response, versatile function, superior safety profiles being demonstrated in early clinical development, and being able to utilize multiple allogeneic cell sources. Efforts on engineering innate immune cells and exploring their therapeutic potential are rapidly emerging. Some of the therapies, such as CD19 CAR natural killer (CAR-NK) cell-based therapy, have demonstrated comparable early efficacy with CD19 CAR-T cells. These studies underscore the significance of developing innate immune cells for cancer therapy. In this review, we focus on the current development of emerging NK cells, γδ T cells, and macrophages. We also present our views on potential challenges and perspectives to overcome these challenges.

Key Points

Innate immune cells can not only target cancer cells directly but also regulate adaptive immune responses.

Therapy with innate immune cells is an emerging area for cancer treatment due to the nature of fast response, off-the-shelf availability, and superior safety profiles.

Ex vivo expansion of immune cells to sustain their innate nature in the tumor microenvironment is a major challenge to overcome.

1 Introduction

Different from adaptive immune responses, innate immune responses are faster responses. Innate immune cells recognize target cells typically through direct ligand-receptor interactions or through pattern recognition. The activation of innate immune cells does not require prior sensitization, although a stronger response with prior sensitization may occur. Some researchers referred to this feature of innate immune cells as 'adaptive' or 'trained' immunity. Among the major innate immune cell types, NK cells, γδ T cells, and macrophages have emerged in many avenues of cancer immunotherapy. One specific aspect of innate immune cell-based therapy is a superior safety profile to T-cell-based therapy. Activation of innate cell types does not rely on self major histocompatibility (MHC) I/II expression and thus avoids graft-versus-host disease (GvHD) in MHC mismatched settings. This unique trait enables current development of allogeneic or off-the-shelf innate cell-based therapy. In this short review, we focus on discussing the current development of harnessing these cell types in therapeutic interventions.

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2 Natural Killer Cell-Based Therapy

Natural killer (NK) cells are one of the key players in innate immune defense. They can sense and eliminate diseased or abnormal cells through an array of surface hard-wired receptors, or 'tentacles'. There are generally two categories of hard-wired NK surface receptors, the activating receptors and the inhibitory receptors [1,2]. Classical NK cell activation through three major pathways within the two classes of receptors: (1) de-activation of the inhibitory pathways; (2) activating of NK cell-activating receptors by induced ligands on abnormal cells; and (3) antibody-mediated antibody-dependent cellular cytotoxicity (ADCC) through engagement of the FcγRIIIa, the CD16a receptor. Utilizing the deficiency of the KIR-MHC I inhibitory pathway is the earliest approach being explored with NK-cell-based therapy for hematological malignancies in the settings of bone marrow transplant [1–5]. Although the initial efficacy was encouraging, durable response was lacking. Data from these pioneer studies suggest that deficiency in the inhibitory pathway cannot explore the full potential of NK antitumor activity. In recent years, significant advancement has been made in understanding the NK cell-activating pathway through the discovery of NK cell activation receptors, predominantly including the c-lectin-type receptor NKG2D and the natural cell cytotoxicity receptors (NCRs) NKp46, NKp44, and NKp30. The new era of NK cell-based therapy started exploring these NK cell-activating receptors and CD16a receptor with either engineered NK cells or NK cell engagers (NKCEs). [6–9]

Non-engineered allogeneic NK cell therapy has demonstrated a superior safety profile and encouraging clinical efficacy in treating hematological malignancies, particularly in the context of using KIR mismatch allogeneic NK cells [10,11]. In the setting of bone marrow transplant to treat hematological malignancies, 'off-the-shelf' allogeneic donor NK cells have been used without inducing GVHD [2,12]. These findings have encouraged the exploration of using engineered 'off-the-shelf' cord blood-expanded allogeneic CAR-NK cells for therapy. In the proof-of-concept phase I/II trial using engineered cord blood-expanded allogeneic HLA-mismatched anti-CD19 CAR-NK cells to treat CD19-positive lymphoid tumors [13], one infusion of anti-CD19 CAR-NK resulted in a 73% (8/11 patients) objective response in the median 13.8 months of follow-up, including seven patients with a complete response. No cytokine release syndrome (CRS), neurotoxicity, hemophagocytic lymphohistiocytosis, or GVHD was present in these patients. In this study, the anti-CD19 CAR was engineered to contain genes encoding for interleukin (IL)-15 and the inducible caspase 9 for eliminating CAR-NK cells upon emergency if severe toxicity occurs. The safety profile and antitumor efficacy of the same engineered allogeneic CAR-NK were confirmed by the phase I/II trial for treating $CD19^+$ B-cell tumors [14]. These positive outcomes have inspired current efforts in 'off-the-shelf' allogeneic NK cell-based therapy for both hematological malignancies and solid tumors. There have been 725 registered clinical trials of NK cell-based therapy for cancers, among which 129 trials are using allogeneic NK cell-based therapy, including combination unmodified NK cell-based therapies, 70 CAR-NK-based therapies, and 5 modified induced pluripotent stem cell (iPSC)-based therapies sponsored by Fate Therapeutics. Eighteen of the current active allogeneic CAR-NK therapies are for advanced solid tumors (Table 1).

Engineering NK cells with T-cell receptors (TCRs) to induce antigen-specific response and avoid endogenous TCR-induced toxicity is a novel approach that is being developed by a number of investigations [15–19], but these are still in the preclinical stages. While the concept to 'dress' NK cells with T-cell capacity is novel, there are important hurdles that need to be understood before the approach can be tested in humans, such as understanding the impact of CD4 or CD8 co-receptors on sustained TCR function, and the uncoordinated function of MHCI in directing TCR function and KIR function.

CD16a-mediated ADCC is one of the critical functions of NK cells in the context of tumor-targeting antibody therapy. A number of bi- or tri-specific antibody-based NKCEs incorporating an CD16a-targeting strategy with anti-CD16a antibody or hIgG1-Fc are in early-stage clinical evaluation as a single agent or for enhancing NK cell therapy. The frontline NKCEs include AFM13 [20], GTB-3550 (known as 151533 TriKE) [21,22], IPH6101 [23], and IPH 6501 [24]. AFM13 is a bi-specific tetravalent CD30/CD16a engager that demonstrated a good safety profile but limited efficacy as a single agent in patients with relapsed or refractory classical Hodgkin lymphoma and other $CD33^+$ malignancies [25,26]. AFM13 was shown to enhance off-the-shelf cord blood-expanded NK cells (CB-NK) presimulated with IL-12/15/18 in preclinical studies [9]. Using AFM13 to enhance allogeneic NK cell therapy is currently in phase I/II evaluation in treating patients with recurrent or refractory $CD30^+$ malignancies (NCT04074746 and NCT05883449). GTB-3550 is a CD16a/IL-15/CD33 tri-specific killer engager for targeting $CD33^+$ hematological malignancies [21,22]. A phase I trial (NCT03214666) indicated that GTB-3550 single agent had a tolerable safety profile with early indication of enhanced NK cell activity in responders. This study was terminated due to a potential improved NKCE in development. IPH6101, also known as SAR443579, an NKp46/CD16a/CD123 tri-specific engager, is currently being evaluated as a single agent in a phase I/II clinical trial for the treatment of relapsed/refractory acute myeloid leukemia (AML; NCT05086315). At the American Society of Hematology (ASH) 2023 annual

Table 1 Currently active CAR-NK clinical trials for solid tumors

NCT number	Indication	Interventions	Study status
NCT04847466	GEJ cancer, advanced HNSCC	PD-L1 t-haNK	Recruiting
NCT05922930	Pancreatic cancer, ovarian cancer, adenocarcinoma	TROP2-CAR-NK	Not yet recruiting
NCT05194709	Advanced solid tumors	Anti-5T4 CAR-NK cells	Recruiting
NCT05686720	Advanced triple-negative breast cancer	SZ011 CAR-NK	Not yet recruiting
NCT03931720	Malignant tumor	BiCAR-NK/T cells (ROBO1 CAR-NK/T cells)	Unknown
NCT03940820	Solid tumor	ROBO1 CAR-NK cells	Unknown
NCT05703854	Advanced renal cell carcinoma, mesothelioma, and osteosarcoma	CAR.70/IL15-transduced CB-derived NK cells	Recruiting
NCT03415100	Solid tumors	CAR-NK cells targeting NKG2D ligands	Unknown
NCT05776355	Ovarian cancer	NKG2D CAR-NK	Recruiting
NCT05410717	Stage IV ovarian cancer, testis cancer, refractory, endometrial cancer	Claudin6 targeting CAR-NK cells	Recruiting
NCT05507593	SCLC, extensive stage	DLL3-CAR-NK cells	Recruiting
NCT03692663	Metastatic castration-resistant prostate cancer	Anti-PSMA CAR NK cell (TABP EIC)	Recruiting
NCT02839954	Multiple solid tumors	Anti-MUC1 CAR-pNK cells	Unknown
NCT05856643	Ovarian epithelial carcinoma	SZ011 CAR-NK	Not yet recruiting
NCT03692637	Epithelial ovarian cancer	Anti-mesothelin CAR-NK cells	Unknown
NCT05213195	Refractory metastatic colorectal cancer	NKG2D CAR-NK	Recruiting
NCT03941457	Pancreatic cancer	BiCAR-NK cells (ROBO1 CAR-NK cells)	Unknown
NCT05845502	Advanced hepatocellular carcinoma	SZ003 CAR-NK	Not yet recruiting

CAR-NK chimeric antigen receptor-natural killer, *GEJ* gastroesophageal junction, *HNSCC* head and neck squamous cell carcinoma, *SCLC* small cell lung cancer

meeting, it was reported that 5/15 patients achieved complete responses at a dose of 1 mg/kg weekly. The drug was well-tolerated up to 6 mg/kg. IPH6105 is the first tera-specific NKCE specific for IL-2Rβ/NKp46/CD16a/CD20 for targeting $CD20^+$ malignancies [24]. Preclinical studies showed that IPH6501 induced NK cell proliferation and accumulation at the tumor bed, as well as the control of local and disseminated tumors [24]. IPH 6501 single agent is currently in a phase I/II clinical trial in patients with relapsed/refractory B-cell non-Hodgkin lymphoma (NCT 06088654). It is perceivable that IPH6101 and IPH6501 will be used to enhance NK cell therapy in future clinical studies once a single-agent safety profile has been established.

It has long been established that activated NK cells shed CD16a to reduce the surface density of CD16a and the capacity to mediate ADCC [27–29]. The activity of a disintegrin and metalloprotease 17 (ADAM17, also known as TACE), which is constitutively expressed on the surface of NK cells, is the primary mediator for CD16a shedding [30,31]. It was shown that inhibiting ADAM17 activity with a highly selective small molecule, BMS566394, or an anti-ADAM17 monoclonal antibody, MEDI3622, could sustain surface CD16a expression on activated NK cells and enhance NK cell ADCC function [29,32]. In IL-2- or IL-15-stimulated human primary NK cells, MT6-MMP, or MMP25, also plays a role in mediating CD16a shedding [28]. The key NK cell survival and activation cytokine IL-2 was shown to increase MT6-MMP expression and translocate MT6-MMP from cytoplasmic to the cell surface upon various stimuli, such as phorbol 12-myristate 13-acetate (PMA), IL-8, and IL-1a [28]. It is noteworthy that small-interfering RNA (siRNA) inhibition of MT6-MMP expression significantly enhanced NK cell ADCC function but did not induce a significant increase in NK cell surface CD16 expression. This suggests that MT6-MMP regulating CD16 surface expression and NK cell ADCC function may intrinsically be a complex. NK cells from patients with solid tumors have lower CD16 expression and function compared with healthy controls [33]. It remains to be tested whether shedding of CD16a may be a potential mechanism in selected cancer patients who are not responsive to tumor surface antigen-targeting monoclonal antibodies, such as trastuzumab and rituximab. Noteworthy, conflicting studies suggested that CD16a shedding or downregulation is potentially an important mechanism for NK cell disengaging immune synapse to enable its ability for serial killing of tumor cell targets [34,35]. The discrepancy among different studies could be due to different sources of NK cells being used in the assays or be suggestive of a more complex underlying biology on how CD16a may dynamically direct NK cell ADCC function in a more delicate manner than our current understandings.

Various strategies are in development to circumvent activation-induced CD16a shedding to sustain NK cell ADCC function. Inhibitors to ADAM17 or ADAM25 to block CD16a shedding would be the logical mechanism-based approach; however, due to the complex roles of these enzymes in normal physiology [36], achieving NK-specific targeting can be challenging in patients. With the identification of ADAM17 target sequence in CD16a [30,31], a high affinity non-cleavable CD16a molecule, hnCD16a, was generated by substituting the serine at position 197 for a proline (S197P) [30]. Further work by Zhu et al. demonstrated that modified iPSC-NK cells expressing hnCD16a (hnCD16-iNK) conferred superior ADCC to unmodified iPSC-NK cells in vitro and in vivo. [37] It was also shown that expression of hnCD16 in iNK cells did not inhibit the detachment of iNK cells from target cells. A further direct comparison in long-term functional assays showed NK cells confer superior ADCC function with hnCD16 expressing compared with wild-type CD16 expressing [37]. In a recent study, iNK engineered with a fusion FcγR composed of the CD64 ectodomain (non-cleavable and high affinity for Fc) and the CD16a transmembrane and cytoplasmic domains exhibited sustained and robust ADCC in targeting ovarian tumors in preclinical models [38]. These studies heightened the potency of CD16 signaling in directing NK cell function and provided a preclinical proof-of-concept for engineering therapeutic NK cells with a high affinity, non-cleavable extracellular moiety to enhance CD16 signaling and thus NK cell ADCC. Noteworthy, iNK cell endogenous wild-type CD16a expression remained intact in these studies. It would be interesting to disrupt endogenous CD16a to further understand whether shedding of the endogenous CD16a would influence the engagement or disengagement of NK cells to or from target cells through the soluble CD16a competitively binding to the Fc of a therapeutic antibody.

NK cells may be effective at treating a wide variety of cancers and may be well-suited to tumors with a cold tumor microenvironment (TME) that are difficult to treat with conventional CAR-T-cell products. Promising CAR-NK cell studies have examined preclinically using immune-deficient mouse models, including studies in glioblastoma, breast cancer, pancreatic cancer and others [39–41]. Frustratingly, there has been little clinical evidence for successful NK cell-based therapy for solid tumor trials to date. These discrepancies in preclinical immune-deficient mouse models and cancer patients suggest that a less immune hostile or 'immune primed' human TME may be critical for NK cell therapy to be effective. Thus, combinatory therapies to prime the TME may be necessary to achieve the full potential of adoptive NK transfer. However, modeling the human TME for combination NK therapy using murine systems is challenging due to inherent differences between human and murine NKs and between the tumors of these species. Studies in a preclinical model system that can resemble human tumor TME would be critical for truly evaluating the approaches of NK cell-based therapy before moving to the clinic.

3 γδ T-Cell-Based Therapy

Arising from the same common multipotent double-negative precursor as the αβ T cells and being differentiated early in the thymus, γδ T cells comprise a heterogeneous group of cells that are considered to be in the interface of innate and adoptive immune responses. The distinctive γδ TCR composed by a γ-chain and a δ-chain defines the characteristics of γδ T cells. Different from the αβ T cells, γδ T cells can be rapidly activated through TCR engagement independent of the MHC complex by directing engaging TCR to an array of specific target molecules on stressed or abnormal cells [42,43]. Similar to NK cells, γδ T cells can respond rapidly to abnormal tissue-stress, such as infection and cancer [44–46]. γδ T cells can sense the stressed or cancer cells based on their damage-associated molecular patterns (DAMPs) [47,48]. These unique features have attracted the potential of using autologous and allogeneic γδ T cells for cancer immune therapy. The MHC complex independent activation suggests that allogeneic γδ T-cell adoptive therapy is less likely to induce GVHD [49], unlike the classic αβ T-cell-based therapy.

γδ T cells can play a critical role in tumor control. The high frequency of γδ T-cell infiltration correlates with better clinical outcomes across many human cancer types [50–55]. Mice that are deficienct of γδ T cells ($TCR\delta^{-/-}$) are more susceptible to aggressive tumor development than their wild-type counterparts [56–58]. γδ T cells are attractive effector cells for cancer immunotherapy due to their MHC-unrestricted antigen recognition and lack of dependence on cancer neoantigens [58]. There are two major subsets of γδ T cells in humans that have been better studied and are thus being explored for cancer therapy, the Vδ1 and Vδ2 subsets. The Vδ1 subset was predominantly distributed in the gut and epithelium, including epithelial-originated tumors [55,59,60], whereas the Vγ9Vδ2 subset was predominantly distributed in the circulating peripheral blood lymphocytes, compositing 90–95% of circulating γδ T cells and 1–10% of circulating lymphocytes in health individuals [59]. The Vδ2 subset has been more extensively developed for cancer therapy than the Vδ1 subset, possibly due to the easy access, well-established culturing conditions, and a better understanding of its activation.

The Vδ2 chain almost exclusively pairs with Vγ9, recognizing butyrophilin (BTN)-bound phosphoantigens (pAgs) [61–64]. The synthetic pAg analogs, mainly bromohydrin pyrophosphate (BrHPP) and 2-methyl-3-butenyl-1-pyrophosphate (2M3B1PP), have been used alone or in

combination with IL-2 to activate Vγ9Vδ2 T cells in situ or during ex vivo expansion [65–70]. IL-21 was shown to increase γδ T-cell cytotoxicity [71–73]; however, the addition of IL-21 to the culture limits the efficacy of ex vivo expansion due to the induction activation of the TIM-3 signaling pathways [74]. Autologous or allogeneic Vγ9Vδ2 T cells being ex vivo expanded and activated with ABP drugs or synthetic pAgs have been tested in the clinic. The safety profile is acceptable; however, the efficacy is limited. Many lymphoid leukemia cells are resistant to fully activated Vγ9Vδ2 T cells [75,76]. While direct administration of activators for Vγ9Vδ2 T cells to patients generated 10–33% objective response in clinical trials [70,77], administration of ex vivo activated for Vγ9Vδ2 T cells did not generate any objective response. [70,77]

The Vδ1 subset of γδ T cells are generally considered to be tissue-resident, supported by recent data confirming expression of tissue retention/homing markers and distinct TCR clones by Vδ1 T cells in human liver [78]. The tissue-resident Vδ1 subset in livers was shown to be more cytotoxic. Although Vγ9Vδ2 can be programmed during ex vivo expansion for tissue homing with aminobisphosphonate zoledronic acid (ZOL), it's functional capacity was shown to be predominantly interferon (IFN)-γ-producing in the tissue rather than cytotoxicity. [79]

Harnessing Vδ1 T cells for cancer immunotherapy only recently emerged due to the high toxic potential and tissue 'resident' or 'homing' nature for epithelial tumors. An NKp46-expressing human gut-resident intraepithelial Vδ1 T-cell subpopulation exhibits high antitumor activity against colorectal cancer (CRC). Higher frequencies of $NKp46^{+}$/Vδ1 intraepithelial lymphocytes (IELs) in tumor-free specimens from CRC patients correlate with a lower risk of developing metastatic stage III/IV disease [80]. Vδ1 T cells can be selectively induced to express NKp30, NKp44 and NKp46 through a process that requires functional phosphatidylinositol 3-kinase (PI-3K)/AKT signaling on stimulation with γ(c) cytokines and TCR agonists. It was shown that the TCR stimulation *in vitro* induces a *de novo* expression of natural cytotoxic receptors (NCRs; mainly NKp30) on circulating Vδ1 T cells, thus remarkably increasing their antitumor effect [81].The stable expression of NCRs was associated with high levels of granzyme B and enhanced cytotoxicity against lymphoid leukemia cells. Specific gain-of-function and loss-of-function experiments demonstrated that NKp30 makes the most important contribution to TCR-independent leukemia cell recognition. It was suggested that $NKp30^{+}$ Vδ1 T cells constitute a novel, inducible, and specialized killer lymphocyte population and a high potential for immunotherapy of human cancer. [81]

Among all the strategies, harnessing the NKG2D/NKG2D ligand pathways is under exploration for enhancing γδ T-cell therapy. The NKG2D receptor is expressed constitutively by both the Vδ1 and Vδ2 subsets of γδ T cells. The ligands, composed of the MHC I chain-related family molecule A and B (MICA and MICB) and the family of UL-16 binding proteins (ULBPs) are restricted to cancerous or pathogenic tissues. It was shown that engagement of NKG2D ligands alone can activate both the NKG2D pathway and TCRs of Vδ1 and Vδ2 [46,82,83]. The mechanism under the due activation is not clear; however, the strategy is emerging in current engineering of γδ T cells. Among 11 registered clinical trials with γδ T cells, two of the three engineered γδ T-cell therapies were NKG2D ligands targeting γδ CAR-T cells (Table 2).

The expansion protocol of Vδ2 has been well-established with the ex vivo engagement of pAgs. However, due to the nature or inherent biodistribution of these cell types, classically in the circulation, not tissue-resident, tissue homing to solid tumors needs to be better understood before the therapy can be effective for solid tumors. Irrespective of hematological malignancies or solid tumors, overstimulation during ex vivo stimulation to induce terminal exhaustion should be considered, which may largely account for lack of durable response in clinics. In addition to exhaustion, ex vivo overstimulation during expansion may also have an 'educational' effect to push these cells into an 'anergic' insensitive state as a self-regulatory mechanism for energy preservation. All these could impact in vivo effector function of these 'pre-activated' Vδ2 cell types. The fundamental biology needs to be better understood before an effective and durable therapeutic platform can be developed for using the Vδ2 subset. As for using the Vδ1 subset for cell-based therapy, considering its tissue-resident nature, efficient ex vivo expansion in suspension culture to maintain its tissue-homing ability and high cytotoxic potential is a challenge. Strategies that can reactivate or potentiate endogenous tissue-resident Vδ1 T cells would have a high viability in the near term.

4 Macrophage-Based Therapy

Macrophages are the essential components of solid TME. When monocytes were recruited to tumors, they further differentiated into macrophages in response to inflammatory cues in the TME. Tumor-associated macrophages (TAMs) possess high functional plasticity in response to tumor environment cues or external stimuli. Within progressive TMEs, TAMs are highly immunosuppressive through secreting cytokines to remodel or reactivate tumor stromal components, facilitating tumor cell proliferation and metastasis, remodeling angiogenesis, and cultivating an immunosuppressive or deprived TME [84–86]. When appropriately stimulated, TAMs can repolarize into immune-activating macrophages to orchestrate antitumor responses through phagocytosis to directly kill tumor cells, presenting antigens

Table 2 Registered γδ T-cell-based clinical trials

NCT number	Indications	Interventions	Phase	Study status
NCT04107142	Multiple solid tumors	NKG2DL-targeting CAR-γδ T cells	I	Unknown
NCT05001451	MRD-positive AML	GDX012 (ex vivo expanded γδ T cell)	I	Terminated
NCT03533816	AML, CML, ALL, MDS	Ex vivo activated and expanded allogenic γδ T-cell infusion	I	Recruiting
NCT05628545	Advanced hepatocellular carcinoma	GDKM-100 allogenic γδ T-cell infusion	I/II	Withdrawn
NCT03790072	AML	Ex vivo expanded γδ T cell	I	Completed
NCT04735471	Lymphoma	CD20-allogenic γδ CAR-T cell	I	Recruiting
NCT05015426	AML	Artificial APC-expanded donor gd T cell	I	Recruiting
NCT05886491	Leukemia	GDX012 (allogenic Vδ1 γδ cell)	I/II	Recruiting
NCT05400603	Refractory relapsed neuroblastoma	Ex vivo expanded allogeneic γδ T cells with anti-GD2	I	Recruiting
NCT05302037	Refractory relapsed cancer	Allogeneic NKG2DL-targeting γδ CAR-T cells (CTM-N2D)	I	Not yet recruiting
NCT04165941	Brain tumor adult	Modified drug-resistant γδ T cells	I	Recruiting

MRD minimal residual disease, *AML* acute myeloid leukemia, *CML* chronic myeloid leukemia, *ALL* acute lymphoblastic leukemia, *MDS* myelodysplastic syndrome

to CD8 T cells, or secreting cytokines/chemokines for NK and CD8T cell recruitment [87–89]. This functional plasticity trait of macrophages is currently being explored for therapy. [90,91]

Three major approaches are currently being explored for macrophage-based therapies: (1) inhibitors to block monocytes or TAM recruitment to tumors, or to block the suppressive function of TAM, such as BAX69 to target macrophage migration inhibitory factor (MIF; NCT02448810, NCT02540356, NCT01765790, NCT03918655) [66,92,93]; (2) in situ reprogramming of TAMs with specific stimuli [94,95]; (3) ex vivo engineering macrophages [96–99]. There are over 100 registered phase I/II clinical trials targeting macrophages through *in situ* reprogramming in solid tumors [84,90]. Among 10 completed trials, all were reported to be safe, but none reached objective responses [100–103]. It is apparent that targeting macrophages in solid tumors has a good safety profile; however, due to the high functional plasticity of macrophages, it would be critical to understand what pathways may drive or 're-mode' macrophages to a *de novo* functional phenotype to co-evolve with tumors. While, conceptually, this is probable and feasible, effective reshaping of macrophages to a sustained antitumor phenotype could be a challenging and windy road. One of the major pitfalls in current preclinical studies is that polarization or programming of macrophages is based on the nature of cytokine-induced inflammatory macrophage polarization to the general M1 phenotype or alternatively activated M2 phenotype in ex vivo settings, without consideration of the complexity of TME or the potential complexity of the TAM functional subtypes in response to specific TME cues [84,104]. Ideally, TAMs in each TME of a particular disease should be fully characterized before a therapeutic intervention is tested.

Using engineered CAR-macrophage (CAR-M) to target solid tumors is still in its infancy but is emerging with new CAR-engineering technologies and iPSC-engineered off-the-shelf CAR-M platform technologies [105–108]. To date, there are only limited active phase I clinical trials with CAR-M (Table 3). Among the registered trials, anti-HER2 mRNA-based CAR-M (CT-0508, NCT04660929) and anti-mesothelin mRNA CAR-PBMC (108 MCY-M11, NCT03608618) were both shown to be safe [109,110]; however, in both studies, the best overall response was stable disease. While these safety outcomes are encouraging, the limited overall efficacy merits further efforts in research and development.

5 Perspectives and Challenges

Innate immune cell-based therapy holds great promise. It can overcome the limitations of T-cell-based therapy: (1) can provide a better safety profile due to MHC-I-independent activation; and (2) can use off-the-shelf product due to the lack of GVHD. However, innate immune cell therapy has its own challenges with many questions to be addressed. One critical question is the persistence of innate immune cells in TME. CAR-T cells can persist in patients from months to years [111,112]. How to enhance innate immune cell persistence in patients and to sustain their effector functions is the foremost challenge. The second challenging aspect is the unstandardized manufacturing process and source of cells, both of which can significantly impact clinical efficacy. The lack of standardization can bring challenges to

Table 3 Registered CAR-macrophage and related trials, to date

NCT number	CAR-target, platform	Source of cells	Indications	Status
NCT04660929	HER2, mRNA CAR (CT-0508)	CAR-M	HER2 overexpressing solid tumors	Phase I/II, outcome reported
NCT03608618	Mesothelin Ab	CAR-PBMC	Advanced ovarian cancer and peritoneal mesothelioma	Phase I, terminated, results reported
NCT05007379	HER2, Ab	CAR-M	Breast cancer	Ex vivo organoids, not yet recruiting
NCT05969041	TROP2, mRNA CAR (MT-302)	CAR-myeloid	Adult advanced or metastatic epithelial tumors	Phase I, recruiting
NCT05138458	CD5, mRNA CAR (MT-101)	CAR-monocytes	CD5+ relapsed/refractory cutaneous T-cell lymphoma	Phase I, suspended, no results posted

HER2 human epidermal growth factor receptor, *CAR* chimeric antigen receptor, *CAR-M* CAR-macrophage, *mRNA* messenger RNA, *CAR-PBMC* CAR peripheral blood mononuclear cell

clinical practice. The third challenge is the functional plasticity of innate immune cells, mostly represented by macrophages and NK cells, both of which can self-reprogram to co-evolve with tumor cells in response to tissue environment cues [113]. Thus, to achieve therapeutic success, it is important to gain a better understanding of how these cells can be rewired in tissues or ex vivo to their fitness, metabolically or epigenetically, to sustain their functional vitality in the hostile TME.

The ability of innate cell types to directly and rapidly kill tumor cells, and their critical roles in sustaining adaptive immune responses, underscore the importance of harnessing these cell types in cancer treatment. With the new technology of single-cell multiomics and machine learning to process the large database of existing patient samples, there will be a rapid advancement in understanding how these innate immune cell types are reprogrammed in the complex TME. Using iPSC as the cell source may facilitate standardization in the manufacturing process once the concept is proven in clinical studies. These advanced scientific knowledge and technologies will shed light on how to overcome current challenges by properly reprograming each of these innate cell types through engineering, manufacturing, or combinatory therapies.

Declarations

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Author's Contributions Jennifer Wu formulated the concept, wrote the manuscript, and prepared all the table illustrations in this manuscript.

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