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Harnessing novel strategies and cell types to overcome immune tolerance during adoptive cell therapy in cancer

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ABSTRACT

Cell therapy encompasses an expanding spectrum of cell-based regimes for the treatment of human ailments, such as the use of immune cells, in particular T cells, for combating tumors and the modulation of inflammatory immune responses. In this review, we focus on cell therapy in the immuno-oncology space, which is largely driven by interests and demands from the clinics for better solutions to target various hard-to-treat cancers. We discuss recent advances in various types of cell therapies, including T cell receptor-T cells, chimeric antigen receptor (CAR)-T cells, tumor-infiltrating lymphocytes and natural killer cells. Particularly, the present review focuses on the strategies to improve therapeutic responses by either enhancing tumor recognition or the resilience of infused immune cells within tumor microenvironment. Finally, we discuss the potential of other innate or innate-like immune cell types currently being explored as promising CAR-cell alternatives that seek to address the limitations of conventional adoptive cell therapies.

INTRODUCTION

The roots of modern immune cell therapy date back more than 40 years when lymphokine-activated killer cells were first generated and subsequently infused into patients harboring various types of metastatic cancers, demonstrating sustainable objective responses.[1] Along with issues of inadequate efficacies being often reported, there are also concerns about its toxicity and the difficulty of obtaining the required cell numbers for transplantation that further drove the development of alternative adoptive cell transfers (ACTs) modalities.[2] Although immune cells can be expanded with considerably high potency in mounting anti-tumor responses, the success of ACT is heavily dependent on critical prerequisites such as a favorable tumor microenvironment (TME) and sufficient tumor immunogenicity. This is why the success of various cell therapies is largely limited to hematological malignancies.[3]

Often in solid tumors, the frailty of immune cells is a result of the harsh TME, which is populated by multiple types of immune-suppressive factors, and the intense competition for nutrients among highly metabolic tumor cells, cancer-associated fibroblasts and inflammatory immune cells.[4] As a result, advancements in conferring cytoprotection and intrinsic resilience to cell therapy products are constantly being developed to overcome such obstacles (figure 1). However, these novel modalities to modify and generate superior immune cells are also hindered by the limited availability of good manufacturing practice (GMP) grade reagents, and the cost-effectiveness of product manufacturing.[5] In contrast to hematological cancers, solid tumors often have high antigenic heterogeneity and lack tumor-specific antigens (TSAs) suitable for targeted-chimeric antigen receptor (CAR) design.[4] Therefore, as seen with combination therapies, means of enhancing anti-tumor responses by epitope spreading are often necessary. Inducing immunogenic cell death, the release of tumor-associated antigens (TAAs) could further boost endogenous immune responses alongside the infused killer cells.[5] In this review, we will not cover in details the description of various cell therapy approaches but rather, focus on highlighting several key strategies to improve existing immune cell therapy by either widening the spectrum of tumor targets or conferring resilience properties to immune cells. Of interest, the emerging potential of alternative immune cell types with intrinsic innate properties is also discussed to overcome existing obstacles in cancer immunotherapy. Other aspects beyond the scope of this review such as treatment-associated toxicities and clinical achievements are briefly mentioned.

GENETICALLY ENGINEERED T CELL RECEPTORS (TCRS) FOR ENHANCED TUMOR RECOGNITION

T cell-mediated anti-tumor immunity is driven by tumor antigen-specific T cells with high avidity that are sufficiently activated for their effector functions. However, such T cells often experience anergy and could be deleted

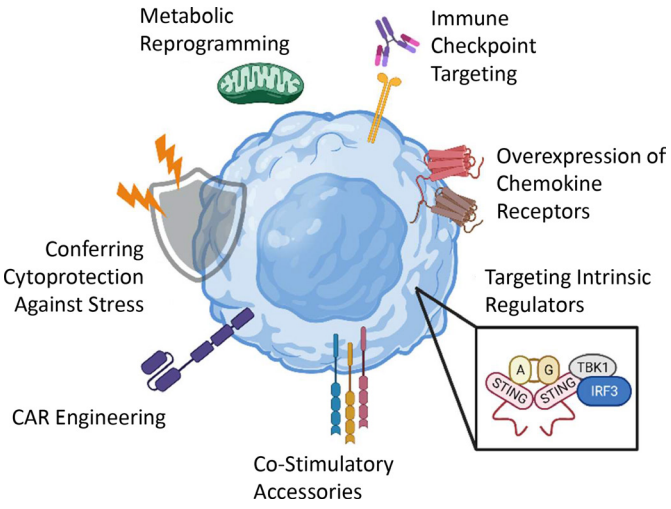

Figure 1 Schematic illustration of modalities to confer both intrinsic and extrinsic resistance to circumvent obstacles in solid tumor treatments. Examples of extrinsic optimization includes inclusion of additional stimulatory domains, CAR transduction, antibody targeting of checkpoints. For intrinsic modulations, genetic and chemical modulations to achieve metabolic reprogramming, downregulation of immune checkpoints and alterations to various cellular signaling pathways are possible (illustrated with Biorender). CAR, chimeric antigen receptor.

during immune tolerance.[6] From the perspective of cell therapy, high-avidity tumor-infiltrating lymphocyte (TIL) could be isolated and selected from the patient's resected tumor for subsequent expansion and autologous infusion. However, such manufacturing processes are often ineffective and particularly infeasible for the treatment of fast-growing tumors.[7] With advances in sequencing platforms driving the identification of TAAs and TSAs, the characterization and cloning of specific TCRs could enable the bulk editing and manufacturing of transgenic T cells with high avidity for effective autologous tumor recognition and targeting.[8]

Selection of antigen for TCR targeting

Ideally, a TCR should be designed to target a 'clean' antigen that is only expressed by tumor tissues. Such neo-antigens usually arise from somatic mutations within the tumor and are ideally able to induce T cell-mediated immune responses on recognition. However, these neo-antigen expressions could be highly variable among patient populations.[9] Hence, commercially feasible tumor targets are still the aberrantly expressed TAAs which could still be expressed on normal tissues. A classic example is New York esophageal squamous cell carcinoma 1 (NY-ESO-1) that is expressed on both melanoma and synovial sarcoma. Relatively high rates of objective clinical responses were achieved in clinical trials for patients with metastatic disease or refractory to standard therapies.[10 11] Given the present lack of druggable TAAs across various cancer types, Cobbold *et al* screened for a group of tumor-associated phosphopeptides, of which 61 out of 95 were specific to the tumor. Interestingly, it was found that many healthy individuals have immunological memory to mount anti-tumor responses against leukemia cells even though such immunity could decline in patients with cancer.[12] The search for novel tumor peptides derived from unique oncogenic posttranslational modifications may indeed be a promising approach ahead but still, TCR-based therapies would similarly encounter issues of immune tolerance in the treatment of cancer.

Mixed chimeric TCRs impede adequate anti-tumoral efficacy

Another major limitation is the presence of endogenous TCRs that could interact with the introduced exogenous construct, limiting the assembly of the anticipated tumor-specific TCRs for adequate anti-tumoral efficacy. Furthermore, there is a high likelihood of generating T cells with chimeric TCR dimers of unexpected new reactivities. These TCRs could engage normal human cells in an HLA-restricted manner and given that TCRs of unknown specificities are not screened for tolerance, there would be a high risk of unpredictable graft-versus-host disease (GVHD) reactions in the recipient hosts. A study in 2010 reported that the transfer of TCR-transduced T cells into wild-type mice resulted in adverse effects such as cachexia and marked bone marrow failure in which the underlying pathophysiology appeared to be IFNγ-dependent.[13] By incorporating an extra disulfide bond between the constant domains of the exogenous TCR, it was demonstrated that 'neo-reactivity' and its associated off-target toxicity could be reduced.[14] A more effective solution to this issue would be the use of genome editing technology such as clustered regularly interspaced short palindromic repeats (CRISPR) or Transcription activator-like effector nucleases (TALENs) to ablate the expression of endogenous TCRs together with the incorporation of tumor-specific TCRs. Using zinc-finger nucleases, Provasi *et al* generated TCR-edited central memory T cells of high avidity that were demonstrated to be well-tolerated in vivo.[7] Likewise, while transduction of viral-based vectors has been the mainstay method for engineering genetically modified T cells, the use of viral-free CRISPR approach is able to simultaneously ablate the expression of endogenous TCR without compromising the viability and functions of the T cells.[15]

Within the highly suppressive TME, TCR-engineered T cells are still prone to regulation by immune checkpoints such as PD-1-mediated immune exhaustion.[16] Additionally, since TCR-mediated targeting remains MHC restricted, another acquired form of tumor resistance could be the downregulation of MHC molecules and the silencing of genes of the antigen presentation machineries.[17] Considering the tumor clonal heterogeneity by introducing a transgenic TCR with a single specificity would be unlikely to mount durable T cell immune responses. Particularly, in the treatment of cancer types with high mutational burden, there is a stronger need for the use of alternative immunotherapies that could harness a broader TCR repertoire.

ADVANCING CAR-T CELL DEVELOPMENTS FOR CANCER TREATMENT

Early efforts aimed at widening the spectrum of anti-tumor specificity of cytotoxic T cells led the development of CAR-T cells which can employ both the humoral and cellular arms of the immune system. In this approach, cytotoxic T cells are endowed with antibody-like specificity to recognize antigens on target cells in the absence of MHC determinants.[18 19] The classic example of CAR would be Kymriah which is designed as a chimeric combination of anti-CD19 scFV fused to TCR-zeta and 4-1BB signaling domains.[20] Despite the remarkable efficacy and durable clinical responses in patients with B cell malignancies, CAR-T therapy similarly faces challenges including life-threatening adverse effects, poor persistence and exhaustion of CAR-T cells, and tumor cell resistance due to antigen escape and modification. Approximately, 77%–93% of patients with leukemia and 37%–93% of patients with lymphoma receiving CAR-T cell therapy experienced cytokine release syndrome which is a systemic inflammatory response that could result in life-threatening clinical manifestations.[20 21] A recent phase-1/2 trial (ZUMA-3) from 2021 reported tolerable safety with anti-CD19 CAR T-cell therapy with a revised clinical management plan for the usage of steroids and Tocilizumab to mitigate the risks of adverse toxicities.[22] Several other strategies have been exploited to limit on-target off-tumor effects. For instance, the antigen-binding affinity of the scFv can be reduced to spare the normal cells with lower antigen expression levels.[23] Additionally, the use of an inducible caspase-9 suicide gene system could be a 'safety switch' to limit CAR-T cells' on-target, off-tumor toxicities.[24] Engineering T cells with two CARs could selectively eradicate dual antigen-expressing tumor cells. A noteworthy example would be the use of anti-CD19/CD22 dual CAR-targeting T cells in patients with B cell Acute Lymphoblastic Leukemia.Although the efficacy was not proven to be more superior than existing CD19 CAR T-cell therapy, its recent phase I trial reported favorable safety profile.[25]

Conferring resilient properties to CAR-T cells

One would expect the engagement of CAR to trigger strong cellular downstream activation signals and render T cells resistant to immune suppressive factors within the TME. However, patients could still experience relapse due to poor CAR-T cell persistence and immune exhaustion. The recent development and design of CAR constructs could bear additional gene-encoding proteins that bestow CAR-T cells survival or cytotoxicity advantages, such as proinflammatory cytokine IL-12,[26] or the immunomodulatory molecules CD40L and 4-1BBL.[27 28] Conversely, constant antigen stimulation could drive the exhaustion of CAR-T cells, leading to defective proliferation and effector functions. To overcome this, T cell-intrinsic pathways are targeted to overcome CAR-T cell exhaustion. The blockade of PD-1 or TGFβR using antibodies or removal of negative signaling modulators for T cell activation such as Cbl-b using CRISPR-Cas9 can effectively reverse exhaustion and restore effector functions of CAR-T cells. Another strategy to prevent CAR-T cell exhaustion is to restrict CAR–antigen interactions with the use of inducible degrading or 'switching-on' CAR expression.[29 30] Similarly, uncoupling the antigen-recognition domain from the CAR activation domain can also be employed to avoid persistent antigen stimulation, thus reducing the risk of CAR-T cell exhaustion.[31] The inherent phenotype of the starting T cells is also a critical attribute in determining the subsequent clinical activity of CAR-T cells. Having T cells with specific phenotypes, such as central memory or stem cell-like memory T cells, or skewing CAR-T cell production towards these memory cell phenotypes might improve the chance of manufacturing successful CAR-T cell products.[32 33]

Expanding the tumor-targeting spectrum and off-the-shelf capabilities of CAR-T cells

Considering that the selection pressure of CAR-T cell surveillance can cause the emergence of low antigen-expressing or even antigen-negative tumor, resistance against CAR-T cell therapy due to tumor antigen escape could be acquired.[34] A common strategy to overcome tumor antigen escape is to target multiple antigens simultaneously by employing either dual CAR constructs or tandem CARs. For solid tumors, HER2/MUC1[35] and HER2/IL13Rα2[36] dual-targeted CAR-T cells have shown superior anti-tumor responses compared with single-targeted therapy in breast cancer and glioblastoma, respectively. Additionally, CAR-T cells could be designed for targeting multi-specificity. Camelids single domains (VHH) which share high homology to human VH domains could be easily manufactured as an alternative to conventional and humanized scFV domains for multi-specific targeting. Oligoclonal CAR-Jurkat cells can be generated to express VHH constructs to target multiple epitopes of HER2, resulting in better proliferation and effector functions on activation.[37]

Interestingly, the possibility of developing off-the-shelf CAR-T cells for allogeneic cell therapy has been explored. Human herpes virus-8 utilizes K3 and K5 E3 ubiquitin ligases to impair the surface expression of MHCI and MHCII molecules of infected cells as a means of immune evasion. With the objective of minimizing host-versus-graft reactions, Wang *et al* developed retroviruses encoding for K3/K5 ligases to downregulate MHC expression in CAR-T cells which in turn inhibited targeted-cytotoxicity by allogeneic T cells in mixed lymphocyte reactions. Subsequently, the authors demonstrated in vivo that the allogeneic-transferred K3-transduced CAR-T cells were well-tolerated and persisted in humanized NSG mice.[38] The use of cytokine-induced killer (CIK) cells could also be an alternative source of immune cell for the development of CAR therapy. Despite being characterized as CD8 TEMRA (T-effector memory reactivated) cells, these CD3+ and CD56+ lymphocytes express high NKG2D and DNAM1, with natural killer (NK)-like capability to kill

tumor target without MHC restriction.[39] Moreover, the use of CIK cells could enable off-the-shelf capabilities given that minimal GVHD reactions were observed in the allogeneic transfer of CIK cells in vivo.[40] The incorporation of CAR into CIK cells has not yet been widely explored. Merker *et al* demonstrated the use of ERBB2-targeted CAR-CIK cells not only displayed promising anti-tumor activity but also led to the expansion of NK cells and NK-T cells in vivo.[41] In terms of recent clinical developments, CD19-targeted CAR-CIK cells were infused into patients with B-ALL and resulted in durable objective responses with minimal toxicities.[42] As novel tumor targets and immune suppression mechanisms continue to emerge, we can expect the growing interest of the biopharmaceutical industry to drive further advancements of CAR therapy to achieve improved anti-tumor efficacies and clinical outcomes.

ADOPTIVE TIL THERAPY FOR CANCER TREATMENT

While conventional immunotherapies such as monoclonal antibodies, immune checkpoint inhibitors and even CAR-T cells are mostly targeted therapies against known tumor markers or antigens, the adoptive transfer of a pool of tumor-reactive TILs with diverse TCR repertoires could serve as a unique form of anti-cancer treatment against highly heterogenous polyclonal tumors.[43] The adoptive transfer of tumor-reactive T cells may not be limited to TILs isolated directly from tumor fragments but could also be expanded from the tumor-draining lymph nodes. Interestingly, tumor-reactive T cells could similarly be isolated from both metastatic and non-metastatic lymph nodes in which suitable clones can be expanded for ACT therapy.[44] The application of adoptive T cell transfers may not be limited to cytotoxic CD8 T cells. Type 1 T helper cells (Th1) can produce growth factor IL-2 and inflammatory cytokines such as IFNγ, both of which could act to prime the TME for adequate responses by cytotoxic lymphocytes. Using cyclophosphamide which is a common lympho-ablative agent to facilitate the homeostatic expansion of transferred T effector cells, Th1 cell therapy resulted in beneficial anti-tumor immunity by enhancing inflammatory responses in vivo.[45 46]

Generation of superior TIL expansions for therapy

While TILs with high tumor antigen avidity are necessary for effective tumor eradication, low-avidity TILs could also contribute to the regression of tumors with high antigen abundance. Tucker *et al* found that IL-12-primed low-avidity T cells were resistant to PD-1-mediated immunosuppression for retained anti-tumor immune responses which could be a better ACT alternative as compared with high-avidity TILs that may give rise to autoimmunity and side effects after administration.[47] One of the challenges in TILs treatment is the selection of tumor-reactive T cell clones from the patient's tumor for subsequent effective expansions. A common marker used for such selection could be PD-1 which is recognized as a marker for reactive T cells. An alternative approach to combined ACT and conventional immune checkpoint therapies was proposed by Chu *et al*. They first isolated and expanded PD-1+TILs from syngeneic implanted mouse tumors and later treated them with anti-PD-1 antibody in vitro. The subsequent infusion of PD-1-antibody bound TILs was found to substantially dampen tumor progression.[48] Likewise, the intrinsic targeting of PD-1 in TILs could be achieved by incorporating stable self-delivering siRNA prior to ACT to counter immune-suppression.[49] Novel interventions integrating magnetic nanoclusters conjugated with anti-PD-1 antibody could be co-administered with ACT which allowed for homing to the target tumor site via MRI guiding.[50] Apart from immune checkpoint targeting approaches, efforts could be made to confer cytoprotection and resilience properties to prime cell therapy products to adapt to the highly suppressive TME. One of the immune-regulatory roles of TGFβ is the activation of miR-23a to repress the expression of BLIMP-1, a critical transcriptional regulator of T cell effector function. Adoptive-transferred T cells can be engineered to withstand suppression within a TGFβ-enriched TME by the direct treatment of miR-23a decoy.[51] Additionally, another study found that coating T cells with a DNA network offered cytoprotection against physical shear stress, radiation and reactive oxygen species (ROS).[52] To address the issue of poor T cell-homing into the solid tumor during cell therapy, T cells can be engineered to highly expressed relevant chemokine receptors for efficient tumor homing and infiltration. Garetto *et al* found that CCL2 is highly expressed in metastatic lymph nodes. Adoptively transferred CCR2-transduced T cells were able to migrate to metastatic lymph nodes to exert their cytotoxic functions, limiting tumor growth.[53] Given that the TLR9 agonist CpG is a common clinical agent to boost immunogenicity, a recent study shows that CpG can be repurposed to expand CD8 TILs by boosting ex vivo T–B cell interactions.[54]

Priming the TME for TIL therapy

Often, the low availability of tumor-reactive TILs is attributed to the 'cold' and poorly immunogenic TME which ought to be modulated for successful immunotherapy. By incorporating the adoptive transfers of dendritic cells (DCs), the immunogenicity of the tumor could be enhanced for better recognition, priming the microenvironment for TIL-mediated immune responses. Patients with advanced melanoma, neuroendocrine carcinoma and hepatocellular carcinoma (HCC) were shown to have prolonged survival on receiving DC-incorporated ACT.[51 55 56] Using a peptide pool of multiple HCC-TAAs, the application of generated DCs allows the priming of autologous T cells for multiple antigen specificities for multiple antigen-stimulating cellular therapies (MASCT). By priming autologous T cells isolated from peripheral blood, MASCT treatment elicited promising anti-tumor responses that are well-tolerated by patients with HCC.[57]

The use of combinatory therapies could also overcome the issue of non-responsiveness of TILs isolated from poorly oncogenic tumors. Oncolytic viruses could be designed to selectively induce immunogenic cell death and the release of TAAs and damage-associated molecular patterns. This triggers DC maturation and promotes antigen presentation.[58] An additional localized injection of an oncolytic virus enhances the fraction of tumor-reactive TILs that can be expanded for subsequent adoptive transfer.[59] Furthermore, these viruses could be custom-engineered to express RANTES and IL-15 for the homing and activation of T cells respectively. The combination treatment of the RANTES/IL-15-armed oncolytic virus together with GD2-targeted CAR T cell therapy prolonged the survival of mice-bearing neuroblastoma.[60]

Apart from soluble inflammatory factors, priming of TME can also be achieved by the induction of immunogenic cell death using pro-apoptotic agents. In the presence of a compound that disrupts mitochondrial functions to drive apoptosis of tumor cells, DCs could upregulate maturation markers such as CD86 and CD80 both in vitro and in vivo which could synergistically work with adoptive T cell therapy to dampen tumor growth.[61] The conventional chemotherapeutic agent, doxorubicin, was reported to increase the susceptibility of tumor cells to immune-mediated killing via the upregulation of the TRAIL receptor. Co-administration of doxorubicin with either an adoptive transfer of NK cells or T cells showed prominent tumor regression.[62] In addition, a localized intratumoral injection of a doxorubicin-containing hydrogel was also demonstrated to modulate the TME wherein immunogenic cell death releases tumor antigens to enhance the efficacy of subsequent ACT.[63] Given that the Wnt/β-catenin signaling pathway is associated with poor prognosis in colorectal cancer, a combinatory treatment of a b-catenin inhibitory peptide, hsBCL9$_{CT}$-24 and EpCAM-targeted CAR T cells synergistically enhanced anti-tumor immunity. Mechanistically, hsBCL9$_{CT}$-24 treatment could result in improved T cell infiltration, increased DCs and decreased regulatory T cell frequencies in the TME.[64] At the same time, hsBCL9$_{CT}$-24 treatment downregulates TGFβ expression observed in tumor tissue, promoting the differentiation and generation of memory T cells accompanied by the upregulation of critical regulators such as BLIMP-1, TCF-1 and EOMES in T cells.[65] Although TILs are seldom enhanced with heavy genetical modifications unlike TCR or CAR-T therapy, the use of adoptive TILs therapy continues to be a highly promising treatment modality for highly heterogenous cancers with the lack of suitable TAAs. Boosting immunogenicity within the TME remains as a mainstay solution to further improve the efficacy of TIL therapy.

NKS—'OFF-THE-SHELF' THERAPEUTICS FOR CANCER TREATMENT

Unlike conventional T cells, NK cell-mediated cytotoxicity does not require prior antigen exposure and is instead, orchestrated by the interplay of inhibitory and activating receptors on the cell surface.[66] From partially HLA-matched umbilical cord blood donors, hematopoietic stem and progenitor cells could be differentiated and expanded into NK cells, which were reported to be well-tolerated as a promising 'off-the-shelf' allogeneic cell therapy for patients with acute myeloid leukemia (AML).[67] Moreover, an NK lymphoma cell line, NK-92 expanded under GMP conditions and infused into patients with either solid tumors or leukemia can be tolerated with minimal side effects.[68] The use of allogeneic NK cells in ACT has been evaluated in an early clinical trial of patients with mixed tumor types in which the in vivo expansion of haplo-identical donor NK cells could be achieved together with complete remissions observed in patients with AML. However, limited responses were observed in patients with metastatic melanoma and renal cell carcinoma who eventually progressed.[69]

NK cells as an emerging alternative for CAR therapy development

Notably, the development of CAR-NK cells for enhanced target recognition is widely explored as an emerging cell therapy modality. While conventional CAR-T cell therapy is frequently associated with bouts of adverse toxicities, the use of CAR-NK promisingly showed no major adverse effects after infusion into patients. One such example would be a recent clinical trial that reported favorable objective response rates in refractory CD19-positive tumors treated with anti-CD19 CAR NK cells in an HLA-mismatched setting.[70] To enhance tumor recognition and sensitivity, the incorporation of both CD19 and BCMA CAR constructs to generate 'dual-CAR' NK cells was shown to outperform single CAR-NK cells in the eradication of myeloma cells in vitro.[71] Additionally, integration of co-stimulatory DNAM1 and 2B4 domains was demonstrated to further boost the killing capacity of GPC3-targeting CAR-NK-92 against hepatocellular cancer cells in vitro.[72] Despite the efforts of gene-editing to ensure tumor recognition, CAR-NK cell therapy has not yielded satisfactory responses in immune-tolerant tumors that may be resistant to NK cell-mediated killing.

Modulating NK cells to circumvent obstacles in solid neoplasms

Like T cells, the successes in NK cell therapy are limited to hematological cancers. Similar issues of poor NK cell infiltration and vulnerability to immune suppression in the TME are outstanding problems to be resolved for solid tumors. Efforts were made to incorporate chemokine receptors on expanded NK cells for adoptive cell therapy. NK cells could downregulate CXCR2 in the presence of tumor cells and intratumoral NK cells in renal cell carcinoma (RCC) had lower CXCR2 expression compared with peripheral blood NK cells. Given that CXCL5 which is a CXCR2 ligand that correlated with increasing infiltration of CXCR2+NK cells into RCC tumors, the study extended to generate CXCR2-transduced NK cells for

enhanced tumor-homing properties that would be critical for future development of ACT targeting RCC and even other solid tumors.[73] A similar strategy was also explored in terms of improving the migratory capacity of NK cells by overexpressing CXCR4 in CAR-NK cells. NK cells with EGFR-targeting CAR with a DNAX-activation domain were co-engineered to express CXCR4 which in turn, responded to CXCL12-secreting glioblastoma tumors and resulted in complete remission of these tumor-bearing mice.[74]

There are also efforts sought to resolve issues of immune resistance. Prior to ACT, inhibitory receptors such as NKG2A could be targeted by monoclonal antibody or silenced by shRNA for enhanced killing capacity.[75 76] PD-L1-targeted CAR-NK cells that overexpress the anti-oxidative protein, peroxiredoxin-1, were observed to be resistant to ROS for better anti-tumor responses.[77] CD73-mediated production of adenosine is another metabolic immune checkpoint known to suppress NK cell functions. Anti-CD73 CAR-NK cells designed to target glioblastoma not only remarkably dampened tumor growth but also reduced levels of intratumoral immune-suppressive adenosine.[78] CAR-NK92 cells designed to target regulatory T cells expressing CD25 could also be a promising approach even though its efficacy in vivo remains to be elucidated.[79]

An emerging field in NK cell therapy is to generate memory-like NK cells primed with superior effector functions and tissue-resilience properties prior for ACT. Similar to T and B cells, NK cells could also undergo clonal expansion and persist as memory-like cells.[80] From the adoptive cell therapy perspective, cytokines such as IL-12, IL-15 and IL-18 could confer memory-like features to NK cells and the delivery of these cells was demonstrated to have better clinical benefit in patients with AML.[81] Moreover, a recent report demonstrated the use of a GMP-compliant expansion protocol that confers NK cells' sustainable adaptive features with robust tumor-killing capacity.[82] Gang *et al* also demonstrated CAR-transduced memory-like NK cells had better anti-tumoral responses compared with conventional CAR-NK cells in the treatment of such NK-resistant lymphoma.[83] Additionally, the targeting of cytokine-inducible SH2-containing protein (CIS) which is a potent negative regulator of IL-15 signaling pathway, would also enhance NK cell functions. CIS-knock out NK cells displayed elevated glycolysis and oxidative phosphorylation together with enhanced anti-tumor responses and in vivo persistence.[84] Nevertheless, these genetic modifications together with the incorporation of CAR constructs remain challenging in NK cells. NK cells are programmed to mount intracellular anti-viral responses which to a certain extent confer resistance to viral transduction. Sutlu *et al* screened for selective inhibitors targeting these pathways and identified a TBK1 inhibitor which could be adopted for improving the efficiency of transducing NK cells for subsequent cell therapy.[85] An alternative solution that is widely explored would be the use of stem cell-derived NK cells generated ex vivo prior to ACT. With the ease of performing efficient genetical engineering of induced pluripotent stem cells (iPSCs), CAR-transfected stem cells could be differentiated into NK cells for adoptive cell therapy. The robustness of such therapeutic application of stem cells enables further development of CAR-NK cell therapy as a universal 'off-the-shelf' product.[86] With the present field heavily focused on further understanding memory-like NK cells in the context of cancer, novel insights could be elucidated to enhance in vivo persistence and tumor-killing capacities.

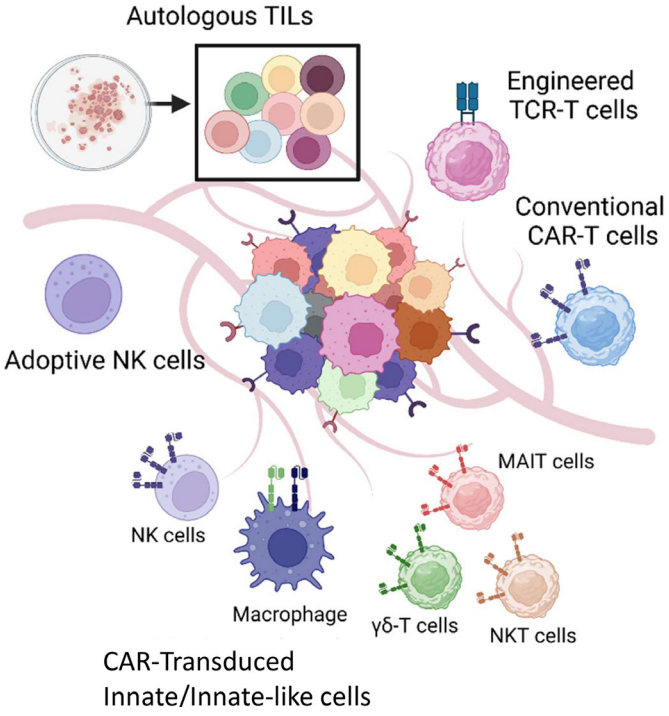

Figure 2 Schematic overview of various immune cell types used in cell therapy development. Cell therapy primarily involves conventional T cells in either in the format of TILs, engineered TCR-T cells or as CAR-transduced T cells. NK cells having considerable potency in tumor eradication is now another developed arm of immune cell therapy yielding promising clinical responses in several recent trials. Other innate-like lymphocytes which are suitable for CAR transduction for potential cell therapy includes invariant T cells encompassing the mucosal-associated invariant T (MAIT) cells, gamma delta-T cells and NK-like T cells. Last but not least, CAR macrophage is another alternative emerging CAR-myeloid cell therapy with remarkable development progress in recent years (illustrated with Biorender). CAR, chimeric antigen receptor; TCR, T cell receptor; TILs, tumor-infiltrating lymphocytes; NK, natural killer.

ENGINEERING MACROPHAGES AND OTHER INNATE-LIKE T CELLS AS ALTERNATIVE IMMUNE CELL THERAPIES

With the limitations of both T and NK cell therapies in solid tumors, other innate or innate-like immune cell types are also being considered for future CAR therapies (figure 2).

The development of the CAR-macrophage platform is another emerging cell therapy modality for its homing properties into tumor tissues and being less susceptible to immune-suppressive TME given that it does not experience similar immune exhaustion processes observed in T and NK cells.[87] Likewise, the sources of CAR macrophages could be either differentiated from monocytes of healthy peripheral blood donors or iPSCs-derived.[88] Zhang *et al* demonstrated CAR-specific anti-tumor responses elicited by iPSC-derived CAR macrophages such as enhanced phagocytosis and secretion of pro-inflammatory factors and cytokines.[89] IFNα is an inflammatory cytokine with known anti-tumor functions but the systemic administration of IFNα in the clinical setting was not satisfactory. By engineering IFNα-expressing vectors into hematopoietic stem cell-derived Tie2+ macrophages with the enhanced homing capability to the tumor metastasis in the liver, the targeted delivery of IFNα could be a promising adjuvant therapeutic strategy to complement existing treatments for metastatic colorectal cancer.[90] Furthermore, CAR macrophages were also shown to provide systemic immunity against metastatic progression. Niu *et al* developed an alternative design of CAR construct that utilizes an intact CCL19 domain for targeting CCR7+ immunosuppressive cells. They evaluated the effectiveness of several intracellular signaling domains, including those derived from TLRs and MerTK, against the standard 4-1BB/CD3ζ domain and found that CAR macrophages engineered with the MerTK domain have the most efficient phagocytic and killing efficacies while simultaneously inflaming the TME to promote T cell infiltration.[91] Another innovative study recently demonstrated the intracavity injection of CAR construct-containing nanoporter-hydrogel to generate CAR macrophages targeting glioblastoma stem cells can prevent tumor relapse.[92]

Likewise, the use of γδT cells as an alternative platform for CAR-T cell therapy is currently being explored as its effector functions are not restricted by MHC barriers, unlike conventional αβT cells. γδT cells express Vγ2Vδ2 that recognizes isopentenyl pyrophosphate (IPP) which is a phosphoantigen highly produced by cancer cells and not normal cells.[93] Moreover, γδT cells do not cause GVHD and could be safely administrated as a form of allogeneic ACT.[94] Rozenbaum *et al* demonstrated a successful rapid expansion of peripheral blood-derived γδT cells transduced with anti-CD19 CAR construct that yield effective tumor clearance in a xenograft setting. Furthermore, the observed anti-tumor effects were further enhanced when mice were treated with zoledronate.[95] Given that zoledronate was previously found to induce the accumulation of IPP,[96] zoledronate indirectly induces γδT cell activation and expansion for potential use in ACT.[97 98] Another interesting work by Capsomidis *et al* demonstrated the application of GD2-targeting CAR γδT cells with an additional capability of cross-presenting antigens to conventional αβT cells.[99]

Other types of innate-like T cells were also studied in the development of CAR-T cell therapy products. Mucosal-associated invariant T cells (MAIT cells) are primarily involved in the recognition of bacterial pyrimidines. The application of mesothelin-targeting CAR-MAIT cells in vitro showed favorable targeting of both tumor cells and immune suppressive macrophages in co-culture settings.[100] The invariant NKT cells are another type of innate-like T cells which are activated by glycolipids restricted by CD1d presentation rather than MHC restriction in conventional T cells. NKT cells have considerable ability to home to tumor sites and kill tumor-associated macrophages with CD1d expression.[101] The co-engineering of GD2-targeting CAR and IL-15 in NKT cells had substantially shown highly robust anti-tumor efficacies in neuroblastoma xenografts, driving progress into the first clinical trial application of CAR-NKT in patients.[102 103]

CONCLUDING REMARKS

The present review highlighted some of the efforts made to overcome obstacles in ACT such as low penetrance into solid tumors, poor target recognition, immune activation and persistence in the TME. While most of this translational research is largely driven by the interest of the biotech and pharmaceutical industries, future studies should also investigate more deeply the various systemic responses of the tumor-bearing host after receiving infusions of customized cell therapy products. Moreover, it should be emphasized that every patient bears a unique tumor composition, let alone responses from the dynamic immune system. Ultimately, the question lies with how effectively we could incorporate the array of recent advances and novel insights to develop tailor-made adoptive cell therapies for every individual. One could envision the future of immunotherapy particularly in adoptive cell therapy, as a personalized medicine against cancer.

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