Cancer Cell

CellPress

Commentary

Breaking the mold: Unconventional T cells in cancer therapy

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https://doi.org/10.1016/j.ccell.2024.11.010

Unconventional T cells, including invariant natural killer T (iNKT) cells, gamma delta (γδ) T cells, and mucosal-associated invariant T (MAIT) cells, play important roles in both innate and adaptive immunity. These cells respond to tumors rapidly and influence the tumor microenvironment (TME). Recent advances in understanding their biology, as well as the development of novel therapeutic approaches, have underscored their potential in cancer immunotherapy. This commentary will assess these advances and translational possibilities in the field.

Unconventional T cells represent a specialized subset of T lymphocytes, distinguished from conventional T cells by their unique T cell receptor (TCR) composition, non-classical antigen recognition mechanisms, and distinct functional properties. Prominent subtypes of unconventional T cells include invariant natural killer T (iNKT) cells, gamma delta (γδ) T cells, and mucosal-associated invariant T (MAIT) cells. These cells are characterized by their distinct TCRs, including the semi-invariant TCRs in MAIT and iNKT cells, as well as the γδ TCR in γδ T cells (Table 1). Unlike conventional αβ T cells, which primarily recognize peptide antigens presented by major histocompatibility complex (MHC) molecules, unconventional T cells utilize alternative antigen-presenting molecules. For example, iNKT cells recognize lipid antigens presented by CD1d, V delta 2 (Vδ2) γδ T cells recognize phosphoantigens presented by BTN3A1/BTN2A1, and MAIT cells recognize microbe-derived riboflavin derivatives presented by MR1 (Table 1). These cells are widely distributed across various tissues, enabling them to mount swift and flexible immune responses to a broader spectrum of pathogens and cellular stress.

Unconventional T cells are distinguished by their ability to bridge innate and adaptive immune responses. These cells mount rapid responses to infections and tumor-associated changes via both TCR signaling and the activation of natural killer receptors (NKRs). Additionally, they play a crucial role in promoting the activation of conventional T and B cells through cytokine secretion, thereby supporting long-term, antigen-specific immune responses. Several characteristics make unconventional T cells particularly promising for cancer therapy (Figure 1). These include their potent antitumor capacity, multiple tumor-targeting mechanisms, efficient infiltration into solid tumors, and ability to modulate the immunosuppressive tumor microenvironment (TME). Moreover, their reduced risk of inducing graft-versus-host disease (GvHD) enhances their suitability for allogeneic use, making them ideal candidates for off-the-shelf cellular therapies. Clinical trials have been initiated to explore the potential of chimeric antigen receptor (CAR)-engineered unconventional T cells, derived from both autologous and allogeneic sources, for cancer treatment (Table 2). These CAR-modified unconventional T cells hold significant promise for addressing the limitations of conventional CAR-T therapies, especially in solid tumors, where traditional CAR-T approaches have been less effective. In this commentary, we explore the exciting advancements in unconventional T cell-based therapies for cancer, focusing on the identification of novel biomarkers, innovative genetic engineering techniques, and cutting-edge biotechnologies to produce these cells. Additionally, we highlight promising clinical outcomes associated with unconventional T cell therapies, underscoring their potential to establish a new frontier in cancer immunotherapy.

iNKT cells: Recent advances in cancer therapy

Early strategies aimed at enhancing endogenous iNKT cell efficacy included the administration of intravenous α-galactosylceramide (αGC) and αGC-pulsed antigen-presenting cells. However, due to the limited abundance of iNKT cells in patients and the rapid depletion of αGC in humans, clinical outcomes were not promising. Despite these challenges, iNKT cells possess unique features, such as potent antitumor activity, multiple tumor-targeting mechanisms through TCR- and NKR-mediated killing, efficient solid tumor infiltration, and the ability to modulate the TME. These characteristics make them promising candidates for the development of adoptive CAR-engineered iNKT (CAR-iNKT) cell therapies. Both preclinical and clinical studies have demonstrated the potent antitumor efficacy and broad tumor-targeting capabilities of CAR-iNKT cells. These include CAR-iNKT cells targeting $CD19^+$ lymphoma and leukemia,[1] B-cell maturation antigen $(BCMA)^+$ multiple myeloma,[2] and $GD2^+$ neuroblastoma.[3,4]

Please cite this article in press as: Li et al., Breaking the mold: Unconventional T cells in cancer therapy, Cancer Cell (2024), https://doi.org/10.1016/j.ccell.2024.11.010

Table 1. Biology of human unconventional T cells

Unconventional T cell types	Subtypes	CD4/CD8 co-receptors	Recognized molecules	Recognized antigens	TCR repertoires	Percentage in human blood	Tissue distribution
NKT cells	Type I NKT (iNKT) cells	Mainly CD4 single positive and CD8 single positive, with some double-negative cells	CD1d	Lipid antigens	α chain: Vα24-Jα18 β chain: mainly Vβ11	~0.001%–1%	Mainly lymphoid tissue, bone marrow, liver, lung, and adipose tissue
	Type II NKT cells		CD1d	Lipid antigens and non-lipidic small molecules	α chain: diverse β chain: diverse	Rare	Mainly liver
γδ T cells	Vδ1 γδ T cells	Mainly double-negative, with some CD8 single-positive cells	CD1a, CD1c, CD1d, MICA, or ULBP	Stress-induced proteins, lipids, and pollen-derived phosphatidylethanolamines	γ chain: Vδ1 δ chain: diverse	~1%	Mainly epithelia, dermis, spleen, liver, and gut
	Vδ2 γδ T cells		BTN2A1/BTN3A1	Phosphoantigens	γ chain: mainly Vγ9 δ chain: Vδ2	~1%–5%	Mainly peripheral blood and lymphoid organs
	Vδ3 γδ T cells		CD1d	Unknown	γ chain: Vγ2/Vγ3 δ chain: Vδ3	Rare	Mainly liver and gut epithelium
	Vδ5 γδ T cells		EPCR	Unknown	γ chain: Vγ4 δ chain: Vδ5	Rare	Unknown
MAIT cells	N/A	Mainly CD8 single-positive or double-negative	MR1	Microbe-derived riboflavin derivatives	α chain: Vα7.2-Jα33/12/20 β chain: mainly Vβ2 and Vβ13	~1%–10%	Mainly lymphoid tissue, lung, liver, gastrointestinal tract, and female genital tract

A recent phase I clinical trial utilizing autologous GD2-targeting IL-15-enhanced CAR-iNKT cells for the treatment of pediatric patients with relapsed/refractory neuroblastoma demonstrated encouraging antitumor activity with a favorable safety profile. No dose-limiting toxicities were observed, and the objective response rate was 25% (3/12), including two partial responses and one complete response.[3] This first-in-human study highlights the promise of CAR-iNKT cells as a therapeutic platform, though further optimization of the cell products is warranted. Potential improvements include selecting donors with high $CD62L^+$ CAR-iNKT cell populations, which are associated with robust *in vivo* expansion and improved clinical outcomes, and knocking down *BTG1*, a gene known to regulate the transition of T cells from a naive to an effector state following antigenic stimulation.[3] Additionally, beyond IL-15, other immune-enhancing molecules such as IL-12 have been explored to promote long-lived, Th1-polarized CAR-iNKT cells without inducing functional exhaustion.[4]

Another unique feature of iNKT cells is that their TCR recognizes the non-polymorphic molecule CD1d, which prevents them from inducing GvHD. This feature makes iNKT cells and their CAR-engineered derivatives ideal candidates for off-the-shelf allogeneic cell therapies to treat cancer. In a recent clinical trial (NCT00840853), researchers developed allogeneic CD19-targeting CAR-iNKT cells for the treatment of patients with relapsed or refractory B cell malignancies. These CAR-iNKT cells were engineered using short hairpin RNA to knock down beta-2 microglobulin and CD74, thereby eliminating HLA-I/II expression and reducing the risk of host T cell-mediated allorejection. These engineered allogeneic CAR-iNKT cells were well tolerated and demonstrated objective responses in patients with relapsed/refractory non-Hodgkin's lymphoma and acute lymphoblastic leukemia, even at low dose levels.

In addition to PBMC-derived CAR-iNKT cell products, Li and colleagues developed allogeneic CAR-iNKT cells from gene-engineered hematopoietic stem and progenitor cells (HSPCs) using a clinically guided culture method.[2] In this preclinical study, researchers successfully generated a variety of CAR-iNKT cell products featuring different CAR constructs, targeting various antigens and utilizing either CD28 or 4-1BB as the co-stimulatory domain. These allogeneic CAR-iNKT cells demonstrated potent antitumor efficacy across multiple tumor models, including multiple myeloma, leukemia, glioblastoma, neuroblastoma, and hepatocellular carcinoma (HCC).[2] Notably, these CAR-iNKT cells also selectively depleted immunosuppressive cells within the TME, including tumor-associated macrophages (TAMs) and myeloid-derived suppressor cells (MDSCs), and countered tumor immune evasion by killing tumor cells via CAR/TCR/NKR-mediated triple targeting mechanisms.[2] These allogeneic CAR-iNKT cells demonstrated a high safety profile, as they did not induce detectable GvHD or cytokine release syndrome (CRS). Additionally, these cells maintained a stable hypoimmunogenic phenotype, making them resistant to allorejection.[2] This study represents a significant advancement in the generation of allogeneic CAR-iNKT cells with high yield and purity, providing strong support for their translational and clinical application in cancer therapy.

In the context of allogeneic cell therapy, host-mediated allorejection is a significant concern for achieving both long-term persistence of allogeneic cells and effective therapeutic efficacy. A study by Rotolo et al. demonstrated that unedited allogeneic iNKT cells can persist for over

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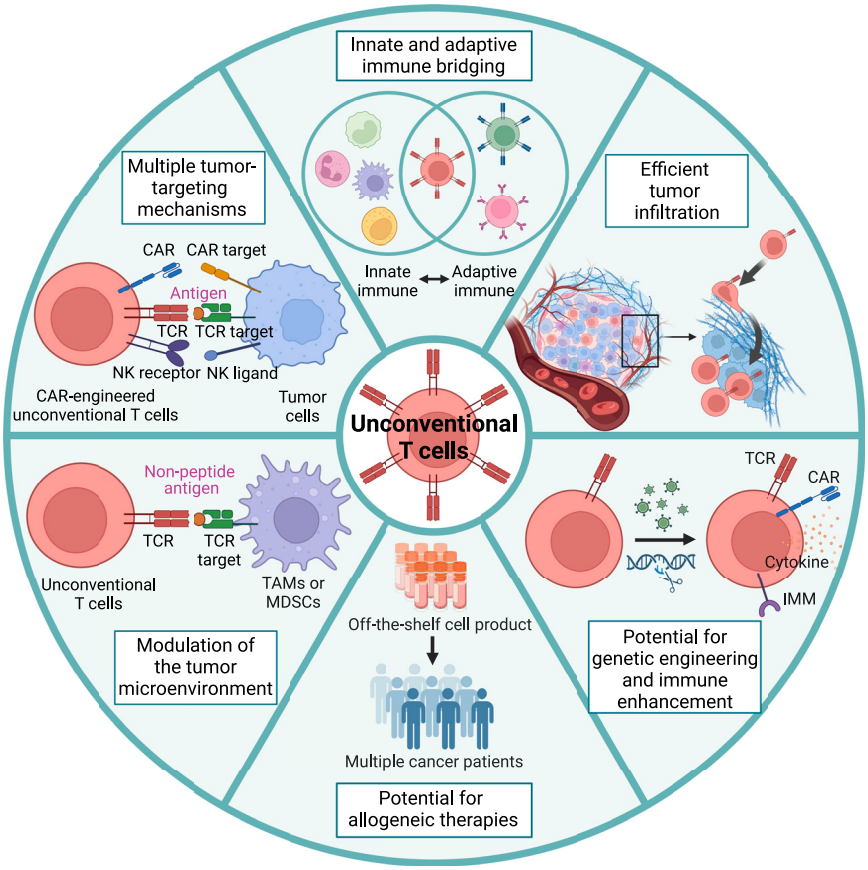

Figure 1. Unique features of unconventional T cells in cancer therapy

Unconventional T cells and their CAR-engineered derivatives exhibit several unique features that make them promising candidates for cancer therapy. These cells act as a bridge between innate and adaptive immunity, enabling them to rapidly respond to tumors while orchestrating an adaptive immune response. Their versatility lies in their ability to recognize and kill tumor cells via multiple pathways, including T cell receptors (TCRs) and natural killer (NK) receptors, allowing them to target a broad range of cancer cells. Additionally, unconventional T cells demonstrate a strong capacity for tumor infiltration, efficiently localizing to inflamed or cancerous tissues, which enhances their therapeutic potential by acting directly within the tumor microenvironment (TME). They also modulate the TME by suppressing immunosuppressive cells such as myeloid-derived suppressor cells (MDSCs) and tumor-associated macrophages (TAMs). Importantly, their resistance to graft-versus-host disease (GvHD) enables the use of allogeneic, off-the-shelf therapies. These cells can be produced in bulk and used across multiple patients without the need for individualized preparations. Furthermore, genetic engineering allows for the expression of chimeric antigen receptors (CARs), enhancing their specificity against cancer cells, and cytokines or immune-modulatory molecules (IMMs) can be introduced to further boost their antitumor activity.

78 days without the need for high-intensity preconditioning while maintaining functional activity in MHC-mismatched canine recipients.[5] This finding supports the potential application of MHC-unedited allogeneic iNKT cells, including CAR-iNKT cells, as a universal accessible platform for treating refractory conditions, such as cancer. Although the mechanisms underlying this persistence were not thoroughly investigated, the authors speculated that low-dose total body irradiation may enhance the allo-tolerizing effects of iNKT cells and facilitate the uptake of αGC-loaded liposomes, thereby promoting consistent iNKT cell activation and stable engraftment in allogeneic settings.[5] This study provides critical insights into the adoptive transfer of human allogeneic iNKT cell products, which may also possess the intrinsic resistance to host-mediated allorejection.

γδ T cells: Unlocking their potential in cancer therapy

γδ T cells have emerged as a promising candidate for cancer immunotherapy due to their intrinsic tumor-fighting capabilities and favorable safety profile in allogeneic settings. These cells are characterized by the expression of NKRs and TCRs composed of γ and δ chains. Unlike conventional αβ TCRs, γδ TCRs recognize a broad range of tumor antigens independent of MHC molecules, reducing the risk of GvHD and supporting their use in allogeneic cell therapy (Table 1). A clinical study of 174 patients undergoing allogeneic hematopoietic stem cell transplantation for hematologic malignancies demonstrated that higher levels of γδ T cells and MAIT cells correlated with lower incidences of acute GvHD, suggesting that these cell populations may confer protective effects against GvHD development.[6]

γδ T cell therapy primarily utilizes two subtypes of γδ T cells: V delta 1 (Vδ1) and Vδ2. These subtypes each have unique tissue distributions and TCR ligands (Table 1). Vδ1T cells are predominately found in epithelial and mucosal tissues and express a TCR composed of the Vδ1 chain and one of the potential six V gamma (Vγ) chains (Vγ2, 3, 4, 5, 8, or 9). These Vδ1 TCRs can recognize stress-inducing ligands such as MHC-I-chain-related genes A and B, cluster of differentiation 1 (CD1a-d), MR1, and heat shock proteins. However, many ligands remain unidentified. Vδ2T cells, which reside in circulation, primarily express the Vγ9Vδ2 TCR. This feature allows them to respond to elevated levels of intracellular phosphoantigens within malignant cells that have dysregulations in the mevalonate pathway.

γδ T cell-based cancer therapies typically consist of two treatment strategies: *in situ* activation of endogenous γδ T cells and the adoptive transfer of *ex vivo* expanded γδ T cells with and without engineering or combination therapy. However, these therapies have only shown mild efficacy and often yield partial responses in cancer patients. This limited clinical success can be attributed to factors such as donor-to-donor variability, short-lived persistence, and potential tumor antigen escape. Next-generation γδ T cell immunotherapies are currently being developed to address these issues and have enhanced anti-tumor functionality. For example, Lee et al. discovered that Vγ9Vδ2T cells with high CD16 expression have enhanced cytotoxicity and identified CD16 as a marker to screen donors with highly functional Vγ9Vδ2T cells.[7] They further enhanced these cells to express a mesothelin-targeting CAR and to constitutively

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Table 2. Clinical trials utilizing allogeneic or autologous CAR-engineered unconventional T cells in cancer treatment

Unconventional T cell types	Allogeneic or autologous	CAR-engineered cell products	Cancer types	ClinicalTrials.gov identifier
iNKT cells	Allogeneic	CD19-targeting CAR-iNKT cells	Refractory/relapsed B cell lymphoma or leukemia	NCT05487651, NCT03774654
	Autologous	GD2-targeting CAR-iNKT cells carrying IL-15	Relapsed/refractory high-risk neuroblastoma	NCT03294954
		CD70-targeting CAR-iNKT cells	Relapsed/metastatic advanced renal cell carcinoma	NCT06182735
		CD70-targeting CAR-iNKT cells	Advanced malignant solid tumor	NCT06394622
γδ T cells	Allogeneic	NKG2DL-targeting CAR-γδ T cells	Advanced solid tumor or hematological malignancies	NCT05302037
		NKG2DL-targeting CAR-γδ T cells	Relapsed or refractory solid tumor	NCT04107142
		HLA-G-targeting CAR-γδ T cells with PD-L1/CD3ε Bispecific T cell engager (BiTE)	Relapsed/refractory triple-negative breast cancer, non-small cell lung cancer, colorectal cancer, or glioblastoma	NCT06150885
		CD20-targeting CAR-γδ T cells	Relapsed/refractory B-cell malignancies	NCT04735471
		B7-H3-targeting CAR-γδ T cells carrying IL-2	Advanced malignant solid tumors	NCT06372236
		B7-H3-targeting CAR-γδT cells carrying IL-2	Relapsed/refractory acute myeloid leukemia	NCT05731219, NCT05722171
		CD123-targeting CAR-γδ T cells	Relapsed acute myeloid leukemia	NCT04796441, NCT05388305
		CD19-targeting CAR-γδ T cells	Relapsed/refractory B cell malignancies	NCT02656147, NCT06092047
		CD7-targeting CAR-γδ T cells	T cell malignancies	NCT04702841
		B7-H3-targeting CAR-γδ T cells	Malignant brain glioma	NCT06018363
		CD19-targeting CAR-γδ T cells	B cell acute lymphoblastic leukemia	NCT06056752
		CD19-targeting CAR-γδ T cells	Relapsed/refractory B cell non-Hodgkin's lymphoma	NCT06503211, NCT05554939
		B7-H3-targeting CAR-γδ T cells	Meningeal metastases	NCT06592092
		BCMA-targeting CAR-γδ T cells	Relapsed/refractory multiple myeloma	NCT06279026
	Autologous	GPC3/Mesothelin-targeting CAR-γδ T cells	Advanced cancer that expresses GPC3 or Mesothelin	NCT06196294

express soluble IL-15. These enhancements minimize the risk of tumor antigen escape and promote persistence. Consequently, engineered-CD16^{high} Vγ9Vδ2T cells demonstrated strong anti-tumor efficacy using *in vitro* and *in vivo* ovarian cancer models and represent a promising γδ T cell immunotherapy.[7] Regrading Vδ1T cell-based therapy, Jiang et al. showed that B7-H3 targeting CAR-Vδ1T cells armored with IL-2 had enhanced anti-tumor efficacy using multiple *in vivo* solid tumor models.[8] Additionally, Makkouk et al. demonstrated that glypican-3-targeting CAR-Vδ1T cells armored with IL-15 effectively controlled liver cancer growth *in vivo*.[9] Overall, these studies highlight the therapeutic potential of Vδ1T and Vδ2T cells and their capacity to treat solid tumors.

To optimize next-generation γδ T cell therapies, a viable approach is to explore the synergistic potential of bispecific T cell engagers or immune-checkpoint blockade.[10] Additionally, exploring the understudied γδ T subtypes Vδ3T and Vδ5T cells may provide additional therapeutic value for cancer immunotherapy.

MAIT cells: Emerging candidates in cancer therapy

Compared to iNKT and γδ T cells, MAIT cells are a relatively newly identified subset of unconventional T cells. MAIT cells are predominantly located in mucosa-rich tissues such as the lungs (~5%), liver (~20%–40%), and intestines (~1%–2%), as well as in peripheral blood (~1%–10%).[11] Their recruitment and retention within tissues are guided by the expression of chemokine receptors (CXCR6$^+$CCR6$^+$CCR9$^+$) and long-term residency markers (CD69^{hi}), while their lack of CCR7 restricts migration through lymphatic tissues. Blood-derived MAIT cells are primarily CD8$^+$αβ and exhibit strong cytotoxic activity upon antigen recognition. In addition to the MAIT TCR-MR1 interaction, these cells express NKRs, such as NKG2D and DNAM-1, enabling them to target MR1-negative tumors.[11]

Characterized by high expression of NK receptors and a tissue-resident effector memory (CD45RA$^-$CD45RO$^+$) T cell phenotype, MAIT cells exhibit potent cytotoxicity and organ infiltration, making them promising candidates for the treatment of solid malignancies. Therapeutic strategies could include reversing their exhausted state within tumors or enhancing their function through *ex vivo* expansion and genetic engineering for adoptive cell transfer. However, our understanding of MAIT cell biology in the context of cancer remains limited. While recent studies have underscored their critical roles in various tumor types,[12–14] the molecular

mechanisms governing MAIT cell-mediated tumor immunity are still largely unclear. The contributions of MAIT cells to tumor immunity likely depend on multiple tumor-related factors, such as cancer type, stage, patient demographics, and TME, as well as the origin and functional subsets of the MAIT cells themselves.

Accumulating evidence suggests that human MAIT cells maintain their mucosal-associated tropism in both healthy and malignant conditions, positioning them as promising candidates for therapeutic applications. Recent clinical studies in HCC,[12] non-small cell lung cancer (NSCLC),[13] and high-grade serous ovarian carcinoma (HGSOC)[14] indicate that tumor-infiltrating or peri-tumoral MAIT cells, despite displaying exhausted phenotypes (PD-1$^+$ and/or LAG3$^+$), serve as strong prognostic markers for positive responses to immune checkpoint blockade and platinum-based chemotherapy. Given that the lack of T cell infiltration is a major limitation for immunotherapy in solid tumors, the proximity of MAIT cells to tumors, combined with their cytotoxic potential, underscores their value as therapeutic targets.

Ruf et al. demonstrated that liver-resident MAIT cells in HCC patients exhibited exhaustion, partly due to impaired tumor infiltration and immunosuppressive interactions with TAMs via the PD-1/PD-L1 axis.[12] *In vivo*, MAIT cell activation, combined with anti-PD-L1 therapy, reduced tumor growth in wild-type mice but not in *Mr1*$^{-/-}$ mice, indicating that MAIT cells are crucial targets for anti-PD-1/PD-L1 immunotherapy in HCC. Notably, our study found that MAIT cells efficiently target TAMs in the solid TME through recognition by both MAIT TCRs and NKRs, thereby enhancing antitumor efficacy.[11] The differing outcomes may be attributed to variations in cancer types and experimental models and the use of endogenous MAIT cells versus *in vitro* engineered MAIT cells. Further investigation into the interaction between MAIT cells and the solid tumor TME, using advanced models and *in vivo* validation, is warranted.

In another study, Qu and colleagues demonstrated that CXCR6$^+$CD8$^+$ MAIT cells are favorable prognostic markers in NSCLC patients responding to anti-PD1 therapy, with upregulation of cytotoxic genes such as *CST7*, *GNLY*, *KLRG1*, *NKG7*, and *PRF1* in responders.[13] Similarly, Zheng et al. identified MAIT cells in the ascites of HGSOC patients as predictors of platinum response, suggesting that immune-activated MAIT cells may enhance chemotherapy efficacy, whereas MAIT cells in non-responsive patients were more likely to be dysfunctional.[14] Furthermore, the study found elevated LAG3 expression on MAIT cells in non-responsive HGSOC patients, indicating the potential of anti-LAG3 therapies to reverse MAIT cell dysfunction and improve treatment outcomes in HGSOC and other solid tumors.[14]

The adoptive transfer of MAIT cells for cancer immunotherapy remains underexplored. As MAIT cells recognize the monomorphic MR1, they hold potential for "off-the-shelf" cell therapies without the risk of GvHD.[11] Dogan et al. have developed CAR-engineered MAIT cells that demonstrate potent *in vitro* antitumor efficacy[15]; however, physiologically relevant studies examining their biodistribution, safety, and effectiveness in human xenograft mouse models are still lacking. Current protocols for MAIT cell engineering and expansion encounter several challenges, including low gene transduction efficiency, suboptimal *in vitro* expansion and cell yield, and the induction of IL-17A production, which may contribute to tumor growth. These limitations must be addressed before the adoptive transfer of MAIT cells and their CAR-engineered derivatives can become a viable option for cancer immunotherapy.

Conclusions

Despite the small patient cohorts in most published clinical trials, unconventional T cells and their CAR-engineered derivatives have shown considerable advantages in cancer therapy as well as in other diseases such as autoimmune disorders (Figure 1). However, one of the significant challenges hindering the widespread application of unconventional T cell therapies is the extremely low frequency of these cells in human blood, making it difficult to reliably expand them to the large numbers required for CAR engineering. To address this, strategies such as genetic modification of stem cells, including HSPCs and induced pluripotent stem cells (iPSCs), along with their directed differentiation into unconventional T cells, have been developed to achieve high yields and purity.[2] This approach aims to produce sufficient numbers of unconventional T cells for therapeutic infusion in cancer patients.

In addition to challenges in expansion, reliable and standardized procedures for engineering and manufacturing these cells need further development. Current protocols vary, particularly in clinical trials. For example, CAR-iNKT cells have been generated by stimulating sorted iNKT cells with α-GC-pulsed PBMCs and culturing them with IL-2 and IL-21[3]; CAR-$\gamma\delta$ T cells were generated by stimulating sorted $\gamma\delta$ T cells with Zoledronic acid monohydrate and culturing them with IL-2.[7] Given the uniqueness of unconventional T cells, manufacturing protocols may need to be tailored for each cell type. However, lessons from the manufacturing, cryopreservation, and administration of conventional CAR-T cells can provide a valuable framework for optimizing these processes.

Finally, various immune-enhancement strategies, such as overexpressing cytokines (e.g., IL-12 and IL-15) or editing immune regulatory molecules (e.g., BTG1),[2–4,7] have been applied to unconventional T cells. Given the relatively smaller body of research on these cells, it will take more time to thoroughly evaluate different strategies and identify the most effective immune enhancements for each unconventional T cell product. Achieving this will require close collaboration between scientists, biotechnology companies, and clinicians to unlock the full therapeutic potential of these cells.

ACKNOWLEDGMENTS

This work was supported by a UCLA BSCRC Innovation Award (to L.Y.) and an Ablon Scholars Award (to L.Y.). Y.-R.L. is a postdoctoral fellow supported by a UCLA MIMG M. John Pickett Post-Doctoral Fellow Award, a CIRM-BSCRC postdoctoral fellowship, and a UCLA Sydney Finegold Postdoctoral Award. K.Z. is a predoctoral fellow supported by a CIRM-BSCRC predoctoral fellowship. Illustrations were created with BioRender.com. Due to reference limitations, the authors apologize for being unable to cite many reports in the literature.

DECLARATION OF INTERESTS

L.Y. is a scientific advisor to AlzChem and Amberstone Biosciences and a cofounder, stockholder, and advisory board member of Appia Bio. None of the declared companies contributed to or directed any of the research reported in this article.

Published date: December 12, 2024

REFERENCES

1. Rotolo, A., Caputo, V.S., Holubova, M., Baxan, N., Dubois, O., Chaudhry, M.S., Xiao, X., Goudevenou, K., Pitcher, D.S., Petevi, K., et al. (2018). Enhanced Anti-lymphoma Activity of CAR19-iNKT Cells Underpinned by Dual CD19 and CD1d Targeting. Cancer Cell *34*, 596–610.e11. https://doi.org/10.1016/j.ccell.2018.08.017.

2. Li, Y.-R., Zhou, Y., Yu, J., Kim, Y.J., Li, M., Lee, D., Zhou, K., Chen, Y., Zhu, Y., Wang, Y.-C., et al. (2024). Generation of allogeneic CAR-NKT cells from hematopoietic stem and progenitor cells using a clinically guided culture method. Nat. Biotechnol. https://doi.org/10.1038/s41587-024-02226-y.

3. Heczey, A., Xu, X., Courtney, A.N., Tian, G., Barragan, G.A., Guo, L., Amador, C.M., Ghatwai, N., Rathi, P., Wood, M.S., et al. (2023). Anti-GD2 CAR-NKT cells in relapsed or refractory neuroblastoma: updated phase 1 trial interim results. Nat. Med. *29*, 1379–1388. https://doi.org/10.1038/s41591-023-02363-y.

4. Landoni, E., Woodcock, M.G., Barragan, G., Casirati, G., Cinella, V., Stucchi, S., Flick, L.M., Withers, T.A., Hudson, H., Casorati, G., et al. (2024). IL-12 reprograms CAR-expressing natural killer T cells to long-lived Th1-polarized cells with potent antitumor activity. Nat. Commun. *15*, 89. https://doi.org/10.1038/s41467-023-44310-y.

5. Rotolo, A., Whelan, E.C., Atherton, M.J., Kulikovskaya, I., Jarocha, D., Fraietta, J.A., Kim, M.M., Diffenderfer, E.S., Cengel, K.A., Piviani, M., et al. (2023). Unedited allogeneic iNKT cells show extended persistence in MHC-mismatched canine recipients. Cell Rep. Med. *4*, 101241. https://doi.org/10.1016/j.xcrm.2023.101241.

6. Andrlová, H., Miltiadous, O., Kousa, A.I., Dai, A., DeWolf, S., Violante, S., Park, H.Y., Janaki-Raman, S., Gardner, R., El Daker, S., et al. (2022). MAIT and Vδ2 unconventional T cells are supported by a diverse intestinal microbiome and correlate with favorable patient outcome after allogeneic HCT. Sci. Transl. Med. *14*, eabj2829. https://doi.org/10.1126/scitranslmed.abj2829.

7. Lee, D., Dunn, Z.S., Guo, W., Rosenthal, C.J., Penn, N.E., Yu, Y., Zhou, K., Li, Z., Ma, F., Li, M., et al. (2023). Unlocking the potential of allogeneic Vδ2 T cells for ovarian cancer therapy through CD16 biomarker selection and CAR/IL-15 engineering. Nat. Commun. *14*, 6942. https://doi.org/10.1038/s41467-023-42619-2.

8. Jiang, L., You, F., Wu, H., Qi, C., Xiang, S., Zhang, P., Meng, H., Wang, M., Huang, J., Li, Y., et al. (2024). B7-H3-Targeted CAR-Vδ1T Cells Exhibit Potent Broad-Spectrum Activity Against Solid Tumors. Cancer Res. https://doi.org/10.1158/0008-5472.CAN-24-0195.

9. Makkouk, A., Yang, X.C., Barca, T., Lucas, A., Turkoz, M., Wong, J.T.S., Nishimoto, K.P., Brodey, M.M., Tabrizizad, M., Gundurao, S.R.Y., et al. (2021). Off-the-shelf Vδ1 gamma delta T cells engineered with glypican-3 (GPC-3)-specific chimeric antigen receptor (CAR) and soluble IL-15 display robust antitumor efficacy against hepatocellular carcinoma. J. Immunother. Cancer *9*, e003441. https://doi.org/10.1136/jitc-2021-003441.

10. Lameris, R., Ruben, J.M., Iglesias-Guimarais, V., de Jong, M., Veth, M., van de Bovenkamp, F.S., de Weerdt, I., Kater, A.P., Zweegman, S., Horbach, S., et al. (2023). A bispecific T cell engager recruits both type 1 NKT and Vγ9Vδ2-T cells for the treatment of CD1d-expressing hematological malignancies. Cell Rep Med. *4*, 100961. https://doi.org/10.1016/j.xcrm.2023.100961.

11. Li, Y.-R., Zhou, K., Wilson, M., Kramer, A., Zhu, Y., Dawson, N., and Yang, L. (2023). Mucosal-associated invariant T cells for cancer immunotherapy. Mol. Ther. *31*, 631–646. https://doi.org/10.1016/j.ymthe.2022.11.019.

12. Ruf, B., Bruhns, M., Babaei, S., Kedei, N., Ma, L., Revsine, M., Benmebarek, M.-R., Ma, C., Heinrich, B., Subramanyam, V., et al. (2023). Tumor-associated macrophages trigger MAIT cell dysfunction at the HCC invasive margin. Cell *186*, 3686–3705.e32. https://doi.org/10.1016/j.cell.2023.07.026.

13. Qu, J., Wu, B., Chen, L., Wen, Z., Fang, L., Zheng, J., Shen, Q., Heng, J., Zhou, J., and Zhou, J. (2024). CXCR6-positive circulating mucosal-associated invariant T cells can identify patients with non-small cell lung cancer responding to anti-PD-1 immunotherapy. J. Exp. Clin. Cancer Res. *43*, 134. https://doi.org/10.1186/s13046-024-03046-3.

14. Zheng, X., Wang, X., Cheng, X., Liu, Z., Yin, Y., Li, X., Huang, Z., Wang, Z., Guo, W., Ginhoux, F., et al. (2023). Single-cell analyses implicate ascites in remodeling the ecosystems of primary and metastatic tumors in ovarian cancer. Nat. Cancer *4*, 1138–1156. https://doi.org/10.1038/s43018-023-00599-8.

15. Dogan, M., Karhan, E., Kozhaya, L., Placek, L., Chen, X., Yigit, M., and Unutmaz, D. (2022). Engineering Human MAIT Cells with Chimeric Antigen Receptors for Cancer Immunotherapy. J. Immunol. *209*, 1523–1531. https://doi.org/10.4049/jimmunol.2100856.