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Review Article

T-cell Receptor Repertoire Analysis in the Context of Transarterial Chemoembolization Synergy with Systemic Therapy for Hepatocellular Carcinoma

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Abstract

T-cell receptor (TCR) sequencing provides a novel platform for insight into and characterization of intricate T-cell profiles, advancing the understanding of tumor immune heterogeneity. Recently, transarterial chemoembolization (TACE) combined with systemic therapy has become the recommended regimen for advanced hepatocellular carcinoma. The regulation of the immune microenvironment after TACE and its impact on tumor progression and recurrence has been a focus of research. By examining and tracking fluctuations in the TCR repertoire following combination treatment, novel perspectives on the modulation of the tumor microenvironment post-TACE and the underlying mechanisms governing tumor progression and recurrence can be gained. Clarifying the distinctive metrics and dynamic alterations of the TCR repertoire within the context of combination therapy is imperative for understanding the mechanisms of anti-tumor immunity, assessing efficacy, exploiting novel treatments, and further advancing precision oncology in the treatment of hepatocellular carcinoma. In this review, we initially summarized the fundamental characteristics of TCR repertoire and depicted immune microenvironment remodeling after TACE. Ultimately, we illustrated the prospective applications of TCR repertoires in TACE combined with systemic therapy.

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Introduction

Hepatocellular carcinoma (HCC) is the most common form of liver cancer and the fourth leading cause of cancer-related death worldwide.[1] Due to the insidious onset of HCC, most patients are diagnosed at intermediate or advanced stages, missing the opportunity for radical surgical treatment.[2] Transarterial chemoembolization (TACE), as a standard treatment regimen, remarkably improves the prognosis of unresectable HCC.[3] With the development of new therapeutic targets, sorafenib is no longer the sole option for advanced HCC.[4] Immune checkpoint inhibitor (ICI) therapy has emerged as a promising pillar for various cancer therapies.[5] Frustratingly, response rates to systemic therapy remain modest, spanning from 5% to 40%.[6] Importantly, no more than 20% of HCC patients benefit from ICI therapy.[7]

The combination of TACE and systemic treatments is a promising option for advanced HCC. Anti-angiogenic drugs effectively block the hypoxia-inducible factor 1α (HIF-1α)/epidermal growth factor receptor pathway and inhibit the proliferation and metastasis of residual tumors following TACE.[8] TACE leads to tumor necrosis and the release of tumor antigens, promoting an anti-tumor immune response that further synergizes with ICI therapy.[9] However, the therapeutic effects of combined treatment are elusive, partly due to liver function,[10] tumor traits,[11] diverse regimens,[12] and adaptive immunity,[13] which lead to distinct clinical outcomes. Previous studies have revealed that T cells in adaptive immunity are crucial and complex in anti-tumor responses, with various T cell subsets and their interactions with other immune cells significantly impacting therapeutic outcomes.[14] As tumor progression occurs, T cell numbers, frequencies, and gene expression profiles become irreversibly exhausted, resulting in heightened immunosuppressive activity. Concurrently, the extensive recruitment of regulatory T cells (Tregs) facilitates HCC immune escape and diminishes ICI therapy efficacy.[15] Furthermore, embolization-induced ischemia-reperfusion injury enhances Th17 cell-mediated recurrence in HCC.[16] Therefore, understanding the heterogeneity and plasticity of the intrahepatic T-cell repertoire is critical for developing immunotherapies and treatment prediction tools for HCC.

Recent strategies have focused on harnessing adaptive immunity by evaluating T-cell receptor (TCR) repertoire to improve systemic therapies.[17,18] The peripheral blood TCR repertoire of HCC patients exhibits distinctive features compared to healthy individuals.[19] Immunogenomic classifica-

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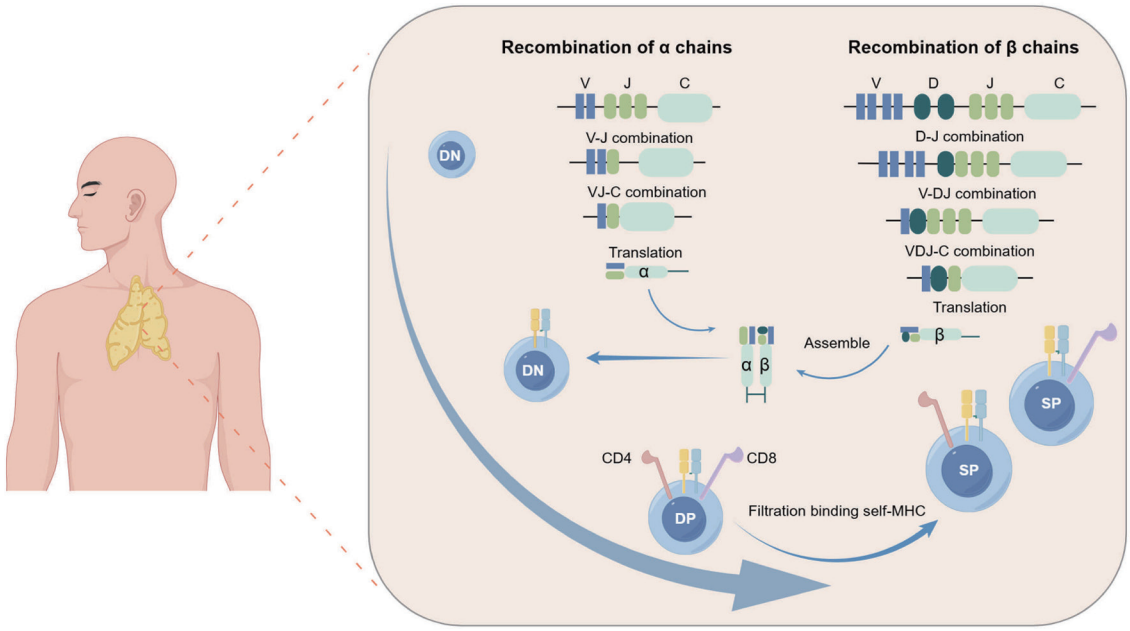

Fig. 1. αβ T cell development and maturation. The initial T cells in the thymus differentiate from a DN T cell state to DP T cells. This process involves the recombination of α and β chains. The α chain is formed through rearrangements of the V and J gene segments, while the β chain is generated through random recombinations of the V, D, and J gene segments. The complete variable region is then linked to the constant region to produce functional α and β chains, ultimately assembling into the full TCRαβ chain. The DP T cells undergo a selection process to ensure that those with TCRs exhibiting high affinity for self-MHC and pMHC complexes are filtered out. The remaining T cells then progress to become SP TCRαβ T cells (by Figdraw). TCR, T-cell Receptor; DN, double-negative; DP, double-positive; self-MHC, self-major histocompatibility complex; SP, single-positive; pMHC, self-peptide MHC.

tion of HCC reveals that the inflamed class possesses more diverse T-cell repertoires and exhibits better responses to ICIs.[20] *De novo* TCR clones have been detected in patients with postoperative HCC recurrence, suggesting neoantigen-induced specific T cells.[21] Additionally, HCC patients responding to pembrolizumab show activated TCR signaling and major histocompatibility complex (MHC) gene expression, indicating heightened T cell cytotoxicity.[22] Notably, TCR-engineered T cell (TCR-T) therapy provides novel treatment options for hepatitis B virus (HBV)-HCC recurrence post-liver transplantation.[23] Here, we initially review the fundamental characteristics of TCR repertoire and depict immune microenvironment remodeling after TACE. Ultimately, we illustrate the prospective applications of TCR repertoires in TACE combined with systemic therapy.

Sequencing and characterization of TCR repertoire

Characterization of TCR repertoire

As widely known, immune cells, most notably infiltrating T lymphocytes, play a fundamental role in tumor surveillance and clearance.[24] Indeed, key roles in T cell-mediated immunity include CD4 and CD8 T cells, referred to as antigen-presenting and cytotoxic cells, respectively. These cells differentiate from the initial CD4/CD8 double-positive state in the thymus.[25,26] They mature into T cells expressing distinct functional TCRs that enable them to identify specific antigens (Fig. 1).[27]

TCRs have been extensively studied as molecular markers for tracking changes in T cells during disease and treatment.[28] The functional TCR is a heterodimer, consisting of either α and β chains or γ and δ chains. The αβ TCR, found in most T cells, recognizes antigens presented on MHC proteins, while the γδ TCR, present in approximately 5% of T cells, functions independently of MHC and is involved in innate immunity.[29] The TCR chain comprises a conserved C-terminal constant region and an N-terminal variable region capable of recognizing antigens (Fig. 2B).[30] The TCR repertoire arises from somatic recombination of V, D, and J gene segments in immature lymphocytes.[31] The variable regions critical for antigen recognition are constructed through V(D)J recombination, a process that combines variable, diversity, and joining gene fragments in a random or ordered fashion, including the recombination of different gene segment alleles (Fig. 2C).[32] The TCR α and γ chains consist of V and J gene segments, whereas the β and δ chains include D gene fragments, enhancing structural diversity (Fig. 1).[33] The combinatorial diversity of gene fragments, along with junctional diversity—resulting from random nucleotide additions or deletions at the junctions of allele segments—endows T cells with extensive antigen specificity, creating approximately 10^{15} to 10^{20} distinct TCR chains.[34] Notably, the transition to different TCR chains is crucial: β chain recombination promotes the maturation of the αβ TCR, while the γδ TCR benefits from the recombination of the γ and δ chains.[35]

The variable structural domains of the TCR chain contain three complementarity-determining regions (CDRs), designated as CDR1, CDR2, and CDR3, with CDR3 exhibiting the greatest variability and determining high TCR chain specificity.[36] CDR1 and CDR2 are encoded by V gene fragments and facilitate interaction between the TCR and MHC primarily through contact with the conserved α-helices of the MHC. CDR3 is encoded by a junction of V and J or D and J gene fragments and contains highly differentiated junctions from V(D)J recombination, implying higher variability. The antigenic specificity of the TCR is mainly determined by CDR3, which is responsible for binding peptide antigens in the MHC-binding groove by encoding the antigen-binding pocket of the TCR (Fig. 2B).[37] Given its inherent variability and direct

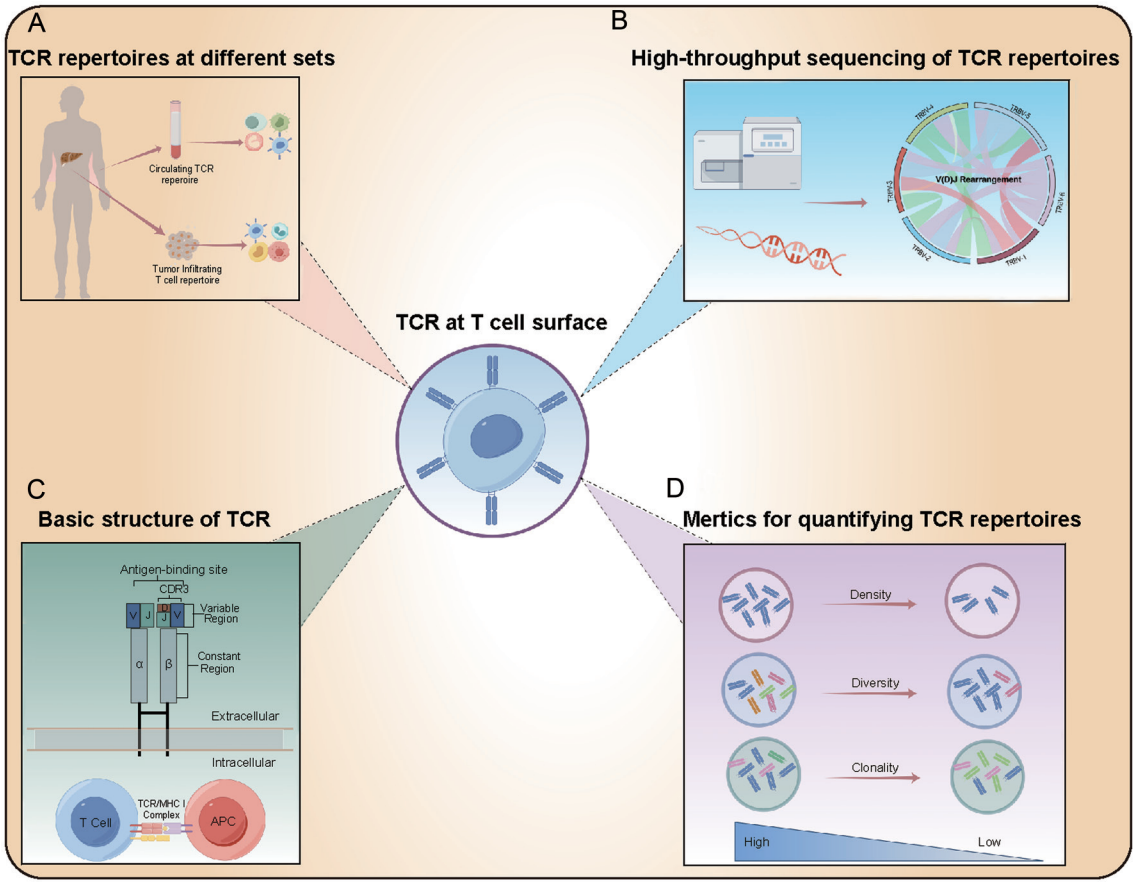

Fig. 2. Characterization of TCR repertoires. (A) TCR repertoires at different sets; (B) High-throughput sequencing of TCR repertoires; (C) Basic structure of TCR and antigen presentation; (D) Metrics for quantifying TCR repertoires (by Figdraw). TCR, T-cell Receptor.

engagement with antigens, CDR3 provides abundant TCR-specific characteristics, serving as a predominant target region in TCR sequencing (TCR-seq). Overall, TCR-seq provides the informational basis for studying T cell changes in disease.

TCR repertoire preparation and sequencing

Initial TCR-seq approaches measured CDR3 diversity by capturing TCRs at the nucleotide level through molecular cloning and Sanger sequencing.[38] Notably, this method only detects minor genomic changes, like substitutions and short indels, and has low throughput, making it inadequate for capturing the vast diversity of TCRs.[27] High-throughput sequencing (HTS) platforms have advanced our understanding of T-cell repertoires by enabling rapid and comprehensive sequencing of genomic DNA or RNA.[39] Before HTS, multiplex PCR and rapid amplification of cDNA ends (5′ RACE) were commonly employed to prepare T-cell repertoires, focusing on amplifying the CDR3 region.[29] Multiplex PCR is often used to amplify genomic DNA or RNA of the CDR3 region, utilizing primers for the J gene segment or TCR constant region along with a mix of primers for known V gene segment alleles.[29] However, multiplex PCR is limited by primer issues and the risk of sequencing bias, errors, and uneven allele amplification, leading to inaccurate TCR diversity and frequency estimates.[34] Strategies have been devised to minimize this bias by using multiplex primers to synthesize TCR molecules and introducing unique molecular identifiers, which help eliminate PCR amplification artifacts.[40,41] The 5′ RACE method uses RNA (reverse transcription) and a single primer pair targeting the TCR chain's constant region and the 5′ mRNA end, enabling the amplification of all TCR rearrangements without the bias present in multiplex PCR.[42] However, 5′ RACE remains prone to PCR-related template switching and sequencing errors. To track amplification bias, researchers added oligonucleotide sequences and unique barcodes to the 3′ and 5′ ends of cDNA.[43]

Bulk and single-cell sequencing have become mainstream methods for analyzing T-cell repertoires. Bulk sequencing commonly targets all TCR chain aggregates in a sample to analyze large-scale TCR diversity and compare patient cohort groups.[34] However, bulk sequencing mainly focuses on the TCRβ chain, overlooking the role of both chains in determining antigenic specificity, which results in underestimating TCR diversity, confusing intraclonal phenotyping, and failing to accurately identify specific T-cell antigens.[38] Conversely, single-cell sequencing provides information on both paired TCR α and β chains, with high read quality and comprehensive coverage of TCR sequences.[44] Additionally, single-cell sequencing focuses on individual T cells or subpopulations, facilitating the identification of rare subpopulations and different TCR cell states.[29] Notably, sequencing results depend on the quantity and quality of cells in the sample and may miss rare clonotypes.[29] Despite advances in TCR-seq that have enhanced our understanding of T-cell repertoires, improvements are still needed in capture efficiency, sensitivity, and cost. Furthermore, droplet-based single-cell sequenc-

Table 1. Metrics for quantifying T-cell Receptor (TCR) repertoires

Metrics	Description	Ref
Hill number	The Hill number, often referred to as the effective species number, serves as a crucial metric for quantifying biodiversity. Within the context of TCR repertoires analysis, Hill numbers can be employed to determine the effective number of distinct clonotypes (i.e., sequences that exhibit equal abundance), thereby enabling an assessment of the TCR repertoires diversity.	47
Shannon entropy	Shannon entropy serves as a metric for assessing diversity, illustrating the variability of complementarity-determining regions (CDRs) while considering both richness and relative abundance. An increase in this index signifies greater diversity and a more varied distribution.	49
Renyi entropy	Renyi entropy serves as a method for assessing biodiversity by analyzing the degree of clonal expansion and distribution. It is influenced by the parameter α. When $\alpha > 1$, there is an increased emphasis on more abundant species—such as TCRs that are highly expanded—whereas an $\alpha < 1$ prioritizes rarer species. This approach offers richer insights compared to using a solitary index, as it accounts for varying weights associated with species abundances.	50
Simpson index	The Simpson index serves as a measure of biodiversity. A high Simpson index signifies an imbalanced distribution of one or a few clones and a less diverse repertoire.	51
Diversity Evenness 50	The Diversity 50 (D50) value is used to assess the diversity of the TCRβ repertoires and is defined as the percentage of dominant TCRβ CDR3 clonotypes that account for 50% of the total TCRβ sequences accumulated in the sample.	28
Morisita-Horn index	The Morisita-Horn Index evaluates the similarity of TCR rearrangements. However, it lacks sensitivity to TCRs that are of low abundance and is typically employed for assessing TCR repertoires with higher abundance.	52
Pielou's evenness index	Pielou's evenness index allows for comparisons among samples that have different total read counts. It ranges from 0 to 1, where a higher score signifies a more uniform distribution. Conversely, a low score reflects clone skewing caused by biased expansion.	53
High expanded clone (HEC)	HEC is employed to characterize the state of the TCR library. It is determined by summing the abundance of all sequences that exceed a specified threshold. Typically, this threshold is set at 0.01% or 0.1%, though it can be modified depending on the requirements of the research.	54
Clonality index	The clonality index can assess clone expansion, reveal the frequency of such expansion, and compare two TCR repertoires with different clone counts. It is derived from the normalized Shannon entropy, which ranges from 0 to 1. A higher value signifies greater clone expansion, while a value of 1 indicates a monoclonal distribution.	55
Jaccard index	The Jaccard index, which is calculated by taking the size of the shared species and dividing it by the total size of the two compared samples, is employed to assess the overlap between T-cell repertoires, but repertoires homology between healthy tumor-adjacent tissues and tumor tissues based only on the Jaccard index is not sufficient to derive any conclusions, and other metrics should be used in parallel.	47
Sorensen index	The Sorensen index serves to measure the similarity between two T-cell repertoires. It is calculated by counting the shared TCR sequences in both repertoires, then multiplying that count by two, and dividing this result by the total number of TCR sequences from both libraries combined. The Sorensen Index produces a value that varies from 0 to 1, where 0 signifies no similarity and 1 indicates complete similarity.	47
Morisita overlap index	The Morisita overlap index (MOI) serves as a quantitative measure for assessing the similarity and overlap of TCR repertoires from two different samples, factoring in both the composition and abundance of T-cell rearrangements. MOI ranges from 0 to 1, with 0 denoting no overlap and 1 indicating total overlap.	56

ing methods capture about 65% of cells, potentially missing specific and rare subpopulations.[45] Single-cell sequencing remains more expensive than bulk sequencing, and advanced single-cell technologies are pricier than traditional methods, limiting some studies.

Metrics for quantifying TCR repertoire

Given the high diversity of TCR sequences, researchers have developed multiple algorithms for computational analysis to obtain valuable information about T cells and to quantify and characterize the TCR repertoire. Commonly used quantitative metrics of TCR repertoires include T cell distribution density, clonality, and diversity (Fig. 2D). T cell density is determined by the total number of T cells within a specific distribution to assess the abundance of T cell infiltration, while diversity reflects the richness and evenness of the TCR repertoire.[46] The term "richness" denotes the diversity of V-J rearrangements, quantified as the ratio of observed to possible combinations of V and J gene segments, indicative of distinct TCR sequences. "Evenness" reflects the similarity of V-J rearrangement frequencies, representing the distribution of unique TCR sequences.[47,48] Researchers have developed numerous quantitative metrics, including the Hill number, Shannon entropy, Rényi entropy, Gini-Simpson index, and Diversity Evenness 50, to assess TCR diversity (Table 1).[28,47,49–56] Clonality integrates density and diversity indicators, evaluating the clonal expansion within T cell populations. Key metrics for assessing TCR repertoire clonality include Shannon entropy and the

Table 2. T-cell Receptor (TCR) and antigen databases

Database	Type	Source	Diseases context	Database size
IEDB	Antibody sequences; T-cell epitope sequences; MHC alleles and ligand sequences; epitope analysis and prediction	Infectious disease; allergy; cancer, autoimmunity; transplantation	Human; non-human primates; other animal species	>6 × 10^6 sequences
McPAS-TCR	TCR sequences; T-cell epitope sequences	Infectious disease; allergy; cancer, autoimmunity; transplantation	Human and mouse	>5 × 10^3 sequences
VDJdb	TCR sequences with known antigen specificity	All	Human; mouse; non-human primates	61,049 sequences
TCRdb	TCRb chain sequences with known antigen specificity	Specific tissue, clinical condition; cell type	Human	>2.77 × 10^5 sequences
10X Genomics Dataset	Paired TCR sequences with known antigen specificity	All	Human	>1.5 × 10^4 sequences
NetTCR-2.0	Paired TCR sequences	All	Human; non-human primates; other animal species	~

Gini-Simpson index.[29] Furthermore, indices such as the Jaccard index, Morisita overlap index, and Sorensen index are utilized to measure TCR repertoire variation and similarity across samples or treatment conditions.[47] Advanced algorithms have been devised for T cell characterization, emphasizing continuous scales of TCR similarity,[57] and prioritizing the biological relevance of TCR sequences over mere clonotype counts.[58] This enhances the efficiency of large-scale TCR-seq analysis and visualization[59,60] and allows for the rapid identification of T cell subset dynamics in longitudinal studies, enabling the tracking and forecasting of immune responses to diseases and treatments.[29] Identifying suitable quantitative metrics for analyzing TCR sequences is essential for understanding the functional and temporal shifts in T cells during disease progression, facilitating disease surveillance and therapy assessment.

TCR and antigen databases

To handle the complex sequencing data from thriving TCR repertoires, researchers have developed various databases to characterize TCR-related information (Table 2). The Immune Epitope Database contains experimentally isolated antigens from various contexts, including infectious agents, allergens, cancer, and autoantigens.[61] McPAS-TCR contains sequences of human and mouse TCRs and T-cell epitopes, providing TCR information for numerous pathologies, including infections and cancer.[62] VDJdb is a database that links TCR sequences with known antigen specificity to their peptide-MHC ligands, facilitating in-depth analysis of TCR interactions.[63] TCRdb utilizes a 10X Genomics single-cell immunoassay dataset with over 270 million TCR sequences from various clinical conditions, tissues, and cell types.[63] Despite researchers' efforts in annotating intricate T-cell repertoires, manually curated previous databases, which contain limited sequences, have become inadequate for high-throughput TCR-seq. New artificial intelligence pipelines have updated existing databases and produced essential tools for analyzing complex TCR repertoires and immune responses.[64]

The dual role of TACE in tumor therapy

As the cornerstone of intermediate and advanced HCC treatment, TACE plays a crucial role in tumor downstaging and improving prognosis.[65] However, debates surrounding the potential adverse effects of TACE are contentious, with concerns primarily focused on liver function impairment and the risk of tumor metastasis and recurrence. While TACE effectively alleviates tumor load, it may also delay surgery for resectable lesions and leave behind more aggressive residual tumor cells, particularly in poorly differentiated HCC.[66] Postoperative adjuvant TACE can further compromise residual liver function and trigger the activation of the HBV, facilitating extrahepatic metastases.[67] For larger lesions, repeated embolization procedures are routinely performed, often resulting in incomplete tumor necrosis, which may reduce the adhesion of tumor cells and allow them free access to the bloodstream, leading to intrahepatic or extrahepatic metastasis.[68] Regarding the tumor microenvironment (TME), TACE induces tumor necrosis and severe hypoxia, both of which upregulate levels of vascular endothelial growth factor (VEGF) and HIF-1α in residual tumors, promoting neovascularization and relapse.[69] Importantly, TACE exacerbates the hypoxic state and the distribution of oxygen gradients within the tumor, fostering tumor plasticity and heterogeneity, and reshaping the TME.[70]

Immune microenvironment restructuring post TACE

TACE has the dual capacity to eradicate tumors and modulate anti-tumor immunity through intricate mechanisms. The ischemia caused by TACE, combined with the cytotoxic impact of chemotherapy, leads to immunogenic cell death (ICD), which releases multiple tumor neoantigens.[71] These antigens are captured by antigen-presenting cells (APCs), which present them to T cells via MHC class I molecules, activating the effector T cells. Previous studies have demonstrated a significant increase in the $CD4^+/CD8^+$ ratio and natural killer cells, alongside a prominent decrease in regulatory $CD4^+/FOXP3^+$ and immune-exhausted $CD8^+/PD\text{-}1^+$ T cells post-TACE, potentially transforming the immunosuppressive microenvironment into an immunosupportive state.[71,72] Notably, immune checkpoints comprising PD-L1, CTLA-4, indoleamine 2,3-dehydrogenase 1, lymphocyte activation gene 3, and T-cell immunoglobulin and mucin domain-containing protein 3 showed no apparent variation.[71] However, TACE also fosters an immunosuppressive TME. Studies revealed a reduction in the clonality of $CD8^+$ T cells, with numerous shared TCR clones detected among $CD8^+$ T cell subsets post-TACE, indicating a substantial degree of homology within these sub-

sets.[71,73] TACE resulted in a significant increase in TREM2$^+$ tumor-associated macrophages (TAMs), which exhibited robust inhibition of cytotoxicity in CD8$^+$ T cells.[74] Moreover, TREM2$^+$ TAMs diminished the secretory release of CXCL9 and facilitated galectin-1-induced PD-L1 overexpression in vascular endothelial cells, further suppressing the migration of CD8$^+$ T cells.[73]

Accumulated evidence underscores the pivotal role of chemotherapeutic agents in bolstering anti-tumor immunity during TACE. These agents increase the immunogenicity of malignant cells via cytostatic/cytotoxicity-inducing ICD while simultaneously disrupting the immunosuppressive pathway to enhance effector T-cell responses.[75] Clinical chemotherapeutics for TACE, including anthracyclines, paclitaxel, and oxaliplatin, are considered ICD inducers, increasing the abundance of intra-tumoral CD8$^+$ T cells.[75,76] Specifically, anthracycline doxorubicin, the preeminent chemotherapeutic agent employed in TACE, induces DNA damage in cancer cells, activating T-cell immunity.[77] Meanwhile, low doses of doxorubicin can reduce myeloid-derived suppressor cells (MDSCs) and Treg cell infiltration.[78,79] Paclitaxel exhibits diverse immunomodulatory effects, enhancing APC phagocytosis, decreasing Treg populations and activity, increasing pro-inflammatory cytokine levels, and boosting dendritic cell-mediated antigen presentation.[80] Paclitaxel can also induce the polarization of M2-like TAMs towards M1-like TAMs.[81] Notably, cisplatin fails to induce ICD but regulates the immune system by releasing tumor antigens and danger-associated molecular patterns in the TME, including the upregulation of MHC-I expression, recruitment and proliferation of effector T cells, and reduction of immunosuppressive factors.[82,83]

Inflammatory responses triggered by TACE not only activate anti-tumor immune responses but also have the potential to trigger various pro-tumorigenic effects, including fostering tumor cell proliferation, initiating the metastatic cascade, promoting angiogenesis, and suppressing adaptive immunity.[84] Previous studies confirmed that elevated CRP levels are linked to reduced CD4$^+$ T lymphocyte infiltration.[85] Moreover, IL-6 promotes tumor immune evasion by upregulating the expression of PD-L1.[86] Overall, the inflammatory response triggered post-TACE facilitates the elimination of tumor necrotic foci and enhances the anti-tumor immune response. However, the persistent inflammatory response resulting from incomplete TACE accelerates the development of an immunosuppressive TME.

Hypoxia after embolization triggers tumor neovascularization, leading to disorganized blood vessels that block T-cell entry into the TME.[87] Moreover, the imbalance between pro- and anti-angiogenic signaling affects blood perfusion in dysfunctional blood vessels. Impaired perfusion, along with subsequent hypoxia and an acidic TME, promotes tumor recurrence, invasion, and metastatic potential by hindering T-cell activity.[87] Notably, VEGF signaling during TACE affects the tumor immune microenvironment (TIME) by inhibiting T cell function, increasing the recruitment of Tregs, MDSCs, and mast cells, and impeding dendritic cell activation.[88] Targeting VEGF signaling can improve ICI treatment efficacy by normalizing the tumor's blood vessels and allowing T cells to penetrate the tumor barrier.[89] Moreover, studies have elucidated that VEGF plays a pivotal role in regulating checkpoint molecule expression, supporting the rationale for combined ICI therapy.[90]

The temporal and spatial variability in the TME after TACE is governed by intricate regulatory mechanisms. Initially, after TACE, tumor cell death releases neoantigens and inflammatory cytokines due to ischemic effects and chemotherapy toxicity. This process initially boosts anti-tumor immunity but later transitions to an immunosuppressive state due to a hypoxic and acidic microenvironment. Moreover, the imbalance between pro- and anti-angiogenic signaling, along with physical compression, causes abnormal tumor blood vessels and reduced blood perfusion. The level of impairment varies based on the tumor's stage and location, exhibiting differences between regions and primary versus metastatic tumors. Overall, the TIME undergoes intricate remodeling after TACE, supporting the heterogeneity of HCC and paving the way for targeted treatments and immunotherapy.

TACE in combination with systemic therapy

Systemic therapies, including tyrosine kinase inhibitors (TKIs)-based targeted therapies and ICI therapies, have reshaped the formulation of therapeutic schemes for HCC. However, only subsets of patients have yielded considerable benefits from ICI and TKI monotherapy.[91] Multiple studies have confirmed that combining ICIs with TKIs improves the prognosis for HCC patients, highlighting the necessity of combination therapy.[92,93] Arterialization is a key hallmark of HCC, and the combination of ICIs and TKIs could potentially improve the TME by normalizing tumor vessels.[94]

Given the intricate nature of anti-tumor immunity, integrating immunotherapy with multiple treatment strategies is essential for effectively eradicating tumors. Merely enhancing the immune response and inhibiting immunosuppressive cells is inadequate, especially in tumors with high tumor mutational burden and actively suppressive immune microenvironments.[25] In this context, the combination of TACE and systemic therapy presents a more effective treatment strategy. TACE induces tumor necrosis and releases tumor-specific antigens (TAAs), thereby activating tumor-specific immune responses. Concurrently, ICIs block inhibitory checkpoints to maintain T-cell effector function. Furthermore, embolization-induced hypoxia elevates the expression of VEGF and PD-L1, indicating potential benefits in combining targeted agents with ICIs.[9] A triple combination of TACE, targeted therapy, and ICI therapy is becoming the primary treatment for advanced HCC. Clinical studies have demonstrated the safety and effectiveness of this strategy, with additional trials underway (Table 3).

As previously stated, numerous tumor antigens are released, which boosts the recruitment of tumor-infiltrating lymphocytes (TILs) and APCs, transforming the TME from "cold" to "hot" and improving the response to immunotherapy. Furthermore, the early establishment of a hypoxic TME enhances drug delivery and elevates the efficacy of systemic therapy.[95] Nevertheless, hypoxia following embolization is a key factor contributing to resistance to systemic therapy. Hypoxia upregulates PD-L1 expression in MDSCS, dendritic cells, and cancer cells, thereby facilitating immune evasion. Simultaneously, hypoxia impedes the activation of T and natural killer cells while augmenting the percentage of Tregs, thereby promoting an immunosuppressive TME.[96] Moreover, hypoxia facilitates the differentiation of M2-TAMs, diminishing the cytotoxic activity of CD8$^+$ T cells.[73] Additionally, hypoxia enhances the expression of VEGF and facilitates the glycolysis of tumor cells, favoring adaptation to hypoxic stress, which ultimately undermines the efficacy of anti-angiogenic treatments.[97] Therefore, the therapeutic outcome of combination treatment will depend on the balance between the positive and negative effects of hypoxia on the TME in a given clinical context.

Recently, researchers have proposed the "Immune Boost TACE" strategy, where the approach shifts from complete embolization to partial embolization techniques aimed at "activating the cancer immune cycle" to amplify the efficacy

Table 3. Ongoing clinical trials for triple therapy

Study design	Experimental arm	Control arm	Disease stage	Primary endpoint	Clinical trials government registration
Phase 3	TACE+Sintilimab+Bevacizumab	Lenvatinib+ TACE	Advanced unresectable HCC	OS	NCT05985798
Phase 2	TACE+Fruquintinib+Sintilimab	None	Unresectable HCC	PFS	NCT05971199
Phase 1/ Phase 2	TACE+Lenvatinib+Tislelizumab	Lenvatinib Plus Tislelizumab	Unresectable HCC	ORR	NCT05842317
Phase 3	TACE+Camrelizumab+ Lenvatinib	None	Advanced HCC	Conversional resection rate	NCT05738616
Observational	TACE+Lenvatinib+Anti-PD-1	None	Unresectable HCC	Conversion resection number	NCT05717738
Phase 3	TACE+Lenvatinib+Sintilimab	Lenvatinib+ TACE	BCLC C	OS	NCT05608200
Phase 2	TACE+Donafenib+Sintilimab	None	Unresectable HCC	ORR	NCT05507632
Phase 2/ Phase 3	TACE+Penpulimab+Anlotinib	Penpulimab+ Anlotinib	Advanced HCC	PFS	NCT05344924
Observational	TACE+PD-1/PD-L1 inhibitors+VEGF-TKI/ bevacizumab	None	Advanced HCC	OS	NCT05332821
Observational	TACE+PD-1/PD-L1 inhibitors+VEGF-TKI/ bevacizumab	None	Intermediate HCC	PFS	NCT05332496
Phase 3	TACE+Camrelizumab+Apatinib mesylate	None	Incurable HCC	PFS	NCT05320692
Phase 3	TAC+Atezolizumab+ Bevacizumab	None	BCLC B	Grade 3 or higher treatment-related adverse events	NCT05320692
Phase 2	TACE+AK104+Lenvatinib	None	Unresectable, non-metastatic hepatocellular carcinoma	PFS	NCT05319431
Phase 2	TACE+Donafenib+Anti-PD-1	None	Advanced HCC	PFS	NCT05262959
Phase 2	TACE+Tilelizumab+Sorafenib	None	BCLC C	1-year survival rate	NCT04992143
Early Phase 1	TACE+Anti-PD-1+Lenvatinib	None	BCLC B/C	Resection rate	NCT04974281
Phase 2	TACE+Sintilimab+Bevacizumab Biosimilar	None	Advanced HCC	ORR	NCT04954794
Phase 3	TACE+Atezolizumab+ Bevacizumab	None	Intermediate HCC	Time to failure of treatment strategy	NCT04803994
Phase 3	TACE+Atezolizumab+ Bevacizumab	None	Incurable HCC	PFS/OS	CTR20202073

TACE, transarterial chemoembolization; HCC, Hepatocellular Carcinoma; BCLC, Barcelona Clinic Liver Cancer; OS, Overall Survival; PFS, Progression-Free Survival; ORR, Objective Response Rate.

of immuno-oncology-based systemic therapies.[98] In a proof-of-concept study for individuals with TACE-unsuitable up-to-seven-out tumors, TACE was administered to partially treat tumors, with atezolizumab and bevacizumab as the primary treatment, resulting in a clinical or pathological complete response in 35% of the patients.[99] Collectively, it is crucial to mitigate the impact of hypoxia following embolization on the immunosuppressive TME and establish sustainable effects in stimulating the cancer immune cycle, thereby enhancing the efficacy of systemic therapy. During this time, substantial quantities of tumor antigens activate the cancer immune response, while reduced hypoxia permits remaining tumor cells to remain functionally quiescent and avoid proliferation.

Rationale for TCR repertoire in combination therapy

With tumor necrosis following TACE, a mass of neoantigens is released, triggering the recognition and expansion of T cells with specific tumor neoantigen TCRs, thereby increasing the diversity and clonality of the TCR repertoire.[71] Subsequently, the ischemic and hypoxic microenvironment after embolization drives tumor immunosuppression and immune escape. The immunoediting hypothesis posits that pro-inflammatory

responses exert selective pressures that force tumors to "evolve" to avoid detection, which includes downregulation of antigenic protein expression and reduced antigen-presenting potential.[100] Tumors may become dormant after TACE but later reactivate with mutations that render them less visible to the immune system. T cells evolve alongside these tumor mutations, leading to specific TCR repertoires. Prolonged exposure to neoantigens can cause cytotoxic T lymphocytes to become exhausted, resulting in decreased function and continued expression of inhibitory receptors.[101] Furthermore, tumor cells activate tolerance mechanisms by expressing self-antigens, depleting antigen-specific T cells.[102] They also downregulate MHC-I expression, which hinders antigen-presenting cell activation and leads to suboptimal activation of tumor-specific T cells.

Variations in T cell populations within the TME and peripheral blood are pivotal for anti-tumor immunity. Studies show that recruiting extra-tumor T cells is critical for ICI response.[103] In mice, T cell proliferation and activation were prevalent in tumor-draining lymph nodes and peripheral blood after PD-1 blockade.[104] Compared to the TME, there was an amplification of peripheral tumor-reactive TCR repertoires in melanoma patients treated with ICIs.[105] Yost *et al*. proposed a model suggesting a connection between the tumor-exogenous T-cell response to PD-1 blockade and the cancer-immune cycle, arguing that the reactivation of tumor-exogenous T cells and preexisting TILs work synergistically to enhance anti-tumor immune clearance during ICI treatment.[103] Previous research revealed that multiple preexisting intra-tumor T cells in patients receiving ICIs failed to exhibit clonal expansion, and preexisting depleted T cell clones remained without reverting to a non-depleted phenotype.[106] T-cell clones that experience expansion within the tumor are shared with both adjacent normal tissue and peripheral blood; however, peripheral blood lacks exhausted TIL clones, indicating that peripheral T cells may serve as a complementary source of non-exhausted TILs.[107]

Altogether, the release of neoantigens due to tumor necrosis and the immune evasion mechanisms alter T cell profiles after triple therapy. Monitoring the TME and peripheral blood TCR repertoire is crucial for assessing the response to and prognosis of combination therapy.

Application of TCR repertoire in the treatment of HCC

TCR repertoire facilitates the development of diagnostic biomarkers and therapeutic strategies

As tumors advance, T cells co-evolute with neoantigens, leading to distinct TCR repertoires. Identifying diverse CDR3 profiles and TCR clonal expansion through TCR-seq is important for predicting HCC progression and treatment outcomes. The TCR repertoire differs between adjacent and tumor tissues in HCC patients, with diminishing diversity as the disease progresses.[108] Li *et al*.[109] observed an increase in the clonality of TCRs in tumor tissue and peripheral blood as the cancer stage progresses. Chen *et al*.[110] found higher levels of certain TCR genes in tumor tissues of patients with HBV-associated HCC compared to adjacent normal tissues. Recent studies indicate that while TRBV CDR3 diversity is similar in tumors and normal tissues, TRBV16 and TRBV7-6, along with various TRBVx/BJx combinations, are more common in tumors.[111] Another study reported higher usage of eight specific Vβ-Jβ pairs in HCC tumor tissue.[19] Additionally, peripheral TCRβ-V-J pairing in HCC shows promise as a non-invasive diagnostic biomarker.[112] Overall, TCR V-D-J rearrangements and specific CDR3 sequences may help differentiate tumors with invasive or metastatic potential. More research is required to understand how clonality and CDR3 sequence diversity influence tumor initiation and progression.

Identifying and defining the immune response to TAAs is a prerequisite for developing cell-based immunotherapy. Introducing genes encoding tumor-specific TCRs to direct patient-derived T cells to target antigens is crucial for tumor immunotherapy.[113] Huang and colleagues[114] identified TCRVβ 7.11_h3f7 as a potential specific target gene for HCC by analyzing TCR Vβ subfamily cloning and sequencing. Furthermore, they demonstrated that peripheral blood mononuclear cells (PBMCs) transfected with the TCRVβ7.1_H3F7 gene displayed targeted killing of HCC cells. The adoptive transfer of PBMCs exhibited significant inhibition of HCC progression in animal models.[114] Transferring TCR gene sequences into T cells to create TCR-T cells that can specifically target tumor cells is a promising strategy.[115] High-affinity TCRs were isolated from TILs or TSA/TAA peptide-induced healthy donor T cells. These T cells were expanded, and their TCR α and β chains were cloned into target T cells for specific tumor antigen recognition (Fig. 3).[116] HBsAg-specific affinity-improved TCR-T cells showed increased sensitivity and cytotoxicity against HCC.[117] Engineered mucosa-associated invariant T cells with HBV-specific TCRs effectively target and destroy HBV-infected hepatocytes.[118] Furthermore, TCR-T cells targeting HLA-A2/α-fetuin amino acids 158-166 exhibited promising anti-tumor efficacy.[119] Table 4 summarizes information on clinical trials of TCR-T therapy in HCC. Altogether, the identification of high-affinity TCR repertoires presents a promising novel avenue for immunotherapy and precision medicine in the treatment of HCC.

TCR repertoire serves as a promising platform to predict the prognosis of HCC

The characterization of the HCC TME and peripheral T-cell repertoires through TCR-seq offers potential biomarkers for prognostic prediction. Lin *et al*.[19] found a positive correlation between the extent of overlap in TCR repertoires between tumor tissue and adjacent tissue and the prognosis of HCC. Theoretically, the evolution of TCR repertoires is closely associated with the development of mutant neoantigens, and an increased overlap in TCR repertoires between paired tissues indicates a reduced burden of tumor mutations and a better prognosis. Additionally, non-tumor-specific TCRs mixed with numerous tumor neoantigen-specific TCRs increase the diversity of T cells, partly explaining the association between TCR diversity and limited prognosis.[120] Compared to non-recurrent patients, recurrent HCC patients showed higher TCR richness in non-tumor tissues and inferior evenness in tumor tissues.[108] Another study found that non-relapsed patients possessed more shared TCR clonotypes in both tumor tissue and PBMC, and the higher frequencies of the top 100 CDR3 sequences were correlated with favorable prognosis.[109] Song *et al*.[121] found a correlation between the high chemical complementarity of TRB CDR3 amino acid sequences in HCC and HBV epitopes with improved prognosis. Additionally, a study demonstrated the predictive value of TCR Vβ-Jβ sequences in the prognosis of NSCLC patients receiving adjuvant therapy, further supporting a prognostic model constructed based on specific V-J combinations.[122] Collectively, TCR repertoire represents a promising tool for predicting survival in HCC patients.

TCR repertoire can predict responses to therapy

Prior studies have demonstrated that the efficacy of TACE is at least partially related to treatment-induced innate immunity and adaptive immune regulation.[2,9] As previously

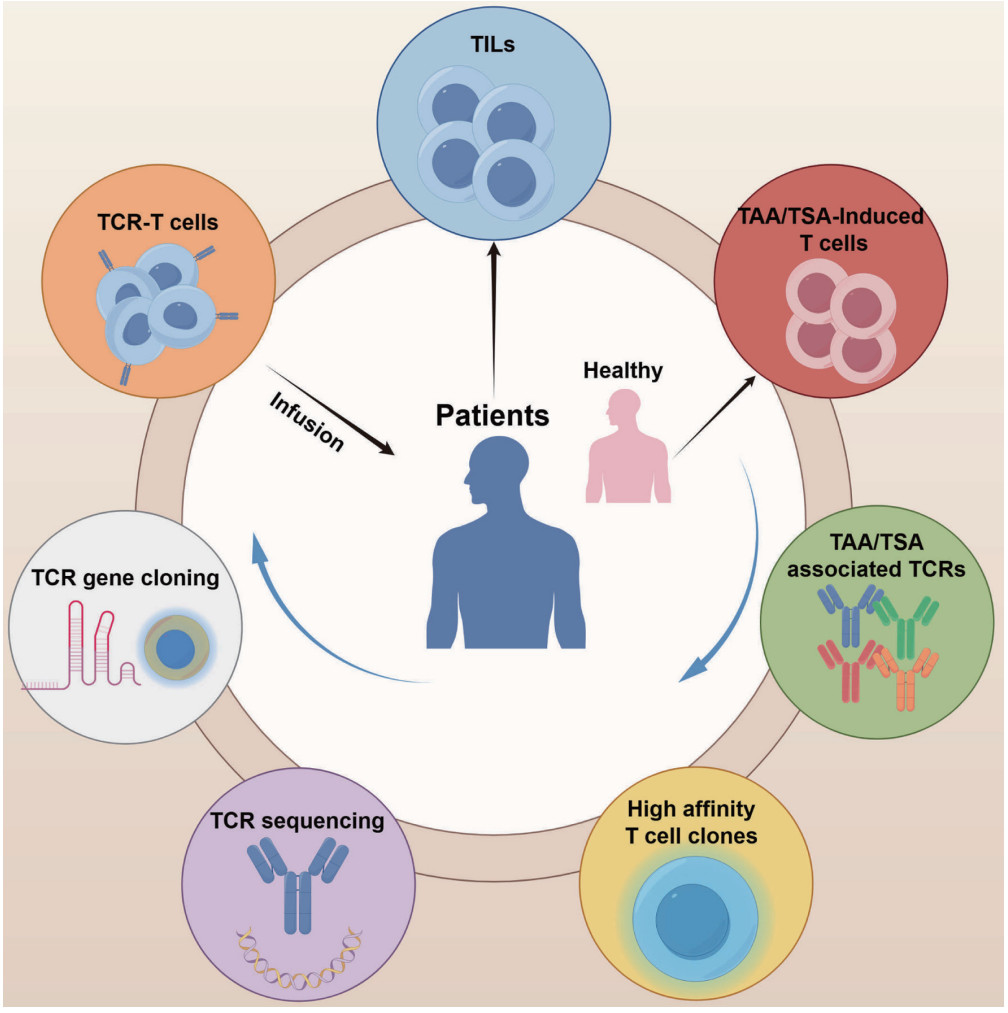

Fig. 3. Summary of TCR-T cell construction for clinical application. TAA, tumor-associated antigens; TSA, tumor-specific antigens; TILs, tumor-infiltrating lymphocytes; TCR, T-cell Receptor.

mentioned, TACE-induced ICD, pro-inflammatory cytokine secretion, and enhanced inflammatory pathways stimulate adaptive immune responses, with spontaneous TACE-induced T-cell responses indicating a favorable prognosis. Thus, characterizing the T-cell repertoires may provide valuable insights into the response to TACE. Prior studies have shown alterations in the clonability of TILs post-TACE, but a definitive correlation between TACE and T cell clonability in tumor infiltration remains unproven.[71] Single-cell analysis revealed that, post-TACE, tumor-infiltrating $CD8^+$ T cells segregated into distinct clusters, with tumor-specific TCRs mainly found in progenitor-exhausted and terminal-exhausted $CD8^+$ T cell populations. Additionally, numerous shared clonotypes were identified among $CD8^+$ T cell subclusters transitioning toward exhaustion phenotypes. Nevertheless, a distinct decrease in shared clonotypes was observed in $CD8^+$ T cell subclusters differentiated toward exhaustion phenotypes within the TACE group compared to the primary tumor group, indicating that TACE may impede $CD8^+$ T cell clone expansion.[73] Consequently, TCR repertoires prove valuable in elucidating the mechanisms of immunomodulation and anti-tumor immunity following TACE, as well as in forecasting therapeutic outcomes.

The combination of ICI therapy and TACE has been shown to effectively rejuvenate the function of exhausted TILs and enhance anti-tumor immunity.[123] The pre-treatment interaction between TCR repertoires and tumor-specific antigens in lymphoid organs promotes the proliferation of tumor-specific T cells, suggesting that the host's existing immune state influences treatment outcomes. Nevertheless, inhibitory receptor upregulation diminishes the anti-tumor cytotoxicity of preexisting tumor-specific T cells, and inhibiting these receptors effectively decreases the activation threshold for T cells stimulated by TCR signaling. After the administration of ICIs, T cells with low-affinity TCRs expand, enhancing T cell clone diversity.[124] Moreover, T cells exhibit a propensity to selectively enhance high-affinity TCR-ligand interactions during T cell responses, while ICIs typically hinder the expansion of cells with strong TCR affinity and promote the diversification of antigen-specific T cell populations. Conversely, inhibitory checkpoint blockade encourages the proliferation of TCR clones with high affinity for tumor-specific antigens, ultimately diminishing the diversity of TILs.[125] Patients with polyclonal TCR repertoires in baseline PBMCs are more likely to benefit from ICI therapy.[48] A study found that the clonality of total circulating TCR enhances the responses to anti-CTLA-4

Table 4. Primary clinical trials of T-cell Receptor (TCR)-T cell therapy in patients with hepatocellular carcinoma (HCC)

Clinical trials	Diseases	Phase	NCT number	Country/government registration	Primary endpoint
Redirected HBV-Specific T Cells in Patients With HBV-related HCC (SAFE-T-HBV) (SAFE-T-HBV)	Hepatocellular carcinoma	Phase 1	NCT04745403	Singapore General Hospital, Singapore	Safety evaluation of mRNA HBV/TCR T-cell treatment; Analysis of modifications of tumor microenvironment caused by mRNA HBV/TCR T-cell treatment
T Cell Receptor-Redirected T Cells Infusions in Subjects With Recurrent HBV-Related Hepatocellular Carcinoma in Post Liver Transplantation	Recurrent hepatocellular carcinoma	Phase 1	NCT02719782	The Third Affiliated Hospital of Sun Yat-sen University, Guangzhou, Guangdong, China	Safety of the TCR-T treatment
T Cell Receptor-Redirected T Cells With Recurrent HBV Treatment in Patients-Related Hepatocellular Carcinoma in Post Liver Transplantation	Recurrent hepatocellular carcinoma	Phase 1	NCT04677088	The First Affiliated Hospital of Sun Yat-sen University, Guangzhou, Guangdong, China	Safety evaluation of the TCR-T treatment
T Cell Receptor-Redirected T Cell Infusion For Prevention of Hepatocellular Carcinoma Recurrence in Subjects With Hepatitis B Virus-Related Hepatocellular Carcinoma Post Liver Transplantation	Hepatocellular carcinoma	Phase 1	NCT02686372	The First Affiliated Hospital of Sun Yat-sen University, Guangzhou, Guangdong, China	To evaluate the safety of the TCR-T treatment
EGFRvIII/DR5/NY-ESO-1/Mesothelin CAR-T/TCR-T Cells Immunotherapy for Solid Malignancies	Advanced - unresectable, relapse/refractory - recurrent hepatocellular carcinoma	Phase 1/Phase 2	NCT03941626	Henan Provincial People's Hospital, Zhengzhou, Henan, China	Number of Participants With Adverse Events evaluated with NCI CTC AE, version 4.0 (Safety evaluation)
AFP Specific T Cell Receptor Transduced T Cells Injection(C-TCR055) in Unresectable Hepatocellular Carcinoma	Hepatocellular carcinoma	Phase 1	NCT03971747	Fudan University Affiliated Zhongshan Hospital, Shanghai, China	Incidence of treatment-related adverse events as assessed by CTCAE v4.0
Personalized New Antigen Reactive Immune Cells (NRT) Combined With Radiotherapy for Advanced Hepatocellular Carcinoma Patients	Hepatocellular carcinoma	Phase 1/Phase 2	NCT03199807	The Affiliated Nanjing Drum Tower Hospital of Nanjing University Medical School, Nanjing, China	Number of participants with Adverse Events
TCR-Redirected T Cells Therapy in Patients with HBV Related HCC	Hepatocellular carcinoma	Phase 1	NCT03899415	Beijing 302 Hospital, Beijing, China	Safety evaluation based on Incidences of adverse events/serious adverse events

HBV, Hepatitis B Virus; SAFE-T-HBV, Safety and Tolerability Study of Redirected HBV-Specific T Cells in Patients with Hepatitis B Virus (HBV)-Related Hepatocellular Carcinoma; NCT, National Cancer Institute; CTACE, Common Terminology Criteria for Adverse Events; CTC, Common Terminology Criteria; AE, Adverse Events.

immunotherapy.[126] Maintaining diverse T-cell repertoires during immune responses allows for greater cross-reactivity to similar antigenic epitopes while avoiding escape by simply mutating a single antigen. Additionally, maintaining T cells expressing low-affinity TCRs may be critical for the production of specific cytokines required for tumor clearance.[125] Notably, the iterative application of ICIs leads to continuous modification of TCR repertoires, characterized by alterations in clonality, which may serve as a predictive tool for evaluating the efficacy of ICI therapy. Overall, the fluctuating T-cell repertoire during treatment offers valuable insights into monitoring and the mechanisms of anti-tumor immunity.

Although TCR repertoires hold promise for HCC applications such as biomarkers, prognostic markers, TCR-T therapies, immune microenvironment dynamics, and immunotherapy response evaluation, clinical management challenges remain. Previous studies showed that TILs derive from peripheral T cells, and liquid biopsies could help address tumor deficiencies and partially explain tumor heterogeneity and plasticity.[127] Although affordable liquid biopsy tubes allow for more frequent immunosurveillance in clinical practice, the sensitivity and accuracy of current assays remain

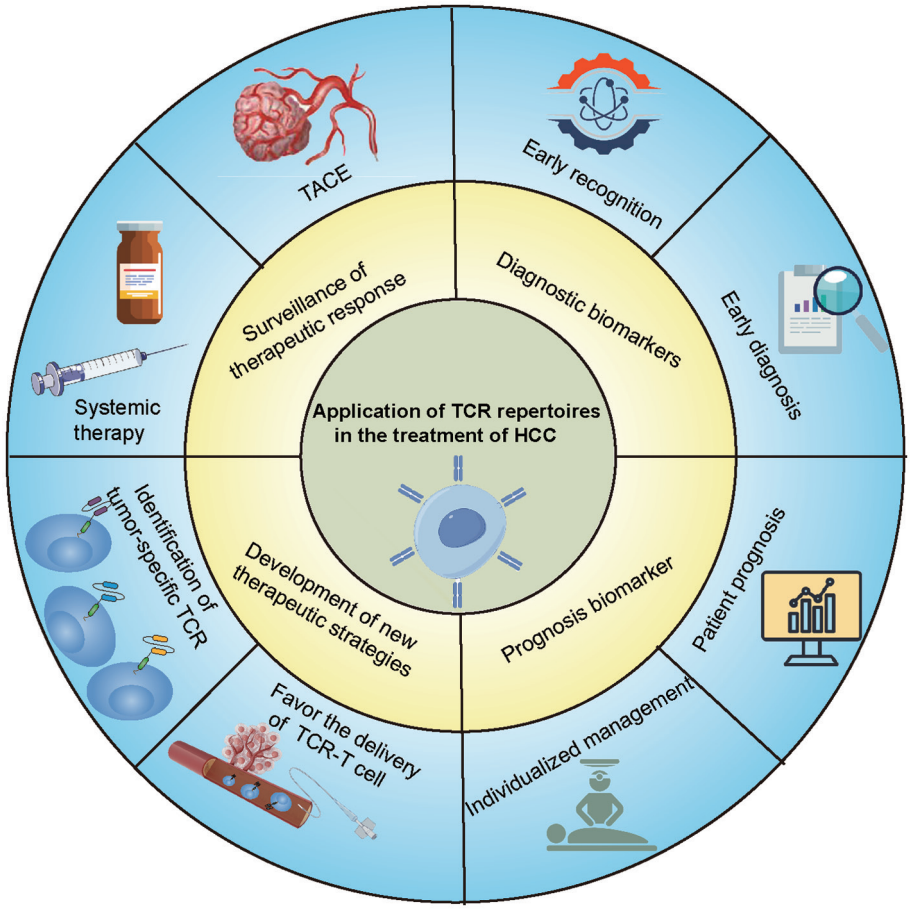

Fig. 4. Application of TCR repertoires in the treatment of HCC. TACE, transarterial chemoembolization; TCR, T-cell Receptor; HCC, hepatocellular carcinoma.

inadequate. Additionally, the correlation between peripheral biomarkers and intratumoral/intertumoral immune heterogeneity remains largely unclear.[128] However, TILs directly interact with tumors, making their TCR repertoire more indicative of responses to tumor-specific antigens. Therefore, combining peripheral blood and tumor tissue TCR-seq could enhance the prediction and treatment of tumor-specific TCRs. A study indicated that identifying overlapping top TCR clones between TILs and peripheral blood improved the prediction of response to cetuximab therapy.[18] Proverbially, tumors reveal a high degree of genetic heterogeneity. Even different regions of the same tumor exhibit varying genetic mutational heterogeneity, resulting in distinct neoantigen repertoires, which ultimately generate TCR repertoires of varying abundance and diversity.[129] Thus, to achieve complete TCR repertoires, multifocal and vascular cancer foci should be sequenced separately, enabling the identification of tissue-specific biomarkers. The model of tumor-exogenous T-cell response to PD-1 blockade highlights the importance of exogenous T-cells as reservoirs of TILs after tumor necrosis, synergizing with pre-existing TILs to enhance anti-tumor immunity.[103] Continuous monitoring of the peripheral T-cell repertoires is a better method than biopsies for evaluating treatment efficacy and disease progression after combination therapy. In clinical practice, TCR repertoires should be analyzed alongside biomarkers like PD-L1 expression and tumor mutational burden to improve immunotherapy prognostic markers.[130] Altogether, TCR repertoires demonstrate promise in predicting and monitoring therapeutic responses and guiding individualized therapy for patients with HCC (Fig. 4).

Future directions

TCR-seq and repertoire analysis offer novel insights into forecasting immunomodulatory mechanisms and therapeutic outcomes of TACE-based triple therapies within the framework of HCC, as well as the formulation of innovative treatment strategies. Clinical and preclinical studies have yielded promising results, and early clinical trials are currently underway. Nevertheless, as we move toward clinical application, it is essential to acknowledge and address potential challenges.

Despite advancements in TCR-seq prompting T-cell research, improving efficiency, sensitivity, and cost-effectiveness—especially in single-cell sequencing—remains imperative. Due to cost constraints, routine TCR-seq is mainly performed using bulk methods. Notably, the utilization of microfluidics holds great potential for enhancing the efficiency and cost-effectiveness of single-cell TCR-seq.[131] Beyond technical challenges, there is a growing interest in integrating spatial transcriptomic two-dimensional information for profiling TCR repertoires, thus achieving *in situ* resolution of T-cell specificities and phenotypes.[132] Additionally, the utilization of lineage tracing alongside TCR analysis to elucidate T cell developmental trajectories and phenotypic plasticity holds great potential for dissecting the varied responses to HCC treatment and the mechanisms involved in remodeling

the immune microenvironment with enhanced resolution.[133] However, immense costs limit the clinical application of these strategies. Furthermore, identifying antigen-specific TCRs faces multiple challenges: first, the frequency of antigen-specific T cells is extremely low[134]; second, the polymorphism of MHC, multispecificity of TCRs, and multiple potential epitopes arising from a single antigen increase the difficulty of resolving the antigen specificity of TCRs[135]; finally, the weak affinity of TCRs to MHCs hampers the selective isolation of antigen-specific T cell populations.[135] On the other hand, considering various tumor antigens and the complex TCR-antigen recognition process, the effectiveness of TCRs in monitoring specific sequences is restricted.[136] Therefore, future work to address these challenges is imperative for advancing TCR-seq in HCC therapy.

Dissecting the heterogeneity of HCC is essential for comprehending and forecasting tumor progression, evaluating treatment responses, and formulating new therapeutic strategies. As an inherent characteristic of tumors, the development of heterogeneity involves variations in the activity of multiple oncogenes and signaling pathways. The multitude of mutations and molecular mechanisms present in these pathways and genes causes various somatic alterations, influencing the diverse behaviors of tumors and responses to therapy.[137] Within the context of tumor immunoediting, tumor cells undergo constant mutational evolution and produce numerous TAAs. While most TAAs are detected, some evade recognition due to a lack of specific TCRs, allowing tumor immune escape, a process that advances tumor heterogeneity.[100] The study of the TIME following TACE has shown that CD8 T cell clonal expansion was impeded after TACE. Additionally, clusters of $CD8^+$ T cells in various functional states exhibit numerous shared TCR clonotypes, indicating the continuous evolution of CD8 cells coinciding with the release of neoantigens.[73] In another study, researchers discovered that immune cells exhibit spatial heterogeneity, transitioning from normal tissue to the leading edge and into tumor regions via single-cell-scale time-of-flight mass cytometry. Further detailed analysis of the T-cell population determined that the leading edge region displays unique T-cell compositions, particularly enriched with double-positive T cells. Furthermore, findings derived from TCR trajectory analysis indicate that tumor-associated double-positive T cells may originate from single-positive T cells.[138] Similarly, HCC with multifocal lesions and intrahepatic metastases also showed significant spatial heterogeneity in the TIME.[139] Overall, the progression of cancer concerning immune regulation is considered a "hallmark of cancer," and the TIME, characterized by its temporal and spatial diversity, partially elucidates the origins of tumor heterogeneity.

TCR-T therapy represents a promising tumor immunotherapy strategy and signifies an advancement in precision medicine. Nevertheless, some inevitable hurdles limit its applications. First, regarding "target antigen selection," TCR-T cells can target both surface and intracellular antigens on tumor cells, allowing for a wider range of targets.[116] Notably, only high-affinity neoantigens can induce phenotypic differentiation and infiltration of primed effector T cells.[120] Secondly, concerning "nonspecific cytotoxicity," the current method of TCR-T delivery, which relies on whole-body circulation, has shown unexpected toxicity attributed to the targeting of TAAs that are overexpressed in tumor cells but minimally expressed in healthy tissue, potentially causing autoimmune reactions. Focusing on neoantigens generated by mutations in tumor genes may enhance TCR-T therapy efficacy by minimizing nonspecific cytotoxic effects.[119] Moreover, leveraging the vascular pathway through TACE and the precise delivery of designated TCR-T cells to the tumor site improves tumor control while minimizing side effects. Thirdly, the "inhibitory TME", along with reduced chemokine expression and increased intercellular adhesion molecules, impedes T cell infiltration within the TME by influencing T cell migration and adhesion. Furthermore, the hypoxic TME increases immune checkpoint molecule expression, causing T cell exhaustion and impairing T cell functionality and cytokine secretion capabilities.[140] A recent study shows that IL-21 signaling boosted the anti-tumor efficacy of AFP-TCR-T by increasing TCR-T cell proliferation, promoting memory differentiation, reducing PD-1 expression, and decreasing apoptosis.[141] Consequently, combined targeted systemic therapy may be a potential strategy to improve the efficacy of TCR-T therapy. Finally, the high cost of TCR-T therapy severely limits its clinical application and promotion. Nevertheless, TCR-T therapy remains a promising strategy for HCC treatment. Future research targeting the identification of highly specific target antigens, minimizing adverse effects, and exploring combination therapies to overcome drug resistance represents areas of interest and potential advancement.

Conclusions

The TCR repertoire provides a novel platform for investigating the modulation of the TME post-TACE and the underlying mechanisms governing tumor development and recurrence. Clarifying the distinctive metrics and dynamic alterations of the TCR repertoire within the context of combination therapy is imperative for understanding the mechanisms of antitumor immunity, assessing efficacy, and further advancing precision oncology in the treatment of HCC.

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Conflict of interest

The authors have no conflict of interests related to this publication.

Author contributions

Provided direction and guidance throughout the preparation of this manuscript (ZL, XL, BW), wrote and edited the manuscript (JL, HY), reviewed and made significant revisions to the manuscript (YZ, LH), collected and prepared the related papers (XL, YL, SY, XY). All authors reviewed and approved the final version and publication of the manuscript.

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