Aging Research

ISSN 2957-8701 (print); 2957-9295 (online)
https://doi.org/10.26599/AGR.2026.9340064

Review

Research progress in enhancing the precision of TCR-T cell therapy targeting cancer-testis antigens for cancer treatment in elderly patients

Zhicheng Cai[1], Yanhao Huang[1,2], Yan Han[3], Zhangyuanzhu Liu[4] (✉), Wei Jiang[3,5] (✉)

[1] *Department of Microbiology and Immunology, School of Medicine; Institute of Geriatric Immunology, School of Medicine, Jinan University, Guangzhou 510632, China*
[2] *The First Affiliated Hospital, Jinan University, Guangzhou 510632, China*
[3] *Affiliated Hospital of Xiangnan University, Chenzhou 423000, China*
[4] *Department of Hepatobiliary Surgery, Guangdong Province Traditional Chinese Medical Hospital, Guangzhou 510180, China*
[5] *Department of General Surgery, Guangdong Provincial Key Laboratory of Precision Medicine for Gastrointestinal Tumor, Nanfang Hospital, Southern Medical University, Guangzhou 510515, China*

Received: 13 October 2025 | **Revised:** 16 December 2025 | **Accepted:** 6 January 2026

ABSTRACT

The elderly population in China is growing increasingly, and elderly individuals are typically characterized by high incidence and mortality rates of cancer. In recent years, adoptive cellular immunotherapy has demonstrated reliable efficacy in cancer treatment. T cell receptor-engineered T (TCR-T) cell therapy, a form of adoptive cellular therapy, demonstrates considerable promise in the treatment of solid tumors. However, the current TCR-T cell-related technologies still face numerous challenges when confronted with issues such as the decline in T cell efficacy and diversity, as well as changes in the tumor microenvironment in elderly cancer patients. Meanwhile, TCR-T cell therapy itself is plagued by the significant challenge of off-target cross-reactivity. To tackle these challenges and develop effective treatment regimens for elderly cancer patients, this review focuses on improving the precision of TCR-T cell therapy and introduces a series of emerging TCR-T-related technologies targeting cancer-testis antigens from various aspects, aiming to offer new strategies for the development of TCR-T cell therapy.

KEYWORDS

T cell receptor-engineered T (TCR-T), cancer-testis antigens, cancer treatment, elderly patients

1 Introduction

China is a country with a large elderly population, and the aging trend is continuing to intensify. Relevant studies predict that by 2050, the population aged 65 and above in China will reach 365 million, accounting for 26.1% of the total national population [1]. However, the incidence of cancer among individuals aged 65 and above is higher than that among younger people. With the continuous extension of life expectancy and the intensification of population aging, both the incidence and mortality rates of cancer in the elderly are expected to increase further [2]. However, over the past few decades, the mainstream tumor treatment methods—surgery, radiotherapy, and chemotherapy—have failed to significantly reduce cancer incidence and mortality, while often compromising patients' quality of life severely due to drug resistance and complications [3–7]. Therefore, the development of new therapeutic approaches is particularly crucial.

T cells can recognize tumor antigen–major histocompatibility complex (MHC) complexes on the cell surface through their surface T cell receptors (TCRs), thereby activating T cells and achieving tumor cell killing. Cancer-testis antigens (CTAs) are a class of antigens encoded by specific genes. These genes are generally not expressed in normal tissues, except in human placental trophoblast tissues and testicular germ cells; however, they are highly expressed in various malignant tumors, including melanoma, bladder cancer, lung cancer, and liver cancer [8]. Since testicular germ cells lack human leukocyte antigens (HLAs), they cannot be recognized by T cells [9]. Currently identified CTAs include

✉ Address correspondence to Zhangyuanzhu Liu, liuzhangyuanzhu@163.com; Wei Jiang, jiangweinf@163.com

Cite this article as Cai, Z. C., et al. *Aging Research*, **2026**, 4: 9340066.

the Melanoma Associated Antigen (MAGE) family, New York Esophageal Squamous cell Carcinoma 1 (NY-ESO-1), the synovial sarcoma on the X chromosome (SSX) family, and the G Antigen Gene (GAGE) family, preferentially expressed antigen in melanoma (PRAME) [10–14] (Table 1).

Over the past decade, adoptive immunotherapy has demonstrated tremendous potential in cancer treatment, particularly in the field of T-cell engineering technology [15]. T-cell engineering technology mainly includes CAR-T cell immunotherapy and TCR-T cell immunotherapy these approaches primarily genetically modify T cells to enable the engineered T cells to specifically recognize and eliminate tumor cells.

Among these, CAR-T cell therapy has proven reliable and effective in the treatment of hematological malignancies [16, 17]. However, its efficacy in the treatment of solid tumors is limited. In contrast, TCR-T can recognize a broad range of potential antigen targets, exhibit more durable killing efficacy, and require a lower density of antigenic epitopes [18].

The development and preclinical evaluation of TCR-T cells typically involve several key stages: first, antigen screening; second, engineering design and manufacturing of TCR-T cells; subsequently, *in vitro* and *in vivo* validation of the prepared engineered TCR-T cells; and finally, biodistribution studies and cross-reactivity tests—only after passing these assessments can the cells be used in clinical trials [19] (Fig. 1).

Among CTAs, the MAGE family and NY-ESO-1 have been extensively studied as tumor targets in TCR-T therapy. The MAGE family is divided into two major subclasses: MAGE-I antigens and MAGE-II antigens. MAGE-II antigens are widely expressed in normal human tissues, whereas only MAGE-I antigens conform to the characteristics of cancer-testis antigens [20, 21]. For instance, MAGE-A4, a member of the MAGE-I family, has been clinically validated as a reliable

Table 1 Introduction and evaluation of partial CTAs

CTA family	Expressed in tumors	Expressed in normal tissues	Clinical significance
MAGE [10]	Melanoma (MM), LC, BC, CRC, etc.	• Testis • Ovary • Placenta	Promoting immunotherapy for various tumors Serving as a marker for tumor progression and prognosis
NY-ESO-1 [11]	MM, epithelial ovarian cancer (EOC), prostate cancer (PCa), etc.	• Ovarian • Endometrial tissues • Adult spermatogonia • Primary spermatocytes	Of great significance in the development of cancer vaccines, targeted therapy, and adoptive cell therapy
SSX [12]	SS, MM, BC, LiC etc.	• The nucleus of spermatogonia • Spermatocytes	Serving as reasonable targets for drug and vaccine therapy
GAGE [13, 25]	MM, LC, BC, LiC, etc.	• Testicular germ cells	Associated with tumor invasiveness Having the potential to serve as a target for tumor immunotherapy
PRAME [14]	MM, BC, non-small cell lung cancer (NSCLC), etc.	• Testis • Ovary • Adrenal gland • Endometrium	Eliciting effective immune responses in melanoma, lung cancer, and other advanced solid tumors. In-depth research will contribute to the development of innovative immunotherapies for solid tumors

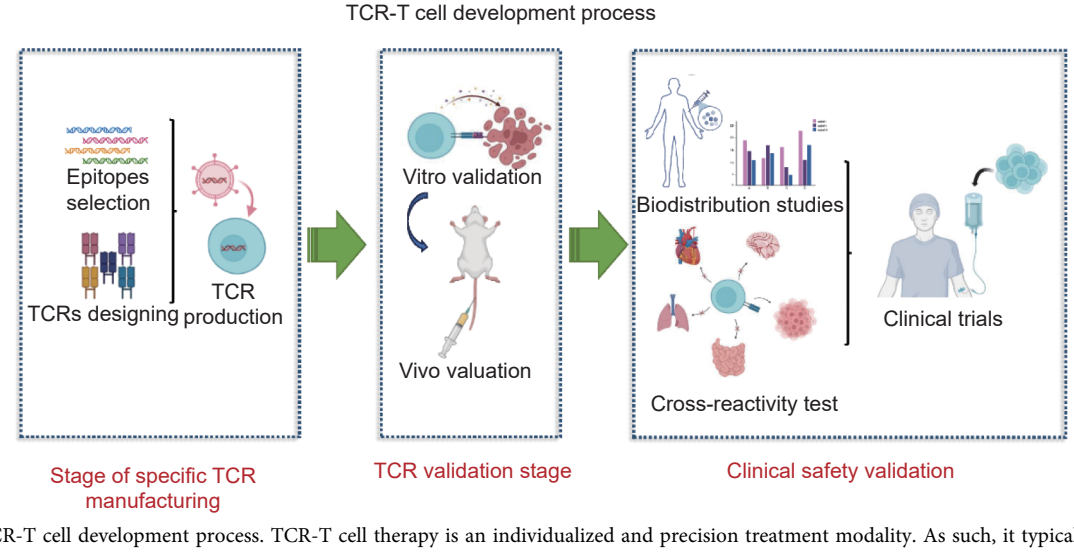

Figure 1 TCR-T cell development process. TCR-T cell therapy is an individualized and precision treatment modality. As such, it typically begins with the screening of tumor-specific antigens that are widely expressed in the patient's body, followed by the identification of TCRs that can bind to these antigens. To ensure the safety of the obtained TCRs, the tumor antigen-specific TCRs are expressed on the surface of T cells, and their functions are validated through cellular or animal experiments. Subsequently, rigorous clinical trials are required to rule out issues such as drug toxicity and cross-reactivity. Only after these steps can a TCR-T cell targeting a specific tumor antigen fragment be successfully developed [19].

TCR-T target [22]. Additionally, in 2015, Robbins et al. successfully treated synovial cell sarcoma and melanoma using TCR-T cell therapy targeting NY-ESO-1 [23].

With the increasing number of studies on the therapeutic efficacy of TCR-T in elderly cancer patients, it has been found that its therapeutic effect is often unsatisfactory [24]. The summarized reasons mainly include the impairment of T cell function and diversity in elderly individuals, changes in the tumor immune microenvironment, and potential threats of cross-reactivity. Therefore, this review mainly discusses and introduces some traditional and emerging technologies in the TCR-T cell therapy process, aiming to improve the therapeutic efficacy of TCR-T cells in elderly cancer patients by enhancing their precision.

2 The current status of TCR-T therapy technologies targeting antigens

The key to TCR-T cell therapy lies in screening out a pair of TCR and tumor antigen with the most appropriate affinity from a vast number of TCRs and tumor antigens. Based on this idea, previous researchers have developed a variety of technical methods. This section will briefly introduce some traditional TCR-T-related technical methods from three aspects: antigen epitope screening, expansion of antigen-specific T cells, and isolation of antigen-specific T cells, and objectively evaluate their limitations and reliability. These studies are helpful for medical researchers to gain inspiration and improve existing protocols, thereby exerting a positive impact on the development and progress of TCR-T immunotherapy in the future.

2.1 Epitopes selection

A critical aspect of TCR-T therapy lies in the TCR's targeting of MHC–antigen complexes carrying specific peptide epitopes to activate T cells. CTAs are typically composed of a broad range of amino acids, presenting only a short fragment on the cell surface. Therefore, identifying the optimal CTAs' epitope with the moderate TCR binding affinity is of paramount importance.

X-Scan is a widely adopted and empirically validated method for epitope screening. Its core principle involves systematically substituting each target residue of the antigen with all possible amino acids and individually assessing these variants to identify optimal target epitopes, cross-reactive epitopes, and TCRs with desired specificity [25]. However, this approach is inherently limited by its low success rate, significantly restricted epitope coverage, and low resolution [26].

An alternative conventional approach involves utilizing the Immune Epitope Database (IEDB) for antigenic epitope prediction. It is a publicly accessible database that integrates experimental data with machine learning algorithms to predict and store antigen epitope–MHC binding interactions. In a TCR-T therapy study targeting non-small cell lung cancer, researchers employed IEDB Recommended 2.22 to screen ten HLA-A2-presented MAGE-A3 epitopes, and subsequent experiments confirmed that the M4 epitope exhibited specific binding to the TCR [27]. However, a critical limitation of this epitope screening approach is its exclusive dependence on computational modeling for epitope prediction, without accounting for the complete physiological processing pathways of epitopes. This makes it incapable of measuring actual immune response data and prone to generating false-positive results.

2.2 Expansion of antigen-specific T cells

TCRs are pivotal protein molecules that recognize peptide-major histocompatibility complexes and initiate T cell activation. Given the immense theoretical diversity of TCRs (up to 1×10^{15}) [28], the enrichment of TCRs that are specific to target antigen peptides represents a critical and necessary step. In this section, we are introducing several conventional methods for expanding antigen-specific TCRs.

2.2.1 Antigen presentation by dendritic cells

Dendritic cells (DCs) play a pivotal role in antitumor immunity by capturing, processing, and presenting antigens to T cells [29]. Therefore, in tumor immunology, DCs are commonly employed to expand and activate antigen-specific T cells [30]. Current strategies include either receptor-mediated antigen delivery or DC genetic modification, both culminating in DC presentation of tumor-specific epitopes to cognate T cells, and significantly enhancing the activation and expansion efficiency of antigen-specific T cells [31, 32]. However, the antigen-presenting defects of DCs, their contradictions with immune cells, and the influence of the tumor microenvironment all contribute to the challenges and limitations faced by DC-based tumor antigen presentation technology in practical applications.

2.2.2 Recombinant protein expression technology

Recombinant protein expression technology refers to the genetic engineering approach of inserting a target protein gene into an expression vector to produce the desired protein in host cells.

As eukaryotic organisms, yeast systems offer superior capability for producing properly folded and functionally intact target proteins, particularly for complex eukaryotic proteins [33]. For example, in one experimental design, peptide (p)-MHC complexes and TCRs were expressed separately in two distinct yeast strains. Through the co-cultivation of these engineered yeast strains, researchers successfully achieved enrichment of antigen-specific TCRs [34].

Baculoviruses are also frequently used as antigen gene vectors because they can efficiently produce large quantities of soluble proteins in insect cells [35]. It has been reported that a p-MHC library was efficiently constructed using the baculovirus system, allowing researchers to compare the recognition preferences of two types of T cells [36].

However, recombinant protein technologies face inherent limitations in both stability and application scope due to their inability to fully recapitulate the complex antigen processing pathways of human cells *in vivo*. These constraints stem primarily from host-specific deficiencies across different expression systems, which collectively compromise protein stability and functional fidelity.

2.2.3 Expansion of human-specific T cells in humanized mice

During thymic selection in humans, T cells with high affinity for self-antigens are typically eliminated, whereas artificially

immunized and MHC-humanized mice can generate T cells with high affinity for human antigens. Therefore, it is feasible to enrich and expand T cells carrying antigen-specific TCRs in mice. Previous studies have reported attempts to generate Latent Membrane Protein 1 $(LMP1)_{166}$-specific $CD8^+$ T cells in HLA-A*0201 transgenic mice for the treatment of Epstein-Barr virus (EBV)-associated tumors [37]. However, humanized mouse models may lead to complications such as graft-versus-host disease (GVHD) or excessive mobilization and depletion of hematopoietic stem cells (HSCs) [38].

2.3 Isolation of antigen-specific T cells

The expanded antigen-specific T cells typically need to be isolated from other eliminated cell populations for further validation. Currently, widely used methods include fluorescence-labeled tetramer staining combined with flow cytometry sorting or magnetic bead-based cell isolation [39, 40]. Both methods maintain high accuracy and efficiency (Table 2).

3 Challenges of TCR-T therapy in the treatment of elderly patients

The efficacy and prognosis of immunotherapy in elderly cancer patients are often suboptimal due to the decline in immune function [24] and the insufficient precision of TCR-T cells. This section examines the detrimental impacts of attenuated T cell function, altered tumor microenvironment, and cross-reactivity on TCR-T immunotherapy efficacy in elderly patients. These findings will actively guide future medical research efforts to continuously refine and improve the therapeutic efficacy of TCR-T cell therapies for elderly cancer patients.

3.1 Reduced T cell ability and diversity

T cells play a pivotal role in human tumor immunity. However, as the human body ages, both the functionality and proliferative capacity of T cells decline [41]. For instance, studies have demonstrated that age-associated impairments in extracellular signal-regulated kinases 2 (ERK2) and c-Jun NH2-terminal kinases (JNK) activation further diminish Interleukin-2 (IL-2) secretion in human T cells from elderly donors [42]. Another study revealed that senescence-induced downregulation of EPAS1 expression compromises the antitumor efficacy of $CD8^+$ T cells in adoptive immunotherapy against solid tumors [43].

The diversity of the TCR repertoire is also essential for the protective functions of the adaptive immune system [44]. Thymic selection constitutes a pivotal determinant shaping the diversity of the TCR repertoire. However, with advancing age, thymic activity progressively declines and eventually ceases, resulting in diminished TCR repertoire diversity in elderly individuals compared to younger populations [45, 46].

Collectively, these findings demonstrate that age-associated T cell dysfunction negatively impacts the therapeutic efficacy of TCR-T cell immunotherapy in elderly cancer patients.

3.2 Modulation of the tumor microenvironment

Tumor cells reside in the tumor immune microenvironment (TME). The TME collectively regulates the intensity and direction of tumor immune responses through mechanisms such as immune cell dysfunction, cytokine network disorder, deterioration of metabolic environment, physical structural barriers, and activation of immune checkpoint molecules [24, 47–50]. The decline in tumor immune efficacy in the elderly is usually associated with changes in the tumor microenvironment. For example, studies have shown that within the myeloid-derived suppressor cell family in the tumor immune microenvironment of elderly individuals, there exists a group of heterogeneous and partially immature cells with strong immunosuppressive functions [51]. Meanwhile, the tumor microenvironment in the elderly can also upregulate pro-inflammatory factors and exacerbate chronic inflammation, thereby increasing the risk of tumorigenesis [52]. Elderly individuals typically have a higher number of senescent T cells [53], and in the tumor microenvironment, these senescent T cells are prone to being induced into inhibitory cells, which exert direct suppressive effects on various types of immune cells and thus become "accomplices" in tumor escape [54].

These studies indicate that changes in the tumor microenvironment of elderly individuals can exacerbate the suppression of immune responses and the escape of tumor cells, making TCR-T immunotherapy more challenging. This also provides a direction for future medical researchers to explore the artificial regulation of the tumor microenvironment to enhance the efficacy of tumor treatment.

Table 2 Current technologies of TCR-T cell therapy targeting antigens

Process	Technology	Evaluation
Epitope screening	X-Scan	• Low resolution • Low success rate • Markedly restricted epitope coverage
	IEDB	• Prone to false positives. • Cannot measure actual immune responses
Antigen-specific T cells expansion	Dendritic cell presents epitopes	• Facing serious problems due to inherent defects and the influence of the immune environment • Facing stability and application scope limitation • Leading to complications
	Recombinant protein expression technology	
	Expansion of human-specific T cells in humanized mice	
Antigen-specific T cell isolation	Magnetic bead-based isolation	• High accuracy and efficiency
	Fluorescence tetramer staining cytometry sorting	

3.3 Cross-reactivity

Currently, the clinical cross-reactivity of TCR-T cells targeting CTAs mainly occurs in MAGE family. Members of the MAGE family exhibit considerable homology in sequences including the EVDPIGHLY epitope [55]. In TCR-T cell therapies targeting the MAGE family, to enhance the affinity for a specific MAGE family member, researchers often modify the structure of TCR through genetic modification techniques. However, such modifications may cause the TCR with increased affinity to mistakenly recognize normal human tissues, resulting in severe consequences. For example, in one study, researchers introduced four mutations into the Complementarity-Determining Region 2 (CDR2) of the α-chain of an HLA-A*01-restricted MAGE-A3 peptide (EVDPIGHLY)-specific TCR, while retaining the wild-type (WT) sequence in the β-chain. However, in subsequent clinical trials, these engineered TCRs mistakenly recognized an unrelated peptide derived from TITIN, a striated muscle-specific protein, leading to cardiogenic shock in two patients and eventually resulting in their death [56]. In another study, researchers substituted alanine with threonine at position 118 of the α-chain in the Complementarity-Determining Region 3 (CDR3) of the TCR targeting MAGE-A3:112–120, which was obtained from human HLA-A*0201 transgenic mice, aiming to enhance its functional affinity in CD4 and CD8 cells [57]. However, in subsequent clinical trials, these engineered TCRs recognized the MAGE-A12 protein expressed in subsets of human brain neurons and activated immune cells to attack the white matter of the brain, resulting in the death of two patients [58].

These experimental results serve as a warning that we must exercise caution in evaluating and modifying TCR affinity, as well as in screening specific antigen epitopes. This also indicates that developing various more reliable methods to assist TCRs in targeting the CTAs with appropriate affinity holds great potential for the future clinical application of TCR-T cell therapy in treating cancer patients (Table 3).

4 Innovative solutions

Given the limitations of conventional TCR-T cell therapy-related technologies, along with the difficulties and challenges faced when applying TCR-T cell therapy to elderly patients, we will focus on enhancing the precision of engineered TCRs in targeting CTAs' epitopes as a key breakthrough. In this chapter, we will present several innovative CTAs' epitopes screening technologies and specific T cell enrichment techniques associated with TCR-T cell therapy. It is anticipated that these technical approaches will provide new technical avenues and methods, and precision therapy will also offer novel pathways for clinical personalized treatment.

4.1 Steady and measured: Strengthening the catch bonds

At the beginning of this century, studies already revealed that the TCR, acting as a mechanosensory, can activate a series of signaling pathways in T cells when it is subjected to mechanical force during the process of binding to p-MHC. [59, 60]. In further research, Baoyu Liu and others found that applying mechanical force to a single agonist TCR–p-MHC bond forms a "catch bond" to prolong its lifespan. Both the magnitude and duration of the mechanical force affect the lifespan of the TCR–p-MHC bond. Only by rapidly accumulating multiple catch bond events within a short time can the signaling pathway of T cells be effectively activated [61]. Wu and colleagues found that cancer-related somatic mutations can inhibit the formation of TCR–p-MHC catch bonds, thereby impairing T cell activation and enabling cancer cells to evade T cell immune surveillance [62]. In a complementary study, Qin et al. elucidated that CD8-mediated amplification of T cell activation signals occurs only when TCR forms a stable catch bond with p-MHC. Notably, either excessively high or low mechanical force exerted on the TCR impairs the synergistic effect of CD8 (Fig. 2). This finding provides a mechanistic framework from the perspective of mechanical–molecular coupling, explaining both how T cells achieve precise antigen discrimination and why TCRs with excessively high affinity tend to exhibit off-target effects [63].

Building on the above research findings, Zhao and colleagues sought to take the engineering of TCR catch bonds as a breakthrough, developing a TCR with low affinity for tumor antigens yet high efficacy. They introduced residue mutations into the α-chain and β-chain of TCR55—a TCR that binds to the HIV peptide presented by HLA-B35 MHC molecules but fails to effectively activate T cells [64]—and constructed a corresponding library. Subsequently, through *in vitro* experiments, they sorted cells with co-staining of low p-MHC tetramer staining and high activation antigen CD69 expression to enrich "low-affinity/high-efficacy" TCR mutants. Eventually, they found that single amino acid positions present on both the α and β chains, acting as catch bond hotspots, can enable TCR mutants to trigger stronger

Table 3 Challenges of TCR-T therapy in elderly patients

Occurrence		Result
Reduced T cell ability and diversity	The functionality of T cells declines [42]	Reducing the ability efficacy of TCR-T cell immunotherapy
	The diversity of T cells declines [46–47]	
Modulation of the TME	There is a group of cells with immunosuppressive functions [52]	Inhibiting immune responses and promote immune escape of tumor cells
	Upregulation of proinflammatory factors increases the risk of tumorigenesis [53]	
	Senescent T cells inhibit other immune cells [55]	
Cross-reactivity	Cross-react with unrelated protein epitopes expressed in cardiac tissue [57]	Severe adverse reactions or even deaths have occurred in clinical patients
	Cross-react with MAGE-A12-derived epitopes in brain tissue [59]	

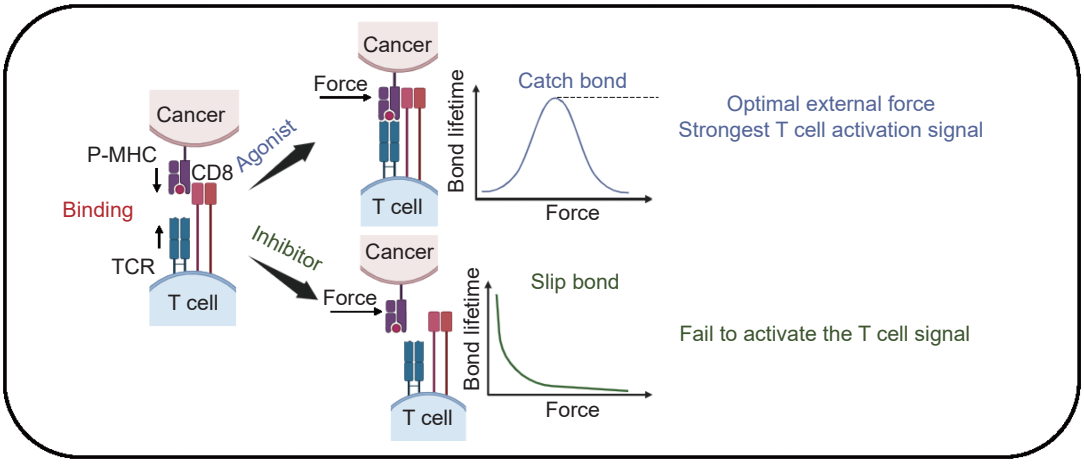

Figure 2 Introduction to the principle of T cell activation via capture key application. Upon binding of the TCR to p-MHC, the application of mechanical force to individual agonist TCR-p-MHC bonds results in the formation of "catch bonds", whereas the application of mechanical force to individual antagonist TCR-p-MHC bonds leads to the formation of "slip bonds". Catch bonds can prolong the lifespan of TCR-p-MHC bonds, while slip bonds exert the opposite effect [61]. The formation of optimal catch bonds and effective T cell activation can be achieved by accumulating sufficient and appropriate mechanical force on the TCR within a short period. Conversely, either excessive or insufficient mechanical force will reduce the lifespan of catch bonds [63].

signal transduction, thereby enhancing their immune function. To promote its clinical application, Zhao and colleagues applied the design strategy of TCR55 to engineer the catch bonds of A3A TCR—a TCR previously reported to cross-react with the TITIN peptide expressed in cardiovascular tissues [56]. They ultimately isolated several TCR mutants with high efficacy yet low affinity for the MAGE-A3 antigen, and found that their cross-reactivity with cells expressing the TITIN peptide was negligible [65].

In summary, studies on how mechanical forces influence TCR-mediated tumor immune responses have provided a new research direction for the precise treatment of TCR-T in the future, and hold valuable guiding significance for the design and modification of engineered TCRs.

4.2 Dimension elevation strike: TCRcost

In the binding process between p-MHC and TCR, the critical region responsible for recognizing antigenic peptides is the CDR3 of the TCR [66]. Therefore, predicting the binding structure between the CDR3 region and peptide fragments is of great significance for TCR-T cell therapy in treating cancer.

TCRcost is a deep learning framework capable of predicting TCR–peptide binding based on TCR–peptide structures, consisting of a structure correction module and a binding prediction module. The correction module enhances structural accuracy and prevents inter-chain interference by adjusting the spatial positions of each atom. It utilizes one-dimensional convolutional neural network (1DCNN) to extract relationships between each adjacent atom, followed by the use of long short-term memory (LSTM) to correct atoms in the main chain and side chains, to combine the two chains into a complete structure, and finally to perform another correction via the LSTM model. The prediction module, on the other hand, extracts information from the atomic features of each atom and the corrected the three-dimensional (3D) coordinates through 3D convolutional neural network (3DCNN), and then uses a fully connected layer to predict TCR-peptide binding. Compared with the original predicted structure, the average accuracy of the corrected structure for TCR-peptide binding prediction using TCRcost increased from 0.375 to 0.762; and the average accuracy of the precise structure was as high as 0.974 [67].

The innovation of TCRcost lies in its deep integration of the three-dimensional structural information of TCR into the deep learning framework. It precisely corrects structural errors and eliminates inter-chain interference through a dedicated structure correction module. Furthermore, by incorporating 3DCNN, it efficiently extracts the three-dimensional spatial interaction features between atoms in the corrected structure. Ultimately, this significantly improves the accuracy of TCR-peptide binding prediction, overcomes the limitations of traditional prediction methods that solely rely on amino acid sequences, and provides a reliable tool for the precise discovery of tumor-specific antigens and their corresponding TCRs.

4.3 Serve as a bridge: ImmTAC

T cell engagers (TCE) are a novel class of bispecific fusion proteins that redirect T cells. Acting like a "bridge", one end binds to antigens on the surface of tumor cells, while the other end binds to CD3 molecules on the surface of T cells, enabling direct activation or inhibition of the immune system [68]. For example, immune mobilising monoclonal TCRs Against Cancer (ImmTAC), as a special type of TCE, combines high-affinity TCRs with optimized anti-CD3 single-chain variable fragments (scFv) in a soluble form. It can effectively redirect T cells to tumor cells that present only 10–50 peptide-HLA (p-HLA) antigens on their surface, and exhibits extremely high affinity and specificity [69] (Fig. 3). Thus, even when the immunosuppressive properties of the TME impair the antigen recognition and activation functions of endogenous TCRs, TCEs can still effectively enhance the tumor-targeting ability and activation efficiency of T cells through their designed properties [70].

ImmTAC has demonstrated favorable performance in practical anti-tumor therapy. In a study on the treatment of metastatic uveal melanoma, Nathan and colleagues achieved positive outcomes in patients with uveal melanoma using

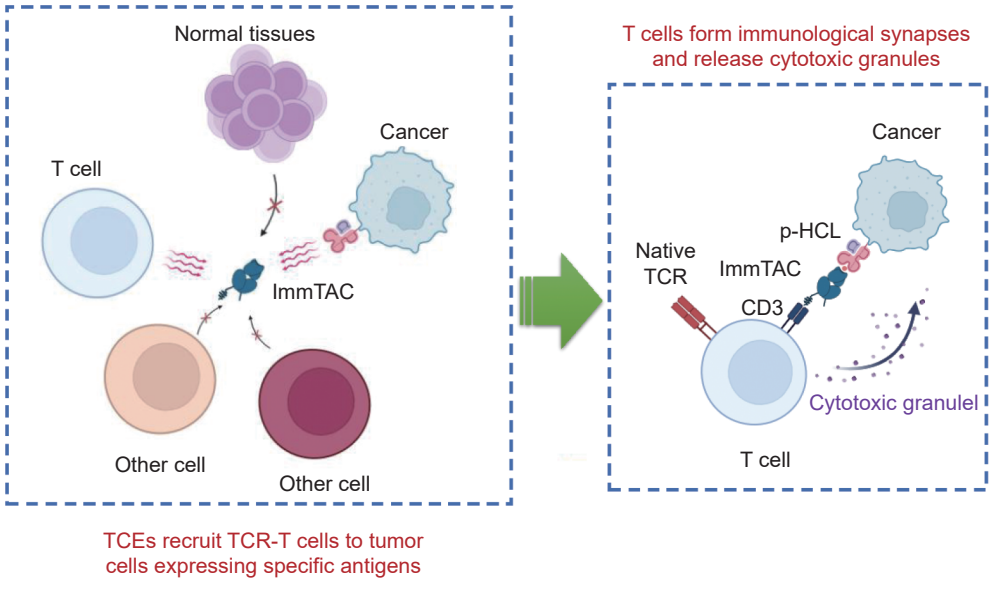

Figure 3 Application of ImmTAC for specific activation of T cells to eliminate tumors. ImmTAC is a type of T-cell engager. By fusing a high-affinity TCR with an anti-CD3 scFv, it acts like a bridge to shorten the physical distance between T cells and tumor cells. The anti-CD3 scFv component of ImmTACs can bind to CD3 on T cells, effectively activating the T cells, which then form immune synapses and release cytotoxic factors to kill tumor cells [69].

Tebentafusp—an ImmTAC consisting of a soluble, affinity-enhanced HLA-A*02:01-restricted T-cell receptor fused to an anti-CD3 scFv, which is specific for the glycoprotein 100 peptide YLEPGPVTA [71]. Building on this, Froning and colleagues aimed to explore more geometric configurations of recombinant T cell receptor (rTCR)/anti-CD3 ImmTAC and compare their ability to redirect T cells to kill tumor cells displaying MAGE-A3/HLA-A1 or NY-ESO-1/HLA-A2 on their surface. Ultimately, their findings indicated that the TCR-Fab format exhibits optimal efficacy, outperforming both the TCR-scFv and TCR-IgG formats. They proposed that future use of mammalian expression systems to produce stable TCR-Fab formats may significantly improve the function and production of rTCR/anti-CD3 TCEs for therapeutic applications [72].

The combined application of ImmTAC and TCR-T cell therapy can achieve multi-dimensional complementarity. When TCR-T cells are used in combination with ImmTAC, ImmTAC can non-specifically activate various T cells, rapidly trigger immune responses, and simultaneously create a favorable immune microenvironment or enhance the immune memory of TCR-T cells. Based on the characteristics of ImmTAC, such as its engineerable nature and specific recognition of tumor cells, it can also be designed into multiple types to assist TCR-T cells and inhibit the immune escape of tumor cells that are not TCR-T targets. In clinical treatment, benefiting from the flexible administration of ImmTAC, different types, doses, and frequencies of ImmTAC can be infused into patients with different conditions to assist TCR-T therapy, thereby achieving personalized treatment (Figure 4).

4.4 One in a million: T-Scan

T-Scan technology is a high-throughput T cell epitope screening technology with two generations of versions available to date: T-Scan-I and T-Scan-II. In T-Scan technology, Granzyme B (GzB)—a key serine protease contained in cytotoxic granules—plays a critical role: upon release, it cleaves various proteins to trigger apoptosis, and can also induce cell death by directly cleaving downstream substrates [73].

T-Scan technology first uses lentiviral transfection to express a candidate antigen library in target cells carrying a GzB reporter (based on Infrared Fluorescent Protein). Subsequently, T cells from patients or volunteers are co-cultured with these target cells. Upon TCR binding to specific candidate antigens, T cells are activated and secrete cytotoxic granules, which activate the reporter gene in target cells and induce fluorescence. Specific antigens can then be isolated via flow cytometry [74, 75]. T-Scan-II, an advancement of T-Scan, is designed for the *de novo* discovery of $CD4^+$ T cell antigens. It integrates technologies such as CD74-based fusions and "centaur" T-cells, enabling both endogenous HLA-II antigen processing in synthetic antigen-presenting cells and TCR signal activation in $CD4^+$ T cells. This allows the simultaneous screening of multiple HLAs and TCRs [76].

Compared with traditional epitope screening methods, T-Scan and T-Scan-II technologies are better able to reflect real physiological processes [26, 27, 77]. Moreover, their combined application enables the comprehensive and accurate identification of $CD8^+$ and $CD4^+$ T cell epitopes, thereby expanding target options for TCR-T therapy. This not only indirectly facilitates the further discovery of TCRs that can precisely target specific epitopes but also plays a positive role in enhancing the ability to cope with tumor heterogeneity caused by aging (Table 4).

5 Conclusions and prospects

With the impairment of human immune function due to aging, changes in the tumor immune microenvironment, and the occurrence of cross-reactivity in clinical TCR-T therapy, the related technologies of TCR-T cell therapy are in urgent need of improvement. Starting from the perspective of enhancing the precision of TCR-T cells targeting CTAs, this

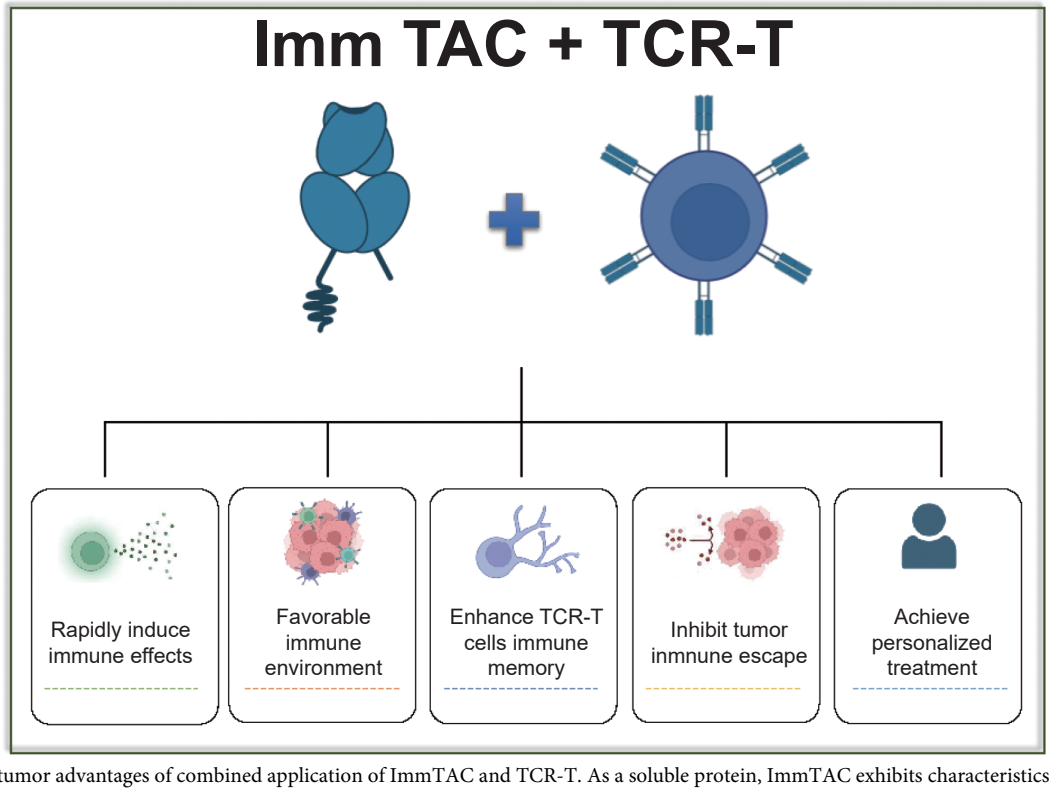

Figure 4 Antitumor advantages of combined application of ImmTAC and TCR-T. As a soluble protein, ImmTAC exhibits characteristics such as flexible administration, proneness to exhaustion, and bispecificity [69]. It holds significant advantages as an adjuvant drug for TCR-T cell therapy in cancer treatment. The non-specific activation of T cells by ImmTAC can rapidly trigger immune responses and extensively enhance the immunological memory of a broad range of T cells. The activation of T cells to release cytokines by ImmTAC helps create a favorable immune environment. The development of multiple types of ImmTACs can broadly inhibit the immune escape of tumor cells. Additionally, its flexible administration allows for personalized treatment.

Table 4 The advantages of T-Scan compared to traditional antigen epitope screening methods

Technology	Comparation	Reference
Traditional technologies	• It requires an antigen epitope prediction step and cannot perform high-throughput sequencing	[27]
	• Limited antigens detected at once fail to address tumor antigen heterogeneity	[26]
	• Be limited to *in vitro* molecular binding and detection of indirect immune responses	[77]
T-Scan	• One-time screening of the whole-genome candidate antigen library	[74, 76]
	• High-throughput whole-genome screening effectively addresses the issue of tumor antigen heterogeneity	[74, 76]
	• Ensure screened epitopes' immunological activity via GZB-mediated reporter in tumor cells *in vivo*.	[75]

review introduces several innovative and high-potential technologies, aiming to improve the efficacy of TCR-T cell immunotherapy for elderly cancer patients and help them recover their health.

It is necessary to conduct further clinical studies on these innovative technologies in the elderly population, including evaluating the safety and efficacy of these innovative methods in the elderly. In addition, the CTAs that are currently widely used in research and have achieved favorable results are limited to the MAGE family and NY-ESO-1. Therefore, in-depth understanding of other cancer-testis antigens will help identify and develop more potential targets and TCRs. The goal of precision therapy is to eliminate off-target cross-reactions of TCR-T, which is also the core practice and key difficulty in personalized therapy. Besides the existing methods such as screening of antigen epitopes and TCRs, as well as using combined drug molecules to create a favorable immune microenvironment and tackle tumor heterogeneity, precision regulatory technologies can be developed in the field of cell signaling pathways in the future. This will enable precise dynamic time control of TCR-T in clinical treatment and improve the precision of therapy.

Ethics approval

Not applicable.

Author contributions

Zhicheng Cai, Zhangyuanzhu Liu, and Wei Jiang conceptualized the review, designed the methodology, and drafted the manuscript. Wei Jiang conducted literature searches, synthesized evidence, and contributed to critical content development. Yanhao Huang and Yan Han coordinated thematic integration and revised the manuscript for intellectual rigor. Zhicheng Cai, Zhangyuanzhu Liu, and Wei Jiang supervised the project, provided structural guidance, and finalized the manuscript for submission.

Acknowledgements

This work was supported by grants from the National Natural Science Foundation of China (No. 82503329), the GuangDong Basic and Applied Basic Research Foundation (No. 2025A1515011769), the Postdoctoral Fellowship Program of CPSF (No. GZC20231069), the Fellowship of China Postdoctoral Science Foundation (No. 2024M751321), the President Foundation of Nanfang Hospital, Southern Medical University (No. 2023B016), the Health Research Project of Hunan Provincial Health Commission (No. 20257625), the Administration of Traditional Chinese Medicine of Guangdong Provincial (No. 20261176), National Innovation and Entrepreneurship Training Program For Undergraduate (NO. 202510559117), the Undergraduate Medical Innovation Capability Expansion Experimental Teaching Demonstration Center of Jinan University.

Conflict of interests

The authors declare that they have no conflicts of interest.

References

[1] Fang, E. F., Xie, C. L., Schenkel, J. A., Wu, C. K., Long, Q., Cui, H. H., Aman, Y., Frank, J., Liao, J., Zou, H. C. et al. A research agenda for ageing in China in the 21st century (2nd edition): Focusing on basic and translational research, long-term care, policy and social networks. *Ageing Research Reviews*, **2020**, 64: 101174. https://doi.org/10.1016/j.arr.2020.101174

[2] Prathap, R., Kirubha, S., Rajan, A. T., Manoharan, S., Elumalai, K. The increasing prevalence of cancer in the elderly: An investigation of epidemiological trends. *Aging Medicine*, **2024**, 7(4): 516–527. https://doi.org/10.1002/agm2.12347

[3] Marin, J., Romero, M., Martinez-Becerra, P., Herraez, E., Briz, O. Overview of the molecular bases of resistance to chemotherapy in liver and gastrointestinal tumours. *Current Molecular Medicine*, **2009**, 9(9): 1108–1129. https://doi.org/10.2174/156652409789839125

[4] Wu, Y., Song, Y. Q., Wang, R. Z., Wang, T. L. Molecular mechanisms of tumor resistance to radiotherapy. *Molecular Cancer*, **2023**, 22(1): 96. https://doi.org/10.1186/s12943-023-01801-2

[5] Skricková, J., Spelda, S., Kaplanova, J. The limits of surgical treatment of non-small cell lung cancer. *Acta Medica Austriaca*, **2001**, 28(2): 43–46. https://doi.org/10.1046/j.1563-2571.2001.01010.x

[6] Lewis, W. G., Williamson, M. E. R., Kuzu, A., Stephenson, B. M., Holdsworth, P. J., Finan, P. J., Ash, D., Johnston, D. Potential disadvantages of post-operative adjuvant radiotherapy after anterior resection for rectal cancer: A pilot study of sphincter function, rectal capacity and clinical outcome. *International Journal of Colorectal Disease*, **1995**, 10(3): 133–137. https://doi.org/10.1007/BF00298533

[7] Li, X. J., Zhang, Y. T., Wang, X. L., Zeng, H., Zhou, L. Y., Huang, G. L., Lin, M. X., Zhuang, B. W., Xie, X. Y., Xu, M. Predicting infectious complications after percutaneous thermal ablation of liver malignancies: A 12-year single-center experience. *Radiology*, **2023**, 308(2): e223091. https://doi.org/10.1148/radiol.223091

[8] Simpson, A. J. G., Caballero, O. L., Jungbluth, A., Chen, Y. T., Old, L. J. Cancer/testis antigens, gametogenesis and cancer. *Nature Reviews Cancer*, **2005**, 5(8): 615–625. https://doi.org/10.1038/nrc1669

[9] Anderson, D. J., Narayan, P., DeWolf, W. C. Major histocompatibility antigens are not detectable on post-meiotic human testicular germ cells. *The Journal of Immunology*, **1984**, 133(4): 1962–1965. https://doi.org/10.4049/jimmunol.133.4.1962

[10] Weon, J. L., Potts, P. R. The MAGE protein family and cancer. *Current Opinion in Cell Biology*, **2015**, 37: 1–8. https://doi.org/10.1016/j.ceb.2015.08.002

[11] Alsalloum, A., Shevchenko, J. A., Sennikov, S. NY-ESO-1 antigen: A promising frontier in cancer immunotherapy. *Clinical and Translational Medicine*, **2024**, 14(9): e70020. https://doi.org/10.1002/ctm2.70020

[12] Smith, H. A., McNeel, D. G. The SSX family of cancer-testis antigens as target proteins for tumor therapy. *Journal of Immunology Research*, **2010**, 2010(1): 150591. https://doi.org/10.1155/2010/150591

[13] Killen, M. W., Taylor, T. L., Stults, D. M., Jin, W., Wang, L. L., Moscow, J. A., Pierce, A. J. Configuration and rearrangement of the human GAGE gene clusters. *American Journal of Translational Research*, **2011**, 3(3): 234–242.

[14] Xu, Y. C., Zou, R. M., Wang, J., Wang, Z. W., Zhu, X. Q. The role of the cancer testis antigen PRAME in tumorigenesis and immunotherapy in human cancer. *Cell Proliferation*, **2020**, 53(3): e12770. https://doi.org/10.1111/cpr.12770

[15] Verdegaal, E. M. Adoptive cell therapy: A highly successful individualized therapy for melanoma with great potential for other malignancies. *Current Opinion in Immunology*, **2016**, 39: 90–95. https://doi.org/10.1016/j.coi.2016.01.004

[16] Li, C. H., Sharma, S., Heczey, A. A., Woods, M. L., Steffin, D. H. M., Louis, C. U., Grilley, B. J., Thakkar, S. G., Wu, M. F., Wang, T. et al. Long-term outcomes of GD2-directed CAR-T cell therapy in patients with neuroblastoma. *Nature Medicine*, **2025**, 31(4): 1125–1129. https://doi.org/10.1038/s41591-025-03513-0

[17] Saez-Ibañez, A. R., Upadhaya, S., Partridge, T., Shah, M., Correa, D., Campbell, J. Landscape of cancer cell therapies: Trends and real-world data. *Nature Reviews Drug Discovery*, **2022**, 21(9): 631–632. https://doi.org/10.1038/d41573-022-00095-1

[18] Chandran, S. S., Klebanoff, C. A. T cell receptor-based cancer immunotherapy: Emerging efficacy and pathways of resistance. *Immunological Reviews*, **2019**, 290(1): 127–147. https://doi.org/10.1111/imr.12772

[19] Golikova, E. A., Alshevskaya, A. A., Alrhmoun, S., Sivitskaya, N. A., Sennikov, S. V. TCR-T cell therapy: Current development approaches, preclinical evaluation, and perspectives on regulatory challenges. *Journal of Translational Medicine*, **2024**, 22(1): 897. https://doi.org/10.1186/s12967-024-05703-9

[20] Chomez, P., De Backer, O., Bertrand, M., De Plaen, E., Boon, T., Lucas, S. An overview of the MAGE gene family with the identification of all human members of the family. *Cancer Research*, **2001**, 61(14): 5544–5551.

[21] Lian, Y. S., Meng, L. J., Ding, P. G., Sang, M. X. Epigenetic regulation of MAGE family in human cancer progression-DNA methylation, histone modification, and non-coding RNAs. *Clinical Epigenetics*, **2018**, 10(1): 115. https://doi.org/10.1186/s13148-018-0550-8

[22] Hong, D. S., Van Tine, B. A., Biswas, S., McAlpine, C., Johnson, M. L., Olszanski, A. J., Clarke, J. M., Araujo, D., Blumenschein, G. R., Kebriaei, P. et al. Autologous T cell therapy for MAGE-A4+ solid cancers in HLA-A*02+ patients: A phase 1 trial. *Nature Medicine*, **2023**, 29(1): 104–114. https://doi.org/10.1038/s41591-022-02128-z

[23] Robbins, P. F., Kassim, S. H., Tran, T. L. N., Crystal, J. S., Morgan, R. A., Feldman, S. A., Yang, J. C., Dudley, M. E., Wunderlich, J. R., Sherry, R. M. et al. A pilot trial using lymphocytes genetically engineered with an NY-ESO-1–reactive T-cell receptor: Long-term follow-up and correlates with response. *Clinical Cancer Research*, **2015**, 21(5): 1019–1027. https://doi.org/10.1158/1078-0432.ccr-14-2708

[24] Li, W. D., Xiao, L., Li, H. Y., Cui, W. Global research trends of immunosenescence and immunotherapy: A bibliometric study. *Human Vaccines & Immunotherapeutics*, **2025**, 21(1): 2469403. https://doi.org/10.1080/21645515.2025.2469403

[25] Border, E. C., Sanderson, J. P., Weissensteiner, T., Gerry, A. B., Pumphrey, N. J. Affinity-enhanced T-cell receptors for adoptive T-cell therapy targeting MAGE-A10: Strategy for selection of an optimal candidate. *OncoImmunology*, **2019**, 8(2): e1532759. https://doi.org/10.1080/2162402X.2018.1532759

[26] Dang, X. B., Guelen, L., Lutje Hulsik, D., Ermakov, G., Hsieh, E. J., Kreijtz, J., Stammen-Vogelzangs, J., Lodewijks, I., Bertens, A.,

Bramer, A. et al. Epitope mapping of monoclonal antibodies: A comprehensive comparison of different technologies. *mAbs*, **2023**, 15(1): 2285285. https://doi.org/10.1080/19420862.2023.2285285

[27] Zhang, B., Ren, Z. Y., Zhao, J. F., Zhu, Y., Huang, B. Y., Xiao, C. C., Zhang, Y., Deng, J. P., Mao, L. P., Tang, L. et al. Global analysis of HLA-A2 restricted MAGE-A3 tumor antigen epitopes and corresponding TCRs in non-small cell lung cancer. *Theranostics*, **2023**, 13(13): 4449–4468. https://doi.org/10.7150/thno.84710

[28] Davis, M. M., Bjorkman, P. J. T-cell antigen receptor genes and T-cell recognition. *Nature*, **1988**, 334(6181): 395–402. https://doi.org/10.1038/334395a0

[29] Steinman, R. M., Hemmi, H. Dendritic cells: Translating innate to adaptive immunity. In: Pulendran, B., Ahmed, R. (eds), *Current Topics in Microbiology and Immunology, vol. 311*. Berlin, Heidelberg: Springer, **2006**: 17–58. https://doi.org/10.1007/3-540-32636-7_2

[30] Palucka, A. K., Ueno, H., Fay, J. W., Banchereau, J. Taming cancer by inducing immunity *via* dendritic cells. *Immunological Reviews*, **2007**, 220(1): 129–150. https://doi.org/10.1111/j.1600-065X.2007.00575.x

[31] Banchereau, J., Palucka, A. K. Dendritic cells as therapeutic vaccines against cancer. *Nature Reviews Immunology*, **2005**, 5(4): 296–306. https://doi.org/10.1038/nri1592

[32] Birkholz, K., Schwenkert, M., Kellner, C., Gross, S., Fey, G., Schuler-Thurner, B., Schuler, G., Schaft, N., Dörrie, J. Targeting of DEC-205 on human dendritic cells results in efficient MHC class II–restricted antigen presentation. *Blood*, **2010**, 116(13): 2277–2285. https://doi.org/10.1182/blood-2010-02-268425

[33] Wang, L., Wang, C. C. Oxidative protein folding fidelity and redoxtasis in the endoplasmic reticulum. *Trends in Biochemical Sciences*, **2023**, 48(1): 40–52. https://doi.org/10.1016/j.tibs.2022.06.011

[34] Wang, L. H., Lan, X. Rapid screening of TCR-pMHC interactions by the YAMTAD system. *Cell Discovery*, **2022**, 8: 30. https://doi.org/10.1038/s41421-022-00386-2

[35] Felberbaum, R. S. The baculovirus expression vector system: A commercial manufacturing platform for viral vaccines and gene therapy vectors. *Biotechnology Journal*, **2015**, 10(5): 702–714. https://doi.org/10.1002/biot.201400438

[36] Crawford, F., Huseby, E., White, J., Marrack, P., Kappler, J. W. Mimotopes for alloreactive and conventional T cells in a peptide-MHC display library. *PLoS Biology*, **2004**, 2(4): e90. https://doi.org/10.1371/journal.pbio.0020090

[37] Cho, H. I., Kim, U. H., Shin, A. R., Won, J. N., Lee, H. J., Sohn, H. J., Kim, T. G. A novel Epstein–Barr virus-latent membrane protein-1-specific T-cell receptor for TCR gene therapy. *British Journal of Cancer*, **2018**, 118(4): 534–545. https://doi.org/10.1038/bjc.2017.475

[38] Zheng, Y. W., Hao, S., Hu, L. P., Cheng, T. Development of immunodeficient mice/humanized mouse models and their applications in hematology research. *Zhonghua Xueyexue Zazhi*, **2015**, 36(11): 966–971. https://doi.org/10.3760/cma.j.issn.0253-2727.2015.11.018

[39] Cheng, Y., Gunasegaran, B., Singh, H. D., Dutertre, C. A., Loh, C. Y., Lim, J. Q., Crawford, J. C., Lee, H. K., Zhang, X. M., Lee, B. et al. Non-terminally exhausted tumor-resident memory HBV-specific T cell responses correlate with relapse-free survival in hepatocellular carcinoma. *Immunity*, **2021**, 54(8): 1825–1840.e7. https://doi.org/10.1016/j.immuni.2021.06.013

[40] Govers, C., Berrevoets, C., Treffers-Westerlaken, E., Broertjes, M., Debets, R. Magnetic-activated cell sorting of TCR-engineered T cells, using tCD34 as a gene marker, but not peptide-MHC multimers, results in significant numbers of functional $CD4^+$ and $CD8^+$ T cells. *Human Gene Therapy Methods*, **2012**, 23(3): 213–224. https://doi.org/10.1089/hgtb.2012.074

[41] Whisler, R. L., Newhouse, Y. G., Bagenstose, S. E. Age-related reductions in the activation of mitogen-activated protein kinases p44mapk/ERK1 and p42mapk/ERK2 in human T cells stimulated *via* ligation of the T cell receptor complex. *Cellular Immunology*, **1996**, 168(2): 201–210. https://doi.org/10.1006/cimm.1996.0067

[42] Liu, B., Carle, K. W., Whisler, R. L. Reductions in the activation of ERK and JNK are associated with decreased IL-2 production in T cells from elderly humans stimulated by the TCR/CD3 complex and costimulatory signals. *Cellular Immunology*, **1997**, 182(2): 79–88. https://doi.org/10.1006/cimm.1997.1226

[43] Kadyrzhanova, G., Tamai, M., Sarkar, S., Kalra, R. S., Ishikawa, H. Aging impairs CD8 T cell responses in adoptive T-cell therapy against solid tumors. *Frontiers in Immunology*, **2025**, 16: 1484303. https://doi.org/10.3389/fimmu.2025.1484303

[44] Mika, J., Yoshida, K., Kusunoki, Y., Candéias, S. M., Polanska, J. Sex- and age-specific aspects of human peripheral T-cell dynamics. *Frontiers in Immunology*, **2023**, 14: 1224304. https://doi.org/10.3389/fimmu.2023.1224304

[45] Goronzy, J. J., Lee, W. W., Weyand, C. M. Aging and T-cell diversity. *Experimental Gerontology*, **2007**, 42(5): 400–406. https://doi.org/10.1016/j.exger.2006.11.016

[46] Naylor, K., Li, G. J., Vallejo, A. N., Lee, W. W., Koetz, K., Bryl, E., Witkowski, J., Fulbright, J., Weyand, C. M., Goronzy, J. J. The influence of age on T cell generation and TCR diversity. *The Journal of Immunology*, **2005**, 174(11): 7446–7452. https://doi.org/10.4049/jimmunol.174.11.7446

[47] Cairns, R., Papandreou, I., Denko, N. Overcoming physiologic barriers to cancer treatment by molecularly targeting the tumor microenvironment. *Molecular Cancer Research*, **2006**, 4(2): 61–70. https://doi.org/10.1158/1541-7786.MCR-06-0002

[48] Yu, Y. R., Ho, P. C. Sculpting tumor microenvironment with immune system: From immunometabolism to immunoediting. *Clinical & Experimental Immunology*, **2019**, 197(2): 153–160. https://doi.org/10.1111/cei.13293

[49] Liu, X., Hoft, D. F., Peng, G. Y. Senescent T cells within suppressive tumor microenvironments: Emerging target for tumor immunotherapy. *Journal of Clinical Investigation*, **2020**, 130(3): 1073–1083. https://doi.org/10.1172/jci133679

[50] Huang, L., Zhang, C. G., Jiang, A. M., Lin, A. Q., Zhu, L. X., Mou, W. M., Zeng, D. Q., Liu, Z. Q., Tang, B. F., Zhang, J. et al. T-cell senescence in the tumor microenvironment. *Cancer Immunology Research*, **2025**, 13(5): 618–632. https://doi.org/10.1158/2326-6066.cir-24-0894

[51] Talarico, G., Orecchioni, S., Falvo, P., Bertolini, F. Myeloid-derived suppressor cells (MDSCs) at the crossroad of senescence and cancer. *Cancers*, **2025**, 17(13): 2251. https://doi.org/10.3390/cancers17132251

[52] You, L., Wu, Q. H. Cellular senescence in tumor immune escape: Mechanisms, implications, and therapeutic potential. *Critical Reviews in Oncology/Hematology*, **2025**, 208: 104628. https://doi.org/10.1016/j.critrevonc.2025.104628

[53] Yang, J., Liu, H. C., Zhang, J. Q., Zou, J. Y., Zhang, X., Chen, W. M., Gu, Y., Hong, H. The effect of metformin on senescence of T lymphocytes. *Immunity & Ageing*, **2023**, 20(1): 73. https://doi.org/10.1186/s12979-023-00394-0

[54] Cheng, J., Zheng, J., Ma, C., Li, Y. Z., Hao, H. T-cell senescence: Unlocking the tumor immune "Dark Box" - A multidimensional analysis from mechanism to tumor immunotherapeutic intervention. *Seminars in Cancer Biology*, **2025**, 113: 190–209. https://doi.org/10.1016/j.semcancer.2025.05.010

[55] Cameron, B. J., Gerry, A. B., Dukes, J., Harper, J. V., Kannan, V., Bianchi, F. C., Grand, F., Brewer, J. E., Gupta, M., Plesa, G. et al. Identification of a titin-derived HLA-A1–presented peptide as a cross-reactive target for engineered MAGE A3–directed T cells. *Science Translational Medicine*, **2013**, 5(197): e3006034. https://doi.org/10.1126/scitranslmed.3006034

[56] Linette, G. P., Stadtmauer, E. A., Maus, M. V., Rapoport, A. P., Levine, B. L., Emery, L., Litzky, L., Bagg, A., Carreno, B. M., Cimino, P. J. et al. Cardiovascular toxicity and titin cross-reactivity of affinity-enhanced T cells in myeloma and melanoma. *Blood*, **2013**, 122(6): 863–871. https://doi.org/10.1182/blood-2013-03-490565

[57] Chinnasamy, N., Wargo, J. A., Yu, Z. Y., Rao, M., Frankel, T. L., Riley, J. P., Hong, J. J., Parkhurst, M. R., Feldman, S. A., Schrump, D. S. et al. A TCR targeting the HLA-A*0201–restricted epitope of MAGE-A3 recognizes multiple epitopes of the MAGE-a antigen

superfamily in several types of cancer. *The Journal of Immunology*, **2011**, 186(2): 685–696. https://doi.org/10.4049/jimmunol.1001775

[58] Morgan, R. A., Chinnasamy, N., Abate-Daga, D., Gros, A., Robbins, P. F., Zheng, Z. L., Dudley, M. E., Feldman, S. A., Yang, J. C., Sherry, R. M. et al. Cancer regression and neurological toxicity following anti-MAGE-A3 TCR gene therapy. *Journal of Immunotherapy*, **2013**, 36(2): 133–151. https://doi.org/10.1097/cji.0b013e3182829903

[59] Kim, S. T., Takeuchi, K., Sun, Z. J., Touma, M., Castro, C. E., Fahmy, A., Lang, M. J., Wagner, G., Reinherz, E. L. The αβ T cell receptor is an anisotropic mechanosensor. *Journal of Biological Chemistry*, **2009**, 284(45): 31028–31037. https://doi.org/10.1074/jbc.m109.052712

[60] Li, Y. C., Chen, B. M., Wu, P. C., Cheng, T. L., Kao, L. S., Tao, M. H., Lieber, A., Roffler, S. R. Cutting edge: Mechanical forces acting on T cells immobilized *via* the TCR complex can trigger TCR signaling. *The Journal of Immunology*, **2010**, 184(11): 5959–5963. https://doi.org/10.4049/jimmunol.0900775

[61] Liu, B. Y., Chen, W., Evavold, B. D., Zhu, C. Accumulation of dynamic catch bonds between TCR and agonist peptide-MHC triggers T cell signaling. *Cell*, **2014**, 157(2): 357–368. https://doi.org/10.1016/j.cell.2014.02.053

[62] Wu, P., Zhang, T. T., Liu, B. Y., Fei, P. Y., Cui, L., Qin, R., Zhu, H. Y., Yao, D. M., Martinez, R. J., Hu, W. et al. Mechano-regulation of peptide-MHC class I conformations determines TCR antigen recognition. *Molecular Cell*, **2019**, 73(5): 1015–1027.e7. https://doi.org/10.1016/j.molcel.2018.12.018

[63] Qin, R., Zhang, Y., Shi, J. W., Wu, P., An, C. Y., Li, Z. H., Liu, N., Wan, Z. Y., Hua, T., Li, X. L. et al. TCR catch bonds nonlinearly control CD8 cooperation to shape T cell specificity. *Cell Research*, **2025**, 35(4): 265–283. https://doi.org/10.1038/s41422-025-01077-9

[64] Sibener, L. V., Fernandes, R. A., Kolawole, E. M., Carbone, C. B., Liu, F., McAffee, D., Birnbaum, M. E., Yang, X. B., Su, L. F., Yu, W. et al. Isolation of a structural mechanism for uncoupling T cell receptor signaling from peptide-MHC binding. *Cell*, **2018**, 174(3): 672–687.e27. https://doi.org/10.1016/j.cell.2018.06.017

[65] Zhao, X., Kolawole, E. M., Chan, W. P., Feng, Y. N., Yang, X. B., Gee, M. H., Jude, K. M., Sibener, L. V., Fordyce, P. M., Germain, R. N. et al. Tuning T cell receptor sensitivity through catch bond engineering. *Science*, **2022**, 376(6589): eabl5282. https://doi.org/10.1126/science.abl5282

[66] Vanhanen, R., Heikkilä, N., Aggarwal, K., Hamm, D., Tarkkila, H., Pätilä, T., Jokiranta, T. S., Saramäki, J., Arstila, T. P. T cell receptor diversity in the human thymus. *Molecular Immunology*, **2016**, 76: 116–122. https://doi.org/10.1016/j.molimm.2016.07.002

[67] Li, F., Qian, X. Y., Zhu, X. Y., Lai, X., Zhang, X. P., Wang, J. Y. TCRcost: A deep learning model utilizing TCR 3D structure for enhanced of TCR–peptide binding. *Frontiers in Genetics*, **2024**, 15: 1346784. https://doi.org/10.3389/fgene.2024.1346784

[68] Berman, D. M., Bell, J. I. Redirecting polyclonal T cells against cancer with soluble T-cell receptors. *Clinical Cancer Research*, **2023**, 29(4): 697–704. https://doi.org/10.1158/1078-0432.CCR-22-0028

[69] Lowe, K. L., Cole, D., Kenefeck, R., OKelly, I., Lepore, M., Jakobsen, B. K. Novel TCR-based biologics: Mobilising T cells to warm 'cold' tumours. *Cancer Treatment Reviews*, **2019**, 77: 35–43. https://doi.org/10.1016/j.ctrv.2019.06.001

[70] Cattaruzza, F., Nazeer, A., To, M., Hammond, M., Koski, C., Liu, L. Y., Pete Yeung, V., Rennerfeldt, D. A., Henkensiefken, A., Fox, M. et al. Precision-activated T-cell engagers targeting HER2 or EGFR and CD3 mitigate on-target, off-tumor toxicity for immunotherapy in solid tumors. *Nature Cancer*, **2023**, 4(4): 485–501. https://doi.org/10.1038/s43018-023-00536-9

[71] Nathan, P., Hassel, J. C., Rutkowski, P., Baurain, J. F., Butler, M. O., Schlaak, M., Sullivan, R. J., Ochsenreither, S., Dummer, R., Kirkwood, J. M. et al. Overall survival benefit with tebentafusp in metastatic uveal melanoma. *New England Journal of Medicine*, **2021**, 385(13): 1196–1206. https://doi.org/10.1056/nejmoa2103485

[72] Froning, K. J., Sereno, A., Huang, F., Demarest, S. J. Generalizable design parameters for soluble T cell receptor-based T cell engagers. *Journal for ImmunoTherapy of Cancer*, **2022**, 10(3): e004281. https://doi.org/10.1136/jitc-2021-004281

[73] Casciola-Rosen, L., Garcia-Calvo, M., Bull, H. G., Becker, J. W., Hines, T., Thornberry, N. A., Rosen, A. Mouse and human granzyme B have distinct tetrapeptide specificities and abilities to recruit the bid pathway. *Journal of Biological Chemistry*, **2007**, 282(7): 4545–4552. https://doi.org/10.1074/jbc.M606564200

[74] Kula, T., Dezfulian, M. H., Wang, C. I., Abdelfattah, N. S., Hartman, Z. C., Wucherpfennig, K. W., Lyerly, H. K., Elledge, S. J. T-scan: A genome-wide method for the systematic discovery of T cell epitopes. *Cell*, **2019**, 178(4): 1016–1028.e13. https://doi.org/10.1016/j.cell.2019.07.009

[75] To, T. L., Piggott, B. J., Makhijani, K., Yu, D., Jan, Y. N., Shu, X. K. Rationally designed fluorogenic protease reporter visualizes spatiotemporal dynamics of apoptosis *in vivo*. *Proceedings of the National Academy of Sciences of the United States of America*, **2015**, 112(11): 3338–3343. https://doi.org/10.1073/pnas.1502857112

[76] Dezfulian, M. H., Kula, T., Pranzatelli, T., Kamitaki, N., Meng, Q. D., Khatri, B., Perez, P., Xu, Q. K., Chang, A. Q., Kohlgruber, A. C. et al. TScan-II: A genome-scale platform for the *de novo* identification of $CD4^+$ T cell epitopes. *Cell*, **2023**, 186(25): 5569–5586.e21. https://doi.org/10.1016/j.cell.2023.10.024

[77] Guo, X. J., Dash, P., Calverley, M., Tomchuck, S., Dallas, M. H., Thomas, P. G. Rapid cloning, expression, and functional characterization of paired αβ and γδ T-cell receptor chains from single-cell analysis. *Molecular Therapy - Methods & Clinical Development*, **2016**, 3: 15054. https://doi.org/10.1038/mtm.2015.54