

REVIEW

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The screening, identification, design and clinical application of tumor-specific neoantigens for TCR-T cells

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Abstract

Recent advances in neoantigen research have accelerated the development of tumor immunotherapies, including adoptive cell therapies (ACTs), cancer vaccines and antibody-based therapies, particularly for solid tumors. With the development of next-generation sequencing and bioinformatics technology, the rapid identification and prediction of tumor-specific antigens (TSAs) has become possible. Compared with tumor-associated antigens (TAAs), highly immunogenic TSAs provide new targets for personalized tumor immunotherapy and can be used as prospective indicators for predicting tumor patient survival, prognosis, and immune checkpoint blockade response. Here, the identification and characterization of neoantigens and the clinical application of neoantigen-based TCR-T immunotherapy strategies are summarized, and the current status, inherent challenges, and clinical translational potential of these strategies are discussed.

Key points

- 1) Established the computational process and platform of neoantigen prediction, and comprehensive prediction and screening of tumor specific neoantigens.
- 2) Rapid identification of neoantigen-specific TCRs for personalized engineered TCR-T cell therapy.
- 3) Classification and characteristics of tumor specific neoantigen-based therapy, and combined antitumor strategies.
- 4) The current status, inherent challenges, and clinical translational potential of TCR-T therapy.

Keywords TSA, Neoantigen, TCR-T, ACT, Immunotherapy

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Background

Major histocompatibility (MHC) molecules of tumor cells can express TSAs or TAAs. TSAs are abnormally expressed in malignant tumor cells or only produced at specific stages of differentiation, and its production is extremely limited in normal tissues. TAAs mainly refer to the antigenic molecules present on normal cells or tumor cells; however, they are not unique to tumor cells. Normal cells can be synthesized in small amounts and are usually highly expressed when tumor cells proliferate (Fig. 1). These tumor-specific polypeptide-MHC (pMHC) complexes are recognized by T cells and trigger an antitumor immune response in the patient. Compared with other types of tumor antigens, such as cancer-testicular antigen (CTA) and TAAs, highly immunogenic and tumor-specific TSAs provide new targets for personalized tumor immunotherapy and can be used as prospective indicators for predicting tumor patient survival, prognosis, and immune checkpoint blockade response. Recent advances in TSA research have accelerated the development and clinical trials of tumor immunotherapies, including adoptive cell therapies, cancer vaccines, and antibody-based therapies. Rapid identification, prediction, and clinical application of TSAs are made possible by advances in next-generation sequencing and bioinformatics.

Various clinical studies have examined the effectiveness of immunotherapies targeting TAAs, such as vaccines against ERBB2, MUC1, and hTERT [1–3]. The prevalence of TAAs among cancer patients makes them a target for quick immunization. Since TAAs are nonmutated autoantigens, central T-cell tolerance may be one of the reasons for the poor T-cell response observed in clinical trials [4]. However, immune escape mechanisms can be reversed by cancer immunotherapy, including the use of tumor vaccines to improve antigen presentation, the increase in antitumor T cells by adoptive metastasis of tumor-infiltrating lymphocytes (TILs) and T-cell

receptor (TCR)-transduced T cells, the restoration of CD8⁺ T-cell effector capacity by immune checkpoint blockades (ICBs), and T cells induced by bispecific antibodies (bsAbs) and chimeric antigen receptors (CARs) to increase the immune recognition of tumors [5–7]. Nevertheless, widespread use of tumor immunotherapy is hampered by a shortage of antigens that target various cancers [5]. In non-virus-associated malignancies, TSAs can be derived from unique proteins or peptides that are produced by abnormal RNA splicing and disrupted post-translational protein modification. For virus-associated cancers, such as HPV-positive cervical cancer and EBV-associated nasopharyngeal carcinoma, TSAs can also be generated through the virus-encoded open reading framework (ORF) [8, 9]. This ability to avoid T-cell tolerance increases the TSA-specific T-cell pool and thus has the potential to enhance the tumor-specific immune response. In addition, immunotherapy enhanced the tolerance of TSA-specific T-cell responses, and the immune memory after treatment offers promise for long-term protection against disease recurrence.

— \ The source of tumor-specific antigens (TSAs)

TSAs are produced due to changes in the genome, transcriptome, and proteome. They are foreign proteins that are not present in normal tissues but can be produced from tumor cells through various mechanisms, such as genomic mutations, abnormal transcriptional mutations, posttranslational modification (PTM) mutations, and viral-encoded open reading framework (ORF) mutations [10] (Fig. 2a, b, c; Table 1).

(—) \ Neoantigens derived from genomic variation

Up to hundreds of nonsynonymous somatic mutations have been recorded in cancer patients, resulting in an average of 150 potential neoantigens per person. Somatic genome changes, including single nucleotide variants

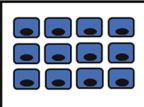
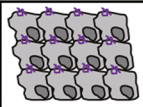
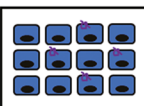
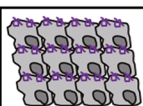
Tumor antigen	Antigen source	Expression in normal tissue	Expression in tumor cells	Features	Representative tumor
Tumor-specific antigen (TSA)	Mutation-associated neoantigens; Oncoviral/Oncobacteria antigens; Alternative tumor-specific antigens			<ul style="list-style-type: none"> - Specific bioinformatic pipeline for alternative antigens + Tumor-specific; Less autoimmune toxicity; Personalized approach; Non-central tolerance 	Lung cancer; Cholangiocarcinoma; Triple-Negative Breast Cancer; HPV ⁺ carcinoma of uterine cervix; EBV ⁺ nasopharyngeal carcinoma; HBV ⁺ hepatocellular carcinoma; and so on
Tumor-associated antigen (TAA)	Cancer germline antigens (CGAs); Tissue differentiation antigens (TDAs); Overexpressed proteins; Lineage-specific differentiation markers			<ul style="list-style-type: none"> - Toxicity on normal tissues; The peripheral and central tolerance + Easy assessment of expression profile (IHC; RNA-seq) 	Esophageal cancer; Embryonal tumours; Gastric cancer; Prostate cancer; Leucocythemia; Lymphoma; and so on

Fig. 1 Characteristics of tumor-specific antigens (TSAs) and tumor-associated antigens (TAAs). Sources of TSA and TAA. In contrast to normal tissues, TSA is only expressed in tumors. TAA is underexpressed in normal tissues and overexpressed in tumors. The advantages and disadvantages of each antigen type of TSA and TAA and its representation of related tumors are shown

(SNVs), base insertions and deletions (INDELs), gene fusion, and structural variants, are major factors that promote TSA production [11].

Single nucleotide variations (SNVs)

SNVs are the most common type of mutation at the genomic level of tumor cells, and they can produce mutant peptides distinct from wild-type peptides, which are presented by the MHC as specific antigens [12]. The neoantigen profiles encoded by SNVs vary greatly between different cancer types and different stages of the same cancer type. Using the TCGA database, 933954 neoantigens were identified in 20 solid tumors. These mutations resulted from somatic mutations with different median frequencies of 893960 neoantigens in different cancers. At least 5% of patients with the same or different cancers share 24 of these neoantigens, including those caused by driver gene mutations such as PIK3CA, RAS and BRAF [13]. SNVs can also appear in mitochondrial DNA (mtDNA). mtDNA is present in most cancer cells and is involved in tumor metabolism and metastasis. Both mouse and human immune systems can recognize and respond to mtDNA-SNP-derived peptides, suggesting that a single SNP in mtDNA is sufficient to produce immunogenic antigens [14]. Thus, nonsynonymous SNPs in mtDNA may produce a large number of mutant peptides, providing an additional source of neoantigens.

Insertions and deletions (INDELs)

INDEL mutations are mainly caused by the insertion or deletion of base pairs in the genome, which frequently lead to nonsynonymous novel ORFs, also known as frameshift mutations. Frameshift INDELs can generate more types of neoantigens with increased MHC-I binding affinity, suggesting a higher immunogenic mutation type compared to SNVs [10]. For example, in renal cell carcinoma with moderate mutation loads, approximately 16% of predicted new peptides came from frameshift insertion, while 21% of new epitopes recognized by T cells came from frameshift insertions [15], suggesting

greater immunogenic potential for frameshift-derived epitopes. Similar to SNV-encoded neoantigens, INDEL-encoded neoantigens are more common in cancers with high microsatellite instability (MSI-H), which is determined by the absence of DNA mismatch repair (MMR) mechanisms. As the evolution of MMR-deficient cancers is mainly triggered by mutations that inactivate tumor suppressor genes (TSGs) containing coding microsatellites, frameshift peptide neoantigens are more frequently shared among MMR-deficient cancers (e.g., endometrial, colorectal, and gastric) than missense mutation-derived neoantigens [16]. Frameshift mutation-encoded neoantigens are more common than missense mutation-derived neoantigens in MMR-deficient cancers, such as breast, colorectal, gastric, and endometrial cancers, and have a strong association with immune responses and a larger proportion of TILs; thus, these neoantigens are excellent candidates for immunotherapy targeting MSI cancers. MSI colorectal cancers with frameshift mutations have a larger proportion of TILs than other colorectal cancers. Similarly, shared immunogenic frameshift peptide neoantigens can be produced as a result of recurrent frameshift mutations, offering excellent candidates for immunotherapy against MSI cancers [17–20]. The results showed that the combination of four frameshift-derived polypeptides could significantly improve the acquired immune response of mice in a VCMsh2-driven intestinal tumor model, reduce the intestinal tumor burden, and prolong overall survival [21]. According to clinical phase I/IIa trials, frameshift mutation-encoded neoantigen vaccines have good systemic tolerance and regularly trigger immune responses, providing a promising new strategy for the treatment and even prevention of MMR-deficient malignancies [22]. These findings suggest that off-the-shelf frameshift mutation neoantigen-driven vaccines are feasible options for the treatment and prevention of MSI cancers. In addition, the frameshift INDEL neoantigen burden is also a novel biomarker for ICB response. INDEL-frameshift mutations are thought to produce more immunogenic neoantigens, thereby improving the

(See figure on next page.)

Fig. 2 Computational workflow of neoantigen prediction. **a** The general route of neoantigen prediction. **b** The workflow of clinical sample collection and single-cell sequencing. **c** The neoantigen sources can develop at the genomic level through SNV mutation, INDEL mutation, fusion mutation, integrated viral ORF and splice variants (the display of prediction software for mutation calling), at the transcriptomic level through alternative splicing, polyadenylation (pA), RNA editing and allegedly noncoding regions, and at the proteomic level through dysregulated translation and PTMs. **d** HLA typing prediction and display by prediction software tools. **e** and **f** Mutant polypeptides are produced by proteasome-mediated decomposition of endogenous proteins, which are subsequently transported to the ER by antigen-processing associated transporters (TAP). They may be loaded into MHC-I and MHC-II for binding to specific peptides produced by mutated proteins that breakdown in the endosomal pathway. These peptide-MHC-II/MHC-I (pMHC) complexes are then transported to the cell surface, where they are recognized by T cells. **g** pMHC complex binding prediction and the display of prediction software tools. **h** The prediction of T-cell recognition of pMHC complexes and the display of prediction software tools. **i** T-cell validation of neoantigens. Coculture of patient TILs or PBMCs with autologous antigen-presenting cells (APCs) expressing candidate neoantigens (TMG or peptides) allows for the identification of neoantigen-reactive T cells based on functional data such as IFN- γ release or 4-1BB expression. On the one hand, it could be injected into patients for cell therapy. On the other hand, the related functions of neoantigen reactive T cells have been verified by different experiments

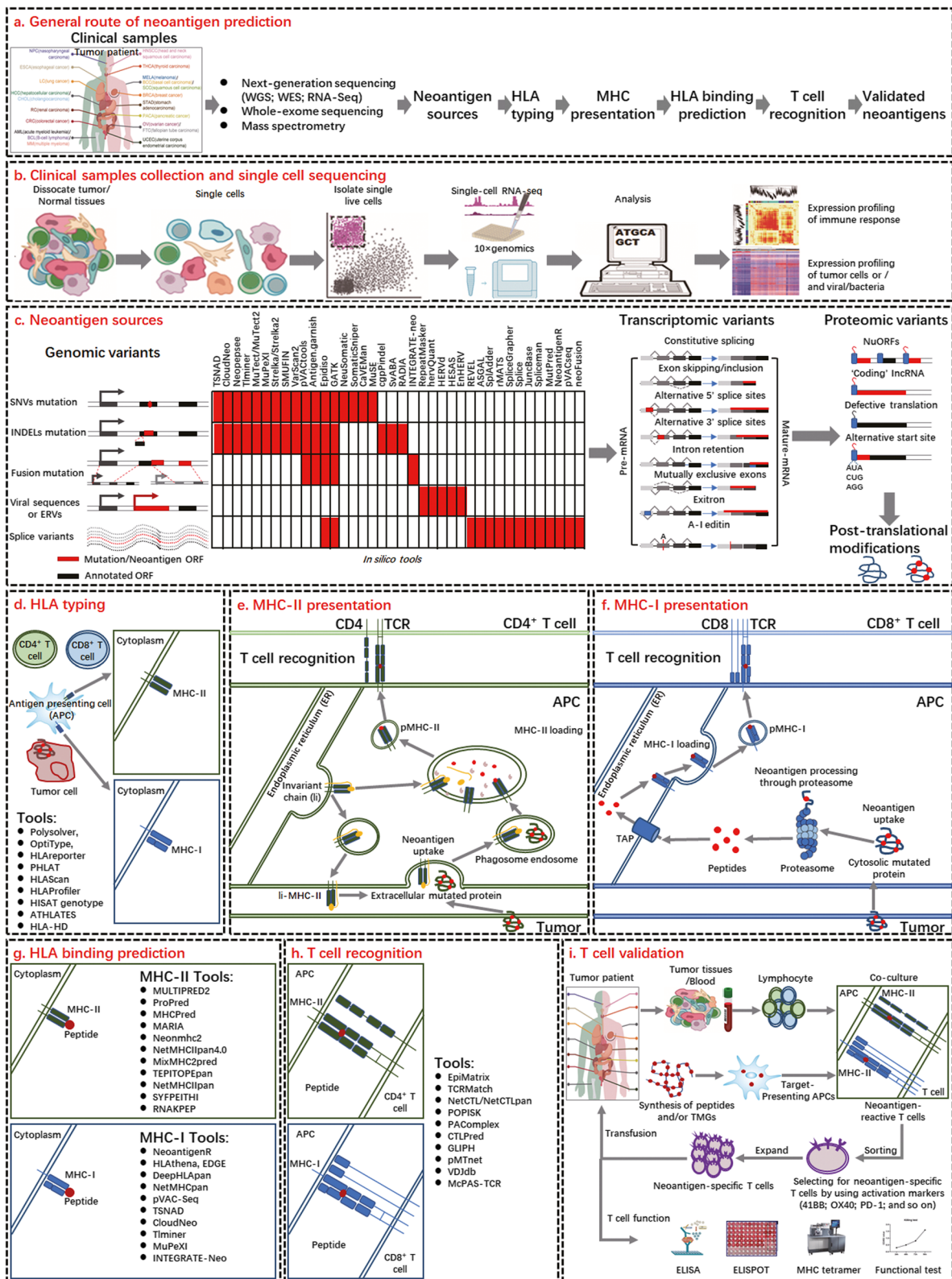


Fig. 2 (See legend on previous page.)

Table 1 The advantages and disadvantages of various neoantigens and associated cancers

Neoantigen classification	Sources	Advantages	Disadvantages	Cancers
Genomic variants	Single-nucleotide variants (SNVs) mutation Insertions and deletions (INDELs) mutation Genes fusion mutation	Relatively high burden; Simple prediction More immunogenic; More potential targets per mutation; More dissimilar from self-antigen More immunogenic; More potential targets per mutation; More dissimilar from self-antigen; Shared targets between tumors	Rarely shared between patients; similar to self-antigen Relatively low burden Relatively low burden	Bladder cancer; Lung cancer (squamous and aseno); Melanoma; Glioblastoma Renal cell carcinomas (Clear cell, chromophobe and papillary); MSI-H tumors Chronic myeloid leukemia; Acute lymphocytic leukemia; Acute myelocytic leukemia; Sarcomas
Transcriptomic variants	Structural variants (SVs) mutation Transcript alternative splicing Polyadenylation (PA) and RNA editing Allegedly non-coding regions RNA splicing	High immunogenicity High immunogenicity; A large number of predicted targets Easy predication More potential targets; Relatively high burden More dissimilar from self-antigen; A large number of predicted targets	Less well studied Less well studied Less well studied Less well studied; Fewer tools available Fewer tools available; Current tools do not account for nonsense mediated decay (NMD); Not well validated in pre-clinical models; Less well studied	Malignant pleural mesothelioma Squamous cell carcinoma of pancreas; Adenocarcinoma of lung Chronic lymphocytic leukemia Acute lymphoblastic leukemias; Lung cancers Chronic lymphocytic leukemia; Acute myelocytic leukemia; Chronic myelocytic leukemia; Myelodysplastic syndrome
Proteomic variants	Post-translational modifications (PTMs) T cell epitopes associated with impaired peptide processing (PEIPP) Transporters associated with antigen processing (TAP) Viral open reading frames (ORFs) or viral sequences or ERVs	Shared between patients TEIPP-specific T cells can escape thymic selection High specificity High immunogenicity; Shared between patients; More dissimilar from self-antigen; Without apparent toxicity to normal tissues High immunogenicity; Strong immunogenicity; Not expressed in normal tissues except testicles and placenta Most are presented by HLA-A2	Less well studied Less well studied; TAP-deficient tumors or Limited in HLA-I low Less well studied; Fewer tools available Limited in specific tumors Less well studied; Limited in specific tumors Less well studied; Cause damage to normal tissue; Inability to effectively present autoantigens	Non-small cell lung cancer; Leukemia; Renal cancer Lung cancer Acute myelocytic leukemia Head and neck cancer; Merkel cell carcinoma; Nasopharyngeal carcinoma; Cervical cancer; Hepatocellular carcinoma; Anal cancers Testicular cancer; Germ cell tumor
Viral-derived neoantigens				
Tumor-testicular antigen				
Tissue differentiation antigens and others				

response to ICB therapy; thus, these mutations can be novel biomarkers for the ICB response. INDEL frameshift mutations are supposed to produce more immunogenic neoantigens, hence improving response to ICBs. When frameshift mutations are present, the progression-free survival of patients receiving ICBs is significantly prolonged [23]. For example, considerable variation in overall response rates and disease control rates has been observed in patients with frameshift-mutated non-small cell lung cancer (NSCLC). Progression-free survival (PFS) was significantly prolonged in patients treated with ICBs when frameshift mutations were present [24]. Therefore, ICBs enhance the immune response to frameshift-driven neoantigens. Pembrolizumab blocks PD-L1 in vivo, restoring frameshift neoantigen-specific T-cell immunity [25].

Gene fusion

Chromosomal translocations or deletions induce DNA rearrangements that can lead to gene fusion, which may produce fusion proteins, another important type of mutation in tumors that may provide many neoantigens [26]. For example, the BCR-ABL fusion protein is produced by translocations between chromosomes 9 and 22 in patients with chronic myelogenous leukemia (CML) [27], and the SYT-SSX1 fusion protein is produced by translocation of chromosomes X and 18 in patients with synovial sarcoma [28]. A further expanded study of 30 different tumor types in the TCGA database found that 24% of cancers expressing fusion proteins contained new epitopes produced by fusion, which were predicted to bind to patient-specific MHC, and peptides from different fusion regions were recognized by the patient's own T cells. At the same time, only 5.8% of fusion-driven neoantigens were consistent between patients, and the immunogenicity of these neoantigens was usually low [29, 30]. A comprehensive analysis of three TCGA databases found that fusion mutations produced more ORFs and approximately 6 and 11 times more neoantigens than SNV and INDEL mutations, had better immunogenicity, and induced a stronger immune response. Similar to the candidate neoantigen load produced by SNV and INDEL mutations, fusion neoantigen load is closely related to fusion mutation load, especially in microsatellite stable tumors with high fusion mutation load [31, 32]. In addition, immune depletion microenvironments or malignancies with HLA loss are more likely to present fusion neoantigens. According to FACETS analysis of the TCGA exome data, HLA heterozygosity loss (LOH) occurred in 18.4% of the cases, which increased the likelihood of the presence of fusion neoantigens [33]. For example, in melanoma treated with

anti-PD-1, the clearance of tumor cells carrying fusion-derived neoantigens showed negative immune surveillance selection pressure against these neoantigens [34]. Neoantigens produced by gene fusion are still able to activate cytotoxic T cells even in some tumors with low TMB and limited immune penetration [29]. These results suggest that gene fusion is important as a source of tumor-specific neoantigens.

Neoantigens derived from gene fusion can induce tumor-specific immune responses. A comprehensive study of 33 tumor types found a variety of common fusion neoantigens, including TMPRSS2-ERG, MYB-NFIB, EML4-ALK, DEK-AFF2, CFBF-MYH11, and SYT-SSX [35–38]. TMPRSS2-ERG is the most common recurrent fusion gene, occurring in 38.2% of prostate cancer patients. Several HLA-restricted epitopes with high affinity were identified from relapsed TMPRSS2-ERG type VI fusion, which bind to HLA-A*02:01 in vitro and are recognized by CD8⁺ T cells [39, 40]. Fusion of the proto-oncogene MYB with the transcription factor NFIB is a biomarker of adenoid cystic carcinoma and occurs in 60% of cases [41]. One NFIB-MYB-derived peptide (MMYSPICLTQT) and three MYB-NFIB-derived peptides (QFIDSSWYL, SLASPLQPT, and SLASPLQSWYL) bind to HLA-A*02:01 to activate the immune system [29, 42]. The EML4-ALK fusion gene is mainly present in 5% of young NSCLC patients who either rarely smoked or never smoked. The use of EML4-ALK-derived peptides can stimulate specific CTL responses and potentially treat EML4-ALK-positive NSCLC patients [43, 44]. A study of head and neck squamous cell carcinoma (HNSCC) found that the immune response to PD-1 treatment is mediated by neoantigens produced by DEK-AFF2 fusion. DEK-AFF2-derived peptides (DKESSEEVV) enhance T-cell activation according to MHC class when delivered to autologous peripheral blood mononuclear cells (PBMCs) [45]. The CFBF-MYH11 fusion antigen is distributed on acute myeloid leukemia (AML) cells and can activate T cells to induce specific killing of tumor cells [46]. SS393 (GYDQIMPVK) and SS391 (PYGYDQIMPVK) are two common SYT-SSX fusion antigens in synovial sarcoma. These neoantigen peptides successfully induce synovial sarcoma-specific CTLs, which specifically kill HLA-A24-positive synovial sarcoma cells containing SYT-SSX neoantigens and target cells with peptide pulses [47, 48]. In addition, FGFR3-TACC3, CCDC6-RET, BCR-ABL, SYT-SSX1/SSX2, PAX3-FOXO1, TPM3/TPM4-ALK, EBS-FLI1 and other fusion antigens are also immunogenic neoantigens, which provide possible targets for tumor immunotherapy [49–54]. Therefore, neoantigens produced by fusion mutations greatly increase the capacity of the tumor-specific antigen library, providing more potential targets for cancer immunotherapy.

Structural variants (SVs)

SVs are one of the most common forms of driver gene mutations in tumors that can lead to changes in genome structure and alter gene expression or function to promote the malignant transformation of tumors [55]. SVs generally refer to genetic variants greater than 50 base pairs, such as insertions, deletions, inversions, translocations, duplications/amplifications, chromosome additions and subtractions, and chromosome rearrangements [56]. For example, in patients with malignant pleural mesothelioma (MPM), potential neoantigens produced by chromosome rearrangements have been identified using a combination of MPseq and RNAseq. Rearranging associated neoantigens can produce MPM-specific immune responses in a manner similar to that of INDEL-induced neoantigens [57, 58]. In particular, neoantigens produced by SVs were predicted to be presented on MHC proteins closely associated with the clonal proliferation of TILs, and effector T cells targeting these neoantigens were found in circulating blood [59]. Therefore, SV-derived neoantigens may also be valuable targets for antitumor immunotherapy.

(二) Neoantigens derived from transcriptome variations

Selective processing of mRNAs, including selective splicing events, polyadenylation (pA), RNA editing, so-called non-coding events, and post-transcriptional events offer the possibility of expanding the neoantigen space and contributing to tumor-specific antigen diversity [60].

Transcript alternative splicing

Abnormal selective mRNA splicing is another source of tumor-specific antigens. In normal cells, RNA splicing is the efficient and realistic conversion of immature mRNA into mature RNA. However, neoantigens derived from the selective splicing of transcripts may be caused by mutations in RNA cis-regulatory elements, trans-regulatory factors, or core spliceosomes [61]. Mutations in cis-acting elements generate potential neoantigens by altering splicing. Extensive studies of TCGA have revealed many exon-exon connections unique to tumors, most of which can express novel neoantigens [62]. Transaction changes, in which somatic mutations in splicing factors lead to splicing mutations, induce the formation of neoantigens across the genome [63]. In hematological malignancies, common mutations in spliceosomal components, including SRSF2, SF3B1, and U2AF1/2, increase the expression of splicing variant mRNA and lead to neoantigen translation and protein expression [64–66]. In cells with normal sense-mediated RNA decay (NMD) function, a first round of translation is required to initiate NMD-mediated degradation of abnormal transcripts, which may also result in the production of small amounts

of neoantigens [67]. For example, mutations in the highly conserved core NMD factor UPF1 are prevalent in pancreatic and lung adenocarcinoma, and these mutations increase the frequency of abnormal transcription and neoantigen production [68, 69]. Highly abnormal splicing events in tumors increase the amount of potential tumor-specific antigens, especially in tumors with low copy number variation and somatic cell mutation rates [30, 60]. In conclusion, these studies emphasize the idea that the alternative splicing of transcripts can promote neoantigen production.

Polyadenylation (pA) and RNA editing

Similar to RNA splicing, polyadenylation (pA) and RNA editing can alter the proteomes of tumor cells, thereby increasing the amount of potential immunotherapeutic targets. However, it must be emphasized that because RNA editing can also occur in normal tissues, these peptides are not necessarily tumor-specific [70, 71]. In tumors, genes upstream of the new pA site and downstream of the nearest 5' splicing site are translated when a specific polyadenylation (IPA) event occurs at the coding region. By comparing RNA-seq data from tumor and normal tissue samples of different cancers, more neoantigens produced by IPA can be identified, and these neoantigens are potential targets for cancer immunotherapy [60]. Similar to splicing and IPA, RNA editing induces nonsynonymous substitution by altering specific nucleotides in the RNA sequence, which can produce new proteins that can be presented by the MHC molecule to further induce activation of specific CD8⁺ T cells. These results suggest that these novel peptides are immunogenic [72].

So-called non-coding events

Recent studies have shown that many regions previously defined as noncoding regions have been shown to have coding functions [60, 73]. The screening of neoantigens from exon-region mutations alone is limited in scope, given that 99% of tumor-specific mutations occur in the noncoding regions of genes and exon regions account for only 2% of the entire human genome [74]. Thus, by studying these newly defined coding genes, many new antigenic peptides that can be presented by MHC have been identified as targets for TIL immunotherapy [75]. These MHC-related peptides (MAPs) from noncoding region genes extend the range of CD8⁺ T-cell immune monitoring from 2% (the proportion of exons in the human genome) to 75% [76]. Using mass spectrometry (MS), it has been found that many noncoding regions produce large amounts of abnormally expressed tumor-specific antigens, most of which result from epigenetic modifications in atypical translation events rather than mutations.

These abnormally expressed tumor-specific antigens are more common than neoantigens produced by coding mutations and can be shared between tumor patients [77–79]. Therefore, there is an urgent need to develop fast and effective computational methods to screen these potential neoantigens and verify their feasibility for use in immunotherapy.

(三) \ Neoantigens derived from proteomic variations

Due to the deletion and replacement of nucleotides encoding a protein during translation, the reading frame is shifted forward, backward or mutated. The functional abnormalities of protein posttranslational modification (PTM), proteasome processing, and antigen-processing related transporter (TAP) ultimately lead to changes in the translated protein sequence, providing an important new source of tumor-specific neoantigens [80, 81].

The presentation of neoantigens by MHC molecules to T cells maintains specific PTMs [82]. Abnormal PTMs, including glycosylation, O-linked beta-n-acetylglucosamine (O-GlcNAc), and phosphorylation, can produce neoantigens presented by the MHC complex in tumors [83]. In addition, an unusually large proportion of mutations may promote the formation of new N-glycosylation sites, leading to the production of neoantigens [84]. Abnormal phosphorylation can produce new antigens by promoting the binding of epitopes to MHC molecules or by altering the antigenicity of the presented epitopes [85]. For example, tumor-associated phosphopeptides derived from BCAR3 and IRS2 are immunogenic to healthy donors and mice [86–88]. Covalent KRAS-G12C inhibitors, such as ARS1620, produce covalently modified peptides that can be presented on MHC to stimulate T-cell responses [89]. These tumor-specific PTMs involve covalent drug-mediated alkylation of mutated cysteine residues on cancer proteins, providing a new source of neoantigens and an easy target for immunotherapy [83].

In addition, another new set of antigenic epitopes is derived from damage that occurs during proteasome processing or the TAP complex. The proteasome processes proteins and converts them into peptides, which are particularly critical for the process of converting proteins to MHC-restricted epitopes. According to preliminary statistical analysis, the proteasome is responsible for splicing approximately one-third of MHC-I-related immune peptides and is capable of activating CD8⁺ T cells [90, 91]. Splicing epitope-specific CD8⁺ T cells from the TILs of human acute myeloid leukemia (AML) patients inhibited the growth of corresponding tumor cells in severe combined immunodeficiency (SCID) mouse models [92]. Neoantigens derived from protein splicing can provide undeveloped or unidentified neoantigens for antitumor vaccines and cancer immunotherapy. However, most

tumors eventually acquire resistance and immune escape mechanisms [93]. Tumors can avoid being recognized by T cells by producing defective HLA-I antigen processing pathways or by downregulating the expression of related genes [94]. A class of neoantigens known as T-cell epitopes associated with impaired peptide processing (TEIPP) has been found in some tumors with low HLA-I/TAP deficiency [95]. They are a class of unmutated antigens from the tumor's own housekeeping proteins that activate TEIPP-specific CD8⁺ T cells and specifically kill these TAP-deficient cancer cells. TEIPP peptides are currently considered immunogenic because they are not presented by normal cells, and TEIPP-specific T cells are not negatively selected for in the thymus [96, 97]. For example, a TEIPP peptide derived from the Lass5 protein, also known as Trh4, was able to activate specific T lymphocytes and inhibit the growth of MHC-I low/TAP deficient tumors in a TCR-transgenic mouse model [98, 99]. In addition, other nonmutated tumor epitopes of TEIPP, including calcitonin (pCT) precursor proteins (ppCT50-59 and ppCT91-100) and procalcitonin (ppCT) (ppCT16-25, ppCT9-17), have been identified. These antigenic peptides based on TEIPP can effectively induce antitumor CTL effects and inhibit tumor growth [100, 101]. Therefore, targeting these TEIPP neoantigens has the potential to provide a promising new immunotherapy approach for TAP-deficient/HLA-low tumors.

(四) \ Neoantigens encoded by viral genes

Viral proteins may be considered another class of neoantigens in virus-induced tumors because they are almost completely different from normal cellular proteins and can trigger high-affinity TCR responses [102]. For example, some solid tumors are caused directly by viral infections, including cervical cancer caused by HPV virus infection [103], nasopharyngeal carcinoma caused by Epstein–Barr virus (EBV) infection [104], and lymphoma caused by EBV infection [105]. These viral genes with carcinogenic properties can be integrated into the cell genome and promote the continuous expression of viral genes, thus leading to the occurrence and development of tumors [106, 107].

Currently, many tumor immunotherapy studies focus on virus-derived tumor antigens [108]. For example, in two of the nine patients with HPV-positive metastatic malignancies, tumor regression continued in the ACT treatment study using TILs [109]. In two separate clinical trials (NCT02280811 and NCT02858310), autologous T cells transduced with anti-E7-TCR responded in 4 of 12 patients, while T cells transduced with anti-E6-TCR responded in all 12 patients [110–112]. ACT therapy for MCPyV and EBV has also achieved promising clinical results in the treatment of corresponding tumors,

although other effective therapies have been implemented in these experimental protocols. None of these clinical trial participants showed significant toxicity to normal tissue [113, 114]. Overall, these trials demonstrate the safety and efficacy of targeting oncogenic viral proteins to treat associated tumors. Given their critical role in tumorigenesis and the fact that they are shared by patients, these neoantigens are ideal targets for cancer immunotherapy, these results support the further development of integrated treatment regimens based on viral protein correlations.

(五) \ Cancer-testicular antigens

Cancer/testis antigens (CTAs) are also known as cancerous testis antigens. These antigens are not expressed in normal tissues except the testis and placenta but are abnormally expressed in a variety of tumors with high tumor specificity and strong immunogenicity [115]. More than 110 CTAs have been identified, which belong to more than ten gene families, such as the MAGE, BAGE, GAGE, SAGE, HAGE, SXX, SCP1, LAGE and NY-ESO-1 families. Among them, MAGE-A, MAGE-B, MAGE-C and NY-ESO-1 family protein molecules are more studied [116, 117]. CTA is an ideal therapeutic target of TCR-T cells. T cells targeting tumor-associated CTAs selectively eliminate tumor cells and with little or no toxicity to normal tissues [118, 119].

(六) \ Tissue differentiation antigens and others

Tissue differentiation antigens are expressed in tumor cells and some normal cells and tissues. Targeting them can cause damage to normal tissue, but these injuries tend not to be fatal [120]. The most famous of these antigens are melanoma differentiation antigens, including Melan-A, Mart-1, gp100, gp75, TRP21 and TRP22 [121]. Melanoma differentiation antigens are only expressed in melanoma and melanocytes, most of which are presented by HLA-A2 [122]. Some lung cancer antigens discovered in recent years are also tissue specific and can also be classified as tissue differentiation antigens [123]. At present, most studies on TCR-T-cell target antigens focus on polypeptide antigens that can be presented by HLA-A2. Only tumor antigens that can be recognized by T cells have been identified in several types of tumors, such as melanoma, while ideal antigens have not been found in the vast majority of tumors, resulting in less ideal tumor-specific or related antigens [124, 125]. Solid tumor cells often have low or no expression of Class HLA-I molecules and cannot effectively present their own antigens, which makes it difficult to find HLA-A2-restricted antigens. In future studies, in addition to continuing to search for Class HLA-I restricted protein antigens, Class HLA-II presented protein antigens will also be studied

[126, 127]. Additionally, attention should be given to other antigens besides protein antigens, such as polysaccharide and lipid antigens [128, 129].

二 \ Identification, prediction and validation of TSAs

Thorough screening of TSAs across the entire cancer spectrum is now possible using the fusion of whole exome sequencing (WES), RNA-seq, and proteome data from TCGA. Based on NGS data, virtual peptide libraries have been created, and potential TSAs have been identified by virtual methods. The typical workflow for TSA prediction can be summarized in the following steps: mutation identification, HLA typing, screening and prioritization of neoantigens based on HLA binding affinity, and experimental validation of immunogenic neoantigens based on T-cell analysis [10] (Fig. 2d, e, f, g, h, i).

(一) \ Identification of somatic mutations

Currently, the initial phase involves mapping tumor-specific genetic abnormalities using the WES of tumor and normal tissue DNA to detect possible neoantigens from NGS data. By comparing NGS data from tumors and normal tissues in the same patient to identify genetic changes, the development of immunogenomics strategies has been greatly accelerated in terms of its ability to predict mutated peptides resulting from somatic mutations [130, 131]. WES is the recommended source of NGS data for neoantigen prediction because it provides the highest mutation coverage by focusing on the protein-coding regions of the genome [132]. RNA-seq data can be combined with WES to determine whether the mutated gene is expressed in the tumor [133]. Additional biological information can also be found in RNA-seq, such as information about copy number changes, microbial contamination, transposable elements, cell types, and neoantigen presence [134]. Mass spectrometry enables high-throughput identification of MHC-binding peptides and direct detection of MHC-binding peptides from immunoprecipitation and extraction. The neoantigens predicted by immunogenomics methods can be verified by comparing the tandem mass spectrometry of the sample with that of the synthetic peptide. Especially for rare HLA allotypes and HLA-II ligands, mapping tumor HLA ligands can help identify neoantigen-specific cancer immunotherapy targets in clinical trials [135]. MS is integrated with NGS to further detect tumor-specific neoantigens produced by somatic mutations, noncoding RNAs, and proteasome splicing, which are omitted by whole-exome- or transcriptome-based neoantigen sequencing techniques [136]. Computational analysis includes data preprocessing and quality control, identification of the variation of somatic mutations, and prediction of altered proteins and functional effects using public genome,

transcriptome, and proteome databases. The immunogenicity of neoantigens, including the rank affinity of mutant and normal peptides, the frequency of mutant alleles, and the amount of gene expression, was evaluated based on the filtration technique of an integrated scoring system and quantitative scoring of the characteristics of the novel peptides to experimentally evaluate the immunogenicity of the peptides [137, 138] (Fig. 2a, b, c; Table 1).

(二) \ HLA typing

Humans have more than 24,000 distinct HLA-I (HLA-A, HLA-B, and HLA-C) and HLA-II (HLA-DR, HLA-DQ, and HLA-DP) alleles, and their mixing results in biological diversity [139]. Similar to other antigens, neoantigens are usually delivered by MHC-II molecules of CD4⁺ T cells and MHC-I molecules of CD8⁺ T cells in a cell-specific manner. The patient's HLA alleles determine their tumor-specific antigen library, which is made available to T cells for recognition. Therefore, one of the most important initial steps in neoantigen prediction is to determine a patient's HLA genotype [126]. Most methods rely on DNA-derived NGS data obtained from WES or WGS to achieve this goal. For example, Optiype and polysolver are excellent tools for identifying Class HLA-I alleles. Other types, such as HISAT-Genotype, ATHLATES, HLA-HD, HLA-Reporter, PHLAT, HLA-Scan and HLA-Profler, can be used for HLA-I and HLA-II type classification [140–142] (Fig. 2d).

(三) \ Prediction of HLA binding and neoantigen presentation

A number of online predictive tools have been created based on the discovery of neoantigens for MHC molecular processing and presentation, including NetChop, NetCTL, and NetCTLpan. By combining HLA ligand set data into computer learning algorithms, such as linear regression and artificial neural networks, their predictive power can be actively improved [143–146]. In vitro peptide-HLA binding datasets are used to train computer learning models, and NetMHCpan and MHCflurry are the main components of the current HLA ligand recognition line. They improve the predictive performance of tumor neoantigens by integrating information from binding affinity data and MS polypeptide set data to generate "panspecific" computer learning strategies for MHC-I [147, 148]. A series of computational techniques for predicting MHC-II binding epitopes have been developed using artificial neural networks, including NetMHCII, NetMHCIIpan, SYFPEITHI, RNAKPEP, MULTIPRED2, ProPred, and MHCpred. However, current computational predictions of binding affinity for MHC-II peptides are less precise than those for MHC-I molecules. First,

compared with MHC-I molecules, MHC-II binding peptides are more promiscuous in terms of peptide length and binding sequence motif. Second, the polymorphism of α and β chains in MHC-II molecules also greatly expands the diversity of peptide binding specificity [149–154]. Given that multiple processes control neoantigen presentation, it can be inferred that increasing binding affinity alone does not accurately reflect cell treatment and T-cell response. Other properties, including proteasome cleavage, transport of peptides into the endoplasmic reticulum, HLA alleles, binding affinity between peptides and MHC molecules, etc., give preference to possible neoantigens [155] (Fig. 2e, f, g).

(四) \ Evaluation and verification of the immunogenicity of candidate neoantigens

It is well known that suitable MHC molecular presentation and effective TCR recognition are prerequisites for immunogenic neoantigens. Most neoantigens predicted by MHC molecular presentation do not trigger an immune response. Therefore, it is critical to consider TCR recognition of pMHC complexes when evaluating the immunogenicity of potential neoantigens [155–157]. There are a number of programs to predict neoantigen-specific T-cell recognition, and the most common methods are NetCTL and NetCTLspan. A composite score is generated based on MHC binding, C-terminal cleavage affinity, and TAP transporter, rather than directly predicting T-cell junctions [145, 158]. Recent studies have used computer learning or deep learning techniques to predict TCR-peptide/-pMHC binding. In addition to identifying TCR-pMHC pairs, clustering methods such as pMTnet and GLIPH can also cluster TCRs that recognize the same epitopes and predict their HLA limitations [159–161]. Predicting the binding affinity of TCR remains challenging due to its low affinity for pMHC ligands (Fig. 2h). Evaluation of the immunogenicity of candidate neoantigens using a variety of screening methods will be critical to more precisely identify and select neoantigens suitable for clinical intervention [162] (Table 2). To more accurately evaluate the potential of neoantigens in immunotherapy, experimental validation of their T-cell reactivity is essential [163]. Neoantigen reactive T cells have been validated or screened by T-cell-based assays, multicolor labeled MHC tetramers, and enzyme-linked immunosorbent spot assays (ELISPOTs).

Validation of the immunogenicity of neoantigen peptides

The specimens used for transplantation of PDTX (Patient-Derived Tumour Xenografts) in humanized mice are directly derived from human tumor tissues and have not been cultured in vitro, stably retaining the genetic, histological, and phenotypic characteristics of

Table 2 Immunological screening assays used to test for neoantigen recognition

Strategy	Advantages	Disadvantages
cDNA libraries	Interrogates all transcribed sequences	Interrogates mutated and non-mutated sequences; Biased toward highly transcribed genes; Influenced by the size, expression levels or GC-richness of transcripts encoding for T-cell epitopes; Labor intensive and time-consuming
Peptide-HLA multimers	Allows the isolation of antigen-specific T cells; Overcomes the need of autologous APCs	Multimers are available for a limited number of HLA molecules; Exclusively interrogates a selected list of mutated epitopes based on in silico prediction or validated by immunopeptidomics; Not optimal for CD4 ⁺ cells
Minimal epitopes	HLA-matched target cells (based on in silico prediction) can be used instead of autologous APCs; Cost-effective	Requires autologous or HLA-matched cells as target cells; Exclusively interrogates a selected list of mutated epitopes based on in silico prediction or validated by immunopeptidomics; Not optimal for CD4 ⁺ cells
Tandem minigenes or peptide pools	Allows potential processing and presentation of candidate neoantigens on HLA-I and HLA-II; Does not require prior knowledge of the minimal epitope or HLA restriction; Can be used to interrogate all or a large portion of mutated epitopes;	Peptide processing by immunoproteasome in APCs might differ from processing by the proteasome in tumor cells; Availability of APCs/effectors can limit this approach, especially when > 250 epitopes are tested; Cost increases in patients with high mutation burden; Requires autologous APCs as target cells;

APC Antigen-presenting cell, HLA Human leukocyte antigen

the tumor, i.e., tumor heterogeneity [164]. Therefore, PDTX can be used to screen the sensitivity or resistance of immune cells by MHC tetramers, and the test results have good clinical predictability. In addition, because PDTX better retains the tumor mesenchymal and stem cell components during the transplantation process, the microenvironment for tumor growth is closer to the actual situation. PDTX can also provide a large number of specimens for the preservation and passage of tumor samples, which can be used before clinical treatment for the efficacy validation of the immunogenicity of cells or vaccine preparations. It can also be used to determine the appropriate sample size and efficacy evaluation for small preclinical trials. The PDTX test can be used to predict and validate the efficacy of neoantigen in advance, improving the effectiveness rate of personalized tumor neoantigen therapy [165–167].

In vitro validation of immunogenicity by ELISPOT test

The T-cell immunogenicity assay is the most direct method to evaluate the immunogenicity of candidate neoantigens. The reactivity of neoantigen-specific T cells amplified *in vitro* was measured by flow cytometry for the T-cell activation markers 4-1BB and OX-40 after polypeptide stimulation or by ELISPOT for IFN- γ production. In organisms, cells will produce cytokines (such as IFN- γ) after stimulation. These cytokines will be captured by specific antibodies and then develop colors to form spots. The number of spots represents the level of immunogenicity of the corresponding neoantigen. This platform will further enhance the accuracy of neoantigen screening at YuceNeo. ELISPOT has little effect on the biological process of cells, and higher sensitivity in cell detection. Based on test on the single-cell level, one IFN- γ -secreting cell can be detected from 200,000 to 300,000 cells through the ELISPOT test. ELISPOT is more sensitive than Elisa and the limiting dilution method [168–172].

In vitro validation of immunogenicity by NEST

In addition, scRNA-seq was used to discover paired TCR sequences associated with cells expressing high levels of IFN- γ in TILs that were cocultured with tandem microgene (TMG)-transfected or peptide-stimulated antigen-presenting cells (APCs) [173]. ELISPOT can be used *in vitro* to analyze which neoantigens can stimulate T cells to secrete cytokines, but it cannot detect the TCR of tumor-specific T cells. YuceNeo's unique NEST (Neoantigen Expansion of Specific T Cells) validation technology integrates TCR sequencing of peptide-stimulated T cell culture with a biological information platform to identify antigen-specific clonal amplification. The NEST platform of YuceNeo compares the TCR databases with

and without stimulation by neoantigen peptides. If a TCR with an increasing frequency can be found, it can indicate that the corresponding neoantigen is immunogenic. The NEST technology platform not only can validate the immunogenicity of neoantigens screened by TruNeo™ in a high-throughput manner, but also can be used to dynamically monitor the effect of tumor immunotherapy [174–176]. Based on WES-guided neoantigen prediction and TCR sequencing of polypeptide-stimulated T-cell cultures, neoantigen-specific clones of TCRV β were sensitively characterized by the mutation-associated neoantigen functional expansion of specific T cells (MANAFEST) assay [177, 178]. In addition to evaluating the tumor specificity of the TCRV β clones, dynamic changes in neoantigen-specific T-cell responses over time can be studied, and the effects of immunotherapy can be monitored using liquid biopsies obtained before or after treatment [179, 180] (Fig. 2i).

三、Enrichment and isolation of tumor antigen-specific T cells

V(D)J recombination of TCR during thymus development leads to great diversity in TCR sequences in the human T-cell pool. It is estimated that in the average adult, there are approximately 4×10^{11} total circulating T cells and approximately 10^{10} unique T-cell clones. Therefore, T-cell clones are specific for the vast majority of nonviral antigens. The frequency of cloning in peripheral blood is much lower than the frequency of isolation of antigen-specific TCRs under current technical conditions [181–183]. Thus, TCR isolation efforts usually begin with methods that allow enrichment of T cells with the desired antigen specificity (Fig. 2i).

(一) Enrichment of antigen-specific T cells

Amplification of TILs

A large number of tumor-infiltrating lymphocytes (TILs) are usually present in some types of solid cancer. Compared with peripheral blood T cells, T cells in tumor tissues are usually enriched in tumor antigen-specific clones [184–186]. Some research groups have used extended TILs as a source for finding tumor-specific TCRs [175, 187]. In addition, TIL amplification *in vitro* is itself an effective adoptive T-cell (ATC) therapy for several types of solid cancer [188, 189]. Studies have shown that Iovance's TIL treatment named Lifileucel has strong efficacy in clinical trials [190, 191].

Vaccination

T cells that are specific to the antigen of interest can be selectively expanded *in vivo* through vaccination strategies. A common approach is to inoculate human HLA transgenic mice with antigens of interest, which results in

robust enrichment of antigen-specific T cells that can be harvested from the lymph nodes and spleen [192, 193]. In certain cases, peripheral blood from patients participating in cancer vaccine trials has been used as a source of antigen-rich T cells for TCR [194].

Selective in vitro expansion of peripheral blood T cells

Peripheral blood T cells were stimulated in vitro in an antigen-specific manner to drive selective T-cell expansion with the desired specificity. Early pioneering work in this area included in vitro stimulation of peripheral blood T cells to preferentially expand virus-specific T cells [195, 196]. These stimulation methods have been used to amplify TAA-rich and neoantigen-specific T cells [197, 198]. These methods usually stimulate T cells by autologous antigen presenting cells (APCs), usually dendritic cells (DCs), in the form of exogenous peptides or by cDNA/RNA delivery pulsed with the antigen of interest [198–202]. Several studies have shown that in the case of patient-derived peripheral blood, initial selection of PD-1⁺ and/or antigenic experience (CD45RO⁺CD62L⁺, CD45RO⁺CD62L⁻ or CD45RO⁻CD62L⁻) T cells can further enhance tumor-specific T-cell enrichment in vitro [203, 204]. To overcome the need to produce autologous mature DCs for antigenic stimulation, so-called artificial antigen presenting cells (aAPCs) have been developed [205]. For example, the myelogenous leukemia cell line K562, a common aAPC system, was used, and this cell line was negative for HLA-A, B and DR. This cell line acts as a modular aAPC by providing the stable transduction of various HLA alleles and costimulatory molecules [206]. Other cell-free aAPC systems have also been developed to conjugate HLA and costimulatory molecules to beads and nanoparticles to stimulate peripheral blood T cells and obtain a large number of clinically needed antigen-specific T cells [207, 208].

(二) \ Isolation of antigen-specific T cells

After obtaining polyclonal T-cell products enriched with target-specific T cells, it is necessary to isolate antigen-specific T cells from a large population of T cells. Approaches in this area usually involve stimulating T cells with homologous antigens of interest and isolating antigen-reactive T cells based on the increased expression of molecules known to be associated with T-cell activation [209]. For example, 4-1BB and OX40 in CD8⁺ T and CD4⁺ T cells allow for the isolation of these cells by FACS sorting or magnetic bead sorting [210]. Alternatively, the use of peptide-HLA polymeric MHC staining followed by FACS sorting or magnetic bead sorting is an effective way to identify and isolate antigen-specific T cells. Although the library of HLA polymeric reagents is expanding, these reagents are still limited to relatively

common HLA alleles [170]. Another approach is IFN- γ capture; IFN- γ is rapidly secreted by antigen-stimulated CD8⁺ T and CD4⁺ Th1 cells, thus, IFN- γ capture identifies and captures antigen-stimulated T cells based on IFN- γ production [211, 212].

四 \ TCR epitope discovery strategy: from TCR to epitope

(一) \ Prediction strategy based on computer algorithms

The binding affinity of HLA and the immunogenicity should be considered in the prediction of antigen epitopes [213]. Antigenic peptides can be run through MHC epitope prediction algorithms to prioritize the list of candidate antigens. The following relevant methods have been used to develop relevant prediction software: structure-based (SB), motif matrices (MM), quantitative affinity matrices, QAM), artificial neural network (ANN), support vector machines (SVM) and so on. The prediction algorithms include NetMHC-4.0, Immune Epitope Database (IEDB), NetMHCpan-4.1, NetMHCIIpan-4.0, SYFPEITHI and HLAthena, which are used to calculate and predict TCR epitopes [150, 214–217].

(二) \ Screening strategies based on tumor cells

HLA peptides were obtained by an immunoprecipitation technique, and the products were acid-eluted to obtain peptides; then, the peptides were identified by mass spectrometry. The quality of peptide segments was determined by primary mass spectrometry, and peptides with high abundance were selected for secondary mass spectrometry. In secondary mass spectrometry, the peptides collided with each other, resulting in amino acid bond breakage, and peptide fragment ions were analyzed by the detector to obtain the amino acid sequence information of screened peptide segments [218, 219]. The technical route is relatively mature, and low-abundance epitopes are difficult to identify.

(三) \ T-cell-based screening strategies

T-Scan The new method of high-throughput, concentrated screening and recognition of T lymphocyte target antigens in the whole genome can be used to identify antigens that can be effectively recognized by T cells. The human whole genome target antigen library was transduced into target cells containing the granzyme reporting system. After coincubation with T cells expressing TCR, the target antigen of the TCR could be enriched and identified. T-scans can be used to recognize functional target antigens of CD8⁺ T cells and can repeatedly detect target antigens from a large number of memory T cells [171, 220]. They have the advantages of genome-wide identification of target antigens, T-cell-based functional tests, and identification of potential off-target antigens.

Cellular system After transduction of the target antigen library into target cells containing the Trophocytosis reporting system and coincubation with T cells expressing TCR, the target antigen of TCR can be enriched and identified. Nibbling is highly specific and occurs only between cells successfully recognized by TCR antigens. TCR-Jurkat T cells expressing the known pairing were injected into a mixture of APC cells with the specific pMHC molecule recognized by the cells at a ratio of 1/10000. By flow cytometry, nibbling was still able to accurately label more than 70% of the APC cells specifically paired with this TCR, while other unrecognized antigen-presenting cells were not [221, 222]. This strategy also has the advantage of genome-wide identification of target antigens, T-cell-based functional tests, and identification of potential off-target antigens.

SABAs system The target antigen library was transduced into target cells containing the TCR-PMHC bifunctional molecule reporting system, and the target antigen of the TCR could be enriched and identified after coincubation with T cells expressing the TCR [223, 224]. Advantages include genome-wide identification of target antigens, T-cell-based functional tests, and identification of potential off-target antigens.

Presenter system After the target cells expressing the antigen library were coincubated with T cells expressing the TCR, the target cells that were killed in significant amounts were identified by high-throughput sequencing to identify the target antigen of the TCR [225].

RootPath system After the TCR sequence was found by single-cell sequencing, the quantized TCR was synthesized. After coculture with target cells expressing MiniGene, tumor reactivity TCR was confirmed by a T-cell reporting system. The T cells carrying this TCR were then assessed to determine if they could recognize the tumor and react with it [110, 226]. This system has the advantages of quantized TCR synthesis and T-cell functional tests.

MHTEM system Libraries of multiple HLAs and the target antigen MiniGene were introduced into target cells. After coculture with T cells, the target cells that were killed in significant amounts were identified by high-throughput sequencing to identify the target antigen of this TCR [227, 228]. The advantages of this system are based on T-cell functional testing, simultaneous screening of multiple HLA presentation epitopes and identification of potential off-target antigens.

(四) \ Strategy based on yeast display

Exogenous target protein gene sequences (exogenous proteins) were fused with specific carrier gene sequences and introduced into yeast cells. The yeast intracellular protein transport mechanism (GPI anchoring) was used to express target proteins and locate them on the yeast cell surface. Yeast was used to display random amino acid epitopes, and the expression of TCR protein was used to enrich potential antigenic epitopes; this system is also known as the 3T system [229, 230].

五 \ TCR cloning strategies: from known epitopes to specific TCRs

The specificity of TCRs is determined by the regional coding of two separate genes, TCR α and TCR β , and presents unique challenges in determining functional TCR sequences from T-cell populations. Accurate sorting of TCRs is the key to designing TCR-T-cell therapy for subsequent treatment [231–233] (Fig. 3).

(一) \ Strategies based on tetramer staining

Multicellular TCR cloning

Multiple cells stained with tetramers positive for specific antigens were selected, and the TCR sequences of these positive cells were amplified. The method is not technically difficult and has a high success rate of amplification. However, a large number of cross-pairing experiments are needed to verify the pairing and specificity of TCR chains, and important TCR chains may be missed [231, 234].

Single cell TCR cloning

Individual cells stained with tetramers positive for specific antigens were selected, and TCR sequences were amplified. Some studies have amplified the TCR- α and - β chains by performing single-cell RT-PCR, thereby avoiding the need to amplify T-cell clones after antigen-specific T-cell isolation. In this method, a single T-cell was sorted by FACS into a well containing RT-PCR buffer, RT-PCR was performed from the single cell, and the TCR- α and - β chains were amplified by PCR. This approach reduces the time and labor required to amplify a single T-cell clone; however, the disadvantage of this approach is that confirmatory assays to assess antigen specificity cannot be performed on T-cell clones prior to sequencing [235, 236]. Single-cell RNA sequencing (scRNAseq) has also emerged as a unique and effective platform for TCR discovery that allows for single-cell evaluation of cellular gene expression and gene transcription. As a result, several recent studies have successfully used the platform for TCR discovery. The method involved stimulation of T cells with the antigen of interest and then conducting

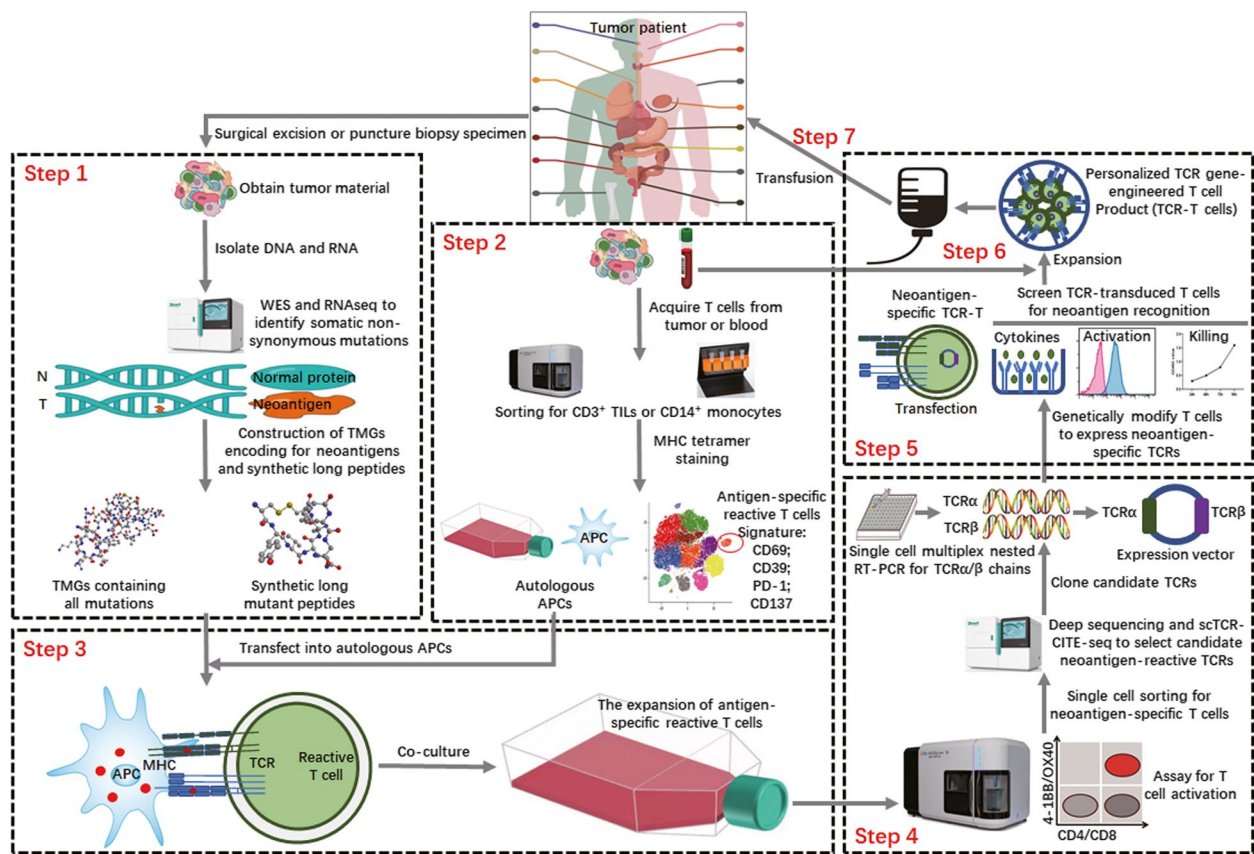


Fig. 3 Rapid identification of neoantigen-specific TCRs for personalized engineered TCR-T-cell therapy. Tumor (T) and normal (N) DNA are used to conduct WES and RNA-seq to identify cancer-specific nonsynonymous mutations. Candidate neoantigens are used to design tandem minigenes (TMGs) encoding mutant peptides and synthesize mutant peptide libraries (Step 1). TILs and PBMCs are isolated from single-cell suspensions taken from patient samples. TILs and PBMCs are analyzed using single-cell TCR-CITE-Seq, and a combined tag of gene and surface protein expression is used to predict candidate neoantigen-reactive T cells (Step 2). Candidate antigen-reactive T cells are cocultured with autologous antigen-presenting cells (APCs) expressing candidate neoantigens (TMG or peptides), and antigen-specific T cells are amplified (Step 3). Antigen-specific T cells are selected by flow cytometry, and neoantigen-reactive TCRs are identified and screened by scTCR-CITE-seq or deep sequencing. Then, the TCR α / β chain is reverse transcribed by single-cell multiplex nested RT-PCR, and its related plasmid is constructed (Step 4). T cells expressing candidate reactive TCRs are generated by cloning the selected TCR sequence into a retrovirus vector and transducing T cells. The recognition of neoantigens by T cells transduced by the TCR α / β chain is verified by different screening experiments (Step 5). PBMCs are obtained from patients, and the reactive T cells of the neoantigen-specific TCR α / β chain are amplified by the above methods (Step 6). Validated neoantigen reactive TCR α / β chains are selected to design final personalized TCR-engineered T (TCR-T) cell products that will be injected into patients for cell therapy (Step 7)

scRNAseq sequencing analysis. Antigen-specific T cells are identified by effector cytokines such as IFN- γ , TNF- α , and/or IL-2. Transcripts of the TCR- α and - β chains of activated cells were then obtained from the same dataset [175, 237]. This method has the advantages of low cost, high speed and high specificity and can directly realize TCR pairing, but it involves relatively complicated technology.

Feature barcoding technology

Tetramer-stained cells were prepared by single-cell droplets, and cell membrane DNA tags, RNA

expression and TCR-V(D)J libraries were constructed and sequenced. 10 \times Feature barcoding technology was used to construct a library by magnetic bead enrichment of tag sequences containing cell surface proteins and antigen-MHC complexes. The relatively long portion is then used to construct an mRNA library. A V(D)J library was constructed by enrichment of the V(D)J region by nested PCR [238, 239]. This method can simultaneously clone the specific TCR of multiple epitopes. However, the disadvantages include the fact that there are limited cell fluxes that can be analyzed, the large amounts of TCR synthesis needed, the limited sources of T cells, high cost and long cycles.

HLA-peptide-cDNA tag complex technology

DNA tags were used to label epitopes with multiple specificities, and tetramers were used to stain multiple epitope-specific T cells simultaneously [240, 241]. This method can be used to simultaneously clone the specific TCRs of multiple antigen epitopes, but it is difficult, and nonspecific reactions are problematic.

(二) \ Strategies based on T-cell functional analysis**Single-cell RNA + VDJ sequencing**

The antigen-stimulated T cells were sequenced by single-cell RNA + VDJ sequencing using 10×RNA expression + TCR VDJ sequencing, independent of tetramer [242, 243]. However, non-specificity increases the subsequent workload, cost and cycle time. Normal T-cell activity is needed, and TILs with poor activity are not appropriate.

Activated marker sorting

Activating molecules (PD1, 4-1BB) were used to sort antigen-activated T cells independently of tetramers [244–246]. This method was dependent on normal T-cell activity and not applicable for TILs with poor activity.

Intracellular factor sorting

T cells were stained with intracellular factors after antigen stimulation, independent of tetramers [247]. This technique relies on normal T-cell activity, and TILs with poor activity are not suitable. The operation is complex and technically difficult.

Lightning optofluidic system

Berkeley Lights' Lightning optofluidic platform enables researchers to precisely study the behavior of single cells within a specified time frame by recording video of the cells throughout the data collection process. The platform works through the microfluidics of a silicon chip the size of a postage stamp that contains tiny nanopens and long, narrow chambers for isolating and growing individual cells. This system can be used to gain unparalleled biological insight. Lightning's new optofluidic technology can isolate and characterize 1,000 single cells on a single platform. Single-cell analysis has traditionally required multiple instruments over a period of several months. The Lightning system allows cells to clone and grow and be analyzed and recovered in a matter of days. After automatic cloning, the cells were individually detected and monitored using bright field and fluorescence techniques. This allows for the creation a visual record of every cell in every experiment, and the understanding of this complex biology at a level that cannot be achieved by any other method. In immunogenomics-related research, the system can be used to selectively derive target T cells/

single T-cell clones after analysis and evaluate them for subsequent downstream sequencing and other experiments. Research at the single-cell level and rapid and efficient construction of CAR/TCR vectors have accelerated the process of T-cell genetic modification [248, 249].

Droplet entrapping microfluidic (DRRM) cell system

This system is an ultrahigh throughput single cell sorting platform developed based on droplet microfluidic technology. By using the latest microfluidic technology, thousands of microdroplets can form every second. Cells are encapsulated in microdroplets and can undergo biochemical processes such as growth, cleavage, metabolism and reaction. Fluorescence signals of different intensities are produced when the cell fully combines with the fluorescent sieve in the droplet. The low- and high-yield cells were separated by fluorescence signals using microdroplet separation technology for high-pass quantification of the separation process. Activated T cells were detected and analyzed by a microfluidic system, and the TCR was cloned. The technique is based on T-cell functional analysis, which reduces nonspecific findings [250, 251]. However, the number of T cells in each analysis was limited, and the system complexity was high.

(三) \ High throughput library strategy

High-throughput human-derived TCR libraries generate single-cell droplets from a large number of patient-derived tumor infiltrating lymphocytes (TILs). TCR- α and - β were amplified in the droplet, and the TCR- α and - β chains were fused in series. TCRs were expressed in series in the vector, TCR plasmid and virus library were formed, and the human TCR library was constructed. After stimulation with the T-cell reporter system and specific antigen epitopes, high-throughput screening of specific TCRs was performed to clone antigen epitopes directly from the TCR library [252–254]. This method is based on the T-cell function of TCR discovery and the specificity is good; this method allows for selection of a specific affinity interval of TCR. The constructed library resources can be regenerated and gradually eliminate TIL resource dependence, but the technical difficulty is high.

六 \ TCR-T cells based on specific TCRs

Adoptive T-cell therapy (ACT) uses the patient's own naturally occurring or genetically engineered antitumor lymphocytes, so neoantigens with high immunogenicity provide an excellent target for ACT therapy [255]. Currently, different ACT technologies are being developed, including tumor-infiltrating lymphocytes (TIL), chimeric antigen receptor T (CAR) cells, and T-cell receptor engineered T cells (TCR-T), which have been successfully used to treat a variety of malignancies [256, 257].

However, the clinical efficacy of TIL and CAR T cells in solid tumors is not ideal due to a number of obstacles, including scarcity of available antigens, tumor heterogeneity, or tumor immunosuppression. Advanced solid tumors are also characterized by connective tissue hyperplasia and abnormal angiogenesis, leading to hypoxia and altered nutrient supply, both of which contribute to poor clinical outcomes [258, 259]. TCR-T-cell therapy is an alternative with several advantages. First, the target antigen pool of TCR-T cells is larger than that of CAR T cells. Second, the epitope density required by TCR-T cells to induce activation is lower than that of classic CAR T cells (1–50 vs. 10^3 epitopes per cell). This increased sensitivity may lead to an improvement in the detection and lethality of tumor cells. Finally, the high affinity of TCR-T cells may also enhance their efficacy. The affinity of TCR-T cells for target cells was lower than that of CAR T cells. It is possible to allow each TCR-T-cell to "scan" and clear several antigen-presenting tumor cells [176, 260] (Fig. 3).

(一) \ Definition of TCR-T cells

TCR is a receptor expressed on the surface of T cells that can specifically recognize related antigens expressed on the surface of tumor cells and presented by MHC, thus mediating antitumor effects. In tumor patients, tumor reactive T cells are present. However, these natural antitumor cells cannot be used for clinical treatment due to their limited source, difficulty in isolation, and difficulty in large-scale expansion *in vitro*. To overcome these difficulties, TCR-coding genes can be isolated from tumor-responsive T cells, introduced into ordinary T cells and imbued with tumor-specific killing capabilities. In this way, a large number of antigen-specific T cells can be rapidly produced to meet application requirements. These T cells that express exogenous TCR and recognize specific epitopes are called TCR-T cells [176, 261]. The main difference from CAR T cells is that only TCR sequences (α and β chains) are introduced into TCR-T cells. TCR signal transduction depends on the host's T-cell signaling pathway. In addition to single-chain variable fragment (scFV) sequences containing specific antigens, CAR molecular sequences also contain functional motifs of costimulatory signaling molecules (CD28 and/or 4-1BB). TCR-transduced T cells can target any surface or intracellular antigen [262]. Several clinical trials have demonstrated the feasibility of effective methods for the identification of new antigens for the design of specific TCR-T cells. When neoantigens are recognized and predicted, new epitope-specific T cells are isolated, and their TCRs are sequenced. Candidate TCR sequences with known neoantigen reactivity can be introduced into T cells by transposon or CRISPR/Cas9 systems. These engineered cells expressing neoantigen-specific TCR

(TCR-T) were injected into patients to determine their antitumor effects after their tumor activity was validated [111, 263–265].

(二) \ Selection of therapeutic target antigens for TCR-T cells

TCR-T cells recognize tumor cells based on the recognition of pMHC/peptides after antigen processing. Thus, antigen specificity determines the accuracy of TCR-T-cell killing [266, 267]. The ideal tumor antigen should be selectively expressed in tumor tissue and not expressed in normal tissue to avoid causing an autoimmune response. At the same time, the targeted antigen should be immunogenic to induce an effective antitumor immune response. The ideal target antigen of TCR-T cells is TSA. TSA is mainly the product of gene mutation. This type of antigen is only present in tumor cells but is not expressed in normal cells and tissues. Theoretically, TSA is an ideal target for TCR-T-cell therapy. The tumor specificity of TSA means that there is no autoimmune tolerance and that the immune response to TSA does not damage normal tissue. However, there are some problems in the study of TCR-targeting TSA. On the one hand, the immunogenicity of antigenic peptides produced by gene mutation is relatively weak, and it is difficult to isolate TCRs with high affinity. On the other hand, TSA is often specific to the individual tumor or even present only at certain points in time. Targeting such antigens requires a very individualized TCR-T-cell preparation process [10, 268–272]. At present, it is necessary to actively search for tumor-specific neoantigens with strong immunogenicity and carry out individualized clinical treatment of TCR-T cells.

(三) \ Antigen presentation by TCR-T cells

The prerequisite for TCR-T cells to recognize target cells is the presence of antigen epitopes on the surface of target cells, which involves antigen presentation [176]. According to the origin, processing method and MHC, antigen processing and presentation can be completed in the cytosol and lysosome, respectively. The processing and presentation of endogenous antigens occurs as follows: the endogenous synthesis of protein molecules includes endogenous antigens, which are also the main targets of TCR-T cells for tumor therapy. The degradation process of endogenous antigens in cells is not fundamentally different from that of ordinary proteins. In fact, endogenous antigen degradation in the cytoplasm utilizes the degradation mechanism of normal intracellular protein conversion. Endogenous antigens are degraded into 5~15 amino acid peptides by proteases in the cytoplasm, which are processed and presented by the cytosol pathway and restricted by MHC-I molecules. Exogenous

antigens are degraded in endosomal lysosomes to produce peptides, some of which are 13–18 or even 0 amino acids in length and can bind to appropriate MHC-II molecules. As the antigens are processed and presented through the lysosome pathway and dependent on MHC-II for delivery to the cell surface. Dendritic cells and macrophages are the main APCs involved in the nonclassical pathway [127, 155, 156, 273]. Only antigens that can be presented are likely to be recognized by T cells. There are MHC differences among different individuals. MHC mismatches between the input cell and the recipient results in immune rejection. As a result, TCR-T cells are mainly transfused after somatic modification. To some extent, the application of TCR-T-cell therapy is limited. T cells recognize target cells mainly through the recognition of specific pMHCs by TCRs. TCR gene rearrangement can lead to approximately 10^{18} TCR sequences, which makes it difficult to identify and isolate specific TCR sequences. However, due to the strict conformational requirements for the binding of peptides and MHC, the same TCR can recognize the same pMHC in different individuals, which also lays the foundation for the universality of TCRs [126, 209, 274]. Therefore, the T-cell memory response is constrained by both antigen specificity and MHC allele specificity.

(四) · The therapeutic process of TCR-T cells

TCRs recognize pMHC/peptides to mediate the recognition and killing of tumors by T cells [176]. A large number of antigen-specific T cells can be generated rapidly by isolating the α and β chains of TCR α and β that are responsive to tumor antigens and transferring them into T cells in a viral or nonvirus-mediated manner. The specific antigen reactive TCR sequence can be isolated, stored and amplified in plasmid form and can be used immediately when needed. This makes the threshold for clinical application of TCR-T cells much lower. The TCR gene can be used as a ready-made reagent for the treatment of tumor patients expressing specific antigens and MHC-restricted molecules [260, 261]. Therefore, the following general process of TCR-T-cell therapy for tumor patients is shown in the figure: ① Peripheral blood was extracted from patients, and T cells were isolated; ② T cells were activated by adding CD3/CD28 beads and IL-2; ③ TCR genes were transferred into activated T cells by virus or other means; ④ T cells (TCR-T cells) with specific TCR transduction were further cultured and expanded to a suitable number; ⑤ Before infusion of TCR-T cells, patients were given moderate chemotherapy or radiotherapy to remove lymphocytes from the body and enhance the therapeutic effect of TCR-T cells; ⑥ A sufficient number of TCR-T cells were injected

intravenously into the patient; and ⑦ The patients were closely observed after transfusion, and the clinical efficacy and related side effects were evaluated later [176, 226].

(五) · Toxicity evaluation and efficacy monitoring of TCR-T-cell therapy

Assessment of the toxicity associated with TCR-T cells included bioinformatics, immunological analyses of transcription and proteome databases, and in vitro tests assessing the ability of TCR-T cells to recognize normal cells or tissues. Off-target toxicity or cross-reaction is associated with TCR recognition of antigens on normal cells that are different from those that are targeted. Therefore, it is necessary to include a strategy for evaluating TCR cross-reactivity at the preclinical level, especially when the TCR sequence has been modified to improve affinity. Reliable immune surveillance is essential to assess whether neoantigen-based immunotherapy can be used to achieve the desired immune effect and to extend the list of effective immune candidates to include larger and suitable patient subpopulations. Methods to predict off-target and off-tumor toxicity (presence of cross-reactivity epitopes in normal tissues) includes the following: In silico prediction, which includes Motif blast, and HLA-binding prediction and expression; structural modeling of the pMHC interface; and TCR clustering. Experimental evaluation includes the following methods: alanine/glycine scan, combination peptide libraries (X-scan) and barcoded peptide libraries; in vitro cytotoxicity testing against primary healthy cells; and TCR-based histological analysis [275–279]. Tumor-reactive T-cell responses are critical to the antitumor effectiveness of various therapies, including cancer vaccines, ACTs, bsAbs, and ICBs. To predict the effect of cancer immunotherapy, the number and quality of tumor-reactive T cells can be measured and tracked. A number of effective markers, including CD39, PD-1, TIM-3, OX40, 4-1BB, IFN- γ , and TNF- α , can be used to determine the proportion of neoantigen-reactive T cells in infusion products and their ability to recognize autologous tumors. CCR5 and CXCL13 can also be used as T-cell intrinsic indicators of CPI sensitivity [244, 280–287]. It should be noted that circulating tumor DNA (ctDNA/cfDNA) levels, as a proxy for tumor load, can be used to dynamically detect neoantigen-producing mutations [288–291]. The characterization of neoantigen-specific TCR clonal types characterizes TILs in tumors, allowing for prediction of TCR based solely on TIL transcription status in neoantigen-specific cancer immunotherapy. These characteristics can provide a degree of clonability. Prediction of the clinical response to various immunotherapies can be performed by identifying anticancer TCRs in the blood, and more

critically, without the need for functional screening of putative neoantigens in the tumor.

(六) Enhanced durability and antitumor function of TCR-T-cell therapy

T-cell persistence is a basic requirement for sustained immunosurveillance. Many clinical trials have shown that most unresponsive patients have no persistence of tumor-specific T cells transfused in vivo. In contrast, patient-engineered T cells that achieved a complete response or had no recurrence and tumor control demonstrated strong proliferative capacity and long-term persistence [168, 292]. To maintain T-cell persistence, multiple cytokines are used in combination to support T-cell survival and expansion. Systemic delivery of IL-2 is known to amplify T cells while maintaining functional activity. Long-lasting regression has been achieved in some patients with metastatic melanoma and kidney cancer and has been approved by the FDA. This strategy is currently used in CAR T and TCR-T-cell immunotherapy [293–296]. IL-7 is a hematopoietic cytokine that regulates many aspects of T-cell biology and is essential for T-cell survival, homeostasis, and proliferation. It promotes the survival of naïve and memory T cells by upregulating the expression of the anti-apoptotic molecule Bcl-2 [297, 298]. IL-12 is a major contributor to effective antitumor immune responses. It activates the effector function of T cells by inducing cytotoxic enzymes such as perforin and cytokines [299, 300]. IL-18 is another cytokine that has the same biological effect as IL-12 but is less toxic [301, 302]. Studies exploring the substitution of IL-18 for IL-12 have shown that CAR T cells designed to secrete IL-18 improve the survival and antitumor activity of CAR T cells in vitro and in vivo by producing IFN- γ and several other cytokines. IL-18 stimulates the expansion of human CD4 cells and activates the endogenous immune system of well-immunized mice [303, 304]. IL-15 is known to stimulate stem cell memory T-cell (Tscm) production, with the potential to maintain long-lasting T-cell responses [305]. IL-7 and IL-15 induce the generation of human memory stem T cells from naïve precursors [306]. Unlike IL-2, IL-15 does not bind to the IL-2R α chain and therefore does not stimulate Tregs and may have a more selective effect. Compared with IL-2, IL-15 tends to enhance the antitumor activity of CAR T cells by preserving their Tscm phenotype [307, 308]. IL-21 is a newly discovered member of the common gamma chain cytokine family. Similar to IL-12 and IL-15, IL-21 does not stimulate Tregs. In contrast, it inhibits the amplification of Tregs by inhibiting Foxp3, thus favoring the enrichment of antigen-specific CD8⁺ T cells [309, 310]. IL-21 promotes the maturation and enhanced killing activity of CD8⁺ T cells and promotes

the differentiation of memory CD8⁺ T cells. IL-21 promotes antigen-specific CD8⁺ CTL production in vitro and performs much better than IL-2 or IL-15 in vivo in mouse models [311, 312]. These results suggest that cytokines can be used alone or in combination with other cytokines to produce tumor-specific T cells with a memory phenotype. Cytokines enhance the persistence, proliferative ability and antitumor effect of adoptive cancer immunotherapy.

(七) Factors influencing TCR-T-cell therapy

Studies have shown that TCR-T cells can produce a large number of inflammatory factors, such as IFN- γ , IL-2 and TNF- α , when they encounter antigen-positive tumor cells. They exhibit antigen-specific cytotoxic effects and induce antigen-stimulated reactive proliferation [261]. With the development of molecular biology, genetic modification methods and transduction technology, the construction system of TCR-T cells has made great progress, which lays a foundation for the further improvement of the therapeutic effect of TCR-T cells. As with other ACT techniques, TCR-T-cell therapy has the following advantages: ① the production of a large number of tumor antigen-specific T cells for treatment in a short period of time; ② T cells can be treated and amplified in vitro to bypass the effects of immune dysfunction in patients, and create a large number of effector cells; ③ the activation and expansion of T cells in vitro prevents the serious side effects caused by the application of biological agents in patients; and ④ by the in vitro culture of T cells, T cells can be differentiated by adding different cytokines or reagents to obtain more powerful TCR-T cells with a more lasting killing effect [226, 260, 268, 313]. However, the therapeutic effect of TCR-T cells, similar to other ACTs, is affected by a variety of factors.

Number of cells transfused

The ratio of TCR-T cells to tumor cells is directly related to the therapeutic effect [261]. Studies have shown that effector cells should account for at least 1%–10% of total T cells. In other words, the number of effector cells injected into the human body should reach $(2–20) \times 10^9$ to effectively control the tumor. However, inputting too many effector cells at a time can lead to serious off-target effects and even death. Therefore, the number of specific T cells in patients can account for 5% of total CD8⁺ T cells through repeated transfusions of a small number of cells each time. Animal experiments have shown that repeated cell inputs can help maintain the continuous attack on tumor cells and play a role in gradually shrinking the tumor [111, 176, 314, 315].

Immunosuppressive factors

When effector cells are imported back into the body, they are inevitably affected by the immunosuppressive state of the body. In early studies, to enhance the activity of transfused cells, the main method was to administer IL-2, IFN- γ and other cytokines simultaneously. However, while enhancing the activity of transfused cells, IL-2 also promotes the expansion of CD4⁺CD25⁺ Treg cells, which are an important class of immunosuppressive cells [293, 316, 317]. In addition, immune checkpoints have been found to be one of the important factors affecting immune cell therapy. Among them, cytotoxic T lymphocyte-associated antigen 4 (CTLA-4) and programmed death receptor 1 (PD-1) had the most significant effects. Based on our experience with CAR T-cell therapy, CTLA-4 and/or PD-1 blocking antibodies can greatly improve clinical outcomes if given to patients at the right time after reinfusion of TCR-T cells [318–320]. In addition to these traditional immunosuppressive cells and molecules, some metabolic regulatory molecules also inhibit the function of effector T cells, such as CD73 and indoleamine-2,3-dioxygenase 1 (IDO1) [321, 322]. These immunosuppressive factors in the tumor microenvironment should be considered in future treatment.

Tumor heterogeneity

Tumor heterogeneity refers to the genetic and biological differences among different malignant cells within the same tumor tissue [323]. In TCR-T-cell therapy, the influence of heterogeneity is mainly reflected in the difference in antigen expression and presentation. The expression level and immunogenicity of tumor antigens are the basis and key of TCR-T-cell therapy. If the tumor cells do not express or present the target antigen, the TCR-T cells cannot attack those tumor cells [226, 261]. In TCR-T-cell therapy, it is hoped that the target antigen will be highly expressed in most, if not all, tumor cells. However, the reality is that a single target antigen is only expressed in a few malignant cells. This requires treatment with TCR-T cells that have two or more targets [10]. In addition, treatments targeting MHC-Class I and Class II molecularly restricted antigens should also be considered. Multitarget combination therapy may yield more positive results [126]. In addition, tumor-specific antigen expression is often downregulated or even terminated after T-cell infiltration. The alteration of antigen expression is associated with the elimination of specific tumor cells. At the same time, it is also related to changes in key genes in tumor cells, such as the expression and modification of antigen genes, MHC genes and genes related to antigen processing and presentation. Fortunately, some

TCR-T-cell target antigens are epigenetically regulated. Demethylation or histone deacetylase drugs can be used to enhance the expression of such antigens and improve the therapeutic effect of T cells [324–327].

The number of effector cells

The degree of differentiation, survival time, ability to move to the tumor site and expression level of TCR cells affect the efficacy of cell therapy. In TCR-T-cell therapy, the T-cell type and differentiation level directly affect the function and action time of effector cells. To a large extent, these factors determine the efficacy of TCR-T-cell therapy [176, 260]. First, T cells are activated and eventually differentiate into effector cells (Teff) and memory cells (Tm). On the one hand, in vitro studies have shown that Teff cells are more effective in short-term killing. On the other hand, clinical studies have shown that the survival time of effector cells in the body largely determines the therapeutic effect. This means that Tm cells will achieve better results when used for treatment. This influencing factor is more obvious in CD8⁺ T-cell selection [328–331]. Naive T cells (Tn) can differentiate into stem cell-like memory T cells (Tscm), central memory T cells (Tcm), effect-memory T cells (Tem) and Teff cells when the initial antigen and costimulatory signal reach a certain quality and quantity. Preclinical studies have shown that CD8⁺ T-cell differentiation and proliferation are antithetical. Therefore, it can be inferred that the differentiation degree of CD8⁺ T cells is inversely related to their persistence and therapeutic effect in vivo [332–334]. Currently, two strategies are available to improve T-cell therapy. One involves adding the right cytokines. For example, the addition of IL-7 + IL-15 or IL-15 + IL-21 resulted in the cultivation of poorly differentiated CD8⁺ T cells [335–337]. The other method involves enriching poorly differentiated T cells according to CD62L expression; these cells then act as receptor cells for gene transfer [338, 339]. The proportion of Tm cells in TCR-T cells can be increased, and the survival time of T cells can be prolonged by adjusting the culture scheme and by appropriate gene modification.

In addition, the type of T-cell subsets injected also directly affects the therapeutic effect. The differentiation of naive T cells into functional T cells is influenced by various cytokines in the surrounding environment. In turn, differentiated T cells affect T-cell function. This variability is particularly common in helper T-cell (Th) differentiation. CD4⁺ T cells can differentiate into a variety of subtypes, including Th1, Th2, Th9, Th17, Th22, Tfh and Treg cells [340–342]. Th1 and Th17 are the most functional cell subtypes in terms of antitumor effects. The addition of CD4⁺ T cells, especially Th1 cells, can prevent the exhaustion of CD8⁺ T

cells and increase the infiltration of CD8⁺ T cells, thus leading to effective tumor killing and removal [343, 344]. Th17 cells enhanced the tumor-clearing effect of TCR-T cells in animal experiments, and this effect of Th17 cells was related to their transformation into Th1 cells [345–347]. In the present study, Th cell activity was effectively activated by transferring MHC-I class molecule-restricted TCR and/or CD8 molecules into CD4⁺ T cells. It is possible to enhance the efficacy of cell therapy by controlling the subpopulation of transfused T cells [348, 349]. These results suggest that the combination of CD8⁺ T cells and Th1/Th17 cells can improve the therapeutic effect of T cells.

Affinity of T-cell infusion

T-cell affinity refers to the ability of T cells to respond to specific concentrations of antigens [189, 350]. In TCR-T cells, imported TCRs often have high affinity. Therefore, the affinity of TCR-T cells is mainly related to the expression level of TCR. The expression of the TCR transgene was enhanced by optimizing the method of gene transfer, including the selection of the gene transfer method, the use of the best vector components and the use of transgenic boxes [351]. Using lentiviral vectors and EF-1 promoters, it is possible to significantly increase the level and proportion of TCR expression based on the experience of CAR T-cell studies [352]. In addition, TCR gene expression can be enhanced by limiting or eliminating TCR mismatch. Strategies to promote the correct pairing of α and β chains of transgenic TCR (to prevent and reduce TCR mismatch) fall into two main categories. First, the mouse modification of TCR was used as the representative, that is, the C region was replaced with the sequence of mouse origin to prevent the mismatch between human transgenic TCR and its own TCR. Second, the correct pairing is achieved through CRISPR/Cas9-mediated endogenous TCR knockout [353–355]. In addition to enhancing TCR surface expression, TCR affinity can also be enhanced through TCR sequence changes and optimization strategies to improve the therapeutic effect. It was found that the high-affinity MART-1-specific TCR-mediated tumor response rate was significantly better than that of low-affinity TCR [356, 357]. Moreover, NY-ESO-1-specific TCR-mediated therapy with enhanced affinity is more effective [358, 359]. In addition, there are different ways to improve TCR affinity. A typical example is to improve affinity by changing the CDR sequence of TCR. TCR affinity can be improved by changing the types of certain amino acids in the CDR region. Although increasing TCR affinity can significantly improve T-cell

function, special attention should be given to potential off-target effects [360, 361]. In clinical trials, enhanced TCR affinity to CEA/HLA-A2, MAGE-A3/HLA-A2, and MAGE-A3/HLA-A1 was found to be associated with toxic reactions in patients [362–364].

Expression of chemokine receptors and immunomodulatory receptors in transfused T cells

At present, TCR-T-cell therapy is achieved through intravenous transfusion. T cells must first travel from blood vessels to the tumor site before they can perform their tumor killing function. The key factors influencing T-cell chemotactic movement are directed movement mediated by chemokines and chemokine receptors (ChRs) [365–367]. Therefore, if TCR-T cells can express an appropriate ChR, the infiltration of T cells into tumors can be increased, and the therapeutic effect of TCR-T cells can be improved. However, the regulatory mechanism of ChR expression is not well understood, and it is difficult to induce specific ChR expression by cytokine or agent addition; although, specific ChR can be introduced by means of genetic engineering [368, 369]. A number of studies have confirmed that ChR gene engineering can improve the directed movement of T cells and improve the effect of immunotherapy [370–372]. It is believed that TCR-T-cell therapies will also benefit from such improvements. In addition to ChR receptors, T cells also express a variety of synergistic signaling receptors that play an immunomodulatory role. The representative costimulatory molecules are CD28, ICOS and 4-1BB. These receptors, when combined with corresponding ligands, can provide costimulatory and coinhibitory signals. In the absence of costimulatory signals, T cells are exhausted under constant antigenic stimulation. T cells show decreased proliferative ability and effector function and upregulation of coinhibitory molecule expression. In theory, it should be possible to enhance T-cell function by activating costimulatory molecules. However, clinical studies using the molecule's activated antibodies against tumors have shown the limited effectiveness of such therapeutic strategies. Thus, this may not be the best option for improving the efficacy of T cells in tumor therapy [373–375]. In contrast, the treatment regimen targeting coinhibitory molecules has achieved better results. The representative coinhibitory molecules are CTLA-4 and PD-1 [376, 377]. Clinical studies have shown that blocking PD-1 or CTLA-4/PD-1 at the same time has a synergistic effect with immunotherapy for multiple solid tumors [378, 379]. Therefore, the combination of T cells and coinhibitory molecules should be considered in TCR-T-cell therapy.

(八) Overview of clinical trials of TCR-T-cell therapy

Clinical trials of TCR-T-cell therapy began as early as 1998, but an increasing number of clinical trials began after Mart-1-specific TCR-T cells were successfully used to treat melanoma in 2006. Currently, TCR-T cells are being tested in clinical trials for melanoma, synovial sarcoma, rectal cancer, esophageal cancer, and myeloma. More than 100 TCR-T-cell clinical trials have been registered on the Clinical Trials website. Among them, some clinical trials have had research results and published articles reporting their efficacy, which provides an important reference for the future adoptive transfusion therapy of TCR-T cells (Table 3). Additionally, a large number of clinical trials targeting TCR-T-cell therapy are currently recruiting (Table 4).

TCR-T cells for hematological tumors

Although few clinical studies have been carried out, TCR-T-cell therapy has achieved good results in hematological tumors. The engineered high-avidity TCRs render CD8⁺ T cells specifically cytotoxic to neoantigen-containing tumors. A 2012 trial (NCT01640301) attempted to treat acute myeloid leukemia with WT1 high-affinity CD8⁺ T cells, but the trial is currently closed for enrollment, and the results have not been reported. In 2018, another clinical study (NCT02770820) used WT1-specific central memory and infantile CD8⁺ TCR-T cells to treat acute myeloid leukemia. At the same time, another clinical trial (NCT03326921) using HA1-specific memory TCR-T cells for the treatment of relapsed acute mixed leukemia enrolled patients. The TCRs specifically targeting recurrent fusion genes CFBF-MYH11 confer CD8⁺ T cells antileukemic activity in vitro and in patient-derived murine xenograft (PDX) models with fusion gene-driven AML [46]. Similarly, peripheral blood lymphocytes transduced with TCRs highly reactive to the mutated KRAS variants G12V and G12D could recognize multiple HLA-A*11:01⁺ pancreatic cell lines bearing the appropriate KRAS mutations in a xenograft model (IND27501) [380]. In 2015, the University of Maryland School of Medicine, the University of Pennsylvania School of Medicine and the Adaptimmune Corporation jointly conducted the study of NY-ESO-1-specific TCR-T-cell therapy. Of the patients with multiple myeloma, 80% had a good clinical response, 70% achieved complete or nearly complete response, and the mean progression-free survival reached 19 months [358].

TCR-T cells for melanoma treatment

Melanoma is one of the most malignant tumors in skin cancer. Once metastasis occurs, it is insensitive to post-operative adjuvant radiotherapy and chemotherapy, and the prognosis is very poor. At present, TCR-T cell

immunotherapy has the most clinical studies in melanoma, and the target is MART-1, P-gp100, NY-ESO-1, MART-A3, and p53 [381]. The large-scale trial of TCR-T cells began in 2006 with two trials targeting metastatic melanoma. Among them, Duval et al. treated 15 patients with melanoma differentiation antigen MART-1-specific TCR-T cells. Although only one patient experienced a partial response, the report demonstrates that TCR-T-cell therapy is safe in humans. Subsequently, Morgan et al. reported the clinical study results of MART-1-specific TCR-T cells used in the treatment of 17 melanoma patients and found that TCR-T cells amplified in vitro could survive in patients for a long time (up to 2 months at the time of detection) and even lasted at a high level in 2 patients for as long as 1 year. Objective responses were achieved in these two patients. Since then, with the deepening of TCR-T-cell research, researchers have realized that TCR affinity is closely related to the therapeutic effect. When high-affinity TCR-T cells were used, patient response rates increased substantially. Johnson et al. used MART-1 high-affinity TCR-T cells to treat 20 patients with metastatic melanoma and found that the objective response rate after treatment reached 30% (NCT02654821; NCT00910650; NCT00509288). Another important factor affecting the efficacy of TCR-T cells is the expression of specific antigens in the tumor. The more widely the antigen is expressed, the better the therapeutic effect of specific TCR-T cells. A typical example is the CT antigen NY-ESO-1. The expression of NY-ESO-1 is very common in tumors. Robbins et al. observed the efficacy of NY-ESO-1-specific TCR-T cells in the treatment of melanoma, and the results showed that most melanoma patients had an objective clinical response, and the 3-year and 5-year survival rates of patients with melanoma were 33%. Since TCR-T cells require costimulatory signals to activate and proliferate, DC cells can enhance the therapeutic effect of TCR-T cells. Chodon et al. observed the effect of a DC vaccine combined with Mart-1-specific TCR-T cells on 13 patients with metastatic melanoma, and the results showed that up to 69% of patients were significantly better (NCT00670748; NCT00670748; NCT02070406; NCT03399448).

In addition, Morgan et al. transferred the TCR gene with MAGE-A3 as the target antigen into T cells for the treatment of metastatic melanoma patients. The results of phase I/II clinical trials showed that the disease response rate was 57% (4/7), of which 1 patient received CR and lasted for 15 months, and 3 patients received PR (of which 2 patients had PR lasting for 4 months and 1 patient had PR lasting for more than 12 months). However, three patients in this clinical trial developed mental disorders caused by brain damage, two of whom

Table 3 List of published TCR-T cell clinical trials

Target antigen	Epitope	HLA type	Cancer type	Number of patients	Number of TCR-T cells	Phase	NCT number	ORR (%)	Clinical response	Toxicities	Author
HPV16-E6	TIHDILECV	HLA-A*02:01	HPV16-positive epithelial cancer	12	0.1–13.4 × 10 ¹⁰	I/II	NCT02280811	2 (17%)	2 PR	None	Doran
HPV16-E7	YMLDLOPET	HLA-A*02:01	HPV16-positive epithelial cancer	12	0.1–12.1 × 10 ¹⁰	I	NCT02858310	6 (50%)	6 PR	None	Nagarsheth
MCPV	KLLEIAPNC	HLA-A*02:01	Merkel cell carcinoma	2	1–9 × 10 ⁸	I	NCT03747484	1 (20%)	0	None	Veatch
HBV	N.A	HLA-A*02/HLA-Cw*0801	HBV-HCC	8	1 × 10 ⁷ / kg-5 × 10 ⁶ /kg	I	NCT03899415	1 (12.5%)	1 PR	Liver toxicity: 1 (12.5%)	Meng
NY-ESO-1	SLLMWTQC	HLA-A*02:01	Melanoma (11); Synovial sarcoma (6)	17 (11 + 6)	0.16–13 × 10 ¹⁰	I	NCT00670748	5 (45%); 4 (67%)	2 CR; 7 PR	None	Robbins
NY-ESO-1	SLLMWTQC	HLA-A*02:01	Melanoma (20); Synovial sarcoma (18)	38 (20 + 18)	0.9–13 × 10 ¹⁰	II	NCT00670748	11 (55%); 11 (61%)	5 CR; 17 PR	None	Robbins
NY-ESO-1	SLLMWTQC	HLA-A*02:01	Melanoma; Synovial sarcoma	10	1 × 10 ⁹	I	NCT02070406	2 (20%)	2 PR	None	Nowicki
NY-ESO-1	SLLMWTQC	HLA-A*02:01/02:06	Synovial sarcoma	12	0.4–14.4 × 10 ⁹	I/II	NCT01343043	6 (50%)	1 CR; 5PR	CRS: 5 (42%)	D'Angelo
NY-ESO-1	SLLMWTQC	HLA-A*02:01/02:06	Synovial sarcoma	30	2.67 × 10 ⁹	I/II	NCT01343043	9 (30%)	9 PR	None	Ramachandran
NY-ESO-1	SLLMWTQC	HLA-A*02:01	Melanoma	3	0.6–7.1 × 10 ⁹	I	NCT03399448	0	0	None	Stadtmauer
MART-1	EAAGIGILTV	HLA-A*02:01	Melanoma	12	0.05–4.56 × 10 ⁹	I/IIa	NCT02654821	2 (16.7%)	2 PR	CRS/sepsis: 1 (8%); Dermatitis: 10 (83%); Uveitis: 2 (17%); Hearing loss: 4 (33%)	Rohaan
MART-1	EAAGIGILTV	HLA-A*02:01	Melanoma	13	0.6–4.8 × 10 ⁹	II	NCT00910650	0	0	Skin rash: 3 (23%); CRS: 2 (15%)	Chodon
MART-1	AAGIGILTV	HLA-A*02:01	Melanoma	20	0.15–10 × 10 ¹⁰	II	NCT00509288	6 (30%)	6 PR	Skin rash: 14 (70%); Uveitis: 11 (50%); Hearing loss: 10 (50%)	Johnson

Table 3 (continued)

Target antigen	Epitope	HLA type	Cancer type	Number of patients	Number of TCR-T cells	Phase	NCT number	ORR (%)	Clinical response	Toxicities	Author
MAGE-A3	KVAELVHFL	HLA-A*02:01	Melanoma (7); Synovial sarcoma (1); Esophageal cancer (1)	9 (7 + 1 + 1)	2.8–7.9 × 10 ¹⁰	I/II	NCT01273181	5 (56%)	1 CR; 4 PR	Severe neurologic toxicity: 3 (33%) including 2 deaths	Morgan
MAGE-A3	EVDPIGHLY	HLA-A*01	Melanoma (1); Myeloma (1)	2 (1 + 1)	2.4–5.3 × 10 ⁹	I	NCT01350401; NCT01352286	0	0	Severe cardiac toxicity and death: 2 (100%) Liver toxicity: 2 (12%)	Linette
MAGE-A3		HLA-DPB1*04:01	Metastatic solid tumors	17	0.01–123 × 10 ⁹	I	NCT02111850	4 (23.5%)	1 CR; 3 PR	Liver toxicity: 2 (12%)	Lu
MAGE-A4	NYKRCFPVI	HLA-A*24:02	Esophageal cancer	10	0.2–5 × 10 ⁹	I	UMIN00002395	0	0	None	Kageyama
MAGE-A4	GVYDGREHTV	HLA-A*02	Advanced solid tumors	38	0.12–10 × 10 ⁹	I	NCT03132922	9 (23.7%)	9 PR	CRS: 19 (50%)	Hong
MAGE-A10	GLYDGM EHL	HLA-A*02:01/02:06	NSCLC	11	0.1–6.77 × 10 ⁹	I	NCT02592577	1 (9%)	1 PR	Neurotoxicity: 1 (9%); CRS: 3 (27%)	Blumenschein
gp100	KTWGQYWQV	HLA-A*02:01	Melanoma	16	0.18–11 × 10 ¹⁰	II	NCT00509496	3 (16%)	1 CR; 2 PR	Skin rash: 15 (94%); Uveitis: 4 (25%); Hearing loss: 5 (31%)	Johnson
CEA	IMIGVLVGV	HLA-A*02:01	Colorectal cancer	3	2–4 × 10 ⁸	I	NCT00923806	1 (33%)	1 PR	Severe transient colitis: 3 (100%)	Parkhurst
TP53	HMTVVRHC	HLA-A*02:01	Metastatic breast cancer	1	5.3 × 10 ¹⁰	I	NCT03412877	1 (100%)	1 PR	CRS	Kim
KRAS G12D	GADG/GKSA/ GADG/GKSAL	HLA-C*08:02	Metastatic pancreatic cancer	1	1.6 × 10 ¹⁰	I	IND27501	1 (100%)	1 PR	None	Leidner
Mutation-associated neoantigens	CRISPR-Cas9 KO-KI	Multiple HLA class I	Metastatic solid tumors	16	0.13–4.0 × 10 ¹⁰	I	NCT03970382	0	0	CRS: 1 (6%); Neurotoxicity: 1 (6%)	Foy
WT1	CMTWNQMNL	HLA-A*24:02	AML; MDS	9	0.12–3.5 × 10 ⁹	I	UMIN000011519	5 (55.6%)	5 PR	None	Isao Tawara

Table 4 List of recruiting TCR-T cell clinical trials

Neoantigen	Cancer type	HLA	Cell source	Date	Phase	NCT number	Conditions
HPV16-E6	Cervical Carcinoma	HLA-A2	PB (Patient)	2022/8/10–2021/8/31	I/II	NCT05357027	Recruiting
HPV16-E7	HPV-associated Cancers	HLA-A*02:01	PB (Patient)	2023/3/7–2025/1/1	II	NCT05686226	Recruiting
	HPV-associated Cancers	HLA-A2	PB (Patient)	2017/1/27–2026/1/1	I/II	NCT02858310	Recruiting
HPV16- CRTE7A2-01	Advanced Cervical, Anal, or Head and Neck Cancers	HLA-A2	PB (Patient)	2022/7/17–2024/12	I	NCT05122221	Recruiting
HPV18- HRYZ-T101	HPV18 positive advanced solid tumor	HLA-A*11:01/02:01/24:02	PB (Patient)	2023/3–2025/9	I	NCT05787535	Recruiting
MAGE	Advanced melanoma (MEL) and head-and-neck carcinoma (HNSCC)	HLA-A2	PB (Patient)	2020/10/20–2027/10/20	I/II	NCT04729543 (MAGE-C2)	Recruiting
	Advanced-Stage/ Metastatic MAGE-A1 ⁺ Solid Tumors	HLA-A*02:01	PB (Patient)	2022/7/29–2037/6	I/II	NCT05430555 (MAGE-A1)	Recruiting
	Advanced Solid Tumors	HLA-A2	PB (Patient)	2019/5/17–2025/7	I/II	NCT03973333 (MAGE-A4)	Recruiting
KRAS	Advanced Pancreatic Cancer	HLA-A*11:01	TIL (Patient)	2021/10/21–2025/4	I/II	NCT04146298 (KRAS G12V)	Recruiting
	Advanced Pancreatic Cancer and Other Solid Tumors	HLA-A*11:01	PB (Patient)	2022/6/7–2025/6/30	I	NCT05438667 (KRAS G12V or G12D)	Recruiting
	Pancreatic/Gastric/ Gastrointestinal / Colon/Rectal Cancer	HLA-A*11:01	PB (Patient)	2017/9/21–2028/7/29	I/II	NCT03190941 (KRAS G12V)	Recruiting
	Pancreatic/Gastric/ Gastrointestinal / Colon/Rectal Cancer	HLA-A*11:01	PB (Patient)	2019/4/16–2028/12/1	I/II	NCT03745326 (KRAS G12D)	Recruiting
NY-ESO-1 (CTAG1A)	Locally Advanced Malignant Neoplasm/ Unresectable Malignant NeoplasmSarcoma	HLA-A*02:01	PB (Patient)	2017/7/26–2024/8/1	I	NCT03240861	Recruiting
	Advanced Soft-tissue Sarcoma	HLA-A*02	PB (Patient)	2022/11/11–2028/12/1	I	NCT05620693	Recruiting
	NY-ESO-1 -Expressing Metastatic Cancers	HLA-A*02	PB (Patient)	2022/41–2027/12/30	I/II	NCT05296564	Recruiting
	Solid Tumors	HLA-A*02:01	PB (Patient)	2022/12–2026/3	I	NCT05648994	Recruiting
AFP	• Soft tissue sarcoma	HLA-A*02:01	PB (Patient)	2022/7/8–2024/9/1	II	NCT05549921	Recruiting
	Advanced hepatocellular carcinoma (HCC) or other AFP expressing tumor Types	HLA-A2	PB (Patient)	2017/4/8–2036/7/7	I	NCT03132792	Recruiting

Table 4 (continued)

Neoantigen	Cancer type	HLA	Cell source	Date	Phase	NCT number	Conditions
HBV	HBV related hepatocellular carcinoma	HLA-A*02:01/24:02	PB (Patient)	2022/4/20–2024/7/1	I	NCT04745403	Recruiting
	Hepatocellular carcinoma	HLA-A2	PB (Patient)	2019/4/20–2024/6/1	I	NCT03899415	Recruiting
	Hepatocellular carcinoma; Liver cancer, Adult; Liver cell carcinoma	HLA-A *02	PB (Patient)	2022/6–2027/5	I/II	NCT05195294	Recruiting
	Hepatitis B Virus related hepatocellular carcinoma; Hepatocellular carcinoma recurrent	HLA-A *02	PB (Patient)	2022/10/26–2025/10	I/II	NCT05417932	Recruiting
	Hepatocellular carcinoma	HLA-A*02	PB (Patient)	2021/4/14–2023/12	I	NCT05339321	Recruiting
gp100 (PMEL)	Advanced Melanoma	HLA-A*02:01	PB (Patient)	2022/12/19–2027/9	II/III	NCT05549297	Recruiting
	Malignant Melanoma	HLA-A*02:01	PB (Patient)	2015/11–2025/1	I/II	NCT02535078	Recruiting
HERV-E	Renal cell carcinoma; Kidney cancer	HLA-A*11:01	PB (Patient)	2018/7/20–2032/12/31	I	NCT03354390	Recruiting
EBV	Nasopharyngeal Carcinoma	HLA-A*02:01/24:02/11:01	PB (Patient)	2022/12–2030/10	I	NCT05587543	Recruiting
	Nasopharyngeal Carcinoma	HLA-A*02:01/24:02/11:01	PB (Patient)	2023/3–2023/8	I/II	NCT04509726	Recruiting
	Head and Neck Squamous Cell Carcinoma	HLA-A*02:01/24:02/11:01	PB (Patient)	2019/3/1–2024/1/1	I/II	NCT04139057	Recruiting
	EBV Lymphoma; Post-transplant lymphoproliferative disease (PTLD)	HLA-A*02:01/24:02/11:01	CTLs (Patient)	2023/5–2025/12	I/II	NCT05688241	Recruiting
	Head and Neck Squamous Cell Carcinoma	HLA-A*02:01/24:02/11:01	PB (Patient)	2023/3/1–2027/6/30	I	NCT05697926	Recruiting
PRAME	Select Advanced Solid Tumors	HLA-A2	PB (Patient)	2020/2/25–2026/2	I/II	NCT04262466	Recruiting
HA-1H	Relapsed or persistent hematologic malignancies	HLA-A*02:01	PB (Patient)	2020/7/2–2025/7	I	NCT04464889	Recruiting
LAGE-1a	Neoplasms	HLA-A*02:01/02:05/02	PB (Patient)	2020/12/21–2025/12/4	I	NCT04526509	Recruiting
	Neoplasms	HLA-A*02	PB (Patient)	2019/12/31–2026/7/31	II	NCT03967223	Recruiting
CMV	CMV Infection After Allogeneic HSCT	HLA-A*02:01/11:01/24:02	PB (Patient)	2021/10/15–2024/12/31	I	NCT05140187	Recruiting
	Allogeneic hematopoietic stem cell transplantation; CMV infection	HLA-A*11:01/02:01/24:02	PB (Patient)	2021/1/6–2023/8	I	NCT05089838	Recruiting
KK-LC-1	Non-small cell lung cancer; Solid tumor, Adult	HLA-A*11	PB (Patient)	2018/12/1–2033/12/30	I	CT03778814	Recruiting
	Gastric/Breast/Cervical/Lung Cancer	HLA-A*01:01 NCT05587543	PB (Patient)	2022/9/26–2028/12/31	I	NCT05483491	Recruiting
	Gastric/Breast/Cervical/Lung and other KK-LC-1 positive epithelial cancers	HLA-A*01	PB (Patient)	2022/3/8–2026/4/6	I	NCT05035407	Recruiting

Table 4 (continued)

Neoantigen	Cancer type	HLA	Cell source	Date	Phase	NCT number	Conditions
Neoantigen	Advanced Solid Tumor	HLA-A*11:01:02:01/24:02	PB (Patient)	2019/1/8–2023/12/1	I	NCT03891706	Recruiting
	Malignant Epithelial Neoplasms	HLA-A*11:01:02:01/24:02	PB (Patient)	2022/2/8–2027/2/28	I	NCT04520711	Recruiting
	Malignant Epithelial Neoplasms	HLA-A*11:01:02:01/24:02	PB (Patient)	2023/3/15–2028/3/15	I	NCT05349890	Recruiting
	Solid tumors	HLA-A*11:01:02:01/24:02	PB (Patient)	2022/10/1–2039/6	I	NCT05292859	Recruiting
	Solid tumors	HLA-A*11:01:02:01/24:02	PB (Patient)	2021/9/24–2028/5/28	I	NCT05124743	Recruiting
	Solid tumors	HLA-A*11:01:02:01/24:02	PB (Patient)	2022/4/4–2029/3	I/II	NCT05194735	Recruiting
KSH01	Refractory/Recurrent solid tumors	HLA-A * 02	PB (Patient)	2022/8/19–2027/7/31	I	NCT05539833	Recruiting
	Solid tumors	HLA-A * 02	PB (Patient)	2022/12/11–2027/12/11	I	NCT05580796	Recruiting
KSX01	Solid tumors	HLA-A * 02	PB (Patient)	2023/3/7–2028/12/31	I	NCT05811975	Recruiting
Mesothelin	Etastatic pancreatic ductal adenocarcinoma	HLA-A*02:01	PB (Patient)	2021/12/14–2024/6/1	I	NCT04809766	Recruiting
MCPyV	Metastatic or unresectable merkel cell cancer	HLA-A*02	PB (Patient)	2019/7/3–2025/12/15	I/II	NCT03747484	Recruiting

developed severe central nervous system damage and died from multifocal necrotizing leukoencephalopathy, which may be caused by neurotoxicity mediated by cross-recognition of the MAGE-A12 antigen in normal brain tissue (NCT01273181; NCT01350401; NCT01352286; NCT02111850). Johnson et al. used melanoma-associated antigen peptides P-gp100 and p53 to prepare TCR-T cells with high expression of TCR-activity for the treatment of metastatic melanoma patients, and observed disease response rates of 18.75% (3/16) and 0% (0/10), respectively. The former had PR in 2 patients and CR in 1 patient, and no adverse reactions were mentioned (NCT00509496; NCT03412877).

TCR-T cells for digestive system tumor treatment

Rosenberg's team used mutant antigen-specific CD4⁺ T cells and CD8⁺ T cells to treat patients with cholangiocarcinoma and colon cancer, respectively, with good results. In 2017, the research team conducted clinical trials of HLA-DPB1-restricted MAGE-A3 antigen-specific TCR-T cells. The study yielded promising results, with one patient with esophageal cancer achieving partial remission for up to four months (NCT01273181). However, there are still significant adverse reactions in clinical trials of TCR-T cells. For example, in a 2011 CEA

antigen-specific TCR-T-cell clinical trial, three patients developed different degrees of colitis, mainly due to the ability of these high-affinity antigen-specific TCR-T cells to recognize normal colon epithelial tissue expressing CEA, in what is known as an off-target phenomenon (NCT00923806).

AFP is the most widely studied target antigen in HCC. AFP is a glycoprotein synthesized in the liver during the fetal period, and its level in adult serum is very low, but the liver cells with malignant changes can recover its synthesis ability. Therefore, AFP can be used as a target of cellular immunotherapy as a specific antigen of liver cancer cells. HLA-A2⁺/AFP specific TCR has been obtained, which can recognize AFP158-166 peptide, and X-scan detection shows that it does not cross-react with other peptides in the human protein database to avoid off-target effects. Clinical trials of TCR-T for HCC are currently underway to confirm its efficacy by examining AFP expression and T cell infiltration in biopsy tissues and closely monitoring biochemical markers in the liver (NCT03132792; NCT02686372; NCT02719782; NCT04677088). The existing NY-ESO-1-specific TCR for the recognition of NY-ESO-1157 is most prominent, and it has been reported that the TCR-T of NY-ESO-1157 can produce a response in HCC patients, especially in patients with Treg cell depletion. In addition, NY-ESO-1

is highly expressed in tumors of some HCC patients. Therefore, some are also used in clinical treatment trials of HCC (NCT01967823; NCT02869217; NCT03159585). Hepatitis B virus (HBV) and hepatitis C virus (HCV) infection is one of the main causes of HCC, and the expression of virus-related proteins in tumor tissues of patients can be used as target antigens for TCR-T therapy. In 2011, researchers identified HBsAg specific TCR and conducted several clinical treatment trials. Because HBsAg is also expressed in non-HCC tissues, its tumor specificity is low, so the above trials are limited to prevention of recurrence in HCC patients after liver transplantation (NCT03899415).

TCR-T cells for the treatment of synovial cell sarcoma

Synovial cell sarcoma is an extremely rare malignant tumor in soft tissue cancer. At present, it is mainly treated by surgery. It is characterized by high local invasiveness and metastasis rate, and the prognosis of patients with metastasis is poor. ROBBINS et al. transferred the TCR gene specific to NY-ESO-1 cancer testis antigen into autologous T cells for the treatment of patients with synovial cell sarcoma. Phase I clinical trial results showed that the disease PR rate was 66.7% (4/6), of which 1 patient's PR duration lasted for 18 months, and no patients who underwent this clinical trial had significant adverse reactions. Four years later, Robbins et al. again used TCR-T cells targeting NY-ESO-1 to treat patients with synovial cell sarcoma, and the results of phase II clinical trial showed that the response rate was 61% (11/18). Among them, 10 patients achieved PR and 1 patient achieved CR more than 20 months; The 3-year and 5-year survival rates were 38% and 14%, respectively, and all patients had transient neutropenia and thrombocytopenia, but no significant adverse effects were found (NCT01343043; NCT03697824; NCT03967223). In addition, Morgan et al. transferred the TCR gene with MAGE-A3 as the target antigen into T cells for the treatment of a patient with synovial cell sarcoma. The results showed that the PR duration of the disease lasted for 5 months without any symptoms of neurotoxicity. TCR-T cell immunotherapy can prolong the survival of patients with synovial cell sarcoma, but there are still some patients who are not responsive to treatment, and the mechanism has not been clarified. Especially for the NYESO-1 target antigen expressed by most malignant tumors, there are still some patients who do not show therapeutic responsiveness when receiving TCR-T cell immunotherapy. This suggests that there may be other factors (such as tumor antigen deletion and low expression, etc.) worthy of consideration and exploration, and finally find an appropriate way to avoid immune escape of TCR-T cells (NCT02869217; NCT03250325).

TCR-T cells for the treatment of other types of tumors

In addition to the treatment of malignant melanoma patients and digestive system tumors, TCR-T cells have achieved good clinical efficacy in the treatment of some other tumors, such as esophageal cancer, multiple myeloma, metastatic cervical cancer, metastatic colorectal cancer, urothelial cancer, osteosarcoma, and breast cancer, and still achieved good clinical efficacy. In the TCR-T clinical study on esophageal cancer, Kageyama et al. transferred the TCR gene with MAGE-A4 as the target antigen into T cells for the treatment of 10 patients with esophageal cancer, among which 3 patients survived for more than 27 months. Seven patients developed disease progression (PD) within 2 months of treatment, but no treatment-related adverse effects were observed. However, similar studies conducted by Davis et al., and Morgan et al., did not find a significant therapeutic effect, and patients in the latter study died after coma. The safety and efficacy of autologous T cells that have been engineered to express TCRs particularly targeting the HLA-A*11:01-presented public neoantigens, KRAS-G12V or G12D, are investigated in a clinical trial enrolling patients with advanced pancreatic cancer (NCT04146298, NCT05438667). Moreover, autologous T cells engineered with personalized neoantigen-specific TCRs are also being conducted in solid tumors, such as ovarian cancer, lung cancer, colorectal cancer, pancreatic cancer, cholangiocarcinoma and gynecologic cancer (NCT05292859, NCT05194735, NCT04520711).

七、Alternative treatment strategies based on neoantigens

Based on the advantages of tumor specificity and immunogenetics, neoantigens can become new targets for cancer immunotherapy, including tumor vaccines, ACT and antibody-based therapies, as well as potential predictors of ICB responses. Target neoantigens include individual neoantigens for each patient or common neoantigens expressed in many patients' cancers. Off-the-shelf therapies based on public neoantigens consume less resources and time than individualized neoantigen therapies. Because personalized neoantigens are patient specific, they cannot be used for large numbers of patients. With recent advances in high-throughput sequencing, personalized neoantigens enable the immune system to target the appropriate immunogenic epitope of a malignant tumor without a predefined public antigen (Fig. 4).

(一) Therapeutic vaccines based on neoantigens

Neoantigen vaccines have the advantages of high feasibility, high safety and simple production processes. They are an effective way to stimulate, enhance and diversify the antitumor T-cell immune response [382, 383]. Different

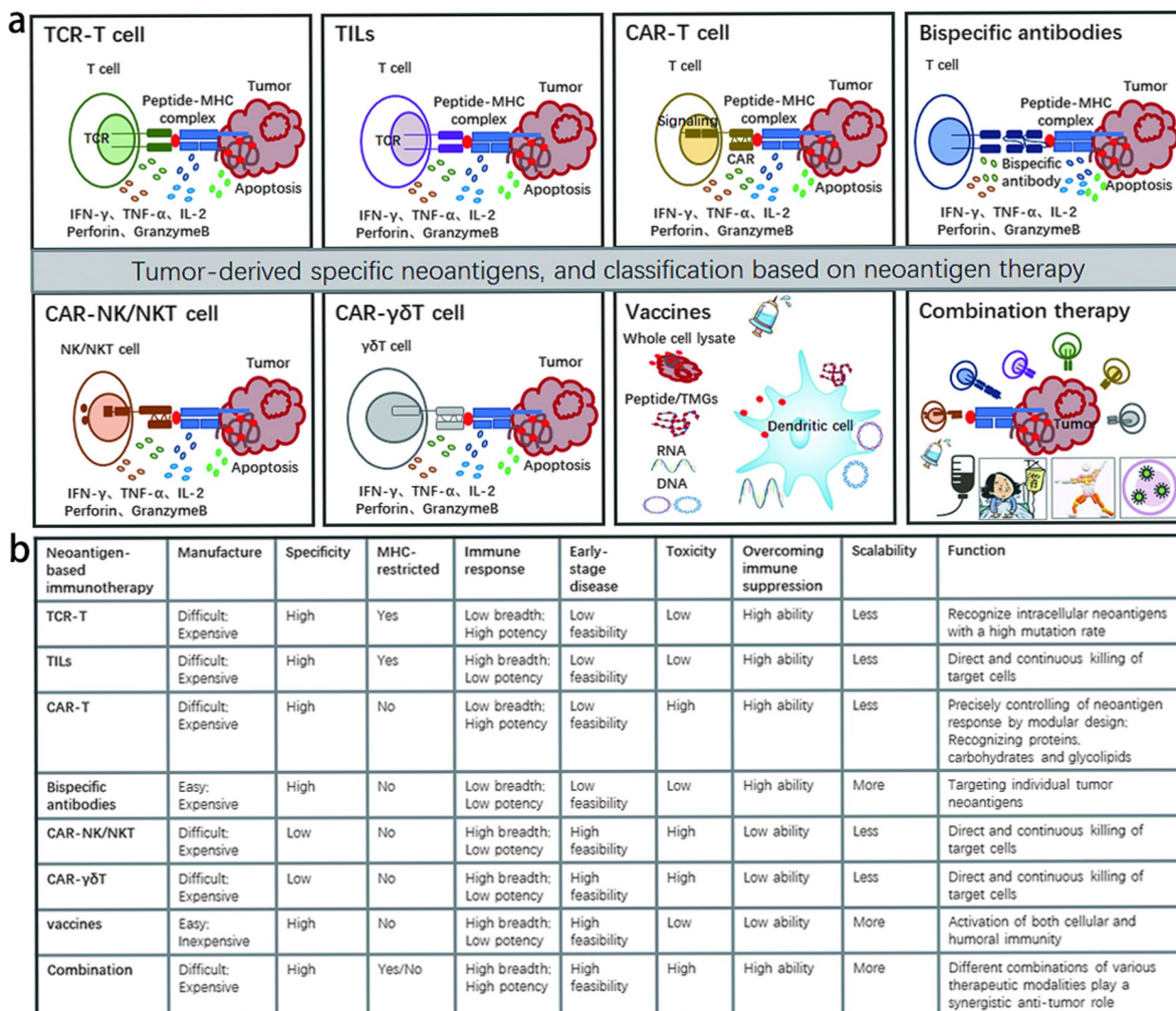


Fig. 4 Classification and characteristics of neoantigen-based therapy. **a** Immunotherapies targeting neoantigens mainly include ACTs (TCR-T, TILs, CAR-T, CAR-NK/NKT, CAR-γδT), bispecific antibodies, cancer vaccines and combination therapy regimens. **b** Advantages and disadvantages of neoantigen-based immunotherapy

forms of neoantigen-based vaccines (such as peptides, nucleic acids, and DC vaccines) are being evaluated in clinical trials in patients with different tumor types. Current peptide and nucleic acid vaccines target neoantigens derived from somatic mutations, including SNVs, frameshift INDELS, and gene fusion. DC vaccines can target selected neoantigens either through the synthesis of peptides or nucleic acid pulses or can target the entire TSA through the introduction of whole cell lysates [384–389]. Therefore, neoantigen vaccines are becoming a new type of tumor immunotherapy.

(二) Adoptive therapy of TILs

CD8⁺ T lymphocytes have the ability to recognize and eliminate cancer cells, and these lymphocytes were discovered more than 50 years ago [390]. Adoptive transfer of auto TILs amplified in vitro without genetic modification has been shown to induce complete remission in some human cancers [257]. These TILs are extracted from patients, amplified under specific circumstances, and prepared to enhance their anticancer activity [391]. The cell product is then injected back into the same patient. These patients had previously received non-myeloablative lymphoid clearance chemotherapy and subsequent cytokine therapy, such as with IL-2, which stimulated a strong antitumor immune response [392,

393]. TILs have a rich specificity for neoantigens and are superior to unselected TILs in achieving complete and lasting tumor regression. Most neoantigen-specific TCRs exhibit significantly higher affinity, even for homologous antigens expressed at relatively low levels, compared to the low affinity of tumor antigen-specific TCRs [350, 354]. Therefore, TILs have been used to treat patients with metastatic malignancies who do not respond to current therapies, including chemotherapy, radiotherapy, and anti-PD-1 therapy.

(三) \ CAR-T therapy

CAR T-cell approaches have other advantages over TCR-T cells. They do not depend on HLA expression and neoantigen presentation, which are mechanisms commonly exploited by cancer cells for immune escape [394, 395]. The engineered expression of CAR molecules includes an intracellular signaling and co-signaling domain, as well as an extracellular antigen-binding domain, that enables CAR T cells to bind to any cell surface protein with antibodies and then activate MHC-independent CAR T cells. Boolean logic gates can be used in CAR T cells even if the antigen is not completely specific. These gates improve the specificity of tumor recognition by activating tumor-specific neoantigens and improves the clearance efficiency of tumor cells by targeting tumor-expressed antigens [396–398]. Early clinical trials using CD19-targeted CAR-T cells for the treatment of B-cell malignancies patients showed outstanding results, while CAR-T cells for the treatment of patients with solid cancer showed poor outcome because of the limited antigens. Tumor neoantigens have inspired creative solutions and given solid tumor patients hope for CAR-T therapy. The limited number of tumor-specific surface neoantigens that are suited for CAR-T can be overcome by integrating a single-chain variable fragment (scFv) that recognizes a neoantigenic pMHC complex on the tumor surface. CAR-T cells redirected at novel neoantigens are being tested in ongoing clinical trials in hematological and solid tumors [399, 400]. A CAR that can recognize the EGFRvIII neoantigen has been created as a part of a lentiviral vector and a truncated EGFR that lacks the ligand binding domain and cytoplasmic kinase domain is incorporated for in vivo tracking and ablation of CAR-T cells in necessary. Human EGFRvIII⁺ xenogeneic subcutaneous and orthotopic models showed that EGFRvIII-directed CAR-T cells could control tumor growth [401]. The T cells can generate CARs that target antigens universally expressed by tumors, like EphA2 and IL13R2, after being primed by a highly tumor-specific neoantigen, like EGFRvIII, and being trained to carry out complete tumor destruction [402]. CAR-NK/NKT

and CAR- $\gamma\delta$ T cells are also being developed as therapies [403].

(四) \ Antibody therapy against neoantigens

In contrast to conventional antibodies that cannot target intracellular proteins, TCR-mimic (TCRm)- or mutation-associated neoantigen (MANA)-specific antibodies can recognize intracellular neoantigens by focusing on the pMHC complex. TCRm antibodies have a stronger binding affinity than TCR, which has been shown to be the key to minimizing on-target, off-tumor effects [404–407]. These antibodies targeting neoantigens are readily converted into a variety of therapeutic forms, including full-length antibodies, antibody–drug conjugates (ADCs), and bispecific antibodies (BsAbs) [408–411]. Phage display, yeast display, and genetic platforms are some of the techniques used to detect human TCRm antibodies that have extremely high specificity for neoantigens presented on HLA. For example, BsAbs can be used to solve the problem of the density of the mutant p53/pMHC complex on the cell surface being insufficient to recruit T lymphocytes to the tumor site [412]. The bispecific T-cell engager (BiTE) is a BsAb constructor that provides an effective and powerful signal for T-cell activation by simultaneously binding neoantigens on tumor cells and CD3 complexes on T cells [413, 414]. Thus, strategies based on TCRm antibodies can be used to target neoantigens derived from oncogene and TSG mutations that are difficult to eradicate using conventional methods. This could lead to more targeted anticancer therapies. The affinity of TCR-Mimic antibodies to polypeptide-HLA molecules is much better than that of natural TCR. To prevent cross-reactivity or the binding of HLA components unrelated to a given peptide, TCR-mimic antibodies must be screened appropriately. Similar to designed TCRs, cross-reactivity can be prevented by negative selection of off-target peptides. At least one of the synthetic agents showed lower cross-reactivity than the equivalent natural receptors [415–417].

(五) \ Neoantigen-based combination therapy

Due to the heterogeneity of neoantigen profiles and the evolving immune escape mechanisms of cancer cells, immunotherapy alone is not effective in the treatment of advanced cancer patients [269, 418]. The combination of several immunotherapies can simultaneously target different stages of the cancer immune cycle, including antigen release and presentation, immune cell initiation and activation, immune cell metastasis and invasion of the tumor, and the recognition and killing of cancer cells, thereby enhancing anticancer efficacy. For example, neoantigen-based immunotherapy combined with ICBs, neoantigen vaccines combined with ACT therapy,

neoantigen-based immunotherapy combined with traditional therapy, etc. Another strategy is to combine therapies with different mechanisms to overcome resistance induced by tumor heterogeneity [269, 292, 407, 419–424]. All targeted cancer cells must have the same pattern of neoantigen expression and presentation; otherwise, resistant clones with no predicted neoantigens can survive and confer clonal growth advantages. Thus, precision immunotherapy can be combined with conventional treatments, such as chemoradiotherapy, to kill cancer cells without relying on neoantigens. This can lead to more significant and lasting therapeutic effects [425, 426]. The "cancer-immune cycle" refers to the sequence of events that must be initiated, conducted, and expanded to achieve an anticancer immune response that effectively eradicates cancer cells. In short, neoantigens produced by tumor formation are released and captured by DCs. The DC transmits the collected neoantigens on the MHC-I and MHC-II molecules to T cells, thereby initiating and activating effector T-cell responses against cancer-specific neoantigens. The activated effector T cells then migrate and infiltrate the tumor bed, where they recognize and ultimately destroy cancer cells. The death of cancer cells produces more tumor-associated neoantigens that amplify and enhance the immune response in subsequent cycles [427–430]. Thus, cancer immunotherapy aims to restart or amplify the self-sustaining cancer immune cycle. A variety of immunotherapies have been developed to target rate-limiting steps in the tumor immune cycle, including enhancing neoantigen release through chemoradiotherapy and oncolytic viruses, increasing the number and quality of tumor-reactive T cells through cancer vaccines and ACTs, and enhancing immune cell invasion and cytotoxic effects through checkpoint inhibitors [108, 431–435]. Therefore, precision immunotherapy can be combined with conventional treatments like radiotherapy and chemotherapy that kills cancer cells independent of the neoantigens, achieving a more prominent and durable therapeutic effect (Fig. 5).

Neoantigen-based immunotherapies and ICBs

Checkpoint inhibitor-based immunotherapy has achieved prolonged anti-tumor effects in several malignancies, including renal cell carcinoma, NSCLC and melanoma. Patients, however, do not react to ICB therapy in the absence of tumor-specific effector T cells. Moreover, ICB therapy only affects one or two phases of the anti-cancer immunity pathways, such as anti-CTLA4 antibodies regulate the immune cell priming and activation, while anti-PD-1/PD-L1 antibodies focus on the final negative regulation of T effector cells [436, 437]. ICBs enhance specific T cell responses by targeting neoantigens, including PRKDC, EVI2B and S100A9, in a relapsed

multiple myeloma patient. For patients with solid tumors who are unresponsive to, or relapsed following anti-PD-1 therapy, mRNA-based neoantigen vaccines, such as mRNA-4157, mRNA-5671, and BNT122, are used together with immune checkpoint inhibitors in multiple clinical trials. The anti-tumor efficacy of CTLs, including those specific for mutation-associated neoantigens, can be further boosted by ICB therapy [438]. In addition, Persistent exposure to TSAs promotes the exhaustion of CD8⁺ T cells, which characteristically expressed high levels of PD-1 and CD39. ICBs can reinvigorate the exhausted neoantigen-specific T cells via overcoming the suppressive microenvironment [439].

Combinations of neoantigen vaccine and ACT

Combinations of neoantigen vaccination and ACT have also been utilized successfully to boost clinical efficacy in tumor treatment. Vaccination can increase the amount of neoantigen-reactive T cells in circulation, possibly by boosting better outgrowth of T lymphocytes. Alternatively, the vaccines can induce de novo T cell responses that overcome the insufficient recognition of neoepitope by T cells due to inadequate cross-presentation of a neoantigen by tumor cells. Vaccine is also used to enhance the efficacy of CAR-T therapy to eliminate solid tumors [440, 441].

Neoantigen-based immunotherapies and conventional therapies

The majority of chemotherapeutic agents and radiation therapy were designed based on their direct cytotoxic effects without considering their impact on immune system. Chemotherapy and radiotherapy can be used to increase the release of tumor-specific neoantigens, circumventing issues such as an insufficient number of neoantigens to stimulate T cell response. During the chemotherapy and targeted therapy, the tumor cells often occur new mutations, including reversion mutation, contributing to drug resistance. Many reversions are predicted to encode tumor-specific neoantigens, offering a potential strategy for combating resistance with CAR-T cell therapies, immune checkpoint inhibitors or anti-cancer vaccines [270, 442, 443].

八、Limitations of TCR-T-cell therapy

Despite success in hematological malignancies and solid tumors, neoantigen-based immunotherapies have shown objective efficacy in only a few documented patient responses. Therefore, a considerable number of changes are needed to improve clinical outcomes, including increasing the accuracy of neoantigen prediction, overcoming immune evasion, and optimizing the production process (Fig. 6).

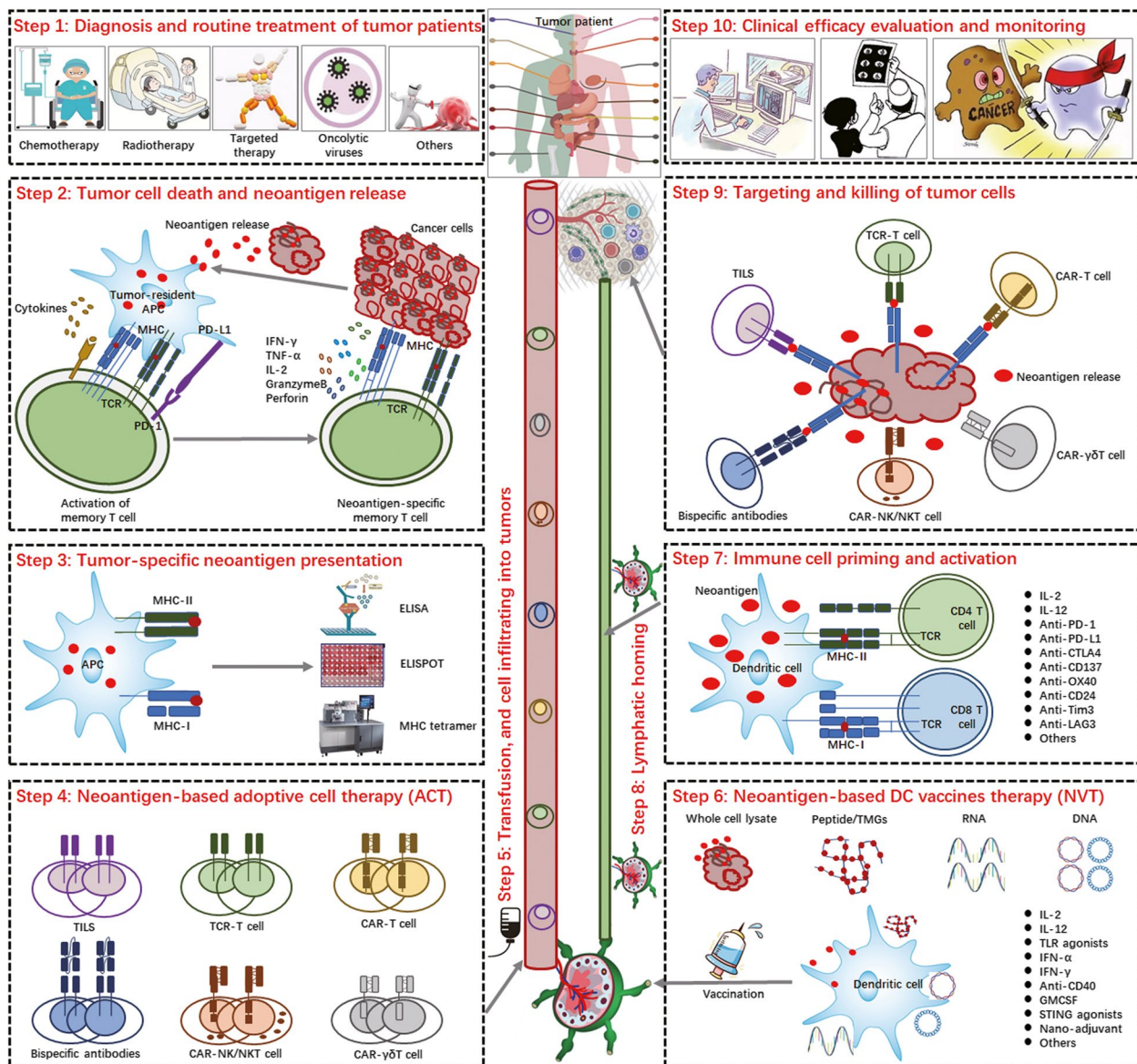


Fig. 5 Combined antitumor strategies based on neoantigens. The diagnosis and routine treatment of tumor patients (Step 1). The formation of tumor cells initiates the immune function of T cells, and the tumor cells die and lyse, resulting in the release of neoantigens (Step 2). Neoantigens produced by tumors are released and captured by DCs. The DC transmits the collected neoantigens on the MHC-I and MHC-II molecules to the T cells (Step 3). Immunotherapies targeting neoantigens (neoantigen-based adoptive cell therapy) mainly include TCR-T cells, TILs, CAR-T cells, CAR-NK/NKT cells, CAR- $\gamma\delta$ T cells and bispecific antibodies (Step 4). Adoptive back transport of ACT cells and chemotaxis into the tumor play an antitumor role (Step 5). Neoantigen-based DC vaccine therapy is also initiated (Step 6). Immune cells are primed and activated in the lymph node (Step 7). Effector cells develop into effector memory cells through lymphatic homing (Step 8). Effector memory ACT cells target and kill tumor cells (Step 9). After a series of treatments, clinical evaluation and efficacy monitoring are performed (Step 10). In brief, the “Cancer-Immunity Cycle” includes enhancing neoantigen release by chemotherapy, radiation therapy and oncolytic viruses, increasing the quantity and quality of tumor-reactive T cells through cancer vaccines and ACTs, and boosting the infiltration and cytotoxicity efficacy of immune cells via checkpoint inhibitors

(一) The accuracy of neoantigen prediction is limited

The widespread use of personalized immunotherapy is limited by limitations in the discovery of neoantigens that target tumors. This is due to the heterogeneity of mutation load and the significant differences in neoantigen

presentation between different tumor types [444]. Therefore, the identification and prediction of neoantigens should be carried out for specific individuals with cancer. The prediction of neoantigens is also limited by genetic heterogeneity, especially different somatic mutations

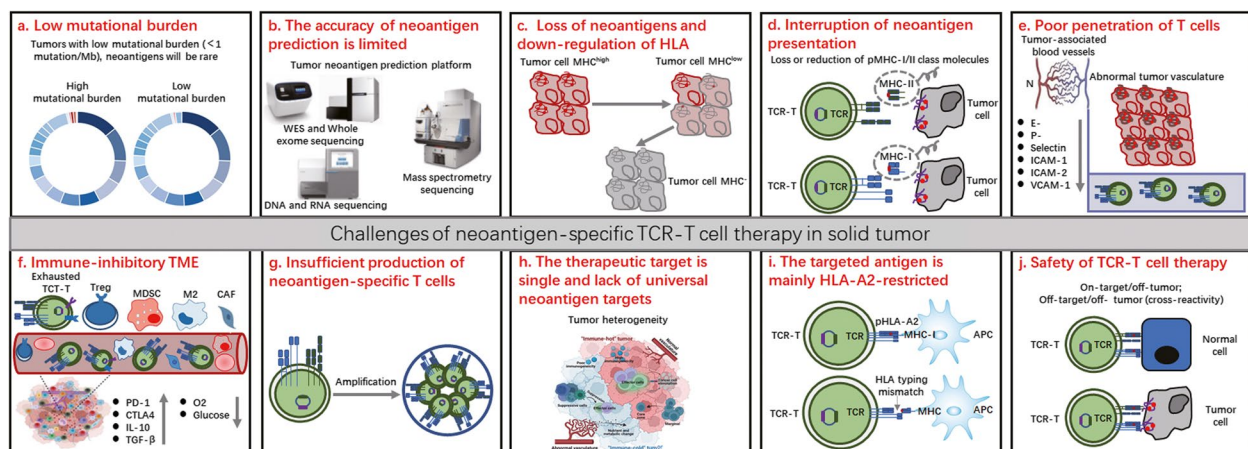


Fig. 6 Challenges in the clinical application of neoantigen TCR-T-cell therapy. **a** Low neoantigen load results in a lack of suitable neoantigen targets. **b** At present, the accuracy of neoantigen prediction technology is limited. **c** Downregulation of MHC expression causes tumor cells to lose neoantigen targets. **d** The loss of pMHC molecules leads to the interruption and reduction of neoantigen presentation. **e** The expression of adhesion molecules and stroma-rich and abnormal blood vessels in tumor tissues is downregulated, which limits the effective penetration of T cells. **f** Immunosuppressive tumor microenvironments inhibit T-cell function. **g** The technical bottleneck of ACT leads to the production of neoantigen-specific T cells. **h** Tumor heterogeneity leads to the singleness of specific tumor therapeutic targets and the absence of universal neoantigen targets. **i** The neoantigen epitopes developed thus far are mainly for HLA-A2 targets. **j** Safety of TCR-T-cell therapy itself

within different cancer types, different individuals and even different tumor subclones. Only 10% of nonsynonymous tumor cell mutations produce mutant peptides with high MHC affinity, and only 1% of MHC-binding peptides are recognized by patient T cells [445–447]. In addition, the heterogeneity of mutations within tumors provides additional complexity to neoantigen prediction. The genome of tumor cells has undergone extensive generation, cloning, alteration and mutation loss. At the same time, the diversity of neoantigen-specific T cells present in patients may not be fully captured by a single resected lesion due to the limited infiltration of T lymphocytes. This limits the library of TCRs that can be built for therapeutic purposes [448, 449]. Theoretically, the higher the TMB, the more neoantigen-specific T cells are detected in the tumor, resulting in a higher immunotherapy response rate. However, low TMB in hematologic malignancies and some epithelial cancers can also produce neoantigen-responsive lymphocytes. Inadequate neoantigen density in low-TMB malignancies requires more robust strategies to accurately identify novel immunogenic epitopes that CD8⁺ T cells can detect [450–453]. Therefore, there may be tumor clones that do not respond to neoantigen-specific T cells. Because of the selection advantage, these cloned cells may outperform other cloned cells, limiting the clinical benefit.

(二) \ Evasion of immune surveillance

Tumors can evade neoantigen-based immunotherapy through many mechanisms, including neoantigen loss,

modification of antigenic peptide presentation, and the immunosuppressive tumor immune microenvironment (TME).

Neoantigen loss

Loss of tumor-specific neoantigens may be an important strategy for tumor immune escape. In particular, many neoantigens are byproducts of tumorigenesis and do not play a critical role in tumor cell survival [454]. Neoantigen depletion may also be an intractable mechanism of antitumor immunity, which limits the application of personalized neoantigen-specific immunotherapy. Neoantigen depletion can have a variety of causes, such as copy number loss, transcriptional inhibition, epigenetic silencing, and posttranslational mechanisms. Neoantigens present only in specific tumor cell subpopulations may also be lost due to eradication of entire subclonal cell populations mediated by CD8⁺ T cells. Many of the missing mutations are recognized by the patient's T cells, and neoantigen coding genes are unlikely to be produced in tumors with widespread immune cell penetration. This suggests that neoantigen-expressing tumor subclones may be preferentially removed by the immune system [455, 456]. Therefore, to compensate for the loss of targeted neoantigens in immunotherapy, personalized neoantigen-specific immunotherapy should

target multiple neoantigens, thereby expanding the range of neoantigen response [292, 459].

Interruption of neoantigen presentation

Tumors may undergo mutations that alter not only neoantigen expression but also HLA heterozygosity and MHC stability in response to tumor immune pressure. These changes impede the processing and presentation of neoantigens, which inhibit T-cell recognition and tumor killing. If the key antigen presenting gene beta-2 microglobulin ($\beta 2m$) is mutated or lacks HLA allelic heterozygosity, the tumor may be able to avoid recognition by adoptive metastatic T lymphocytes [460–462]. The second demonstrated mechanism of epitope loss is the downregulation of MHC molecule expression in tumor cells due to abnormal transcription, translation, or protein stability events [463]. Together, these mechanisms may help partly explain why higher neoantigen loads in some cancers are not associated with better prognosis due to reduced neoantigen presentation. Based on these findings, ICBs may be more effective in cancer treatment if MHC-I presentation is activated using splicing inhibitors or autophagy inhibitors [464–466].

Immunosuppressive TME

Tumor cells live in a heterogeneous microenvironment composed of invasive and resident host cells, secretory factors, and extracellular matrix. Infiltrating cells in the TME include T cells (TILs and Tregs), B cells, fibroblasts, macrophages (M1 and M2), MDSCs and other immune cells and secretion factors, including the immunosuppressive cytokines IL-10 and TGF β . These components can interact with each other to induce a supportive environment for malignant cell growth, migration, and metastasis, thereby escaping the immune system and tumor-specific CTLs [467–469]. Immunosuppressive TME processes can also impair neoantigen recognition and T-cell activation, including suppression of immune checkpoints, immunosuppressive effects of various TME cells, and release of ions or proteins within tumor cells. Immunosuppressive checkpoint ligand molecules (such as PD-L1 and CTLA-4) can biologically limit T-cell growth and function and are commonly increased in tumor cells during immunotherapy [470–473]. Induced neoantigen expression and combined CAR and epitope diffusion are compensation strategies that can be used to address the immune escape of tumor cells. MHC-I immune epitopes can expand by splicing new epitopes due to defective interaction of the splicing complex with RNA, incorrect degradation of auxiliary splicing factors, or abnormal splicing factor PTM [273, 474, 475]. Most tumor stromal cells in the TME express the immunosuppressive checkpoint ligand PD-L1, which

can interact with PD-1 expressed on T cells, leading to antitumor functional inhibition and depletion of adoptive metastatic TILs, CAR T cells and TCR-T cells. This effect can be mitigated by blocking checkpoints with anti-PD-1 and anti-PD-L1 antibodies [377, 470]. CTLA-4 expressed on activated T cells has a similar effect because CTLA-4 binds to CD80/86 on antigen-presenting cells with a higher affinity, competing with the T-cell costimulatory molecule CD28 to suppress antitumor immunity. Anti-CTLA4 antibodies not only block the interaction between CTLA4 and CD80/86 but also consume Tregs, thereby promoting the costimulation and amplification of tumor-specific CTLs and improving clinical benefits [376, 476].

Among the multiple immunosuppressive factors secreted by the TME, TGF- β plays a central role in driving tumor signaling, remodeling, and metabolism. TGF- β is produced by many cell types, including tumor cells, stromal cells, and Tregs. It stimulates autocrine and paracrine signaling to promote angiogenesis, inhibits the antitumor response of CD8⁺ and Th1 cells, and induces the epithelial-mesenchymal transformation of tumor cells, thus promoting tumor invasion. Thus, blocking TGF- β signaling in the TME enhances the antitumor response [477, 478]. Indeed, one possible mechanism for anti-CTLA4 antibody therapy is the depletion of immunosuppressive TGF- β -producing Treg cells, thereby promoting the costimulation and amplification of tumor-specific CTLs [479]. To further improve the efficacy of checkpoint inhibitor antibodies, bifunctional antibody ligands have been produced that consist of antibodies targeting CTLA-4 or PD-L1 fused into the TGF β receptor II extracellular domain (TGF β RIIecd), where TGF β RIIecd isolates TGF- β secretion in the TME, while checkpoint inhibitor antibodies consume Tregs and promote CTL costimulation [480]. This dual strategy may be more effective against cancers resistant to checkpoint inhibitors alone. As an alternative to TGF- β isolation, tumor-reactive T cells can be transduced with dominant negative TGF- β receptor-II (DNTGf- β rii) to produce TGF- β -resistant antitumor T cells. TCR-T cells expressing dnTGF β RII showed complete tumor regression and prolonged survival in mouse models of advanced and invasive prostate cancer [481, 482]. To take advantage of the high concentration of TGF- β in the TME, a study fused the extracellular domain of TGF- β receptor II (TGF- β RII) into the intracellular domain of 4-1BB. This strategy converted the immunosuppressive effect of TGF- β into an immunostimulatory signal. Coexpression of this transgene receptor produces additive effects and improves the amplification, persistence, tumor lysis, and selective antitumor activity in vivo [483]. Similar to TGF- β , Fas ligand-mediated T-cell death signals that are highly

expressed in the TME can also be transformed into pro-survival signals via CSR. These signals enhance the proliferation and antitumor function of engineered T cells by fusing the Fas extracellular domain with the 4-1BB intracellular domain [484].

(三) \ Insufficient production of neoantigen-specific T cells

Immunotherapies, including vaccination, adoptive TIL and TCR-T cells, and ICBs, all rely on neoantigen-specific T cells [163, 459]. Direct detection of neoantigens presented by tumor cells may be the most effective way to build neoantigen-reactive T cells, ensuring that they recognize new epitopes in the body [175]. However, the implementation of TIL therapy will be hampered by the scarcity of fresh tumor samples and low TIL cold tumors. First, the acquisition of TILs requires invasive surgery to remove resectable lesions, making it suitable for only some patients. Second, inhibition of the TME in cold tumors may reduce the efficiency and number of neoantigen-specific T cells derived from TILs. Readily available peripheral blood may be a suitable source for generating large numbers of neoantigen reactive T cells for ACTs. However, it should be emphasized that large amounts of *in vitro* amplification can increase both the further differentiation of T cells and the false-positive neoantigen response of T cells. Most cancer patients cannot be treated with TILs due to inadequate TCR libraries [485–487]. Efforts are underway to develop effective strategies to isolate and rapidly amplify neoantigen-specific T cells, which may benefit neoantigen-based behavior. TILs contain a large number of neoantigen-reactive T cells, making them a valuable source of T lymphocytes for ACT [237, 255, 391]. Currently, mature DC or EBV-transformed B-cell lines that transfect APCs with peptide pulses or TMGs are used to present antigens to T cells [488, 489]. mRNA transfection of DCs expressing neoantigens was also used to activate autologous naive CD8⁺ T lymphocytes from healthy donors who had not been exposed to the immunosuppressive environment of the tumor host. However, TCRs triggered by APCs that encode mRNA or synthesize peptide pulses may not endogenously recognize antigen-presenting tumor cells [490]. Naive and very early memory T cells with stem-like properties can be generated using induced pluripotent stem cell (iPSC) techniques. Immature T-cell lines derived from iPSCs can now be further differentiated into neoantigen-specific T cells based on a unique three-dimensional thymus organ culture system that fully generalizes disease *in vitro* [491, 492]. Another benefit is that iPSCs can be cloned from a single neoantigen-specific $\alpha\beta$ and re-differentiated into a large number of CTLs. In the near future, iPSCs from single CTL clones will be used to generate sufficient numbers of neoantigen-specific

TCR-T cells. These cells remain naive and contain an endogenous state of TCR, thereby significantly increasing the efficacy of neoantigen-based immunotherapy [493].

(四) \ The singleness of therapeutic targets

In current clinical studies on TCR-T cells, only TCR-T cells attacking a single epitope have been utilized [176]. Heterogeneity is an important characteristic of tumors. This means that this unique target of T-cell attack may be decreased or even disappear, leading to T-cell therapy failure [260, 323]. The use of single TCR-T cells may also be an important reason for the poor results of such clinical trials. To achieve better tumor treatment results, combination therapy with TCR-T cells targeting different sites must be used to prevent immune escape [10].

(五) \ Immunotherapy resistance

The mechanism of primary and secondary resistance to TCR-based immunotherapy may manifest as the low expression or heterogeneity of target antigens in tumor cells or the intrinsic resistance of tumor cells to T-cell-mediated cytotoxicity [5, 397]. The main mechanism of resistance to TCR-T-cell therapy is the loss or reduction of MHC-I/II class molecules on the surface of tumor cells, which prevents TCR-T cells from recognizing target epitopes [85, 126, 141, 161, 353, 395].

(六) \ The targeted antigen is mainly HLA-A2-restricted

Most of the antigen recognition in clinical trials of TCR-T cells is HLA-A2-mediated [119, 494, 495]. Although HLA-A2 is the main MHC molecule in the population, the positive rate is high (approximately 1/3 of the Chinese population expresses HLA-A2). However, TCR-T cells that rely solely on the recognition of HLA-A2 presenting antigens are not applicable in a significant number of patients. The search for only HLA-A2-dependent TCRs also limits the search for targeted antigens [496, 497]. Many of the antigens suitable for TCR-T-cell therapy may be presented by other MHC molecules [263, 315]. Monoclonal or oligoclonal neoantigen-reactive T cells made by amplification would also be an alternative route [10]. Therefore, other HLA-polypeptide tetramers must be developed to broaden the search for antigenic and reactive TCRs.

(七) \ The safety of TCR-T-cell therapy

Safety is an issue that must be addressed before TCR-T cells can be used in clinical therapy [498, 499]. There have been cases of death in clinical trials of TCR-T cells. The reason may be that the enhanced affinity of TCR leads T cells to recognize autoantigens with high homology and then attack normal cells and tissues, eventually leading to death [500, 501]. Thus, the balance between functionality

and security must be considered in TCR transformation. The safety of TCR-T-cell therapy should be carefully determined by sequence alignment, cell experiments and animal experiments [502]. Another way to ensure the safety of TCR-T cells is to introduce the suicide gene. TCR-T cells with the suicide gene can perform the killing function normally. However, after the injection of suicide gene activating drugs, T cells undergo apoptosis to avoid the nonspecific killing of T cells [503–505].

九、Advances in TCR-T-cell therapy

In clinical studies, the safety and efficacy of TCR-T-cell therapy have shown major problems. To solve these problems, in the field of TCR-T-cell research, the search for safe and effective target antigens to improve the affinity and efficiency of TCRs has been the focus. However, it is worth noting that in recent years, an increasing number of studies have been conducted to search for reactive TCRs using neoantigens as targets, and good results have been achieved.

(一) Identification of neoantigen reactive TCRs

Neoantigens are abnormal peptides produced by genetic mutations. These antigens are often tumor specific, making them good therapeutic targets [163]. In recent years, with the advancement of sequencing technology, it has become possible to conduct high-throughput sequencing of tumor tissue and normal tissue to look for neoantigens [426, 506, 507]. After the neoantigen mutation site is identified, the neoantigen reactive TCR can be found by coinubation with tumor-infiltrating T cells by using the presenting cells to express multiple antigenic peptides at different locations of the mutant epitopes [159, 350, 508]. In addition, the expression of coinhibitory molecules such as PD-1, 4-1BB and ICOS is also helpful in searching for neoantigen-reactive TCRs [244, 280, 375]. Targeting neoantigens is an attractive therapeutic strategy, but it falls into the category of individualized therapy and is costly. At present, it is not the mainstream strategy of TCR-T-cell therapy [176]. However, TCR-T-cell therapy targeting neoantigens will be a direction for development in the future, and it could even be the main direction of research [260].

(二) The therapeutic effect of CD4⁺ TCR-T cells

CD8⁺ T cells are traditionally considered to be the primary tumor killer cells [188]. Therefore, the role of CD4⁺ T cells in tumor therapy has not received much attention. In recent years, however, it has been found that CD4⁺ T cells also play an important role in tumor killing [280, 282, 348]. In the identification of TCRs, we have always been searching for MHC-I molecular-restricted TCR sequences. The transfer of such TCRs into CD4⁺ T

cells can also promote the function of the latter. The role of CD4⁺ T cells in tumor control has also been directly demonstrated in clinical trials. A reduction in tumor charge was also observed after patients received neoantigen-reactive CD4⁺ T cells. However, due to the lack of the stabilizing effect of the CD4 molecule, it is difficult for these CD4⁺ T cells to play a long-lasting role in the body [263, 509]. In general, it is thought that the recognition of antigens by TCR is MHC-restricted, that is, it recognizes antigens presented by either MHC-I or MHC-II molecules [126]. However, studies have shown that some TCRs can recognize both MHC-I and MHC-II molecular presented antigens. This means that such TCRs may be stabilized by both CD8 and CD4. The therapeutic effect of CD4⁺ TCR-T cells will be improved by using such TCRs for treatment [510].

(三) The selection of TCR α and β chain connection sequences

The TCR consists of alpha and beta chains. In natural T cells, alpha and beta chains are encoded by two loci, each transcribed and translated, and then the TCR is assembled [511]. However, in TCR-T cells, to synthesize functional exogenous TCRs, it is necessary to consider introducing both α -chain and β -chain genes into the same T cell. In this process, two vectors or two promoters in the same vector can be considered to express the α or β chains. However, this may lead to an imbalance in the α chain and β chain expression of exogenous TCR, increasing the probability of TCR mismatch [232, 512]. Therefore, a better solution is to make the α and β chains under the control of a promoter by using the linking sequence to ensure the balance of molecular translation. The internal ribosome entry site sequence (IRES) is a widely used bicistronic junction sequence that enables coexpression of the IRES. However, the efficiency of IRES-mediated translation is low, resulting in low expression levels of genes located behind IRESs. If IRES sequences are used to connect the alpha and beta chains of TCRs, mismatches may still occur [513–515]. In the TCR expression system, the 2A peptide sequence derived from picornavirus or porcine enterovirus is a good choice. Peptide 2A has a self-cutting function. In other words, when the α - β chain is connected by the 2A sequence, the two chains are transcribed and then broken into separate peptide segments after translation. The amount (mole) of TCR chain can be expressed by using the 2A sequence to reduce the probability of mismatch. Notably, the function of TCR is not affected by the 2A binding sequence despite the addition of several additional amino acids to the α and β chains of TCR [515, 516].

(四) Improvement of the surface expression efficiency of the TCR membrane

When a sufficient number of TCRs recognize and bind to pMHC, T cells are activated to function. The amount of TCR required for T-cell activation can be reduced by costimulation. However, the continued activation of T cells still depends on the number and affinity of TCRs on the cell surface [517, 518]. The assembly of TCR and the localization of the membrane surface are complicated processes. The translated α and β chains are assembled to form heterodimers, which bind to multiple CD3 subtype molecules (γ , δ , ϵ , and ζ). The amount of CD3 molecules, especially of the ζ subtype, is constant in the cell. If a complete complex cannot be formed with the CD3 molecule, the excess TCR is degraded [511, 519]. The problem is particularly acute in TCR-T cells. The use of a strong promoter and 2A sequence can ensure the efficient expression of exogenous TCR in host cells. However, the number of TCRs that can bind to CD3 is limited, and there is competition to bind to endogenous TCRs. This will result in the degradation of a large number of TCR transgene products that are unable to form functional TCR-CD3 complexes [516, 520, 521]. To solve this problem, TCR and CD3 molecules can be expressed together. Animal experiments have shown that when TCR is coexpressed with CD3 subtype molecules, the expression of TCR on the T-cell surface increases by tens of times. This increases the affinity of T cells to target antigens while improving the efficiency of tumor clearance and memory response [521]. In addition, appropriate modifications of TCR coding sequences, such as the deletion of unstable mRNA sequences and splicing sites, can also upregulate the expression of TCR transgenes and enhance the anti-cancer activity of TCR-T cells [513, 522].

(五) Selection of the TCR constant region (C region)

The C region of TCRs plays an important role in the correct pairing of alpha and beta chains. If the C region of exogenous TCR can be made different from the C region of the host itself, the correct heterodimer formation of exogenous TCR can be guaranteed [520]. The experimental observation showed that the expression efficiency of mouse TCR in human T cells was higher than that of human TCR, suggesting that mouse TCR could be correctly assembled and bind to CD3 molecules [523]. The C region of mouse TCR plays an important role in this phenomenon. Based on this orientation, a hybrid TCR containing the human V region and mouse C region was developed and studied. The results showed that the function of the hybrid TCR was stronger than that of the fully human TCR in human T cells. These results indicated that the C region of mice ensured the preferential pairing of exogenous mixed TCR itself and the

stability of the mixed Tcr-CD3 complex while improving the membrane surface expression efficiency of TCR [524–526]. Although the introduction of mouse region C induces immune rejection, the immunogenicity of the mixed TCR can be significantly reduced by modifying a few amino acids in mouse region C. In addition to introducing mouse sequences, amino acid sequences in the C region can also be modified to improve the accuracy of TCR pairing. Cysteine plays an important role in the pairing and stabilization of the alpha and beta chains of TCR [527]. Using a point mutation technique, the threonine at position 48 of the alpha chain and the serine at position 57 of the beta chain were replaced with cysteine, forming an additional disulfide bond in the C region. This change enhanced the pairing efficiency and stability of the exogenous α and β chains, reduced the occurrence of mismatch, increased the expression of exogenous TCR on the cell surface, and enhanced the antigen-specific response. Studies have shown that this strategy can reduce the autoimmune pathological reactions caused by TCR mismatch [528].

(六) Other strategies to reduce TCR mismatch

Endogenous TCR expression can be reduced using siRNA or CRISPR/cas9 techniques. This can not only prevent TCR mismatch but also reduce the competition between TCR and CD3 molecules [355, 516]. Several studies have shown the feasibility of this strategy. For example, T cells have coexpressed MAGE-A4-specific TCRs (whose codon-optimized C region is different from that of the wild type) and siRNA targeting conserved sequences in the C region of wild-type TCRs. The results showed that the surface expression of the TCR transgene membrane in human T lymphocytes transduced by the MAGE-A4-TCR/siRNA vector was upregulated [529, 530]. In addition, the transfer of the TCR gene into $\gamma\delta$ T cells can also prevent the mismatch of endogenous TCR. However, $\gamma\delta$ T cells did not express CD4 and CD8 molecules. The use of $\gamma\delta$ T cells to express functional α/β TCR requires simultaneous transduction of CD4 or CD8 molecules to enhance the antigen-specific immune response of $\gamma\delta$ T cells [531–533].

(七) Enhanced T-cell activation signals

CD3 ζ and costimulatory or coinhibitory molecules are the main signaling molecules that directly affect the activity of TCR-T cells [534]. The function of TCR-T cells is affected by altering the expression and function of CD3 ζ and/or costimulatory/coinhibitory molecules. By coexpressing CD3 ζ in series with the α and β chains of TCR, it can increase the membrane surface expression of TCR and enhance the function of antigen-specific T cells [535]. In addition, the costimulatory signal CD28 can

be introduced into the TCR sequence to enhance TCR signal transduction. This method requires the complete removal of the C region and its replacement with the transmembrane region of CD28. Therefore, such TCRs do not pair with endogenous TCRs and have a stronger activation capacity. However, this method directly connects $V\alpha$ and $V\beta$ through flexible sequences, which destroys the original TCR sequences and may not be suitable for all TCR sequences, limiting the application of this modification strategy [373, 536]. In recent years, PD-1 has received great attention. If the PD-1 inhibition of T cells can be removed, the function of TCR-T cells can be effectively improved. Currently, CRISPR/Cas9 can be used to knockdown PD-1 expression or express specific antibodies to block PD-1. It can not only make TCR-T cells have tumor killing specificity but also block the cosuppression signal to avoid the suppression of tumor killing function [537–541].

(八) Improved TCR affinity

The affinity between TCR and antigen determines the ability of T cells to recognize and kill tumor cells, which is the main focus of TCR-T cell research [350]. (1) The isolation of high-affinity TCR from artificial nontolerant environments is performed as follows: This approach utilizes humanized mice to produce high-affinity T cells [542]. $CD8^+$ T cells in the body that have a high affinity for the tumor-associated antigen p53 are often deleted. Immunizing transgenic mice expressing human HLA-A2 with human p53 peptide induced amplification of $CD8^+$ T cells with high affinity for human p53 [543, 544]. When isolated from these high-affinity TCR sequences, these peptides can be used to redirect human $CD8^+$ or $CD4^+$ T cells. Using the same method, T-cell clones with high affinity for other tumor-associated antigens, including MDM2, CEA, and gp100, have been isolated [545–547]. (2) The isolation of high affinity MHC-restricted TCR from MHC-mismatched donors can be performed as follows: T-cell clones with high affinity for the HLA-A2 presenting antigen can be isolated from the natural lymphocyte pool of HLA-A2 negative donors. Using this method, human T-cell clones with high affinity for tumor-associated antigens, such as cyclin D1, WT1, and MDM2, were isolated, and these high-affinity TCR genes were cloned into vectors for gene therapy [548–551]. In mouse models, human T cells with allogeneic HLA-A2 antigen-specific TCR gene transduction were shown to clear tumor cells [549, 552]. (3) TCR sequences can be modified to improve affinity as follows: Improving affinity by changing the sequence of the TCR is an effective way to improve the effects of TCR-T-cell therapy. The CDR region responsible for binding to pMHC determines the affinity of TCR, and modification of the CDR region is

expected to improve T-cell function [520, 527, 553]. Substituting an amino acid at site 107 in TCR has been shown to improve the stability of the CDR3 β ring structure and increase the antigenic specificity of TCR [554, 555]. In screening TCRs with high affinity mutations, point mutation techniques can be used to construct a variety of TCR α chain and β chain libraries. The assembled TCRs can then be displayed using phages, yeast, or T cells, and affinity screening is performed [556–558]. MHC-polypeptide tetramers are an important tool in affinity screening. High affinity TCRs can even be screened from natural TCRs by using tetramers. The high-affinity TCRs screened can theoretically enhance the antigenic reactivity of T cells [170, 559]. However, some studies have shown that T cells transduced with high-affinity TCRs do not respond to antigens or even have negative reactions. Another concern is that high-affinity TCRs can misrecognize antigens, resulting in off-target effects [350, 560, 561].

(九) $CD4^+$ T cells are dependent on MHC-I molecule-restricted TCRs

Early studies on TCR-T cells focused on how to construct antigen-specific $CD8^+$ T cells [176]. Currently, the influence and role of $CD4^+$ T cells is also being considered. $CD4^+$ T cells recognize the MHC-II class molecular delivery of antigenic peptides. Unlike $CD8^+$ T cells, which mainly exert cytotoxic effects, $CD4^+$ T cells mainly regulate the adaptive immune system, enhance the function of $CD8^+$ T cells, and induce the long-term memory of T cells [267, 340, 341]. Although the isolated TCRs with high affinity for tumor antigens were mostly MHC-I molecule restricted, they performed best in the presence of $CD8$ coreceptors [282]. Studies have shown that these TCRs can function in $CD4^+$ T cells even in the absence of $CD8$ coreceptors. Tumor-specific $CD4^+$ T cells can be generated by using MHC-I molecular restrictive TCR, which enhances the tumor-killing ability of specific $CD8^+$ T cells. The reason for this phenomenon may be related to the secretion of various immune factors by $CD4^+$ T cells [344, 349, 562]. In addition, antigen-specific $CD4^+$ T cells have also been used to treat tumor patients [563–565]. These results suggest that attention should be given to the role of $CD4^+$ T cells in TCR-T-cell therapy.

(十) Prolonging the survival time of TCR-T cells in vivo

A common challenge faced by adoptive cell therapy is the survival time of the transfused cells in vivo [256]. Studies of TCR-T cells and CAR T cells have demonstrated that T-cell infusion can lead to the formation of memory and these cells can survive long-term in vivo [176, 257, 398]. However, the number of such cells is too small to play a role in the treatment of solid tumors. At present, the

common ways to maintain the survival of T cells include the administration of exogenous IL-2 and the removal of lymphocytes from the body by radiotherapy or chemotherapy [255–257]. Among them, IL-2 injection has relatively obvious toxicity, especially in the application of high doses, and adverse reactions are more obvious. The adverse effects of IL-2 limit its use. To address this limitation, researchers have tried to express IL-2 in T cells, but the results were not good [566]. Another approach is to use chemoradiotherapy to eliminate lymphocytes, reduce the number of endogenous T cells, and avoid endogenous T cells competing for cell growth factors [567]. Animal experiments have shown that without preconditioning, transfused T cells cannot survive or clear tumors. Lymphocyte elimination preconditioning has become the standard practice for infusion cell therapy, including for TCR-T cells [268]. The TCR sequence can also be transferred into less differentiated T cells or memory T cells. In addition to treating T cells directly, TCRs can also be transferred into hematopoietic stem cells (HSCs). Transgenic HSCs can develop into mature CD8⁺ T cells through positive selection and produce a rapid antigen-specific response [568, 569]. In addition, it is possible to prolong the survival of TCR-T cells in vivo by influencing T-cell differentiation through metabolically regulated drugs, such as metformin [570].

† Summary and outlook

Neoantigens play a key role in cancer immunotherapy, including cancer vaccines, ACTs, antibody-based therapies, and ICBs [571]. Therapeutic strategies targeting these cancer-specific neoantigens without destroying normal tissue provide a strong theoretical basis to support the relevance of neoantigens in supporting clinically successful immunotherapies. Many initiatives are underway to develop personalized or off-the-shelf anticancer drugs based on neoantigens. However, to address the timing and economics of advanced personalized neoantigen immunotherapy, experimental and theoretical improvements are needed, including effective patient recruitment, optimized sequencing techniques and neoantigen prediction algorithms, and off-the-shelf therapies targeting common neoantigens [272].

Effective patient recruitment is key to neoantigen-based immunotherapy. First, early excision may give clinicians more time to carefully design, produce and test neoantigen-based therapeutics to improve clinical outcomes. Second, early excisions make it easier to select patients who may be eligible for off-the-shelf therapies, including vaccines, TCR or TCRm antibodies that target a well-defined cancer-driving mutation. Third, cancer treatments, including chemotherapy, radiation, and ICBs, can stimulate the over-differentiation of T cells.

Immediately isolating autologous T cells, or TILs, as soon as a patient is diagnosed with cancer allows for the highest quality and least differentiated T cells to be collected from the patient. In addition, neoantigen-based therapy in patients who were recruited early can allow for the effective infusion of high-quality neoantigen-based cell products and reduce the severity of comorbidities caused by advanced metastatic cancer clones.

Accurate identification of immunogenic neoantigens and their homologous TCRs is a key and rate-limiting step in the development of personalized cancer immunotherapy [163]. Immunogenic neoantigens can be identified by the immunogenomics methods of constructing virtual peptides based on NGS data and the immunolabeling methods of analyzing MHC-loaded peptides using MS. Genome and transcriptional sequencing data are also combined with MS maps of HLA-related peptides to improve the sensitivity and specificity of neoantigen identification [292]. However, neoantigen-based therapies may be quite affordable due to the application of more economical high-throughput sequencing and powerful deep learning algorithms. Comprehensive and efficient one-stop computational workflows or classification benchmarks available in computerized neoantigen assay methods are still necessary for clinical applications. Efficient one-stop computing methods can also use large data queues to determine the potential of neoantigens as biomarkers for patient prognosis or ICB response prediction. Most critically, the accuracy of these epitope prediction methods should also be confirmed by thorough immune surveillance in early clinical studies to facilitate the development of neoantigen-based cancer therapies [163, 171, 253]. In addition to these computerized methods for predicting immunogenic neoantigens and homologous TCRs based on high-throughput sequencing data, several T-cell antigen discovery strategies have recently been developed to unbiasedly identify immunogenic neoantigens. A variety of pMHC libraries have been established, including yeast display libraries, SABRs, BATLLES, etc., which allow the flexible and extensible screening of antigen epitopes. By relying on the physiological activity of T-cell killing rather than evaluating the binding affinity of TCR-pMHC, T-Scan is able to query a significantly larger antigen space independent of predictive algorithms [171, 229, 230, 572]. Therefore, the simplicity and scalability of T-cell ligand discovery techniques will facilitate study of the immunogenicity of candidate neoantigens and contribute to the development of new neoantigen-based immunotherapies.

Off-the-shelf precision immunotherapies targeting common neoantigens is another possible strategy to overcome the time and funding issues in individualized therapy based on personalized neoantigens. Common

neoantigens shared between patients will be produced by driver genes or TSGs with hot mutation polypeptides presented by relatively common HLA alleles. Common neoantigens are more likely to be clonally conserved in metastatic tumors and to reappear systematically in patients. Many general-purpose therapeutic techniques readily target common neoantigens, including vaccines, BSABs, CTLs, and adoptive metastasis of TCR-T cells. Subsequently, TCR libraries that specifically target shared neoantigens in an HLA-specific manner are developed for advanced cancer patients. As more common neoantigens and corresponding TCRs are discovered, more patients with frequent genetic changes that lead to cancer will benefit from the public neoantigen response TCR library. In addition, the widespread use of cancer genome sequencing and neoantigen prediction methods will help to match patients to treatments that target common neoantigens in their tumors [5, 573–575]. Therefore, this off-the-shelf strategy based on common neoantigens is expected to shorten the time required for neoantigen identification and widespread T-cell culture, increasing the use of neoantigen-based therapies in a significant proportion of patients.

In addition to neoantigens produced by spontaneous mutations during carcinogenesis, some covalent molecules can induce the production of tumor-specific public neoantigens by modifying hotspot residues in tumors with highly repetitive somatic mutations. Covalent KRAS-G12C inhibitors, such as ARS1620, irreversibly modify mutant cysteines. Hapten peptides carrying covalently linked small molecules can be presented on the cell surface via MHC-I. Hapten peptide-MHC complexes can act as tumor-specific neoantigens to trigger cytotoxic T-cell responses [89, 576, 577]. Based on this principle, mutant tumor suppressor proteins can be targeted by a new class of molecules. These molecules induce neoantigen production and trigger specific immune responses by covalently modifying hotspot residues, such as TP53 Y220C and TP53 R273C [578, 579]. Thus, the range of tumor-specific neoantigens suitable for therapeutic targeting can be significantly expanded by specifically modifying haptens of mutated cancer proteins rather than acting as drug inhibitors.

These neoantigens provide powerful targets for tumor vaccines that not only accurately eliminate residual tumor lesions but also effectively target distant metastatic cells due to their systemic properties. Personalized neoantigen vaccines are produced according to the individual tumor through the following steps: the collection of tumor tissue and normal samples, sequencing and analysis of unique mutations, prediction and validation of immunogenic neoantigens, and vaccine design and production. A variety of platforms, including peptides, nucleic acids and

DCS, can be used to develop vaccines based on predicted personalized or matched shared neoantigens. Neoantigen vaccines based on peptides, RNA and DNA are feasible, safe and economical vaccines [192, 292, 382, 386]. However, most patients cannot reliably produce a substantial neoantigen-specific CD8⁺ T-cell response by peptide-based neoantigen vaccines. All active components of tumor vaccines, such as neoantigens, formulations, and delivery systems, are constantly being improved. Synthetic self-amplified mRNAs (samRNAs) containing replicase genes encoding RNA-dependent RNA polymerase (RdRp) have attracted much attention due to their higher and longer-lasting antigenic expression than traditional mRNAs. In vivo expression of vaccine neoantigens can also be enhanced by vectors that naturally carry genetic instructions, including adenoviruses (Ads), retroviruses, and adeno-associated viruses (AAV). In addition, various nanoparticles are formed, such as lipid nanoparticles, exosomes, viral-like particles, cage protein nanoparticles, bacterial membrane material-based nanocarriers, high-density lipoprotein mimic nanodisks, polymers and polymer nanoparticles, to enhance transport and tissue penetration capabilities and improve the immunogenicity of personalized vaccines. Compared with viral vectors, nanoparticles can also effectively deliver vaccines and immune adjuvants together to lymphatic organs to increase neoantigen presentation [292, 382, 580].

Monocytes or hematopoietic progenitor cells isolated from blood loaded with tumor neoantigens in vitro can effectively improve the anticancer effect of neoantigen vaccines. Autologous DCs can be loaded with neoantigens in the form of peptides, RNA and DNA [382, 581]. Compared with the time-consuming and costly sequencing and computational analysis of patient-specific neoantigens, autologous dendritic cell (DC) vaccines with whole-tumor lysate (WTL) induce a T-cell-mediated antitumor response in vitro and are a more convenient and economical method to induce neoantigen-specific immune responses. The entire tumor cell contains both MANAs and nonmutated TAAs, which may overcome potential immune escape and resistance mechanisms. However, the higher abundance of nonimmunogenic autoantigens may limit the ability of neoantigens to induce immune responses. Various immunosuppressive factors also exist in WTL, which inhibit DC maturation and T-cell activation [582–585]. To overcome these problems, extracellular vesicles (EVs) produced by tumor cells have recently been shown to be a vaccination platform that supports DC maturation and neoantigen presentation. Tumor cell-derived EVs can deliver tumor antigen libraries to DCs and promote the cross-presentation of neoantigens. EVs also have high levels of immune stimulators, which can trigger DCs to release natural

immune signals. In addition to tumor cell-derived EVs, DC-derived EVs can also serve as neoantigen-presenting units for immune cells. EV-based vaccines can transform "cold" tumors into "hot" tumors by modulating the tumor immune microenvironment and systemic immune response [586–589]. Thus, EVs may provide an alternative form of neoantigen-based cancer vaccine that might be administered orally.

Although neoantigen-based immunotherapy has shown promising results in early preclinical and clinical studies, significant progress remains to be made, especially in patients with epithelial malignancies. Cancer cells have evolved built-in defenses to evade immune recognition at every stage of the cancer immune cycle. Given the complex mechanisms of immune escape in cancer, combination therapies that simultaneously target different stages of the cancer immune cycle may be more effective. Cell death following chemotherapy, radiotherapy, targeted therapy, photodynamic therapy, and oncolytic virus therapy will promote the production and release of neoantigens, thereby further enhancing the antitumor immune cycle [10, 590]. The use of IFN- α , GM-CSF, anti-CD40, TLR agonists, and STING agonists promoted neoantigen presentation [591–594]. To promote the infiltration of immune cells into the tumor, TME modifications and intracellular cytokines can be used. ICBs and IDO inhibitors will also alter immunosuppressive TMEs to enhance neoantigen-based immunotherapy [322, 378]. Nano and EV drug delivery systems have recently been used as an integrated platform for the simultaneous administration of many drugs or therapeutic drugs that work synergistically to activate different stages of the cancer-immune cycle, reverse immunosuppression and create immune support TMEs [595, 596]. These combination strategies using therapeutic agents with different mechanisms of action induce a strong, effective, durable, and tumor-specific immune response in cancer patients.

Conclusion

The viability of TCR-T cells in treating tumors has been demonstrated. At present, although there are still some problems in TCR-T-cell therapy, more attention should be given to the powerful clinical application prospects of this kind of therapy. With advances in tumor immunology and genetic engineering, TCR-T-cell therapy will become more individualized. At present, TCR-T cells are mainly focused on "universal targets" for treatment. When used for treatment, TCR-T cells only consider the expression of antigen and MHC molecules. When these two conditions are met, they are used for the treatment of multiple patients. However, the actual situation of these patients varies greatly, and the low selectivity of TCR-T-cell therapy may be related to poor or ineffective

treatment response. Studies have shown that the development of TCR-T cancer immunotherapy that targets certain tumors with intracellular antigens has become a research hotspot in recent years. If individual TCR-T cells can be designed according to the expression of patients' tumor-associated antigens or even neoantigens, it is possible to significantly improve the efficacy and safety of treatment. The maturation of sequencing technology and advances in cell culture technology have laid the foundation for this scenario. On the other hand, the use of under-differentiated T cells or hematopoietic stem cells to produce TCR-T cells with longer in vivo survival will also improve the effectiveness of such treatments. In addition, TCR-T cells can be used in combination with immune checkpoint blockers to improve the efficacy of TCR-T cell treatments. In summary, TCR-T cells will play an increasingly important role in tumor therapy, and their development will bring more hope to tumor patients.

Authors' contributions

Jiangping Li conceived and designed the review; Jiangping Li, Zhiwen Xiao, Donghui Wang, Lei Jia, Shihong Nie, Xingda Zeng and Wei Hu wrote and revised the manuscript; Jiangping Li provided funding and supervision. All authors read and approved the final manuscript.

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Availability of data and materials

All the data that support the findings of this study are available from the corresponding authors upon reasonable request.

Declarations

Ethics approval and consent to participate

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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References

1. Disis M, Guthrie KA, Liu Y, Coveler AL, Higgins DM, Childs JS, Dang Y, Salazar LG. Safety and outcomes of a plasmid DNA vaccine encoding the ERBB2 intracellular domain in patients with advanced-stage ERBB2-positive breast cancer: a phase 1 nonrandomized clinical trial. *JAMA Oncol.* 2023;9:71–8.
2. Gao T, Cen Q, Lei H. A review on development of MUC1-based cancer vaccine. *Biomed Pharmacother.* 2020;132:110888.
3. Ellingsen EB, Aamdal E, Guren T, Lilleby W, Brunsvig PF, Mangsbo SM, Aamdal S, Hovig E, Mensali N, Gaudernack G, Inderberg EM. Durable and dynamic hTERT immune responses following vaccination with the long-peptide cancer vaccine UV1: long-term follow-up of three phase I clinical trials. *J Immunother Cancer.* 2022;10(5):e004345.

4. Zhao Y, Baldin AV, Isayev O, Werner J, Zamyatin AJ, Bazhin AV. Cancer vaccines: antigen selection strategy. *Vaccines* (Basel). 2021;9(2):85.
5. Hegde PS, Chen DS. Top 10 challenges in cancer immunotherapy. *Immunity*. 2020;52:17–35.
6. Beatty GL, Gladney WL. Immune escape mechanisms as a guide for cancer immunotherapy. *Clin Cancer Res*. 2015;21:687–92.
7. Aktar N, Yueting C, Abbas M, Zafar H, Paiva-Santos AC, Zhang Q, Chen T, Ahmed M, Raza F, Zhou X. Understanding of immune escape mechanisms and advances in cancer immunotherapy. *J Oncol*. 2022;2022:8901326.
8. Shang S, Zhao Y, Qian K, Qin Y, Zhang X, Li T, Shan L, Wei M, Xi J, Tang B. The role of neoantigens in tumor immunotherapy. *Biomed Pharmacother*. 2022;151:113118.
9. Dong S, Guo X, Han F, He Z, Wang Y. Emerging role of natural products in cancer immunotherapy. *Acta Pharm Sin B*. 2022;12:1163–85.
10. Xie N, Shen G, Gao W, Huang Z, Huang C, Fu L. Neoantigens: promising targets for cancer therapy. *Signal Transduct Target Ther*. 2023;8:9.
11. De Mattos-Arruda L, Vazquez M, Finotello F, Lepore R, Porta E, Hundal J, Amengual-Rigo P, Ng C, Valencia A, Carrillo J, et al. Neoantigen prediction and computational perspectives towards clinical benefit: recommendations from the ESMO Precision Medicine Working Group. *Ann Oncol*. 2020;31:978–90.
12. Abi A, Safavi A. Targeted detection of single-nucleotide variations: progress and promise. *ACS Sens*. 2019;4:792–807.
13. Ma X, Liu Y, Liu Y, Alexandrov LB, Edmonson MN, Gawad C, Zhou X, Li Y, Rusch MC, Easton J, et al. Pan-cancer genome and transcriptome analyses of 1,699 paediatric leukaemias and solid tumours. *Nature*. 2018;555:371–6.
14. Paulsen T, Kumar P, Koseoglu MM, Dutta A. Discoveries of extrachromosomal circles of DNA in normal and tumor cells. *Trends Genet*. 2018;34:270–8.
15. Turajlic S, Litchfield K, Xu H, Rosenthal R, McGranahan N, Reading JL, Wong Y, Rowan A, Kanu N, Al BM, et al. Insertion-and-deletion-derived tumour-specific neoantigens and the immunogenic phenotype: a pan-cancer analysis. *Lancet Oncol*. 2017;18:1009–21.
16. Chen PJ, Hussmann JA, Yan J, Knipping F, Ravisankar P, Chen PF, Chen C, Nelson JW, Newby GA, Sahin M, et al. Enhanced prime editing systems by manipulating cellular determinants of editing outcomes. *Cell*. 2021;184:5635–52.
17. Ozcan D, Lade-Keller J, Tramm T. Can evaluation of mismatch repair defect and TILs increase the number of triple-negative breast cancer patients eligible for immunotherapy? *Pathol Res Pract*. 2021;226:153606.
18. Zurlo IV, Schino M, Strippoli A, Calegari MA, Cocomazzi A, Cassano A, Pozzo C, Di Salvatore M, Ricci R, Barone C, et al. Predictive value of NLR, TILs (CD4+/CD8+) and PD-L1 expression for prognosis and response to preoperative chemotherapy in gastric cancer. *Cancer Immunol Immunother*. 2022;71:45–55.
19. Williams DS, Mouradov D, Jorissen RN, Newman MR, Amini E, Nickless DK, Teague JA, Fang CG, Palmieri M, Parsons MJ, et al. Lymphocytic response to tumour and deficient DNA mismatch repair identify subtypes of stage II/III colorectal cancer associated with patient outcomes. *Gut*. 2019;68:465–74.
20. Raffone A, Travaglino A, Raimondo D, Boccellino MP, Maletta M, Borghese G, Casadio P, Insabato L, Mollo A, Zullo F, Seracchioli R. Tumor-infiltrating lymphocytes and POLE mutation in endometrial carcinoma. *Gynecol Oncol*. 2021;161:621–8.
21. Gebert J, Gelincik O, Oezcan-Wahlbrink M, Marshall JD, Hernandez-Sanchez A, Urban K, Long M, Cortes E, Tosti E, Katzenmaier EM, et al. Recurrent frameshift neoantigen vaccine elicits protective immunity with reduced tumor burden and improved overall survival in a lynch syndrome mouse model. *Gastroenterology*. 2021;161:1288–302.
22. Roudko V, Cimen BC, Greenbaum B, Lucas A, Samstein R, Bhardwaj N. Lynch syndrome and MSI-H cancers: from mechanisms to “Off-The-Shelf” cancer vaccines. *Front Immunol*. 2021;12:757804.
23. Budczies J, Allgauer M, Litchfield K, Rempel E, Christopoulos P, Kazdal D, Endris V, Thomas M, Frohling S, Peters S, et al. Optimizing panel-based tumor mutational burden (TMB) measurement. *Ann Oncol*. 2019;30:1496–506.
24. Govindan R, Ding L, Griffith M, Subramanian J, Dees ND, Kanchi KL, Maher CA, Fulton R, Fulton L, Wallis J, et al. Genomic landscape of non-small cell lung cancer in smokers and never-smokers. *Cell*. 2012;150:1121–34.
25. Shi Y, Lei Y, Liu L, Zhang S, Wang W, Zhao J, Zhao S, Dong X, Yao M, Wang K, Zhou Q. Integration of comprehensive genomic profiling, tumor mutational burden, and PD-L1 expression to identify novel biomarkers of immunotherapy in non-small cell lung cancer. *Cancer Med*. 2021;10:2216–31.
26. Zhang J, Mardis ER, Maher CA. INTEGRATE-neo: a pipeline for personalized gene fusion neoantigen discovery. *Bioinformatics*. 2017;33:555–7.
27. Rossari F, Minutolo F, Orciolo E. Past, present, and future of Bcr-Abl inhibitors: from chemical development to clinical efficacy. *J Hematol Oncol*. 2018;11:84.
28. Qi Y, Dong SS, He YL, Liu ZH, Huang YL, Wang N, Zhang Z, Li Z, Shi M, Feng X, et al. SYT-SSX1 enhances the invasiveness and maintains stem-like cell properties in synovial sarcoma via induction of TGF-beta1/Smad signaling. *BMC Cancer*. 2022;22:166.
29. Yang W, Lee KW, Srivastava RM, Kuo F, Krishna C, Chowell D, Makarov V, Hoen D, Dalin MG, Wexler L, et al. Immunogenic neoantigens derived from gene fusions stimulate T cell responses. *Nat Med*. 2019;25:767–75.
30. Frankiw L, Baltimore D, Li G. Alternative mRNA splicing in cancer immunotherapy. *Nat Rev Immunol*. 2019;19:675–87.
31. Wang L, Shamardani K, Babikir H, Catalan F, Nejo T, Chang S, Phillips JJ, Okada H, Diaz AA. The evolution of alternative splicing in glioblastoma under therapy. *Genome Biol*. 2021;22:48.
32. Wei Z, Zhou C, Zhang Z, Guan M, Zhang C, Liu Z, Liu Q. The landscape of tumor fusion neoantigens: a pan-cancer analysis. *iScience*. 2019;21:249–60.
33. Shen R, Seshan VE. FACETS: allele-specific copy number and clonal heterogeneity analysis tool for high-throughput DNA sequencing. *Nucleic Acids Res*. 2016;44:e131.
34. Wei M, Liu X, Cao C, Yang J, Lv Y, Huang J, Wang Y, Qin Y. An engineered PD-1-based and MMP-2/9-oriented fusion protein exerts potent antitumor effects against melanoma. *BMB Rep*. 2018;51:572–7.
35. Yang J, Annala M, Ji P, Wang G, Zheng H, Codgell D, Du X, Fang Z, Sun B, Nykter M, et al. Recurrent LRP1-SNRNP25 and KCNMB4-CCND3 fusion genes promote tumor cell motility in human osteosarcoma. *J Hematol Oncol*. 2014;7:76.
36. Clark RE, Dodi IA, Hill SC, Lill JR, Aubert G, Macintyre AR, Rojas J, Bourdon A, Bonner PL, Wang L, et al. Direct evidence that leukemic cells present HLA-associated immunogenic peptides derived from the BCR-ABL b3a2 fusion protein. *Blood*. 2001;98:2887–93.
37. Velaga R, Koo KM, Mainwaring PN. Harnessing gene fusion-derived neoantigens for “cold” breast and prostate tumor immunotherapy. *Immunotherapy*. 2022;14:1165–79.
38. Fotakis G, Rieder D, Haider M, Trajanoski Z, Finotello F. NeoFuse: predicting fusion neoantigens from RNA sequencing data. *Bioinformatics*. 2020;36:2260–1.
39. Kobelyatskaya AA, Pudova EA, Snezhkina AV, Fedorova MS, Pavlov VS, Guvatova ZG, Savvateeva MV, Melnikova NV, Dmitriev AA, Trofimov DY, et al. Impact TMPRSS2-ERG molecular subtype on prostate cancer recurrence. *Life* (Basel). 2021;11(6):588.
40. Wang Z, Wang Y, Zhang J, Hu Q, Zhi F, Zhang S, Mao D, Zhang Y, Liang H. Significance of the TMPRSS2:ERG gene fusion in prostate cancer. *Mol Med Rep*. 2017;16:5450–8.
41. de Almeida-Pinto YD, Costa S, de Andrade B, Altmani A, Vargas PA, Abreu LG, Fonseca FP. t(6;9)(MYB-NFIB) in head and neck adenoid cystic carcinoma: A systematic review with meta-analysis. *Oral Dis*. 2019;25:1277–82.
42. Srivastava RM, Purohit TA, Chan TA. Diverse neoantigens and the development of cancer therapies. *Semin Radiat Oncol*. 2020;30:113–28.
43. Camidge DR, Dziadziuszko R, Peters S, Mok T, Noe J, Nowicka M, Gadgeel SM, Cheema P, Pavlakis N, de Marinis F, et al. Updated efficacy and safety data and impact of the EML4-ALK fusion variant on the efficacy of Alectinib in untreated ALK-Positive advanced non-small cell lung cancer in the global phase III ALEX study. *J Thorac Oncol*. 2019;14:1233–43.
44. Lin JJ, Zhu VW, Yoda S, Yeap BY, Schrock AB, Dagogo-Jack I, Jessop NA, Jiang GY, Le LP, Gowen K, et al. Impact of EML4-ALK variant on resistance mechanisms and clinical outcomes in ALK-Positive lung cancer. *J Clin Oncol*. 2018;36:1199–206.

45. Ruangritchankul K, Sandison A. DEK::AFF2 fusion carcinomas of head and neck. *Adv Anat Pathol.* 2023;30:86–94.
46. Biernacki MA, Foster KA, Woodward KB, Coon ME, Cummings C, Cunningham TM, Dossa RG, Brault M, Stokke J, Olsen TM, et al. CBFβ-MYH11 fusion neoantigen enables T cell recognition and killing of acute myeloid leukemia. *J Clin Invest.* 2020;130:5127–41.
47. Kawaguchi S, Wada T, Ida K, Sato Y, Nagoya S, Tsukahara T, Kimura S, Sahara H, Ikeda H, Shimozawa K, et al. Phase I vaccination trial of SYT-SSX junction peptide in patients with disseminated synovial sarcoma. *J Transl Med.* 2005;3:1.
48. Sato Y, Nabeta Y, Tsukahara T, Hirohashi Y, Syunsui R, Maeda A, Sahara H, Ikeda H, Torigoe T, Ichimiya S, et al. Detection and induction of CTLs specific for SYT-SSX-derived peptides in HLA-A24(+) patients with synovial sarcoma. *J Immunol.* 2002;169:1611–8.
49. Frattini V, Pagnotta SM, Tala, Fan JJ, Russo MV, Lee SB, Garofano L, Zhang J, Shi P, Lewis G, et al. A metabolic function of FGFR3-TACC3 gene fusions in cancer. *Nature.* 2018;553:222–7.
50. Shields MD, Hicks JK, Boyle TA, Haura EB, Creelan BC. Selpercatinib overcomes CCDC6-RET-mediated resistance to Osimertinib. *J Thorac Oncol.* 2021;16:e15–7.
51. Kawakami S, Tsuma-Kaneko M, Sawanobori M, Uno T, Nakamura Y, Matsuzawa H, Suzuki R, Onizuka M, Yahata T, Naka K, et al. Pterostilbene downregulates BCR/ABL and induces apoptosis of T3151-mutated BCR/ABL-positive leukemic cells. *Sci Rep.* 2022;12:704.
52. Saito T, Nagai M, Ladanyi M. SYT-SSX1 and SYT-SSX2 interfere with repression of E-cadherin by snail and slug: a potential mechanism for aberrant mesenchymal to epithelial transition in human synovial sarcoma. *Cancer Res.* 2006;66:6919–27.
53. Gryder BE, Yohe ME, Chou HC, Zhang X, Marques J, Wachtel M, Schaefer B, Sen N, Song Y, Gualtieri A, et al. PAX3-FOXO1 establishes myogenic super enhancers and confers BET bromodomain vulnerability. *Cancer Discov.* 2017;7:884–99.
54. Gorthi A, Romero JC, Loranc E, Cao L, Lawrence LA, Goodale E, Iniguez AB, Bernard X, Masamsetti VP, Roston S, et al. EWS-FL11 increases transcription to cause R-loops and block BRCA1 repair in Ewing sarcoma. *Nature.* 2018;555:387–91.
55. Hadi K, Yao X, Behr JM, Deshpande A, Xanthopoulos C, Tian H, Kudman S, Rosiene J, Darmofal M, DeRose J, et al. Distinct classes of complex structural variation uncovered across thousands of cancer genome graphs. *Cell.* 2020;183:197–210.
56. Gawronski AR, Lin YY, McConeghy B, LeBihan S, Asghari H, Kockan C, Orabi B, Adra N, Pili R, Collins CC, et al. Structural variation and fusion detection using targeted sequencing data from circulating cell free DNA. *Nucleic Acids Res.* 2019;47:e38.
57. Knelson EH, Ivanova EV, Tarannum M, Campisi M, Lizotte PH, Booker MA, Ozgenc I, Noureddine M, Meisenheimer B, Chen M, et al. Activation of tumor-Cell STING primes NK-Cell therapy. *Cancer Immunol Res.* 2022;10:947–61.
58. Adusumilli PS, Zauderer MG, Riviere I, Solomon SB, Rusch VW, O’Cearbhaill RE, Zhu A, Cheema W, Chintala NK, Halton E, et al. A phase I trial of regional mesothelin-targeted CART-cell therapy in patients with malignant pleural disease, in combination with the Anti-PD-1 agent pembrolizumab. *Cancer Discov.* 2021;11:2748–63.
59. Fennell DA, Dulloo S, Harber J. Immunotherapy approaches for malignant pleural mesothelioma. *Nat Rev Clin Oncol.* 2022;19:573–84.
60. Zaccara S, Ries RJ, Jaffrey SR. Reading, writing and erasing mRNA methylation. *Nat Rev Mol Cell Biol.* 2019;20:608–24.
61. Damo M, Fitzgerald B, Lu Y, Nader M, William I, Cheung JF, Connolly KA, Foster GG, Akama-Garren E, Lee DY, et al. Inducible de novo expression of neoantigens in tumor cells and mice. *Nat Biotechnol.* 2021;39:64–73.
62. Kahles A, Lehmann KV, Toussaint NC, Huser M, Stark SG, Sachsensberg T, Stegle O, Kohlbacher O, Sander C, Ratsch G. Comprehensive analysis of alternative splicing across tumors from 8,705 patients. *Cancer Cell.* 2018;34:211–24.
63. Wang ET, Sandberg R, Luo S, Khrebukova I, Zhang L, Mayr C, Kingsmore SF, Schroth GP, Burge CB. Alternative isoform regulation in human tissue transcriptomes. *Nature.* 2008;456:470–6.
64. Rahman MA, Lin KT, Bradley RK, Abdel-Wahab O, Krainer AR. Recurrent SRSF2 mutations in MDS affect both splicing and NMD. *Genes Dev.* 2020;34:413–27.
65. Malcovati L, Stevenson K, Papaemmanuil E, Neuberger D, Bejar R, Boulwood J, Bowen DT, Campbell PJ, Ebert BL, Fenaux P, et al. SF3B1-mutant MDS as a distinct disease subtype: a proposal from the international working group for the prognosis of MDS. *Blood.* 2020;136:157–70.
66. Gill H, Leung AY, Kwong YL. Molecular and cellular mechanisms of myelodysplastic syndrome: implications on targeted therapy. *Int J Mol Sci.* 2016;17:440.
67. Supek F, Lehner B, Lindeboom R. To NMD or not To NMD: nonsense-mediated mRNA decay in cancer and other genetic diseases. *Trends Genet.* 2021;37:657–68.
68. Wang M, Zhang P, Zhu Y, Kong X, Zhang Z, Hu L. Assessing the activity of nonsense-mediated mRNA decay in lung cancer. *BMC Med Genomics.* 2017;10:55.
69. Polaski JT, Udy DB, Escobar-Hoyos LF, Askan G, Leach SD, Ventura A, Kannan R, Bradley RK. The origins and consequences of UPF1 variants in pancreatic adenocarcinoma. *Elife.* 2021;10:e62209.
70. Chen H, Yao J, Bao R, Dong Y, Zhang T, Du Y, Wang G, Ni D, Xun Z, Niu X, et al. Cross-talk of four types of RNA modification writers defines tumor microenvironment and pharmacogenomic landscape in colorectal cancer. *Mol Cancer.* 2021;20:29.
71. Zhou B, Bie F, Zang R, Zhang M, Song P, Liu L, Peng Y, Bai G, Zhao J, Gao S. RNA modification writer expression profiles predict clinical outcomes and guide neoadjuvant immunotherapy in non-small cell lung cancer. *EBioMedicine.* 2022;84:104268.
72. Zhou C, Wei Z, Zhang L, Yang Z, Liu Q. Systematically characterizing A-to-I RNA editing neoantigens in cancer. *Front Oncol.* 2020;10:593989.
73. Wilkes MC, Repellin CE, Sakamoto KM. Beyond mRNA: The role of non-coding RNAs in normal and aberrant hematopoiesis. *Mol Genet Metab.* 2017;122:28–38.
74. Diederichs S, Bartsch L, Berkmann JC, Frose K, Heitmann J, Hoppe C, Iggena D, Jazmati D, Karschnia P, Linsenmeier M, et al. The dark matter of the cancer genome: aberrations in regulatory elements, untranslated regions, splice sites, non-coding RNA and synonymous mutations. *EMBO Mol Med.* 2016;8:442–57.
75. Li H, Xiong HG, Xiao Y, Yang QC, Yang SC, Tang HC, Zhang WF, Sun ZJ. Long non-coding RNA LINC02195 as a regulator of MHC I molecules and favorable prognostic marker for head and neck squamous cell carcinoma. *Front Oncol.* 2020;10:615.
76. Laumont CM, Perreault C. Exploiting non-canonical translation to identify new targets for T cell-based cancer immunotherapy. *Cell Mol Life Sci.* 2018;75:607–21.
77. Gerstberger S, Hafner M, Tuschl T. A census of human RNA-binding proteins. *Nat Rev Genet.* 2014;15:829–45.
78. Gaston KW, Limbach PA. The identification and characterization of non-coding and coding RNAs and their modified nucleosides by mass spectrometry. *RNA Biol.* 2014;11:1568–85.
79. Giambruno R, Mihailovich M, Bonaldi T. Mass spectrometry-based proteomics to unveil the non-coding RNA World. *Front Mol Biosci.* 2018;5:90.
80. Chick JM, Munger SC, Simecek P, Huttlin EL, Choi K, Gatti DM, Raghupathy N, Svenson KL, Churchill GA, Gygi SP. Defining the consequences of genetic variation on a proteome-wide scale. *Nature.* 2016;534:500–5.
81. Lundberg E, Borner G. Spatial proteomics: a powerful discovery tool for cell biology. *Nat Rev Mol Cell Biol.* 2019;20:285–302.
82. De Bousser E, Meuris L, Callewaert N, Festjens N. Human T cell glycosylation and implications on immune therapy for cancer. *Hum Vaccin Immunother.* 2020;16:2374–88.
83. Han ZJ, Feng YH, Gu BH, Li YM, Chen H. The post-translational modification, SUMOylation, and cancer (Review). *Int J Oncol.* 2018;52:1081–94.
84. Fu C, Zhao H, Wang Y, Cai H, Xiao Y, Zeng Y, Chen H. Tumor-associated antigens: Tn antigen, sTn antigen, and T antigen. *HLA.* 2016;88:275–86.
85. Mohammed F, Cobbold M, Zarling AL, Salim M, Barrett-Wilt GA, Shabanowitz J, Hunt DF, Engelhard VH, Willcox BE. Phosphorylation-dependent interaction between antigenic peptides and MHC class I: a molecular basis for the presentation of transformed self. *Nat Immunol.* 2008;9:1236–43.
86. Zhang Z, Wang Y, Wang Y, Wang C, Shuai Y, Luo J, Liu R. BCAR3 promotes head and neck cancer growth and is associated with poor prognosis. *Cell Death Discov.* 2021;7:316.
87. Arras J, Thomas KS, Myers PJ, Cross AM, Osei AD, Vazquez GE, Atkins KA, Conaway MR, Jones MK, Lazzara MJ, Bouton AH. Breast Cancer

- Antiestrogen Resistance 3 (BCAR3) promotes tumor growth and progression in triple-negative breast cancer. *Am J Cancer Res.* 2021;11:4768–87.
88. Dao T, Mun SS, Molvi Z, Korontsvit T, Klatt MG, Khan AG, Nyakatura EK, Pohl MA, White TE, Balderes PJ, et al. A TCR mimic monoclonal antibody reactive with the “public” phospho-Neoantigen pIR52/HLA-A*02:01 complex. *JCI Insight.* 2022;7(5):e151624.
 89. Zhang Z, Rohweder PJ, Ongpipattanakul C, Basu K, Bohn MF, Dugan EJ, Steri V, Hann B, Shokat KM, Craik CS. A covalent inhibitor of K-Ras(G12C) induces MHC class I presentation of haptenated peptide neoepitopes targetable by immunotherapy. *Cancer Cell.* 2022;40:1060–9.
 90. Santambrogio L, Berendam SJ, Engelhard VH. The antigen processing and presentation machinery in lymphatic endothelial cells. *Front Immunol.* 2019;10:1033.
 91. Lehnert E, Tampe R. Structure and dynamics of antigenic peptides in complex with TAP. *Front Immunol.* 2017;8:10.
 92. Leick MB, Silva H, Scarfo I, Larson R, Choi BD, Bouffard AA, Gallagher K, Schmidts A, Bailey SR, Kann MC, et al. Non-cleavable hinge enhances avidity and expansion of CAR-T cells for acute myeloid leukemia. *Cancer Cell.* 2022;40:494–508.
 93. Amaro A, Gangemi R, Piaggio F, Angelini G, Barisione G, Ferrini S, Pfeffer U. The biology of uveal melanoma. *Cancer Metastasis Rev.* 2017;36:109–40.
 94. Marijt KA, Doorduijn EM, van Hall T. TEIPP antigens for T-cell based immunotherapy of immune-edited HLA class I(low) cancers. *Mol Immunol.* 2019;113:43–9.
 95. Marijt KA, Van Der Burg SH, van Hall T. TEIPP peptides: exploration of unTAPped cancer antigens. *Oncoimmunology.* 2019;8:1599639.
 96. Gigoux M, Wolchok J. Refusing to TAP out: 16 new human TEIPPs identified. *J Exp Med.* 2018;215:2233–4.
 97. Kiessling R. TAP-ing into TIEPPs for cancer immunotherapy. *J Clin Invest.* 2016;126:480–2.
 98. Hafstrand I, Doorduijn EM, Duru AD, Buratto J, Oliveira CC, Sandalova T, van Hall T, Achour A. The MHC class I cancer-associated neoepitope Trh4 linked with impaired peptide processing induces a unique noncanonical TCR conformer. *J Immunol.* 2016;196:2327–34.
 99. van Hall T, Wolpert EZ, van Veelen P, Laban S, van der Veer M, Roseboom M, Bres S, Grufman P, de Ru A, Meiring H, et al. Selective cytotoxic T-lymphocyte targeting of tumor immune escape variants. *Nat Med.* 2006;12:417–24.
 100. Caviezel C, Schuetz P, Gerdes S, Gambazzi F. Procalcitonin as preoperative marker for surgery in advanced parapneumonic empyema. *J Thorac Dis.* 2017;9:734–41.
 101. Qu SY, Zhang Y, Wu S, Wang MM, Liu LL, Yang XM, Shan HL, Yang JL, Chen P, Ti XY. Combined analysis of C-reactive protein in pleural fluid and serum is effective in the differential diagnosis of exudative pleural effusions. *Ann Transl Med.* 2021;9:1183.
 102. Pietropaolo V, Prezioso C, Moens U. Role of virus-induced host cell epigenetic changes in cancer. *Int J Mol Sci.* 2021;22(15):8346.
 103. Hu Z, Ma D. The precision prevention and therapy of HPV-related cervical cancer: new concepts and clinical implications. *Cancer Med.* 2018;7:5217–36.
 104. Chua M, Wee J, Hui EP, Chan A. Nasopharyngeal carcinoma. *Lancet.* 2016;387:1012–24.
 105. Shannon-Lowe C, Rickinson AB, Bell AI. Epstein-Barr virus-associated lymphomas. *Philos Trans R Soc Lond B Biol Sci.* 2017;372(1732):20160271.
 106. Farrell PJ. Epstein-Barr virus and cancer. *Annu Rev Pathol.* 2019;14:29–53.
 107. Marur S, D’Souza G, Westra WH, Forastiere AA. HPV-associated head and neck cancer: a virus-related cancer epidemic. *Lancet Oncol.* 2010;11:781–9.
 108. Ma R, Li Z, Chioccia EA, Caligiuri MA, Yu J. The emerging field of oncolytic virus-based cancer immunotherapy. *Trends Cancer.* 2023;9:122–39.
 109. Stevanovic S, Helman SR, Wunderlich JR, Langhan MM, Doran SL, Kwong M, Somerville R, Klebanoff CA, Kammula US, Sherry RM, et al. A Phase II study of tumor-infiltrating lymphocyte therapy for human papillomavirus-associated epithelial cancers. *Clin Cancer Res.* 2019;25:1486–93.
 110. Nagarsheth NB, Norberg SM, Sinkoe AL, Adhikary S, Meyer TJ, Lack JB, Warner AC, Schweitzer C, Doran SL, Korrapati S, et al. TCR-engineered T cells targeting E7 for patients with metastatic HPV-associated epithelial cancers. *Nat Med.* 2021;27:419–25.
 111. Jin BY, Campbell TE, Draper LM, Stevanovic S, Weissbrich B, Yu Z, Restifo NP, Rosenberg SA, Trimble CL, Hinrichs CS. Engineered T cells targeting E7 mediate regression of human papillomavirus cancers in a murine model. *JCI Insight.* 2018;3(8):e99488.
 112. Burki TK. TCR gene therapy for HPV-associated epithelial cancers. *Lancet Oncol.* 2019;20:e474.
 113. Miller NJ, Church CD, Dong L, Crispin D, Fitzgibbon MP, Lachance K, Jing L, Shinohara M, Gavvovidis I, Willimsky G, et al. Tumor-infiltrating merkel cell polyomavirus-specific T cells are diverse and associated with improved patient survival. *Cancer Immunol Res.* 2017;5:137–47.
 114. Zhang C, Tan Q, Li S, Shen L, Zhang J, Liu Y, Yang W, Lu Z. Induction of EBV latent membrane protein-2A (LMP2A)-specific T cells and construction of individualized TCR-engineered T cells for EBV-associated malignancies. *J Immunother Cancer.* 2021;9(7):e002516.
 115. Meng X, Sun X, Liu Z, He Y. A novel era of cancer/testis antigen in cancer immunotherapy. *Int Immunopharmacol.* 2021;98:107889.
 116. Thomas R, Al-Khadairi G, Roelands J, Hendrickx W, Dermime S, Bedognetti D, Decock J. NY-ESO-1 based immunotherapy of cancer: current perspectives. *Front Immunol.* 2018;9:947.
 117. Das B, Senapati S. Immunological and functional aspects of MAGEA3 cancer/testis antigen. *Adv Protein Chem Struct Biol.* 2021;125:121–47.
 118. Zhao J, Xu Z, Liu Y, Wang X, Liu X, Gao Y, Jin Y. The expression of cancer-testis antigen in ovarian cancer and the development of immunotherapy. *Am J Cancer Res.* 2022;12:681–94.
 119. Davari K, Holland T, Prassmayer L, Longinotti G, Ganley KP, Pechilis LJ, Diaconu I, Nambiar PR, Magee MS, Schendel DJ, et al. Development of a CD8 co-receptor independent T-cell receptor specific for tumor-associated antigen MAGE-A4 for next generation T-cell-based immunotherapy. *J Immunother Cancer.* 2021;9(3):e002035.
 120. Trefzer U, Hofmann M, Reinke S, Guo YJ, Audring H, Spagnoli G, Sterry W. Concordant loss of melanoma differentiation antigens in synchronous and asynchronous melanoma metastases: implications for immunotherapy. *Melanoma Res.* 2006;16:137–45.
 121. Takeuchi H, Kuo C, Morton DL, Wang HJ, Hoon DS. Expression of differentiation melanoma-associated antigen genes is associated with favorable disease outcome in advanced-stage melanomas. *Cancer Res.* 2003;63:441–8.
 122. Park J, Talukder AH, Lim SA, Kim K, Pan K, Melendez B, Bradley SD, Jackson KR, Khalili JS, Wang J, et al. SLC45A2: a melanoma antigen with high tumor selectivity and reduced potential for autoimmune toxicity. *Cancer Immunol Res.* 2017;5:618–29.
 123. Yao V, Parwani A, Maier C, Heston WD, Bacich DJ. Moderate expression of prostate-specific membrane antigen, a tissue differentiation antigen and folate hydrolase, facilitates prostate carcinogenesis. *Cancer Res.* 2008;68:9070–7.
 124. Middleton MR, McAlpine C, Woodcock VK, Corrie P, Infante JR, Steven NM, Evans T, Anthoney A, Shoushtari AN, Hamid O, et al. Tebentafusp, A TCR/Anti-CD3 bispecific fusion protein targeting gp100, potently activated antitumor immune responses in patients with metastatic melanoma. *Clin Cancer Res.* 2020;26:5869–78.
 125. Coccors M, Straetemans T, Govers C, Lamers C, Sleijfer S, Debets R. T cell receptor (TCR) gene therapy to treat melanoma: lessons from clinical and preclinical studies. *Expert Opin Biol Ther.* 2010;10:547–62.
 126. Neeffes J, Jongma ML, Paul P, Bakke O. Towards a systems understanding of MHC class I and MHC class II antigen presentation. *Nat Rev Immunol.* 2011;11:823–36.
 127. Pishesha N, Harmand TJ, Ploegh HL. A guide to antigen processing and presentation. *Nat Rev Immunol.* 2022;22:751–64.
 128. Lang GA, Shrestha B, Amadou AS, Shadid TM, Ballard JD, Lang ML. alpha-Galactosylceramide-Reactive NKT Cells Increase IgG1 Class Switch against a Clostridioides difficile Polysaccharide Antigen and Enhance Immunity against a Live Pathogen Challenge. *Infect Immun.* 2021;89:e43821.
 129. Shahine A. The intricacies of self-lipid antigen presentation by CD1b. *Mol Immunol.* 2018;104:27–36.
 130. Setliff I, Shiakolas AR, Pilewski KA, Murji AA, Mapengo RE, Janowska K, Richardson S, Oosthuysen C, Raju N, Ronsard L, et al. High-throughput mapping of B cell receptor sequences to antigen specificity. *Cell.* 2019;179:1636–46.

131. Lancaster EM, Jablons D, Kratz JR. Applications of next-generation sequencing in neoantigen prediction and cancer vaccine development. *Genet Test Mol Biomarkers*. 2020;24:59–66.
132. Xiao W, Ren L, Chen Z, Fang LT, Zhao Y, Lack J, Guan M, Zhu B, Jaeger E, Kerrigan L, et al. Toward best practice in cancer mutation detection with whole-genome and whole-exome sequencing. *Nat Biotechnol*. 2021;39:1141–50.
133. Newman S, Nakitandwe J, Kesserwan CA, Azzato EM, Wheeler DA, Rusch M, Shurtliff S, Hedges DJ, Hamilton KV, Foy SG, et al. Genomes for kids: the scope of pathogenic mutations in pediatric cancer revealed by comprehensive DNA and RNA sequencing. *Cancer Discov*. 2021;11:3008–27.
134. Chen G, Ning B, Shi T. Single-Cell RNA-Seq technologies and related computational data analysis. *Front Genet*. 2019;10:317.
135. Purcell AW, Ramarathinam SH, Ternette N. Mass spectrometry-based identification of MHC-bound peptides for immunopeptidomics. *Nat Protoc*. 2019;14:1687–707.
136. Bennett HM, Stephenson W, Rose CM, Darmanis S. Single-cell proteomics enabled by next-generation sequencing or mass spectrometry. *Nat Methods*. 2023;20:363–74.
137. Mayakonda A, Lin DC, Assenov Y, Plass C, Koeffler HP. Maftools: efficient and comprehensive analysis of somatic variants in cancer. *Genome Res*. 2018;28:1747–56.
138. Nik-Zainal S, Davies H, Staaf J, Ramakrishna M, Glodzik D, Zou X, Martincorena I, Alexandrov LB, Martin S, Wedge DC, et al. Landscape of somatic mutations in 560 breast cancer whole-genome sequences. *Nature*. 2016;534:47–54.
139. Profaizer T, Kumanovics A. Human leukocyte antigen typing by next-generation sequencing. *Clin Lab Med*. 2018;38:565–78.
140. Kim D, Paggi JM, Park C, Bennett C, Salzberg SL. Graph-based genome alignment and genotyping with HISAT2 and HISAT-genotype. *Nat Biotechnol*. 2019;37:907–15.
141. Abelin JG, Harjanto D, Malloy M, Suri P, Colson T, Goulding SP, Creech AL, Serrano LR, Nasir G, Nasrullah Y, et al. Defining HLA-II ligand processing and binding rules with mass spectrometry enhances cancer epitope prediction. *Immunity*. 2019;51:766–79.
142. Kiyotani K, Mai TH, Nakamura Y. Comparison of exome-based HLA class I genotyping tools: identification of platform-specific genotyping errors. *J Hum Genet*. 2017;62:397–405.
143. Ibrahim HS, Kafi SK. A computational vaccine designing approach for MERS-CoV infections. *Methods Mol Biol*. 2020;2131:39–145.
144. Gomez-Perosanz M, Ras-Carmona A, Reche PA. PCPS: a web server to predict proteasomal cleavage sites. *Methods Mol Biol*. 2020;2131:399–406.
145. Stranzl T, Larsen MV, Lundegaard C, Nielsen M. NetCTLpan: pan-specific MHC class I pathway epitope predictions. *Immunogenetics*. 2010;62:357–68.
146. Larsen MV, Lundegaard C, Lamberth K, Buus S, Lund O, Nielsen M. Large-scale validation of methods for cytotoxic T-lymphocyte epitope prediction. *BMC Bioinformatics*. 2007;8:424.
147. Jurtz V, Paul S, Andreatta M, Marcatili P, Peters B, Nielsen M. NetMHCpan-4.0: improved peptide-MHC class I interaction predictions integrating eluted ligand and peptide binding affinity data. *J Immunol*. 2017;199:3360–8.
148. O'Donnell TJ, Rubinsteyn A, Bonsack M, Riemer AB, Laserson U, Hammerbacher J. MHCflurry: open-source class I MHC binding affinity prediction. *Cell Syst*. 2018;7:129–32.
149. Jensen KK, Andreatta M, Marcatili P, Buus S, Greenbaum JA, Yan Z, Sette A, Peters B, Nielsen M. Improved methods for predicting peptide binding affinity to MHC class II molecules. *Immunology*. 2018;154:394–406.
150. Schuler MM, Nastke MD, Stevanovic S. SYFPEITHI: database for searching and T-cell epitope prediction. *Methods Mol Biol*. 2007;409:75–93.
151. Reche PA, Glutting JP, Zhang H, Reinherz EL. Enhancement to the RANK-PEP resource for the prediction of peptide binding to MHC molecules using profiles. *Immunogenetics*. 2004;56:405–19.
152. Zhang GL, DeLuca DS, Keskin DB, Chitkushev L, Zlateva T, Lund O, Reinherz EL, Brusci V. MULTIPRED2: a computational system for large-scale identification of peptides predicted to bind to HLA supertypes and alleles. *J Immunol Methods*. 2011;374:53–61.
153. Singh H, Raghava GP. ProPred: prediction of HLA-DR binding sites. *Bioinformatics*. 2001;17:1236–7.
154. Guan P, Doytchinova IA, Zygouri C, Flower DR. MHCpred: a server for quantitative prediction of peptide-MHC binding. *Nucleic Acids Res*. 2003;31:3621–4.
155. Lee MY, Jeon JW, Sievers C, Allen CT. Antigen processing and presentation in cancer immunotherapy. *J Immunother Cancer*. 2020;8(2):e001111.
156. Wang S, He Z, Wang X, Li H, Liu XS. Antigen presentation and tumor immunogenicity in cancer immunotherapy response prediction. *Elife*. 2019;8:e49020.
157. Kotsias F, Cebrian I, Alloati A. Antigen processing and presentation. *Int Rev Cell Mol Biol*. 2019;348:69–121.
158. Chong LC, Khan AM. Identification of highly conserved, serotype-specific dengue virus sequences: implications for vaccine design. *BMC Genomics*. 2019;20:921.
159. Glanville J, Huang H, Nau A, Hatton O, Wagar LE, Rubelt F, Ji X, Han A, Krams SM, Pettus C, et al. Identifying specificity groups in the T cell receptor repertoire. *Nature*. 2017;547:94–8.
160. Hayashi F, Isobe N, Glanville J, Matsushita T, Maimaitijiang G, Fukumoto S, Watanabe M, Masaki K, Kira JI. A new clustering method identifies multiple sclerosis-specific T-cell receptors. *Ann Clin Transl Neurol*. 2021;8:163–76.
161. Lu T, Zhang Z, Zhu J, Wang Y, Jiang P, Xiao X, Bernatchez C, Heymach JV, Gibbons DL, Wang J, et al. Deep learning-based prediction of the T cell receptor-antigen binding specificity. *Nat Mach Intell*. 2021;3:864–75.
162. Garcia-Garjito A, Fajardo CA, Gros A. Determinants for neoantigen identification. *Front Immunol*. 2019;10:1392.
163. Schumacher TN, Schreiber RD. Neoantigens in cancer immunotherapy. *Science*. 2015;348:69–74.
164. Okada S, Vaeteewoottacharn K, Kariya R. Application of highly immunocompromised mice for the establishment of Patient-Derived Xenograft (PDX) models. *Cells*. 2019;8(8):889.
165. Corsaro B, Yang TY, Murphy R, Sonderegger I, Exley A, Bertholet S, Dakappagari N, Dessy F, Garofolo F, Kierstead L, et al. 2020 white paper on recent issues in Bioanalysis: vaccine assay validation, qPCR assay validation, QC for CAR-T flow cytometry, NAb Assay harmonization and ELISpot validation (Part 3 - recommendations on immunogenicity assay strategies, NAb assays, biosimilars and FDA/EMA immunogenicity guidance/guideline, gene & cell therapy and vaccine assays). *Bioanalysis*. 2021;13:415–63.
166. Abdolahi S, Ghazvinian Z, Muhammadnejad S, Saleh M, Asadzadeh AH, Baghaei K. Patient-derived xenograft (PDX) models, applications and challenges in cancer research. *J Transl Med*. 2022;20:206.
167. Zanella ER, Grassi E, Trusolino L. Towards precision oncology with patient-derived xenografts. *Nat Rev Clin Oncol*. 2022;19:719–32.
168. Stronen E, Toebe M, Kelderman S, van Buuren MM, Yang W, van Rooij N, Donia M, Boschen ML, Lund-Johansen F, Olweus J, Schumacher TN. Targeting of cancer neoantigens with donor-derived T cell receptor repertoires. *Science*. 2016;352:1337–41.
169. Slota M, Lim JB, Dang Y, Disis ML. ELISpot for measuring human immune responses to vaccines. *Expert Rev Vaccines*. 2011;10:299–306.
170. Abdelaal HM, Cartwright EK, Skinner PJ. Detection of antigen-specific T cells using in situ MHC tetramer staining. *Int J Mol Sci*. 2019;20(20):5165.
171. Kula T, Dezfulian MH, Wang CI, Abdelfattah NS, Hartman ZC, Wucherpfennig KW, Lyerly HK, Elledge SJ. T-Scan: a genome-wide method for the systematic discovery of T cell epitopes. *Cell*. 2019;178:1016–28.
172. Parkhurst M, Gros A, Pasetto A, Prickett T, Crystal JS, Robbins P, Rosenberg SA. Isolation of T-Cell receptors specifically reactive with mutated tumor-associated antigens from tumor-infiltrating lymphocytes based on CD137 expression. *Clin Cancer Res*. 2017;23:2491–505.
173. Tu AA, Gierahn TM, Monian B, Morgan DM, Mehta NK, Ruitter B, Shreffler WG, Shalek AK, Love JC. TCR sequencing paired with massively parallel 3' RNA-seq reveals clonotypic T cell signatures. *Nat Immunol*. 2019;20:1692–9.
174. Schmidt J, Smith AR, Magnin M, Racle J, Devlin JR, Bobisse S, Cesbron J, Bonnet V, Carmona SJ, Huber F, et al. Prediction of neo-epitope immunogenicity reveals TCR recognition determinants and provides insight into immunoeediting. *Cell Rep Med*. 2021;2:100194.
175. Lowery FJ, Krishna S, Yossef R, Parikh NB, Chatani PD, Zacharakis N, Parkhurst MR, Levin N, Sindiri S, Sachs A, et al. Molecular signatures of antitumor neoantigen-reactive T cells from metastatic human cancers. *Science*. 2022;375:877–84.

176. He J, Xiong X, Yang H, Li D, Liu X, Li S, Liao S, Chen S, Wen X, Yu K, et al. Defined tumor antigen-specific T cells potentiate personalized TCR-T cell therapy and prediction of immunotherapy response. *Cell Res*. 2022;32:530–42.
177. Danilova L, Anagnostou V, Caushi JX, Sidhom JW, Guo H, Chan HY, Suri P, Tam A, Zhang J, Asmar ME, et al. The mutation-associated neoantigen functional expansion of specific T cells (MANAFEST) assay: a sensitive platform for monitoring antitumor immunity. *Cancer Immunol Res*. 2018;6:888–99.
178. Chan HY, Zhang J, Garliss CC, Kwaa AK, Blankson JN, Smith KN. A T cell receptor sequencing-based assay identifies cross-reactive recall CD8(+) T cell clonotypes against autologous HIV-1 epitope variants. *Front Immunol*. 2020;11:591.
179. Ojler AK, Nebdal D, Snapkov I, Olsen V, Kidman J, Greiff V, Chee J, Helland A. Dynamic changes in the T cell receptor repertoire during treatment with radiotherapy combined with an immune checkpoint inhibitor. *Mol Oncol*. 2021;15:2958–68.
180. Chen R, Qin S, Zhu H, Chang G, Li M, Lu H, Shen M, Gao Q, Lin X. Dynamic monitoring of circulating CD8(+) T and NK cell function in patients with septic shock. *Immunol Lett*. 2022;243:61–8.
181. Kumar BV, Connors TJ, Farber DL. Human T cell development, localization, and function throughout life. *Immunity*. 2018;48:202–13.
182. Seddon B, Yates AJ. The natural history of naive T cells from birth to maturity. *Immunol Rev*. 2018;285:218–32.
183. Shah DK, Zuniga-Pflucker JC. An overview of the intrathymic intricacies of T cell development. *J Immunol*. 2014;192:4017–23.
184. Le Gal FA, Widmer VM, Dutoit V, Rubio-Godoy V, Schrenzel J, Walker PR, Romero PJ, Valmori D, Speiser DE, Dietrich PY. Tissue homing and persistence of defined antigen-specific CD8+ tumor-reactive T-cell clones in long-term melanoma survivors. *J Invest Dermatol*. 2007;127:622–9.
185. Schumacher TN, Thommen DS. Tertiary lymphoid structures in cancer. *Science*. 2022;375: f9419.
186. Sautes-Fridman C, Pettiprez F, Calderaro J, Fridman WH. Tertiary lymphoid structures in the era of cancer immunotherapy. *Nat Rev Cancer*. 2019;19:307–25.
187. Neal JT, Li X, Zhu J, Giangarra V, Grzeskowiak CL, Ju J, Liu IH, Chiou SH, Salahudeen AA, Smith AR, et al. Organoid modeling of the tumor immune microenvironment. *Cell*. 2018;175:1972–88.
188. Krishna S, Lowery FJ, Copeland AR, Bahadiroglu E, Mukherjee R, Jia L, Anibal JT, Sachs A, Adebola SO, Gurusamy D, et al. Stem-like CD8 T cells mediate response of adoptive cell immunotherapy against human cancer. *Science*. 2020;370:1328–34.
189. Sim M, Lu J, Spencer M, Hopkins F, Tran E, Rosenberg SA, Long EO, Sun PD. High-affinity oligoclonal TCRs define effective adoptive T cell therapy targeting mutant KRAS-G12D. *Proc Natl Acad Sci U S A*. 2020;117:12826–35.
190. Sarnaik AA, Hamid O, Khushalani NI, Lewis KD, Medina T, Kluger HM, Thomas SS, Domingo-Musibay E, Pavlick AC, Whitman ED, et al. Lifileucel, a tumor-infiltrating lymphocyte therapy, in metastatic melanoma. *J Clin Oncol*. 2021;39:2656–66.
191. Chesney J, Lewis KD, Kluger H, Hamid O, Whitman E, Thomas S, Wermke M, Cusnir M, Domingo-Musibay E, Phan GQ, et al. Efficacy and safety of lifileucel, a one-time autologous tumor-infiltrating lymphocyte (TIL) cell therapy, in patients with advanced melanoma after progression on immune checkpoint inhibitors and targeted therapies: pooled analysis of consecutive cohorts of the C-144-01 study. *J Immunother Cancer*. 2022;10(12):e005755.
192. Liu S, Jiang Q, Zhao X, Zhao R, Wang Y, Wang Y, Liu J, Shang Y, Zhao S, Wu T, et al. A DNA nanodevice-based vaccine for cancer immunotherapy. *Nat Mater*. 2021;20:421–30.
193. Saif JM, Vadakekolathu J, Rane SS, McDonald D, Ahmad M, Mathieu M, Pockley AG, Durrant L, Metheringham R, Rees RC, McArdle SE. Novel prostate acid phosphatase-based peptide vaccination strategy induces antigen-specific T-cell responses and limits tumour growth in mice. *Eur J Immunol*. 2014;44:994–1004.
194. Berzofsky JA, Terabe M, Trepel JB, Pastan I, Stronck DF, Morris JC, Wood LV. Cancer vaccine strategies: translation from mice to human clinical trials. *Cancer Immunol Immunother*. 2018;67:1863–9.
195. Larsson M, Wilkens DT, Fonteneau JF, Beadle TJ, Merritt MJ, Kost RG, Haslett PA, Cu-Uvin S, Bhardwaj N, Nixon DF, Shacklett BL. Amplification of low-frequency antiviral CD8 T cell responses using autologous dendritic cells. *AIDS*. 2002;16:171–80.
196. Hammer MH, Brestrich G, Mittenzweig A, Roemhild A, Zwinger S, Subklewe M, Beier C, Kurtz A, Babel N, Volk HD, Reinke P. Generation of EBV-specific T cells for adoptive immunotherapy: a novel protocol using formalin-fixed stimulator cells to increase biosafety. *J Immunother*. 2007;30:817–24.
197. Carreno BM, Magrini V, Becker-Hapak M, Kaabinejadian S, Hundal J, Petti AA, Ly A, Lie WR, Hildebrand WH, Mardis ER, Linette GP. Cancer immunotherapy. A dendritic cell vaccine increases the breadth and diversity of melanoma neoantigen-specific T cells. *Science*. 2015;348:803–8.
198. Wei T, Leisegang M, Xia M, Kiyotani K, Li N, Zeng C, Deng C, Jiang J, Harada M, Agrawal N, et al. Generation of neoantigen-specific T cells for adoptive cell transfer for treating head and neck squamous cell carcinoma. *Oncoimmunology*. 2021;10:1929726.
199. Ichimiya S, Fujimura A, Masuda M, Masuda S, Yasumatsu R, Umabayashi M, Tanaka H, Koya N, Nakagawa S, Yew PY, et al. Contribution of pre-existing neoantigen-specific T cells to a durable complete response after tumor-pulsed dendritic cell vaccine plus nivolumab therapy in a patient with metastatic salivary duct carcinoma. *Immunol Invest*. 2022;51:1498–514.
200. Tiriveedhi V, Fleming TP, Goedegebuure PS, Naughton M, Ma C, Lockhart C, Gao F, Gillanders WE, Mohanakumar T. Mammaglobin-A cDNA vaccination of breast cancer patients induces antigen-specific cytotoxic CD4+ICOShi T cells. *Breast Cancer Res Treat*. 2013;138:109–18.
201. Painter MM, Mathew D, Goel RR, Apostolidis SA, Pattekar A, Kuthuru O, Baxter AE, Herati RS, Oldridge DA, Gouma S, et al. Rapid induction of antigen-specific CD4(+) T cells is associated with coordinated humoral and cellular immunity to SARS-CoV-2 mRNA vaccination. *Immunity*. 2021;54:2133–42.
202. Kramer KJ, Wilfong EM, Voss K, Barone SM, Shiakolas AR, Raju N, Roe CE, Suryadevara N, Walker LM, Wall SC, et al. Single-cell profiling of the antigen-specific response to BNT162b2 SARS-CoV-2 RNA vaccine. *Nat Commun*. 2022;13:3466.
203. Siddiqui I, Schaeuble K, Chennupati V, Fuentes MS, Calderon-Copete S, Pais FD, Carmona SJ, Scarpellino L, Gfeller D, Pradervand S, et al. Intratumoral Tcf1(+)PD-1(+)CD8(+) T cells with stem-like properties promote tumor control in response to vaccination and checkpoint blockade immunotherapy. *Immunity*. 2019;50:195–211.
204. Yost KE, Satpathy AT, Wells DK, Qi Y, Wang C, Kageyama R, McNamara KL, Granja JM, Sarin KY, Brown RA, et al. Clonal replacement of tumor-specific T cells following PD-1 blockade. *Nat Med*. 2019;25:1251–9.
205. Butler MO, Hirano N. Human cell-based artificial antigen-presenting cells for cancer immunotherapy. *Immunol Rev*. 2014;257:191–209.
206. Vidard L, Dureuil C, Baudhuin J, Vescovi L, Durand L, Sierra V, Parmantier E. CD137 (4-1BB) engagement fine-tunes synergistic IL-15- and IL-21-driven NK cell proliferation. *J Immunol*. 2019;203:676–85.
207. Xiao P, Wang J, Zhao Z, Liu X, Sun X, Wang D, Li Y. Engineering nanoscale artificial antigen-presenting cells by metabolic dendritic cell labeling to potentiate cancer immunotherapy. *Nano Lett*. 2021;21:2094–103.
208. Rhodes KR, Green JJ. Nanoscale artificial antigen presenting cells for cancer immunotherapy. *Mol Immunol*. 2018;98:13–8.
209. Huang J, Zeng X, Sigal N, Lund PJ, Su LF, Huang H, Chien YH, Davis MM. Detection, phenotyping, and quantification of antigen-specific T cells using a peptide-MHC dodecamer. *Proc Natl Acad Sci U S A*. 2016;113:E1890–7.
210. Melake MJ, Smith HG, Mansfield D, Davies E, Dillon MT, Wilkins AC, Patin EC, Pedersen M, Buus R, Melcher AA, et al. OX40 and 4-1BB delineate distinct immune profiles in sarcoma. *Oncoimmunology*. 2022;11:2066050.
211. Yang J, Lemas VM, Flinn IW, Krone C, Ambinder RF. Application of the ELISPOT assay to the characterization of CD8(+) responses to Epstein-Barr virus antigens. *Blood*. 2000;95:241–8.
212. Akache B, McCluskie MJ. The quantification of antigen-specific T cells by IFN-gamma ELISPOT. *Methods Mol Biol*. 2021;2183:525–36.
213. Gong X, Karchin R. Pan-cancer HLA gene-mediated tumor immunogenicity and immune evasion. *Mol Cancer Res*. 2022;20:1272–83.
214. Teku GN, Vihinen M. Pan-cancer analysis of neoepitopes. *Sci Rep*. 2018;8:12735.

215. Vita R, Mahajan S, Overton JA, Dhanda SK, Martini S, Cantrell JR, Wheeler DK, Sette A, Peters B. The Immune Epitope Database (IEDB): 2018 update. *Nucleic Acids Res.* 2019;47:D339–43.
216. Reynisson B, Alvarez B, Paul S, Peters B, Nielsen M. NetMHCpan-4.1 and NetMHCIIpan-4.0: improved predictions of MHC antigen presentation by concurrent motif deconvolution and integration of MS MHC eluted ligand data. *Nucleic Acids Res.* 2020;48:W449–54.
217. Sarkizova S, Klaeger S, Le PM, Li LW, Oliveira G, Keshishian H, Hartigan CR, Zhang W, Braun DA, Ligon KL, et al. A large peptidome dataset improves HLA class I epitope prediction across most of the human population. *Nat Biotechnol.* 2020;38:199–209.
218. Kalaora S, Samuëls Y. Cancer exome-based identification of tumor neo-antigens using mass spectrometry. *Methods Mol Biol.* 2019;1884:203–14.
219. Blatnik R, Mohan N, Bonsack M, Falkenby LG, Hoppe S, Josef K, Steinbach A, Becker S, Nadler WM, Rucevic M, et al. A targeted LC-MS strategy for low-abundant HLA class-I-presented peptide detection identifies novel human papillomavirus T-Cell epitopes. *Proteomics.* 2018;18:e1700390.
220. Wang Z, Cheng G, Li G. TCR ligand discovery via T-Scan. *Trends Immunol.* 2019;40:1075–7.
221. Hamieh M, Dobrin A, Cabriolu A, van der Stegen S, Giavridis T, Mansilla-Soto J, Eyquem J, Zhao Z, Whitlock BM, Miele MM, et al. CAR T cell trogocytosis and cooperative killing regulate tumour antigen escape. *Nature.* 2019;568:112–6.
222. Li G, Bethune MT, Wong S, Joglekar AV, Leonard MT, Wang JK, Kim JT, Cheng D, Peng S, Zaretsky JM, et al. T cell antigen discovery via trogocytosis. *Nat Methods.* 2019;16:183–90.
223. Janson C, Menzies-Gow A, Nan C, Nuevo J, Papi A, Quint JK, Quirce S, Vogelmeier CF. SABINA: an overview of short-acting beta(2)-agonist use in asthma in European Countries. *Adv Ther.* 2020;37:1124–35.
224. Vahatalo I, Lehtimäki L, Tuomisto LE, Karjalainen J, Niemela O, Ilmarinen P, Kankaanranta H. Long-term use of short-acting beta(2)-agonists in patients with adult-onset asthma. *J Allergy Clin Immunol Pract.* 2022;10:2074–83.
225. Cohly HH, Morrison DR, Atassi MZ. Antigen presentation by non-immune B-cell hybridoma clones: presentation of synthetic antigenic sites reveals clones that exhibit no specificity and clones that present only one epitope. *Immunol Invest.* 1989;18:987–92.
226. Shafer P, Kelly LM, Hoyos V. Cancer therapy with TCR-engineered t cells: current strategies, challenges, and prospects. *Front Immunol.* 2022;13:835762.
227. Schirmer D, Grunewald TG, Klar R, Schmidt O, Wohlleber D, Rubio RA, Uckert W, Thiel U, Bohne F, Busch DH, et al. Transgenic antigen-specific, HLA-A*02:01-allele-restricted cytotoxic T cells recognize tumor-associated target antigen STEAP1 with high specificity. *Oncoimmunology.* 2016;5:e1175795.
228. Liu X, Xu Y, Xiong W, Yin B, Huang Y, Chu J, Xing C, Qian C, Du Y, Duan T, et al. Development of a TCR-like antibody and chimeric antigen receptor against NY-ESO-1/HLA-A2 for cancer immunotherapy. *J Immunother Cancer.* 2022;10(3):e004035.
229. Huisman BD, Balivada PA, Birnbaum ME. Yeast display platform with expression of linear peptide epitopes for high-throughput assessment of peptide-MHC-II binding. *J Biol Chem.* 2023;299:102913.
230. Gera N, Hussain M, Rao BM. Protein selection using yeast surface display. *Methods.* 2013;60:15–26.
231. Paria BC, Levin N, Lowery FJ, Pasetto A, Deniger DC, Parkhurst MR, Yossef R, Kim SP, Florentin M, Ngo LT, et al. Rapid identification and evaluation of neoantigen-reactive T-Cell receptors from single cells. *J Immunother.* 2021;44:1–8.
232. Kuball J, Dossett ML, Wolf M, Ho WY, Voss RH, Fowler C, Greenberg PD. Facilitating matched pairing and expression of TCR chains introduced into human T cells. *Blood.* 2007;109:2331–8.
233. Kim SM, Bhonsle L, Besgen P, Nickel J, Backes A, Held K, Vollmer S, Dornmair K, Prinz JC. Analysis of the paired TCR alpha- and beta-chains of single human T cells. *PLoS ONE.* 2012;7:e37338.
234. Lu YC, Zheng Z, Lowery FJ, Gartner JJ, Prickett TD, Robbins PF, Rosenberg SA. Direct identification of neoantigen-specific TCRs from tumor specimens by high-throughput single-cell sequencing. *J Immunother Cancer.* 2021;9(7):e002595.
235. Leidner R, Sanjuan SN, Huang H, Sprott D, Zheng C, Shih YP, Leung A, Payne R, Sutcliffe K, Cramer J, et al. Neoantigen T-Cell receptor gene therapy in pancreatic cancer. *N Engl J Med.* 2022;386:2112–9.
236. Gao S, Wu Z, Arnold B, Diamond C, Batchu S, Giudice V, Alemu L, Raffo DQ, Feng X, Kajigaya S, et al. Single-cell RNA sequencing coupled to TCR profiling of large granular lymphocyte leukemia T cells. *Nat Commun.* 1982;2022:13.
237. Hanada KI, Zhao C, Gil-Hoyos R, Gartner JJ, Chow-Parmer C, Lowery FJ, Krishna S, Prickett TD, Kivitz S, Parkhurst MR, et al. A phenotypic signature that identifies neoantigen-reactive T cells in fresh human lung cancers. *Cancer Cell.* 2022;40:479–93.
238. Yoshida M, Worlock KB, Huang N, Lindeboom R, Butler CR, Kumasaka N, Dominguez CC, Mamanova L, Bolt L, Richardson L, et al. Local and systemic responses to SARS-CoV-2 infection in children and adults. *Nature.* 2022;602:321–7.
239. Cao Y, Yisimayi A, Jian F, Song W, Xiao T, Wang L, Du S, Wang J, Li Q, Chen X, et al. BA.2.12.1, BA.4 and BA.5 escape antibodies elicited by Omicron infection. *Nature.* 2022;608:593–602.
240. Lank SM, Golbach BA, Creager HM, Wiseman RW, Keskin DB, Reinherz EL, Brusic V, O'Connor DH. Ultra-high resolution HLA genotyping and allele discovery by highly multiplexed cDNA amplicon pyrosequencing. *BMC Genomics.* 2012;13:378.
241. Cole C, Byrne A, Adams M, Volden R, Vollmers C. Complete characterization of the human immune cell transcriptome using accurate full-length cDNA sequencing. *Genome Res.* 2020;30:589–601.
242. Cao Y, Su B, Guo X, Sun W, Deng Y, Bao L, Zhu Q, Zhang X, Zheng Y, Geng C, et al. Potent neutralizing antibodies against SARS-CoV-2 identified by high-throughput single-cell sequencing of convalescent patients' B cells. *Cell.* 2020;182:73–84.
243. Dominguez CC, Xu C, Jarvis LB, Rainbow DB, Wells SB, Gomes T, Howlett SK, Suchanek O, Polanski K, King HW, et al. Cross-tissue immune cell analysis reveals tissue-specific features in humans. *Science.* 2022;376:15197.
244. Gros A, Robbins PF, Yao X, Li YF, Turcotte S, Tran E, Wunderlich JR, Mixon A, Farid S, Dudley ME, et al. PD-1 identifies the patient-specific CD8(+) tumor-reactive repertoire infiltrating human tumors. *J Clin Invest.* 2014;124:2246–59.
245. Gros A, Tran E, Parkhurst MR, Ilyas S, Pasetto A, Groh EM, Robbins PF, Yossef R, Garcia-Garjito A, Fajardo CA, et al. Recognition of human gastrointestinal cancer neoantigens by circulating PD-1+ lymphocytes. *J Clin Invest.* 2019;129:4992–5004.
246. Trub M, Uhlenbrock F, Claus C, Herzog P, Thelen M, Karanikas V, Bacac M, Amann M, Albrecht R, Ferrara-Koller C, et al. Fibroblast activation protein-targeted 4-1BB ligand agonist amplifies effector functions of intratumoral T cells in human cancer. *J Immunother Cancer.* 2020;8(2):e000238.
247. Hiltbold EM, Vlad AM, Ciborowski P, Watkins SC, Finn OJ. The mechanism of unresponsiveness to circulating tumor antigen MUC1 is a block in intracellular sorting and processing by dendritic cells. *J Immunol.* 2000;165:3730–41.
248. Hall CA, Kravitz RH, Johnson KF, Sanek NA, Maiti P, Ziemba KR, Liu J, Andreev DO, Chrostowski VL, Collins IJ, Bleck GT. Shortening the biologicals clinical timeline with a novel method for generating stable, high-producing cell pools and clones. *Biotechnol Bioeng.* 2022;120:1746–61.
249. Bunse L, Green EW, Platten M. High-throughput discovery of cancer-targeting TCRs. *Methods Enzymol.* 2019;629:401–17.
250. Wheeler MA, Clark IC, Lee HG, Li Z, Linnerbauer M, Rone JM, Blain M, Akl CF, Piester G, Giovannoni F, et al. Droplet-based forward genetic screening of astrocyte-microglia cross-talk. *Science.* 2023;379:1023–30.
251. Shono M, Ito R, Fujita F, Sakuta H, Yoshikawa K. Emergence of uniform linearly-arranged micro-droplets entrapping DNA and living cells through water/water phase-separation. *Sci Rep.* 2021;11:23570.
252. Shalem O, Sanjana NE, Zhang F. High-throughput functional genomics using CRISPR-Cas9. *Nat Rev Genet.* 2015;16:299–311.
253. Linnemann C, Heemskerk B, Kvistborg P, Kluijn RJ, Bolotin DA, Chen X, Bresser K, Nieuwland M, Schotte R, Michels S, et al. High-throughput identification of antigen-specific TCRs by TCR gene capture. *Nat Med.* 2013;19:1534–41.
254. Lossius A, Johansen JN, Vartdal F, Robins H, Jurate SB, Holmoy T, Olweus J. High-throughput sequencing of TCR repertoires in multiple sclerosis

- reveals intrathecal enrichment of EBV-reactive CD8+ T cells. *Eur J Immunol.* 2014;44:3439–52.
255. Leon E, Ranganathan R, Savoldo B. Adoptive T cell therapy: boosting the immune system to fight cancer. *Semin Immunol.* 2020;49:101437.
 256. Chan JD, Lai J, Slaney CY, Kallies A, Beavis PA, Darcy PK. Cellular networks controlling T cell persistence in adoptive cell therapy. *Nat Rev Immunol.* 2021;21:769–84.
 257. Met O, Jensen KM, Chamberlain CA, Donia M, Svane IM. Principles of adoptive T cell therapy in cancer. *Semin Immunopathol.* 2019;41:49–58.
 258. Majzner RG, Mackall CL. Tumor antigen escape from CAR T-cell therapy. *Cancer Discov.* 2018;8:1219–26.
 259. Wei J, Guo Y, Wang Y, Wu Z, Bo J, Zhang B, Zhu J, Han W. Clinical development of CAR T cell therapy in China: 2020 update. *Cell Mol Immunol.* 2021;18:792–804.
 260. Xu R, Du S, Zhu J, Meng F, Liu B. Neoantigen-targeted TCR-T cell therapy for solid tumors: how far from clinical application. *Cancer Lett.* 2022;546:215840.
 261. Baulu E, Gardet C, Chuvain N, Depil S. TCR-engineered T cell therapy in solid tumors: state of the art and perspectives. *Sci Adv.* 2023;9:f3700.
 262. Wachsmann T, Wouters AK, Remst D, Hagedoorn RS, Meeuwssen MH, van Diest E, Leusen J, Kuball J, Falkenburg J, Heemskerk M. Comparing CAR and TCR engineered T cell performance as a function of tumor cell exposure. *Oncoimmunology.* 2022;11:2033528.
 263. Ishihara M, Kitano S, Kageyama S, Miyahara Y, Yamamoto N, Kato H, Mishima H, Hattori H, Funakoshi T, Kojima T, et al. NY-ESO-1-specific redirected T cells with endogenous TCR knockdown mediate tumor response and cytokine release syndrome. *J Immunother Cancer.* 2022;10(6):e003811.
 264. van Balen P, Jedema I, van Loenen MM, de Boer R, van Egmond HM, Hagedoorn RS, Hoogstaten C, Veld S, Hageman L, van Liempt P, et al. HA-1H T-cell receptor gene transfer to redirect virus-specific T cells for treatment of hematological malignancies after allogeneic stem cell transplantation: a phase 1 clinical study. *Front Immunol.* 1804;2020:11.
 265. Hafezi M, Lin M, Chia A, Chua A, Ho ZZ, Fam R, Tan D, Aw J, Pavesi A, Krishnamoorthy TL, et al. Immunosuppressive drug-resistant armored T-cell receptor T cells for immune therapy of HCC in liver transplant patients. *Hepatology.* 2021;74:200–13.
 266. Gannon PO, Wieckowski S, Baumgaertner P, Hebeisen M, Allard M, Speiser DE, Rufer N. Quantitative TCR:pMHC dissociation rate assessment by NTAmers reveals antineoplastic T cell repertoires enriched for high functional competence. *J Immunol.* 2015;195:356–66.
 267. Rockinger GA, Guillaume P, Cachot A, Saillard M, Speiser DE, Coukos G, Harari A, Romero PJ, Schmidt J, Jandus C. Optimized combinatorial pMHC class II multimer labeling for precision immune monitoring of tumor-specific CD4 T cells in patients. *J Immunother Cancer.* 2020;8(1):e000435.
 268. Wang Z, Cao YJ. Adoptive cell therapy targeting neoantigens: a frontier for cancer research. *Front Immunol.* 2020;11:176.
 269. Zhao X, Pan X, Wang Y, Zhang Y. Targeting neoantigens for cancer immunotherapy. *Biomark Res.* 2021;9:61.
 270. Zheng Y, Fu Y, Wang PP, Ding ZY. Neoantigen: a promising target for the immunotherapy of colorectal cancer. *Dis Markers.* 2022;2022:8270305.
 271. Gupta RG, Li F, Roszik J, Lizee G. Exploiting tumor neoantigens to target cancer evolution: current challenges and promising therapeutic approaches. *Cancer Discov.* 2021;11:1024–39.
 272. Lybaert L, Lefever S, Fant B, Smits E, De Geest B, Breckpot K, Dirix L, Feldman SA, van Criekinge W, Thielemans K, et al. Challenges in neoantigen-directed therapeutics. *Cancer Cell.* 2023;41:15–40.
 273. Jhunjhunwala S, Hammer C, Delamarre L. Antigen presentation in cancer: insights into tumour immunogenicity and immune evasion. *Nat Rev Cancer.* 2021;21:298–312.
 274. Truong HV, Sgourakis NG. Dynamics of MHC-I molecules in the antigen processing and presentation pathway. *Curr Opin Immunol.* 2021;70:122–8.
 275. Kristensen NP, Heeke C, Tvingsholm SA, Borch A, Draghi A, Crowther MD, Carri I, Munk KK, Holm JS, Bjerregaard AM, et al. Neoantigen-reactive CD8+ T cells affect clinical outcome of adoptive cell therapy with tumor-infiltrating lymphocytes in melanoma. *J Clin Invest.* 2022;132(2):e150535.
 276. Qi Y, Zhao M, Hu Y, Wang Y, Li P, Cao J, Shi M, Tan J, Zhang M, Xiao X, et al. Efficacy and safety of CD19-specific CAR T cell-based therapy in B-cell acute lymphoblastic leukemia patients with CNSL. *Blood.* 2022;139:3376–86.
 277. Zhang J, Hu Y, Yang J, Li W, Zhang M, Wang Q, Zhang L, Wei G, Tian Y, Zhao K, et al. Non-viral, specifically targeted CAR-T cells achieve high safety and efficacy in B-NHL. *Nature.* 2022;609:369–74.
 278. Lu Y, Xue J, Deng T, Zhou X, Yu K, Deng L, Huang M, Yi X, Liang M, Wang Y, et al. Safety and feasibility of CRISPR-edited T cells in patients with refractory non-small-cell lung cancer. *Nat Med.* 2020;26:732–40.
 279. Song Q, Yang B, Sheng W, Zhou Z, Zhang T, Qin B, Ji L, Li P, Wang D, Zhang X, et al. Safety and efficacy of mutant neoantigen-specific T-cell treatment combined anti-PD-1 therapy in stage IV solid tumors. *Immunotherapy.* 2022;14:553–65.
 280. Duhon R, Fesneau O, Samson KA, Frye AK, Beymer M, Rajamanickam V, Ross D, Tran E, Bernard B, Weinberg AD, Duhon T. PD-1 and ICOS coexpression identifies tumor-reactive CD4+ T cells in human solid tumors. *J Clin Invest.* 2022;132(12):e156821.
 281. Liu B, Zhang Y, Wang D, Hu X, Zhang Z. Single-cell meta-analyses reveal responses of tumor-reactive CXCL13(+) T cells to immune-checkpoint blockade. *Nat Cancer.* 2022;3:1123–36.
 282. Veatch JR, Lee SM, Shasha C, Singhi N, Szeto JL, Moshiri AS, Kim TS, Smythe K, Kong P, Fitzgibbon M, et al. Neoantigen-specific CD4(+) T cells in human melanoma have diverse differentiation states and correlate with CD8(+) T cell, macrophage, and B cell function. *Cancer Cell.* 2022;40:393–409.
 283. Moesta AK, Li XY, Smyth MJ. Targeting CD39 in cancer. *Nat Rev Immunol.* 2020;20:739–55.
 284. Dixon KO, Tabaka M, Schramm MA, Xiao S, Tang R, Dionne D, Anderson AC, Rozenblatt-Rosen O, Regev A, Kuchroo VK. TIM-3 restrains anti-tumour immunity by regulating inflammasome activation. *Nature.* 2021;595:101–6.
 285. Buchan SL, Rogel A, Al-Shamkhani A. The immunobiology of CD27 and OX40 and their potential as targets for cancer immunotherapy. *Blood.* 2018;131:39–48.
 286. Muik A, Garralda E, Altintas I, Gieseke F, Geva R, Ben-Ami E, Maurice-Dror C, Calvo E, LoRusso PM, Alonso G, et al. Preclinical characterization and phase I trial results of a bispecific antibody targeting PD-L1 and 4–1BB (GEN1046) in patients with advanced refractory solid tumors. *Cancer Discov.* 2022;12:1248–65.
 287. Jiao X, Nawab O, Patel T, Kossenkov AV, Halama N, Jaeger D, Pestell RG. Recent advances targeting CCR5 for cancer and its role in immunology. *Cancer Res.* 2019;79:4801–7.
 288. Stadler JC, Belloum Y, Deitert B, Sementsov M, Heidrich I, Gebhardt C, Keller L, Pantel K. Current and future clinical applications of ctDNA in immuno-oncology. *Cancer Res.* 2022;82:349–58.
 289. Abbosh C, Birkbak NJ, Wilson GA, Jamal-Hanjani M, Constantin T, Salari R, Le Quesne J, Moore DA, Veeriah S, Rosenthal R, et al. Phylogenetic ctDNA analysis depicts early-stage lung cancer evolution. *Nature.* 2017;545:446–51.
 290. Chemi F, Pearce SP, Clipson A, Hill SM, Conway AM, Richardson SA, Kamieniecka K, Caesar R, White DJ, Mohan S, et al. cfDNA methylome profiling for detection and subtyping of small cell lung cancers. *Nat Cancer.* 2022;3:1260–70.
 291. Romero D. A cfDNA-based approach is feasible. *Nat Rev Clin Oncol.* 2021;18:603.
 292. Blass E, Ott PA. Advances in the development of personalized neoantigen-based therapeutic cancer vaccines. *Nat Rev Clin Oncol.* 2021;18:215–29.
 293. Hernandez R, Poder J, LaPorte KM, Malek TR. Engineering IL-2 for immunotherapy of autoimmunity and cancer. *Nat Rev Immunol.* 2022;22:614–28.
 294. Baek AE. Improving IL-2 for T cell therapy. *Sci Signal.* 2021;14:m6438.
 295. Moschos SJ, Eroglu Z, Khushalani NI, Kendra KL, Anstas G, In GK, Wang P, Liu G, Collichio FA, Google PB, et al. Targeting the IL-2 inducible kinase in melanoma; a phase 2 study of ibrutinib in systemic treatment-refractory distant metastatic cutaneous melanoma: preclinical rationale, biology, and clinical activity (NCI9922). *Melanoma Res.* 2021;31:162–72.
 296. Thoma C. Kidney cancer: combination of HDAC inhibitor with IL-2 promising. *Nat Rev Urol.* 2017;14:639.
 297. Barata JT, Durum SK, Seddon B. Flip the coin: IL-7 and IL-7R in health and disease. *Nat Immunol.* 2019;20:1584–93.

298. Lin J, Zhu Z, Xiao H, Wakefield MR, Ding VA, Bai Q, Fang Y. The role of IL-7 in immunity and cancer. *Anticancer Res.* 2017;37:963–7.
299. Garris CS, Arlauckas SP, Kohler RH, Trefny MP, Garren S, Piot C, Engblom C, Pfirschke C, Siwicki M, Gungabeesoon J, et al. Successful Anti-PD-1 cancer immunotherapy requires T cell-dendritic cell crosstalk involving the cytokines IFN-gamma and IL-12. *Immunity.* 2018;49:1148–61.
300. Alsaieedi A, Holler A, Velica P, Bendle G, Stauss HJ. Safety and efficacy of Tet-regulated IL-12 expression in cancer-specific T cells. *Oncoimmunology.* 2019;8:1542917.
301. Becker-Hapak MK, Shrestha N, McClain E, Dee MJ, Chaturvedi P, Leclerc GM, Marsala LI, Foster M, Schappe T, Tran J, et al. A fusion protein complex that combines IL-12, IL-15, and IL-18 signaling to induce memory-like NK cells for cancer immunotherapy. *Cancer Immunol Res.* 2021;9:1071–87.
302. Mierzejewska J, Wegierek-Ciura K, Rossowska J, Szczygiel A, Anger-Gora N, Szermer-Olearnik B, Geneja M, Pajtasz-Piasecka E. The beneficial effect of IL-12 and IL-18 transduced dendritic cells stimulated with tumor antigens on generation of an antitumor response in a mouse colon carcinoma model. *J Immunol Res.* 2022;2022:7508928.
303. Huang Y, Li D, Zhang PF, Liu M, Liang X, Yang X, Jiang L, Zhang LF, Zhou WL, Su JH, et al. IL-18R-dependent and independent pathways account for IL-18-enhanced antitumor ability of CAR-T cells. *FASEB J.* 2020;34:1768–82.
304. Jaspers JE, Khan JF, Godfrey WD, Lopez AV, Ciampricotti M, Rudin CM, Brentjens RJ. IL-18-secreting CART cells targeting DLL3 are highly effective in small cell lung cancer models. *J Clin Invest.* 2023;133(9):e166028.
305. Guo J, Liang Y, Xue D, Shen J, Cai Y, Zhu J, Fu YX, Peng H. Tumor-conditional IL-15 pro-cytokine reactivates anti-tumor immunity with limited toxicity. *Cell Res.* 2021;31:1190–8.
306. Cieri N, Camisa B, Cocchiarella F, Forcato M, Oliveira G, Provasi E, Bondanza A, Bordignon C, Peccatori J, Ciceri F, et al. IL-7 and IL-15 instruct the generation of human memory stem T cells from naive precursors. *Blood.* 2013;121:573–84.
307. Silva DA, Yu S, Ulge UY, Spangler JB, Jude KM, Labao-Almeida C, Ali LR, Quijano-Rubio A, Ruterbusch M, Leung I, et al. De novo design of potent and selective mimics of IL-2 and IL-15. *Nature.* 2019;565:186–91.
308. Waldmann TA, Dubois S, Miljkovic MD, Conlon KC. IL-15 in the combination immunotherapy of cancer. *Front Immunol.* 2020;11:868.
309. Shi Y, Chen Z, Zhao Z, Yu Y, Fan H, Xu X, Bu X, Gu J. IL-21 induces an imbalance of Th17/Treg cells in moderate-to-severe plaque psoriasis patients. *Front Immunol.* 1865;2019:10.
310. Attridge K, Wang CJ, Wardzinski L, Kenefeck R, Chamberlain JL, Manzotti C, Kopf M, Walker LS. IL-21 inhibits T cell IL-2 production and impairs Treg homeostasis. *Blood.* 2012;119:4656–64.
311. Davis MR, Zhu Z, Hansen DM, Bai Q, Fang Y. The role of IL-21 in immunity and cancer. *Cancer Lett.* 2015;358:107–14.
312. Hermans D, Gautam S, Garcia-Canaveras JC, Gromer D, Mitra S, Spolski R, Li P, Christensen S, Nguyen R, Lin JX, et al. Lactate dehydrogenase inhibition synergizes with IL-21 to promote CD8(+) T cell stemness and antitumor immunity. *Proc Natl Acad Sci U S A.* 2020;117:6047–55.
313. Wei F, Cheng XX, Xue JZ, Xue SA. Emerging strategies in TCR-Engineered T Cells. *Front Immunol.* 2022;13:850358.
314. Meng F, Zhao J, Tan AT, Hu W, Wang SY, Jin J, Wu J, Li Y, Shi L, Fu JL, et al. Immunotherapy of HBV-related advanced hepatocellular carcinoma with short-term HBV-specific TCR expressed T cells: results of dose escalation, phase I trial. *Hepatol Int.* 2021;15:1402–12.
315. Tawara I, Kageyama S, Miyahara Y, Fujiwara H, Nishida T, Akatsuka Y, Ikeda H, Tanimoto K, Terakura S, Murata M, et al. Safety and persistence of WT1-specific T-cell receptor gene-transduced lymphocytes in patients with AML and MDS. *Blood.* 2017;130:1985–94.
316. Cho H, Wang Z, Levy D. Study of dose-dependent combination immunotherapy using engineered T cells and IL-2 in cervical cancer. *J Theor Biol.* 2020;505:110403.
317. Harris F, Berdugo Y, Tree T. IL-2-based approaches to Treg enhancement. *Clin Exp Immunol.* 2023;211:149–63.
318. Shi LZ, Goswami S, Fu T, Guan B, Chen J, Xiong L, Zhang J, Ng TD, Zhang X, Vence L, et al. Blockade of CTLA-4 and PD-1 enhances adoptive T-cell therapy efficacy in an ICOS-Mediated manner. *Cancer Immunol Res.* 2019;7:1803–12.
319. Winge-Main AK, Walchli S, Inderberg EM. T cell receptor therapy against melanoma-Immunotherapy for the future? *Scand J Immunol.* 2020;92:e12927.
320. Smith C, McGrath M, Neller MA, Matthews KK, Crooks P, Le Texier L, Panizza B, Porceddu S, Khanna R. Complete response to PD-1 blockade following EBV-specific T-cell therapy in metastatic nasopharyngeal carcinoma. *NPJ Precis Oncol.* 2021;5:24.
321. Allard B, Longhi MS, Robson SC, Stagg J. The eutonucleotidases CD39 and CD73: novel checkpoint inhibitor targets. *Immunol Rev.* 2017;276:121–44.
322. Zhai L, Ladomersky E, Lenzen A, Nguyen B, Patel R, Lauing KL, Wu M, Wainwright DA. IDO1 in cancer: a Gemini of immune checkpoints. *Cell Mol Immunol.* 2018;15:447–57.
323. McGranahan N, Swanton C. Clonal heterogeneity and tumor evolution: past, present, and the future. *Cell.* 2017;168:613–28.
324. Han D, Liu J, Chen C, Dong L, Liu Y, Chang R, Huang X, Liu Y, Wang J, Dougherty U, et al. Anti-tumour immunity controlled through mRNA m(6A) methylation and YTHDF1 in dendritic cells. *Nature.* 2019;566:270–4.
325. Dufva O, Polonen P, Bruck O, Keranen M, Klievink J, Mehtonen J, Huuhtanen J, Kumar A, Malani D, Siitonen S, et al. Immunogenomic landscape of hematological malignancies. *Cancer Cell.* 2020;38:380–99.
326. Khan AN, Gregorie CJ, Tomasi TB. Histone deacetylase inhibitors induce TAP, LMP, Tapasin genes and MHC class I antigen presentation by melanoma cells. *Cancer Immunol Immunother.* 2008;57:647–54.
327. McCaw TR, Randall TD, Forero A, Buchsbaum DJ. Modulation of antitumor immunity with histone deacetylase inhibitors. *Immunotherapy.* 2017;9:1359–72.
328. Lim AR, Rathmell WK, Rathmell JC. The tumor microenvironment as a metabolic barrier to effector T cells and immunotherapy. *Elife.* 2020;9:e55185.
329. Klein GR, Edwards-Hicks J, Apostolova P, O'Sullivan D, Sanin DE, Patterson AE, Puleston DJ, Lighthart N, Buescher JM, Grzes KM, et al. Metabolic conditioning of CD8(+) effector T cells for adoptive cell therapy. *Nat Metab.* 2020;2:703–16.
330. Liu Q, Sun Z, Chen L. Memory T cells: strategies for optimizing tumor immunotherapy. *Protein Cell.* 2020;11:549–64.
331. Okla K, Farber DL, Zou W. Tissue-resident memory T cells in tumor immunity and immunotherapy. *J Exp Med.* 2021;218(4):e20201605.
332. Wang Y, Qiu F, Xu Y, Hou X, Zhang Z, Huang L, Wang H, Xing H, Wu S. Stem cell-like memory T cells: the generation and application. *J Leukoc Biol.* 2021;110:1209–23.
333. Sung JH, Zhang H, Moseman EA, Alvarez D, Iannacone M, Henrickson SE, de la Torre JC, Groom JR, Luster AD, von Andrian UH. Chemokine guidance of central memory T cells is critical for antiviral recall responses in lymph nodes. *Cell.* 2012;150:1249–63.
334. Konduri V, Joseph SK, Byrd TT, Nawas Z, Vazquez-Perez J, Hofferek CJ, Halpern MM, Liu D, Liang Z, Baig Y, et al. A subset of cytotoxic effector memory T cells enhances CAR T cell efficacy in a model of pancreatic ductal adenocarcinoma. *Sci Transl Med.* 2021;13(592):eabc3196.
335. Xu Y, Zhang M, Ramos CA, Durett A, Liu E, Dakhova O, Liu H, Creighton CJ, Gee AP, Heslop HE, et al. Closely related T-memory stem cells correlate with in vivo expansion of CAR-CD19-T cells and are preserved by IL-7 and IL-15. *Blood.* 2014;123:3750–9.
336. Chamucero-Millares JA, Bernal-Estevéz DA, Parra-Lopez CA. Usefulness of IL-21, IL-7, and IL-15 conditioned media for expansion of antigen-specific CD8+ T cells from healthy donor-PBMCs suitable for immunotherapy. *Cell Immunol.* 2021;360:104257.
337. Rodrigues L, Bonorino C. Role of IL-15 and IL-21 in viral immunity: applications for vaccines and therapies. *Expert Rev Vaccines.* 2009;8:167–77.
338. Hengel RL, Thaker V, Pavlick MV, Metcalf JA, Dennis GJ, Yang J, Lempicki RA, Sereti I, Lane HC. Cutting edge: L-selectin (CD62L) expression distinguishes small resting memory CD4+ T cells that preferentially respond to recall antigen. *J Immunol.* 2003;170:28–32.
339. Nakajima Y, Chamoto K, Oura T, Honjo T. Critical role of the CD44(low) CD62L(low) CD8(+) T cell subset in restoring antitumor immunity in aged mice. *Proc Natl Acad Sci U S A.* 2021;118(23):e2103730118.
340. Zhu J, Yamane H, Paul WE. Differentiation of effector CD4 T cell populations (*). *Annu Rev Immunol.* 2010;28:445–89.
341. Taniuchi I. CD4 helper and CD8 cytotoxic T cell differentiation. *Annu Rev Immunol.* 2018;36:579–601.

342. Hilligan KL, Ronchese F. Antigen presentation by dendritic cells and their instruction of CD4+ T helper cell responses. *Cell Mol Immunol*. 2020;17:587–99.
343. Lu YJ, Barreira-Silva P, Boyce S, Powers J, Cavallo K, Behar SM. CD4 T cell help prevents CD8 T cell exhaustion and promotes control of Mycobacterium tuberculosis infection. *Cell Rep*. 2021;36:109696.
344. Kmieciak M, Worschech A, Nikizad H, Gowda M, Habibi M, Depczynski A, Wang E, Godder K, Holt SE, Marincola FM, Manjili MH. CD4+ T cells inhibit the neu-specific CD8+ T-cell exhaustion during the priming phase of immune responses against breast cancer. *Breast Cancer Res Treat*. 2011;126:385–94.
345. Lai P, Chen X, Wang Y, Wang J, Zhang Y, Geng S, Li P, Du X, Weng J, Pei D. C3aR costimulation enhances the antitumor efficacy of CAR-T cell therapy through Th17 expansion and memory T cell induction. *J Hematol Oncol*. 2022;15:68.
346. Fiering SN, Ho GW. Speed kills: advancement in Th17 cell adoptive cell therapy for solid tumors. *Cancer Res*. 2020;80:3795–6.
347. Johnson MO, Wolf MM, Madden MZ, Andrejeva G, Sugiura A, Contreras DC, Maseda D, Liberti MV, Paz K, Kishon RJ, et al. Distinct regulation of Th17 and Th1 cell differentiation by glutaminase-dependent metabolism. *Cell*. 2018;175:1780–95.
348. Oh DY, Kwek SS, Raju SS, Li T, McCarthy E, Chow E, Aran D, Ilano A, Pai CS, Rancan C, et al. Intratumoral CD4(+) T cells mediate anti-tumor cytotoxicity in human bladder cancer. *Cell*. 2020;181:1612–25.
349. Chhabra A. MHC class I TCR engineered anti-tumor CD4 T cells: implications for cancer immunotherapy. *Endocr Metab Immune Disord Drug Targets*. 2009;9:344–52.
350. Spear TT, Evavold BD, Baker BM, Nishimura MI. Understanding TCR affinity, antigen specificity, and cross-reactivity to improve TCR gene-modified T cells for cancer immunotherapy. *Cancer Immunol Immunother*. 2019;68:1881–9.
351. Liu Q, Tian Y, Li Y, Zhang W, Cai W, Liu Y, Ren Y, Liang Z, Zhou P, Zhang Y, et al. In vivo therapeutic effects of affinity-improved-TCR engineered T-cells on HBV-related hepatocellular carcinoma. *J Immunother Cancer*. 2020;8(2):e001748.
352. Hudecek M, Lupo-Stanghellini MT, Kosasih PL, Sommermeyer D, Jensen MC, Rader C, Riddell SR. Receptor affinity and extracellular domain modifications affect tumor recognition by ROR1-specific chimeric antigen receptor T cells. *Clin Cancer Res*. 2013;19:3153–64.
353. Depoil D, Dustin ML. Force and affinity in ligand discrimination by the TCR. *Trends Immunol*. 2014;35:597–603.
354. Miller AM, Bahmanof M, Zehn D, Cohen E, Schoenberger SP. Leveraging TCR affinity in adoptive immunotherapy against shared tumor/self-antigens. *Cancer Immunol Res*. 2019;7:40–9.
355. Stenger D, Stief TA, Kaeuferle T, Willier S, Rataj F, Schober K, Vick B, Lotfi R, Wagner B, Grunewald T, et al. Endogenous TCR promotes in vivo persistence of CD19-CAR-T cells compared to a CRISPR/Cas9-mediated TCR knockout CAR. *Blood*. 2020;136:1407–18.
356. Rohaan MW, Gomez-Eerland R, van den Berg JH, Geukes FM, van Zon M, Raud B, Jedema I, Scheij S, de Boer R, Bakker N, et al. MART-1 TCR gene-modified peripheral blood T cells for the treatment of metastatic melanoma: a phase I/IIa clinical trial. *Immuno-oncol Technol*. 2022;15:100089.
357. Pinto S, Sommermeyer D, Michel C, Wilde S, Schendel D, Uckert W, Blankenstein T, Kyewski B. Misinitiation of intrathymic MART-1 transcription and biased TCR usage explain the high frequency of MART-1-specific T cells. *Eur J Immunol*. 2014;44:2811–21.
358. Rapoport AP, Stadtmayer EA, Binder-Scholl GK, Goloubeva O, Vogl DT, Lacey SF, Badros AZ, Garfall A, Weiss B, Finklestein J, et al. NY-ESO-1-specific TCR-engineered T cells mediate sustained antigen-specific antitumor effects in myeloma. *Nat Med*. 2015;21:914–21.
359. Shenderov E, Kandasamy M, Gileadi U, Chen J, Shepherd D, Gibbs J, Prota G, Silk JD, Yewdell JW, Cerundolo V. Generation and characterization of HLA-A2 transgenic mice expressing the human TCR 1G4 specific for the HLA-A2 restricted NY-ESO-1(157–165) tumor-specific peptide. *J Immunother Cancer*. 2021;9(6):e002544.
360. Thakkar N, Bailey-Kellogg C. Balancing sensitivity and specificity in distinguishing TCR groups by CDR sequence similarity. *BMC Bioinformatics*. 2019;20:241.
361. Toivonen R, Arstila TP, Hanninen A. Islet-associated T-cell receptor-beta CDR sequence repertoire in prediabetic NOD mice reveals antigen-driven T-cell expansion and shared usage of Vbeta1beta TCR chains. *Mol Immunol*. 2015;64:127–35.
362. Saha A, Bhattacharya-Chatterjee M, Foon KA, Celis E, Chatterjee SK. Stimulatory effects of CpG oligodeoxynucleotide on dendritic cell-based immunotherapy of colon cancer in CEA/HLA-A2 transgenic mice. *Int J Cancer*. 2009;124:877–88.
363. Zhou D, Zheng H, Liu Q, Lu X, Deng X, Jiang L, Hou B, Fu Y, Zhu F, Ding Y, et al. Attenuated plasmodium sporozoite expressing MAGE-A3 induces antigen-specific CD8+ T cell response against lung cancer in mice. *Cancer Biol Med*. 2019;16:288–98.
364. Chinnasamy N, Wargo JA, Yu Z, Rao M, Frankel TL, Riley JP, Hong JJ, Parkhurst MR, Feldman SA, Schrupp DS, 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. *J Immunol*. 2011;186:685–96.
365. Chimen M, Apta BH, Mcgettrick HM. Introduction: T Cell Trafficking in Inflammation and Immunity. *Methods Mol Biol*. 2017;1591:73–84.
366. Ortiz ZN, Bemelmans MP, Handel TM, de Visser KE, Heitman LH. Anti-cancer opportunities at every stage of chemokine function. *Trends Pharmacol Sci*. 2021;42:912–28.
367. Lacalle RA, Blanco R, Carmona-Rodriguez L, Martin-Leal A, Mira E, Manes S. Chemokine receptor signaling and the hallmarks of cancer. *Int Rev Cell Mol Biol*. 2017;331:181–244.
368. Lai WY, Mueller A. Latest update on chemokine receptors as therapeutic targets. *Biochem Soc Trans*. 2021;49:1385–95.
369. Kraus S, Kolman T, Yeung A, Deming D. Chemokine receptor antagonists: role in oncology. *Curr Oncol Rep*. 2021;23:131.
370. Doi T, Muro K, Ishii H, Kato T, Tsushima T, Takenoyama M, Oizumi S, Gemmoto K, Suna H, Enokitani K, et al. A Phase I study of the anti-CC chemokine receptor 4 antibody, mogamulizumab, in combination with nivolumab in patients with advanced or metastatic solid tumors. *Clin Cancer Res*. 2019;25:6614–22.
371. Li YL, Shi ZH, Wang X, Gu KS, Zhai ZM. Prognostic significance of monocyte chemoattractant protein-1 and CC chemokine receptor 2 in diffuse large B cell lymphoma. *Ann Hematol*. 2019;98:413–22.
372. Correale P, Rotundo MS, Botta C, Del VM, Ginanneschi C, Licchetta A, Conca R, Apollinari S, De Luca F, Tassone P, Tagliaferri P. Tumor infiltration by T lymphocytes expressing chemokine receptor 7 (CCR7) is predictive of favorable outcome in patients with advanced colorectal carcinoma. *Clin Cancer Res*. 2012;18:850–7.
373. Esensten JH, Helou YA, Chopra G, Weiss A, Bluestone JA. CD28 costimulation: from mechanism to therapy. *Immunity*. 2016;44:973–88.
374. Amatore F, Gorvel L, Olive D. Role of Inducible Co-Stimulator (ICOS) in cancer immunotherapy. *Expert Opin Biol Ther*. 2020;20:141–50.
375. Chester C, Sanmamed MF, Wang J, Melero I. Immunotherapy targeting 4-1BB: mechanistic rationale, clinical results, and future strategies. *Blood*. 2018;131:49–57.
376. Rowshanravan B, Halliday N, Sansom DM. CTLA-4: a moving target in immunotherapy. *Blood*. 2018;131:58–67.
377. Wu X, Gu Z, Chen Y, Chen B, Chen W, Weng L, Liu X. Application of PD-1 blockade in cancer immunotherapy. *Comput Struct Biotechnol J*. 2019;17:661–74.
378. Forde PM, Chaft JE, Smith KN, Anagnostou V, Cottrell TR, Hellmann MD, Zahurak M, Yang SC, Jones DR, Broderick S, et al. Neoadjuvant PD-1 blockade in resectable lung cancer. *N Engl J Med*. 2018;378:1976–86.
379. O'Malley DM, Neffa M, Monk BJ, Melkadze T, Huang M, Kryzhanivska A, Bulat I, Meniawy TM, Bagameri A, Wang EW, et al. Dual PD-1 and CTLA-4 checkpoint blockade using balstilimab and zalifrelimab combination as second-line treatment for advanced cervical cancer: an open-label phase II study. *J Clin Oncol*. 2022;40:762–71.
380. Poole A, Karupiah V, Hartt A, Haidar JN, Moureau S, Dobrzycki T, Hayes C, Rowley C, Dias J, Harper S, et al. Therapeutic high affinity T cell receptor targeting a KRAS(G12D) cancer neoantigen. *Nat Commun*. 2022;13:5333.
381. Simon B, Harter DC, Thirion C, Schuler-Thurner B, Schuler G, Uslu U. Enhancing lentiviral transduction to generate melanoma-specific human T cells for cancer immunotherapy. *J Immunol Methods*. 2019;472:55–64.
382. Peng M, Mo Y, Wang Y, Wu P, Zhang Y, Xiong F, Guo C, Wu X, Li Y, Li X, et al. Neoantigen vaccine: an emerging tumor immunotherapy. *Mol Cancer*. 2019;18:128.

383. Sellars MC, Wu CJ, Fritsch EF. Cancer vaccines: building a bridge over troubled waters. *Cell*. 2022;185:2770–88.
384. Cafri G, Gartner JJ, Zaks T, Hopson K, Levin N, Paria BC, Parkhurst MR, Yossef R, Lowery FJ, Jafferji MS, et al. mRNA vaccine-induced neoantigen-specific T cell immunity in patients with gastrointestinal cancer. *J Clin Invest*. 2020;130:5976–88.
385. Keskin DB, Anandappa AJ, Sun J, Tirosh I, Mathewson ND, Li S, Oliveira G, Giobbie-Hurder A, Felt K, Gjini E, et al. Neoantigen vaccine generates intratumoral T cell responses in phase Ib glioblastoma trial. *Nature*. 2019;565:234–9.
386. Palmer CD, Rappaport AR, Davis MJ, Hart MG, Scallan CD, Hong SJ, Gitlin L, Kraemer LD, Kounlavouth S, Yang A, et al. Individualized, heterologous chimpanzee adenovirus and self-amplifying mRNA neoantigen vaccine for advanced metastatic solid tumors: phase 1 trial interim results. *Nat Med*. 2022;28:1619–29.
387. Platten M, Bunse L, Wick A, Bunse T, Le Cornet L, Harting I, Sahn F, Sanghvi K, Tan CL, Poschke I, et al. A vaccine targeting mutant IDH1 in newly diagnosed glioma. *Nature*. 2021;592:463–8.
388. Kloor M, Reuschenbach M, Pauligk C, Karbach J, Rafiyan MR, Al-Batran SE, Tariverdian M, Jager E, von Knebel DM. A frameshift peptide neoantigen-based vaccine for mismatch repair-deficient cancers: a phase I/IIa clinical trial. *Clin Cancer Res*. 2020;26:4503–10.
389. Shou J, Mo F, Zhang S, Lu L, Han N, Liu L, Qiu M, Li H, Han W, Ma D, et al. Combination treatment of radiofrequency ablation and peptide neoantigen vaccination: Promising modality for future cancer immunotherapy. *Front Immunol*. 2022;13:1000681.
390. Park J, Hsueh PC, Li Z, Ho PC. Microenvironment-driven metabolic adaptations guiding CD8(+) T cell anti-tumor immunity. *Immunity*. 2023;56:32–42.
391. Hall M, Liu H, Malafa M, Centeno B, Hodul PJ, Pimiento J, Pilon-Thomas S, Sarnaik AA. Expansion of tumor-infiltrating lymphocytes (TIL) from human pancreatic tumors. *J Immunother Cancer*. 2016;4:61.
392. Dafni U, Michielin O, Lluésma SM, Tsourtzi Z, Polydoropoulou V, Karlis D, Besser MJ, Haanen J, Svane IM, Ohashi PS, et al. Efficacy of adoptive therapy with tumor-infiltrating lymphocytes and recombinant interleukin-2 in advanced cutaneous melanoma: a systematic review and meta-analysis. *Ann Oncol*. 2019;30:1902–13.
393. Hsu EJ, Cao X, Moon B, Bae J, Sun Z, Liu Z, Fu YX. A cytokine receptor-masked IL2 prodrug selectively activates tumor-infiltrating lymphocytes for potent antitumor therapy. *Nat Commun*. 2021;12:2768.
394. Hiltensperger M, Krackhardt AM. Current and future concepts for the generation and application of genetically engineered CAR-T and TCR-T cells. *Front Immunol*. 2023;14:1121030.
395. Shevryev DV, Tereshchenko VP, Sennikov SV. The enigmatic nature of the TCR-pMHC interaction: implications for CAR-T and TCR-T engineering. *Int J Mol Sci*. 2022;23(23):14728.
396. June CH, O'Connor RS, Kawalekar OU, Ghassemi S, Milone MC. CAR T cell immunotherapy for human cancer. *Science*. 2018;359:1361–5.
397. Shah NN, Fry TJ. Mechanisms of resistance to CAR T cell therapy. *Nat Rev Clin Oncol*. 2019;16:372–85.
398. Newick K, O'Brien S, Moon E, Albelda SM. CAR T cell therapy for solid tumors. *Annu Rev Med*. 2017;68:139–52.
399. Ying Z, Huang XF, Xiang X, Liu Y, Kang X, Song Y, Guo X, Liu H, Ding N, Zhang T, et al. A safe and potent anti-CD19 CAR T cell therapy. *Nat Med*. 2019;25:947–53.
400. Brudno JN, Lam N, Vanasse D, Shen YW, Rose JJ, Rossi J, Xue A, Bot A, Scholler N, Mikkilineni L, et al. Safety and feasibility of anti-CD19 CAR T cells with fully human binding domains in patients with B-cell lymphoma. *Nat Med*. 2020;26:270–80.
401. O'Rourke DM, Nasrallah MP, Desai A, Melenhorst JJ, Mansfield K, Morrisette J, Martinez-Lage M, Brem S, Maloney E, Shen A, et al. A single dose of peripherally infused EGFRvIII-directed CAR T cells mediates antigen loss and induces adaptive resistance in patients with recurrent glioblastoma. *Sci Transl Med*. 2017;9(399):eaaa0984.
402. Muhammad N, Wang R, Li W, Zhang Z, Chang Y, Hu Y, Zhao J, Zheng X, Mao Q, Xia H. A novel TanCAR targeting IL13Ralpha2 and EphA2 for enhanced glioblastoma therapy. *Mol Ther Oncolytics*. 2022;24:729–41.
403. Pan K, Farrukh H, Chittepu V, Xu H, Pan CX, Zhu Z. CAR race to cancer immunotherapy: from CAR T, CAR NK to CAR macrophage therapy. *J Exp Clin Cancer Res*. 2022;41:119.
404. Sengupta S, Board NL, Wu F, Moskovljevic M, Douglass J, Zhang J, Reinhold BR, Duke-Cohan J, Yu J, Reed MC, et al. TCR-mimic bispecific antibodies to target the HIV-1 reservoir. *Proc Natl Acad Sci U S A*. 2022;119:e2123406119.
405. Gerber HP, Presta LG. TCR mimic compounds for pHLA targeting with high potency modalities in oncology. *Front Oncol*. 2022;12:1027548.
406. Klatt MG, Dao T, Yang Z, Liu J, Mun SS, Dacek MM, Luo H, Gardner TJ, Bourne C, Peraro L, et al. A TCR mimic CAR T cell specific for NDC80 is broadly reactive with solid tumors and hematologic malignancies. *Blood*. 2022;140:861–74.
407. Caushi JX, Zhang J, Ji Z, Vaghasia A, Zhang B, Hsiue EH, Mog BJ, Hou W, Justesen S, Blosser R, et al. Transcriptional programs of neoantigen-specific TIL in anti-PD-1-treated lung cancers. *Nature*. 2021;596:126–32.
408. Shen Y, Li YM, Zhou JJ, Zhou Z, Xu YC, Zhao WB, Chen SQ. The antitumor activity of TCR-mimic antibody-drug conjugates (TCRm-ADCs) targeting the intracellular wilms tumor 1 (WT1) oncoprotein. *Int J Mol Sci*. 2019;20(16):3912.
409. Shen Y, Wei X, Jin S, Wu Y, Zhao W, Xu Y, Pan L, Zhou Z, Chen S. TCR-mimic antibody-drug conjugates targeting intracellular tumor-specific mutant antigen KRAS G12V mutation. *Asian J Pharm Sci*. 2020;15:777–85.
410. Labrijn AF, Janmaat ML, Reichert JM, Parren P. Bispecific antibodies: a mechanistic review of the pipeline. *Nat Rev Drug Discov*. 2019;18:585–608.
411. Thakur A, Huang M, Lum LG. Bispecific antibody based therapeutics: strengths and challenges. *Blood Rev*. 2018;32:339–47.
412. Groeneveldt C, Kinderman P, van den Wollenberg D, van den Oever RL, Middelburg J, Mustafa D, Hoeben RC, van der Burg SH, van Hall T, van Montfort N. Preconditioning of the tumor microenvironment with oncolytic reovirus converts CD3-bispecific antibody treatment into effective immunotherapy. *J Immunother Cancer*. 2020;8:e001191.
413. Zhao L, Li S, Wei X, Qi X, Liu D, Liu L, Wen F, Zhang JS, Wang F, Liu ZL, Cao YJ. A novel CD19/CD22/CD3 trispecific antibody enhances therapeutic efficacy and overcomes immune escape against B-ALL. *Blood*. 2022;140:1790–802.
414. Robinson HR, Qi J, Cook EM, Nichols C, Dadashian EL, Underbayev C, Herman S, Saba NS, Keyvanfar K, Sun C, et al. A CD19/CD3 bispecific antibody for effective immunotherapy of chronic lymphocytic leukemia in the ibrutinib era. *Blood*. 2018;132:521–32.
415. Gejman RS, Jones HF, Klatt MG, Chang AY, Oh CY, Chandran SS, Korontsvit T, Zakahleva V, Dao T, Klebanoff CA, Scheinberg DA. Identification of the targets of T-cell receptor therapeutic agents and cells by use of a high-throughput genetic platform. *Cancer Immunol Res*. 2020;8:672–84.
416. Kurosawa N, Midorikawa A, Ida K, Fudaba YW, Isobe M. Development of a T-cell receptor mimic antibody targeting a novel Wilms tumor 1-derived peptide and analysis of its specificity. *Cancer Sci*. 2020;111:3516–26.
417. Ahmed M, Lopez-Albaiteo A, Pankov D, Santich BH, Liu H, Yan S, Xiang J, Wang P, Hasan AN, Selvakumar A, et al. TCR-mimic bispecific antibodies targeting LMP2A show potent activity against EBV malignancies. *JCI Insight*. 2018;3(4):e97805.
418. Ma W, Pham B, Li T. Cancer neoantigens as potential targets for immunotherapy. *Clin Exp Metastasis*. 2022;39:51–60.
419. Peng S, Chen S, Hu W, Mei J, Zeng X, Su T, Wang W, Chen Z, Xiao H, Zhou Q, et al. Combination neoantigen-based dendritic cell vaccination and adoptive T-Cell transfer induces antitumor responses against recurrence of hepatocellular carcinoma. *Cancer Immunol Res*. 2022;10:728–44.
420. Ott PA, Dotti G, Yee C, Goff SL. An update on adoptive T-Cell therapy and neoantigen vaccines. *Am Soc Clin Oncol Educ Book*. 2019;39:e70–8.
421. Awad MM, Govindan R, Balogh KN, Spigel DR, Garon EB, Bushway ME, Poran A, Sheen JH, Kohler V, Esaulova E, et al. Personalized neoantigen vaccine NEO-PV-01 with chemotherapy and anti-PD-1 as first-line treatment for non-squamous non-small cell lung cancer. *Cancer Cell*. 2022;40:1010–26.
422. Fang X, Guo Z, Liang J, Wen J, Liu Y, Guan X, Li H. Neoantigens and their potential applications in tumor immunotherapy. *Oncol Lett*. 2022;23:88.
423. Ott PA, Hu-Lieskovan S, Chmielowski B, Govindan R, Naing A, Bhardwaj N, Margolin K, Awad MM, Hellmann MD, Lin JJ, et al. A Phase Ib trial

- of personalized neoantigen therapy plus anti-PD-1 in patients with advanced melanoma, non-small cell lung cancer, or bladder cancer. *Cell*. 2020;183:347–62.
424. Ding Z, Li Q, Zhang R, Xie L, Shu Y, Gao S, Wang P, Su X, Qin Y, Wang Y, et al. Personalized neoantigen pulsed dendritic cell vaccine for advanced lung cancer. *Signal Transduct Target Ther*. 2021;6:26.
 425. Gronholm M, Feodoroff M, Antignani G, Martins B, Hamdan F, Cerullo V. Patient-derived organoids for precision cancer immunotherapy. *Cancer Res*. 2021;81:3149–55.
 426. Zhang Q, Jia Q, Zhang J, Zhu B. Neoantigens in precision cancer immunotherapy: from identification to clinical applications. *Chin Med J (Engl)*. 2022;135:1285–98.
 427. Hao Y, Hao S, Andersen-Nissen E, Mauck WR, Zheng S, Butler A, Lee MJ, Wilk AJ, Darby C, Zager M, et al. Integrated analysis of multimodal single-cell data. *Cell*. 2021;184:3573–87.
 428. Dieu-Nosjean MC, Giraldo NA, Kaplon H, Germain C, Fridman WH, Sautes-Fridman C. Tertiary lymphoid structures, drivers of the anti-tumor responses in human cancers. *Immunol Rev*. 2016;271:260–75.
 429. Xie F, Zhou X, Fang M, Li H, Su P, Tu Y, Zhang L, Zhou F. Extracellular vesicles in cancer immune microenvironment and cancer immunotherapy. *Adv Sci (Weinh)*. 2019;6:1901779.
 430. Flores B, Chawla S, Ma N, Sanada C, Kujur PK, Yeung R, Bellon MB, Hukari K, Fowler B, Lynch M, et al. Microfluidic live tracking and transcriptomics of cancer-immune cell doublets link intercellular proximity and gene regulation. *Commun Biol*. 2022;5:1231.
 431. Wedge ME, Jennings VA, Crupi M, Poutou J, Jamieson T, Pelin A, Pugliese G, de Souza CT, Petryk J, Laight BJ, et al. Virally programmed extracellular vesicles sensitize cancer cells to oncolytic virus and small molecule therapy. *Nat Commun*. 1898;2022:13.
 432. Spiotto M, Fu YX, Weichselbaum RR. The intersection of radiotherapy and immunotherapy: mechanisms and clinical implications. *Sci Immunol*. 2016;1(3):EAAG1266.
 433. Voronova V, Vislobokova A, Mutig K, Samsonov M, Peskov K, Sekacheva M, Materenchuk M, Bunyatyan N, Lebedeva S. Combination of immune checkpoint inhibitors with radiation therapy in cancer: a hammer breaking the wall of resistance. *Front Oncol*. 2022;12:1035884.
 434. Zhang L, Feng D, Yu LX, Tsung K, Norton JA. Preexisting antitumor immunity augments the antitumor effects of chemotherapy. *Cancer Immunol Immunother*. 2013;62:1061–71.
 435. Sahin U, Oehm P, Derhovanessian E, Jabulowsky RA, Vormehr M, Gold M, Maurus D, Schwarck-Kokarakis D, Kuhn AN, Omokoko T, et al. An RNA vaccine drives immunity in checkpoint-inhibitor-treated melanoma. *Nature*. 2020;585:107–12.
 436. Chae YK, Arya A, Iams W, Cruz MR, Chandra S, Choi J, Giles F. Current landscape and future of dual anti-CTLA4 and PD-1/PD-L1 blockade immunotherapy in cancer; lessons learned from clinical trials with melanoma and non-small cell lung cancer (NSCLC). *J Immunother Cancer*. 2018;6:39.
 437. Mondini M, Levy A, Meziani L, Milliat F, Deutsch E. Radiotherapy-immunotherapy combinations - perspectives and challenges. *Mol Oncol*. 2020;14:1529–37.
 438. Wang L, Ge X, Zhang Z, Ye Y, Zhou Z, Li M, Yan H, Wu L, Bai Q, Li J, et al. Identification of a ferroptosis-related long noncoding RNA prognostic signature and its predictive ability to immunotherapy in hepatocellular carcinoma. *Front Genet*. 2021;12:628082.
 439. Balanca CC, Salvioni A, Scarlata CM, Michelas M, Martinez-Gomez C, Gomez-Roca C, Sarradin V, Tosolini M, Valle C, Pont F, et al. PD-1 blockade restores helper activity of tumor-infiltrating, exhausted PD-1hiCD39+ CD4 T cells. *JCI Insight*. 2021;6(2):e142513.
 440. Faghfuri E, Shadbad MA, Faghfouri AH, Soozangar N. Cellular immunotherapy in gastric cancer: adoptive cell therapy and dendritic cell-based vaccination. *Immunotherapy*. 2022;14:475–88.
 441. Lahiri A, Maji A, Potdar PD, Singh N, Parikh P, Bisht B, Mukherjee A, Paul MK. Lung cancer immunotherapy: progress, pitfalls, and promises. *Mol Cancer*. 2023;22:40.
 442. Luo Q, Zhang L, Luo C, Jiang M. Emerging strategies in cancer therapy combining chemotherapy with immunotherapy. *Cancer Lett*. 2019;454:191–203.
 443. Yi M, Qin S, Zhao W, Yu S, Chu Q, Wu K. The role of neoantigen in immune checkpoint blockade therapy. *Exp Hematol Oncol*. 2018;7:28.
 444. Kiyotani K, Chan HT, Nakamura Y. Immunopharmacogenomics towards personalized cancer immunotherapy targeting neoantigens. *Cancer Sci*. 2018;109:542–9.
 445. Ellis GI, Sheppard NC, Riley JL. Genetic engineering of T cells for immunotherapy. *Nat Rev Genet*. 2021;22:427–47.
 446. Grasso CS, Giannakis M, Wells DK, Hamada T, Mu XJ, Quist M, Nowak JA, Nishihara R, Qian ZR, Inamura K, et al. Genetic mechanisms of immune evasion in colorectal cancer. *Cancer Discov*. 2018;8:730–49.
 447. Sivapalan L, Anagnostou V. Genetic variation in antigen presentation and cancer immunotherapy. *Immunity*. 2022;55:3–6.
 448. Wolf Y, Bartok O, Patkar S, Eli GB, Cohen S, Litchfield K, Levy R, Jimenez-Sanchez A, Trabish S, Lee JS, et al. UVB-induced tumor heterogeneity diminishes immune response in melanoma. *Cell*. 2019;179:219–35.
 449. Dao T, Klatt MG, Korontsvit T, Mun SS, Guzman S, Mattar M, Zivanovic O, Kyi CK, Socci ND, O'Ceirbhail RE, Scheinberg DA. Impact of tumor heterogeneity and microenvironment in identifying neoantigens in a patient with ovarian cancer. *Cancer Immunol Immunother*. 2021;70:1189–202.
 450. McCann K, von Witzleben A, Thomas J, Wang C, Wood O, Singh D, Boukas K, Bendjama K, Silvestre N, Nielsen FC, et al. Targeting the tumor mutanome for personalized vaccination in a TMB low non-small cell lung cancer. *J Immunother Cancer*. 2022;10(3):e003821.
 451. Newell F, Pires DSI, Johansson PA, Menzies AM, Wilmott JS, Addala V, Carlino MS, Rizos H, Nones K, Edwards JJ, et al. Multiomic profiling of checkpoint inhibitor-treated melanoma: Identifying predictors of response and resistance, and markers of biological discordance. *Cancer Cell*. 2022;40:88–102.
 452. Liu T, Tan J, Wu M, Fan W, Wei J, Zhu B, Guo J, Wang S, Zhou P, Zhang H, et al. High-affinity neoantigens correlate with better prognosis and trigger potent antihepatocellular carcinoma (HCC) activity by activating CD39(+)/CD8(+)-T cells. *Gut*. 2021;70:1965–77.
 453. Subudhi SK, Vence L, Zhao H, Blando J, Yadav SS, Xiong Q, Reuben A, Aparicio A, Corn PG, Chapin BF, et al. Neoantigen responses, immune correlates, and favorable outcomes after ipilimumab treatment of patients with prostate cancer. *Sci Transl Med*. 2020;12(537):eaaz3577.
 454. Rosenthal R, Cadieux EL, Salgado R, Bakir MA, Moore DA, Hiley CT, Lund T, Tanic M, Reading JL, Joshi K, et al. Neoantigen-directed immune escape in lung cancer evolution. *Nature*. 2019;567:479–85.
 455. Angelova M, Mlecnik B, Vasaturo A, Bindea G, Fredriksen T, Lafontaine L, Buttard B, Morgand E, Bruni D, Jouret-Mourin A, et al. Evolution of metastases in space and time under immune selection. *Cell*. 2018;175:751–65.
 456. Lin M, Zhang XL, You R, Yang Q, Zou X, Yu K, Liu YP, Zou RH, Hua YJ, Huang PY, et al. Neoantigen landscape in metastatic nasopharyngeal carcinoma. *Theranostics*. 2021;11:6427–44.
 457. Vesely MD, Zhang T, Chen L. Resistance mechanisms to Anti-PD-1 cancer immunotherapy. *Annu Rev Immunol*. 2022;40:45–74.
 458. Lu C, Guan J, Lu S, Jin Q, Rousseau B, Lu T, Stephens D, Zhang H, Zhu J, Yang M, et al. DNA sensing in mismatch repair-deficient tumor cells is essential for anti-tumor immunity. *Cancer Cell*. 2021;39:96–108.
 459. Chen F, Zou Z, Du J, Su S, Shao J, Meng F, Yang J, Xu Q, Ding N, Yang Y, et al. Neoantigen identification strategies enable personalized immunotherapy in refractory solid tumors. *J Clin Invest*. 2019;129:2056–70.
 460. McGranahan N, Rosenthal R, Hiley CT, Rowan AJ, Watkins T, Wilson GA, Birnbak NJ, Veeriah S, Van Loo P, Herrero J, Swanton C. Allele-specific HLA loss and immune escape in lung cancer evolution. *Cell*. 2017;171:1259–71.
 461. Blaha DT, Anderson SD, Yoakum DM, Hager MV, Zha Y, Gajewski TF, Kranz DM. High-throughput stability screening of neoantigen/HLA complexes improves immunogenicity predictions. *Cancer Immunol Res*. 2019;7:50–61.
 462. Del CA, Carretero J, Munoz JA, Zinchenko S, Ruiz-Cabello F, Gonzalez-Aseguiolaza G, Garrido F, Aptsiauri N. Adenovirus expressing beta2-microglobulin recovers HLA class I expression and antitumor immunity by increasing T-cell recognition. *Cancer Gene Ther*. 2014;21:317–32.
 463. Taylor BC, Balko JM. Mechanisms of MHC-I downregulation and role in immunotherapy response. *Front Immunol*. 2022;13:844866.
 464. Lu SX, De Neef E, Thomas JD, Sabio E, Rousseau B, Gigoux M, Knorr DA, Greenbaum B, Elhanati Y, Hogg SJ, et al. Pharmacologic modulation of RNA splicing enhances anti-tumor immunity. *Cell*. 2021;184:4032–47.

465. Liang J, Wang L, Wang C, Shen J, Su B, Marisetty AL, Fang D, Kassab C, Jeong KJ, Zhao W, et al. Verteporfin inhibits PD-L1 through autophagy and the STAT1-IRF1-TRIM28 signaling axis, exerting antitumor efficacy. *Cancer Immunol Res.* 2020;8:952–65.
466. Koustas E, Trifylli EM, Sarantis P, Papadopoulos N, Papanikolopoulos K, Aloizos G, Damaskos C, Gampis N, Gampis A, Matthaos D, Karamouzis MV. Exploiting autophagy-dependent neoantigen presentation in tumor microenvironment. *Genes (Basel).* 2023;14(2):474.
467. Gajewski TF, Schreiber H, Fu YX. Innate and adaptive immune cells in the tumor microenvironment. *Nat Immunol.* 2013;14:1014–22.
468. Lei X, Lei Y, Li JK, Du WX, Li RG, Yang J, Li J, Li F, Tan HB. Immune cells within the tumor microenvironment: Biological functions and roles in cancer immunotherapy. *Cancer Lett.* 2020;470:126–33.
469. Mao X, Xu J, Wang W, Liang C, Hua J, Liu J, Zhang B, Meng Q, Yu X, Shi S. Crosstalk between cancer-associated fibroblasts and immune cells in the tumor microenvironment: new findings and future perspectives. *Mol Cancer.* 2021;20:131.
470. Jiang X, Wang J, Deng X, Xiong F, Ge J, Xiang B, Wu X, Ma J, Zhou M, Li X, et al. Role of the tumor microenvironment in PD-L1/PD-1-mediated tumor immune escape. *Mol Cancer.* 2019;18:10.
471. Zhang C, Chen J, Song Q, Sun X, Xue M, Yang Z, Shang J. Comprehensive analysis of CTLA-4 in the tumor immune microenvironment of 33 cancer types. *Int Immunopharmacol.* 2020;85:106633.
472. Reticker-Flynn NE, Zhang W, Belk JA, Basto PA, Escalante NK, Pilarowski G, Bejnood A, Martins MM, Kenkel JA, Linde IL, et al. Lymph node colonization induces tumor-immune tolerance to promote distant metastasis. *Cell.* 2022;185:1924–42.
473. Joyce JA, Fearon DT. T cell exclusion, immune privilege, and the tumor microenvironment. *Science.* 2015;348:74–80.
474. Leone RD, Zhao L, Englert JM, Sun IM, Oh MH, Sun IH, Arwood ML, Bettencourt IA, Patel CH, Wen J, et al. Glutamine blockade induces divergent metabolic programs to overcome tumor immune evasion. *Science.* 2019;366:1013–21.
475. Walsh SR, Simovic B, Chen L, Bastin D, Nguyen A, Stephenson K, Mandur TS, Bramson JL, Lichty BD, Wan Y. Endogenous T cells prevent tumor immune escape following adoptive T cell therapy. *J Clin Invest.* 2019;129:5400–10.
476. Zappasodi R, Serganova I, Cohen IJ, Maeda M, Shindo M, Senbabaoglu Y, Watson MJ, Leftin A, Maniyar R, Verma S, et al. CTLA-4 blockade drives loss of T(reg) stability in glycolysis-low tumours. *Nature.* 2021;591:652–8.
477. Li S, Liu M, Do MH, Chou C, Stamatides EG, Nixon BG, Shi W, Zhang X, Li P, Gao S, et al. Cancer immunotherapy via targeted TGF-beta signaling blockade in T(H) cells. *Nature.* 2020;587:121–5.
478. Derynck R, Turley SJ, Akhurst RJ. TGFbeta biology in cancer progression and immunotherapy. *Nat Rev Clin Oncol.* 2021;18:9–34.
479. Li H, Li J, Zhang C, Zhang C, Wang H. TERT mutations correlate with higher TMB value and unique tumor microenvironment and may be a potential biomarker for anti-CTLA4 treatment. *Cancer Med.* 2020;9:7151–60.
480. Lind H, Gameiro SR, Jochems C, Donahue RN, Strauss J, Gulley JLM, Palena C, Schlom J. Dual targeting of TGF-beta and PD-L1 via a bifunctional anti-PD-L1/TGF-betaRII agent: status of preclinical and clinical advances. *J Immunother Cancer.* 2020;8(1):e000433.
481. Pu H, Begemann DE, Kyprianou N. Aberrant TGF-beta signaling drives castration-resistant prostate cancer in a male mouse model of prostate tumorigenesis. *Endocrinology.* 2017;158:1612–22.
482. Paller C, Pu H, Begemann DE, Wade CA, Hensley PJ, Kyprianou N. TGF-beta receptor I inhibitor enhances response to enzalutamide in a pre-clinical model of advanced prostate cancer. *Prostate.* 2019;79:31–43.
483. Kloss CC, Lee J, Zhang A, Chen F, Melenhorst JJ, Lacey SF, Maus MV, Fraietta JA, Zhao Y, June CH. Dominant-negative TGF-beta receptor enhances PSMA-targeted human CAR T cell proliferation and augments prostate cancer eradication. *Mol Ther.* 2018;26:1855–66.
484. Oda SK, Anderson KG, Ravikumar P, Bonson P, Garcia NM, Jenkins CM, Zhuang S, Daman AW, Chiu EY, Bates BM, Greenberg PD. A Fas-4-1BB fusion protein converts a death to a pro-survival signal and enhances T cell therapy. *J Exp Med.* 2020;217(12):e20191166.
485. Yu P, Fu YX. Tumor-infiltrating T lymphocytes: friends or foes? *Lab Invest.* 2006;86:231–45.
486. Kumar A, Watkins R, Vilgelm AE. Cell therapy with TILs: training and taming t cells to fight cancer. *Front Immunol.* 2021;12:690499.
487. Kazemi MH, Sadri M, Najafi A, Rahimi A, Baghernejadan Z, Khorramdelazad H, Falak R. Tumor-infiltrating lymphocytes for treatment of solid tumors: It takes two to tango? *Front Immunol.* 2022;13:1018962.
488. Burton EM, Voyer J, Gewurz BE. Epstein-Barr virus latency programs dynamically sensitize B cells to ferroptosis. *Proc Natl Acad Sci U S A.* 2022;119:e2118300119.
489. Tan Q, Ku W, Zhang C, Heyilimu P, Tian Y, Ke Y, Lu Z. Mutation analysis of the EBV-lymphoblastoid cell line cautions their use as antigen-presenting cells. *Immunol Cell Biol.* 2018;96:204–11.
490. Dorrie J, Schaft N, Schuler G, Schuler-Thurner B. Therapeutic cancer vaccination with ex vivo RNA-transfected dendritic cells—an update. *Pharmaceutics.* 2020;12(2):92.
491. Wang Z, McWilliams-Koepfen HP, Reza H, Ostberg JR, Chen W, Wang X, Huynh C, Vyas V, Chang WC, Starr R, et al. 3D-organoid culture supports differentiation of human CAR(+) iPSCs into highly functional CAR T cells. *Cell Stem Cell.* 2022;29:515–27.
492. Minagawa A, Kaneko S. Rise of iPSCs as a cell source for adoptive immunotherapy. *Hum Cell.* 2014;27:47–50.
493. Lu F, Ma XJ, Jin WL, Luo Y, Li X. Neoantigen specific T cells derived from T cell-derived induced pluripotent stem cells for the treatment of hepatocellular carcinoma: potential and challenges. *Front Immunol.* 2021;12:690565.
494. Zhu W, Peng Y, Wang L, Hong Y, Jiang X, Li Q, Liu H, Huang L, Wu J, Celis E, et al. Identification of alpha-fetoprotein-specific T-cell receptors for hepatocellular carcinoma immunotherapy. *Hepatology.* 2018;68:574–89.
495. Zhang H, Sun M, Wang J, Zeng B, Cao X, Han Y, Tan S, Gao GF. Identification of NY-ESO-1(157–165) specific murine T cell receptors with distinct recognition pattern for tumor immunotherapy. *Front Immunol.* 2021;12:644520.
496. Fujiwara K, Shao Y, Niu N, Zhang T, Herbst B, Henderson M, Muth S, Zhang P, Zheng L. Direct identification of HLA class I and class II-restricted T cell epitopes in pancreatic cancer tissues by mass spectrometry. *J Hematol Oncol.* 2022;15:154.
497. He Q, Jiang X, Zhou X, Weng J. Targeting cancers through TCR-peptide/MHC interactions. *J Hematol Oncol.* 2019;12:139.
498. Pavesi A, Tan AT, Koh S, Chia A, Colombo M, Antonocchia E, Miccolis C, Ceccarello E, Adriani G, Raimondi MT, et al. A 3D microfluidic model for preclinical evaluation of TCR-engineered T cells against solid tumors. *JCI Insight.* 2017;2(12):e89762.
499. Owen K, Ghaly R, Shohdy KS, Thistlethwaite F. Lymphodepleting chemotherapy practices and effect on safety and efficacy outcomes in patients with solid tumours undergoing T cell receptor-engineered T cell (TCR-T) Therapy: a systematic review and meta-analysis. *Cancer Immunol Immunother.* 2023;72:805–14.
500. Ni PP, Solomon B, Hsieh CS, Allen PM, Morris GP. The ability to rearrange dual TCRs enhances positive selection, leading to increased Allo- and Autoreactive T cell repertoires. *J Immunol.* 2014;193:1778–86.
501. Hale JS, Nelson LT, Simmons KB, Fink PJ. Bcl-2-interacting mediator of cell death influences autoantigen-driven deletion and TCR revision. *J Immunol.* 2011;186:799–806.
502. Lebrech H, Maier CC, Maki K, Ponce R, Shenton J, Green S. Nonclinical safety assessment of engineered T cell therapies. *Regul Toxicol Pharmacol.* 2021;127:105064.
503. Jones BS, Lamb LS, Goldman F, Di Stasi A. Improving the safety of cell therapy products by suicide gene transfer. *Front Pharmacol.* 2014;5:254.
504. Gschwend EH, McCracken MN, Kaufman ML, Ho M, Hollis RP, Wang X, Saini N, Koya RC, Chodon T, Ribas A, et al. HSV-sr39TK positron emission tomography and suicide gene elimination of human hematopoietic stem cells and their progeny in humanized mice. *Cancer Res.* 2014;74:5173–83.
505. Puig-Saus C, Parisi G, Garcia-Diaz A, Krystofinski PE, Sandoval S, Zhang R, Champhekar AS, McCabe J, Cheung-Lau GC, Truong NA, et al. IND-enabling studies for a clinical trial to genetically program a persistent cancer-targeted immune system. *Clin Cancer Res.* 2019;25:1000–11.
506. Ye B, Smerin D, Gao Q, Kang C, Xiong X. High-throughput sequencing of the immune repertoire in oncology: applications for clinical diagnosis, monitoring, and immunotherapies. *Cancer Lett.* 2018;416:42–56.
507. Holtstrater C, Schrors B, Bukur T, Lower M. Bioinformatics for cancer immunotherapy. *Methods Mol Biol.* 2020;2120:1–9.

508. Zhang H, Liu L, Zhang J, Chen J, Ye J, Shukla S, Qiao J, Zhan X, Chen H, Wu CJ, et al. Investigation of antigen-specific t-cell receptor clusters in human cancers. *Clin Cancer Res.* 2020;26:1359–71.
509. Liang Y, Xu Q, Liu S, Li J, Wang F, Li Z, Liao L, Lu Y, Li Y, Mu F, et al. Single-cell transcriptomics reveals killing mechanisms of antitumor cytotoxic CD4(+) TCR-T cells. *Front Immunol.* 2022;13:939940.
510. Asnagli H, Schmitt-Verhulst AM, Guimezanes A. Class I- and class II-reactive TCRs coexpressed on CD4+ T cells both trigger CD4/CD8-shared and CD4-unique functions. *J Immunol.* 1997;158:4533–42.
511. Wang JH, Reinherz EL. The structural basis of alphabeta T-lineage immune recognition: TCR docking topologies, mechanotransduction, and co-receptor function. *Immunol Rev.* 2012;250:102–19.
512. Carter JA, Preall JB, Grigaityte K, Goldfless SJ, Jeffery E, Briggs AW, Vigneault F, Atwal GS. Single T cell sequencing demonstrates the functional role of alphabeta TCR pairing in cell lineage and antigen specificity. *Front Immunol.* 2019;10:1516.
513. Scholten KB, Kramer D, Kueter EW, Graf M, Schoedl T, Meijer CJ, Schreurs MW, Hooijberg E. Codon modification of T cell receptors allows enhanced functional expression in transgenic human T cells. *Clin Immunol.* 2006;119:135–45.
514. Jones S, Peng PD, Yang S, Hsu C, Cohen CJ, Zhao Y, Abad J, Zheng Z, Rosenberg SA, Morgan RA. Lentiviral vector design for optimal T cell receptor gene expression in the transduction of peripheral blood lymphocytes and tumor-infiltrating lymphocytes. *Hum Gene Ther.* 2009;20:630–40.
515. Leisegang M, Engels B, Meyerhuber P, Kieback E, Sommermeyer D, Xue SA, Reuss S, Stauss H, Uckert W. Enhanced functionality of T cell receptor-redirection T cells is defined by the transgene cassette. *J Mol Med (Berl).* 2008;86:573–83.
516. Okamoto S, Amaishi Y, Goto Y, Ikeda H, Fujiwara H, Kuzushima K, Yasukawa M, Shiku H, Mineno J. A promising vector for TCR gene therapy: differential effect of siRNA, 2A peptide, and disulfide bond on the introduced TCR expression. *Mol Ther Nucleic Acids.* 2012;1:e63.
517. Susac L, Vuong MT, Thomas C, von Bulow S, O'Brien-Ball C, Santos AM, Fernandes RA, Hummer G, Tampe R, Davis SJ. Structure of a fully assembled tumor-specific T cell receptor ligated by pMHC. *Cell.* 2022;185:3201–13.
518. Zhao X, Kolawole EM, Chan W, Feng Y, Yang X, Gee MH, Jude KM, Sibener LV, Fordyce PM, Germain RN, et al. Tuning T cell receptor sensitivity through catch bond engineering. *Science.* 2022;376:15282.
519. Birnbaum ME, Berry R, Hsiao YS, Chen Z, Shingu-Vazquez MA, Yu X, Waghray D, Fischer S, McCluskey J, Rossjohn J, et al. Molecular architecture of the alphabeta T cell receptor-CD3 complex. *Proc Natl Acad Sci U S A.* 2014;111:17576–81.
520. Kuhns MS, Davis MM, Garcia KC. Deconstructing the form and function of the TCR/CD3 complex. *Immunity.* 2006;24:133–9.
521. Dong D, Zheng L, Lin J, Zhang B, Zhu Y, Li N, Xie S, Wang Y, Gao N, Huang Z. Structural basis of assembly of the human T cell receptor-CD3 complex. *Nature.* 2019;573:546–52.
522. Liu Y, Yan X, Zhang F, Zhang X, Tang F, Han Z, Li Y. TCR-T immunotherapy: the challenges and solutions. *Front Oncol.* 2021;11:794183.
523. Johnson LA, Morgan RA, Dudley ME, Cassard L, Yang JC, Hughes MS, Kammula US, Royal RE, Sherry RM, Wunderlich JR, et al. Gene therapy with human and mouse T-cell receptors mediates cancer regression and targets normal tissues expressing cognate antigen. *Blood.* 2009;114:535–46.
524. Yu B, Shi Q, Belk JA, Yost KE, Parker KR, Li R, Liu BB, Huang H, Lingwood D, Greenleaf WJ, et al. Engineered cell entry links receptor biology with single-cell genomics. *Cell.* 2022;185:4904–20.
525. Zhang M, Racine JJ, Lin Q, Liu Y, Tang S, Qin Q, Qi T, Riggs AD, Zeng D. MHC-mismatched mixed chimerism restores peripheral tolerance of noncross-reactive autoreactive T cells in NOD mice. *Proc Natl Acad Sci U S A.* 2018;115:E2329–37.
526. Tao C, Shao H, Yuan Y, Wang H, Zhang W, Zheng W, Ma W, Huang S. Imaging of T-cell receptor fused to CD3zeta reveals enhanced expression and improved pairing in living cells. *Int J Mol Med.* 2014;34:849–55.
527. Szeto C, Zareie P, Wirasinha RC, Zhang JB, Nguyen AT, Riboldi-Tunnlicliff A, La Gruta NL, Gras S, Daley SR. Covalent TCR-peptide-MHC interactions induce T cell activation and redirect T cell fate in the thymus. *Nat Commun.* 2022;13:4951.
528. Schultz A, Schnurra M, El-Bizri A, Woessner NM, Hartmann S, Hartig R, Minguet S, Schraven B, Simeoni L. A cysteine residue within the kinase domain of Zap70 regulates Lck activity and proximal TCR signaling. *Cells.* 2022;11(17):2723.
529. Sun Q, Zhang X, Wang L, Gao X, Xiong Y, Liu L, Wei F, Yang L, Ren X. T-cell receptor gene therapy targeting melanoma-associated antigen-A4 by silencing of endogenous TCR inhibits tumor growth in mice and human. *Cell Death Dis.* 2019;10:475.
530. Okamoto S, Mineno J, Ikeda H, Fujiwara H, Yasukawa M, Shiku H, Kato I. Improved expression and reactivity of transduced tumor-specific TCRs in human lymphocytes by specific silencing of endogenous TCR. *Cancer Res.* 2009;69:9003–11.
531. Morandi F, Yazdanifar M, Cocco C, Bertaina A, Airoidi I. Engineering the bridge between innate and adaptive immunity for cancer immunotherapy: focus on gammadelta T and NK cells. *Cells.* 2020;9(8):1757.
532. Li X, Zhu X, Zhang X, Wang W. Successful treatment of a pure red-cell aplasia patient with gammadeltaT cells and clonal TCR gene rearrangement: a case report. *Front Immunol.* 2022;13:1103448.
533. Ferry GM, Agbuduwe C, Forrester M, Dunlop S, Chester K, Fisher J, Anderson J, Barisa M. A simple and robust single-step method for CAR-Vdelta1 gammadeltaT cell expansion and transduction for cancer immunotherapy. *Front Immunol.* 2022;13:863155.
534. Saito T. Molecular dynamics of co-signal molecules in T-cell activation. *Adv Exp Med Biol.* 2019;1189:135–52.
535. Dong G, Kalifa R, Nath PR, Babichev Y, Gelkop S, Isakov N. Crk adaptor proteins regulate CD3zeta chain phosphorylation and TCR/CD3 down-modulation in activated T cells. *Cell Signal.* 2017;36:117–26.
536. Kagoya Y, Tanaka S, Guo T, Anczurowski M, Wang CH, Saso K, Butler MO, Minden MD, Hirano N. A novel chimeric antigen receptor containing a JAK-STAT signaling domain mediates superior antitumor effects. *Nat Med.* 2018;24:352–9.
537. Xu Y, Chen C, Guo Y, Hu S, Sun Z. Effect of CRISPR/Cas9-Edited PD-1/PD-L1 on tumor immunity and immunotherapy. *Front Immunol.* 2022;13:848327.
538. Liu Q. World-first phase I clinical trial for CRISPR-Cas9 PD-1-Edited T-Cells in advanced nonsmall cell lung cancer. *Glob Med Genet.* 2020;7:73–4.
539. Choi BD, Yu X, Castano AP, Darr H, Henderson DB, Bouffard AA, Larson RC, Scarfo I, Bailey SR, Gerhard GM, et al. CRISPR-Cas9 disruption of PD-1 enhances activity of universal EGFRvIII CART cells in a preclinical model of human glioblastoma. *J Immunother Cancer.* 2019;7:304.
540. Wang TW, Johmura Y, Suzuki N, Omori S, Migita T, Yamaguchi K, Hatakeyama S, Yamazaki S, Shimizu E, Imoto S, et al. Blocking PD-L1-PD-1 improves senescence surveillance and ageing phenotypes. *Nature.* 2022;611:358–64.
541. Li C, Zhang N, Zhou J, Ding C, Jin Y, Cui X, Pu K, Zhu Y. Peptide blocking of PD-1/PD-L1 interaction for cancer immunotherapy. *Cancer Immunol Res.* 2018;6:178–88.
542. Jing Y, Kong Y, McGinty J, Blahnik-Fagan G, Lee T, Orozco-Figueroa S, Bettini ML, James EA, Bettini M. T-Cell Receptor/HLA humanized mice reveal reduced tolerance and increased immunogenicity of posttranslationally modified GAD65 epitope. *Diabetes.* 2022;71:1012–22.
543. Andrade FP, Ito D, Deleo AB, Ferris RL. CD8+ T cell recognition of polymorphic wild-type sequence p53(65–73) peptides in squamous cell carcinoma of the head and neck. *Cancer Immunol Immunother.* 2010;59:1561–8.
544. Sorensen RB, Andersen RS, Svane IM, Engell-Noerregaard L, Hadrup SR, Balslev E, Andersen MH, Thor SP. CD8 T-cell responses against cyclin B1 in breast cancer patients with tumors overexpressing p53. *Clin Cancer Res.* 2009;15:1543–9.
545. Mayr C, Bund D, Schlee M, Bamberger M, Kofler DM, Hallek M, Wendtner CM. MDM2 is recognized as a tumor-associated antigen in chronic lymphocytic leukemia by CD8+ autologous T lymphocytes. *Exp Hematol.* 2006;34:44–53.
546. Antonilli M, Rahimi H, Visconti V, Napoletano C, Ruscito I, Zizzari IG, Caponnetto S, Barchiesi G, Iadarola R, Pierelli L, et al. Triple peptide vaccination as consolidation treatment in women affected by ovarian and breast cancer: Clinical and immunological data of a phase I/II clinical trial. *Int J Oncol.* 2016;48:1369–78.
547. Smith JN, Walker EB, Fox BA, Haley D, Wisner KP, Doran T, Fisher B, Justice L, Wood W, Vetto J, et al. Adjuvant immunization of HLA-A2-positive

- melanoma patients with a modified gp100 peptide induces peptide-specific CD8⁺ T-cell responses. *J Clin Oncol.* 2003;21:1562–73.
548. Chapuis AG, Egan DN, Bar M, Schmitt TM, McAfee MS, Paulson KG, Voillet V, Gottardo R, Ragnarsson GB, Bleakley M, et al. T cell receptor gene therapy targeting WT1 prevents acute myeloid leukemia relapse post-transplant. *Nat Med.* 2019;25:1064–72.
549. Arber C, Feng X, Abhyankar H, Romero E, Wu MF, Heslop HE, Barth P, Dotti G, Savoldo B. Survivin-specific T cell receptor targets tumor but not T cells. *J Clin Invest.* 2015;125:157–68.
550. Stanislawski T, Voss RH, Lotz C, Sadovnikova E, Willemsen RA, Kuball J, Ruppert T, Bolhuis RL, Melief CJ, Huber C, et al. Circumventing tolerance to a human MDM2-derived tumor antigen by TCR gene transfer. *Nat Immunol.* 2001;2:962–70.
551. Asai T, Storkus WJ, Mueller-Berghaus J, Knapp W, DeLeo AB, Chikamatsu K, Whiteside TL. In vitro generated cytolytic T lymphocytes reactive against head and neck cancer recognize multiple epitopes presented by HLA-A2, including peptides derived from the p53 and MDM-2 proteins. *Cancer Immun.* 2002;2:3.
552. Mensali N, Ying F, Sheng VO, Yang W, Walseng E, Kumari S, Fallang LE, Kolstad A, Uckert W, Malmberg KJ, et al. Targeting B-cell neoplasia with T-cell receptors recognizing a CD20-derived peptide on patient-specific HLA. *Oncoimmunology.* 2016;5:e1138199.
553. Yang X, Nishimiya D, Lochte S, Jude KM, Borowska M, Savvides CS, Dougan M, Su L, Zhao X, Piehler J, Garcia KC. Facile repurposing of peptide-MHC-restricted antibodies for cancer immunotherapy. *Nat Biotechnol.* 2023;41(7):932–43.
554. Yu K, Shi J, Lu D, Yang Q. Comparative analysis of CDR3 regions in paired human alpha beta CD8 T cells. *FEBS Open Bio.* 2019;9:1450–9.
555. Alli R, Zhang ZM, Nguyen P, Zheng JJ, Geiger TL. Rational design of T cell receptors with enhanced sensitivity for antigen. *PLoS ONE.* 2011;6:e18027.
556. Kamimura D, Atsumi T, Stofkova A, Nishikawa N, Ohki T, Suzuki H, Katsunuma K, Jiang JJ, Bando H, Meng J, et al. Naive T cell homeostasis regulated by stress responses and TCR signaling. *Front Immunol.* 2015;6:638.
557. Ch'Ng A, Lam P, Alassiri M, Lim TS. Application of phage display for T-cell receptor discovery. *Biotechnol Adv.* 2022;54:107870.
558. Birnbaum ME, Mendoza JL, Sethi DK, Dong S, Glanville J, Dobbins J, Ozkan E, Davis MM, Wucherpfennig KW, Garcia KC. Deconstructing the peptide-MHC specificity of T cell recognition. *Cell.* 2014;157:1073–87.
559. Pan YG, Aiamkitumrit B, Bartolo L, Wang Y, Lavery C, Marc A, Holec PV, Rappazzo CG, Eilola T, Gimotty PA, et al. Vaccination reshapes the virus-specific T cell repertoire in unexposed adults. *Immunity.* 2021;54:1245–56.
560. Sun Y, Li F, Sonnemann H, Jackson KR, Talukder AH, Katailaha AS, Lizee G. Evolution of CD8⁺ T cell receptor (TCR) engineered therapies for the treatment of cancer. *Cells.* 2021;10(9):2379.
561. Jin Y, Dong Y, Zhang J, Sun J, Liu Y, Chen Y. The toxicity of cell therapy: mechanism, manifestations, and challenges. *J Appl Toxicol.* 2021;41:659–67.
562. Anderson VE, Brilha SS, Weber AM, Pachnio A, Wiedermann GE, Dauleh S, Ahmed T, Pope GR, Quinn LL, Docta RY, et al. Enhancing efficacy of TCR-engineered CD4⁺ T cells via coexpression of CD8alpha. *J Immunother.* 2023;46(4):132–44.
563. Chhabra A, Mukherji B. Death receptor-independent activation-induced cell death in human melanoma antigen-specific MHC class I-restricted TCR-engineered CD4 T cells. *J Immunol.* 2013;191:3471–7.
564. Dillard P, Koksai H, Maggadottir SM, Winge-Main A, Pollmann S, Menard M, Myhre MR, Maeldansmo GM, Florenes VA, Gaudernack G, et al. Targeting telomerase with an HLA class II-restricted TCR for cancer immunotherapy. *Mol Ther.* 2021;29:1199–213.
565. Poncette L, Chen X, Lorenz FK, Blankenstein T. Effective NY-ESO-1-specific MHC II-restricted T cell receptors on antigen-negative hosts enhance tumor regression. *J Clin Invest.* 2019;129:324–35.
566. Allen GM, Frankel NW, Reddy NR, Bhargava HK, Yoshida MA, Stark SR, Puri M, Lee J, Yee JL, Yu W, et al. Synthetic cytokine circuits that drive T cells into immune-excluded tumors. *Science.* 2022;378:a1624.
567. Schizas D, Charalampakis N, Kole C, Economopoulou P, Koustas E, Gkotsis E, Ziogas D, Psyrri A, Karamouzis MV. Immunotherapy for pancreatic cancer: a 2020 update. *Cancer Treat Rev.* 2020;86:102016.
568. Mastaglio S, Genovese P, Magnani Z, Ruggiero E, Landoni E, Camisa B, Schirolli G, Provasi E, Lombardo A, Reik A, et al. NY-ESO-1 TCR single edited stem and central memory T cells to treat multiple myeloma without graft-versus-host disease. *Blood.* 2017;130:606–18.
569. Starck L, Popp K, Pircher H, Uckert W. Immunotherapy with TCR-redirectioned T cells: comparison of TCR-transduced and TCR-engineered hematopoietic stem cell-derived T cells. *J Immunol.* 2014;192:206–13.
570. Zhang Z, Li F, Tian Y, Cao L, Gao Q, Zhang C, Zhang K, Shen C, Ping Y, Maimela NR, et al. Metformin enhances the antitumor activity of CD8⁺ T lymphocytes via the AMPK-miR-107-Eomes-PD-1 pathway. *J Immunol.* 2020;204:2575–88.
571. Kishton RJ, Lynn RC, Restifo NP. Strength in numbers: identifying neoantigen targets for cancer immunotherapy. *Cell.* 2020;183:591–3.
572. Joglekar AV, Leonard MT, Jeppson JD, Swift M, Li G, Wong S, Peng S, Zaretsky JM, Heath JR, Ribas A, et al. T cell antigen discovery via signaling and antigen-presenting bifunctional receptors. *Nat Methods.* 2019;16:191–8.
573. Chandran SS, Ma J, Klatt MG, Dundar F, Bandlamudi C, Razavi P, Wen HY, Weigelt B, Zumbo P, Fu SN, et al. Immunogenicity and therapeutic targeting of a public neoantigen derived from mutated PIK3CA. *Nat Med.* 2022;28:946–57.
574. Pearlman AH, Hwang MS, Konig MF, Hsue EH, Douglass J, DiNapoli SR, Mog BJ, Bettegowda C, Pardoll DM, Gabelli SB, et al. Targeting public neoantigens for cancer immunotherapy. *Nat Cancer.* 2021;2:487–97.
575. Armistead PM. Cellular therapy against public neoantigens. *J Clin Invest.* 2019;129:506–8.
576. Janes MR, Zhang J, Li LS, Hansen R, Peters U, Guo X, Chen Y, Babbar A, Firdaus SJ, Darjania L, et al. Targeting KRAS mutant cancers with a covalent G12C-specific inhibitor. *Cell.* 2018;172:578–89.
577. Lai LP, Fer N, Burgan W, Wall VE, Xu B, Soppet D, Esposito D, Nissley DV, McCormick F. Classical RAS proteins are not essential for paradoxical ERK activation induced by RAF inhibitors. *Proc Natl Acad Sci U S A.* 2022;119(5):e2113491119.
578. Ye C, Lin S, Hong J, Liu F, Guo G, Chen S, Zhou Z, Lin W, Li J, Ye Y. Altered HLA-A2-restricted TP53 epitope induces specific CTL cytotoxicity against hepatocellular carcinoma. *Eur J Immunol.* 2023;53(5):e2250054.
579. Malekzadeh P, Yossef R, Cafri G, Paria BC, Lowery FJ, Jafferji M, Good ML, Sachs A, Copeland AR, Kim SP, et al. Antigen experienced T cells from peripheral blood recognize p53 neoantigens. *Clin Cancer Res.* 2020;26:1267–76.
580. Guo Y, Lei K, Tang L. Neoantigen vaccine delivery for personalized anticancer immunotherapy. *Front Immunol.* 2018;9:1499.
581. Tang L, Zhang R, Zhang X, Yang L. Personalized neoantigen-pulsed DC vaccines: advances in clinical applications. *Front Oncol.* 2021;11:701777.
582. Yu J, Sun H, Cao W, Song Y, Jiang Z. Research progress on dendritic cell vaccines in cancer immunotherapy. *Exp Hematol Oncol.* 2022;11:3.
583. Tanyi JL, Bobisse S, Ophir E, Tuytaerts S, Roberti A, Genolet R, Baumgartner P, Stevenson BJ, Iseli C, Dangaj D, et al. Personalized cancer vaccine effectively mobilizes antitumor T cell immunity in ovarian cancer. *Sci Transl Med.* 2018;10(436):eaao5931.
584. El-Ashrawy NE, El-Zamarany EA, Khedr EG, El-Bahrawy HA, El-Feky OA. Immunotherapeutic strategies for treatment of hepatocellular carcinoma with antigen-loaded dendritic cells: in vivo study. *Clin Exp Med.* 2018;18:535–46.
585. El-Ashrawy NE, El-Zamarany EA, Khedr EG, El-Bahrawy HA, El-Feky OA. Antigen-loaded dendritic cells triggers a specific cytotoxic T lymphocytes immune response against hepatocellular carcinoma: in vitro study. *Clin Transl Oncol.* 2019;21:636–45.
586. Fernandez-Delgado I, Calzada-Fraile D, Sanchez-Madrid F. Immune regulation by dendritic cell extracellular vesicles in cancer immunotherapy and vaccines. *Cancers (Basel).* 2020;12(12):3558.
587. Kowal J, Tkach M. Dendritic cell extracellular vesicles. *Int Rev Cell Mol Biol.* 2019;349:213–49.
588. Schierer S, Ostalecki C, Zinser E, Lamprecht R, Plosnita B, Stich L, Dorrie J, Lutz MB, Schuler G, Baur AS. Extracellular vesicles from mature dendritic cells (DC) differentiate monocytes into immature DC. *Life Sci Alliance.* 2018;1:e201800093.

589. Silva AM, Almeida MI, Teixeira JH, Maia AF, Calin GA, Barbosa MA, Santos SG. Dendritic cell-derived extracellular vesicles mediate mesenchymal stem/stromal cell recruitment. *Sci Rep.* 2017;7:1667.
590. Richard G, Princiotta MF, Bridon D, Martin WD, Steinberg GD, De Groot AS. Neoantigen-based personalized cancer vaccines: the emergence of precision cancer immunotherapy. *Expert Rev Vaccines.* 2022;21:173–84.
591. Lee SE, Jang GY, Lee JW, Park SH, Han HD, Park YM, Kang TH. Improvement of STING-mediated cancer immunotherapy using immune checkpoint inhibitors as a game-changer. *Cancer Immunol Immunother.* 2022;71:3029–42.
592. Na IK, Keilholz U, Letsch A, Bauer S, Asemissen AM, Nagorsen D, Thiel E, Scheibenbogen C. Addition of GM-CSF to a peptide/KLH vaccine results in increased frequencies of CXCR3-expressing KLH-specific T cells. *Cancer Immunol Immunother.* 2007;56:391–6.
593. Lookian PP, Zhao D, Medina R, Wang H, Zenka J, Gilbert MR, Pacak K, Zhuang Z. Mannan-BAM, TLR Ligands, Anti-CD40 Antibody (MBTA) vaccine immunotherapy: a review of current evidence and applications in Glioblastoma. *Int J Mol Sci.* 2021;22(7):3455.
594. Zhou L, Hou B, Wang D, Sun F, Song R, Shao Q, Wang H, Yu H, Li Y. Engineering polymeric prodrug nanoplatfor for vaccination immunotherapy of cancer. *Nano Lett.* 2020;20:4393–402.
595. Ni Q, Zhang F, Liu Y, Wang Z, Yu G, Liang B, Niu G, Su T, Zhu G, Lu G, et al. A bi-adjuvant nanovaccine that potentiates immunogenicity of neoantigen for combination immunotherapy of colorectal cancer. *Sci Adv.* 2020;6:w6071.
596. Xiong X, Ke X, Wang L, Lin Y, Wang S, Yao Z, Li K, Luo Y, Liu F, Pan Y, et al. Neoantigen-based cancer vaccination using chimeric RNA-loaded dendritic cell-derived extracellular vesicles. *J Extracell Vesicles.* 2022;11:e12243.

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