Review Article

Clinical research progress of telomerase targeted cancer immunotherapy: a literature review

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Background and Objective: Telomerase is activated or overexpressed in 85–90% of tumors, which maintains the length of telomere and has become an important anti-cancer target. Increasing clinical and preclinical data suggest that telomerase-targeted cancer immunotherapy could achieve effective killing of tumor cells *in vivo*. This article reviews the research progress of telomerase targeted cancer immunotherapy in clinical and pre-clinical trials, aiming to provide a reference for further clinical research and treatment of cancers.

Methods: We investigated the research progress of telomerase immunotherapy in the last 20 years from four electronic databases.

Key Content and Findings: Telomerase-targeted immunotherapies have been developed with the arising of a new era in immuno-oncology, including peptide vaccines, DNA vaccines, dendritic cells (DCs), adoptive cell transfer (ACT) therapies, antibodies, etc. Some of them have been approved for undergoing clinical trials by the Food and Drug Administration (FDA) for the treatment of various cancers, such as pancreatic cancer, non-small cell lung cancer, melanoma, leukaemia. Of all the treatment modalities, vaccines are the primary treatment methods, some of which have been even entered into phase III clinical trials. The main clinical application direction of telomerase vaccine is the combination with other drugs and treatment modalities, including combination with other vaccines targeting human telomerase reverse transcriptase (hTERT), traditional chemotherapy drugs and immunosuppressors. We also summarized the recent findings of immunotherapy targeting hTERT, focusing on various vaccines and the current status of associated clinical trials. We further discussed the advantages, disadvantages and potential developmental directions of various telomerase-targeted immunotherapies.

Conclusions: Telomerase-targeted cancer immunotherapy has promising prospects in improving patient survival expectancy. This review may provide data support and design ideas for all researchers and pharmaceutical enterprises in this field.

Keywords: Telomerase; immunotherapy; vaccine; cancer; targeted therapy

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Introduction

Cancer immunotherapy is a treatment approach that aims to arm patients with cancer-fighting immunity (1). Unlike traditional treatments (surgery, chemotherapy and radiotherapy), immunotherapy focuses on the patient's own immune system rather than cancer cells themselves or pathogenic microorganisms, resulting in fewer side effects, better survival, and a wider range of oncology indications. The key to its success lies in the identification of effective tumor-associated antigens (TAAs) (2).

The human cancer-fighting immune system includes both innate and adaptive immune responses. The innate immune response, where TAAs can directly or indirectly activate macrophages and natural killer (NK) cells and induce their anticancer functions, emerges earlier than the adaptive immune response after the stimulation of TAAs (3,4). However, the adaptive immune response including cellular immunity and humoral immunity, plays a more important role in specific anticancer responses and can be capable of producing an immunological memory effect. The cellular immunity is the main force, while the humoral immunity usually plays a synergistic role in some cases relying on antibodies produced by plasmocytes. In human cellular immunity, antigen-presenting cells (APCs) are able to internalize, process and degrade TAAs into peptide segments of different length, which can bind to major histocompatibility complex (MHC) class I or II molecules, for display on the surface of the APCs. The T-cell receptor (TCR) on the surface of $CD8^+$ or $CD4^+$ T cells recognizes antigenic peptide-MHC class I or II complexes, resulting in the activation of cytotoxic T lymphocytes (CTLs) and T helper cells (Th cells) (5). Therefore, TAAs can initiate multiple immune responses against cancer cells. Identifying safe and efficient TAAs is crucial for cancer immunotherapy.

Telomerase is a ribonucleoprotein complex composed of RNA (hTR) and protein (human telomerase reverse transcriptase, hTERT) that has the functions of maintaining telomere length and genome stability (6). Telomerase is mainly expressed in germ and stem cells and is not expressed or expressed at only low levels in normal somatic cells. However, it is highly expressed in over 85% of cancer cells, enabling cells to undergo immortalization, which is the necessary for cellular carcinogenesis (7,8). Studies have shown that the complexes formed by short and long peptides derived from hTERT combined with MHC class I and II molecules are able to induce CTL- and Th cell-mediated cellular immune responses *in vitro* (9,10). Therefore, hTERT is considered a universal TAA and could be an ideal target for cancer immunotherapy (11) (*Figure 1*). In fact, this conclusion has been confirmed by many preclinical and clinical studies (*Table 1*) (12,13).

Current hTERT-based cancer immunotherapies include therapeutic vaccines, cellular therapies, and antibodies. All of these types of approaches include some agents that have entered clinical trials approved by the Food and Drug Administration (FDA), including phase III trials. Phase I clinical trials include preliminary clinical pharmacology and human safety evaluation tests to provide a basis for the formulation of a dosing regimen. Phase II clinical trials assessing the safety and tolerability of the drug, are usually larger than phase I studies. Phase III clinical trials are conducted in a larger and often more diverse target population in order to demonstrate efficacy and to estimate the incidence of common adverse reactions (28). Although no hTERT-based cancer immunotherapy has been authorized for commercial use, hTERT-based immunotherapy is still a focus of many pharmaceutical companies, and more than ten clinical trials are currently recruiting (3,29). This review focuses on the therapeutic strategies and current evidence of hTERT-based cancer immunotherapy and discusses its future perspectives. We present this article in accordance with the Narrative Review reporting checklist (available at https://tcr.amegroups.com/article/view/10.21037/tcr-24-196/rc).

Methods

We searched and studied relevant literature in the databases PubMed, CNKI, and ClinicalTrials.gov using the keywords "telomerase", "cancer" or "tumor", "immunotherapy", etc. We read both English and Chinese research literatures, but only adopted research articles written in English. In addition, secondary references cited in the articles retrieved by PubMed were also retrieved. *Table 2* summarizes the search methodology.

Therapeutic vaccines

Therapeutic vaccines, as an active immunotherapy, aims to using TAAs to induce durable and specific anti-tumor immune responses, which are well tolerated and almost no dose-related toxicity (30). Current therapeutic vaccines include peptide vaccine, mRNA vaccine, cell vaccine, virus vector vaccine and so on. For hTERT, the main types of therapeutic cancer vaccines are peptide vaccines and DNA

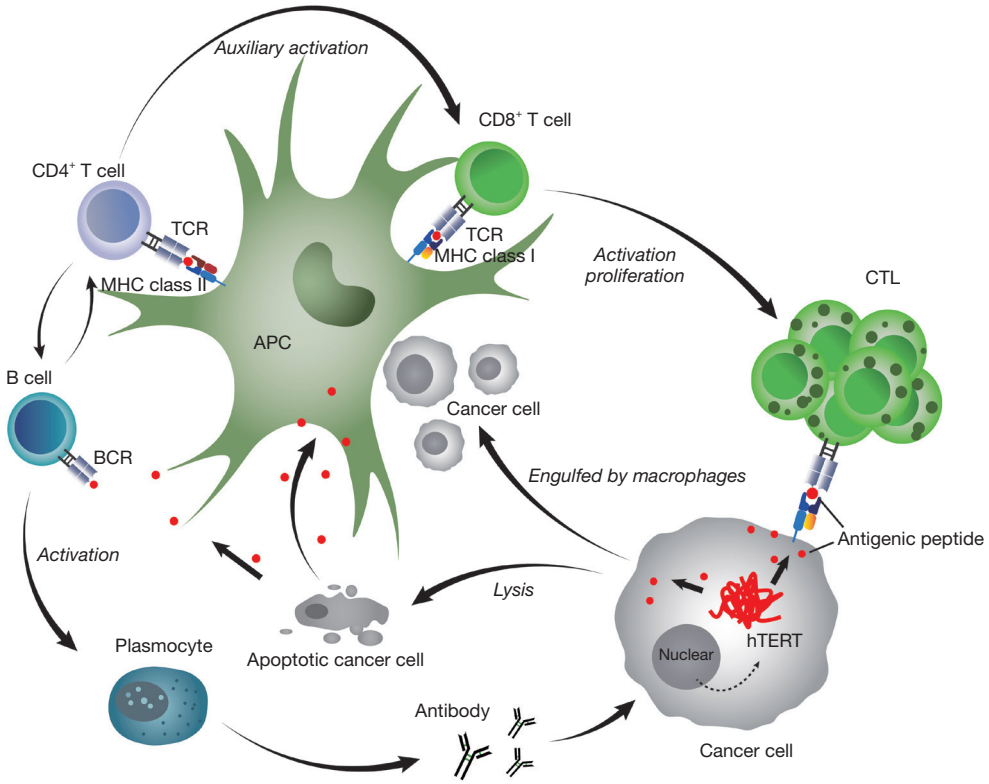

Figure 1 Cellular and humoral immune responses induced by hTERT. The hTERT protein produced by cancer cells binds to MHC class I molecules in tumor cells to form antigenic peptide-MHC class I molecule complexes that are presented on the cell surface. CTLs recognize and bind to these complexes and subsequently release perforin, granzyme, cytokines, etc. to kill cancer cells. Following cell death by apoptosis or necrosis, cancer cells release tumor antigens that are phagocytosed by APCs (including DCs and B lymphocytes). APCs present antigenic peptides (derived from hTERT)-MHC class I or II complexes to $CD8^+$ T cells and $CD4^+$ T cells, respectively. Activated $CD8^+$ T cells differentiate into CTLs and proliferate; these cells are recruited to the tumor site and further kill cancer cells. In contrast, $CD4^+$ T cells can differentiate into various types of Th cells and release various cytokines to help activate $CD8^+$ T cells and B cells and enhance the killing activity of CTLs. B cells are activated into plasmocyte under the combined action of antigenic peptides and Th cells, and plasmocytes synthesize and secrete a large number of antibodies to exert antitumor effect. TCR, T cell receptor; MHC, major histocompatibility complex; APC, antigen-presenting cell; CTL, cytotoxic T lymphocyte; BCR, B cell receptor; hTERT, human telomerase reverse transcriptase; DC, dendritic cell.

vaccines, which are steadily entering clinical trials and have achieved good clinical therapeutic effects (31).

Peptide vaccines

Peptide vaccines are the most widely studied and applied telomerase-related therapeutic vaccines to date. After investigated *in vitro* (cell-based) as well as *in vivo* (animal experiment), the hTERT-derived peptide vaccine is inoculated into humans. After uptake and internalization by APCs, it is presented on the APC surface in the form of antigenic peptide-MHC complexes to induce T-cell activation and proliferation, thereby initiating and enhancing adaptive immune responses. Initially, it was generally believed that MHC class I-restricted $CD8^+$ CTLs were the main effector cells involved in killing cancer cells; therefore, early vaccines were usually short peptides (usually ≤10 aa) (32). Later, with advancements in research on the immune system, Th1 cells were shown to exert multiple functions in the entire cancer-immune cycle including seven steps (T cells neither respond nor work on their own, but exist in the context of a series of steps, some of which are even extrinsic to the immune system and the cancer) (33), making long-peptide vaccines widely studied. These

Table 1 Clinical trials of peptide vaccines related to hTERT

Name	Cancer targeted	Clinical trial phase	Clinical results/status	NCT number	Year*	Ref.
GV1001	BPH	Phase II	Effective and well tolerated/completed		2018	(12)
		Phase III	No clear clinical results/completed	NCT04032067	2022	
	Melanoma	Phase I/II	Considerable immune responses and well tolerated/completed	NCT01247623	2011	(13)
	NSCLC	Phase II	Well-tolerated and mild toxic reaction/unknown	NCT01579188	2011	(14)
	HCC	Phase II	Tolerated but no considerable immune responses/completed	NCT00444782	2010	(15)
	Colorectal cancer	Phase II	Tolerated but no considerable immune responses/completed		2022	(16)
	Pancreatic cancer	Phase III	No improvement in overall survival/completed	NCT00425360	2014	(17)
p540	Brain and central nervous system tumors/gastrointestinal stromal tumor/sarcoma	Phase I	No clear clinical results/completed	NCT00069940	2010	
	Cutaneous melanoma	Phase I	Immune responses and well tolerated/completed		2011	(18)
	Metastatic cancer	Phase II	No clear clinical results/completed	NCT00021164	2013	
	Breast cancer	Phase I	No clear clinical results/completed	NCT00079157	2020	
UV1	NSCLC	Phase II	Immune responses and well tolerated/active, not recruiting	NCT01789099	2020	(19)
	Malignant mesothelioma	Phase II	Well tolerated/recruiting	NCT04300244	2021	(20)
	Metastatic melanoma	Phase I/II	Well tolerated and mild vaccination-related adverse events/completed	NCT03538314	2021	(21)
	Malignant melanoma/NSCLC/prostate cancer	Phase I	Considerable immune responses, well tolerated and safe/completed		2022	(22)
	NSCLC/melanoma/ovarian cancer/head and neck squamous cell carcinoma	Phase II	No clear clinical results/recruiting	NCT05344209/NCT04382664/NCT04742075/NCT05075122	2022	
GX301	Prostate and renal cancer	Phase I/II	Immune responses and well tolerated/completed		2013	(23)
	mCRPC	Phase II	Considerable immune responses positively correlated with dosage and safe/completed	NCT02293707	2021	(24)
Vx-001	NSCLC	Phase II	Specific CD8 immune response induction but failed to meet its primary endpoint/completed	NCT01935154	2020	(25)
UCPVax	NSCLC	Phase I/II	Considerable immune responses and safe/active, not recruiting	NCT02818426	2023	(26)
	HPV-positive cancers (squamous cell carcinoma of the head and neck/anal canal cancer/cervical cancer)/HCC/glioblastoma/NSCLC	Phase II	No clear clinical results/recruiting	NCT03946358/NCT05528952/NCT04280848/NCT04263051	2022	(27)

*, year represents either the year of publication or year of the last trial update. hTERT, human telomerase reverse transcriptase; BPH, benign prostatic hyperplasia; NSCLC, non-small cell lung cancer; HCC, hepatocellular carcinoma; mCRPC, metastatic castration-resistant prostate cancer; HPV, human papillomavirus; NCT, National Clinical Trial.

Table 2 The search strategy summary

Items	Specification
Date of search	29/1/2024
Databases and other sources searched	PubMed, CNKI, ClinicalTrials.gov
Search terms used	"Telomerase", "hTERT", "cancer or tumor", "immunotherapy", "peptide vaccine", "DNA vaccine", "cellular therapy", "adoptive cell transfer therapy", etc.
Timeframe	27/9/1994–29/1/2024
Inclusion	In terms of content, we first selected the relevant review literature in the past 5 years, and then searched the relevant clinical research results according to each therapy. In languages, we read English and Chinese research literatures
Selection process	Four authors selected studies together

hTERT, human telomerase reverse transcriptase.

vaccines can not only activate $CD4^+$ Th cells but may also generate a more comprehensive and efficient cellular immune response through cross-presentation by APCs (34,35). Compared with DNA and cell-based vaccines, peptide vaccines are safer and easier to synthesize and administer and have a longer shelf life; thus, they are often used as the first choice for vaccines. In addition, a variety of hTERT-derived peptide vaccines, such as GV1001, GX301, and GRNVAC1, have been designed, and these vaccines have been preclinically screened and approved for evaluation in a variety of tumors in clinical trials, with some peptide vaccines even entering phase III clinical trials (36).

GV1001

GV1001 is an MHC class II-restricted peptide vaccine containing 16 amino acids from the active site of hTERT (hTERT611-626, EARPALLTSRLRFIPK) (37). GV1001 is the only market-approved hTERT vaccine and was the first clinically evaluated therapeutic vaccine targeting telomerase. Because more than 90% of cancers have been shown to exhibit high expression of hTERT, GV1001 was originally developed as a broad-spectrum peptide vaccine for cancers and has been found to be safe in several clinical trials in pancreatic cancer (38), prostate cancer (39,40), melanoma (41), non-small cell lung cancer (NSCLC) (14) and hepatocellular carcinoma (HCC) (15). The results of a study on renal cell carcinoma patients showed that GV1001 could induce apoptosis by significantly reduced angiogenesis through regulation of hypoxia-inducible factor *in vitro* and *in vivo*, indicating that GV1001 may be an effective therapeutic target for renal cell carcinoma (42). In addition, GV1001 was found to harbour various human leukocyte antigen (HLA)-class I or II epitopes, which means inoculation of GV1001 into lung cancer patients can potentially elicit combined $CD8^+$ CTL and $CD4^+$ Th cell immune responses (43).

The combined application of GV1001 with other hTERT peptide vaccines, chemotherapeutic drugs or radiotherapy results in improved clinical therapeutic effects. A clinical phase I/II study of patients with NSCLC showed that the combination of GV1001 and HR2822 (hTERT540-548, ILAKFLHWL) induced a safer and more effective immune response, with no significant effect on the bone marrow, suggesting that combination therapy with an hTERT peptide vaccine may be more effective than vaccination alone (44). In addition, the combination of GV1001 with granulocyte-macrophage colony-stimulating factor (GM-CSF) and temozolomide has shown certain benefits (18,45). For instance, a clinical trial examined the effects in 25 melanoma patients treated with GV1001 and temozolomide. In that study, as measured by assessment of delayed-type hypersensitivity, T-cell proliferation, and cytokine levels, the patient immune response rate was considerable, with low toxicity, supporting the benefit of the combination of cancer vaccines with chemotherapy (13). To evaluate the immune response, toxicity, and clinical outcomes following GV1001 vaccination, Brunsvig *et al.* (14) performed an 8-year phase II clinical trial of patients with NSCLC using a two treatment regimen, one of which was radiation therapy and chemotherapy followed by GV1001 administration, and the other was a combination of GV1001 and p540 peptides. The results verified that GV1001 vaccination was well tolerated and could induce immune responses in most NSCLC patients and establish

a durable T-cell memory, with a considerable immune response rate and low toxicity, supporting the application of chemoradiotherapy in conjunction with vaccination (14). In a recent phase II clinical trial, 56 patients with metastatic colorectal cancer (CRC) from seven hospitals in Korea who received GV1001 in combination with chemotherapy showed tolerance of the regimen, which was associated with modest efficacy outcomes. Although no obvious immune response was observed, this was the first clinical trial of a telomerase vaccine in CRC patients (16). Another study showed that GV1001 in combination with gemcitabine resulted in a significant loss of intratumoral fibrous tissue and death of pancreatic ductal cancer cells (46). However, in this phase III clinical trial, GV1001 in combination with gemcitabine and capecitabine did not improve the survival of patients with advanced pancreatic cancer (17).

P540

P540, hTERT540-548 (ILAKFLHWL), was the first reported immunogenic hTERT peptide. Minev *et al.* showed that p540-specific CTLs could be generated *in vitro* and recognize tumor cells derived from different tissue types, resulting in strong specific CTL responses in mice (47). Ayyoub *et al.* stimulated highly enriched $CD8^+$ cells from melanoma patients PBMC (peripheral blood mononuclear cells) with synthetic hTERT540 peptide (the hTERT-derived peptide 540-548aa) in the presence of autologous APCs and cytokines. While the hTERT540 peptide was recognized by HLA-A0201-restricted T cell lines derived from healthy donors, these specific $CD8^+$ T cells failed to recognize HLA-$A0201^+$ target cells expressing hTERT in melanoma patients, which may be related to improper handling of antigenic peptides (48). Therefore, there is a high degree of contradiction in the data on the immunogenicity of P540. Based on these findings, Wenandy *et al.* analyzed whether there are p540-specific T cells in the peripheral blood lymphocytes of patients with renal cell carcinoma, prostate cancer or melanoma, and the results showed that p540-specific T cells were present in only a few patients (49). In conclusion, p540 is immunogenic to humans, but the effect of triggering an immune response needs to be confirmed by more experiments, and the combination of p540 with other peptide vaccines is a future direction for clinical application (50).

UV1

UV1 consists of the three most common hTERT-derived peptides: hTERT691-705 (RTFVLRVRAQDPPPE), hTERT660-689 (ALFSVLNYERARRPGLLGASVLGL DDIHRA) and hTERT652-665 (AERLTSRVKALFSVL). These three peptides are unique to long-term survivors of cancer (51), indicating that UV1 can prolong patient survival. A phase I study demonstrated that NSCLC patients treated by UV1 can be well tolerated without serious adverse events and the combination of UV1 with immune checkpoint inhibitors might have improved clinical efficacy (19). Haakensen *et al.* used ipilimumab (CTL-associated antigen-4 blocker, CTLA-4 blocker) and nivolumab (programmed death 1 blocker, PD-1 blocker) combined with UV1 to treat patients with malignant mesothelioma and found that UV1 combined with ipilimumab induced more rapid and frequent immune responses (20). Similarly, another study examined UV1 in combination with ipilimumab in patients with metastatic melanoma, and the 5-year follow-up showed that the treatment was well tolerated and that UV1-specific Th1 cells expanded rapidly in most patients (21). These findings demonstrate a synergistic effect between UV1 vaccines and immune checkpoint inhibitors, with the combination of the two being a beneficial clinical treatment, and provide a scientific basis for the combination of telomerase therapeutic vaccines and checkpoint inhibitors to improve the treatment of cancer patients.

In addition, a recent study evaluated three approaches: UV1 treatment alone in lung cancer patients, combination of this treatment with androgen blockade in prostate cancer patients, and ipilimumab monotherapy in melanoma patients 1 week after a UV1 vaccination. Patients have been followed for survival for a median of 28.2 months (range, 4.7–87.3 months), 61.8 months (range, 11.7–96.3 months), and 55.7 months (range, 3.5–79.5 months), respectively. During 8 years of follow-up, 78.4% of the patients developed a UV1-related telomerase peptide-specific T-cell response in the blood, and the associated immune response lasted for up to 7.5 years. The combination of UV1 and ipilimumab increased the speed and frequency of successful immune response induction (22). This demonstrates not only the functional advantage of T cells in long-term survivors but also the feasibility and efficacy of combination therapeutic strategies. Currently, UV1-related phase II clinical trials in patients with cancers such as malignant mesothelioma (NCT03538314), NSCLC (NCT01789099/ NCT05344209), ovarian cancer (NCT04742075), and head and neck squamous cell carcinoma (NCT05075122) are active or recruiting.

GX301

GX301 is a vaccine consisting of 4 hTERT-derived peptides [hTERT540-548 (ILAKFLHWL), hTERT611-626 (EARPALLTSRLRFIPK), hTERT672-686 (RPGLLGASVLGLDDI), and hTERT766-780 (LTDLQPYMRQFVAHL)], which is compatible with MHC class I and II molecules and contains the two adjuvants Montanide ISA-51 and imiquimod (52). In phase I/II clinical trials in patients with prostate or kidney cancer, GX301 was administered by intradermally injecting 500 μg of each peptide (dissolved in Montanide ISA-51) in the skin of the abdomen. And patients were clinically and immunologically monitored up to 6 months from the first immunization. Researchers isolated patient peripheral blood mononuclear cells treated with single or multiple GX301 component peptides and observed the responses of these immune cells to tumor cells (23). The results showed that on the one hand, all patients developed an immune response to at least one peptide, suggesting specific selection of antigenic peptides by patients; on the other hand, the immune responses to multiplex peptides and the number of responders were more considerable than those for single peptides, indicating that multi-peptide vaccines are more effective than single-peptide vaccines. In addition, in a recent phase II clinical trial of patients with metastatic castration-resistant prostate cancer (mCRPC), 98 mCRPC patients were injected with GX301 at different times, and GX301 elicited at least one vaccine-specific immune response in 95% of the patients without major side effects. The above research results show that GX301 has certain safety and immunogenicity characteristics (24). Based on clinical results, increasing the number of administrations improves the immune response and this vaccine may have certain prognostic value. Furthermore, combination therapy with immune checkpoint inhibitors may produce further improvements.

Vx-001

Vx-001 consists of two hTERT-derived peptides (hTERT572-580) each containing 9 amino acids: one is a wild-type low-affinity cryptic hTERT peptide (RLFFYRKSV) whose functional peptide is hidden inside the protein, and the other is a mutant optimized hTERT peptide (YLFFYRKSV) that is an immunodominant peptide (53). Replacement of the first amino acid of the wild-type peptide with tyrosine enhances the affinity of the antigenic peptide for MHC class I molecules, thereby amplifying hTERT-specific CTL responses (54,55). The antitumor efficacy of Vx-001 against different types of cancer, such as NSCLC, melanoma, breast cancer, and cholangiocarcinoma, has been confirmed in phase I/II clinical trials. The vaccine not only induces a strong hTERT-specific immune response but is also well tolerated with only mild side effects (25,56,57). A recent study reported that Vx-001 showed clinical benefits in cancer patients deficient in tumor-infiltrating lymphocytes (TILs) but not in patients with abundant TILs (58). This indicates that there is an inverse correlation between cancer therapeutic vaccines and TILs. This may be because the activity of TILs is inhibited by different inhibitory pathways in immunogenic tumors, including the PD-1/PD-L1 pathway, which leads to a failure to control tumor growth. These findings suggest that patients with poor TIL infiltration (resistance to immune checkpoint inhibitor therapy or with a poor prognosis) may be the population that benefits most from therapeutic cancer vaccines.

UCP-Vax

Universal cancer peptides (UCPs), consisting of UCP1 (PAAFRALVAQCLVCV), UCP2 (KSVWSKLQSIGIRQH), UCP3 (GTAFVQMPAHGLFPW) and UCP4 (SLCYSILKAKNAGMS), are a novel type of MHC class II-restricted peptide composed of four hTERT peptides (59). UCP-Vax, composed of UCP2 and UCP4, has entered clinical trials. Phase I trial results demonstrated the safety and immunogenicity of UCP-Vax at the efficacious dose and showed good tolerance in patients. UCP-Vax is currently undergoing phase I/II clinical studies (26), and the results for 59 NSCLC patients grouped to receive three doses (0.25 mg, 0.5 mg, 1 mg) of UCP-Vax showed high safety and immunogenicity, with the induction of a specific immune response, though the immune effect was not related to dose. Additionally, phase II clinical trials in HPV-related tumors, HCC, glioblastoma and other cancers are actively recruiting (27).

DNA vaccines

Plasmid DNA can be transfected into mammalian cells by intramuscular injection to achieve stable long-term amounts of proteins or polypeptides to induce specific humoral and cellular immune responses (60,61), which together lay the physiological foundation for the emergence and development of DNA vaccines. Compared with other vaccines, DNA vaccines can continuously express TAA protein and stimulate the body to produce extensive

humoral and cellular immune responses. Moreover, DNA vaccines are well tolerated by patients without causing serious adverse reactions (62,63). In addition, the particularity of DNA vaccines is that they contain multiple antigenic epitopes, which greatly increases immunogenicity and can circumvent the variability of antigenic peptide presentation and the limitation of a single MHC molecule type, thereby producing more effective antitumor immunity and indicating great clinical potential. However, DNA vaccines also have certain limitations. Foreign DNA may integrate into the host cell's own DNA, resulting in insertional mutations, and because the introduction of DNA plasmids into cells often requires electroporation to increase *in vivo* delivery of pDNA to tissues, primarily skin and skeletal muscle, following a direct SC or IM injection (64), stimulation with high-voltage electrical pulses may lead to strong muscle contractions, electrode displacement and other safety issues. Therefore, DNA vaccines require particular attention to safety.

phTERT

Recombinant DNA technology has been used to construct DNA vectors that contain the full-length hTERT gene incorporating two mutations (R589Y and D1005Y) which was subcloned into pGX0001 and named as phTERT. The sequence of phTERT, a DNA vaccine that is highly optimized to produce a more efficient antigenic epitope, can improve the recognition and binding rate of T cells. Yan *et al.* inoculated this vaccine into mice and rhesus monkeys by intramuscular injection followed by electroporation, and the results showed that phTERT elicited a broad and potent hTERT-specific $CD8^+$ T-cell response in these animals. A large number of molecules, such as CD107a, IFN-γ and TNF-α, were secreted by T cells. In addition, significant IFN-γ responses and antigen-specific perforin release were observed in immunized monkeys, suggesting that this vaccine overcomes immune tolerance and induces strong cytotoxic responses in a relevant model of the human immune system (65). Additionally, some studies have reported the use of HPV16-related tumor models to explore the preventive and therapeutic potential of phTERT and found that the vaccine reduced the rate of tumor cell proliferation and improved the survival rate to a certain extent (66,67).

The INO-1400 and INO-1401 plasmids can express full-length hTERT mutants generated by optimizing the low-affinity protein sequence of the wild-type hTERT protein. INO-1400 maintains more than 99% homology with the original hTERT sequence, and the similarity between INO-1401 and the original hTERT protein is approximately 95%, showing acceptable safety in patients with solid tumors (68). This latest clinical research has examined the effects of INO-1400 and INO-1401 in patients with various types of cancer (breast cancer, lung cancer, pancreatic cancer, ovarian cancer, CRC, gastric cancer, esophageal cancer, and HCC). The results showed that these plasmids could elicit cellular immune responses in patients and that the induction rate was 96% (88/92), which was much higher than that of the cellular response elicited by the wild-type hTERT protein, indicating that INO-1400 and INO-1401 have certain immunogenicity in patients with solid tumors (68). In addition, related clinical trials for glioblastoma and urothelial carcinoma (NCT03502785 and NCT03491683) are actively underway.

INVAC-1

INVAC-1 is a DNA plasmid encoding an inactive form of hTERT that can be administered by intradermal injection to elicit a cellular immune response. In a variety of mouse models, INVAC-1 has been shown to induce hTERT-specific $CD4^+$ and $CD8^+$ T-cell responses, and in a mouse model of melanoma, INVAC-1 increased survival by 50% compared with controls and significantly delayed tumor growth (69). In recent years, some success has been achieved in INVAC-1-related phase I clinical trials (NCT02327468), and phase II trials for solid tumors, chronic lymphocytic leukemia, etc., are being carried out (70) (*Table 3*). The results of the latest phase I clinical trial showed that this vaccine had good safety, tolerability and immunogenicity in patients with relapsed or refractory solid tumors and that most patients maintained a stable condition during and after treatment (71). However, the specific $CD8^+$ T-cell response in this study was low; therefore, it is speculated that using INVAC-1 in the early stages of disease or making it target to specific indications with high telomerase activity may yield greater clinical benefits.

Cell-based immunotherapy

Cell-based immunotherapy is on the basis of the administration of living immune cells to patients, aiming to boost the immune system (83). Researchers collect human autospecific immune cells (such as DCs and T cells), culturing them *in vitro* to amplify them in thousands of times. And then, the amplified cells will be infused into the human body to enhance the immune activity of the body to

Table 3 Clinical trials of DNA vaccines, cell-based immunotherapies and antibodies related to hTERT

Approach	Name	Cancer targeted	Clinical trial phase	Clinical results/status	NCT number	Year*	Ref.
DNA vaccine	INVAC-1	Solid tumor	Phase I	Immune responses, well tolerated and safe/completed	NCT02301754/ NCT04515043	2020	(71)
	phTERT (INO-1400/1401/9012/5401)	Solid tumors	Phase I	Immune responses and well tolerated/completed	NCT02960594	2021	
		Glioblastoma	Phase I/II	No clear clinical results/active, not recruiting	NCT03491683	2022	
		Urothelial carcinoma	Phase I/II	No clear clinical results/active, not recruiting	NCT03502785	2022	
DCs	GRNVAC 1	Metastatic prostate cancer	Phase I/II	Immune responses and well tolerated/completed		2005	(72)
		Acute myeloid leukemia	Phase II	Immune responses and well tolerated/completed	NCT00510133	2017	(73)
	Other DCs	Metastatic melanoma	Phase I/II	Immune responses/completed		2016	(74)
		Pancreatic cancer	Phase I	Immune responses and well tolerated/completed		2017	(75)
		Breast cancer	Phase I/II	Immune responses and safe/ completed		2004	(76)
		Renal cell carcinoma	Phase I/II	No clear clinical results/ completed	NCT00197860	2011	
	TAPCells	Melanoma and prostate cancer	Phase I/II	Immune responses and well tolerated/completed		2013	(77)
ACT	TILs	Breast cancer	Phase I/II	Immune responses and safe/ completed		2007	(78)
		Spinal chordoma	Preclinical trials	hTERT protein expression increased in chordoma tissues/ completed		2016	(79)
	TLI	Prostatic neoplasms	Phase I	No clear clinical results/ completed	NCT00061035	2003	(80)
		Skin melanoma	Phase II	No clear clinical results/unknown	NCT00925314	2012	
	Radium-4	Solid tumor	Preclinical trials	Immune responses and safe/ completed		2021	(81)
Antibody	TMab-6	Glioma	Preclinical trials	Immune responses/completed		2018	(82)

*, year represents either the year of publication or year of the last trial update. DC, dendritic cell; ACT, adoptive cell transfer; TIL, tumor-infiltrating lymphocyte; TLI, transgenic lymphocyte immunization; NCT, National Clinical Trial.

target kill tumor cells (84). It solves the serious problems of poor immunity and low quality of life in patients treated with radiotherapy and chemotherapy to a certain extent and may greatly extend the survival time of patients. Cell-based immunotherapy is based on DCs and lymphocytes (adoptive cell transfer, ACT). ACT therapies mainly include TILs, transgenic lymphocyte immunization (TLI), T-cell receptor-engineered T (TCR-T) cells, chimeric antigen receptor T (CAR-T) cells and bispecific T-cell engagers (BiTEs), but only the first three have been reported in

research related to hTERT (85).

hTERT-targeting DCs

DCs are the most important APCs in the body and play important roles in the induction of innate and adaptive immunity (86). The basic application principle of DC-based therapies is that the patient's own DCs were isolated *in vitro* and loaded with TAA by various means, such as co-incubation, cell fusion, transfection, after which the DCs containing TAA antigenic specificity would be reinfused into hosts to induce specific anticancer immune responses. Current loading hTERT on DC vaccines have been mainly applied in two ways: one is to stimulate DCs with hTERT protein *in vitro* to sensitize the DCs (87), the other is to acquire and present hTERT-derived antigens by overexpressing the hTERT protein (73).

GRNVAC1 and GRNVAC2

GRNVAC1 is a therapeutic agent comprising hTERT-targeting DCs, which are generated by isolating mature DCs from the patient's own blood and transfecting them with mRNA encoding hTERT and lysosome-associated membrane protein 1 (LAMP1) (53). The cytoplasmic domain of LAMP-1 contains the amino acid sequence Tyr-Gln-Thr-lle, whose structure conforms to Tyr-Xaa-Xaa-hydrophobic amino acid motif that mediates cell membrane internalization and possibly lysosomal targeting of several cell surface receptors (88). Under the protection and guidance of LAMP1, hTERT enters the lysosomes, where it is degraded into small peptides that can be presented as antigenic epitopes representing different parts of hTERT peptides on the surface of DCs, thereby triggering a polyclonal immune response. In addition, the results of a study showed that chimeric vaccines containing LAMP1 have been shown to induce stronger T-cell responses in patients with metastatic prostate cancer than non-chimeric vaccines. And the vaccine was clinically well tolerated for 3 or 6 weeks after injection into patients with metastatic prostate cancer, eliciting strong $CD8^+$ and $CD4^+$ T-cell responses but no significant autoimmune symptoms (72). In addition, a phase II clinical trial showed that GRNVAC1 was well tolerated in patients with acute myeloid leukemia without severe toxicity and that 58% of patients developed hTERT-specific T-cell responses after use (73).

Different from GRNVAC1, GRNVAC2 is an allogenic dendritic cell (DC) product derived from human embryonic stem cells (89). Because it is not limited by MHC type, GRNVAC2 may have great potential for the treatment of tumors with unknown T-cell epitopes (53).

Other DC-based immunotherapies

Sioud *et al.* (74) vaccinated a metastatic melanoma patient who has treated with ipilimumab, with indoleamine 2,3-dioxygenase (IDO)-silenced DC vaccines comprised DCs cotransfected with mRNA for survivin (left arm) or hTERT tumor antigen (right arm). During the vaccination period, T cells generated immune responses to survivin and the hTERT tumor antigens, significantly reducing lung, liver, and skin cancer metastasis and resulting in certain clinical benefits (74). In addition, T-cell responses to MART-1 and NY-ESO-1 were detected in the peripheral blood of the patient, and the patient developed antibody responses to several melanoma proteins, indicating the diversity of antitumor immunity in the patient. In another study, the transfection of DCs with a recombinant adenovirus encoding hTERT cDNA and expressing an hTERT antigen activated autologous T cells *in vitro*, generating hTERT-specific CTLs and significantly improving the CTL immune response against a variety of tumour cell types (90). Moreover, in a recent phase I clinical trial, a vaccine was prepared by pulsing DCs with three HLA-A2-restricted peptides [hTERT (TERT572Y), CEA (Cap1-6D), and survivin (SRV. A2)], and its therapeutic effects on pancreatic cancer were evaluated. The results showed that these DCs induced specific T-cell responses and were well tolerated in patients. The most common side effects of the treatment were transient fatigue and flu-like symptoms (75). In addition, this telomerase peptide-pulsed DC vaccine has shown clinical efficacy in patients with breast cancer (76), renal cell carcinoma (91).

Tumor antigen-presenting cells (TAPCells)

Salazar-Onfray *et al.* used human metastatic melanoma-derived cell lysates (TRIMEL) as the antigen source and activator for *ex vivo* stimulation and activation of peripheral blood leukocytes to obtain highly active DCs for infusion into patients to induce antitumor immune responses (77). This production of therapeutic DC cells, named TAPCells, have been evaluated in phase I/II clinical trials with more than 120 melanoma patients and 20 prostate cancer patients. In these trials, the vaccine significantly improved the rate of survival in melanoma patients, prolonged the serum prostate specific antigen doubling time (PSADT) and induced biochemical and memory immune response in castration-resistant prostate cancer patients, implying

that the treatment enhanced antitumor immune memory associated with clinical efficacy (92,93).

ACT therapy

ACT therapy originally referred to the isolation of TILs from cancer patients, followed by *in vitro* activation, expansion and functional identification prior to return to the patient, which resulted in restraining the growth and survival of tumor cells (78,94). However, TIL therapy has certain limitation in terms of efficacy, safety and accessibility. On the one hand, fresh tumor samples and TILs with strong antitumor activity and a strong proliferative ability are difficult to obtain (in general, only through surgery). On the other hand, TIL expansion *in vitro* and reinfusion are costly and time consuming (95). To solve these problems and increase the breadth of cancer applications, researchers have tried to use genetic engineering to improve TILs or generate T cells with surface expression of specific TCRs. Many novel therapies, such as TCR-T cells, CAR-T cells and BiTEs, have emerged (96).

TLI therapy was developed to introduce and integrate part of a TAA gene into patients' isolated lymphocytes, and then these cells were reinfused into the patients to trigger a targeted immune response. American Cosmo Bioscience has conducted phase I and II clinical trials in prostate cancer and melanoma patients (NCT00061035 and NCT00925314) (79,80).

TCR-engineered T cell therapy aims to screening for TCR αβ chain sequences highly specific for hTERT and uses a viral vector to transfect the TCR αβ chain sequence into a patient's isolated T cells *in vitro* to obtain TCR-engineered T cells that can specifically recognize tumor antigens. The modified TCR-engineered T cells are cultured and expanded *in vitro* and then infused back into the patient, specifically targeting and killing cancer cells with high hTERT expression (97-100). Dillard *et al.* isolated an hTERT-specific TCR sequence targeting MHC class II molecules, which was called Radium-4, from the pancreatic cancer patient who developed an immune response to the hTERT peptide. TCR-engineered T cells containing Radium-4, including both $CD4^+$ T cells and $CD8^+$ T cells, were able to kill melanoma cells *in vitro* without toxicity to bone marrow stem cells or mature hematopoietic cells, indicating that redirected T cells are effective and safe and have good prospects for the treatment of solid tumors (81). There are still challenges regarding TCR-engineered T cell therapy, for example, persistence of T cells *in vivo* (101), tumor escape due to the expression of cancer-related antigens varying in different tumor cells (102) and a limited eligible population with identical MHC expression, that need to be addressed with further experimental research.

CAR-T-cell therapy involves the construction of a chimeric antigen receptor (CAR) by combining a single-chain antibody (ScFv) that can recognize a TAA of interest with signaling molecules of the T cells (CD3, CD28, etc.) that are required for T-cell activation. The CAR is transfected into T cells, endowing the CAR-T cells with the ability to recognize the TAA without MHC restriction (103). These cells then kill cancer cells through the perforin and granzyme axis, the Fas and Fas ligand axis, as well as the cytokines (104).

BiTEs connect a single-chain antibody against a tumor antigen in conjunction with a single-chain antibody against a T-cell surface molecule to express bispecific antibody components; thus, BiTEs can not only effectively activate T cells but also recognize tumor cells (105). Unfortunately, there is a lack of clear research results for the application of telomerase as a TAA for CAR-T cells and BiTEs at present, which may provide an opportunity for future research directions.

Immunological methods based on other cells

Mu *et al.* transfected human umbilical vein endothelial cells (HUVECs) with an hTERT-carrying lentivirus to immortalize the cells and explored the antitumor immunity induced by hTERT-expressing human umbilical vein endothelial cells (HUVEC-TERTs) (106). The results showed that HUVEC-TERTs had high telomerase activity and expressed CD31, vascular endothelial growth factor receptor-2 (VEGFR-2) and integrin α5. These cells had antiangiogenic and tumor microenvironment (TME)-regulating activity that could induce certain humoral immunity and cellular immunity, which may lead to the large-scale application of HUVECs in cancer immunotherapy.

Anti-hTERT antibodies

In addition to cell-mediated immune responses, hTERT antibodies in the circulating blood of cancer patients have also been detected by researchers, suggesting that antibody-mediated humoral immunity against hTERT is also a specific anticancer approach (107). Kenta Masui *et al.* developed an hTERT-specific monoclonal antibody, TMab-6 (IgM, kappa), specific for cancer patients (82). Recent studies have shown that the key epitope for TMab-6 binding to hTERT is the PSTSRPRPWD sequence of hTERT and that Thr310 and Ser311 of hTERT are important amino acids recognized by

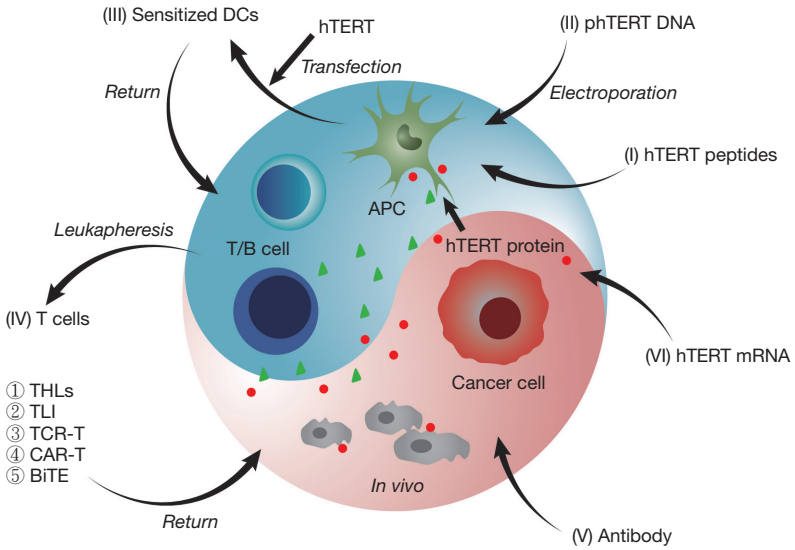

Figure 2 Types of telomerase-targeted cancer immunotherapies. (I) Peptide vaccines—single or multiple hTERT antigen peptides with different fragments are injected into the human body and recognized by APCs, which subsequently activate T and B cells. (II) DNA vaccines—plasmids carrying hTERT DNA with special epitopes are usually electroporated to sensitize APCs, which process and present antigens to activate T and B cells. (III) hTERT-targeting DCs—DCs are isolated from patient blood, hTERT antigen peptides are used to stimulate the DCs *in vitro*, and the synthetic vaccine is injected back into the patient to activate T and B cells. (IV) ACT therapies—leukapheresis, performing using apheresis equipment to separate leukocytes from peripheral blood, at the same time returning autologous plasma, platelets and erythrocytes to the patient, is utilized to activate autologous lymphocytes and induce their proliferation *in vitro*, and expanded cells are then reinfused into the patient to directly kill cancer cells; ACT mainly includes TILs, TCR-T cells, CAR-T cells, and BiTE therapy. TILs directly perform functions and expand. TLI therapy was developed to introduce and integrate part of a TAA gene into patients' isolated lymphocytes, and then these cells were reinfused into the patients to trigger a targeted immune response. TCR-T-cell therapy involves the integration of the hTERT gene into a TCR sequence and subsequent transfection into T cells, which can then express the TCR that specifically recognizes hTERT. CAR-T-cell therapy involves the combination of an hTERT antigen fragment with T-cell surface molecular signals (CD3, etc.) for subsequent transfection into T cells, which can express the CAR. BiTE therapy involves the combination of a single-chain antibody specific for a T-cell surface molecule with a single-chain antibody specific for a tumor cell surface antigen; the antibody chains are linked to form an hTERT-specific BiTE, and transfected T cells can target, bind, and kill tumor cells. (V) Antibody therapy—direct injection of anti-hTERT antibodies. (VI) hTERT mRNA may be introduced into the human body and after being engulfed by the APC, it is directly translated into the hTERT protein to induce an immune response. But we should pay some attention to underlying side effects. DC, dendritic cell; hTERT, human telomerase reverse transcriptase; phTERT, a synthetic highly optimized full-length hTERT DNA vaccine; APC, antigen-presenting cell; TIL, tumor-infiltrating lymphocyte; TLI, transgenic lymphocyte immunization; TCR-T, T-cell receptor-engineered T; CAR-T, chimeric antigen receptor T; BiTE, bispecific T-cell engager; TAA, tumor-associated antigen; CAR, chimeric antigen receptor.

TMab-6 (108). Compared with antigen vaccines based on cellular immunity, antibodies are more suitable for individuals who are immunosuppressed and have difficulty producing immune responses, especially those who need immediate protection. After all, the generation of memory immune responses after antigen vaccination usually requires two to six weeks, but antibody therapy allows the body to directly neutralize antigens, resulting in immediate passive immunity. However, the specific application mechanisms of antibodies targeting hTERT have not been fully elucidated, and related clinical trials are also lacking.

Conclusions

Targeting telomerase is a focus and hotspot in cancer immunotherapy (109). Current immunotherapies targeting hTERT include peptide vaccines, DNA vaccines, DCs, ACT therapies, antibodies and so on (*Figure 2*). Many of

these approaches have been approved by the FDA and other countries to enter clinical trials, and some of these therapies, such as "RIAVAX™ (GV1001)" (Samsung Pharmaceutical Co., Ltd., NCT02854072), GV1001 (GemVax & Kael, NCT04032067), for the treatment of pancreatic cancer (110) and benign prostatic hyperplasia (BPH), respectively, have entered phase III clinical trials. And RZ-001 (Rznomics, Inc., NCT05595473) for the treatment of HCC have entered phase I/II clinical trials. This fully indicates that immunotherapy targeting hTERT has promising application prospects. Hematopoietic stem cells and T and B lymphocytes have relatively high telomerase activity, suggesting that cancer immunotherapy targeting hTERT can kill not only cancer cells but also these lymphocytes, which in turn damages the immune system. Therefore, it is necessary to pay attention to host immune system abnormalities, though notably no serious adverse reactions have been observed in the above mentioned immunotherapies targeting hTERT (8).

Peptide vaccines, most of which include a single antigenic epitope, were the first to enter clinical practice. Clinical trial results showed that they induced only weak immune responses in patients. Recently, some researchers have created combination peptide vaccines targeting multiple hTERT epitopes to treat patients, while other researchers have developed DNA vaccines based on the full-length hTERT protein, and their results showed that a stronger patient immune response could be induced with this approach (5,10). However, the administration of these vaccines still did not eliminate solid tumors effectively. Accumulating clinical trial evidence indicates that the combination of telomerase-targeted cancer immunotherapy and other therapies, including immunotherapies, molecularly targeted drugs and chemotherapy, can improve therapeutic effects and prognosis in cancer patients (4). These results suggest that the application direction of telomerase-targeted cancer immunotherapy may lie in improving the immunogenicity of vaccines or administering them with other anticancer therapies. One of the reasons why the therapeutic effect of hTERT-derived vaccines is not effective may be that the hTERT protein is a self-antigen in the human body with only low affinity recognition by TCRs (111). Therefore, hTERT-derived vaccines have difficulty inducing sufficient corresponding effector T cells in patients, resulting in a relatively weak antitumor effect. ACT technologies, especially TCR-T-cell therapy, which introduces a large number of T cells carrying modified high-affinity TCRs into the body to generate a strong immune response in a short period of time, may solve this problem (112,113).

Coronavirus disease 2019 (COVID-19) mRNA vaccines were first approved by the FDA for clinical use at an unprecedented speed given the urgent need to combat severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) in 2020 and have shown a very strong protective effect, leading to a rapid expansion of mRNA vaccines in the biomedical field (114). Perhaps an mRNA vaccine that targets hTERT will have the same excellent biological efficacy. Compared with traditional vaccines, mRNA vaccines are synthesized at a faster rate, and their sequences can be precisely designed and modified. The translated proteins have stable immunogenicity, and the exposed antigenic sites can also be diverse. Moreover, mRNA does not integrate into the genome, avoiding the risk of mutagenesis (115,116). The outstanding advantages of mRNA vaccines in delivering the code of antigens and inducing immune responses are potentially greatly conducive to the treatment of many severe diseases.

Future research on hTERT-targeted immunotherapeutic approaches may be devoted to the following four aspects: (I) further exploration of the regulation mechanism of hTERT and its application in anti-tumor research; (II) screening and optimization of hTERT-based TAA molecules and development of novel vaccines that induce a stronger immune response; (III) design of novel vaccines, such as mRNA vaccines, or ACT cells to improve the intensity and duration of the immune response; and (IV) development and selection of novel adjuvants to enhance vaccine immunogenicity and reduce toxic side effects. With the development of the above technologies, we believe that telomerase-targeted cancer immunotherapy will achieve effective killing of tumor cells in patients and improve patient survival rate and quality of life.

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Footnote

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