

Targeting Telomere Dynamics as an Effective Approach for the Development of Cancer Therapeutics

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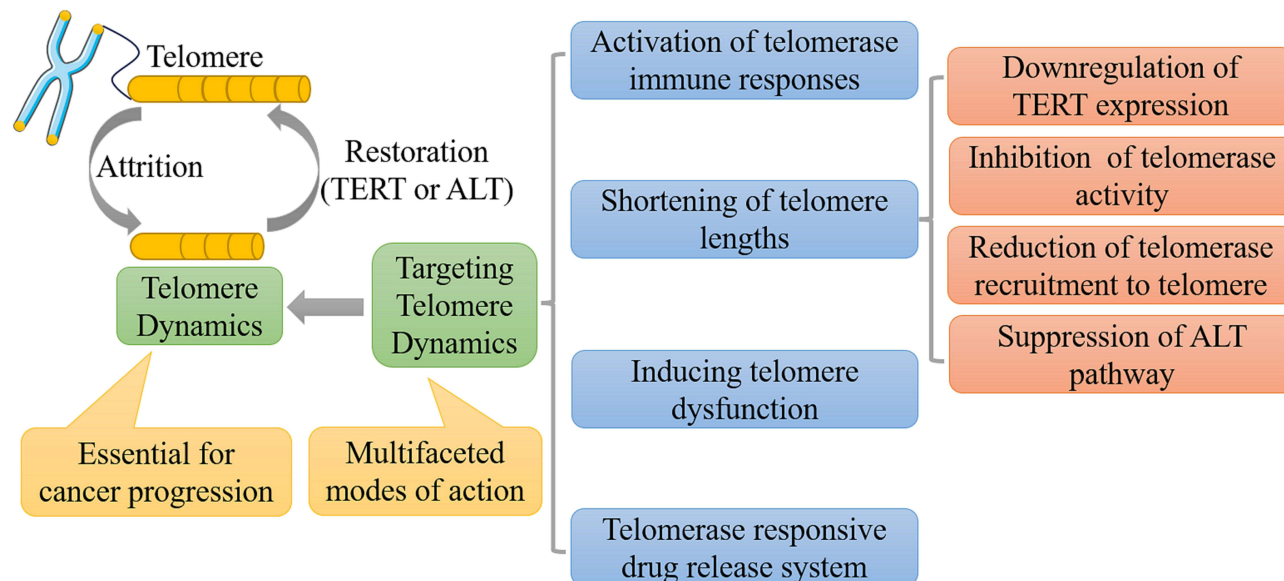
Abstract: Telomere is a protective structure located at the end of chromosomes of eukaryotes, involved in maintaining the integrity and stability of the genome. Telomeres play an essential role in cancer progression; accordingly, targeting telomere dynamics emerges as an effective approach for the development of cancer therapeutics. Targeting telomere dynamics may work through multifaceted molecular mechanisms; those include the activation of anti-telomerase immune responses, shortening of telomere lengths, induction of telomere dysfunction and constitution of telomerase-responsive drug release systems. In this review, we summarize a wide variety of telomere dynamics-targeted agents in preclinical studies and clinical trials, and reveal their promising therapeutic potential in cancer therapy. As shown, telomere dynamics-active agents are effective as anti-cancer chemotherapeutics and immunotherapeutics. Notably, these agents may display efficacy against cancer stem cells, reducing cancer stem levels. Furthermore, these agents can be integrated with the capability of tumor-specific drug delivery by the constitution of related nanoparticles, antibody drug conjugates and HSA-based drugs.

Keywords: telomere dynamics, telomerase, alternative lengthening of telomeres (ALT), cancer therapy

Introduction

Telomere is a protective structure located at the end of the linear chromosome of eukaryotes, which is made up of tandem repetitive DNA sequences and related proteins. It is essential for maintaining the integrity and stability of the genome. A cancer cell, originated from the aggregates of abnormal mutated cells, is characterized by uncontrollably infinite proliferation, infiltrating into surrounding tissues, further metastasizing to distant organs and ultimately leading to the death of host organisms.¹ Remarkable advances in the field of telomere biology have revealed the paramount importance of telomere in cancer progression. However, slightly different from the general hypothesis, several analyses focused on the correlation of telomere lengths and cancer incidence reported that both longer and shorter telomeres were correlated with cancer development.² A systematic analysis of telomere lengths of 18,430 samples covering tumor and normal tissues from 31 types of cancers revealed that 70% of samples displayed shortening telomere lengths in comparison with matched normal tissues and 30% showed elongating telomere lengths. In detail, elongated telomeres existed in testicular germ cell tumors, low grade glioma and sarcoma, whereas cervical cancer, endometrial cancer, uveal melanoma, lymphoma, kidney papillary and kidney chromophobe carcinoma showed shortened telomeres.³ With the participation of telomerase and alternative lengthening of telomeres (ALT) pathway, longer telomeres indicate more replicative times and provide favorable opportunity for the unlimited proliferation of cancer cells. Meanwhile, shortened telomeres may cause the telomere crisis and genomic instability, which also facilitate tumor progression.^{4,5} Thus, instead of merely affecting telomere lengths, seeking versatile mechanisms which refer to targeting telomere dynamics may achieve satisfactory results in cancer therapy. The emerging multifaceted strategy as chemotherapeutic, immunotherapeutic and

Graphical Abstract



nanomedicine, includes activating anti-telomerase immune responses, shortening telomere lengths, inducing telomere dysfunction and constituting telomerase-responsive drug release system.

In this review, the correlation of telomere dynamics and cancer is illustrated, and current anticancer therapeutics that interfere in telomere dynamics are summarized in detail. Meanwhile, the advantages and disadvantages of those therapeutics and their future directions are also critically discussed to fully exploit their potential in cancer therapy.

Telomere Dynamics as Drug Target

“Telomere” was originally named by Hermann Muller in 1938, based on the Greek words for “end” (telos) and “part” (meros). He discovered that the free end of chromosomes presented a cap-like structure which made it resistant to X-rays.⁶ The human telomere was first sequenced as tandem 5'-TTAGGG-3' repeats in 1988⁷ and the same repeated 5'-TTAGGG-3' sequence exists among 91 vertebrate species.⁸ As a capping structure at the chromosome termini, telomere consists of tandem repeated 5'-(TTAGGG)_n-3' double stranded DNA sequence (15–20 kb in human telomere) with a terminus of G-rich single stranded 3'-overhang (50–200 nucleotide) and a multiprotein complex that binds to the telomere.⁹ The 3'-overhang invades and folds back onto the telomeric dsDNA region to form a stable telomeric loop (T-loop) structure with a single-stranded displacement D-loop at the invasion site.¹⁰ The multiprotein complex, called shelterin or telosome, is made up of six proteins viz. telomeric repeat factor 1/2 (TRF1/2), TERC-interacting nuclear factor 2 (TIN2), repressor activator protein 1 (RAP1), protection of telomere 1 (POT1) and tripeptidyl peptidase 1 (TPP1).¹¹ As known, TRF1 and TRF2 bind directly to the double-stranded region of the telomere as homodimers, and subsequently RAP1 is localized to the telomeric DNA duplex by binding to TRF2. In addition, TPP1 forms a heterodimer with POT1, which binds specifically to the single stranded G-rich telomere sequence via POT1. Furthermore, TIN2 binds to TRF1/2 in the telomere double-stranded region and TPP1 in the single-stranded region to stabilize the entire shelterin complex. The shelterin plays an important role in avoiding telomere from being recognized as DNA damage sites, recruiting telomerase to telomere ends, maintaining telomere length, promoting T-loop formation and stabilizing telomere structures.^{12,13}

Telomere becomes shorter by 50–150 bp during each cell division. In normal somatic cells, the ever-worsening telomere erosion ultimately elicits telomere crisis, which is characterized by replicative senescence, genome instability and cell death.¹⁴ Therein, scarce cells that accumulate oncogenic mutations can survive in the telomere crisis and become

immortalized with malignant phenotypes via the activation of telomere maintaining mechanisms.¹⁵ In short, telomere possesses vital biological functions in sustaining the genomic stability; those include protecting chromosome terminus from nuclease degradation or DNA damage repair, ensuring complete end-replication in linear chromosomes, and thus limiting the number of cell divisions and preventing cell canceration.¹⁶

Telomere dynamics refer to the shortening and lengthening of telomeres during the process of cell growth. Most human cells display progressive telomere shortening with each division and eventually walk to inevitable senescence and even cell death. Whereas, telomere lengths in embryonic stem cells and germ cells do not shorten obviously due to their strong telomerase activity, ensuring a longer lifespan for these cells. Telomerase activity was first identified in tetrahymena extracts in 1985, which was called telomere terminal transferase at that time.¹⁷ Cancer cells featured by infinite proliferation and immortalization counteract the telomere attritions with the assistance of telomerase. As reported, telomerase could be reactivated or upregulated in the majority of cancers (85–90%).¹⁸ Moreover, the telomerase negative cancers (10–15%) could employ alternative lengthening telomeres (ALT) mechanisms to elongate the telomeres.¹⁹

Telomerase is a ribonucleoprotein complex with reverse transcriptase efficacy, which is composed of a catalytic subunit (telomere reverse transcriptase, TERT, encoded by the hTERT gene located at the human chromosome 5p15.33), an RNA template (telomerase RNA component, TERC, originated from the hTERC gene positioned at the human chromosome 3q26) and a number of accessory protein subunits with regulatory functions. Those accessory protein subunits include dyskerin, TCAB1, NHP2, NOP10, pontin and reptin. Telomerase can use its own RNA as a template to add telomeric DNA repeated sequence to the 3' end of chromosomes for maintaining the relative stability of telomere length.^{20,21} Therein, TERT and TERC are core components of the human telomerase holoenzyme, whereas the accessory proteins participate in regulating the assembly and localization of telomerase *in vivo*.²² Besides maintaining the lengths of telomeres, telomerase also plays a pivotal role in some non-telomeric actions such as promoting the inflammation and immunosuppressive tumor microenvironment through the NF- κ B and cGAS-STING pathway,^{23,24} accelerating tumor angiogenesis with activation of VEGF,²⁵ maintaining the stemness and multipotency of cancer stem cells through upregulating expressions of Oct3/4, NANOG, Sox-2 and LGR5,²⁶ and preventing cells from oxidative stress and DNA damage through the decrease of mitochondrial ROS production.²⁷

The alternative lengthening of telomeres (ALT) mechanism elongates the attrited telomeres based on the DNA homologous recombination (HR) pathways.²⁸ ALT is characterized by highly heterogeneous telomere lengths, frequent exchanges between telomeres and sister chromatids, existence of ALT associated promyelocytic leukemia bodies (APBs) and extra-chromosomal repeated telomeric DNA circles.²⁹ ALT is highly prevalent in cancers derived from mesenchymal tissues, viz neuroendocrine system, soft tissues, and peripheral and central nervous systems.³⁰

To sum up, as shown in Figure 1, telomerase and ALT are jointly involved in modulating telomere dynamics. However, the interaction between two pathways demands further investigations, on account of the phenomenon that anti-telomerase therapies usually facilitate the generation of ALT.³¹

Based on the process of telomere dynamics in cancer cells mentioned above, targeting telomere dynamics has emerged as a promising approach for cancer therapy. As shown in Figure 2, targeting telomere dynamics mainly involves the inhibition of telomerase activity, downregulation of telomerase expression, shortening of telomere lengths, activation of anti-telomerase immune responses, induction of telomere dysfunction, and suppression of the alternative lengthening of telomeres (ALT); as well as the constitution of telomerase-responsive drug release system. Those are elaborated in detail in the following sections on active agents in clinical trials and in preclinical studies.

Active Agents in Clinical Trials

Currently, telomere has emerged as a promising target in cancer therapy and some telomere-targeted therapeutics have been assessed in the clinical trials for various types of cancers since 2003, as shown in Table 1. Therein, the telomere-targeted drugs approved in the clinical trials mainly fall into two categories: chemotherapeutic and immunotherapeutic agents. Chemotherapeutics are further divided into telomerase inhibitors such as imetelstat.^{32–38} and KML-001,³⁹ and telomere dysfunction inducers such as nucleoside analogue 6-thio-2'-deoxyguanosine.⁴⁰ Immunotherapeutics primarily include telomerase-specific oncolytic adenoviruses, such as OBP-301^{41–44} and KH901,⁴⁵ hTERT peptide vaccines, such as GV1001,^{46–56} UCPVax,^{57–61} VX-001,^{62–65} UVI^{66–69} and GX301,⁷⁰ hTERT^{540–548} peptide,^{71–73} hTERT^{572Y} peptide^{74,75} and hTERT and

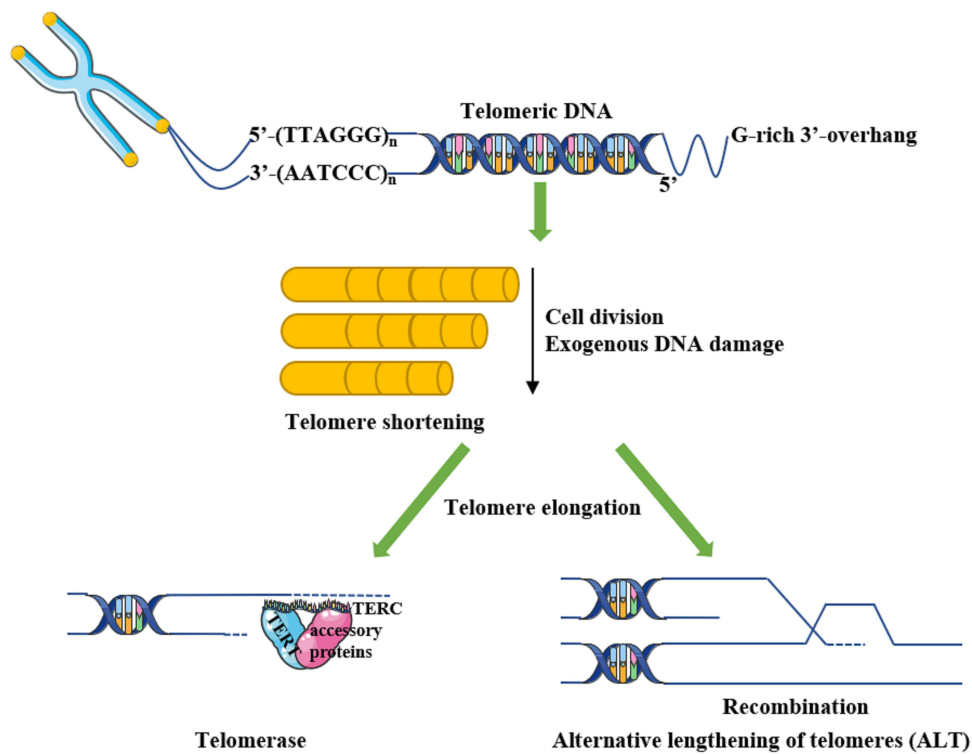


Figure 1 Telomere and telomere dynamics. Telomere, a capping structure at the chromosome termini, consists of tandem repeated 5'-(TTAGGG)_n-3' double stranded DNA sequence (15–20 kb in human telomere) with a terminus of G-rich single stranded 3'-overhang (50–200 nucleotides) and a multiprotein complex that binds to the telomere. Telomere dynamics refer to the shortening and lengthening of telomeres. Specifically, telomeres are shortened during cell division or exogenous DNA damage, meanwhile elongated with telomerase action or alternative lengthening telomeres (ALT) mechanisms.

survivin multi-peptide;^{76,77} hTERT DNA vaccines such as INVAC-1,⁷⁸ V934/V935⁷⁹ and INO-1400/1401/5401;^{80–82} hTERT mRNA vaccines;⁸³ dendritic cell vaccines, such as hTERT RNA transfected DCs^{84–86} and hTERT peptide pulsed DCs,^{87–92} as well as transgenic lymphocyte immunization vaccines against telomerase.^{93,94}

Several clinical trials indicated that imetelstat could inhibit telomerase activity in peripheral blood mononuclear cells (PBMCs)^{32,33} and tumor cells,³³ but caused severe side effects in children with recurrent central nervous system (CNS) tumors.³³ Meanwhile, imetelstat, as a maintenance therapy with platinum-based chemotherapy, displayed an improvement in overall survival (OS) and median progression-free survival (PFS) in non-small cell lung cancer (NSCLC) patients with short telomere length.³⁶ Similarly, the clinical trial of KML-001 (sodium metaarsenite, a telomerase inhibitor) also demonstrated the potential of combination of KML-001 and platinum agents.³⁹

Clinical trials have shown that OBP-301 (telomelysin, a telomerase specific replication-competent oncolytic adenovirus) possessed favorable antitumor efficacy and tolerated toxicity against various solid tumors,⁴⁴ and esophageal cancer in combination with radiotherapy.⁴³ Analogously, KH901, an oncolytic adenovirus conditionally replicating in telomerase-positive tumor cells and expressing granulocyte macrophage colony-stimulating factor (GM-CSF), provided clinical benefits in patients with recurrent head and neck cancer, and revealed the possibility of combination with chemotherapy.⁴⁵

Immune vaccines that have been developed as telomerase targeting agents principally cover hTERT peptide, hTERT DNA, hTERT mRNA, dendritic cell and transgenic lymphocyte immunization vaccines. To speak of peptide vaccines, several clinical trials have examined the safety and efficacy of the combination of GV1001 with radiotherapy or chemotherapy such as gemcitabine, cyclophosphamide, temozolomide, tuberculin and docetaxel.^{51–61} The results showed that the combinations were well tolerated in patients with non-small cell lung cancer,^{55–60} inoperable pancreatic cancer⁵⁶ and melanoma;^{58,59} and induced GV1001 specific immune responses, which further led to the tumor responses manifested in prolonged survival. Similarly, Vx-001 or UV1 (combination with/without ipilimumab) also developed immunological responses and long-term clinical outcomes without severe side effects in advanced non-small cell lung cancer,^{67,70,72} unresectable or metastatic malignant melanoma,⁷³ metastatic hormone-naive prostate cancer⁷⁴ and other solid tumors.⁶⁸

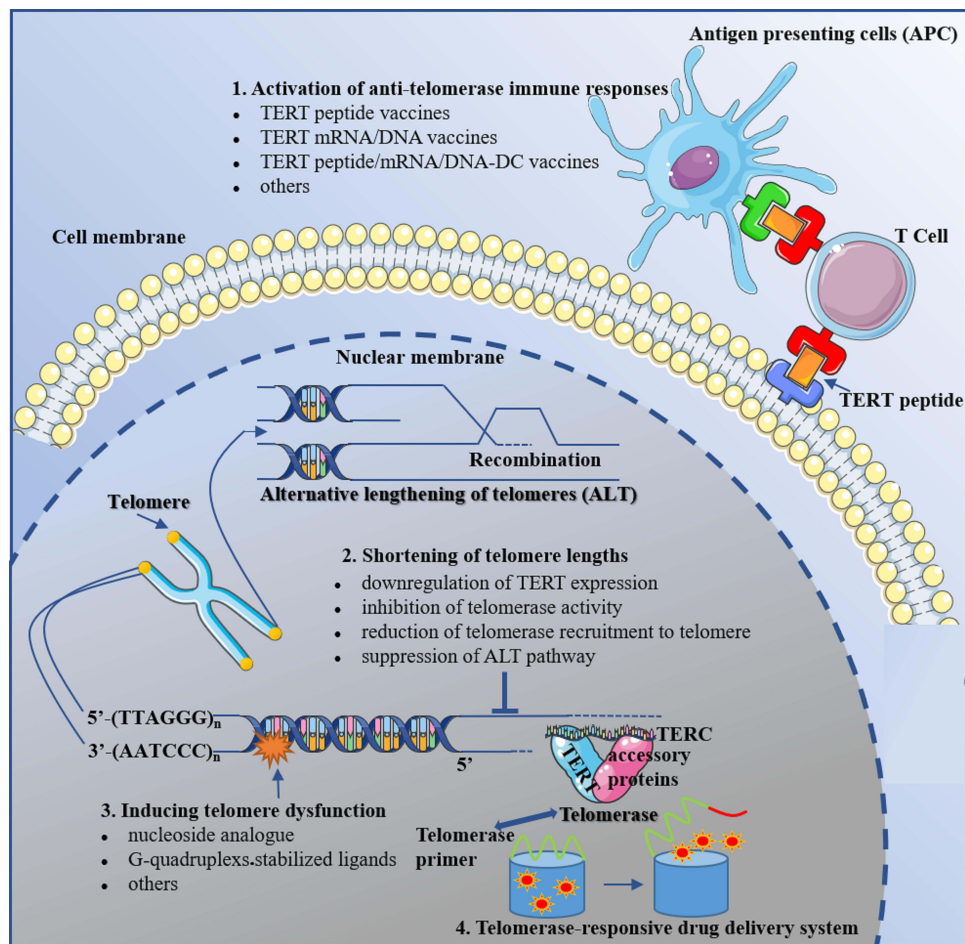


Figure 2 Anti-cancer therapeutics targeting telomere dynamics. Considering the pivotal role telomere plays in cancer progression, targeting telomere dynamics may hold great potential in cancer therapy, which covers those of activating anti-telomerase immune responses, shortening telomere lengths, inducing telomere dysfunction and constituting telomerase-responsive drug release system.

Referring to DNA and mRNA vaccines, the delivery of hTERT-encoded DNA plasmids with or without interleukin-12 plasmid via intramuscular electroporation could display favorable safety and efficacy in patients with pancreatic cancer, in association with positive immune responses represented by the increased production of hTERT-specific IFN- γ and activation of hTERT-specific T cells.⁸⁶ Analogously, intradermal injection of naked mRNA coding for tumor-associated antigens such as telomerase, with GM-CSF as adjuvant, could perform well in renal cell cancer patients, with median survival of 24–26 months.⁸⁹

As to DC vaccines, several clinical trials have demonstrated that the vaccinations with transfected telomerase RNA or the telomerase peptide pulsed DC could be well tolerated and exert beneficial clinical effects in patients with renal cancer,^{89,90,92} pancreatic adenocarcinoma,⁹³ malignant melanoma⁹¹ and prostate or breast cancer.⁹²

Active Agents in Preclinical Studies

In addition to the above clinical trials of telomere-targeted therapeutics, a variety of agents that modulate telomere dynamics have been in preclinical investigations, striving to obtain satisfactory achievements in cancer therapy, as shown in Table 2. As depicted in Figure 2, anti-cancer drugs currently under investigation that target telomere dynamics primarily cover those of activating anti-telomerase immune responses, shortening telomere lengths, inducing telomere dysfunction and constituting telomerase-responsive drug-release system.

Table 1 Ongoing/Completed Clinical Trials of Telomere-Related Anti-Cancer Therapeutics

	Identifier Code	Intervention/Treatment	Approved Application/ Indication	Study Start Year	Phase	Ref
1	NA	Imetelstat	Children with refractory or recurrent solid tumors	2013	I	[32]
2	NA	Imetelstat	Children with recurrent or refractory central nervous system malignancies	2017	II	[33]
3	NCT01273090	Imetelstat sodium	Children with refractory or recurrent solid tumors or lymphoma	2011	I	[34]
4	NCT01265927	GRN163L and Trastuzumab	HER2 ⁺ breast cancer	2010	I	[35]
5	NCT01137968	Imetelstat and bevacizumab	Advanced non-small cell lung cancer	2010	II	[36]
6	NCT01256762	Imetelstat; paclitaxel with or without bevacizumab	Locally recurrent or metastatic breast cancer	2010	II	[37]
7	NCT01242930	Imetelstat with or without lenalidomide	Multiple myeloma	2010	II	[38]
8	NCT01110226	KML-001 (sodium metaarsenite) and cisplatin	Advanced solid tumors	2010	I	[39]
9	NCT05208944	THIO (6-Thio-2'-Deoxyguanosine); Cemiplimab	Advanced non-small cell lung cancer	2022	II	[40]
10	NCT04391049	OBP-301 (telomerase-specific type 5 adenovirus); carboplatin; paclitaxel and radiation therapy	Locally advanced esophageal and gastroesophageal cancer	2020	I	[41]
11	NCT03190824	OBP-301 (telomerase specific replication-competent oncolytic adenovirus)	Unresectable metastatic melanoma	2016	II	[42]
12	NCT03213054	OBP-301 (telomelysin) with radiotherapy	Esophageal cancer	2017	I	[43]
13	NA	Telomelysin (hTERT promoter driven modified oncolytic adenovirus)	Solid tumors	2010	I	[44]
14	NA	KH901 (oncolytic adenovirus, replicates in and lyses telomerase-positive tumor cells and expresses GM-CSF)	Head and neck cancers	2009	I	[45]
15	NCT01579188	GV1001 (after GM-CSF)	Inoperable stage III non-small cell lung cancer	2012	III	[46]
16	NCT00425360	GV1001; sargramostim; capecitabine and gemcitabine hydrochloride	Locally advanced or metastatic pancreatic cancer	2007	III	[47]
17	NCT00509457	GV1001 (after radiotherapy and docetaxel)	Locally advanced non-small cell lung cancer	2007	II	[48]
18	NCT00444782	GV1001; cyclophosphamide and GM-CSF	Advanced hepatocellular carcinoma	2007	II	[49]
19	NA	GV1001 (hTERT: 611–626); HR2822 (hTERT: 540–548) and GM-CSF	Non-small cell lung cancer	2006	I/II	[50]
20	NA	GV1001 and GM-CSF	Inoperable pancreatic cancer	2006	I/II	[51]
21	NCT01247623	GV1001 and temozolomide	Advanced malignant melanoma	2010	I/II	[52]
22	NA	Temozolomide and GV1001	Stage IV melanoma	2011	I/II	[53]
23	NA	GV1001 (hTERT: 611–626); p540 (hTERT: 540–548) and GM-CSF or tuberculin	Cutaneous melanoma	2011	I	[54]
24	CTN-2000; CTN-2006	GV1001 (after radiotherapy and docetaxel)	Non-small cell lung cancer	2011	I/II	[55]
25	ISRCTN4382138	GV1001 (after gemcitabine and capecitabine)	Advanced pancreatic ductal adenocarcinoma	2015	III	[56]
26	NCT02818426	UCPVax	Metastatic non-small cell lung cancer	2016	I/II	[57]
27	NCT05528952	UCPVax (emulsified in montanide ISA-51); atezolizumab and bevacizumab	Unresectable hepatocellular carcinoma	2022	II	[58]
28	NCT04280848	UCPVax; radiotherapy and temozolomide	Glioblastoma	2020	II	[59]

(Continued)

Table I (Continued).

	Identifier Code	Intervention/Treatment	Approved Application/ Indication	Study Start Year	Phase	Ref
29	NCT03946358	UCPVax and atezolizumab	Human papilloma virus positive cancers	2019	II	[60]
30	NCT04263051	UCPVax and nivolumab	Advanced non-small cell lung cancer	2020	II	[61]
31	NCT01935154	VX-001	Stage IV non-small cell lung cancer	2013	II	[62]
32	NA	Vx-001	Advanced solid tumors	2012	II	[63]
33	NA	Vx-001 (Montanide ISA51)	Advanced non-small cell lung cancer	2013	II	[64]
34	NA	Vx-001	Advanced non-small cell lung cancer	2014	II	[65]
35	NCT04300244	Nivolumab and ipilimumab with or without UVI	Inoperable malignant pleural mesothelioma after first-line platinum-based chemotherapy	2020	II	[66]
36	NCT01789099	UVI and GM-CSF (leukine)	Non-small cell lung cancer	2013	I/II	[67]
37	NCT02275416	UVI; GM-CSF and ipilimumab	Unresectable or metastatic malignant melanoma	2014	I/II	[68]
38	NCT01784913	UVI and GM-CSF	Men with metastatic hormone-naive prostate cancer	2013	I/II	[69]
39	NCT02293707	GX301 (emulsified in montanide ISA-51 and imiquimod 5% cream)	Castration-resistant prostate cancer	2014	II	[70]
40	NCT00079157	Telomerase: 540–548 peptide vaccine emulsified in montanide ISA-51 and sargramostim (GM-CSF)	Stage IV breast cancer	2004	I	[71]
41	NCT00021164	Telomerase: 540–548 peptide vaccine (emulsified in montanide ISA-51) and aldesleukin	Metastatic cancer	2003	II	[72]
42	NCT00069940	Telomerase: 540–548 peptide vaccine and sargramostim (GM-CSF)	Sarcoma or brain tumor	2003	I	[73]
43	NA	TERT _{572Y} peptide vaccine (emulsified in montanide ISA51)	Advanced cancer	2006	I	[74]
44	NA	TERT _{572Y} peptide vaccine	Advanced non-small cell lung cancer	2007	I	[75]
45	NCT00573495	hTERT/survivin multi-peptide vaccine; daclizumab and prevnar	Metastatic breast cancer	2007	I	[76]
46	NCT00499577	TERT and surviving multi-peptide vaccine	Myeloma	2011	I/II	[77]
47	NCT04515043	INVAC-I	Solid tumor	2020	I	[78]
48	NCT00753415	V934/V935 hTERT DNA vaccines	Solid tumors	2008	I	[79]
49	NCT02960594	INO-1400 or INO-1401 (hTERT) with or without INO-9012 (IL-12 DNA) delivered by electroporation	Solid tumors	2016	I	[80]
50	NCT03502785	INO-5401 (WT1, PSMA and hTERT); INO-9012 (IL-12) and atezolizumab	Locally advanced unresectable or metastatic/recurrent urothelial carcinoma	2018	I/II	[81]
51	NCT03491683	INO-5401; INO-9012; cemiplimab (REGN2810); radiation and chemotherapy (temozolomide)	Newly-diagnosed glioblastoma	2018	I/II	[82]
52	NA	mRNA (MUC1, CEA, Her-2, telomerase, surviving and MAGE-A1) and GM-CSF	Stage IV renal cell cancer	2011	I/II	[83]
53	NCT00510133	GRNVAC I	Acute myelogenous leukemia	2007	II	[84]
54	NCT01153113	hTERT mRNA DCs	Metastatic prostate cancer	2010	I/II	[85]
55	NA	Tumor RNA-transfected dendritic cells	Renal cancer	2003	I	[86]

(Continued)

Table 1 (Continued).

	Identifier Code	Intervention/Treatment	Approved Application/ Indication	Study Start Year	Phase	Ref
56	NCT01410968	Poly-ICLC and peptide-pulsed dendritic cells	Metastatic, locally advanced, unresectable, or recurrent pancreatic adenocarcinoma	2011	I	[87]
57	NCT00197912	p53, survivin and telomerase peptide-pulsed dendritic cells	Advanced melanoma	2005	I/II	[88]
58	NCT00197860	Survivin and telomerase peptides or tumor lysate DCs and low-dose IL-2	Advanced renal cell carcinoma	2005	I/II	[89]
59	NA	Survivin and telomerase peptide-pulsed dendritic cells and low-dose IL-2	Metastatic renal cell carcinoma (mRCC)	2009	I/II	[90]
60	NA	P53, survivin and telomerase peptide-pulsed dendritic cells; interleukin (IL)-2 and interferon (IFN)- α 2b	Malignant melanoma	2010	I/II	[91]
61	NA	hTERT I540 peptide and keyhole limpet hemocyanin (KLH) autologous DCs	Prostate or breast cancer	2005	I	[92]
62	NCT00061035	Transgenic lymphocyte immunization vaccine	Prostate adenocarcinoma	2003	I	[93]
63	NCT00925314	CB-10-01 (transgenic lymphocyte immunization)	Stage III Melanoma	2009	II	[94]

Abbreviation: NA, not available.

Table 2 Preclinical Researches of Telomere-Related Anti-Cancer Therapeutics

	Drug	Tumor Models	Descriptions	Year	Ref
1	GV1001; GEM; GM-CSF	Patients with pancreatic adenocarcinomas	Inducing telomerase specific immune responses	2014	[95]
2	GV1001	MCF7 (human breast adenocarcinoma cell line), Jurkat (human T-cell leukemia cell line), MC38 (murine colon adenocarcinoma) and HeLa (human cervical adenocarcinoma) cells	Reducing HSP70/90, HIF-1 α and VEGF	2014	[96]
3	GV1001; GEM	Pancreatic ductal adenocarcinomas (PDACs)	Reducing fibrosis with decreasing TNF- α , interleukin (IL)-6 and IL-1 β	2016	[97]
4	GV1001	Prostate cancer	Binding and antagonizing GnRHR through activation of Gas/cAMP pathway and downregulating releasing of G α q-coupled Ca ²⁺	2019	[98]
5	hTERT peptides	BI6F10 melanoma	Inducing high avidity CD4 ⁺ T _H 1 cells, activating dendritic cells, enhancing primary and memory CTL responses	2012	[99]
6	hTERT peptides	Hepatocellular carcinoma	Positive T cell responses	2018	[100]
7	rAAV-/rAdv viral cocktail expressing hTERTC27	Melanoma	Activation of NK cells	2010	[101]
8	hTERT with a lentiviral vector system	Melanoma	CD8 ⁺ T cell responses	2010	[102]
9	hTERT and HER-2/neu multi-peptides	Various cancers	Stimulating specific CTLs	2002	[103]
10	TERT and HCV multi-peptides; taxanes and alkylating agents	Hepatocellular carcinoma	Enhancing specific T cell responses and reducing Treg frequency	2015	[104]

(Continued)

Table 2 (Continued).

	Drug	Tumor Models	Descriptions	Year	Ref
11	TERT and HCV DNA	Liver cancer	Inducing multi-cytokine response of CD4 ⁺ and CD8 ⁺ T cells	2021	[105]
12	CCL21-TERT-Fc DNA; anti-4-1BB mAbs	Various cancers	Enhancing the immune responses of NK, CD4 ⁺ and CD8 ⁺ T cell	2006	[106]
13	TERT DNA and α -CTLA-4/ α -PD-1	TC-I tumor	Improving antigen-specific immune responses	2018	[107]
14	TERT DNA and CCL21	Breast cancer	Augmenting antigen-specific immunity	2007	[108]
15	lenti-hTERT vector-transduced DC vaccines	Hepatocellular carcinoma	Stimulating CTLs	2011	[109]
16	rAd-hTERT transduced DCs	Various tumors	Inducing CTLs and increasing IFN- γ	2006	[110]
17	Lipid-mediated transfection of hTERT DNA into DCs	Various tumors	Inducing specific T-cell-mediated tumor immunity	2003	[111]
18	TERT mRNA transfected DCs	Melanoma	Inducing IFN- γ secreting CTLs	2007	[112]
19	hTERT and survivin mRNA, IDO siRNA transfected DCs	Ovarian cancer	Inducing antitumor immunity.	2013	[113]
20	hTERT TCR	Melanoma	Redirecting CD4 ⁺ and CD8 ⁺ T cells and promoting inflammatory cytokines	2021	[114]
21	TERT expressed with Mannan-modified adenovirus	Melanoma	Inducing antitumor immunity	2007	[115]
22	TERT and VEGFR-2 expressed with mannan-modified adenovirus	Breast and colon cancer	Displaying synergistic antitumor immune responses and reducing angiogenesis	2015	[116]
23	5-aza-2'-deoxycytidine	Chronic myelogenous leukemia	Decreasing hTERT expression through reducing the binding of c-myc with hTERT promoter	2014	[117]
24	Perylene derivates PM2 and PIPER	Lung cancer	Downregulation of hTERT through inducing G-quadruplex formation in telomere and hTERT promoter regions	2013	[118]
25	A retrovirus vector with full-length hTERT antisense complementary DNA	Ovarian cancer	Decreasing hTERT expression and telomerase activity	2016	[119]
26	ZD55-hTERT (an oncolytic adenovirus-based shRNA delivery system)	Renal cancer	Silencing hTERT expression	2009	[120]
27	PEG-CMCS/CaP nanoparticles loaded with hTERT siRNA	Hepatocellular carcinoma	Silencing hTERT expression	2014	[121]
28	Chelidonine	Breast cancer	Inhibiting telomerase activity	2018	[122]
29	Rhodospirillum rubrum L-asparaginase mutant RrA	Various cancers	Reducing telomerase activity	2017	[123]
30	Suramin	Various cancers	Inhibiting telomerase activity	2015	[124]
31	2'-O-methyl-RNA delivered with chitosan-coated poly(lactide-co-glycolide) (PLGA) nanoparticles	Lung cancer	Inhibiting telomerase activity	2010	[125]
32	Gold nanoparticles (AuNPs) functionalized with ¹¹¹ In-labelled oligonucleotides	Various cancers	Inhibiting telomerase activity	2021	[126]

(Continued)

Table 2 (Continued).

	Drug	Tumor Models	Descriptions	Year	Ref
33	BIBRI 1532	Cervical cancer	Evoking TEL mutation and reducing the recruitment of telomerase to telomere	2013	[127]
34	TPPI-OB domain overexpression with lentivirus transduction	Lung cancer	Inhibiting the recruitment of telomerase to telomeres and enhancing the sensitivity to paclitaxel	2019	[128]
35	Cisplatin derivate Tetra-Pt	Various cancers	Inhibiting ALT pathway	2017	[129]
36	Withaferin-A	Various cancers	Blocking ALT pathway	2017	[130]
37	TMPyP4	Osteosarcoma	Blocking ALT pathway and inducing formation of telomere and FAK G-quadruplex	2021	[131]
38	6-thio-2'-deoxyguanosine (6-thio-dG)	Lung cancer	Inducing telomere dysfunction	2015	[132]
39	6-thio-dG	Melanoma	Inducing telomere dysfunction and overcoming therapy resistance	2018	[133]
40	Tolyl terpyridin-Pt complex (Pt-tppy)	Ovarian carcinoma	Inducing telomere dysfunction through binding to G-quadruplex (G4) structures	2021	[134]
41	Terpyridine platinum (Pt-tpy)	Fibrosarcoma	Inducing telomere dysfunction	2021	[135]
42	Ru ^{II} -Pt ^{II} complexes encapsulated with biotin-functionalized DNA cages	Various cancers	Inducing telomere dysfunction through stabilizing G-quadruplex	2022	[136]
43	BRACO-19	Glioblastoma	Inducing telomere dysfunction through binding to G-quadruplex	2016	[137]
44	Telomestatin	Glioblastoma	Inducing telomere dysfunction through stabilizing G-quadruplex	2016	[138]
45	Schizocommunin derivative	Various cancers	Inducing telomere dysfunction through formation of G-quadruplex	2018	[139]
46	Pyridostatin analogues	Various cancers	Inducing telomere dysfunction through stabilizing G-quadruplex	2012	[140]
47	Lpid nanoparticles (LNPs) containing miR-182-3p	Breast cancer	Inducing telomere dysfunction	2023	[141]
48	5-azacytidine (5-AZA)	Acute myeloid leukemia	Concomitantly inducing telomere dysfunction and telomere length shortening	2015	[142]
49	Bortezomib	Leukemic and gastric cancer	Concomitantly inducing telomere dysfunction and telomere length shortening	2015	[143]
50	MST-312	Breast cancer	Concomitantly inducing telomere dysfunction and telomere length shortening	2014	[144]
51	Au@Ag nanorods loaded with DOX	Cervical cancer	Releasing DOX to telomerase positive cells	2014	[145]
52	MSNP-NH ₂ -DOX-DNA system	Various cancers	Slow and sustained releasing of DOX to telomerase positive cells	2018	[146]
53	dCas9-MSNs/DOX/DNA	Cervical cancer	Releasing DOX into the nuclei of telomerase positive cells	2021	[147]
54	PtNPs@DNA	Gastric cancer	Releasing PtNPs to telomerase positive cells and overcoming drug resistance	2018	[148]
55	AS1411/nanotube/RTA	Various cancers	Releasing RTA to telomerase positive cells	2021	[149]

(Continued)

Table 2 (Continued).

	Drug	Tumor Models	Descriptions	Year	Ref
56	Self-assembled DNA polymer	Various cancers	Detecting and in situ monitoring telomerase activity of cancer cells	2018	[150]
57	Dox-AuNP-MB	Various cancers	Detecting the intracellular telomerase activity and releasing DOX to telomerase positive cells	2016	[151]
58	Au-MPs-DOX	Various cancers	Detecting the intracellular telomerase activity and releasing DOX to telomerase positive cells	2017	[152]

Activation of Anti-Telomerase Immune Responses

As the majority of telomere dynamics targeted anticancer therapeutics applied in the clinical trial, anti-telomerase immunotherapeutics such as OBP-301, KH901, GV1001, VX-001 and UV1 have achieved impressive efficacy in various tumors with favorable tolerance as illustrated in the previous section. Currently, the agents that activate the anti-telomerase immune responses could be divided into TERT peptide vaccines, TERT mRNA/DNA vaccines, TERT peptide/mRNA/DNA-DC vaccines and others, according to the diverse types of agents and mechanisms of action.

TERT Peptide Vaccines

As described above, several clinical trials reported that combinations of GV1001 (a 16 amino acid hTERT peptide including positions 611–626, EARPALLTSRLRFIPK) with radiotherapy or chemotherapy such as gemcitabine have exhibited superior safety and efficacy in patients with various tumors. Staff et al discovered that GV1001 in combination with gemcitabine and GM-CSF, caused telomerase-specific immune responses and mild adverse effects in 10/17 patients with pancreatic adenocarcinomas.⁹⁵ In addition to telomerase-related immune responses, GV1001 displayed potential antitumor efficacy, such as inhibiting angiogenesis via the suppression of HSP90/HSP70/HIF-1 α /VEGF pathway,⁹⁶ reducing fibrosis in pancreatic ductal adenocarcinomas (PDACs) via the decrease of TNF- α , interleukin (IL)-6 and IL-1 β ,⁹⁷ and antagonizing the gonadotropin-releasing hormone receptor (GnRHR) agonist via the direct binding to GnRHR and activation of G α s/cAMP pathway.⁹⁸ Apart from several hTERT peptides,^{99,100} delivering hTERT peptide vaccines with recombinant adenovirus and adeno-associated virus¹⁰¹ or a lentiviral vector¹⁰² led to stronger and more sustained antitumor immune responses and enhanced immunological tolerance. Moreover, multi-peptide vaccines consisting of hTERT and HER-2/neu¹⁰³ or hepatitis C virus (HCV)¹⁰⁴ also exerted prominent anti-tumor effects in antigen-expressing cancer cells.

TERT mRNA/DNA Vaccines

Jansons et al have elucidated that a DNA vaccine derived from rat TERT contributed to the antitumor responses by inducing the release of IFN- γ /IL-2/TNF- α from CD4⁺ and CD8⁺ T cells.¹⁰⁵ For the purpose of improving the antigen-specific antitumor immunity of DNA vaccines, bringing in the adjuvants has gained extensive attraction. The DNA vaccine (pCCL21-Te-Fc) was based on plasmids and constructed by linking human CCL21 and IgG Fc to two hTERT fragments. As shown, the vaccine could inhibit tumor growth through the CD8⁺ T cells mediated antitumor immune responses. Furthermore, the combination of pCCL21-Te-Fc and anti-4-1BB monoclonal antibodies (mAbs) could augment immune-responses and significantly prolong the survival of tumor-bearing mice, with 75% by contrast to 25% of mice surviving longer than 120 days.¹⁰⁶ Homoplastically, the TERT DNA vaccines combined with immune checkpoint inhibitors¹⁰⁷ or CCL21 chemokine¹⁰⁸ could prolong survival, enhance immune responses and restrain tumor growth of cervical cancer and breast cancer.

TERT Peptide/mRNA/DNA-DC Vaccines

Besides the free TERT antigen from TERT peptide and the expressed TERT from mRNA or DNA, the primed DCs could also activate specific anti-telomerase immunity. Cui et al constructed a lentivirus which contains hTERT cDNA fragments

and transduced the lenti-hTERT vectors into DCs. The lenti-hTERT vector-transduced DC vaccines dramatically evoked anticancer immunity in telomerase positive HepG2 cells.¹⁰⁹ Analogously, DC vaccines that expressed hTERT through the transduction with an hTERT recombinant adenoviral vector¹¹⁰ or the transfection with lipid-mediated hTERT plasmid,¹¹¹ could arouse antitumor immune reactions by the induction of hTERT specific cytotoxic T lymphocytes and the increased production of IFN- γ in hTERT-positive cancer cells.¹¹⁰ Moreover, DCs transfected with TERT mRNA could markedly restrain the TERT-positive tumor growth and improve the survival of tumor-bearing mice, through the induction of antitumor immunity.¹¹² Furthermore, DC vaccines transfected with indoleamine 2, 3-dioxygenase (IDO) siRNA, hTERT, and survivin mRNA also exerted enhanced antitumor effects.¹¹³

Others

With the development of T cell receptor (TCR)-engineered T cell anticancer therapy, Radium-4, an hTERT specific TCR sequence derived from the patient who has been vaccinated with hTERT peptide, could suppress tumor growth in hTERT⁺ melanoma and prolong survival through directing CD4⁺ and CD8⁺ T cells, and secreting pro-inflammatory cytokines.¹¹⁴ Considering that the mannan receptors are expressed on the surface of antigen presenting cells (APCs), mannan has been applied to modify the immune-stimulatory viruses, for the sake of inducing antitumor immune responses through activation of APCs. Ding et al constructed a telomerase-expressing adenovirus which was modified with mannan; evidently, the adenovirus successfully delivered the telomerase antigen to DCs and thus activated the CD4⁺ and CD8⁺ T cells based antitumor immunity, and remarkably inhibited tumor growth.¹¹⁵ Furthermore, the combination of mannan-modified adenoviruses expressing TERT and vascular endothelial growth factor receptor-2 (VEGFR-2) displayed notably synergistic antitumor immune responses and reduced intratumoral angiogenesis in murine breast and colon cancer models.¹¹⁶

Shortening of Telomere Lengths

In view of the paramount role telomere lengths play in cancer progression, some telomere dynamics-targeted anticancer therapeutics pursue the shortening of telomere lengths. As illustrated in the following section, those anti-cancer therapeutic agents can be divided into direct shortening telomere lengths and indirect shortening telomere lengths. For the latter, the indirect shortening of telomere length may be induced by various mechanisms, such as the downregulation of TERT expression, inhibition of telomerase activity, reduction of telomerase recruitment to telomere, and suppression of ALT pathway. On account of the difficulty to distinguish whether the telomere length shortening was the consequence of the direct drug action, the following section focused on the illustration of the drugs that indirectly affect the telomere lengths through telomerase or ALT pathway.

Downregulation of TERT Expression

Current studies have demonstrated that several signaling pathway inhibitors or gene therapy technology could achieve the downregulation of TERT expression and thus shortening telomere lengths. Grandjette et al found that 5-aza-2'-deoxycytidine (DAC, a DNA demethylating agent) decreased the lengths of telomeres through the downregulation of TERT expression which was triggered by reducing the binding of c-myc with hTERT promoter.¹¹⁷ Moreover, the perylene derivatives PM2 and PIPER evoked the formation of G-quadruplex in regions of both telomere and hTERT promoter in A549 cells, which thereby decreased the hTERT expression. The hTERT downregulation led to significant telomere shortening and ultimately suppressed the proliferation and tumorigenicity of A549 cancer cells.¹¹⁸ Furthermore, Qi et al has created a recombinant retrovirus vector carrying a full-length hTERT antisense complementary DNA. The vector system could obviously downregulate the expression of hTERT gene and telomerase activity, eventually restraining the ovarian tumor growth and prolonging the mice survival time.¹¹⁹ Similarly, the ZD55-hTERT (an adenovirus-based shRNA delivery system)¹²⁰ and the PEGylated carboxymethyl chitosan/calcium phosphate (PEG-CMCS/CaP) hybrid anionic nanoparticles loaded with hTERT siRNA¹²¹ could markedly silence hTERT and thus inhibit tumor growth.

Inhibition of Telomerase Activity

As elaborated above, the telomerase activity inhibitors imetelstat³⁶ and KML-001³⁹ have showed favorable anti-cancer efficacy in combination with platinum-based chemotherapy in the clinical trials. The therapeutics inhibited the activity of

telomerase through binding to the telomerase subunits or stabilizing the G-quadruplex, etc. Nouredini et al disclosed that the chelidone (a benzylisoquinoline alkaloid) could make the telomeres shortened to approximately 30% of that in untreated breast cancer MCF7 cells through inhibiting telomerase activity (with an IC_{50} value of $0.45 \pm 0.08 \mu\text{M}$ at 48 h exposure time).¹²² Analogously, the *Rhodospirillum rubrum* L-asparaginase mutant RrA, could gradually decrease the telomere lengths from $10,105 \pm 2530$ bp to 1233 ± 636 bp after 35 days of treatment; in association, the telomerase activity reduced to less than $29.63 \pm 12.3\%$ of control.¹²³ Besides, suramin could enhance the chemosensitivity of cancer cells via inhibiting telomerase (with IC_{50} values of 1–3 μM at 24 h); and subsequently, induce gradual telomere shortening (by more than 40% reduction with continuous exposure for 6 weeks).¹²⁴ Apart from the above-mentioned chemotherapeutics, several nanoparticles are also active in the inhibition of telomerase activity. As reported, utilizing chitosan-coated poly(lactide-co-glycolide) (PLGA) nanoparticles to deliver 2'-O-methyl-RNA to human lung cancer cells could suppress telomerase with an inhibition rate of about 80% and shorten telomere lengths from 5.9 kb down to 4 kb.¹²⁵ Similarly, gold nanoparticles (AuNPs) functionalized with ^{111}In -labelled oligonucleotides and cell-penetrating peptide Tat could enhance the uptake of oligonucleotides and inhibit telomerase activity.¹²⁶

Reducing the Recruitment of Telomerase to Telomere

The TPP1 protein was essential for the recruitment of telomerase to telomeres through the binding of an N-terminal oligonucleotide/oligosaccharide-binding (OB) domain on TPP1 to telomerase. The TPP1 glutamate (E) and leucine (L)-rich (TEL) patch on the surface of TPP1-OB mediates the interaction between TPP1 and telomerase, and thus achieves the recruitment of telomerase to telomeres, leading to telomere elongation.¹⁵³ Nakashima et al discovered that BIBR1532 could evoke the mutations of TEL patch, shorten the telomere lengths, and thereby induce apoptosis in HeLa cells.¹²⁷ Considering the crucial role of TPP1-OB, Zhu et al have generated the TPP1-OB domain-overexpressed lung cancer cells via the lentivirus transduction. As shown, TPP1-OB domain overexpression could competitively disrupt the interaction of endogenous TPP1 and telomerase, thus impeding the localization and binding of telomerase to telomeres. The results showed that TPP1-OB could inhibit both lung cancer cell proliferation in vitro and tumor growth in vivo accompanied with shortened telomere lengths.¹²⁸

Suppression of ALT Pathway

Independent of telomerase, Zheng et al revealed that the derivate of cisplatin Tetra-Pt (bpy) observably could suppress tumor growth through inhibiting ALT pathway in ALT-activated U2OS cancer cells and shorten telomere lengths with a mean length of 943.16 ± 121.65 TFU in treated cells compared with 1396.09 ± 278.16 TFU in untreated cells. Therein, ALT-positive cells treated with Tetra-Pt (bpy) could present fewer ALT-associated promyelocytic leukemia bodies, telomere sister chromatid exchanges and extrachromosomal C-circles due to the reduction of telomeric homologous recombination.¹²⁹ Similarly, withaferin-A¹³⁰ and TMPyP4¹³¹ also displayed prominent cytotoxicity towards ALT-activated cancer cells through blocking ALT pathway, manifested as fewer extrachromosomal C-circles and ALT-associated promyelocytic leukemia bodies.

Inducing Telomere Dysfunction

Except for the progressive shortening of telomeres, the induction of telomere dysfunction also offers a novel strategy to suppress cancer progress through affecting telomere dynamics. Telomere dysfunction refers to a process of telomere-correlated DNA damages, manifested as chromosomal instability leaded by double-stranded breaks (DSBs) located at telomeres. In the evaluation of drug activity, immune-FISH (fluorescence in situ hybridization) assay was usually utilized to visualize the telomere dysfunction marker (telomere induced DNA damage foci, TIFs or telomere associated DNA damage response foci, TAFs), which was the colocalization of telomeres and DNA damage response proteins such as $\gamma\text{H}_2\text{AX}$, ATM and 53BP1.¹⁵⁴ Currently, anti-cancer chemotherapeutics that induce telomere dysfunction mainly include nucleoside analogues, G-quadruplex ligands and others.

Ilgén et al discovered that 6-thio-2'-deoxyguanosine (6-thio-dG), a nucleoside analogue originated from the approved drug 6-thioguanine, acting as the substrate of telomerase, could be inserted into the newly synthesized telomere sequence, resulting in the disruption and dysfunction of telomeres. As shown, 6-thio-dG could inhibit the proliferation, induce the generation of

TIFs and shorten telomeres in various telomerase-positive cancer cells.¹³² Moreover, 6-thio-dG exhibited ascendant efficacy in impairing cell viability, and inducing telomere dysfunction in telomerase-positive cancer cells and BRAF-mutated or therapy-resistant melanoma cells.¹³³ As elaborated above, 6-thio-dG was applied in a Phase II clinical trial to examine the efficacy and safety of its combination with cemiplimab (a PD-1 inhibitor) on non-small cell lung cancer.⁴⁰

Telomeres might form four stranded structures named G-quadruplexes (G4s) with the G-rich repeated sequences. The G4s could hinder the recognition and interaction of telomerase and telomere. The ligands of G4s could stabilize the structure of G4s and thus lead to genomic instability.¹⁵⁵ Ali et al reported that the tolyl terpyridin-Pt complex (Pt-ttpty) irreversibly bound to G4s with metal coordination features, and thereby brought about the displacement of TRF2 from telomere and telomeric DNA damage and telomere length shortening.¹³⁴ Similarly, terpyridine platinum (Pt-tpy) and its derivatives increased TIFs and led to chromosomal instability by triggering the formation of micronucleus and chromatin bridges in late mitosis.¹³⁵ Based on the above-mentioned studies of Pt complex, a chiral Ru^{II}-Pt^{II} complexes encapsulated with biotin-functionalized DNA cages could enhance the sub-cellular localization and cancer selectivity, meanwhile retaining the activity of G-quadruplex stabilizing.¹³⁶ Zhou et al demonstrated that BRACO-19 could induce telomeric DNA damage, telomere uncapping and T-loop disassembly characterized by the dissociation of TRF2 and POT1 from telomeres, and simultaneously restrain telomerase activity in human glioblastoma cells.¹³⁷ Besides, telomestatin, an antibiotic isolated from *Streptomyces anulatus* 3533-SV4, could stabilize the G4s and contribute to telomere dysfunction and delocalization of TRF2 from telomeres, ultimately inhibiting the growth of glioma stem cells (GSCs) both in vitro and in vivo.¹³⁸ Furthermore, the novel schizocommunin derivative¹³⁹ and pyridostatin analogues¹⁴⁰ could also cause telomere dysfunction and finally restrain tumor growth through stabilizing G4s.

Besides disrupting the structure of telomeres, impacting on the expression of telomere complex could also lead to telomere dysfunction. Dinami et al discovered that miR-182-3p delivered with lipid nanoparticles (LNPs) could suppress breast cancer through reducing TRF2 expression and inducing DNA damage at telomeric sites. Moreover, the LNPs could cross the blood-brain barrier and reduce the metastatic tumor lesions in the brain.¹⁴¹ Several chemotherapeutics could concomitantly induce telomere dysfunction and telomere length shortening. Zhang et al found that 5-azacytidine (5-AZA, a DNA methyltransferase inhibitor) could trigger telomere dysfunction with the increased number of 53-BP1 and telomere colocalization foci, concurrently downregulating TERT expression and shortening telomeres, and eventually inducing apoptosis of acute myeloid leukemia (AML) cells.¹⁴² Analogously, bortezomib (a ubiquitin-proteasome pathway inhibitor¹⁴³) and MST-312 (a chemically modified analogue from epigallocatechin gallate¹⁴⁴) could decrease TERT expression, suppress telomerase activity, shorten telomere lengths; and thus cause telomere dysfunction in leukemia, gastric cancer and breast cancer cells, respectively.

Telomerase-Responsive Drug Release System

Currently, stimuli-activatable design based on endogenous factors (tumor specific features such as low pH and over-expressed enzymes) or exogenous stimuli (light, ultrasound, magnet, and radiation) has emerged as a promising strategy for improving nanomedicines in cancer diagnosis and therapy.¹⁵⁶ Considering the general upregulation or activation of telomerase in multifarious cancers, telomerase was chosen as a responsive target for nano-delivery systems of anti-cancer drugs. The telomerase-responsive delivery system could specifically deliver and release drugs to telomerase positive cancers, greatly reduce the toxic and side effects of drugs on normal cells and provide more opportunities for the practical drug application.

Zong et al designed and prepared a telomerase-triggered drug releasing nanocarrier system, in which the nanocarrier displayed a core-shell structure with mesoporous silica nanoparticles as drug loading shell and Au@Ag nanorods (NRs) as the surface enhanced Raman scattering (SERS) active core. The nanocarrier pores were loaded with doxorubicin (DOX) and blocked by an oligonucleotide (CAP1) containing a telomeric repeated complementary sequence and a telomerase substrate primer sequence. The hairpin structure was formed by lengthened telomeric sequences of CAP1, separated from the nanocarrier and thus released DOX from the opened pores. As predicted, the results showed that DOX were only released and diffused into the nuclei of telomerase positive HeLa cells.¹⁴⁵ Analogously, Srivastava et al constructed the MSNP-NH₂-DOX-DNA system, and discovered that the system displayed slow and sustained release of DOX in telomerase positive MCF-7, K-562, and DL cells, with remarkable inhibition of proliferation and

induction of apoptosis; and the efficacy was stronger than that of free DOX.¹⁴⁶ Moreover, in order to achieve the active targeting capability of the telomerase-responsive systems, Ma et al generated a CRISPR-dCas9 guided nanosystem dCas9-MSNs/DOX/DNA. This system could release DOX into the nuclei of telomerase overexpressed HeLa cells, and exert enhanced anticancer effects both *in vitro* and *in vivo* (with a tumor inhibition rate of 88%).¹⁴⁷

Besides the above-mentioned delivery systems based on mesoporous silica nanoparticles, the nanostructures of DNA materials have been applied in the telomerase-responsive delivery system. PtNPs@DNA, a self-assembled DNA icosahedra nanoparticles encapsulated with platinum, could specifically release PtNPs to cancer cells with high telomerase activity, overcome the drug resistance and alleviate systemic toxicity in BCG823/DDP (cisplatin-resistant human gastric cancer) cells.¹⁴⁸ Similarly, AS1411/nanotube/RTA, a DNA nanotube modified with targeted aptamer of nucleolin (AS1411) and loaded with ricin A chain (RTA), could accumulate and release RTA more intensively in tumor cells with high expression of telomerase, while showed favorable safety.¹⁴⁹

Apart from specifically releasing drugs to telomerase positive cancer cells, the telomerase-responsive delivery system has been utilized as signal probes to dynamically monitor the telomerase activity in living cells, displaying the potential in diagnostic and biological applications. Zhu et al have developed a multivalent self-assembled DNA polymer that was constructed through telomerase primer chain and two hairpin probes functioned with tumor targeting aptamer and signal probe. The DNA polymer performed well in detecting and *in situ* monitoring the telomerase activity of cancer cells.¹⁵⁰ Furthermore, Dox-AuNP-MB, a gold nanoparticle-based molecular beacon conjugated with FITC-labeled telomerase primer hybridized hairpin DNA sequences and loaded with DOX, could detect the intracellular telomerase activity of living cells and precisely release DOX to cancer cells without toxicity to normal cells.¹⁵¹ Similarly, Au-MPs-DOX, a gold nanoparticle-conjugated with carboxyfluorescein (FAM)-fluorescence biopolymer initiated by telomere extension and loaded with DOX, could have superior efficiency for detection of telomerase activity and drug delivery to telomerase-expressed cells.¹⁵²

Conclusions and Future Directions

Telomeres play a pivotal role in cancer progression; accordingly, targeting telomere dynamics is an effective way for development of novel cancer therapeutics. A variety of telomere-active agents have been extensively investigated; furthermore, the diversity of molecular mechanisms of action are elucidated. The studies of telomere-based cancer therapeutics have achieved substantial progress. Although none of the telomere dynamics-targeted therapeutics has been approved in the clinical application of anticancer therapy, studies have achieved favorable advances in the preclinical studies and early phase of clinical trials, as chemotherapeutics and immunotherapeutics. The main problems impeding the clinical utilization of the telomere dynamics-targeted therapeutic agents include the long lag time between drug administration and the emergence of clinical responses, and related side effects.

Currently, numerous telomere dynamics-targeted therapeutics such as telomerase inhibitors work through shortening telomere lengths. Nevertheless, the tumor suppression effects appear only after the telomeres shortened to the critical lengths, which may demand a long time and depend on the cancer initial telomere length. Meanwhile, the remanent telomerase activity or even the activation of ALT in cancer cells after treatment with telomerase inhibitors may also delay the process of telomere shortening. Hence, telomere dynamics-targeted therapeutics that indirectly shorten telomere lengths may be more suitable for the treatment of cancer patients with short original telomere length, or used as an adjuvant therapy and maintenance therapy to prevent cancer recurrence after conventional surgery, radiotherapy or chemotherapy. In addition to extending the telomere lengths, telomerase may also exert a wide variety of effects, such as maintaining the quantity and multipotency of cancer stem cells through upregulating expressions of CD117, Oct4 and Sox-2,¹⁵⁷ promoting cancer cell migration and invasion through increasing MMP9, TGF- β 1, integrin β 1 (ITGB1), heparinase and VEGF and activating Wnt/ β -catenin axis,¹⁵⁸ and preventing cell cycle arrest caused by DNA damage through blocking checkpoint signal transduction.¹⁵⁹ Therefore, all those mentioned above lay a foundation for the combinations of telomere dynamics-targeted therapeutics and various chemotherapeutics.

Meanwhile, many telomere dynamics-targeted therapeutics may act as immunotherapeutics or immune-modulating agents through eliciting immune responses. As reported, 6-thio-dG could induce telomere stress which activates antitumor immunity via cGAS/STING/IFN-I pathway, and also synergize with immune checkpoint inhibitors.¹⁶⁰

What's more, telomere dysfunction leads to activation of innate immunity through the TERRA-ZBP1 complex induced by the cGAS/STING pathway.¹⁶¹ Furthermore, a prospective cohort of 70 bladder cancer patients reveals the positive correlation between the expression of TERT and PD-L1/2.¹⁶² Thus, more and more studies have provided the basis for the combination of telomere dynamics-targeted therapeutics and immunotherapeutics.

Instead of merely shortening telomere lengths, multiple action mechanisms involve in the suppression of telomere dynamics. The diversified strategies for development of telomere-related therapeutics cover the suppression of telomerase activity, downregulation of TERT expression, reduction of telomerase recruitment to telomeres, induction of telomere dysfunction and disruption of alternative lengthening of telomeres (ALT) pathway. The diversity of action mechanisms indicates that a great variety of telomere dynamics-targeted agents including small molecule compounds, ligand-based peptides, recombinant fusion proteins, and monoclonal antibodies could be generated and evaluated.

For the sake of enhancing therapeutic efficacy and reducing toxicity, the telomere targeted agents may be modified and reconstituted. Consequently, the prepared telomere targeted agents are integrated with the capability of tumor-specific drug delivery. These kinds of tumor microenvironment (TME)-oriented therapeutics are generated on the basis of the active targeting delivery systems such as antibody drug conjugates (ADCs) and the passive targeting delivery systems such as human serum albumin (HSA)-based drug conjugates or nanoparticles. In our research, a recombinant EGFR targeted fusion protein conjugate induces telomere length shortening, telomere dysfunction and telomerase downregulation. The conjugate possesses the active targeting capability of Fv fragment of an anti-EGFR monoclonal antibody and the passive targeting capability of the HSA domain.¹⁶³

Overall, targeting telomere dynamics has emerged as an effective approach for the discovery and development of cancer therapeutics. A wide variety of active agents have been under preclinical investigation and clinical trials. Telomere dynamics-active agents are potentially effective as anti-cancer chemotherapeutics or immunotherapeutics. Therefore, the therapeutics acting through the activation of anti-telomerase immune responses can be developed via the combination with immunotherapeutic agents. The therapeutics working through shortening telomere lengths should be precisely applied in the patients with initial short telomeres, or in the adjuvant and maintenance therapy. Although there are disadvantages, telomere dynamics-targeted therapeutics have shown great potential in cancer therapy.

Disclosure

The authors report no conflicts of interest in this work.

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