

TELOMERASE IS A GOOD TARGET FOR CANCER TREATMENT**Priyanka V. Bandivadekar*, Shraddha A. Phadke, Sunita Patil and Tejshree Sonawne**

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University.**ABSTRACT**

Telomerase, the ribonucleoprotein enzyme maintaining the telomeres of eukaryotic chromosomes, is active in most human cancers and in germ line cells. Telomerase plays a key role in cell fate: loss of telomerase in normal differentiated cells heralds senescence and limits cell division, whereas reactivation of telomerase sustains proliferation and potentiates mutagenesis and transformation. Given this pivotal role, Telomerase has been the subject of intense investigation in the field of developmental cancer therapeutics which lead to telomere shortening and cessation of unrestrained proliferation. A broad spectrum of therapeutic strategies has been developed, ranging from direct targeting or reprogramming of the enzyme, to immune or virus-mediated targeting of cells expressing

telomerase, to strategies focusing on the telomeres themselves. We will review the mechanistic rationale and preclinical and clinical state of development of the various telomerase-based therapeutic approaches.

KEYWORDS: telomerase, senescence, proliferation, mutagenesis.**INTRODUCTION****Telomeres, telomerase & their structure****• TELOMERES**

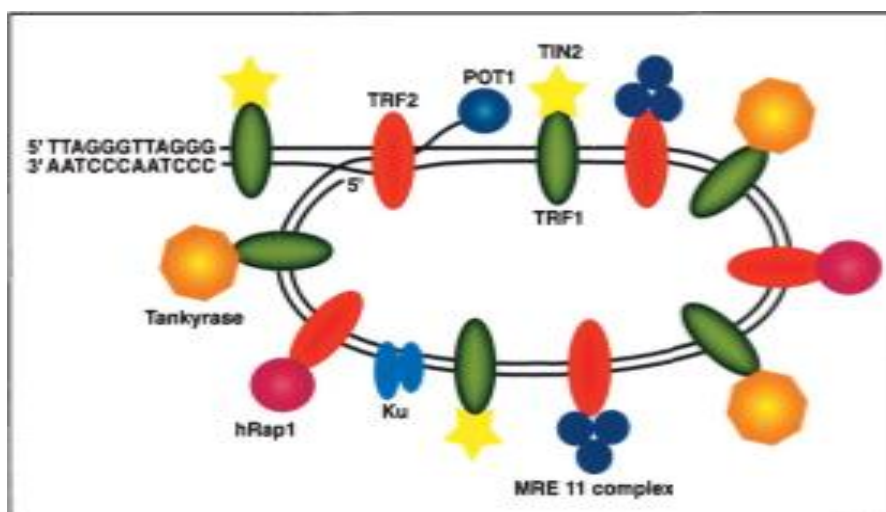
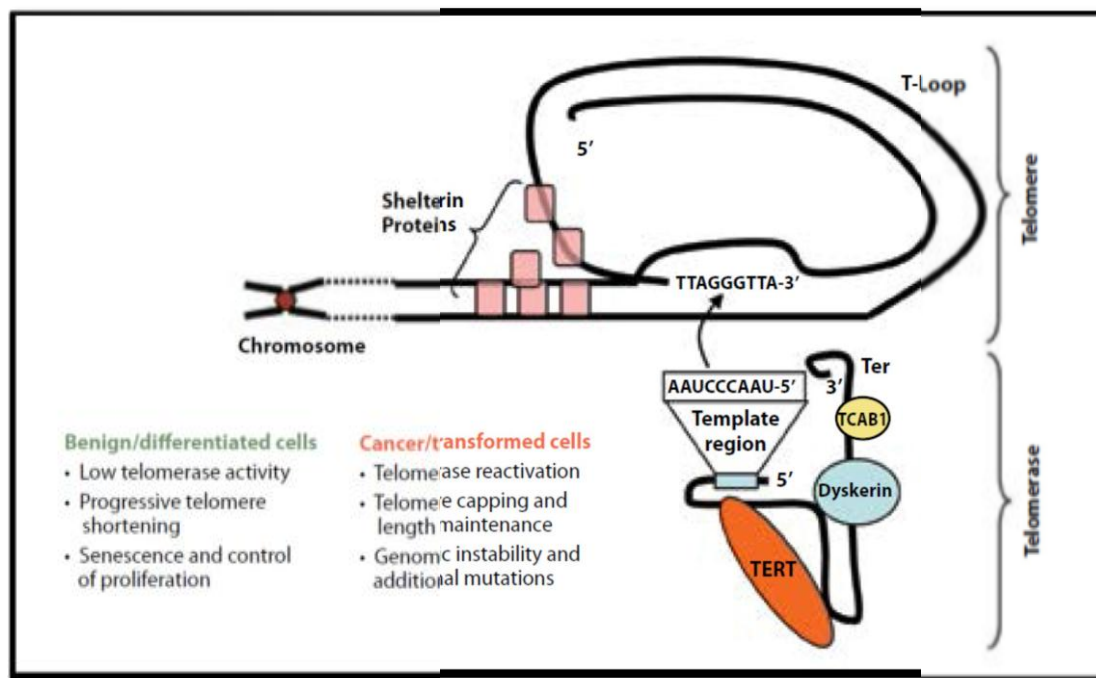
Telomeres consist of both repeated DNA elements and specific DNA-binding proteins, forming ends of the chromosomes.^[1] Telomeres composed of short guanine-rich repetitive hexameric DNA sequence (TTAGGG / AATCCC for human telomeres) protect chromosomes from degradation and loss of essential genes and allow cell to distinguish between double stranded DNA & natural protein.^[2-3] Without telomeres, chromosomes are -leads to degradation, recombination and fusion by DNA repaired mechanism.^[4] The

ends of telomeres forms a lariat like structure called t-loop. A six-protein complex known as “shelterin” has remarkable specificity for telomeres and has function to maintain telomere length, promote t-loop formation, protect end of chromosomes from damage.^[5] When the t-loop structure is disturbed, the growth of cell is arrested and the cell cycle checkpoint p53 and pRB are activated. There are three possible things that occurred when the checkpoints are active.

- 1) The cell cannot bypass both checkpoints, the growth of the cell senescence occurs.
- 2) If only pRB checkpoint is bypassed, then intact p53 checkpoint will cause apoptosis.
- 3) If both checkpoints are bypassed, the cell may continue to grow infinitely, resulting in genomic instability.^[6]

Telomere-specific proteins such as protection of telomeres-1 (POT-1), telomeric repeat-binding factor-1 (TRF-1), telomeric-binding factor-2 (TRF-2) bind double- and single-stranded telomeric regions to form a complex, protect ends of the chromosomes.^[7-8] Two proteins bind TRF1, the poly(adenosine diphosphate) ribosylase trypsinase and the TRF-interacting nuclear protein serve to regulate TRF1 function. TRF2 also interacts with several proteins, including the human Rap 1 protein and the Mre11 complex made up of Mre11, Red50 and the nijmegen breakage syndrome protein, which is implicated in cellular response to agents that damage DNA. Mre11 and ku complex involving certain DNA double strand break repair, localizes to the telomere.^[9-11]

Telomere erosion takes place as an organism ages, the telomere is known as “mitotic clock” is the set for the onset of senescence.^[12] Telomeric maintenance can also be taken place by ALT (alternative lengthening of telomeres). The another possible conformation for end protection is a formation of G-quadruplexes. These are planar stacks of G residues formed by intra- or intermolecular interaction of the G-rich single stranded telomere ends.

FIG NO.1^[1]

• TELOMERASE

A specialized RNA-dependent, DNA polymerase, called telomerase that extends the 3' ends of linear chromosomes, which would actually diminish in size with every cell division, eventually leading to cellular ageing and maintains telomeric DNA.^[13-14] Tumors, the ability to activate telomerase which in turn promote the immortalization and spread of rapidly dividing cancer cell.^[15]

The catalytic subunit of telomerase is structurally similar to retro-viral reverse transcriptase, viral RNA polymerase and lesser extend of bacteriophages B-family DNA polymerases.^[16]

Telomerase activity is not detected in most somatic cells, proliferative cells of renewal tissues and male germline cells.^[17]

This ribonucleoprotein enzyme is a reverse transcriptase composed of two essential subunits, human telomerase RNA component (hTERC) and human telomerase catalytic component (hTERT). hTERC, the RNA subunit, provides the template for the telomere synthesis reaction and expressed in mammalian cells.^[18] The expression of the catalytic subunit of telomerase, hTERT, is restricted only to cells that exhibit telomerase activity, indicating that hTERT is the rate-limiting component of the telomerase enzyme. Recent observations indicate that telomerase exists as a complex tetramer composed of two RNA subunits and two catalytic subunits.^[19-24] These subunits act in concert to elongate telomeres by reading from the RNA template sequence carried by the RNA subunit and synthesizing a complementary DNA strand. Telomerase catalyzing the addition of a single telomere repeat, and translocating to the new terminus. This processive cycle continues until the holoenzyme dissociates from the telomere. Shelterin protein at telomere serve as negative regulators of telomerase extension of telomeres. Telomerase remain associated with the chromosomal end until several telomeric repeats, a process that is highly regulated by telomere binding proteins, such as TPIP1-POT1 complex in humans.^[25-27]

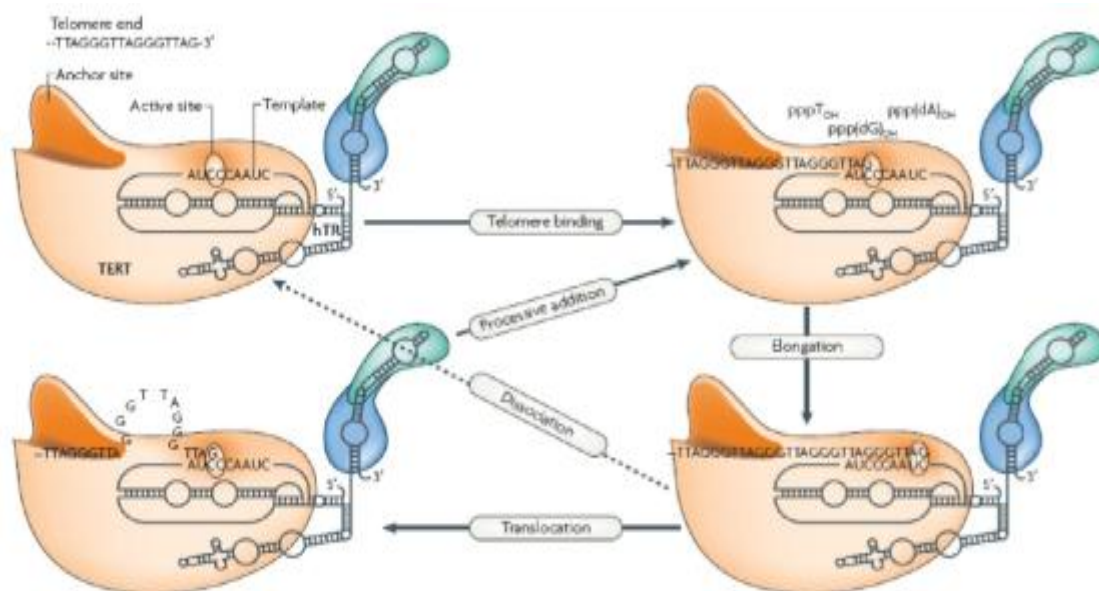
FUNCTION OF TELOMERASE

One of the primary functions of the telomere is to protect linear chromosomes from damage and degradation. Disruption of telomeric structure directly or through the interruption of the homeostatic mechanisms that maintain telomere length rapidly leads to the accumulation of illegitimate chromosomal associations, the total number of telomeric repeats plays an important role in this protective function.^[28-30] The TRF2 protein binds the Mre11 complex and that Ku associates with the telomere raise the possibility that there is an active interplay between the telomere and the cellular response to DNA damage.^[31-32]

Normal human cells exhibit a limited proliferative capacity in culture. After extended passage in vitro, normal human cells enter an irreversible growth arrest called replicative senescence.^[33-34]

Human cells must bypass two proliferative barriers to achieve immortalization.^[35] The first of the barriers is, as described above, replicative senescence, a state of arrested

proliferation but continued cell metabolism. Although expression of hTERT leading to activation of telomerase is one method to bypass senescence in some human cell types, simultaneous inactivation of the retinoblastoma (pRB) and p53 tumor-suppressor pathways also allows human cells to avoid replicative senescence.^[36] An enzyme called telomerase is activated to extend telomeres and to prevent senescence. TRF1 and 2, can inhibit telomere elongation by looping the telomere and preventing telomerase from binding to the telomere. By inhibiting the binding of TRF1 to the telomere, telomerase can access the telomere and telomere elongation occurs.^[37-38]



ROLE OF TELOMERASE IN PROPAGATION OF CANCER

Telomerase is expressed in embryonic cells and in adult male germ line cells, but is undetectable in normal somatic cells with the exception of the proliferative cells of renewal tissues.^[39]

In present human cells, telomerase activity is repressed, and the telomeres in these cells shorten with successive cell divisions. Consistent with these observations, inhibition of telomerase in immortal cancer cell lines by genetic, antisense, or pharmacologic methods results in telomere shortening and eventually halts cell proliferation.^[40-41] In most normal human cells lacked telomerase activity. The only exceptions to this correlation were normal cells whose functions required ongoing proliferation, such as lymphocytes, basal keratinocytes, intestinal crypt cells, CD34-expressing peripheral-blood stem cells, and immortal cells that exhibited the ALT phenotype.^[42-49] Subsequent studies have

confirmed that telomerase activity and the expression of *hTERT* correlates strongly with histological evidence of malignant cells in many different human tissues.^[50] A model that emerges from these types of observations indicates that nascent cancer cells acquire replicative immortality by acquiring the ability to maintain telomere length, usually through the activation of *hTERT* and telomerase. Thus, during the early stages of cell transformation, telomere attrition suppresses malignant transformation by limiting cell life span; whereas, telomere maintenance by telomerase or ALT in later stages of cancer development facilitates oncogenesis. (Table 1)^[51]

In all normal somatic cells, even those with detectable telomerase activity, progressive telomere shortening is observed, eventually leading to greatly shortened telomeres and To limited replicative capacity. Introduction of *hTERT* into telomerase-silent cells is sufficient to reactivate telomerase, elongate or maintain telomeres, and to result in the bypass of both M1 and mortality stage 2 (M2)(BOX 1).^[52-53] Telomeres are therefore effectively molecular clocks that count the number of times a cell has divided, And determine when cellular senescence (M1) and crisis (M2) occurs Expression of viral oncoproteins in human cells extends the cultured lifespan of normal cells, but does not directly immortalize the cells. Rather than entering a period of prolonged quiescence as do normal cells at the limit of their proliferative capacity (mortality stage 1 (M1)), cells expressing such viral proteins enter a state known as crisis (mortality stage 2, (M2)). Crisis occurs when cells enter a state such that the population size initially ceases to increase and the population cell growth is balanced by cell death/ apoptosis.

Occasionally, as a very rare event, an immortal cell emerges from crisis (see figure). The two-stage model of cellular senescence in which M1 and M2 represent independent mechanisms limiting the capacity of normal cells to continue dividing, helps explain this behavior. M1 (normal replicative senescence) occurs when a few short telomeres elicit a DNA-damage signal resulting in growth arrest. However, the damage signal initiated by a few short telomeres at M1 can be ignored in cells that have inactivated important cellcycle checkpoint genes, such as p53, that normally act to stop cell-cycle progression.^[54-55] If M1 is bypassed or abrogated, cells enter an extended period of proliferation and telomeres continue to shorten in the period between M1 and M2. When telomeres become so short that they fail to protect the ends of the chromosomes, the ends become ligated to produce dicentric chromosomes, with a consequent mitotic catastrophe at M2. As a very rare event (1

in 10^{-6} in epithelial cells and 1 in 10^{-7} in human fibroblasts), cells can escape M2, leading to an immortal cell and cancer cell progression. Both M1 and M2 can therefore be thought of as potent initial barriers to continued cell division (for example, a tumour-suppressor pathway), even though at crisis the end fusions and ensuing chromosome rearrangements might in some instances contribute to the genomic instability that characterizes most cancer cells. Recent work using human and rodent models of cancer provides clear experimental support for this paradigm for telomere biology and cancer.^[56]

Dual role of telomerase and telomere in cancer development

	Telomere loss	Telomerase activation
Tumor suppression	Limits cell lifespan (telomeres long)	Protect chromosomes end
Tumor promotion	Includes genomic instability (telomeres short)	Promotes immortalization

Telomeres play second critical role in promoting malignant transformation.^[57]

TELOMERASE INHIBITORS

As the ends of chromosomes depending upon the telomerase function telomerase inhibitor is innovative approach to fight cancer. The most appealing feature of telomerase inhibitors, which distinguishes them from Conventional anti-cancer drugs, is probably seen in their intrinsic non-toxicity to normal cells.^[58] Nevertheless, efficient delivery to the target cells, i.e. to the tumor. Telomerase inhibitors largely fall in three classes of compounds: small synthetic molecules, nucleotide-based biological and some natural food compounds.

Approach to inhibit telomerase is to block the access of telomerase to the telomere. In vitro, telomeric G-rich DNA forms compact structures most easily explained by the formation of guanosine (G) quartets. Recently, two groups demonstrated that expression

of altered hTERT mutants into human cancer cells resulted in immediate telomere degradation and apoptotic cell death, indicating that specific alteration of telomere structure may also be a strategy to target cancer cells.^[59]

However, because the expression of such hTERT mutants in cells expressing telomerase would be expected to have dramatic consequences, proper targeting of this type of agent will be required to avoid undesirable side effects. The main points of effect are the catalytic subunit hTERT, the template RNA hTR or the telomere structure, respectively. anti-cancer therapy is the induction of an immune response that kills the tumor cells. Using the protein subunit of telomerase (hTERT) as an antigen to activate T cells will give rise to T cells that are specific for killing cells with high levels of hTERT expression.^[60]

Since most normal somatic cells do not express hTERT, tumor cells with high level of hTERT are the preferential target for these T-cells. In synthetic telomerase inhibitor, there are following drugs mainly used they are.

1. Oligonucleotide: GRN163L, BIBR1532
2. G quadruplex stabilizer: (stabilising agents-BRACO19 TMPyP4, RHPS4, Telomestatin)
3. Immunotherapy: vaccines like GV1001
4. Natural compounds: polyphenols

Oligonucleotide: These are the modified oligonucleotide with olimerse.^[61-62] The template region of the telomerase RNA (hTR) offers an accessible substrate for direct enzymatic inhibition using oligonucleotide-based small molecule Inhibitors oligonucleotides that can hybridize to the 11-base hTR template region act as competitive telomerase inhibitors (not antisense targeting messenger RNA).^[63] The template region of hTR must be accessible to bind to the telomeric repeats, and therefore must be exposed to targeted oligonucleotides. These modified molecules bind RNA sequences with improved selectivity, enhanced efficacy and improved pharmacological properties.

GRN163 is one of oligonucleotide RNA (hTR) templet antagonist agent recently in clinical trial phase. Currently known as Imetelstat, is a lipidated N3-P5 thio-phosphoramidate 13-mer. The thio-phosphoramidate backbone causes the oligonucleotide to be water soluble, acid stable, nuclease resistant and to form stable RNA duplexes.^{[64-}

^{66]} The sequence 5'-Palm-TAGGGTTAGACAA-3' is complementary to a 13-nucleotide-

long region partially overlapping and extending by four nucleotides beyond the 5'-boundary of the template region of hTR.^[67-70] The GRN163L sequence is apparently unique in the human transcriptome, and shows greatly enhanced stability as well as extremely specific and high-affinity binding to telomerase, while the lipid modification on GRN163L significantly improves its potency and biodistribution. GRN163L-induced telomere shortening initiates cellular crisis caused by chromosomal fusions, anaphase bridges and subsequent apoptosis. In mice with human tumor xenografts, GRN163L was well-tolerated and induced telomerase inhibition in doses ranging from 5 mg/kg to 1000 mg/kg.^[71] Xenograft models showed that GRN163L works to inhibit tumor growth, prevent growth of metastases, and sensitizes tumors to conventional chemotherapy agents.^[71,72]

GRN163L also is able to cross the blood-brain barrier to target glioblastoma xenograft tumors, supporting the further study of using already completed several Phase I trials in patients with chronic lymphocytic leukemia and solid tumors such as breast cancer and non small cell lung cancer.^[73] These Phase I trials showed that intravenously infused GRN163L has excellent bioavailability, pharmacokinetics and tolerability, and after dose-escalation studies, a dose of 9.4 mg/kg was chosen for Phase II clinical trials. Phase II trials of GRN163L alone, in combination with other chemotherapy and targeted drugs, or in a maintenance setting after standard chemotherapy are now being conducted for patients with NSCLC, advanced breast cancer, Chronic leukemia, essential thrombocythemia and multiple myeloma.^[74]

The second one is BIBR1532. Beside those nucleotide based biologicals, the small synthetic inhibitor BIBR1532, 2-((E)-3-naphthalene-2-yl-but-2-enoylamino) benzoic acid, is a potent hTERT inhibitor. BIBR1532 acts as a non-competitive inhibitor of telomerase, which specifically blocks the elongation of DNA. Treatment of different human cancer cell lines *in vitro* resulted in reduced telomere length, inhibition of cell proliferation and cellular senescence. If BIBR1532 pretreated cells were implanted into nude mice, tumor growth was delayed compared to control group. A further treatment of the animal with BIBR1532 did not provide any additional benefit.^[75]

Telomeric DNA of vertebrates consist of repeats of the sequence d(TTAGGG) these G rich ends can fold up into four-stranded G-quadruplex structure. According to its biological and structural methods the central units of G-quadruplexs are hydrogen bounded arrays of guanine base with several G-quadruplexes held together by – stacking interactions. These

DNA structure suggest a straight forward path for telomeric folding and unfolding and to recognize telomeric associate proteins. To exploit the potential telomeric uncapping and shortening of the G-quadruplex phenomenon, several G quadruplex stabilizing agents like TMPyP4, telomestatin, BRACO19 etc. were play important role in anti tumor activity in both in-vivo and in-vitro.^[76-79]

Cancer immunotherapy given its large expression in human tumor and its immunogenicity by targeting hTERT. Telomerase is an intracellular molecule consist of intracellular proteosomes, presented in the context of major histocompatibility molecules as antigen recognize by cytotoxic T lymphocyte (CTL).

CTL kill TERT-positive tumor cells. Several phase 1 clinical trials testing hTERT as a cancer vaccine target have shown the induction of T-cell immune response with minimal toxicity in patients with multiple types of cancer. At present, several clinical trials involving telomerase vaccination are now open that will test the feasibility, toxicity and efficacy of antitelomerase immunotherapy.^[80]

Naturally occurring inhibitor

Various studies report that some compounds, in particular, natural compounds, inhibit Telomerase activity and induce apoptosis. The researchers conducted generally emphasize on two beneficial effects of natural compounds. These beneficial effects of natural compounds are potential anti-carcinogenic effects and few or milder side effects in normal cells. Telomerase regulation which is quite important for formation of human cancers and on mechanisms that result in apoptosis and impacts and inter-relations of resveratrol, quercetin and tannic acid. In addition to some polyphenols focused on here, relationships of telomerase inhibition and apoptosis induction of other polyphenols with cancer. The other telomere proteins which compose this complex and their duties are summarized. The molecular structure of these compounds is shown below.^[81]

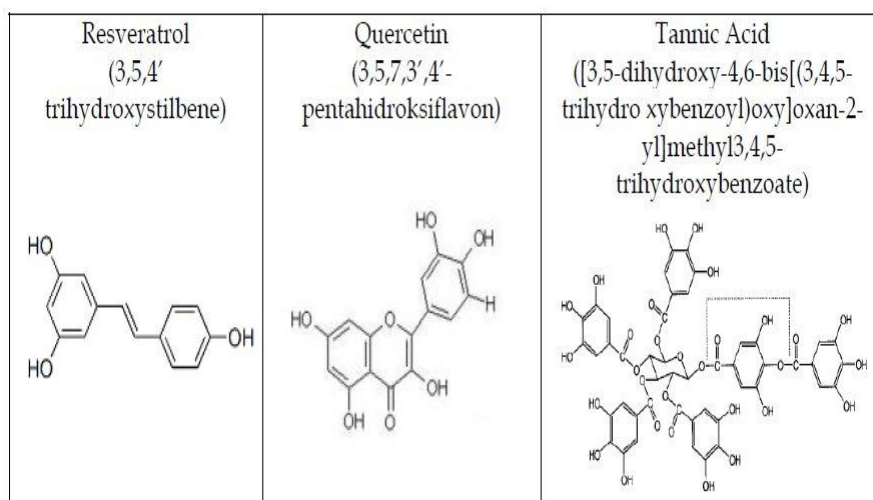
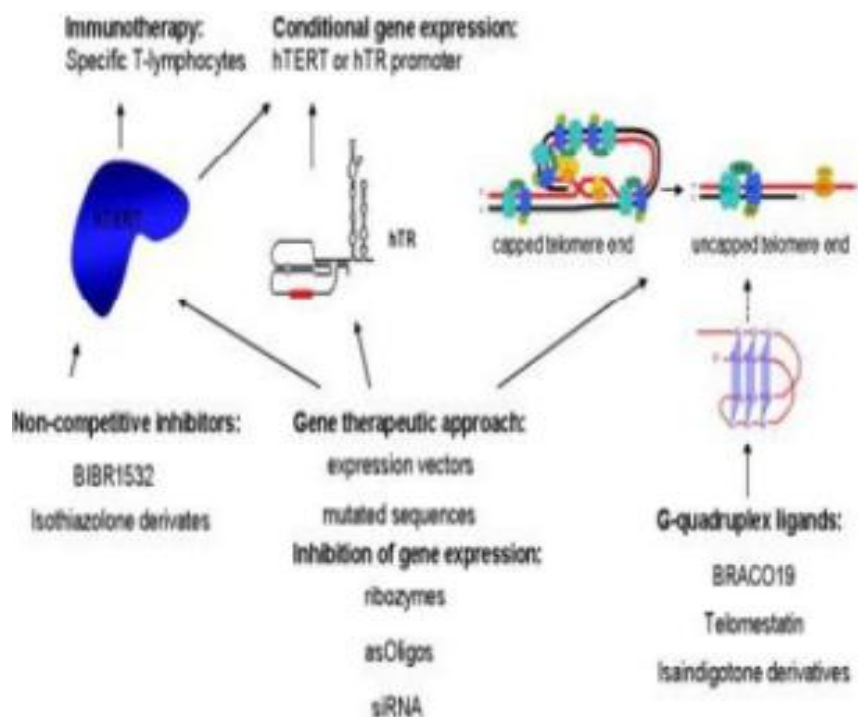


Fig. 2. Name of phenolic compounds and chemical formulas.

Products	Cells	Mechanisms of action
Curcumin	MCF-10A Human mammary epithelial cells MCF-7 Human breast cancer cells	Inhibits telomerase activity by down-regulating hTERT expression.
	HL-60 Human acute myeloblastic leukemia cells	Induced apoptosis. Inhibition of telomerase activity in a dose dependent manner.
	K562 Human chronic myelogenous leukaemia cells	Induced apoptosis. Inhibits telomerase activity.
Ginsenoside Rk1	HepG2 Human hepatocellular liver carcinoma cells	Inhibited cell growth. Induce apoptosis. Inhibit telomerase activity.
Verbascoside	MKN45 Human gastric carcinoma cells	Inhibited telomerase activity. Reduced telomere length. Arrested tumor cell cycle in G2/M phase.

Proteins	Functions
Rap 1 (Repressor activator protein 1)	Mammalian Rap1, whose function is still unclear, TRF2-binding protein, negative regulator of telomere length.
TIN2 (TRF1 Interacting Nuclear protein 2)	TRF1-TRF2 binding protein, negative regulator of telomere length.
TPP1 (previously called TINT1 [Houghtaling et al. 2004], PTOP [Liu et al. 2004], and PIP1 [Ye et al. 2004b])	Plays a role in telomere capping by interacting with TIN2 and POT1.
POT 1 (Protection of telomeres 1)	Binds single-stranded TTAGGG repeats, necessary for telomere-length maintenance and telomere protection.
TANK1 and TANK2 Tankyrase (TANK) telomere-associated poly (ADP-ribose) polymerase (PARP) 1	Positive regulator of telomere length through inhibition of TRF1
PINX1 (PIN2-interacting protein X1)	Potential telomerase inhibitor, negatively regulating telomere length by interacting with TRF1.
Ku86	Negative regulator of telomere length, role in telomere capping, regulation of telomerase recruitment.
DNA-PK (DNA-dependent protein kinase)	Plays a role in telomere capping, putative role in post-replicative processing of telomeres.



CONCLUSION AND RESULT

There have been many recent significant developments in the telomere/telomerase fields of research, but there are still many gaps in our understanding. More preclinical proof-of-efficacy studies and additional clinical trials are required. The progress made in the past 2 years has been impressive and there is an emerging general consensus that telomerase-targeted therapies are a promising and novel approach to cancer therapeutics that could lead to effective interventions for the treatment of cancer & the aging process with minimal side effects.

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