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Review

Assessment of telomerase as drug target in breast cancer

RISHI KUMAR JAISWAL[1] and PRAMOD KUMAR YADAVA[1,2]*

[1]*Applied Molecular Biology Laboratory, School of Life Sciences, Jawaharlal Nehru University, New Delhi 110 067, India*

[2]*Present Address: Department of Biological Sciences, Indian Institute of Science Education and Research, Berhampur, Odisha 760 010, India*

**Corresponding author (Email, pkyadava1953@gmail.com)*

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Telomerase is a specialized enzyme which maintains telomere length at the extreme end of the chromosome. Over 90% of all cases of cancer show high expression of telomerase while in normal cells, its expression is extremely low or undetectable. Detection of telomerase activity in a wide range of breast cancer makes telomerase an interesting target for diagnosis and therapy. In this review, we have aimed to describe telomerase as a therapeutic and accurate diagnostic target in breast cancer. Telomerase performs many extracurricular activities apart from maintaining telomere length; here, we have also tried to address its role in epithelial-mesenchymal transition (EMT) of breast cancer progression.

Keywords. AZT; breast cancer; EMT; hTERT; TRAP assay

1. Introduction

Telomeres are the ends of chromosomes comprising multiple repeats of G/T-rich hexameric to octomeric sequences associated with telomere-specific proteins. Along with the bound proteins, telomere forms a protective cap and inhibits the recognition of chromosome ends as a double-strand break and prevents an end to end fusion among them (Jaiswal *et al.* 2013). DNA polymerase is incapable to completely replicate the 3′ end of the template strand; hence a specialized reverse transcriptase known as telomerase elongates the end by adding tandemly repeated oligomeric sequences using an RNA template (Jaiswal *et al.* 2013). Telomerase complex consists of six different subunits, viz., heat shock protein 90 (hsp90), human telomerase RNA component (hTERC), dyskerin, telomerase-associated protein 1 (TEP1), p23, and human telomerase reverse transcriptase (hTERT) among other constituents (Jaiswal *et al.* 2017). It is very well established now that hTERT and hTERC are essential and sufficient for in vitro reconstitution of telomerase activity (Jaiswal *et al.* 2013, 2017). Moreover, hTERC expression is detectable in both normal somatic cells and tumor cells, while the expression of hTERT is detected only in actively dividing normal cells and cancer cells (Baykal *et al.* 2004). Furthermore, overexpression of hTERT in telomerase negative cells is sufficient to detect telomerase activity and extended life span of the cancer cells (Baykal *et al.* 2004). Around 90% of cancerous cells show high telomerase expression while the rest 10% have ALT (alternative lengthening of telomere) pathway to maintain their telomere length (Wang *et al.* 2018). A wide range of human cancers has been detected with high telomerase activity. Telomerase activity was discovered in 80% of pulmonary cancers, 94% of neuroblastomas, 73% of breast carcinomas, and 85% of hepatocellular carcinomas (Kim *et al.* 1994). It is not very well known how telomerase is activated in cancer cells while its activation ensures that cancer cells become immortal. Development of any cancer, including breast cancer, is a complex process and is characterized by the expression of different markers. Moreover, up and down-regulation of these markers in

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breast cancer suggest their probable involvement in the control of telomerase activity. It is reported that p53 downregulation enhances the telomerase activity and hence, the proliferation rate while its up-regulation leads to the reduced expression of hTERT mRNA, justifying tumor suppressor nature of p53 (Xu *et al.* 2000). It has been shown that c-Myc enhances the transcription of hTERT by directly binding to the E-box of the hTERT promoter (Wu *et al.* 1999; Greenberg *et al.* 1999; Kyo *et al.* 2000). Also, BRCA1 binding with c-Myc leads to the down-regulation of hTERT (Zhou and Liu 2003). These reports suggest a direct connection between breast cancer immortalization and tumor suppression by BRCA1. Protein kinase Cα seems to control telomerase expression in breast cancer cells since human telomerase associated protein 1 (hTEP1) and hTERT, are basically phosphoproteins, and their phosphorylation is required for the activation of telomerase in breast cancer ("Telomerase Is Controlled by Protein Kinase Cα in Human Breast Cancer Cells") (Li *et al.* 1998).

Special relevance of telomerase in context with breast cancer: Breast cancer is becoming the main cause of mortality among women globally, and hence requires proper diagnosis and treatment (Becker 2015). As demonstrated by many studies, around 85% of cancers express telomerase and provide immortality to cancer cells by maintaining telomere length. Remaining 15% of cancers either use a homologous based alternative lengthening of telomeres (ALT) pathway to maintain their telomere length or do not maintain telomere length (Holysz *et al.* 2013). However, almost 100% of breast cancer cells maintain their telomere length by expressing telomerase (Holysz *et al.* 2013). In addition to that, Denis G *et al.* reported that both ER 81 (a factor involved in breast tumor formation) and HER2 are involved in the synergistic induction of hTERT at the transcriptional level in breast cancer patients (Bosc and Janknecht 2002). Moreover, leptin (a breast cancer risk factor), estrogen receptors ERa and ERb, and epidermal growth factor receptor (EGFR) were also found to enhance telomere expression (Holysz *et al.* 2013). Divella *et al.* observed that the diagnostic value of TERT DNA is much better than carbohydrate antigen CA 15.3 in early breast cancer disease (Divella *et al.* 2009). Interestingly, telomere length in breast cancer cells is quite short as compared to the normal cells, and the breast cancer cells with the short telomere length and rapid rate of cell division make telomerase a potential target for breast cancer therapy (Holysz *et al.* 2013). However, stem cells with telomerase expression might also be initially affected by telomerase inhibition. Still, most stem cells are quiescent and do not undergo cell division, and at the same time, stem cells have longer telomere, which is way above the critically short telomere of the breast cancer cell (Holysz *et al.* 2013). The role of BRCA1 and BRCA2 in telomere structure and function is also reported, which makes telomerase an attractive target (Uziel *et al.* 2015).

2. Telomerase diagnosis in breast cancer

Several studies have shown that in some cancers, there is a high expression of hTERC (Cong *et al.* 1999). However, hTERC cannot be a rate-limiting factor for the enzymatic activity of telomerase due to its presence in most normal tissues (Wojtyla *et al.* 2011). In breast cancer cells, telomerase activity can be detected by many methods such as, by checking the expression of hTERT mRNA and protein expression of tumor cell extracts by real-time PCR and western blot, respectively (figure 1). (Baykal *et al.* 2004). By measuring the level of hTERT mRNA by qRT-PCR, a statistical correlation between breast tumor aggressiveness and hTERT mRNA has been found (Bièche *et al.* 2000). In situ hybridization with a labeled probe for hTERT mRNAs has also been used (figure 1). (Yashima *et al.* 1998; Soder *et al.* 1998) although this method has difficulties in precisely quantifying the level of mRNA expression (Soder *et al.* 1998). Alternative methods like immunohistochemistry for detecting the hTERT protein also are limited in quantitative interpretation (figure 1). (Hiyama *et al.* 2001). Moreover, hTERT mRNA has multiple splice variants, and some of them can be present in cells lacking telomerase activity. (Ulaner *et al.* 1998). The detection of telomerase activity was revolutionized by Kim *et al.* (1994) with the development of a PCR based assay termed as TRAP (telomerase repeat amplification protocol) assay (figure 1). (Quach *et al.* 2013). The TRAP assay consists of three different steps, i.e., the extension of an oligonucleotide substrate (TS), amplification, and detection. In extension, telomeric repeats are added to a non-telomeric oligonucleotide, by telomerase. In the amplification step, two different primers are used (Upstream primer TS and downstream primes ACX) to amplify extension product. In detection, step electrophoresis is performed to analyze the PCR products (Kim *et al.* 1994). Detection of telomerase can be based on either radioactively labeled probe or fluorescence due to SYBR green or ethidium bromide (Baykal *et al.* 2004). A study has shown that all

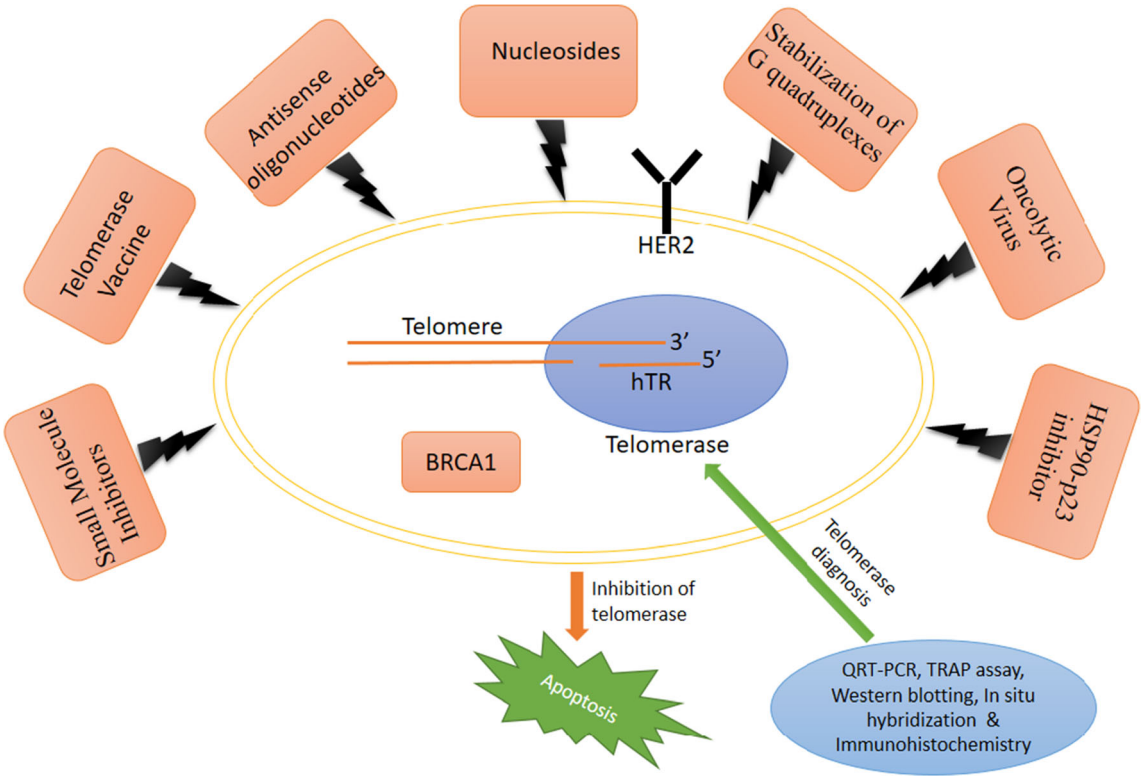

Figure 1. Schematic representation of different classes of telomerase inhibitors and various methods developed to diagnose telomerase in cancer cells.

stages of breast cancer were around 88% positive in TRAP assay. This value might be close to 95% if negative samples were carefully handled (Shay and Bacchetti 1997). Similar to other methods, TRAP assay has its own limitations. The marked heterogeneity of breast lesions at the microscopic level cannot be differentiated using TRAP assay. The lesions include mixtures of in situ and invasive carcinoma components and other cell types that are telomerase positive, like activated lymphocytes and proliferative stem cells, and these make the analysis more complicated. (Hiyama *et al.* 1995, 1996; Wright *et al.* 1996).

To improve the accuracy of diagnosis in breast neoplasms, Hiyama *et al.* used preoperative specimens such as fine-needle aspirates (FNAs) and extracts derived from 1000 cells to detect telomerase activity. They observed telomerase reactivation in 15 of the total of 33 FNA specimens analyzed (Hiyama *et al.* 2000). Additionally, Poremba *et al.* determined telomerase activity in 172 preoperative breast specimens and found that 92% FNA from breast cancer specimens were positive for telomerase activity while 94% of FNAs were telomerase negative from patients with benign breast lesions (Poremba *et al.* 1999).

3. Activity and prognosis of telomerase in breast cancer

As the number of breast cancer cases are on the rise, it has become all the more important to have accurate screening procedure. A marker is required to differentiate subsequent invasive breast cancer risk. In breast cancer, telomerase activity determination by TRAP assay showed a very high positive impact in comparison to other breast cancer markers. Hoos *et al.* (1998) reported that tumor aggressiveness is positively correlated with telomerase activity (Hoos *et al.* 1998). They analyzed 25 breast cancer patients for telomerase activity in untreated, and chemotherapy treated tumors and detected telomerase activity in all breast cancers (Hoos *et al.* 1998). They also established a substantial association between tumor size, lymph node stage, and telomerase activity (Hoos *et al.* 1998). An analysis of malignant breast tumor and healthy breast tissues by TRAP assay showed that 77 out of 102 malignant breast lesions had telomerase activity while it was totally absent in the healthy breast tissues (Kulić *et al.* 2016). An important correlation was found between tumor size, historical grade, telomerase activity, the aggressiveness of the tumor, and expression of the

nuclear antigen Ki-67 (Kulić *et al.* 2016). Liu *et al.* published that, telomerase knockdown in breast cancer cells leads to the reduction of cell proliferation, migration, and invasive ability of breast cancer cells (Liu *et al.* 2013). They have also shown that telomerase knockdown makes cancer cells more prone to radio and chemotherapy. These reports are making telomerase a key prognostic marker in breast cancer research (Liu *et al.* 2013).

4. Telomere length and breast cancer

Telomere shortening is a key event which contributes to genomic instability and results in malignant transformation. It is frequently noticed with breast cancer and can be correlated with cancer stages and prognosis (Takubo *et al.* 1998). Breast cancer tissues have shorter telomere and are more prone to induce cancer progression (Odagiri *et al.* 1994). However, there is apparently no significant difference in telomere length among tissues with fibroadenomas, or gynecomastia and breast carcinomas (Odagiri *et al.* 1994). They further correlated the degree of telomeric shortening with histologic grade and confirmed Grade 3 breast carcinoma as most conspicuous in these terms (Odagiri *et al.* 1994). A marked telomere shortening was observed in a majority of invasive breast cancers while a smaller subset showed only moderate telomere shortening (Meeker *et al.* 2004). Based on a study of telomere length and other tumor characteristics, out of the total 103 specimens collected at the Johns Hopkins Hospital, breast tumors were categorized into luminal A, luminal B, HER-2-positives and triple-negative carcinomas. Luminal B, HER-2-positives, and triple-negative cases had shorter telomere than a major portion of luminal A, which had normal or long telomere length (Meeker *et al.* 2004). In contrast, it has been shown that telomere length has no effect on disease outcome and is not associated with any of the pathological or clinical features (Lu *et al.* 2011).

5. Telomerase and EMT in breast cancer

Metastasis is the leading cause of human death by cancer, and the principal mechanisms responsible for this involves an epithelial-mesenchymal transition (EMT). EMT plays an important role in embryonic development and is described by the gain of mesenchymal markers like N-cadherin and vimentin, loss of epithelial markers like E-cadherin, loss of cell-to-cell adhesion, loss of apico-basal polarity, resistance to apoptosis and capacity to invade. (Jaiswal *et al.* 2018a). There is a big debate on the contribution of EMT heterogeneity to disease progression. A recent study profiling EMT status of cultured circulating and disseminated tumor cells (CTCs/DTCs) showed strongest lung metastases formation ability in Epithelial-type CTCs with a limited mesenchymal transition, while mesenchymal-type CTCs had limited metastatic ability (Liu *et al.* 2019). EpCAM (epithelial cell adhesion molecule) expression was used to evaluate the EMT heterogeneity of clinical samples from metastatic breast cancer cells (MBC). It suggests heterogeneous EMT phenotypes as an essential factor in tumor progression. (Liu *et al.* 2019). The classical function of telomerase is to maintain telomere length to confer replicative immortality on cells. Acquiring immortality does not mean that cells become cancerous; they need other factors to get completely transformed. Transforming factors like SV 40 large T antigen seem to be sufficient to transform a cell (Jaiswal *et al.* 2018b). The function of telomerase is not limited to only maintain the telomere length but also involves many other functions such as regulation of gene expression, RNA-dependent RNA polymerase activity, RNA-dependent DNA polymerase activity, apoptosis, DNA repair, and EMT, and these functions together constitute the extracurricular activities of telomerase (Jaiswal *et al.* 2013). The role of telomerase in EMT is not very well known and is an emerging field (Yadav *et al.* 2018). However, there are many reports which indicate a prominent role of telomerase in EMT of breast cancer. Pan *et al.* recently published a paper in which they have shown that ZEB1 positively regulates hTERT in breast invasive ductal carcinoma samples probably by recruiting a coactivator YAP resulting in transcriptional activation of hTERT (Yu *et al.* 2018). Moreover, this study confirms an earlier finding that hTERT/ZEB1 complex suppresses E-cadherin expression directly to enhance EMT in colorectal cancer" (Qin *et al.* 2015). E-cadherin down-regulation leads to overexpression of hTERT in SW480 and HCT116 cells, although E-cadherin expression returned to normal level when ZEB1 expression was diminished even in hTERT overexpressing cells (Qin *et al.* 2015). This indicated that ZEB1 is needed for hTERT dependent down-regulation of E-cadherin. Telomerase also regulates the EMT in breast cancer stem cells (CSCs) (El-Badawy *et al.* 2018). Despite having a higher concentration of telomerase, breast cancer stem cells have shorter telomeres than normal cells again suggesting that the role of telomerase is not limited to maintaining telomere length only. Knocking

Table 1. Drugs and vaccines targeting telomerase in various phases of clinical trial

Agent	Clinical status	Cancer type	Type of inhibitor	NCT Identifier
Imetelstat sodium	Phase I Completed	Refractory or Recurrent Solid Tumors or Lymphoma	Telomerase inhibitor	NCT01273090
Imetelstat	Phase I Withdrawn	Recurrent Solid Tumors and Lymphoma	Telomerase inhibitor	NCT01568632
Imetelstat sodium	Phase II Withdrawn	Relapsed or Refractory Solid Tumors	Telomerase inhibitor	NCT02011126
Imetelstat sodium, Paclitaxel and/or Bevacizumab	Phase II Completed	Locally Recurrent or Metastatic Breast Cancer	Telomerase inhibitor	NCT01256762
Imetelstat	Phase II Completed	Essential Thrombocythemia or Polycythemia Vera	Telomerase inhibitor	NCT01243073
Imetelstat sodium	Phase II Terminated	Young patients with recurrent or refractory brain tumors	Telomerase inhibitor	NCT01836549
Imetelstat	Phase I Completed	Chronic lymphoproliferative disease	Telomerase inhibitor	NCT00124189
Imetelstat	Phase I Withdrawn	Neuroblastoma	Telomerase inhibitor	NCT01916187
Imetelstat	Phase I Completed	Breast cancer	Telomerase inhibitor	NCT00732056
Imetelstat and trastuzumab	Phase I Completed	HER2+ metastatic breast cancer	Telomerase inhibitor	NCT01265927
Imetelstat	Phase II, recruiting	Myelofibrosis	Telomerase inhibitor	NCT02426086
Imetelstat	Phase I Completed	Solid tumor	Telomerase inhibitor	NCT00310895
Imetelstat	Phase II Active, not recruiting	Myelofibrosis	Telomerase inhibitor	NCT01731951
Imetelstat	Phase I Completed	Lung cancer	Telomerase inhibitor	NCT00510445
Imetelstat and Bevacizumab	Phase II Completed	Non-small Cell Lung Cancer	Telomerase inhibitor	NCT01137968
Imetelstat	Phase III, recruiting	Myelodysplastic syndrome	Telomerase inhibitor	NCT02598661
Imetelstat	Phase I Completed	Melanoma	Telomerase inhibitor	NCT00718601
Imetelstat	Phase I Completed	Myeloma	Telomerase inhibitor	NCT00594126
Imetelstat	Phase II Completed	Previously Treated Multiple Myeloma	Telomerase inhibitor	NCT01242930
Telomerase based vaccine in clinical trial:				
GV1001	Phase I/II, Completed	Advanced Malignant Melanoma	Telomerase Vaccine	NCT01247623
GV1001	Phase III, unknown	Non-small Cell Lung Cancer	Telomerase Vaccine	NCT01579188
GV1001	Phase I, Recruiting	Carcinoma	Telomerase Vaccine	NCT01223209
GV1001, capecitabine, gemcitabine hydrochloride	Phase III, Completed	Advanced or Metastatic Pancreatic Cancer	Telomerase Vaccine	NCT00425360
GV1001	Not applicable Completed	Non-Small-Cell Lung carcinoma	Telomerase Vaccine	NCT00509457
Tedalafil and GV1001	Phase I, Completed	Pancreatic Cancer	Telomerase Vaccine	NCT01342224
VX-001	Phase II, Active, not recruiting	Non-small-cell lung cancer	Telomerase Vaccine	NCT01935154
GV1001	Phase II, Completed	Hepatocellular carcinoma	Telomerase Vaccine	NCT00444782
GV1001	Phase III, terminated	Pancreatic cancer	Telomerase Vaccine	NCT00358566
Gemcitabine and/or capecitabine in combination with GV1001	Phase III, Ongoing	Advanced and metastatic pancreatic cancer	Telomerase Vaccine	2006-000461-10
GX-301	Phase II, recruiting	Prostate cancer	Telomerase Vaccine	NCT02293707
hTERT multi peptide	Phase I, Completed	Breast cancer	Telomerase Vaccine	NCT00573495
hTERT 540–548 peptide	Phase I, Completed	Brain tumor, Sarcoma	Telomerase Vaccine	NCT00069940
hTERT 540–548 peptide	Phase I, Not available	Breast cancer	Telomerase Vaccine	NCT00079157
hTERT 540–548 peptide	Phase II, Completed	Melanoma, solid tumor	Telomerase Vaccine	NCT00021164

Table 1 (continued)

Agent	Clinical status	Cancer type	Type of inhibitor	NCT Identifier
hTERT multi peptide	Phase I/II, Not available	Myeloma	Telomerase Vaccine	NCT00834665
hTERT multi peptide	Phase I/II, Completed	Myloma, plasma cell neoplasm	Telomerase Vaccine	NCT00499577
DC pulsed with hTERT 540–548 peptide	Phase I, not available	Breast and Prostate cancer	Telomerase Vaccine	Not available
DC pulsed with hTERT mRNA	Phase I/II, Withdrawn	Prostate cancer	Telomerase Vaccine	NCT01153113
GRNVAC1/ AST-VAC1	Phase II, Completed	Acute myeloid leukemia	Telomerase Vaccine	NCT00510133
DC pulsed with Telomerase peptide or tumor lysates	Phase I/II, Completed	Renal cell carcinoma	Telomerase Vaccine	NCT00197860
AST-VAC2	Phase I/II, not available	Non-small-cell lung cancer	Telomerase Vaccine	Not available
TLI (hTERT DNA fragment)	Phase I, Completed	Prostate cancer	Telomerase Vaccine	NCT00061035
DC pulsed with Telomerase peptide or tumor lysates	Phase I/II, Completed	Melanoma	Telomerase Vaccine	NCT00197912
Completed and ongoing clinical trials of other agents in cancer patients				
hTERT peptides	Phase I, Ongoing	Advanced malignancies; No longer responding to cancer treatments	Telomerase Vaccine	2014-003025-18
INVAC-1	Phase II, Recruiting	Chronic Lymphocytic Leukemia	DNA Vaccines	NCT03265717
INO-1400 and INO-1401	Phase I, Completed	Adults with Solid Tumors at High Risk of Relapse	DNA Vaccines	NCT02960594
UCPVax	Phase not applicable	Metastatic Lung Cancer	Telomerase Vaccine	NCT02846103
UCPVax	Phase not applicable	Melanoma	Telomerase Vaccine	NCT02838433
UCPVax	Phase I/II, Recruiting	Metastatic Non-small Cell Lung Cancer	Telomerase Vaccine	NCT02818426
Procure®	Phase I, Unknown	Ovarian cancer	Telomerase Vaccine	NCT01456065
KML-001, Cisplatin	Phase I, Unknown status	Non-Small Cell Lung Cancer, Small Cell Lung Cancer	Telomerase inhibitor	NCT01110226
CB-10-01	Phase II, unknown status	Skin Melanoma	Telomerase Vaccine	NCT00925314
540-548 peptide vaccine	Phase I, unknown status	Breast Cancer	Telomerase Vaccine	NCT00079157
GX-301	Phase II, Ongoing	Castration-Resistant Prostate Cancer	Telomerase Vaccine	2014-000095-26
Other telomerase inhibitors				
Agent	Clinical status	Cancer type	Type of inhibitors	
BIBR 1532	Not available	Breast, prostate and fibrosarcoma cancer cell lines	Small Molecule Inhibitors	
RHPS 4 methosulfate	Not available	Uterus carcinoma cell line, Brain Tumor Cells	Stabilization of G Cuadruplexes	
Telomelysin/ OBP-301	Phase I/II, Recruiting	Hepatocellular carcinoma	Oncolytic Virus	
Azidothymidine (A)ZT	Phase I/II clinical trials recruiting	Breast Cancer	Telomerase inhibitor	
Azidothymidine (AZT)	Phase I/II, active, not recruiting	T-cell Lymphoma Leukaemia	Telomerase inhibitor	
DN-hTERT	Not available	Breast cancer, human acute leukemia cells	Telomerase inhibitor	
Antisense oligonucleotides	Not available	Breast cancer, cervical cancer, Lung cancer, etc.	Telomerase inhibitor	

Table 1 (continued)

Agent	Clinical status	Cancer type	Type of inhibitor	NCT Identifier
Ribozymes	Not available	Human Melanoma Cells, cervical cancer	Telomerase inhibitor	
Carbovir	Not available	Immortal mouse fibroblasts	Nucleosides	
AZddG	Not available	Human leukemia cell line	Nucleosides	
C.OXT-GTP	Not available	Human leukemia cell line	Nucleosides	
CBV-TP(active metabolite of Abacavir)	Not available	Cervical cancer cell line	Nucleosides	
PMEGpp	Not available	Human leukemia cell line	Nucleosides	
Acyclovir	Completed	Hepatocellular carcinoma	Nucleosides	
Ganciclovir	Not available	Osteosarcoma cells	Nucleosides	
Penciclovir	Not available	Hepatocellular carcinoma	Nucleosides	
Valacyclovir	Recruiting	Metastatic Non-small Cell Lung Cancer, Metastatic Triple-negative Breast Cancer	Nucleosides	
AZddGTP	Not available	Hepatocellular carcinoma	Nucleosides	
TDG-TP	Not available	Unknown	Nucleosides	
7-deaza-2'- deoxypurine nucleoside triphosphates	Not available	Unknown	Nucleosides	
PU27	Preclinical stage	Leukemia, prostate cancer, renal cancer and breast cancer	Antisense oligonucleotides	
Telomestatin	Under clinical trial	Unknown	Stabilization of G Cuadruplexes	
Distamycin	Not available	Human melanoma cell	Stabilization of G Cuadruplexes	
Quarfloxin	Phase II, Completed	Neuroendocrine Tumors, Advanced Solid Tumors, Carcinoid Tumor, Lymphoma	Stabilization of G Cuadruplexes	
Ascididemin	Not available	Breast cancer, Lung cancer	Stabilization of G Cuadruplexes	
Meridine	Not available	Breast cancer, Lung cancer	Stabilization of G Cuadruplexes	
17-AAG	Clinical trials at the moment	Human melanoma cells	HSP90-p23 inhibitor	
17- DAG	Clinical trials at the moment	Human melanoma cells	HSP90-p23 inhibitor	
Rubromycins	Not available	Human neonatal keratinocytes	Telomerase inhibitor	
Diazaphilonic acid	Not available	Leukemia	Telomerase inhibitor	
Helenalin	Not available	Hematopoietic cancer cells.	Telomerase inhibitor	

down hTERT apparently reverses EMT in breast CSCs. hTERT regulates the expression of key breast CSCs marker like CDC133 and induces β-catenin nuclear localization (El-Badawy *et al.* 2018).

6. Targeting telomerase in breast cancer

The advantage of targeting telomerase over other cellular targets is that telomerase is absent in differentiated somatic cells. Brittney-Shea *et al.* explained a few important criteria one should consider before targeting telomerase (Herbert *et al.* 2001). Almost all telomerase inhibitors should affect telomerase activity without initially compromising cell proliferation and should not show immediate toxic effect. After losing telomerase activity, telomere should gradually shorten with each cell division. Eventually, the cells should die or experience growth check and the time required calculated to initial telomere length. (Herbert *et al.* 2001). There are two core subunits of telomerase, the catalytic part hTERT, and its RNA template hTERC. Many breast cancer studies have employed antisense nucleotides against both the core subunit and showed effective inhibition of cell growth. Out of these two subunits, hTERT is rate-limiting part of telomerase and hence is the preferred target. Xiangxia *et al.* used interfering p Super-retro-puro-hTERT-RNA to downregulate hTERT in MCF-7 cells and MDA-MB-231 cells and found reduced migration, invasion, and proliferation of these cells (Liu *et al.* 2013). The pSUPER RNAi System is frequently used by researchers to specifically and efficiently down-regulate expression of a gene by stable expression of siRNAs, which leads to the functional inactivation of the targeted genes (Soddu 2012).

Apart from antisense oligonucleotides, many drugs, vaccines and natural-derived compounds were reported to have antitumor activity by targeting telomerase (figure 1, table 1). Moon *et al.* discovered butein (3,4,2′,4′-tetrahydroxychalcone) compound that suppressed hTERT gene expression in MCF-7 cells (Moon *et al.* 2009). Pectenotoxin-2 is another compound which reduces hTERT gene expression, and this suppression of hTERT was mediated through Sp1 and c-Myc binding on the regulatory region of hTERT (Kim *et al.* 2008). Another promising compound, genistein was found to target telomerase activity and induce apoptosis of the breast cancer cells (Li *et al.* 2009). It was believed that genistein affects epigenetic pathways because genistein treatment results in a decrease of three DNA methyltransferases. (Li *et al.* 2009). Azido-deoxythymidine (AZT) is the most potent telomerase inhibitor known so far. It is reported that AZT prevents telomerase activity and breast cancer cell growth at lower doses than in normal cells (Multani *et al.* 1998). AZT treatment in MCF-7 cells leads to the reduction in the telomeric signal in fluorescence in situ hybridization (Multani *et al.* 1998). The dominant negative mutant of hTERT can also be very effective in breast cancer cells (Rao *et al.* 2016). Till now, several classes of telomerase inhibitors have been discovered to inhibit telomerase in cancer cells (Harley 2008; Xu and Goldkorn 2016; Relitti *et al.* 2020): ribozymes against hTERT, antisense oligonucleotides, nucleosides, oncolytic virus, different telomerase vaccine, inhibitors isolated from many natural products, etc., are in various phases of clinical trials and a few have completed their clinical trials (figure 1).

A list of telomerase inhibitors in various phases of clinical trials is presented in table 1 (Xu and Goldkorn 2016, Mizukoshi and Kaneko 2019; Relitti *et al.* 2020)

7. Telomerase inhibition with breast cancer subtypes

Based on the presence of ER (estrogen receptor), PR (progesterone receptor), and HER2 gene, four different molecular subtypes of breast cancer with distinct biological features have been identified. These four types are luminal A (both ER+ and PR+ are present, and there is absent of HER2- gene), luminal B (ER+, PR- and HER2+), HER2 positive (ER-, PR- and HER2+) and basal-like (ER-, PR-, and HER2-) (Kammori *et al.* 2015). Heaphy *et al.* reported the association between telomere shortening with different subtypes of breast cancer (Heaphy *et al.* 2011). They reported that ER+ and PR+ breast cancers have longer telomere than ER- and PR- cancers, which suggest that telomere length is a prognostic marker of breast cancer (Heaphy *et al.* 2011). In contrast to that, Kammori *et al.* (2015) demonstrated that ER+ and PR+ breast cancer had shorter telomere than ER- and PR- breast cancers (Kammori *et al.* 2015). The difference between these two studies may be due to inclusion of fewer patients, and both ER+ and PR+ groups also involved pN3 cases, which had very short telomeres. As demonstrated by many studies, different breast cancer subtypes have different responses to treatment and different survival outcomes. Hence, it is required to discover new potential targets in breast cancer treatment. Among other different molecular targets, telomerase is one of the most potential targets for breast cancer therapy. In breast cancer, telomerase activity determination by

TRAP assay showed a very high positive impact in comparison to other breast cancer markers. Considering the fact that HER-2, luminal B, and triple-negative breast cancer subtypes have shorter telomere in comparison with normal cells make telomerase even more attractive target for their treatment (Heaphy *et al.* 2011). So far, several classes of telomerase inhibitors against different cancer types, and different breast cancer subtypes have been discovered (table 1). Imetelstat (GRN163L) is one of the best telomerase inhibitors known so far (Hochreiter *et al.* 2006). Hochreiter *et al.* demonstrated the effect of Imetelstat on different breast cancer cell lines representing different breast cancer subtypes. They found that telomerase inhibition with Imetelstat causes telomere shortening and growth arrest of different breast cancer subtypes (Hochreiter *et al.* 2006). Many other factors also suppress telomerase in breast cancer. It has been shown that c-Myc enhances the transcription of hTERT by directly binding to the E-box of the hTERT promoter (Wu *et al.* 1999; Greenberg *et al.* 1999; Kyo *et al.* 2000). Also, BRCA1 binding with c-Myc leads to the down-regulation of hTERT (Zhou and Liu 2003). These reports suggest a direct connection between breast cancer immortalization and tumor suppression by BRCA1. However, Xiong *et al.* demonstrated BRCA1 involvement in telomere shortening independent of telomerase (Xiong *et al.* 2003). It is also found that BRCA1 regulates the length of 3′ G-rich overhang by its interaction with two shelterin complex proteins TRF1 and TRF2 (Xiong *et al.* 2003). Telomerase inhibition by Imetelstat has been evaluated in primary ductal carcinoma, which has BRCA1 mutation (BRCA1mut, ER-, p53-, basal-like), and it is found that telomerase inhibition in breast cancer subtypes leads to telomere shortening and growth arrest (Hochreiter *et al.* 2006). Pongsavee reported the effect of curcumin on telomerase inhibition in 744ins20–ter240 *BRCA1* mutation cancer cells. He discovered that curcumin treatment results in a decrease in cell viability of 744ins20–ter240 *BRCA1* mutation cancer cells (Harley 2008; Xu and Goldkorn 2016; Mizukoshi and Kaneko 2019; Relitti *et al.* 2020). In summary, we can conclude that telomerase in an excellent target not only in different subtypes of breast cancer but also in various cancer types.

8. Conclusions

Telomerase is an important target for diagnosis and therapy of breast carcinoma. Telomerase expression in a broad range of breast cancer and its absence in most of the normal cells make it distinct target for anticancer therapeutics. In contrast, many other anticancer therapies also affect normal cells and hence are not very specific. The discovery of high telomerase activity in breast cancers validates the role of telomerase in the immortalization of cancer cell and projects this enzyme as a perfect therapeutic target. Telomerase inhibition in cancer cells induces shortening of telomere length, and once it reaches the critically short telomere stage, a DNA damage response is induced. Initial telomere length would be the most critical factor to determine the effectiveness and suitability of telomerase inhibitor as already short telomere containing breast cancer cells would presumably be most susceptible. The role of telomerase is not limited only to maintain telomere length, and its role in EMT suggests that a combined therapy by targeting ZEB1 and telomerase would be a good strategy to control cancer cell proliferation.

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Corresponding editor: Tapas Kumar Kundu