

# Is Telomerase a Novel Target for Metastatic Colon Cancer?

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Most current therapies for advanced colon cancer lead to the emergence of drug-resistant cells and tumor recurrences. Targeted cancer therapeutics seek to identify pathways that are more tumor specific, resulting in fewer side effects, and that may produce long-term durable responses. Telomerase is a cellular reverse transcriptase that maintains the ends of chromosomes (telomeres). Telomerase is activated in the vast majority of human cancers, including colon cancer, and telomeres are maintained at short but stable lengths. In normal tissues, telomerase is not expressed or is expressed at very low levels that do not fully maintain telomeres. This suggests that telomerase may be a novel cancer target, and approaches to inhibit telomerase for cancer therapy are an attractive option because of its distinct pattern of expression. This article reviews the background of telomeres and telomerase in colon cancer and the use of telomerase inhibitors in the treatment of cancer.

## Introduction

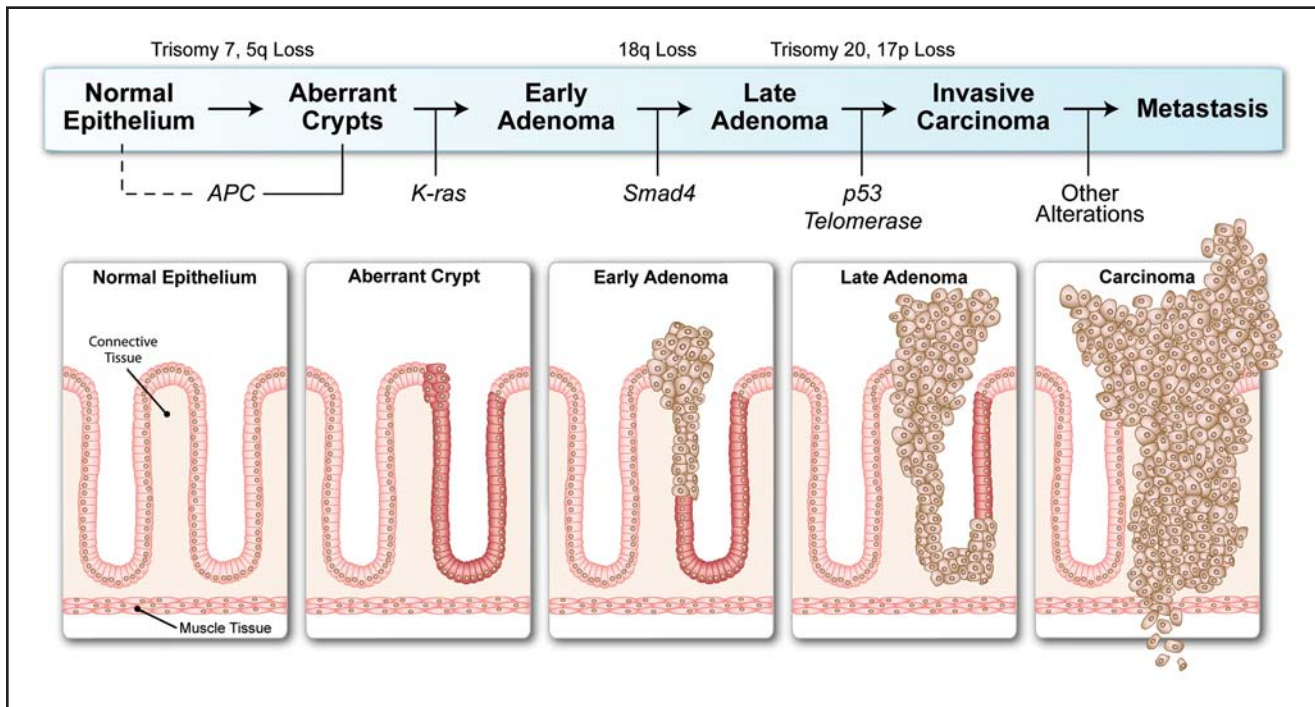
Telomerase is an RNA-containing enzyme that synthesizes DNA onto the ends of chromosomes, helping to maintain the integrity of the genome. In the 1930s, scientists observed that protective caps at the ends of linear chromosomes helped ensure the faithful propagation of chromosomes during cell division by preventing them from inappropriately fusing with one another. Hermann Muller [1] and Barbara McClintock [2] inferred that the ends or termini of chromosomes should have special characteristics. Thus the idea emerged that a distinct structure must protect linear chromosome ends not only from fusing to each other but also from eliciting a DNA damage signal that would cause cells to stop dividing. Muller [1] termed

the chromosome termini *telomeres* from the Greek *telos* for “end” and *meros* for “part” or “segment.” However, for more than 30 years it was a mystery as to what made telomeres different from randomly generated DNA ends.

In 1978, Elizabeth Blackburn [3] showed that simple repeated DNA sequences were at the chromosome ends in unicellular organisms and that these repeats stabilized chromosomes inside cells. Although this work predicted the existence of an enzyme that would maintain sequences at telomeres, it was not until 1985 that Carol Greider and Elizabeth Blackburn [4] biochemically identified the activity and also showed that it was a ribonucleoprotein enzyme complex. More than a decade later, the two essential genes that constitute the cellular ribonucleoprotein enzyme complex (*TERC* [5] and *TERT* [6]) were cloned.

Before the cloning of the components of telomerase, it was shown that human telomeres consist of repetitive TTAGGG DNA sequences [7,8], provide genomic stability (protect the ends from being recognized as DNA breaks needing repair), and are a source of expendable DNA [8–10]. The ability to lose telomeres without initially affecting the function of cells provided a solution to the inability of the replication machinery to copy the extreme ends of chromosomes, often referred to as the end replication problem [11]. Thus, almost all normal human cells show progressive telomere shortening with ongoing cell division until telomeres reach a critically shortened length, leading to the induction of a DNA damage signal often referred to as replicative senescence or cellular aging [12]. Some of the earliest evidence to support the loss of telomeric DNA during aging of primary cells in humans was from a study by Cooke and Smith [13], who showed the telomeric fragments of chromosomes were longer in the germline than in the peripheral blood or gastrointestinal tissue.

Research during the past decade has led to increased knowledge of the structure and function of telomeres. Telomeres end in G-rich single-stranded 3' overhangs, which can form a lariat structure called a t-loop [14]. The chromosome ends are protected by a group of telomere proteins called the shelterin complex [15]. Some of the six members of the shelterin complex bind to double-stranded telomeric repeats, others bind to these DNA binding proteins, and at least one, POT1 (*protection of telomeres*), binds directly to the G-rich single-stranded overhang



**Figure 1.** Activation of telomerase in the multistep model of human colon cancer progression. The timing of telomerase upregulation or reactivation is surprisingly late in colon cancer progression. Less than half of late adenomas express any detectable telomerase activity, whereas more than 90% of colorectal carcinomas are strongly positive [20,34]. This is in contrast to normal tissues and tissues that are adjacent to tumor tissues, in which only 16% to 25% of specimens have any detectable telomerase activity [20,34]. Studies also have shown that the prognosis of patients with high telomerase activity is significantly worse than that of patients with moderate or low telomerase activity [31]. Many genetic and epigenetic changes occur during colon cancer progression, including nonrandom chromosome numeric changes (eg, trisomy 7 and 20) and loss/deletions in 5q, 18q, and 17p. Some of these cytogenetic changes may correlate with loss of tumor suppressor gene function or abnormal expression of other genes. Targeted therapeutics of these cancer-driven pathways, such as inhibition of telomerase, continues to be an area of active interest.

protecting the mammalian chromosome ends from the ATR-dependent DNA damage responses as well as being involved in telomerase-mediated telomere extension [16]. Proof that telomere shortening and cellular aging are causally and not just correlatively related was provided in 1998 when Bodnar et al. [17] showed that introduction of *hTERT* into normal telomerase silent human cells was sufficient to activate telomerase activity, bypass senescence, and lead to cell immortalization.

### Telomerase as a Diagnostic Biomarker in Early Cancer Detection

There is a rich literature on the potential of telomerase to serve as a sensitive biomarker for early cancer detection and prognosis and to monitor for residual disease [17–23]. The development of a critically important assay for the field, the Telomeric Repeat Amplification Protocol (TRAP), led to an expansion in the ability to detect telomerase activity in small numbers of human cancer cells [19]. This sensitive polymerase chain reaction–based assay detects as few as 1 to 10 positive cells in a mixed population and may permit the detection of early cancer lesions before the onset of tissue invasion. Telomerase assays almost always show increased sensitivity and specificity compared with many other early cancer detection molecular assays. However,

US Food and Drug Administration–approved clinical telomerase diagnostics for screening or monitoring patients are still undergoing validation and standardization studies. In some cancers, such as lung and breast cancers, telomerase is upregulated or reactivated early in disease progression [20,23]. In colon cancer, telomerase is not detected at the earliest stages of disease progression but generally at the adenoma/carcinoma transition (Fig. 1) [20,23]. One recent approach currently in preclinical testing is the Telomerase Biosensor Technology (TBT; Sienna Cancer Diagnostics, Melbourne, Australia). This assay is predicted to allow 95% specificity (few false positives) and 95% sensitivity (few false negatives) for bladder cancer, melanoma, and colorectal cancer. Detecting lesions that may have prognostic value before the onset of tissue invasion, such as those seen in frozen sections, fine-needle aspirates, circulating tumor cells, normal secretions, pathologic fluids, aspirates, and washes and brushes, is one important goal of telomerase screening [23].

### Telomeres and Telomerase in Colon Cancer

Telomerase activity and telomere length in colorectal cancer and noncancerous colonic mucosa specimens have been examined in several studies [24–31,32•,33–35]. In the absence of a mechanism to slow telomere loss, the

shortening of telomeres would be predicted to occur in rapidly proliferating cells of the skin, gastrointestinal system, and blood. One mechanism to solve this problem is to regulate the expression of telomerase in these tissues with high rates of cellular turnover. Although some telomerase activity is detected in the colonic crypts [24,25], this activity is not sufficient to fully maintain telomere length, thus telomeres progressively shorten with increased age in the gastrointestinal tract [26]. Tissues with a high degree of cellular turnover, such as gastrointestinal tract tissue, have engaged a mechanism to slow the rate of, but not stop, progressive telomere loss (ie, regulated telomerase activity). Very little is known about how proliferative stem cells regulate telomerase activity. In the intestinal cell renewal system, the putative stem cells are located in the lower regions of the intestinal crypts and their descendant cells migrate up the crypt–villus axis, losing proliferative ability, differentiating, and finally becoming lost at the villus tip. Telomerase activity is detected only in the lower proliferative zone of the intestinal crypt, where putative stem cells and/or their progenitors are located [24,25]. Similar stem cell architecture occurs in the human colon.

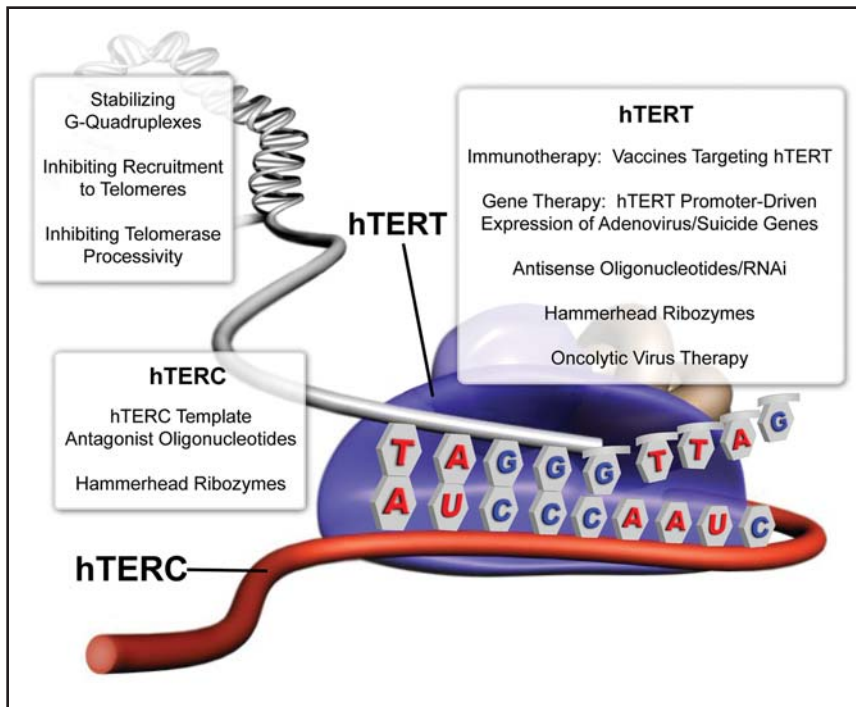
There have been several studies on telomerase activity in colon cancer progression [27–31,32•,33,34]. One study's objective was to determine whether the level of telomerase activity or telomere length is a prognostic indicator of patient outcome [31]. In this study, patients were followed up more than 3 years after surgery. Among 100 primary colorectal cancer specimens, 96 had telomerase activity. Because noncancerous mucosa can express some detectable telomerase [24,25], the levels of telomerase activity were divided into three categories in this study: high (> 50-fold more than that in noncancerous mucosa); moderate (10- to 50-fold); and low (< 10-fold) levels [31]. Among the 100 colon cancer specimens, 28 showed moderate telomerase activity and 44 showed high telomerase activity. The frequency of tumors with moderate or high telomerase activity showed no significant relationship to other clinicopathologic factors. However, the prognosis of the patients with high telomerase activity was significantly worse than that of patients with moderate and low telomerase activity ( $P < 0.01$ ). Among the 87 patients with surgically resectable and potentially curative tumors, the disease-free survival rate of those with high telomerase activity also was significantly poorer ( $P < 0.01$ ). These results indicate that a high level of telomerase activity may be an independent prognostic factor in patients with colorectal cancer and suggest that inhibitors of telomerase may prove efficacious in treating patients with advanced disease. An increased level of telomerase activity was a prognostic indicator, independent of disease stage and Dukes' classification. Thus, high telomerase activity may risk-stratify patients who are likely to have cancer recurrence and may give an indication for postoperative standard chemoadjuvant therapy or future telomerase-targeting therapy. The exclusion of low-risk patients from postoperative chemoadjuvant therapy could spare serious side effects.

In a similar study, investigators observed strong telomerase activity in 32 of 35 colon cancer specimens (92%) but did not observe activity in 30 of 35 matched normal colonic tissue specimens (86%) [34]. Interestingly, four of seven specimens from patients with ulcerative colitis and two of five Crohn's disease lesions were negative for telomerase, and the rest were only weakly positive for telomerase activity. These investigators also assayed for telomerase in exfoliated cells in luminal washings from colectomy specimens from 15 patients with colon carcinoma and 9 with inflammatory bowel disease. Telomerase was detected in washings from 9 of the 15 colon carcinoma cases (60%) but not in any of the inflammatory bowel disease cases. This suggests that telomerase could be a marker for risk-stratifying patients with colon cancer detected during colonoscopy.

In a more recent study, investigators showed that hTERT mRNA was expressed in all colorectal tumor samples [32•]. The level of expression in the colorectal adenocarcinomas was significantly higher compared with the corresponding nontumorigenic mucosa. Interestingly, a statistically significantly higher level of hTERT expression was found in the adenocarcinomas arising from the left colon and rectum compared with those from the right colon. One additional study demonstrated that telomeres in colorectal carcinoma tissues were significantly shorter than those in adjacent normal mucosa. In multivariate analysis, the telomere length ratio proved to be of independent prognostic value, with shorter tumor telomeres having worse outcomes. This also was noted in another study [35]. The correlation of short telomeres with tumor stage and patient survival suggests that hTERT-mediated telomere stabilization may be critical for progression and prognosis of colorectal carcinoma [33].

### Telomerase Inhibition as an Anticancer Approach

Approximately 90% of human cancers express telomerase activity [19,20]. Many approaches are being pursued to inhibit telomerase as a highly specific anticancer strategy [36–41,42•,43•], and several clinical trials are now in progress. The average telomere length in 90% of cancer cells is usually well below that of normal cells [35]. This difference in telomere length between normal tissue stem cells and cancer cells should provide a window of therapeutic opportunity to force cancer cells into apoptosis, with only limited side effects on normal cells. It is believed that treatment with telomerase inhibitors may result in fewer toxicities compared with other therapeutic agents because telomerase is absent in most somatic cells. Although normal proliferating telomerase-positive stem cells may also be affected initially, their telomeres are well above the critically short length that induces a DNA damage/growth arrest mechanism. Furthermore, most normal stem cells are quiescent, and telomere shortening normally occurs only with cell division. Because most cancer cells have



**Figure 2.** Approaches to targeting telomerase in cancer cells. Some approaches to targeting telomerase might lead the already shortened cancer telomeres to get even shorter, eventually resulting in apoptotic cell death. Germline and normal adult stem cells that have regulated telomerase almost always contain longer telomeres compared with most cancer cells. There is no evidence that cancer stem or cancer-initiating cells have longer telomeres than the bulk of the cancer cells. Thus, there may be a therapeutic window to drive cancer cells into apoptosis without major side effects on normal cells. Clinical trials are using a small molecule oligonucleotide inhibitor of telomerase (GRN163L, imetelstat) [43•], an immunotherapy peptide approach (GV1001) [46], and a gene therapy approach using a conditionally restricted, replication-competent adenovirus containing the *hTERT* (telomerase) promoter driving the adenoviral genes *E1A* and *E1B* (Telomelysin, OBP-301). Other preclinical approaches shown here are detailed in another recent review [42•]. The hope is that novel targeted therapeutic interventions, such as telomerase inhibitors, in combination with surgery and postoperative chemotherapy may improve survival outcomes for patients with colorectal cancer.

very short telomeres, treatment with telomerase inhibitors should lead to growth arrest and cell death. There is only limited information on whether cancer stem cells can become quiescent, but several studies have indicated that cancer cells with stem cell markers are telomerase positive [44,45]. Thus, telomerase not only may affect the bulk of the tumor cells, it also may target cancer stem cells.

### Clinical Advances in the Development of Telomerase Inhibitors

There is a large amount of knowledge regarding how telomerase inhibitors should act when used in clinical trials. Clearly it will be important to confirm that telomerase inhibitors are acting specifically via a telomere-dependent mechanism. First, not only should telomerase inhibitors reduce telomerase activity, initially they should not affect cell proliferation. Only after a subset of telomeres in cancer cells reaches a critically shortened length would cell proliferation be affected and tumor volume decrease. Importantly, without telomerase activity, telomeres should shorten progressively with each cell division. Cells should ultimately die or undergo growth arrest, and the time required should be related to initial telomere length.

One small molecule, GRN163L (Geron Corporation, Menlo Park, CA), has been investigated extensively. Based on broad *in vivo* and *in vitro* proof-of-concept experiments, good safety profiles, and adequate pharmacokinetics and biodistribution properties, several clinical trials are currently in progress. These studies include phase 1/2 for treating patients with chronic lymphocytic leukemia, phase 1/2 for multiple myeloma, phase 1 for

solid tumors (safety, tolerability, maximum tolerated dose), phase 1/2 for breast cancer (GRN163L plus paclitaxel and bevacizumab for local recurrent or metastatic breast cancer), and phase 1/2 for non-small cell lung cancer (GRN163L plus paclitaxel and carboplatin for patients with stage IIIb disease with pleural effusion, stage IV disease, or recurrent disease).

In addition to enzymatic inhibition of telomerase, a variety of other approaches are being investigated (Fig. 2). Ongoing immunotherapy trials are targeting *TERT* epitopes expressed on the surface of cancer cells but not normal stem cells. A peptide vaccination approach (GV1001) has had some encouraging preliminary results, with no evidence of serious adverse effects, such as bone marrow repression or evidence of autoimmune disease [46,47]. TeloVac, a large ongoing randomized phase 3 trial involving 80 cancer centers in the United Kingdom, is targeting advanced pancreatic cancer. This study is comparing standard chemotherapy with a combination of chemotherapy (gemcitabine/capecitabine) and the *TERT* peptide vaccine. The TeloVac trial, sponsored by Kael Co., Ltd. (Daejeon, South Korea), has recruited approximately 400 of 1100 patients to date.

Telomerase inhibitors likely will have the most impact in patients with minimal residual disease. In these cases, GRN163L may prove efficacious when provided as maintenance therapy after tumor debulking by surgery or chemotherapy or in combination with adjuvant cytotoxic chemotherapy or radiotherapy. Although it is too early to know the best methods for using telomerase inhibitors to treat patients with advanced disease, the ongoing trials will attempt to determine whether telomerase is an

effective target in combination therapy or after debulking chemotherapy, and whether there is an additive or synergistic effect when a telomerase inhibitor is combined with chemotherapy. The most appropriate ways to combine telomerase inhibitors with established therapies are not yet known. However, emerging data from early clinical trials will aid this stage of development. Clearly, treating patients with advanced and metastatic cancer is a challenge; therefore, the best use of telomerase inhibitors may be as adjuvant therapy in earlier-stage disease or even as primary prevention in high-risk patient populations, or in cancer patients at high risk for disease recurrence.

Another approach that has just entered phase 1 solid tumor clinical trials involves OBP-301 (Telomelysin; Oncolys BioPharma, Tokyo, Japan), a conditionally restricted, replication-competent adenovirus containing the *hTERT* promoter driving the adenoviral genes *E1A* and *E1B*. Telomelysin is administered locally into the tumor; this approach combines the advantages of tumor-selective gene expression, using the *hTERT* promoter, with the beneficial effects of an oncolytic virus. Newer approaches inserting a suicide gene (gene-directed enzyme prodrug therapy) into the oncolytic virus (also with the *hTERT* promoter) are nearing clinical trials. With this strategy, once the gene therapy is administered and the introduced enzyme is expressed in cancer cells, the prodrug can be administered to convert the enzyme into a cytotoxic drug [42•]. Although these approaches using telomerase-specific oncolytic viruses may be useful for local disease control, systemic therapy has a greater potential to affect telomerase-competent proliferating stem cells.

## Conclusions

Telomerase is an attractive target for cancer diagnosis and therapy because it is expressed in approximately 90% of primary tumor cells but not in most normal tissues. Inhibition of telomerase in vitro leads to progressive telomere shortening, inducing a DNA damage response because of the critically short telomeres and eventually leading to growth arrest or cell death. Human diseases of telomerase provide compelling evidence that haploinsufficiency for telomerase leads to premature aging syndromes associated with stem cell depletion [48,49•,50]. Thus, the vast majority of cancer cells with already shortened telomeres should be most affected by telomerase inhibitors, whereas normal stem cells with longer telomeres should be relatively resistant. The effects of telomerase inhibitors likely depend on the initial telomere length and rate of cell division, and it may take months of treatment to observe changes in tumor size. Thus, combining telomerase inhibitors with current therapies to reduce tumor burden may provide a better regimen, leading to durable remissions. There still are many unanswered questions with respect to key safety concerns, such as the effect of telomerase inhibitors on normal stem cells that express some telomerase. Although there is mounting evidence that cancer cells enriched for

stem cell markers express telomerase activity, it is not known if they will be resistant to telomerase inhibition therapy. One issue is whether cancer stem cells are more quiescent than the bulk of the more differentiated tumor cells. If so, then telomerase inhibitors may be less effective. Finally, will human cancers become resistant to telomerase inhibitors? Clearly there is still much to be discovered in this rapidly evolving area of research. The telomerase field has benefited greatly from years of research in yeast, protozoa, and plants, and it is appropriate to remember the importance of basic research in developing new insights and approaches to treating human diseases.

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## Disclosure

Dr. Wright and Dr. Shay hold stock in Geron Corporation and stock options in Sienna Cancer Diagnostics. No other potential conflicts of interest relevant to this article were reported.

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