

Telomerase Targeted Therapy in Cancer and Cancer Stem Cells

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Abstract: 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. To date, 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. The recent discovery and growing interest in cancer stem cells has thrust telomerase therapy into new relief as an approach that may be uniquely suited to neutralizing this treatment-resistant subpopulation of cancer cells. Here we will review the mechanistic rationale and preclinical and clinical state of development of the various telomerase-based therapeutic approaches, with emphasis on the role of telomerase in cancer stem cell biology and its implications for therapeutic efforts.

Introduction

In 2009, the Nobel Prize in Physiology or Medicine was awarded to Elizabeth H. Blackburn, Carol W. Greider, and Jack W. Szostak for their discovery of telomerase a quarter century ago.¹⁻² Since that time, the telomerase field has advanced by leaps and bounds, currently boasting hundreds of studies each year seeking to elucidate basic telomerase structure/function and to parlay these insights into biomedical applications. The latter goal has perhaps been closest to the hearts of investigators in the fields of oncology and developmental therapeutics—who for the past 15 years have striven to deliver on the promise of telomerase as a nearly universal cancer target that plays a critical role in virtually every common malignancy. Most recently, efforts to develop telomerase-based therapies have been reinvigorated by their potential efficacy against cancer stem cells, subpopulations of cancer cells that are highly tumorigenic and generally resistant to standard therapies. In this review, we will outline the principal features of telomerase biology and its role in cancer, and we will review the main strategies under-

Keywords

Telomerase, cancer stem cell, targeted therapy, telomeres

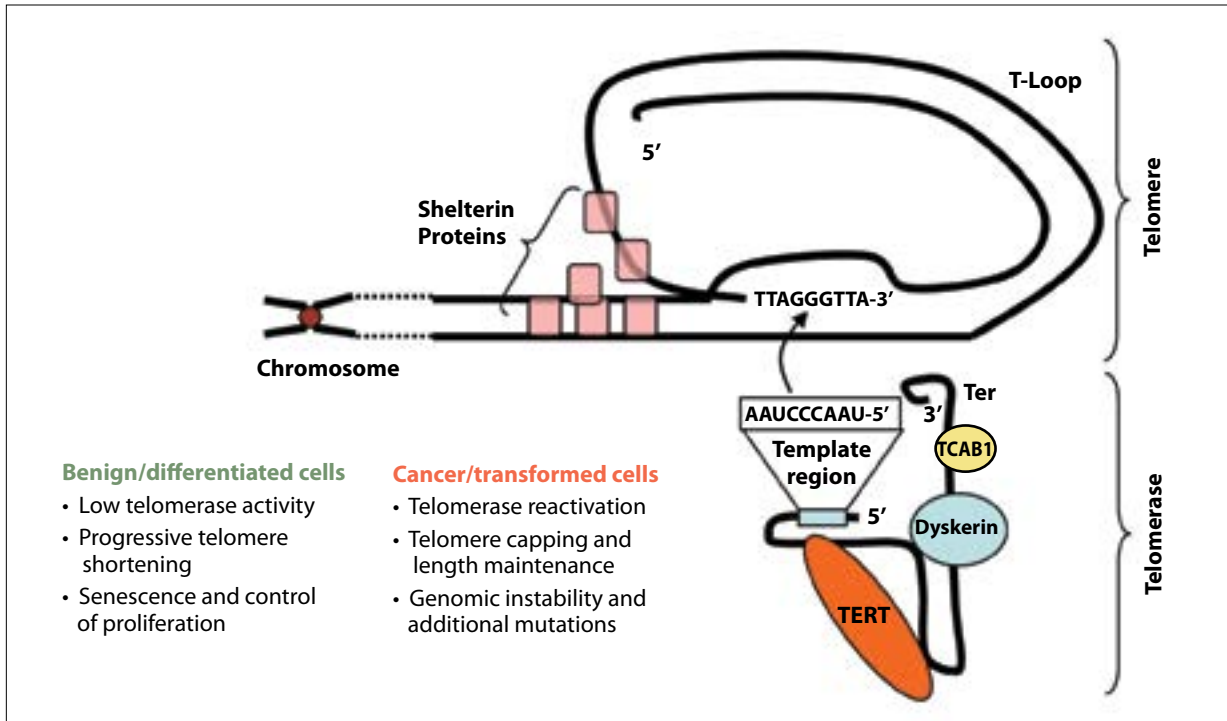


Figure 1. Telomeres and telomerase. Schematic depicting the main components of telomeres (top) and of the telomerase ribonucleoprotein (bottom).

TCAB1=telomerase Cajal protein body 1; TERT=telomerase reverse transcriptase; Ter=telomerase RNA.

taken to date for targeting cancer vis-à-vis telomerase. Also, we will discuss the evolving concept of cancer stem cells and recent observations made by our group and others about the biologic role and therapeutic potential of telomerase in this special subpopulation of cells.

Biology of Telomeres and Telomerase

Telomere Biology

The well-established canonical function of the telomerase enzyme is the maintenance and lengthening of telomeres, the tandem repetitive DNA sequences located at the ends of human chromosomes.³ The 3' telomeric strand consists of G-rich tandem repeats (TTAGGG) terminating in a single stranded 3'-overhang with a lariat structure that often loops back and reinserts as a terminal T-loop into the double-stranded telomeric region (Figure 1).⁴ The 2 essential functions of telomeres are protecting chromosome ends (the “capping” function of telomeres) and facilitating their complete replication. The average human telomere length at birth is approximately 15–20 kb⁵⁻⁶; however, as a result of telomerase down-regulation in normal somatic cells, human chromosomes can lose up to 50–200 nucleotides of telomeric sequence per cell division.⁷⁻⁸ Such shortening of telomeres is attributed to the so-called “end replication problem,” wherein spaces

left by RNA primers during lagging strand replication lead to progressive shortening with each division/replication cycle.⁹ The resulting telomeric shortening has been proposed to be a mitotic clock that monitors cell division, and sufficiently short telomeres in the absence of telomerase may signal replicative senescence at approximately 4–6 kb, known as mortality stage 1 (M1).^{6,7,10} Some cells may bypass M1 via inactivation of p53 or the retinoblastoma protein (RB1) and enter mortality stage 2 (M2 or crisis), manifested by genomic instability and fusion/breakage mutagenic events and massive cell death. Activation of telomerase at M1 or M2 can stabilize telomere length and immortalize cells, which may potentiate cancer formation as cells proliferate beyond M2.^{6,11}

Although telomerase plays a central role in telomere maintenance, it is important to note that other factors also contribute significantly to telomere biology. Numerous proteins have been shown to interact with telomeres, among them the 6 members of the shelterin complex (TRF1, TRF2, Pot1, Tin2, Rap1, TPP1),¹² which interact directly or indirectly with telomeric DNA to regulate telomere length and recruit telomerase and additional proteins to single-stranded or double-stranded telomeric regions. Moreover, a small but significant group of benign and malignant cell types (eg, some fibroblasts and sarcomas, respectively) do not activate telomerase at

all but rather rely on telomeric recombination—so-called alternative lengthening of telomeres (ALT)—to maintain telomere lengths.¹³ Cancers that employ ALT rather than telomerase for telomere maintenance are few in number and therefore have not significantly dampened the enthusiasm for telomerase-based approaches; however, it is conceivable that ALT may in time emerge as a potential resistance mechanism in telomerase-positive cancers treated with telomerase-based therapies.^{14,15}

Telomerase Biology

The telomerase core ribonucleoprotein (RNP) consists of 2 components: a reverse transcriptase protein (telomerase reverse transcriptase [TERT], 127 kD in humans) and an intrinsic telomerase RNA molecule (Ter, 153kD, and 451 nt in humans; Figure 1).¹⁶ Ter contains a short template sequence used by TERT to reverse transcribe telomeric DNA.¹⁷ The secondary and tertiary structures of TERT and Ter and the elucidation of their functional domains are the subject of ongoing investigation and are beyond the scope of this review.¹⁸⁻¹⁹ Several additional proteins that associate with the core RNP have been identified,^{16,20} among them dyskerin and telomerase Cajal body protein 1,²¹ both of which play a pivotal role in telomerase biogenesis and function. Mutations in dyskerin are implicated in the telomerase dysfunction disease dyskeratosis congenital.^{22,23} Although these proteins play a critical role in telomerase holoenzyme biogenesis and function, their potential as therapeutic targets has not been extensively explored to date.

Telomerase in Cancer

Expression of telomerase protein (TERT) is tightly regulated at the transcriptional level; with the exception of renewable progenitor compartments (hematopoietic, epidermal, gastrointestinal), most benign, terminally differentiated tissues have extremely low telomerase activity.^{24,25} In contrast, malignant cells from as many as 90% of all human cancers—including prostate, melanoma, breast, colon, sarcoma, and ovarian—have significant telomerase expression and telomerase activity levels that correlate directly with malignant/metastatic potential by enabling continued proliferation and telomere stabilization beyond M1 and M2/crisis.²⁶⁻³² As a result of this sharp phenotypic dichotomy between benign and malignant tissues (Figure 1), telomerase has been recognized as a highly promising cancer therapeutic target: minimally toxic to host tissues and potentially efficacious against a majority of malignancies. Indeed, early *in vitro* studies demonstrated that activation of telomerase by ectopic expression of TERT, combined with expression of SV40 antigen (inactivates pRB and p53) and H-ras, was sufficient to transform benign cells in culture.^{33,34} Conversely, attempts to

attenuate telomerase function in cell culture led to not only telomere shortening³⁵ but also cellular apoptosis and inhibition of cancer cell growth *in vitro*,^{36,37} thus providing additional compelling mechanistic evidence that telomerase-dependent telomere maintenance is essential for cancer cell immortalization, tumor progression, and disease metastasis.

Telomerase Therapeutics

The pivotal role of telomeres and telomerase both in early carcinogenesis and in advanced malignancy across a majority of cancer types has stimulated efforts to develop therapies aimed at disrupting their functions. Over the past decade, some telomerase-based therapeutic strategies have progressed into clinical trials, whereas others are still undergoing *in vitro* study and preclinical development. Testing these novel therapeutics preclinically and then clinically requires concurrent use of informative pharmacodynamic endpoints that confirm effective drug-on-target effects, such as inhibition of telomerase activity, alteration of telomere lengths, or induction of apoptosis in the cancer cells being targeted. Because host regenerative compartments (eg, hematopoietic, gastrointestinal) possess low levels of telomerase activity, cells from these tissues must also be assayed to quantify off-target toxicities. As with other targeted therapies, some forms of telomerase targeting may exert tumoristatic rather than tumoricidal effects. Thus, it would require that clinical trials include not only radiographic endpoints of tumor response, but also clinical endpoints of disease progression and survival, as well as correlative biologic endpoints (eg, post-treatment tumor specimens, circulating tumor cells, host lymphocytes) in order to document disease response. Furthermore, because some telomerase therapeutics may preferentially eliminate particular cancer subpopulations such as cancer stem cells, ultimately their optimal therapeutic efficacy may be in combination with more standard chemotherapies, radiotherapies, or other targeted agents.

For the purposes of this review, the main therapeutic approaches will be discussed based on their general mechanism of action: 1) approaches that directly target the enzymatic function of telomerase; 2) approaches that target telomerase as a cancer-specific marker; and 3) approaches aimed at targeting telomeres in order to disrupt telomerase function (Figure 2).

Targeting the Enzymatic Function of Telomerase

Telomerase Inhibition Perhaps the most straightforward therapeutic strategy seeks to inhibit the enzymatic activity of telomerase, thus abolishing its telomere-lengthening function, leaving telomeres to shorten with subsequent cell divisions, ultimately resulting in senescence or

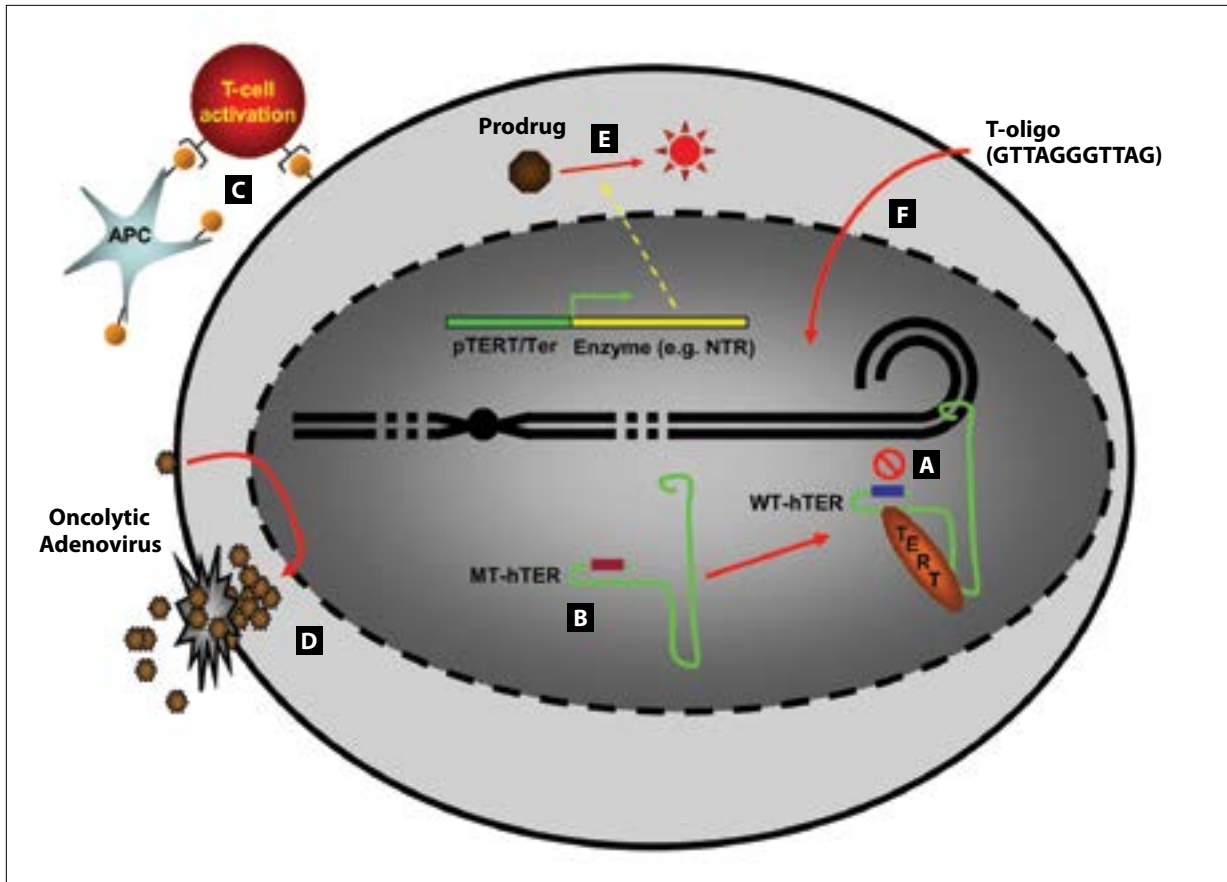


Figure 2. Overview of telomerase therapeutic strategies. Various telomerase-based therapeutic approaches have been developed: (A) direct telomerase inhibition via an oligonucleotide (GRN163) that binds hTer template; (B) telomerase interference (MT-hTer) that reprograms telomerase enzyme to add incorrect telomeric repeats; (C) telomerase vaccines (various) that induce cytotoxic T lymphocyte by either direct inoculation or ex-vivo activation; (D) oncolytic viruses (various) that cause tumor-specific cell lysis; (E) suicide gene therapy that employs telomerase RNA and telomerase reverse transcriptase promoter-driven activation of pro-drugs; (F) telomeric oligos (T-oligos) that mimic uncapped telomeres.

NTR=nitroreductase; TERT=telomerase reverse transcriptase.

apoptosis. Significant efforts to identify small molecule inhibitors of telomerase reverse transcriptase function have failed to yield an agent with adequate efficacy and specificity. However, an alternative tact undertaken by Geron Corp. has yielded an inhibitor, which has been the single most clinically tested telomerase therapeutic to date (Figure 2A). Imetelstat (GRN163L) is an oligonucleotide with the sequence TAGGGTTAGACAA that is complementary to the 11-nucleotide hTer template, the highly conserved region of telomerase RNA used by the RNP to reverse transcribe telomeric repeats. Binding of the hTer template region by imetelstat blocks the biogenesis of an active telomerase RNP and results in progressive telomere shortening, cellular senescence, or apoptosis, and inhibition of cancer proliferation—either alone or in combination with standard therapies—in a variety of in vitro and mouse cancer models.³⁸⁻⁴¹ Modi-

fication of the imetelstat oligonucleotide backbone via an N3' to P5' thio-phosphoramidate (NPS) transition stabilizes oligonucleotide-hTER duplex formation, and addition of a lipid group at 5' terminus of imetelstat facilitates cellular and tissue penetration. Currently, imetelstat is undergoing extensive phase I/II clinical testing in breast cancer, lung cancer, multiple myeloma, and chronic myeloproliferative diseases (Table 1, www.clinicaltrials.gov). Preliminary reports cite cytopenias, prolonged clotting, gastrointestinal side effects, fatigue, and peripheral neuropathy as the most common toxicities.^{42,43} As it proceeds towards additional phase II and upcoming phase III trials, imetelstat continues to be a very promising agent, and is among the most highly developed of the telomerase therapeutics. One theoretical concern about this drug stems from its mechanism of action. After imetelstat inhibited telom-

Table 1. Telomerase Therapeutics Currently in Clinical Development

Telomerase Inhibitor				
Agent (Sponsor)	Trial	Status	Results	NCT Identifier
GRN163L (Geron)	Ph II: Breast cancer	Recruiting	N/A	NCT01256762
GRN163L (Geron)	Ph II: NSCLC	Recruiting	N/A	NCT01137968
GRN163L (Geron)	Ph I: CLD	Active, not recruiting	N/A	NCT00124189
GRN163L (Geron)	Ph I: NSCLC	Active, not recruiting	N/A	NCT00510445
GRN163L (Geron)	Ph I: Melanoma	Active, not recruiting	N/A	NCT00718601
GRN163L (Geron) ¹⁵⁴	Ph I: Solid tumor	Active, not recruiting	Thrombocytopenia at doses >3.2 mg/kg/wk	NCT00310895
GRN163L (Indiana U.)	Ph I: Breast cancer	Not recruiting	N/A	NCT01265927
GRN163L (Geron) ⁴²	Ph I/II: Breast cancer	Active, not recruiting	No DLTs, cytopenias	NCT00732056
GRN163L (Geron)	Ph II: ET	Recruiting	N/A	NCT01243073
GRN163L (Geron)	Ph I: Myeloma	Active, not recruiting	N/A	NCT00594126
GRN163L (Geron)	Ph II: Myeloma	Recruiting	N/A	NCT01242930
Telomerase Vaccine				
Agent (Sponsor)	Trial	Status	Results	NCT Identifier
GV1001 (Lytix Biopharma)	Ph I: Carcinoma	Recruiting	N/A	NCT01223209
GV1001 (Pharmexa) ⁸⁹	Ph II: HCC	Completed	Well tolerated, mild injection site reaction; no antitumor immune response	NCT00444782
GV1001 (Oslo U. H.) ⁸⁷	Ph I/II: NSCLC	Completed	Minor side effects, no bone marrow toxicity; immune response in 13/24 pts	NCT00509457
GV1001 (Oslo U. H.) ⁸⁸	Ph I/II: Melanoma	Completed	Well tolerated with neutropenia in 1/14 pts; immune response in 17/21 pts	NCT01247623
GV1001 (Pharmexa) ⁹⁰	Ph III: Pancreatic cancer	Terminated	No survival benefit	NCT00358566
GV1001 (Royal Liverpool U. H.)	Ph III: Pancreatic cancer	Recruiting	N/A	NCT00425360
hTERT 540-548 peptide (NCI) ¹⁵⁵	Ph II: Melanoma, solid tumor	Completed	No immune response against hTERT+ tumor	NCT00021164
hTERT 540-548 peptide (DFCI)	Ph I: Brain tumor, sarcoma	Active, not recruiting	N/A	NCT00069940
hTERT 540-548 peptide (UPenn) ⁹⁴	Ph I: Breast cancer	Active, not recruiting	Injection site reactions; suggestion of prolonged survival in immune responders	NCT00079157
hTERT multi-peptide (UMGCC) ¹⁵⁶	Ph I/II: Myeloma	Completed	Mild to moderate chills and rigors; antitumor immunity in 10/28 pts	NCT00499577

(Table continues on following page)

Table 1. (Continued) Telomerase Therapeutics Currently in Clinical Development

Telomerase Vaccine (continued)				
Agent (Sponsor)	Trial	Status	Results	NCT Identifier
hTERT multi-peptide (UPenn) ¹⁵⁷	Ph I: Breast cancer	Recruiting	Well tolerated; immune response in 80% of pts	NCT00573495
hTERT multi-peptide (UPenn)	Ph I/II: Myeloma	Active, not recruiting	N/A	NCT00834665
DC pulsed with hTERT 540-548 peptide (DFCI) ⁹³	Ph I: Breast and prostate cancer	N/A	Well tolerated, no changes in B cell number; immune response in 4/7 pts	
DC pulsed with telomerase peptide or tumor lysates (Herlev H.) ¹⁵⁸	Ph I/II: Melanoma	Completed	TERT immune response and disease stabilization in subset of patients	NCT00197912
DC pulsed with telomerase peptide or tumor lysates (Herlev H.) ¹⁵⁹	Ph I/II: RCC	Active, not recruiting	Well tolerated without severe toxicities; disease stabilized in half of pts	NCT00197860
DC pulsed with hTERT mRNA (UF) ¹⁶⁰	Ph I/II: Prostate cancer	Active, not recruiting	Fatigue or flu-like symptoms, erythema/induration; immune response in 19/20 pts	NCT01153113
GRNVAC1 (DC pulsed with hTERT/hTERT-LAMP mRNA, Geron)	Ph II: AML	Active, not recruiting	N/A	NCT00510133
TLI (hTERT DNA fragment, Cosmo Bioscience) ⁹⁶	Ph I: Prostate cancer	Completed	Feasible and safe: immune response by single-dose TLI	NCT00061035
Oncolytic Virus				
Agent (Sponsor)	Trial	Status	Results	NCT Identifier
Telomelysin (Oncolys BioPharma) ⁹⁹	Ph I: Solid tumor	N/A	Pain at injection site, fevers, chills; detected viral DNA in 13/16 pts	

AML=acute myeloid leukemia; CLD=chronic lymphoproliferative disease; DC=dendritic cell; DFCI=Dana-Farber Cancer Institute; DLT=dose-limiting toxicity; ET=essential thrombocythemia; HCC=hepatocellular carcinoma; N/A=not available; NCI=National Cancer Institute; NCT=National Clinical Trials; NSCLC=non-small-cell lung cancer; pts=patients; Ph=phase; RCC=renal cell carcinoma; TLI=transgenic lymphocyte immunization; UF=University of Florida; UMGCC=University of Maryland Greenebaum Cancer Center; UPenn=University of Pennsylvania.

erase in some preclinical studies, multiple cell divisions with progressive telomere shortening had to occur over several weeks before inhibition of cancer proliferation was observed.^{44,45} This “phenotypic delay” raises the possibility that some cancer cells might have the opportunity to develop resistance mechanisms, such as upregulation of TERT or alternative maintenance of telomeres via recombination.¹³ Whether such phenomena will play a clinical role or will impact the efficacy of imetelstat will soon be addressed in additional phase II and upcoming phase III trials.

Telomerase Interference A different approach, which directly targets telomerase, involves telomerase interference, which refers to altering the template region of hTer to reprogram the RNP (Figure 2B). This strategy, which was initially developed in the laboratory of Dr. Elizabeth

Blackburn as a tool to dissect telomerase reverse transcriptase function in ciliates,⁴⁶ was subsequently noted to exert an inhibitory effect on cancer cells.⁴⁷⁻⁵² Specifically, endogenous wild-type hTer is depleted using a short hairpin RNA knockdown, and simultaneously, an hTer with a mutated template region (MT-hTer) is ectopically introduced in its place. We and others have shown that MT-hTer is incorporated into active telomerase in cancer cells, where it essentially “reprograms” the enzyme to add incorrect telomeric tandem repeats. These altered telomeric repeats are recognized as “uncapped” telomeres, eliciting a rapid DNA damage response and apoptotic cascade, culminating in inhibition of proliferation.^{49,50,53} A potential strength of telomerase interference is its immediate, dominant effects. Telomerase reprogramming is not dependent on subsequent telomeric shortening and therefore has an almost immediate effect on cancer cells

by uncapping their telomeres within 1 or 2 cell divisions, manifested by significant apoptosis and growth inhibition within 48 hours of treatment. Moreover, cancer cells cannot upregulate TERT expression as a resistance mechanism, because increased levels of TERT actually potentiate the pro-apoptotic effects of MT-hTer by offering more enzyme to reprogram, and thus even more dramatic telomeric uncapping. On the other hand, the effects of telomerase reprogramming may be so rapid and pervasive as to raise concerns about telomeric uncapping and toxicity in normal stem cells that rely on telomerase activation to sustain progenitor tissue compartments. A second, more practical obstacle is the challenge of effective systemic delivery, as telomerase reprogramming currently is achieved via expression of the entire 451-nt MT-hTer from a DNA plasmid, making this an ineffective approach for systemic treatment. The challenges of systemic delivery and possible stem cell toxicity are being addressed in ongoing studies; our group has recently validated murine-targeting MT-mTer and shRNA constructs⁵¹ that are being used to address these questions in mouse models of malignancy.

Targeting Telomerase as a Unique Cancer Marker

Telomerase as a Cancer Biomarker Telomerase expression and activity are high in most cancer types but low in benign, differentiated cells, a specificity that has been exploited diagnostically and prognostically by quantifying telomerase in primary tumor tissues and metastases, and more recently in peripheral blood circulating tumor cells.^{24,54-61}

Diagnostic In prostate cancer, several studies assaying telomerase activity from expressed prostatic secretions have demonstrated cancer detection rates approaching 90%, as reviewed by Meeker.⁵⁸ Multiple other studies of diagnostic utility have demonstrated a high sensitivity and specificity of detection in numerous cancer types, including bladder, breast, lung, pancreatic, hepatocellular, and gastric.^{56,62-72}

Prognostic In breast cancer, 1 large study of nearly 400 patients found that increased telomerase activity levels correlated with decreased disease-free survival and overall survival.⁷³ Another breast cancer study using a tissue microarray of over 600 breast cancer specimens found a strong correlation ($P < .001$) between telomerase mRNA (TERT and hTer) expression and overall survival.³⁰ In non-small-cell lung cancer, the levels of telomerase activity correlated with the overall survival and disease-free survival in stage I patients who underwent curative resection.⁵⁷ In another lung cancer study, high levels of telomerase activity in transthoracic fine-needle biopsy

specimens were associated with an increased risk of disease recurrence and death.⁷⁴ Additional studies have shown a strong prognostic value for telomerase in gastric and colon cancer and in neuroblastoma.^{28,59,61,75-78}

Telomerase in Circulating Tumor Cells Telomerase also has been used to detect circulating tumor cells (CTC) in the blood of cancer patients. In one study, cells were isolated from the blood of women with metastatic breast cancer using magnetic beads coated with antibodies that bind epithelial cell adhesion molecule (EpCAM). Isolated cells were subjected to polymerase chain reaction (PCR)-based telomerase activity assays, which detected telomerase activity in 21 of 25 breast cancer patients and in none of 9 healthy volunteers.⁷⁹ The same group later applied this approach in a larger study of more than 100 men with prostate cancer and detected CTC by telomerase measurement from a majority of patients with advanced and local disease, but none in normal healthy controls.⁸⁰ Although these results constituted major strides in the application of telomerase as a CTC biomarker, they relied on EpCAM binding for CTC isolation; during dissemination, cancer cells frequently downregulate EpCAM, and several important malignancies (including HCC, sarcomas, and melanoma) express low levels of EpCAM to begin with.⁸¹⁻⁸⁶ To address these limitations, our group recently developed, in collaboration with Caltech, a novel cancer detection platform that measures telomerase activity from live CTCs captured by size segregation on a parylene-C slot microfilter. Using a constant low-pressure delivery system, this platform achieved rapid CTC capture with high efficiency and viability, and telomerase activity was detected by real time quantitative PCR from as few as 25 cancer cells added to 7.5 mL of whole blood. Moreover, significant telomerase activity elevation was also measured from patients' blood samples and from single cancer cells lifted off of the microfilter.⁵⁴

Thus, telomerase has been validated repeatedly as a valuable cancer biomarker for disease detection, diagnosis, and prognosis across a broad spectrum of malignancy types and stages. Similarly, the cancer-specificity of telomerase (TERT) expression has been exploited therapeutically as a homing beacon for immune and virus-mediated strategies.

Immunotherapy That Exploits Telomerase as a Unique Cancer Marker Telomerase-positive cancer cells display TERT peptide fragments on their surface in association with major histocompatibility complex (MHC) class I molecules; hence, TERT vaccines aim to break immune tolerance and induce a TERT-specific cytotoxic T lymphocyte (CTL) response (Figure 2C). There are 2 main vaccine strategies: 1) direct inoculation with antigen or 2)

ex-vivo activation of autologous antigen-presenting cells (APC, dendritic cells) or of B lymphocytes.

Direct Inoculation With Antigen The most clinically advanced in this group is GV1001 (GemVax, Denmark), a TERT-derived p611–626 16-mer with the sequence EARPALLTSRLRFIPK that binds to and is subsequently presented by MHC class I. Multiple phase I/II studies have been conducted in pancreatic cancer, malignant melanoma, and non-small-cell lung carcinoma wherein patients received intradermal injections of GV1001 (Table 1). Toxicities were relatively limited (local pain and inflammation at injection site, fevers, chills), and a majority of patients developed quantifiable immune responses (CTL which recognize TERT), with a suggestion of prolonged survival in immune responders.^{87,88} However, to date, larger follow-up phase II and III studies combining GV1001 with single-agent chemotherapy in hepatocellular and pancreatic cancer have demonstrated no survival benefit.^{89,90} Another phase III study of GV1001 in advanced pancreatic cancer (in combination with gemcitabine and capecitabine) is still ongoing. A similar approach using a TERT-derived p540–548 ILAKFLHWL peptide is also currently in early phase clinical trials, where it has been well-tolerated and was shown to generate hTERT-specific CD8 positive CTL, with tumor infiltration and partial tumor regression observed in some cases.^{87,91–94}

Ex-Vivo Pulsing of Antigen Presenting Cells (APC, Dendritic Cells) or of B Lymphocytes Here, autologous immune cells are isolated from the cancer patient, activated ex-vivo with TERT-derived peptides and reinfused into the patient. Several phase I/II trials using this approach have reported good tolerability, induction of immune response (TERT-targeting CTL), and some instances of disease stabilization (Table 1). A dendritic cell approach using RNA-based ex vivo activation (GRNVAC1, Geron Corp.) offers the advantage of encoding multiple epitopes (compared to 1 epitope with peptide pulsing), thus extending the scope of vaccination to strengthen the immune response.⁹⁵ In a phase I/II trial in patients with prostate cancer, GRNVAC1 was well tolerated and hTERT-specific CD8-positive cells were detected in 19 of 20 patients.⁹⁶ In a similar approach using ex vivo DNA-pulsed autologous B lymphocytes, there were no observed toxicities and no vaccination-induced TERT-specific T-cell responses.⁹⁷

In summary, TERT-targeting vaccine strategies have been aggressively developed in the past decade, driven by enthusiasm for TERT's specificity and ubiquity across a majority of malignancies. Early phase I/II trials have demonstrated that these vaccines are able to break tolerance

and activate a TERT-specific immune response, resulting in tumor infiltration and a suggestion of clinical response in some cases. These promising results are tempered by preliminary data from early phase III trials, which failed to show clinical benefit. There are 2 possible hurdles that may underlie these modest clinical results: 1) cancer patients are relatively immune-suppressed, a condition attributed to the cytokine milieu elaborated to varying degrees by their tumors; therefore, some patients may have difficulty “breaking tolerance” and mounting a clinically significant response to the vaccine; 2) the absolute level of TERT even in telomerase-positive cancer cells is quite low, approximately 100 molecules per cell^{16,97}; therefore, even if CTL are activated by the vaccine, the levels of TERT peptides displayed on tumor cells may not constitute a sufficient “homing beacon” for a clinically significant immune response. Newer vaccine strategies aimed at maximizing the immune response are currently in preclinical development, and additional phase III trials are ongoing.

Virus-Mediated Therapy That Exploits Telomerase Upregulation in Cancer

Oncolytic Viruses A variety of preclinical viral and suicide gene strategies have been developed to exploit the active hTERT promoter in cancer cells. Of these, the furthest advanced in clinical development is the telomerase-specific oncolytic virus (Figure 2D). A conditionally replicative adenovirus is created by inserting the adenovirus E1A and E1B genes downstream of the hTERT promoter, thus inducing adenoviral replication and cellular lysis in a tumor-specific manner.⁹⁸ Telomelysin (OBP-301) is the first telomerase-specific oncolytic adenovirus to enter phase I study (Table 1). Patients with various solid tumors were administered a single intratumoral injection of telomelysin, which was associated with grade 1 and 2 toxicities (pain at the injection site, fevers, chills). Of 16 enrolled subjects, 13 were shown to have viral DNA in plasma, and 1 patient experienced partial response at the injected malignant lesion at day 56 after injection.⁹⁹ These early results are promising, and the presence of viral DNA in plasma suggests a potential for therapeutic benefit beyond the local intratumoral injection site; however, the clinical benefit of this approach in the metastatic disease setting currently awaits further clinical testing.

Suicide Gene Therapy A related therapeutic strategy, suicide gene therapy, entails delivery of a toxic gene to the cancer cells, where it is selectively activated to kill the cells (Figure 2E). Bilslund and colleagues developed such a system, wherein the nitroreductase (NTR) gene was packaged into an adenoviral vector under the transcriptional control of the hTERT and hTer promoters.¹⁰⁰

When introduced into cancer cell lines in cell culture or in tumor xenografts, the high levels of hTERT and hTer promoter activity present in tumor cells induced hTERT and hTer promoter-driven transcription of NTR, a bacterial enzyme that bioactivates the prodrug CB1954 into an active cytotoxic alkylating agent. This in turn led to preferential chemosensitivity and killing of the cancer cells in response to CB1954. Although clinical data using this approach are not yet available, these promising preliminary results illustrate the potential strength of suicide gene therapy for exploiting the upregulation of telomerase as a unique cancer marker in a broad spectrum of malignancies.

Telomere-Based Therapeutic Strategies

Telomere targeting efforts have sought to interfere directly with the telomeric sequences themselves rather than with telomerase. Telomere-based approaches have the potential benefit of selective toxicity to cancer cells, which often possess telomeres that are already much shorter than benign cells. Moreover, telomere-based approaches may prove beneficial even in the small but significant proportion of cancers (eg, sarcomas) that rely on alternative lengthening of telomeres rather than on telomerase for their sustained proliferation.

G-quadruplex Stabilizers The G-rich (TTAGGG) single stranded 3' overhangs of telomeres have been observed to form intramolecular 4-stranded ribbon-like structures termed G-quadruplexes.¹⁰¹ Such structures, when stabilized by small molecular compounds, prevent access of telomerase to telomeres, thus inhibiting the canonical telomere lengthening and capping process. To exploit the potential telomeric uncapping and shortening of the G-quadruplex phenomenon, several G-quadruplex stabilizing agents, such as TMPyP4,¹⁰² RHPS4,¹⁰³ BRACO-19,^{104,105} and telomestatin,^{106,107} were developed and shown to exert significant antitumor efficacy both in vitro and in vivo. Studies of RHPS4 and telomestatin have demonstrated displacement of shelterin components (Pot1 and TRF2) from telomeres associated with the uncapping DNA damage response.¹⁰⁸⁻¹¹¹ However, to date, no telomere-specific G-quadruplex agents have entered clinical trials, although some (eg, telomestatin) are expected to enter phase I testing shortly. One general concern about this class of agents is a potential lack of specificity, because the G-quadruplex structure is not unique to telomeres and is shared by other genomic entities such as the c-MYC promoter, the VEGF promoter, and guanine-rich genomic sequences.¹¹²⁻¹¹⁴ Accordingly, it is worth noting that the only G-quadruplex-targeting agent to have entered phase I/II clinical testing—CX-3543 (Quarfloxin, Cylene Pharmaceuticals)—in fact does not target telomeric G-quadruplexes, but

rather is designed to disrupt nucleolin/rDNA G-quadruplex complexes. Preclinical studies of CX-3543 showed anticancer efficacy that was not associated with altered telomere biology,¹¹⁵ but rather with disruption of nucleolin-rDNA interaction and inhibition of rRNA biosynthesis.

Telomeric Oligonucleotides In an effort to mimic the telomeric uncapping and DNA damage induced by telomerase interference (MT-hTer) and by G-quadruplex stabilizers, investigators have attempted to treat cancer cells with DNA oligonucleotides homologous to the uncapped TTAGGG telomeric repeats (Figure 2F).^{116,117} Introduction of so-called T-oligos directly into cancer cells has induced apoptosis, autophagy, and senescence, both in vitro and in vivo.¹¹⁸⁻¹²¹ Although these inhibitory effects were shown to be more pronounced in cancer than in benign cells, their degree of cancer specificity is the subject of continuing investigation, and they have not yet advanced into the clinical trial arena.

Telomerase and Cancer Stem Cells

Cancer Stem Cells: A New Cancer Paradigm?

It has been proposed that tumor formation and dissemination may be caused by cancer stem cells (CSC)—pluripotent cells with the capacity to differentiate and give rise to entire new tumors—much the same as normal tissue stem cells are able to differentiate and regenerate normal tissues (Figure 3). CSC are characterized by a relatively long life span, activation of pathways necessary for self-renewal (eg, Wnt/ β -catenin, Notch, Shh, BMI1), relative resistance to standard chemotherapy, and high tumorigenicity relative to unselected tumor cells. CSC subpopulations were first isolated in acute myeloid leukemia, followed by breast cancer and glioblastoma, and more recently reported in prostate, pancreatic, colon, and bladder cancers.¹²²⁻¹²⁷ Their resistance to therapy is attributed to high expression of ATP-binding cassette (ABC) drug transporters (eg, ABCG2, ABCB1), which can actively pump out many chemotherapeutic agents.¹²⁸⁻¹³² Given these properties, CSC have become an important therapeutic target, and their biology and role in cancer progression are under intense investigation.

Recently, it has been postulated that CSC are not a static “seed population” as traditionally assumed, but rather a dynamic phenotype that can be displayed by any cancer cell given the right stimulus.^{126,133,134} In support of this hypothesis, it has been shown in noncancer models that differentiated cells can be reprogrammed into a so-called “induced progenitor state” (iPS) by ectopic overexpression of several genes.^{135,136} Similarly in cancer models, differentiated cancer cells induced with cytokines to undergo epithelial to mesenchymal transformation

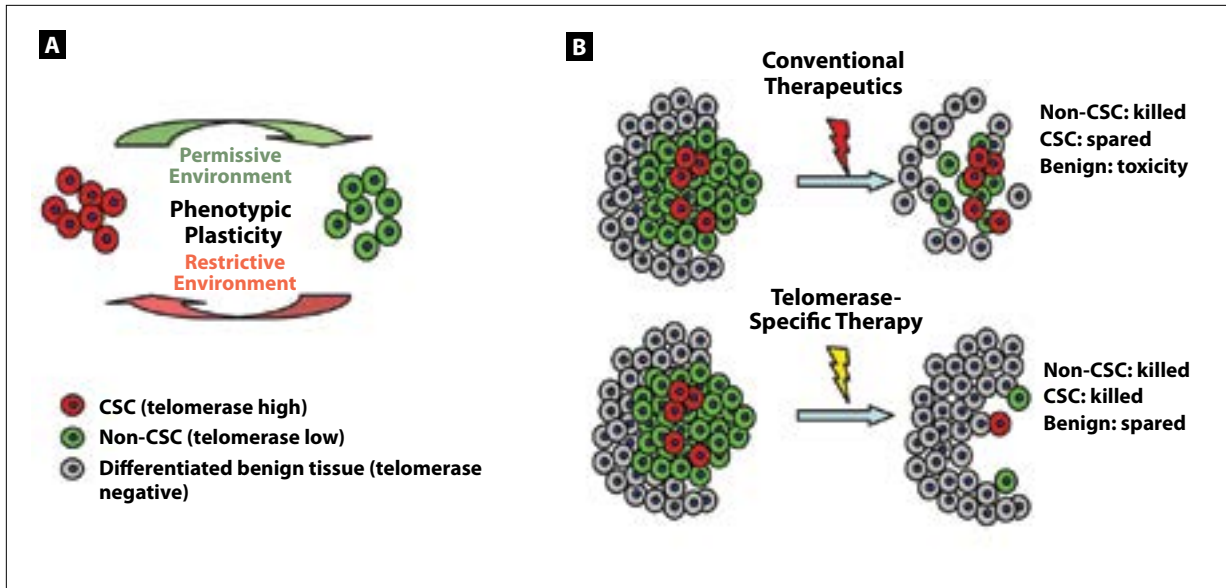


Figure 3. Cancer stem cells (CSC) and telomerase. A) A high degree of phenotypic plasticity in some cancer models whereby cancer cells may alternately lose or re-acquire cancer stem-like features. B) Potential benefit of telomerase targeting as a therapeutic strategy aimed at cancer stem cells.

(EMT) take on a phenotype with many similarities to CSC.⁸² In other studies, cells with CSC features (drug resistance, high tumorigenicity) were able to emerge de novo under certain conditions from cancer cell populations initially lacking these phenotypes.¹³⁷⁻¹³⁹ In 2 recent reports, this plasticity was associated with expression of the H3K4 demethylases JARID1A and JARID1B,^{140,141} raising the intriguing possibility of epigenetic regulation of the CSC phenotype. Our group investigated when and how CSC arise by characterizing and tracking CSC-like subpopulations in vitro and in vivo over time. Using flow cytometry with Hoechst dye and GFP labeling, we observed a dynamic 2-way equilibrium between the CSC-like and non-CSC-like subpopulations in cell culture and in tumor xenografts.¹⁴² Specifically, the highly tumorigenic, drug-resistant CSC-like subpopulations first became depleted by differentiation into non-CSC-like cells, and subsequently the CSC-like subpopulation was reconstituted by direct conversion of numerous non-CSC-like cells simultaneously back to the CSC-like phenotype. These transitions occurred spontaneously in the course of proliferation without exogenous selection pressures or separation into constituent subpopulations. Our findings demonstrated that intact cancer cell lines exhibit continuous, spontaneous plasticity, whereby large numbers of cells lose and subsequently regain a drug-resistant highly tumorigenic CSC-like phenotype in a cyclical manner. Our findings reaffirmed the possibility that CSC do not represent a static, progenitor seed

population, but rather a transient phenotype, which the bulk of cancer cells can reacquire, perhaps in response to specific environmental stimuli encountered during proliferation (Figure 3A).

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The discovery of CSC and the recognition of their potential role in cancer formation and progression have prompted a broad search for therapies capable of targeting this cancer phenotype.^{125,126,143} Intriguingly, telomerase targeting may offer this therapeutic benefit. Traditionally, telomerase activity has been considered a nearly universal characteristic of cancer cells, an assumption that may exist because early surveys of telomerase activity were conducted indiscriminately from lysates of entire cancer populations,⁶⁰ and because the oncogenic role of telomerase was demonstrated by ectopically introducing the enzyme into unselected cell populations.^{33,144} Contrary to this model of homogeneous telomerase activation, studies of normal tissue stem cell compartments have demonstrated a unique role for telomerase in stem cell activation,^{145,146} raising the possibility that perhaps telomerase plays a parallel unique role in CSC. In support of such a role, ectopic overexpression of telomerase in cancer cell lines has indeed been shown to enhance tumor initiation, perhaps reflecting a potentiation of the CSC phenotype.^{147,148}

Our group has investigated the relative telomerase activity and expression levels within CSC and non-CSC subpopulations isolated (using flow cytometry)

from freshly resected human prostate tumors and from prostate cancer cell lines. Remarkably, both in tumors and cell lines, CSC possessed markedly elevated levels of telomerase expression and activity compared with non-CSC. Moreover, induction of telomerase interference via ectopic expression of MT-hTer/siRNA (described earlier) effectively reprogrammed the active telomerase of prostate CSC to induce rapid apoptosis and abrogate tumor initiation.¹⁴⁹ Hence, these results demonstrated that telomerase expression and activity may not be a uniform phenotype common to all cancer cells, but rather may be concentrated in a subpopulation of cells with CSC-like properties, which in turn renders these cells exceedingly susceptible to telomerase interference. Our observations were consistent with findings from a handful of other recent studies. Elevated telomerase activity was observed in CSC-like subpopulations in breast and lung cancers,^{150,151} and the telomerase inhibitor imetelstat was shown to effectively inhibit the proliferation of CSC-like cells in prostate cancer and glioblastoma models.^{152,153} Collectively, these studies suggest that targeting telomerase may constitute a promising new therapeutic strategy for neutralizing CSC (Figure 3B).

Conclusion

The unique role of telomeres and telomerase in cellular immortality and carcinogenesis has spurred an entire new field driven by the promise of potent cancer therapeutics with broad efficacy and minimal toxicity. Currently, a broad array of approaches is being pursued, each marked by its own unique balance of clinical potential and technical challenges. The recent focus on CSC and their role in tumor formation, therapy resistance, and cancer progression, have recast telomerase therapeutics in a new light. Preliminary studies of CSC subpopulations have demonstrated that, much like normal stem cells, these cancer cells do have high levels of telomerase expression and activity that may constitute an “Achilles heel” for neutralizing these cells. Moreover, intriguing new insights into cancer stem cell and telomerase biology suggest a greater degree of plasticity than previously realized. Cancer cells may have the capacity to transition in and out of the cancer stem cell phenotype, and such phenotypic changes may in fact be facilitated by a noncanonical signaling role only now being discovered for telomerase. These previously unsuspected roles for telomerase in cancer offer entirely new mechanisms and therapeutic possibilities, and reinvigorate our continuing efforts to develop telomere- and telomerase-based cures.

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