Telomerase therapeutics for cancer: challenges and new directions

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Abstract | It has been approximately a decade since telomerase was described as an almost universal marker for human cancer. Most human tumours not only express telomerase but also have very short telomeres, whereas telomerase activity is either reduced or absent in normal tissues, making the inhibition of telomerase an attractive target for cancer therapeutics. Here we review the current status of telomerase therapeutics and discuss future opportunities and challenges for telomerase research, including a possible relationship with cancer stem cells that could be a source of chemo-/radioresistance development in many advanced cancers.

Replicative senescence
The process by which most normal human cells 'count' the number of times they have divided, eventually undergoing an irreversible growth arrest due to telomere shortening on a few chromosome ends.

Mitotic catastrophe
A response to abnormal mitotic DNA damage, leading to cell death. Normal cells avoid mitotic catastrophe by activating different cell-cycle checkpoint genes, which allows cells to repair the damage before mitosis; this mechanism is absent in checkpoint-deficient cells with critically shortened telomeres.

Telomerase reverse transcriptase
(hTERT). The catalytic subunit of telomerase (an RNA-dependent DNA polymerase) that synthesizes telomeric repeats onto the end of telomeres using the integral RNA (hTR) component as a template.

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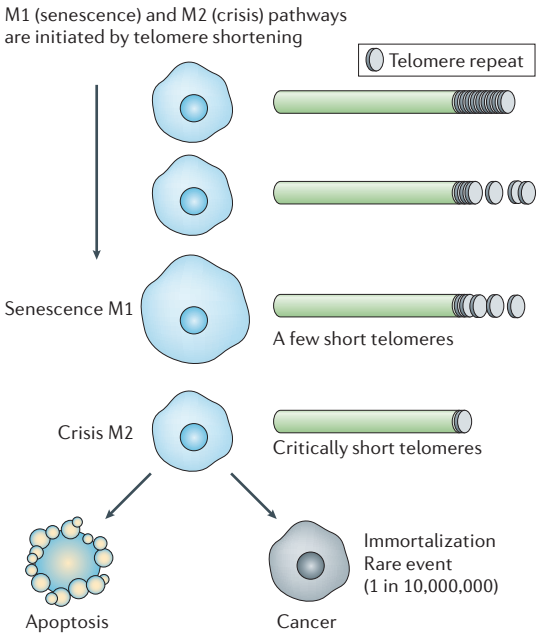
Telomeres are tracts of repetitive DNA (TTAGGG/AATCCC for human telomeres) that protect chromosomes from degradation and loss of essential genes, and allow the cell to distinguish between double-strand breaks and natural chromosome ends. Human telomeres at birth contain 15–20-kilobase pairs of the repetitive sequence TTAGGG[1–2] followed by a 3′ single-strand overhang on the G-rich strand, which is believed to be inserted within the double-stranded region to give a lariat-like structure called a t-loop[3–4]. Telomeres progressively shorten in most human cells with increased age, and telomere length in almost all middle-aged human tissues is approximately half that of the new born length. Telomere-specific proteins (such as protection of telomeres-1 (POT1), telomeric repeat-binding factor-1 (TRF1) and TRF2) bind directly to the single- and double-strand telomere regions to form a complex, providing a cap over the ends of the chromosomes that protects chromosome termini from degradation, recombination and end-joining reactions[2–5].

Functional telomeres are essential for continued cell proliferation. As a result of the incomplete replication of lagging-strand DNA synthesis and other end-processing events, telomeres progressively shorten in all somatic cells with each cell division[2,5]. When telomeres become short, cells usually undergo replicative senescence (mortality stage 1 (M1)) (BOX 1)[6]. In addition to progressive telomere shortening (leading to replicative senescence), telomere dysfunction can be caused by a change of state (uncapping) that leads to a rapid induction of growth arrest (involving end fusions and fusion–bridge–breakage cycles that can lead to mitotic catastrophe). Therefore when the telomeric DNA sequence or structure is altered, or telomere proteins are mutated or depleted, cells generally undergo chromosome end associations and fusions that lead to growth arrest or death.

Telomere length is maintained by a balance between processes that lengthen telomeres, such as the activity of the cellular ribonucleoprotein enzyme complex telomerase, and processes that shorten telomeres, such as incomplete synthesis of the lagging DNA strand and end-processing events. Telomerase stabilizes telomere length by adding TTAGGG repeats onto the telomeric ends of the chromosomes, thereby compensating for the continued erosion of telomeres that occurs in its absence[7–11]. Human telomerase contains two essential components, a telomerase reverse transcriptase catalytic subunit (hTERT)[8] and a functional telomerase RNA (hTR, also known as TERC)[7], as shown in FIG. 1. Telomerase is expressed in embryonic cells and in adult male germline cells[12], but is undetectable in normal somatic cells with the exception of the proliferative cells of renewal tissues[12–14]. In all normal somatic cells, even those with detectable telomerase activity, progressive telomere shortening is observed, eventually leading to greatly shortened telomeres and to limited replicative capacity. Introduction of hTERT into telomerase-silent cells is sufficient to reactivate telomerase, elongate or maintain telomeres, and to result in the bypass of both M1 and mortality stage 2 (M2) (BOX 1)[15]. Telomeres are therefore effectively molecular clocks that count the number of times a cell has divided, and determine when cellular senescence (M1) and crisis (M2) occurs[16–18]. In the absence of other genetic or epigenetic changes, telomeres can serve as a potent tumour-suppressor mechanism. Several human diseases of telomere dysfunction[19] have been discovered (BOX 2), and it is becoming clear that individuals born

Box 1 | **The two-stage M1/M2 model of senescence**

Expression of viral oncoproteins in human cells extends the cultured lifespan of normal cells, but does not directly immortalize the cells. Rather than entering a period of prolonged quiescence as do normal cells at the limit of their proliferative capacity (mortality stage 1 (M1)), cells expressing such viral proteins enter a state known as crisis (mortality stage 2, (M2)). Crisis occurs when cells enter a state such that the population size initially ceases to increase and the population cell growth is balanced by cell death/apoptosis. Occasionally, as a very rare event, an immortal cell emerges from crisis (see figure). The two-stage model of cellular senescence[6,79–81], in which M1 and M2 represent independent mechanisms limiting the capacity of normal cells to continue dividing, helps explain this behaviour. M1 (normal replicative senescence) occurs when a few short telomeres elicit a DNA-damage signal resulting in growth arrest[82]. However, the damage signal initiated by a few short telomeres at M1 can be ignored in cells that have inactivated important cell-cycle checkpoint genes, such as p53, that normally act to stop cell-cycle progression. If M1 is bypassed or abrogated, cells enter an extended period of proliferation and telomeres continue to shorten in the period between M1 and M2. When telomeres become so short that they fail to protect the ends of the chromosomes, the ends become ligated to produce dicentric chromosomes, with a consequent mitotic catastrophe at M2. As a very rare event (1 in 10^{-6} in epithelial cells and 1 in 10^{-7} in human fibroblasts), cells can escape M2, leading to an immortal cell and cancer cell progression. Both M1 and M2 can therefore be thought of as potent initial barriers to continued cell division (for example, a tumour-suppressor pathway[81]), even though at crisis the end fusions and ensuing chromosome rearrangements might in some instances contribute to the genomic instability that characterizes most cancer cells[83–85].



with reduced levels of telomerase have short telomeres that lead to telomere dysfunction in highly proliferative cells such as the bone marrow, resulting in diseases such as aplastic anaemia. This suggests that more detailed knowledge of telomerase and telomere function might provide insights into human diseases. Re-expression or up-regulation of telomerase is associated with telomere stabilization in tumour cells, making telomerase a rational target for cancer therapeutics. In this review, we describe the connection between telomerase activity and tumour maintenance, and evaluate progress in the development of different strategies to target telomerase in human tumours. Finally, we discuss recent results that suggest that targeting telomerase activity in cancer stem cells might provide additional benefit.

Telomerase and cancer

In contrast to normal cells, tumour cells generally have short telomere lengths and show no net loss of average telomere length with successive cell divisions, suggesting that telomere stability might be required for cells to escape from replicative senescence and proliferate indefinitely. Most, but not necessarily all, malignant tumours might need to become immortal to sustain their growth[20] and telomerase activity could therefore be a rate-limiting step required for the continuing proliferation of advanced cancers[21]. The telomere/telomerase hypothesis of ageing and cancer is based on the findings that most human tumours have telomerase activity whereas adjacent normal human somatic cells do not[22,23]. Therefore, a therapeutic window exists in which cancer cells can be efficiently targeted by telomerase inhibitors, while normal telomerase-expressing cells, such as stem and germline cells, remain unaffected as a result of their longer telomere lengths and slower rates of cell division (FIG. 2). Numerous approaches to target telomeres and telomerase activity have been described[24–43] (BOX 3) and there are several late-stage trials in the pipeline. In this review we provide an update on telomerase-specific therapeutics and describe only the most advanced therapeutic strategies in detail.

Immunotherapy: vaccines targeting telomerase

Even though the intricacies of telomere function have not been fully elucidated, the clinical application of antitelomerase therapies for cancer has been making rapid progress in the past 2–3 years. One of the most fully developed approaches relies on the observation that telomerase is over-expressed in nearly all cancers, making it a natural target for strategies that bolster the immune system to attack cancer cells, such as vaccines. Because the long-term growth of telomerase-expressing (hTERT-positive) tumour cells requires functionally active telomerase, hTERT is believed to be a prototypic, perhaps universal, immune target[44–49]. hTERT-specific epitopes are expressed on cancer cells but not on normal cells.

Investigators have stimulated an immune response to cancer cells in 26 unselected patients with advanced/metastatic lung cancer[48] using two different hTERT peptide fragments in an injectable vaccine (along with 30 μg of granulocyte–macrophage colony-stimulating factor (GM-CSF) to recruit antigen-presenting cells). The protocol gave weekly intra-dermal (paraumbilical area) vaccines for the first 10 weeks followed by monthly booster vaccines. The synthetic 16-amino-acid peptide GV1001 contained residues 611–626 (EARPALLTSRLRFIPK), whereas the 9-amino-acid peptide HR2822 contained residues 540–548 (ILAKFLHWL) of the hTERT protein. Each patient received a low dose of HR2822 (60 nM) and either a low (60 nM) or intermediate dose (300 nM) of GV1001. As a result of the initially advanced disease state of the patients only 14 patients completed the treatment protocol. Importantly, 12 of the 14 patients demonstrated an immune response[48].

In another dose-escalation study, 47 patients with newly diagnosed, histologically confirmed, non-resectable pancreatic cancer were included[50]. None of the patients received prior or concomitant chemotherapy. The peptide was injected intradermally 8 times over a period of 10 weeks and monthly booster vaccinations were

Telomerase RNA
(hTR/hTERC). The integral RNA that provides an 11-bp template complementary to the telomeric repeats to be added to the chromosome.

Crisis
A balance between cell growth and cell death. When cells bypass replicative senescence, telomeres continue to shorten, eventually leading to mitotic catastrophe. These cells die or, rarely, reactivate telomerase, leading to an immortalized cell line.

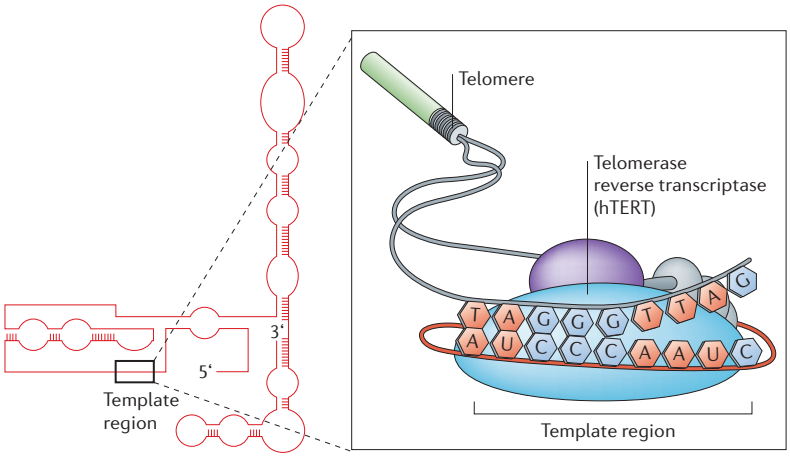


Figure 1 | **Telomerase components.** Human telomerase is a cellular reverse transcriptase. It is composed of two essential components: telomerase reverse transcriptase catalytic subunit (hTERT) and functional telomerase RNA (hTR), which serves as a template for the addition of telomeric repeats (left side). The maintenance of telomeres by telomerase is conserved in most eukaryotes, and although much is now known about telomerase biochemistry and biogenesis, and telomerase-associated proteins, many details about the higher-order structure, regulation and recruitment of telomerase to telomeres are still not resolved[88,89].

given thereafter. The study revealed a strong correlation between vaccine dose, number of responders and survival. Three of ten patients who received the low dose responded, compared with 13 out of 17 patients at the intermediate dose level. The median survival times of the two groups were 3.5 months for the low-dose group and 9.8 months for the intermediate-dose group[51]. These results demonstrate that immunity to hTERT can be generated safely and effectively in patients and encourages further trials of these therapeutics.

Importantly the results of immunotherapy trials to date have not revealed any serious adverse effects, with only a subset reporting flu-like symptoms consisting of mild episodes of fever and chills. There was no evidence of depression of stem cells in bone marrow, and no evidence of autoimmune disease in long-term survivors who received the monthly booster vaccines (some in excess of 2 years). These studies have helped to identify the best way to vaccinate and the optimal clinical setting for testing immunotherapy against hTERT. On the basis of these studies, GemVax AS (recently acquired by Pharmexa A/S), has moved the vaccine GV1001 into two Phase III studies that will begin in 2006 (Telovax and Primovax). It is expected that up to 1,800 patients will be enrolled in these trials. Because pancreatic cancer is such a lethal disease with minimal clinical options, orphan drug status will be filed, perhaps resulting in more rapid approval within a few years. In addition, a Phase II trial involving treating patients with hepatocellular carcinoma will be conducted in 2006.

The immunological and biological effects of vaccinating metastatic breast cancer patients and hormone-independent prostate cancer patients using a human leukocyte antigen (HLA)-A2-restricted hTERT peptide has been studied by another group[46,47]. In one study, 11 HLA-A2-positive women with metastatic breast cancer were vaccinated subcutaneously. As in the other trials, no serious adverse events were observed, including no evidence of bone-marrow toxicity. Grade 1 and 2 injection-site reactions were observed in most patients. Interestingly, some patients reported a syndrome of pain or itchiness at the site of metastatic tumour after three or four vaccines, and in one patient there seemed to be a clinical response. In summary, vaccination of metastatic breast cancer patients against hTERT induces hTERT-specific T cells that can be identified in peripheral blood and tumours without major toxicity. Tumour necrosis is observed after vaccination and dose-escalation results in an enhanced immunological response.

In a slight variation of the above trials, a third group primed potent antigen-presenting dendritic cells *ex vivo* with hTERT mRNA in an attempt to get the body's own immune system to attack cancer cells[49]. A Phase I safety trial, which enrolled 20 advanced prostate cancer patients, has been completed. All patients but one had strong telomerase-specific cellular immune responses and no patients had treatment-related side effects. Although this initial study was designed to study patient safety and dosing schemes, the preliminary clinical results were highly promising. Evidence suggestive of a clinical effect included a reduction of serum prostate-specific antigen (PSA) that suggests a slowdown of tumour progression. In this study there was a statistically significant prolongation of PSA doubling time associated with the presence of antitelomerase T cells[49].

The development of these promising approaches for a telomerase-based universal cancer vaccine is encouraging and might be even more effective when used to treat patients with less advanced disease. It is hoped that randomized Phase II and Phase III clinical trials will show objective responses in patients and continue to move this approach forward.

Oligonucleotide-based therapeutics

It has been suggested that the 11-base template region of telomerase RNA (hTR) should be an excellent target for direct enzymatic inhibition of telomerase activity[52]. Regardless of the conformation of the telomerase holoenzyme, the template region of hTR must be accessible to bind to the telomeric repeats, and therefore must be exposed to targeted oligonucleotides. The major challenge for this class of drugs is access and stability — how to get the oligonucleotides into the cell and then to the enzyme without being degraded by nucleases. Oligomers with modified oligonucleotides containing novel bond linkages have been reviewed recently[34–36]. In brief, these modified molecules bind RNA sequences with improved selectivity, enhanced efficacy and improved pharmacological properties. One such compound (GRN163L, a telomerase RNA (hTR) template antagonist agent[53–55]) has recently entered into Phase I/II clinical trials in patients with chronic lymphocytic leukaemia. The sequence 5′-Palm-TAGGGTTAGACAA-3′ is complementary to a 13-nucleotide-long region partially overlapping and extending by four nucleotides beyond the 5′-boundary of the template region of hTR[53–59].

Cancer stem cells
A small subset of tumour cells that can recreate and sustain (re-initiate, re-populate) the tumour in a functional transplant assay. It is believed that cancer stem cells have multilineage potential and might be responsible for the failure of current therapies.

Prostate-specific antigen
A serine protease in the kallikrein gene family that is secreted into seminal fluid by prostatic epithelial cells and found in the serum. As it is almost exclusively a product of prostate cells, measurement in blood has proved to be exceptionally useful as a tumour marker for diagnosis of prostate cancer and monitoring the effectiveness of treatment.

Box 2 | Human diseases associated with telomerase components

Individuals with shorter than age-matched control telomeres are prone to disease (reviewed in REF. 23). For example, patients with atherosclerotic heart disease have shorter telomeres compared with healthy aged-matched controls, although it is not yet established whether this is a cause or consequence of the disease state. Telomere length can also be a major independent predictor for overall mortality. Elderly patients with the shortest quartile of blood telomeres have a higher mortality due to infectious disease compared with those in the other quartiles. In dyskeratosis congenita (DKC) bone marrow failure or pulmonary complications are the major cause of death[86]. The X-linked form of DKC is due to mutations in the dyskerin gene, which is involved in processing the RNA component (hTR) of telomerase. X-linked DKC patients have reduced hTR levels (and therefore less telomerase activity). The autosomal dominant form of DKC generally occurs with a later age of onset, and is directly attributable to dysfunction of telomere maintenance because deletion or mutation in the *hTR* gene itself is observed. It is thought that haploinsufficiency can cause DKC (that is, when one copy of *hTR* is mutated, it produces cells having less hTR and therefore less telomerase activity to maintain proliferative stem-like cells). Disease anticipation (in which the onset of disease occurs at progressively younger ages in successive generations) occurs in autosomal dominant DKC families, and this correlates with progressive telomere shortening in successive generations. DKC patients have to inherit both short telomeres and be heterozygous for *hTR* (for example, *hTR*+/–) in order to show anticipation[86]. In another study examining patients with sporadic aplastic anaemia, mutations in the human telomerase reverse transcriptase catalytic subunit gene (*hTERT*) were sometimes found. Collectively, these studies lend support to the hypothesis that short telomeres correlate with disease. Showing cause and effect will require demonstrating that slowing down the rate of telomere loss or resetting the telomere clock reverses or delays the onset of disease.

This lipidated 13-mer *thio*-phosphoramidate targets the hTR component of telomerase, preventing it from forming an active complex with hTERT. In its current formulation, it does not require a lipid carrier, because the lipid palmitate moiety is built into the molecule. The GRN163L sequence is apparently unique in the human transcriptome, and shows greatly enhanced stability as well as extremely specific and high-affinity binding to telomerase, while the lipid modification on GRN163L significantly improves its potency and biodistribution. Importantly, duplexes with GRN163L are not substrates for RNase H hydrolysis and function as competitive enzymatic inhibitors, in contrast to standard antisense approaches that target messenger RNA.

Xenograft
Transplantation of tissue or cells from one species to another. In cancer research, most xenografts are human cancer cell lines or human tumours that have been transplanted to immune-deficient rodents.

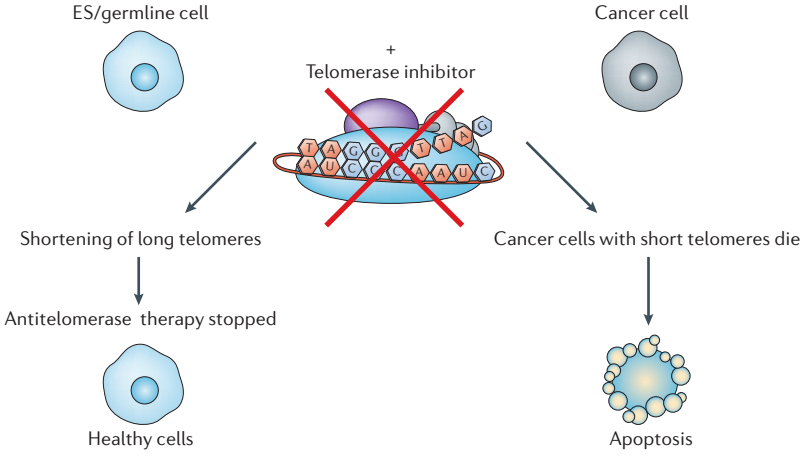


Figure 2 | **Comparing telomerase inhibition in normal versus cancer cells.** Proliferative male germline and embryonic stem (ES) cells fully maintain telomeres and express telomerase. Other proliferative stem cells that express telomerase progressively shorten telomeres and eventually undergo senescence. Cancer cells that escape from senescence express telomerase activity and almost universally have short telomeres. This means that there might be a therapeutic window to treat cancer cells with telomerase inhibitors without serious side effects to normal germline and stem cells.

In addition to inhibiting telomerase, GRN163L[34,53,54] and similar oligonucleotides[50] can cause off-target effects on cell shape/attachment in tumour cells. Perhaps related to this effect, preclinical experiments in xenograft models of human lung cancer showed that GRN163L rapidly reduced or prevented lung cancer metastasis at pharmacological doses[53]. This property increases the chance of therapeutic value with relatively low doses of the drug. The growing body of human xenograft efficacy data in rodents suggests that intermittent intravenous dosing of GRN163L should achieve therapeutic tissue levels of the drug in cancer patients, while maintaining an acceptable safety profile. In summary, GRN163L is one of the first generation of small-molecule telomerase inhibitors for the treatment of cancer. Ongoing clinical trial results should provide valuable information on risk (such as toxicity, which is expected to be low) versus benefit in humans, but current data suggest it has the potential to be a universal anticancer agent with minimal side effects.

Gene therapy: telomerase oncolytic virus

Vector-mediated approaches have been devised to kill telomerase-positive cancer cells. These include methods to block telomerase assembly, disrupt telomerase function, and to use telomerase promoter-driven genes or viruses to trigger suicide or cell death[60–68]. In addition, conditionally replicating oncolytic viruses offer a promising modality for cancer treatment. Oncolytic viruses (tumour-selective viruses that mediate oncolytic effects on tumours) are genetically modified viruses engineered to replicate in and kill targeted cancer cells. Among this novel group of therapeutics are oncolytic adenoviruses engineered with tumour- or tissue-specific transcriptional response elements that control essential genes. One new oncolytic virus, CG5757 (Cell Genesys), has demonstrated a high degree of specificity and effectiveness in xenograft models[34]. This virus was generated by replacing the *E1a* and *E1b* endogenous promoters with promoters derived from the human *E2F1* and the *hTERT* genes, respectively. The *E2F1* promoter is activated in retinoblastoma (Rb)-defective tumour types, a pathway mutated in ~85% of all cancers. Likewise, telomerase is aberrantly expressed in ~90% of tumours. CG5757 shows strong tumour selectivity and antitumour efficacy. *In vitro*, expression of *E1a* and *E1b* genes was restricted to Rb-defective and hTERT-positive cancer cells and the virus did not replicate in normal cells. CG5757 replicates similarly to wild-type virus in tumour cells, but its replication is, on average, 1,000-times less efficient than wild-type virus in normal cells. In a viral cytotoxicity assay, CG5757 destroys tumour cells 100–10,000-times more efficiently than normal cells. *In vivo*, strong antitumour activity was seen using CG5757 in nude mice injected with a variety of human cancer cell lines. In the bladder cancer 253J B-V mouse model, 4 weeks after treatment the average tumour volume in animals treated with 4 consecutive daily intratumoural injections

Box 3 | **Additional targets for telomerase inhibition**

In addition to the major approaches for telomerase inhibition that are currently in clinical trials and described in this review, there are several additional areas of investigation for targeting telomerase activity that are in various stages of preclinical testing[25–43].

An alternative to targeting telomerase is to target telomeres themselves. Several classes of small molecules have been described that stabilize the folding of the G-rich telomere strand into G-quadruplex structures. It is believed that such folding prevents telomerase from functioning at the telomere, but the effect of these compounds on normal cells has not been adequately studied. These molecules have rapid 'uncapping' effects on cells leading to growth arrest. One such molecule, BRACO19 (Antisoma), has been shown to be effective in xenograft tumour models and is entering clinical trials[27,30,40]. Another G-quadruplex ligand might affect telomerase via human telomerase reverse transcriptase catalytic subunit (hTERT) RNA alternative splicing[87].

Gene transfection of a mutated form of hTR results in the synthesis of mutant sequences at telomeres and a rapid growth arrest because the mutant sequence does not interact with telomere-binding proteins. resulting in rapid uncapping of telomeres. Again, effects on normal cells have not been adequately examined[43].

Heat-shock protein-90 (HSP90) inhibitors are in clinical trials for other purposes, and it has been shown that HSP90/p23 is required for telomerase assembly, suggesting these therapeutics might also affect telomerase as well as other targets[28,29].

Finally, there is evidence that some chemotherapeutic agents and specific types of radiation therapy preferentially affect telomeres, but these areas have not been well defined.

Agents that target telomeres have an additional burden of proof that is often not addressed in most primary reports. To be a cancer-specific therapeutic, the agent should have minimal toxicity on normal cells. Given that telomeres are present in both normal and cancer cells, agents that specifically target telomeres need to be shown to have a differential effect on normal proliferating cells and proliferating cancer cells. Even with this cautionary note, some advanced quadraplex stabilizers have been reported to demonstrate *in vivo* activity in mice bearing human tumour xenografts at less toxic doses[40]. This suggests that some telomere-specific compounds could potentially inactivate telomerase by sequestration or other mechanisms that are still not clearly understood.

of CG5757 (4×10^8 particles per mm^3 of tumour) decreased to 72% of baseline, whereas the control group had an increase to 944% of baseline. Furthermore, 50% of treated animals had complete regression of the 253J B-V tumour xenografts. These data demonstrate the potential therapeutic efficacy of such dual-promoter-controlled oncolytic adenoviruses in cancers that are Rb-defective and hTERT (telomerase)-positive.

Combination therapy

As depicted in FIG. 3a, most, but not necessarily all, anti-telomerase therapies might require a period of time to drive already short telomeres into a state of crisis and apoptotic cell death. During this treatment period, telomeres would gradually shorten but the tumour mass would also continue to increase. A telomerase inhibitor might therefore not be effective by itself, because cell death would only occur after many divisions had taken place. In contrast, current chemotherapy approaches result in an immediate reduction in tumour burden (FIG. 3b), but, even with continued treatments, tumour relapse and therapy resistance occurs. As outlined below, many believe that this could be due to resistance of cancer stem cells in the mixed population of cancer cells. In addition, current chemotherapy approaches would not be predicted to directly affect telomere length. As outlined above, using small-molecule telomerase inhibitors such as GRN163L in combination with telomerase vaccines or oncolytic viruses could eventually be important. Using conventional therapies (standard chemotherapy/radiation therapy) to produce a setting of minimal residual disease, and combining this approach with single or combination telomerase inhibitors, could improve overall survival or at least delay the time to tumour regrowth (FIG. 3c). After initial dose-escalation and toxicity trials, telomerase inhibitors are therefore likely to be administered as an adjuvant therapy in combination with standard therapeutic regimens. An important challenge for basic and preclinical work will be to develop the best strategies to design more effective clinical trials. Knowledge of the factors influencing the recruitment to, or action of, telomerase on telomeres is increasing, and there is evidence that inhibiting 'recruitment' enhances the efficacy of telomerase inhibitors[13,17]. It is important to develop combination therapies to best enhance the erosion of telomeres to cause a more rapid decrease in cancer cell proliferation without affecting normal cell telomeres.

New directions — cancer stem cells

A major problem associated with current cancer therapeutics is the eventual development of resistance in residual cells that remain after the bulk of the tumour cells have been destroyed. New ideas and approaches are needed to treat human metastatic disease that is essentially incurable with today's therapeutics. It is becoming more generally accepted that many cancers can be sustained by a small subset of rare cells, called cancer stem cells (CSCs)[69–76]. CSCs are a biologically and molecularly distinct cell type within a tumour mass. A CSC is most likely an immortal, largely quiescent (with lower rates of cell division), pluripotent cell type that is capable of self-renewal and the capacity to give rise to phenotypic and functional tumour heterogeneity. Although the bulk of cancer masses display the phenotypic characteristics that are frequently reported in gene expression and proteomics analyses, it is entirely possible that scientists have missed characterizing the much smaller subset of CSCs. As such, CSCs might possess properties that, if more fully understood, would permit the rational design of novel therapeutic approaches for cancer. Studies have suggested that more differentiated cancer cells might not give rise to tumours when depleted of the CSC compartment, and therefore the main population of cancer cells might not be responsible for resistance to treatment[69,71,72]. Given that CSCs are believed to be quite rare (estimated to be perhaps 1 in 1,500 cells in primary human breast carcinomas[51] and 10^{-4} or 10^{-5} in haematological malignancies[69,71]), it could be proposed that standard cancer therapies might not be targeting CSCs. There are a variety of markers and techniques to isolate these rare CSCs[74–76] and in primary breast cancer it has been demonstrated that they are telomerase positive[76]. The telomerase inhibitors being developed might therefore target CSCs as well as the more mature cancer cells. At present, it is not known whether the regulation of telomerase in CSCs is the same or different from that of more differentiated cancer cells or normal stem cells in renewal tissues.

In contrast to studies that have suggested an up-regulation of telomerase in tumour cells, a recent report has demonstrated that, in comparison with haematopoietic stem cells from normal individuals, hTERT was downregulated in the haematopoietic ($CD34^+$) stem cells of patients with chronic myeloid leukaemia (CML)[77]. One possible explanation of these findings, suggested by the authors of the study, is that the downregulation of c-MYC (a hTERT transcriptional activator), in the presence of the BCR–ABL fusion product present in CML, causes haematopoietic CSCs to cycle slightly slower. This could lead to slowly proliferating stem cells with lower telomerase activity and shorter telomeres than normal stem cells, potentially leading to increased genomic instability and disease progression. These observations would be consistent with the popular notion that stem cells enter the cell cycle less frequently than the majority of tumour cells, and that both normal and cancer stem cells might therefore be predicted to be enriched for cells that retain labels such as BrdU owing to their slow rate of division. An alternative viewpoint is that, when measuring populations of cells based on one marker (such as CD34), although the vast majority of the cells, in the presence of potentially lower levels of telomerase, are gradually shortening their telomeres and are not able to maintain their proliferative capacity, there could exist a rare (yet to be identified) subset of CD34+ haematopoietic CSCs that have high levels of telomerase and that are responsible for tumour maintenance. However, even if the first scenario is correct and the rare cancer stem cell is perhaps more quiescent compared with the bulk of the tumour cells, it can be predicted that, if the CSCs express telomerase, then telomerase antagonists would not only target these cells but could potentially shift these cells out of their stem compartment (niche) making them more vulnerable to standard therapeutic approaches.

With mounting evidence that CSCs are telomerase-positive, one would predict that combining standard chemotherapy with telomerase inhibitors would drive telomeres to shorten in a setting of minimal residual disease and also target CSCs (FIG. 3c). Even if CSCs are more quiescent than the bulk of the tumour cells, eventually they have to divide to maintain the tumour. If a telomerase inhibitor is present when a CSC divides, then telomeres should shorten and perhaps even shift the entire pool into the more differentiated state.

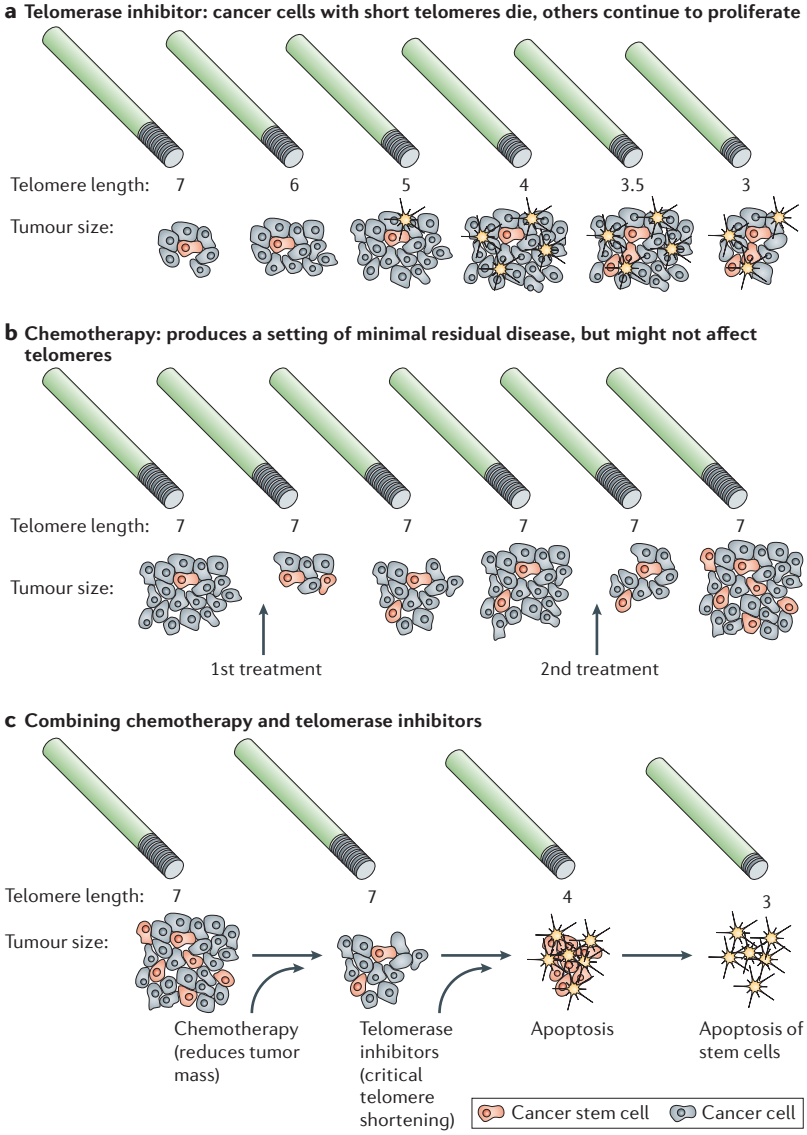


Figure 3 | **Predicted outcomes of telomerase therapy. a** | Inhibition of telomerase would not usually be predicted to have an immediate effect on tumour size. Although telomeres progressively shorten in the presence of a telomerase inhibitor, tumour size would only start decreasing once some telomeres in the cancer cell population became short enough to cause increased DNA-damage signalling or end-fusions. **b** | Chemotherapy treatment of cancer cells reduces tumour size but does not affect telomere length; residual tumour cells therefore continue to grow and can become resistant to therapy. **c** | Combining chemotherapy with telomerase therapy would be predicted to both shorten telomeres and reduce tumour burden. Even if rare cancer stem cells are quiescent, eventually they will have to proliferate to maintain the growth of the tumour. The presence of a non-toxic or minimally toxic dose of a telomerase inhibitor should then affect the telomerase-positive cancer stem cells and eventually lead to apoptosis.

Conclusions and perspectives

There have been many recent significant developments in the telomere/telomerase fields of research, but there are still many gaps in our understanding. More preclinical proof-of-efficacy studies and additional clinical trials are required. The progress made in the past 2 years has been impressive and there is an emerging general consensus that telomerase-targeted therapies are a promising and novel approach to cancer therapeutics that could lead to effective interventions for the treatment of cancer with minimal side effects. Although one can always make arguments for and against any novel cancer therapeutic, the preclinical and emerging clinical experimental evidence for telomerase as a relatively universal target for cancer therapy is encouraging, and targeting telomere-maintenance mechanisms continues to be an exciting prospect in our repertoire of future cancer strategies. However, many gaps remain in our understanding of the complexities of the expression and regulation of telomerase and telomere length control in normal human cells[78]. Importantly, we need to establish how ageing (and cellular replicative senescence) contribute to actual human physiology and how its dysregulation can contribute to cancer progression.

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Competing interests statement
The authors declare competing financial interests: see Web version for details.

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