Telomerase: A target for cancer therapeutics

Jerry W. Shay[1] and Woodring E. Wright

The University of Texas Southwestern Medical Center at Dallas, 5323 Harry Hines Boulevard, Dallas, Texas 75309
[1]Correspondence: jerry.shay@utsouthwestern.edu

The continuous growth of advanced malignancies almost universally correlates with the reactivation of telomerase. While there is still a great deal of basic and applied research to be done, telomerase remains a very attractive novel target for cancer therapeutics. In this review, we will discuss the challenges and the pros and cons of the most promising antitelomerase approaches currently being investigated.

Introduction: Telomeres, telomerase, and cancer

The ends of linear eukaryotic chromosomes contain specialized structures called telomeres (reviewed in McEachern et al., 2000; Collins, 2000). Human telomeres consist of tandem arrays of the hexameric sequence TTAGGG, with overall telomere sizes ranging from about 15–20 kbp at birth to sometimes less than 5 kbp in chronic disease states (Aisner et al., 2002; Forsyth et al., 2002; Collins, 2000; Kakuo et al., 1999; Brummendorf et al., 2000, 2001; Boultwood et al., 1997). The telomeric repeats help maintain chromosomal integrity and provide a buffer of expendable DNA (Blackburn, 2000; Fajkus et al., 2002). The ends of telomeres are protected and regulated by telomere binding proteins and form a t loop structure (Griffith et al., 1999). This packaging is thought to mask telomeres from being recognized as broken or damaged DNA (Van Steensel et al., 1998; Smogorzewska et al., 2000; de Lange, 2002). The inability of DNA polymerase to replicate the end of the chromosome during lagging strand synthesis ("end replication problem"), coupled with various possible processing events, results in the loss of telomeric repeats each time a cell divides. Cells that lack a compensatory mechanism to counteract this gradual loss exhibit progressive telomere shortening. This eventually results in a growth arrest (called replicative senescence) that is thought to occur when one or more critically short telomeres trigger a p53 (and perhaps RB) -regulated DNA damage response (Sherr and McCormick, 2002; Smogorzewska and de Lange, 2002). Human cells can temporarily bypass this growth arrest when RB and p53 are disabled (Wright et al., 1989; Shay et al., 1991a, 1991b; Counter et al., 1992), but ultimately so many telomeres become critically shortened that multiple chromosome end fusions occur, resulting in loss of cell viability ("crisis").

Telomeres provide a barrier to cancer progression by preventing immortalization. Replicative senescence is postulated to be a tumor-protective mechanism in vivo, where the number of cell divisions required for the accumulation of multiple mutations would erode existing telomeres as tumorigenesis progressed, eventually driving premalignant cells into senescence (Wright and Shay, 2000). However, persuasive evidence that this occurs in vivo remains elusive (Stewart and Weinberg, 2002). The ability to bypass replicative senescence by circumventing telomere-based growth limitations is thought to be one critical rate-limiting step in the evolution of most malignancies (Harley, 1991; Shay et al., 1991b). Clinically significant tumors must have a mechanism for telomere maintenance to have the unlimited proliferative capacity that appears to be required for most advanced cancers. The almost universal path to overcoming the telomere mitotic clock is the reactivation or upregulation of telomerase (Kim et al., 1994; Shay and Bacchetti, 1997). Telomerase is a cellular ribonucleoprotein enzyme responsible for adding TTAGGG repeats onto the 3′ ends of chromosomes (Greider and Blackburn, 1985; Morin, 1989; Collins and Mitchell, 2002; Lingner and Cech, 1998; Nugent and Lundblad, 1998). It has two major components: the enzymatic reverse transcriptase catalytic subunit hTERT (Nakamura et al., 1997) and the integral RNA component (hTR or hTERC) that provides an 11 bp template encoding the telomeric repeats to be added to the chromosome (Feng et al., 1995). The technically correct nomenclature for the RNA component, hTERC, is easily confused when spoken with hTERT, the catalytic protein component, so most scientists have adopted the use of hTR to designate the functional or template RNA component. Direct evidence linking telomerase to replicative senescence was demonstrated (Bodnar et al., 1998) by producing telomerase activity in telomerase-negative cells following the introduction of only the hTERT component. This resulted in telomere length stabilization and the direct immortalization of cells without oncogenic transformation (Morales et al., 1999; Harley, 2002) as long as cells are cultured in adequate conditions (Ramirez et al., 2001).

Concerns about telomeres and telomerase as anticancer targets

There are several potential concerns that have been raised about telomerase as an anticancer target. First, with many (but not all) telomerase therapeutic approaches, one expects there will be a lag phase between the time telomerase is inhibited and the time telomeres of the cancer cells will have shortened sufficiently to produce detrimental effects on cellular proliferation. If this is correct, it would suggest that telomerase inhibitors might be most effective in combinations with other conventional or experimental cancer treatments (Figure 1) or in a setting of minimal residual disease (White et al., 2001). It is possible that in primary human tumors there will be some very short telomeres in most cells and that the lag phase before telomerase inhibitors produce proliferative deficiencies in vivo may be more rapid than that observed in tumor cell line preclinical models.

Second, while telomerase is not detected in most normal tissues (Wright et al., 1996; Forsyth et al., 2002), inhibitors of telomerase would potentially have detrimental effects on those human cells that do express telomerase, such as hematopoetic progenitor cells, germline cells, and other cells of the renewal tissues such as the epidermis and intestinal crypts (Forsyth et al., 2002). Although so-called transient amplifying cells divide rapidly, the deeper stem cells from which they derive only rarely enter the proliferative compartment (Mahmud et al., 2001).

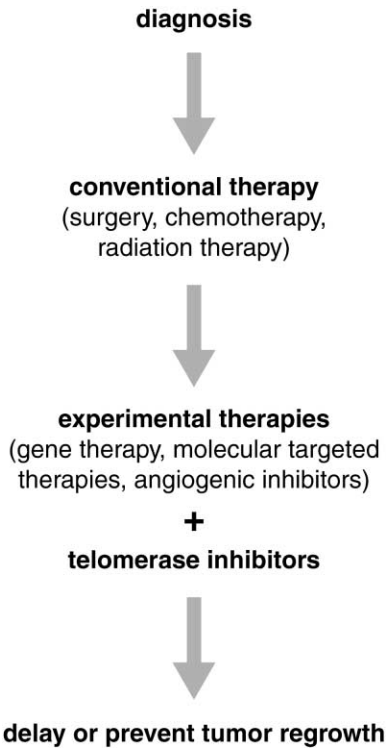

Figure 1. Theoretical approach to managing the care of cancer patients with combinations of conventional and experimental therapies, including telomerase inhibitors

During the time that these stem cells are quiescent, telomerase activity is negligible (Forsyth et al., 2002). Telomerase inhibitor effects on stem cells may thus be minor because these telomerase-competent cells only proliferate intermittently and typically have much longer telomeres than cancer cells. In addition, although telomerase-specific inhibitors would shorten the telomeres in the proliferating transient amplifying stem cells, it would not cause their immediate death; thus, typical therapeutic side effects such as hair loss or nausea are unlikely to be major immediate side effects of antitelomerase cancer therapy.

A third issue that has been raised about telomerase inhibitors is that alternative mechanisms for telomere maintenance have been reported in other organisms, in experimentally derived human immortalized cell lines, and in some rare human cancers (Lundblad, 2002; Lundblad and Blackburn, 1993; Bryan et al., 1995, 1997; Bryan and Reddel, 1997; Henson et al., 2002). Telomerase inhibitors might thus result in the emergence of drug-resistant telomerase-independent cancer cells. While this is certainly a possibility, there have been no published reports of telomerase-positive human tumor cells being experimentally converted to a telomerase-independent pathway using telomerase inhibitors. However, the lack of ALT activation in the setting of telomerase inhibition in cell culture could relate to the inability of cell culture to fully recapitulate what happens in vivo. In addition, some people believe that advanced human malignancies may contain rare telomerase-independent variants. With the long-term use of telomerase-specific inhibitors, these cells may have a selective growth advantage.

A final concern is that telomerase inhibitors could lead to increased genomic instability in surviving cells (Hackett and Greider, 2002; Harrington and Robinson, 2002), leading to more advanced or aggressive tumors. While there is some experimental animal model evidence in support of this, especially in p53-deficient tumors (Chin et al., 1999; Gonzalez-Suarez et al., 2001), most of the experiments treating human cancer cell lines with telomerase inhibitors and experimental models producing progressively shortened telomeres indicate that these approaches generally lead to a less tumorigenic phenotype even, in some instances, in the absence of normal p53 function (Herbert et al., 1999; Zhang et al., 1999; Kim et al., 2001; Boklan et al., 2002; Gonzalez-Suarez et al., 2000; Greenberg et al., 1999; de Lange and Jacks, 1999; Franco et al., 2002; Hahn et al., 1999; Goytisolo and Blasco, 2002).

Telomerase is unusual among cancer molecular targets because a large body of outstanding basic science in telomere biology has preceded development of effective lead compounds, allowing potential problems to be anticipated before evidence of efficacy in model systems is in hand, exactly the opposite of the situation faced during most drug development. Even though telomerase does not cause cancer and its role in cancer is most probably permissive, cancer therapy directed at telomerase has advanced in some instances to clinical trials to validate safety and specificity (Vonderheide, 2002). While there are many potential ways to interfere with normal telomerase function, only a few of the most promising approaches in human preclinical and clinical trials will be described in this review. For information on mouse models of telomere function and the connection between chromosomes, crisis, and cancer, the reader is referred to a recent review (Maser and DePinho, 2002). Other areas of telomerase inhibition (such as downregulating the hTR and hTERT genes at the promoter level, the use of a dominant-negative hTERT gene delivery [Hahn et al., 1999; Zhang et al., 1999], inhibition of telomerase assembly [e.g., interfering with p23 hsp 90 (Holt et al., 1999)], telomerase-specific phosphorylation inhibitors, blocking telomerase accessibility [G-quadraplex stabilizers (Riou et al., 2002; Gowan et al., 2002; Sun et al., 1997; Read et al., 1999)], hammerhead ribozymes directed against hTR [Yokoyama et al., 1998], mutant template RNA gene therapy [Kim et al., 2001], and reverse transcriptase inhibitor approaches [Murakami et al., 1998; Strahl and Blackburn, 1996; Gomez et al., 1998]) will not be covered and the reader is referred to the original reports or other recent reviews (Pathak et al., 2002; Hodes, 2001; Folini et al., 2000; Kelland, 2001; Helder et al., 2002; Granger et al., 2002; McKenzie et al., 1999; Neidle and Parkinson, 2002). In this review, we will consider four areas: a gene therapy approach that uses the proximal hTERT promoter to make a general cancer-specific oncolytic virus; a telomerase-specific immunotherapy; the use of telomerase template antagonists; and the potential role of telomerase inhibitors as chemopreventive agents. Cancer therapy directed at frequently occurring molecular alterations in cancer (Hanahan and Weinberg, 2000) are starting to show initial successes in clinical trials (Shawyer et al., 2002). We anticipate that after a new wave of target-specific drugs (including those that inhibit telomerase) are developed in early-phase clinical trials, that combination of treatments (Figure 1) will lead to prolonged periods of cancer remission and overall better cancer patient management.

Telomerase-specific oncolytic virus

The use of tissue- or cell-specific promoters for targeting cancer cells, while appealing, has some limitations, such as lack of

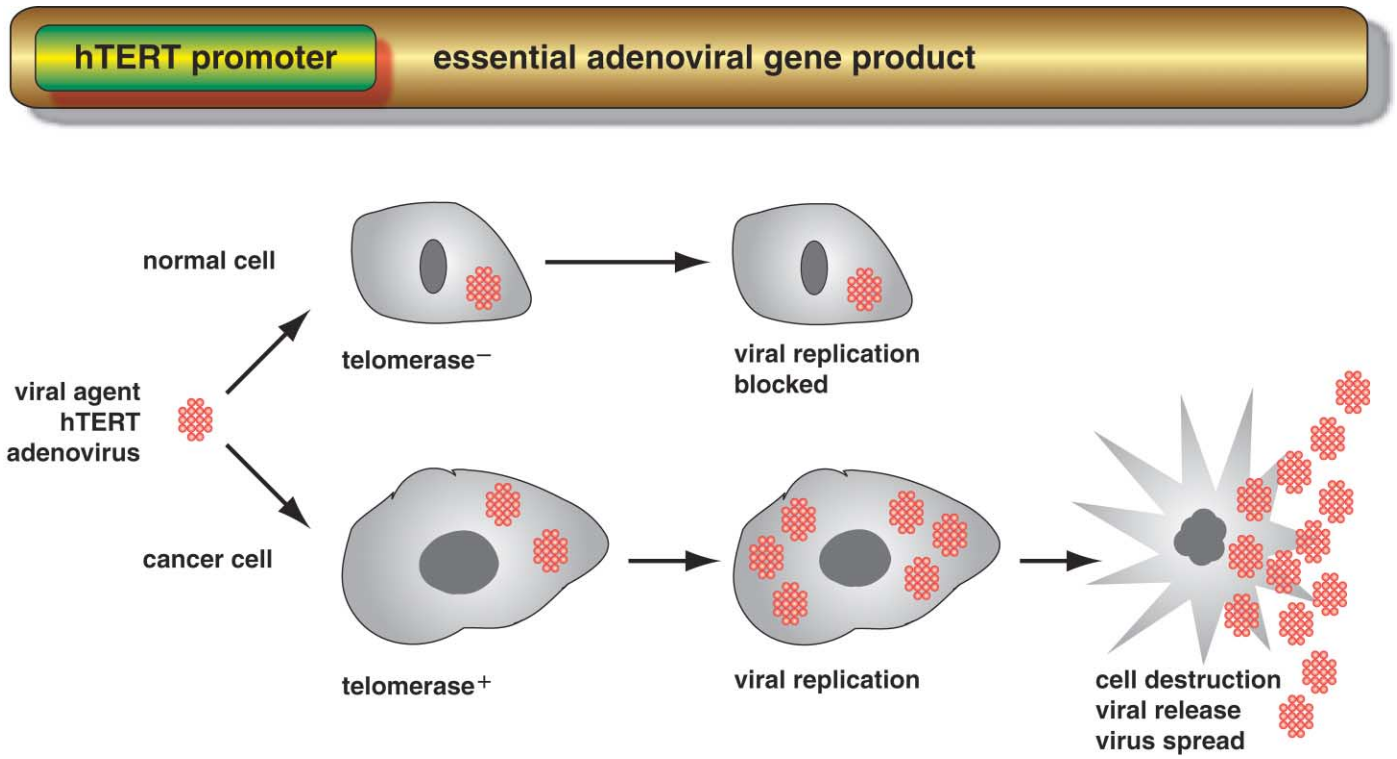

Figure 2. Telomerase promoter-dependent adenoviral therapy

The systemic use of the hTERT proximal promoter in a replication-competent adenoviral should have minimal effects on normal cells that do not express telomerase activity. Cancer cells expressing telomerase activity transcriptionally activate the hTERT promoter, permitting adenoviral replication, lysis, and spread to adjacent cells.

expression in diverse tumor types and toxic effects on normal cells expressing the promoter. The hTERT proximal promoter (from –1 to about –200 to –400) can be used to produce a more universal gene therapy system, since almost all advanced human cancer cells express telomerase and most normal cells do not. Because the hTERT gene is regulated at the transcription level, only tumor cells expressing telomerase activity would activate the promoter. The approaches using "suicide gene strategies" described so far appear promising and include gene transfer via direct intratumor injections of plasmids or adenoviral vectors containing the human telomerase promoter upstream from proapoptotic genes such as the *FADD* gene (Koga et al., 2001), *Caspase 6* and *8* genes (Komata et al., 2001; Koga et al., 2000), and the *Bax* gene (Gu et al., 2001, 2002). The use of replication-deficient adenoviral vectors containing the hTERT promoter are limited as a cancer therapy, since most tumor cells will not be transduced and thus only a subset of cancer cells will be eliminated. However, there is recent progress using the telomerase hTERT promoter (hTERTp) to drive a *T*umor-specific *R*eplication competent *AD*enoviral (hTERTp-TRAD) gene therapy approach. In this approach (Figure 2), an introduced adenoviral vector could infect both normal and tumor cells, but the virus would only replicate in those cells that have robust telomerase activity. Thus, the virus would replicate and eventually lyse the telomerase-expressing tumor cells and then spread to adjacent cells over the few weeks that adenovirus is active. There are limited normal stem-like cells that express telomerase in the brain (Komata et al., 2001), and thus hTERTp-TRAD therapy for gliomas may have few serious side effects. Immune cells that express telomerase are not easily infected by adenovirus (Gu et al., 2001; Wantanabe et al., 1996), so hematopoietic cells are unlikely to be affected. Systemic hTERTp-TRAD might be expected to have some immediate side effects on transient amplifying stem cells such as proliferating spermatocytes in the testes, cells in the crypts of the intestine, and a subset of cells in the basal and suprabasal layer of the epidermis (Holt and Shay, 1999; White et al., 2001; Forsyth et al., 2002). However, we do not expect this to be any more detrimental than conventional cytotoxic drugs that affect all proliferating cells. In initial experiments, the use of intratumoral hTERTp-TRAD injections may limit the ability of the adenoviral vectors to go to distant sites, and this approach could be useful for short-term immediate tumor burden reduction. Surprisingly, in studies comparing a CMV-LacZ to hTERT-lacZ adenoviral vector by direct injections into the liver and spleen of mice (tissues that are telomerase-positive in mice), there was essentially no β-galactosidase activity with the hTERT vector but high levels with the constitutive CMV promoter (Gu et al., 2001). Although this may be due to the human TERT promoter not working well in murine cells, it may be that normal cells in most organs do not express telomerase at sufficiently high levels to produce functional levels of downstream effector genes.

In another approach, the transcriptional regulatory sequences from the hTERT or hTR genes were used to regulate the expression of the bacterial nitroreductase enzyme in combination with a prodrug, CB1954 (Plumb et al., 2001). This approach sensitized the cancer cells so that the prodrug very efficiently killed the cancer cells. This approach was apparently

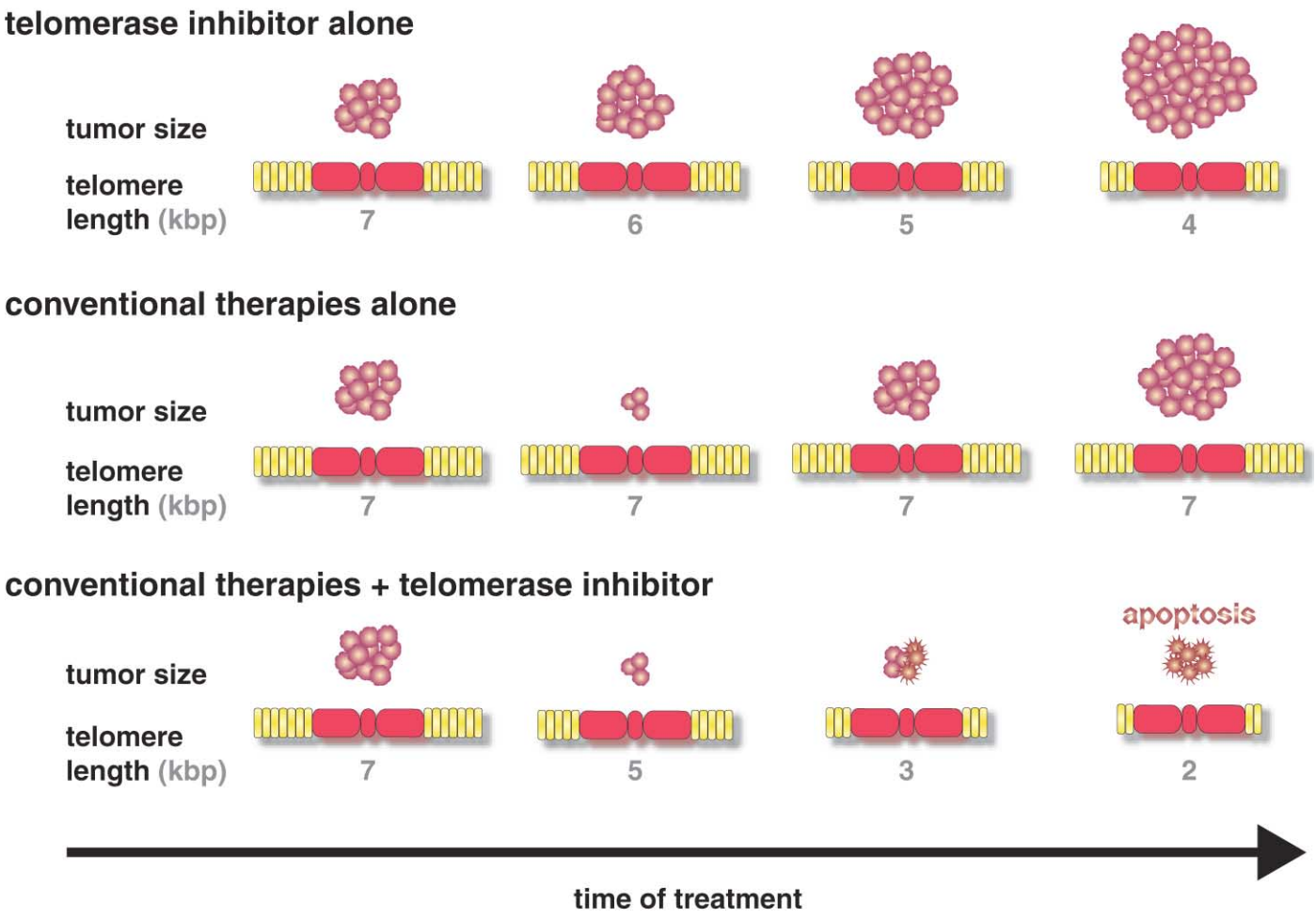

Figure 3. Telomerase inhibitors and conventional therapies

A telomerase inhibitor used as a single agent will progressively reduce telomere length, but there is an expected time delay until cell death. Conventional cytoxic therapies will initially reduce tumor burden but not affect telomere length. Combinations of conventional therapies with telomerase inhibitors would be predicted to both reduce tumor burden and shorten telomeres, potentially preventing or delaying tumor recurrences.

much more effective compared to toxins/apoptotic genes or the thymidine kinase/gancyclovir combination (Abdul-Ghani et al., 2000; Majumdar et al., 2001).

hTERT immunotherapy

Tumor-associated antigens have been shown to mediate specific anticancer T lymphocyte responses resulting in tumor regression (reviewed in Vonderheide, 2002). Most previous research has focused on tumor-associated antigens that were restricted to only a few tumor types. The catalytic protein component of telomerase (hTERT) qualifies as an attractive candidate as a nearly universal tumor-associated antigen. hTERT protein is naturally processed and hTERT peptides are presented as epitopes by MHC, eliciting CTL responses and protective immunity against tumors (Nair et al., 2000; Vonderheide et al., 2001; Minev et al., 2000). These early in vitro studies on the immunogenicity of hTERT peptides provide a good rationale for using this approach for telomerase inhibition. The advantage, as in the hTERTp-TRAD approach, is that there would be no lag period required for telomere shortening prior to observation of cell growth arrest and death. The major disadvantage would be that normal cells expressing high levels of telomerase might also be affected. However, while investigators were able to elicit a specific CTL killing of tumor cells of prostate, lung, breast, colon, and melanoma, they did not observe a CTL effect on telomerase-positive $CD34^+$ hematopoetic cells, suggesting that hTERT is a poor autoantigen in stem cells (Vonderheide et al., 2001). This may be due to the relatively low level of telomerase expression in these cells as compared with tumor cells and the fact that they do not continuously express telomerase. In ongoing phase I clinical trials to establish safety of the approach, there was measurable immune reactivity without any high-grade toxic side effects (Vonderheide, 2002). While vaccinations in patients with high-grade tumors are unlikely to be clinically effective, preventative immunotherapy could be a viable option (if there are minimal toxicities) in patients with minimal residual disease or in patients with a high risk for cancer development.

Targeting the RNA component of telomerase (telomerase template antagonists)

Oligonucleotide strategies for cancer therapy directed against a variety of molecular targets are currently in late-stage clinical trials. The initial results are encouraging and indicate that the oligonucleotides delivered systemically can enter human tumors

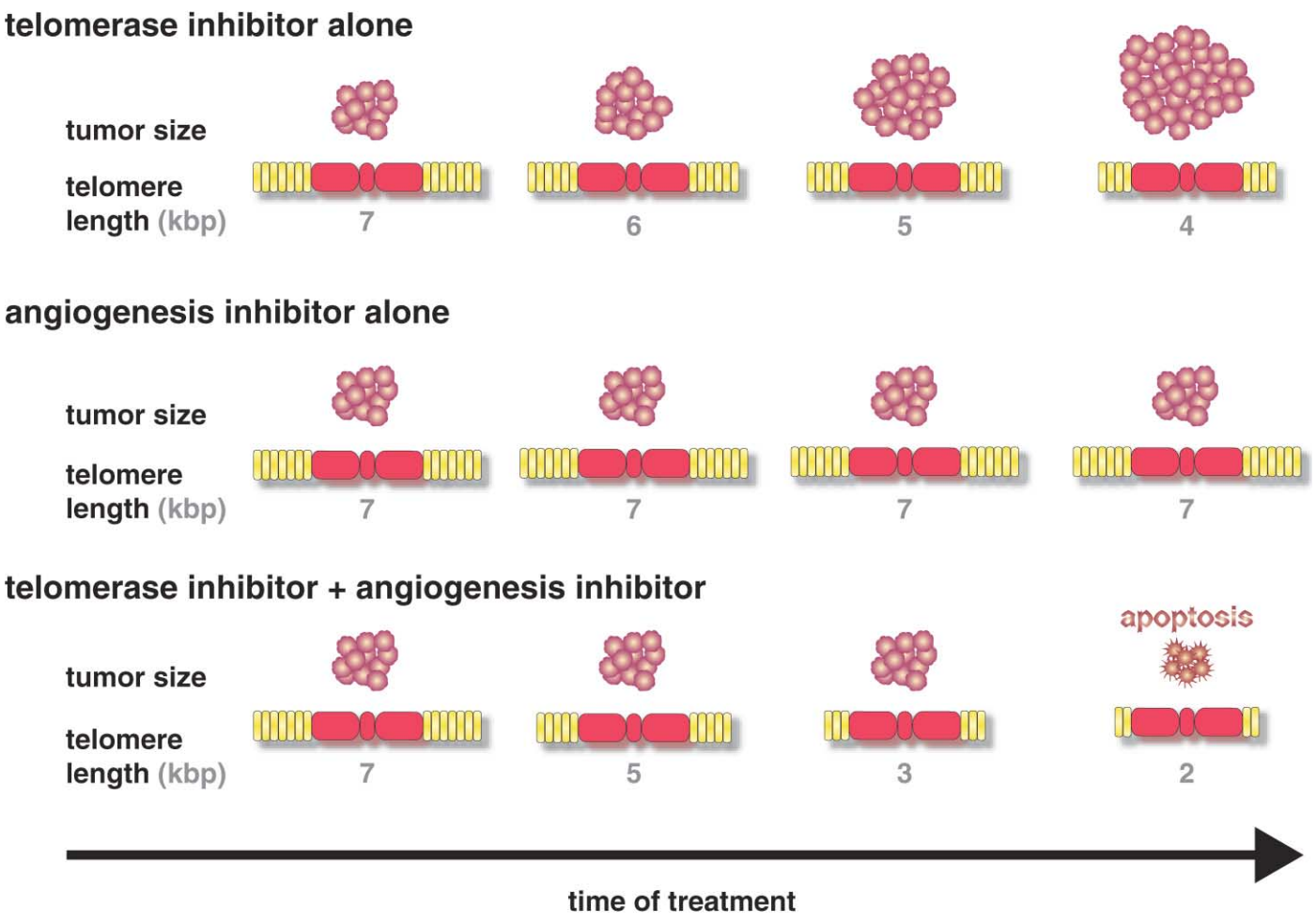

Figure 4. Telomerase inhibitors and experimental angiogenesis inhibitors

A telomerase inhibitor used as a single agent will progressively reduce telomere length, but there is an expected time delay until cell death. Some angiogenesis inhibitors will maintain a reduced tumor burden but will not eradicate the tumor or affect telomere length. Combinations of angiogenesis inhibitors with telomerase inhibitors would be predicted to maintain a reduced tumor burden while producing progressively shortened telomeres, potentially leading to preventing or delaying tumor recurrences.

in patients, find their targets, and in some instances result in improved survival (Corey, 2000, 2002). Oligonucleotides complementary to the template region of the RNA component of telomerase (hTR) offer certain advantages as well as disadvantages as a cancer therapeutic. The major disadvantage is that, compared to telomerase promoter oncolytic viruses or the hTERT immunotherapy, inhibiting telomerase will take time before telomere shortening affects tumor cell survival. However, telomerase is an intriguing target since the template region of hTR must be exposed in order to add new telomeric repeats onto the chromosomes, making it an accessible target for oligonucleotides. Rather than acting by "antisense" mechanisms to degrade mRNA or inhibit translation, oligonucleotides targeting the hTR template region function as classical enzymatic inhibitors of telomerase activity. With typical mRNA targeting, as much as 90% or more inhibition may be necessary to observe an effect, while hTR inhibitors may be effective at much lower efficiencies.

Several classes of oligonucleotides (such a phosphodiester DNA, phosphoramidates, peptide nucleic acids, locked nucleic acids, 2′ O-methyl-RNA, and 2′-O-methoxyethyl RNA) targeting telomerase have been reported (Norton et al., 1996; Pitts and Corey, 1998; Corey, 2000, 2002; Herbert et al., 2002; Hamilton et al., 1999; Gryaznov et al., 2001; Elayadi and Corey, 2001). Almost all these chemistries can effectively inhibit telomerase in intact cells. Importantly, most of these chemistries work at pharmacological concentrations. Replacing some of the phosphodiester bonds with phosphorothioate linkages results in greater resistance to nucleases but unfortunately leads to loss of specificity (Matthes and Lehmann, 1999). In some experiments, telomerase template antagonists administered to intact cancer cells have been shown to reduce telomerase activity, lead to progressive shortening of telomeres, and cause cell proliferation to decrease and apoptosis to increase in a time period proportional to initial telomere length (Herbert et al., 1999). Importantly, chemically related molecules that did not inhibit telomerase did not cause decreased cell proliferation or telomere shortening.

When the telomerase template antagonist was removed from the cells in culture, the surviving cells regained baseline telomerase activity and their telomeres grew back to their original lengths, supporting the assumption that the mechanism of action was through a competitive inhibition of the telomerase

enzyme (Herbert et al., 1999) and that the agents will most likely have to be administered to cancer patients for an extended period. During this study (Herbert et al., 1999), there was no evidence of any emergence of an alternative pathway for telomere elongation. There have not been any studies on the long-term effects of these template antagonists on normal somatic cells.

Inhibiting telomerase in precancerous cells: A novel cancer prevention approach

Chemopreventive agents act by preventing the occurrence or recurrence of cancer. Discovery of new chemopreventive agents and assays to quantitatively analyze these agents is therefore of vital importance. The effective use of chemopreventive agents ultimately depends on the understanding of the mechanism of action. A mechanism-based assay has been developed using cultures of human breast-derived, epithelial cells that spontaneously immortalize in cell culture (Herbert et al., 2001). The endpoint, determining the frequency of cell immortalization in the presence of chemopreventive agents, has been validated using telomerase inhibitors (Herbert et al., 2001). Cells were exposed to an oligonucleotide-based telomerase antagonist using 2′ O-methyl-RNA targeting the human telomerase RNA template region or infected with a retrovirus expressing a dominant-negative mutant of the human telomerase catalytic subunit. Both of these agents significantly reduced the frequency of spontaneous immortalization of the breast epithelial cells. In addition to preventing immortalization with known telomerase inhibitors, other validated chemopreventive agents were also shown to inhibit immortalization (Herbert et al., 2001). The future identification of novel chemopreventive agents that prevent the upregulation of telomerase may have important clinical implications. These studies validate the application of chemopreventive agents in a mechanism-based assay, using cell immortalization as an endpoint. The results also suggest that the telomerase ribonucleoprotein complex may be an important target for breast cancer prevention.

Conclusions/future perspectives

Cancer remains a major cause of death in spite of substantial progress in understanding its molecular mechanisms and the development of an array of powerful treatments. The discovery of validated targets and new drugs is therefore a high priority. Telomerase-based drugs have the potential to act by novel mechanisms that will provide new options for cancer therapy and allow for unprecedented therapeutic specificity and efficacy. Telomerase inhibitors, for example, might not only directly limit or stop the growth of human tumors, but also might act in a synergistic fashion with existing therapeutic modalities and amplify their effectiveness. After initial chemotherapy or surgery, telomerase inhibitors might be used to inhibit the recovery of residual cancer cells, making them more susceptible to attack by the immune system or killing by existing chemotherapeutic agents or other novel therapies as discussed in this review (Figures 3). In addition, the combination of angiogenesis inhibitors with telomerase inhibitors could keep the tumor burden small as well as lead to progressive telomere shortening (Figure 4).

In summary, we have described some of the major avenues of targeting telomerase in human cancer cells. Immunotherapy directed against telomerase-positive cells as well as strategies using a suicide gene promoter targeted to hTERT-expressing cells are currently under active investigation and in some instances in early-stage clinical trials. These modalities have the advantage of abolishing the lag phase that is required with the classic mode of telomerase inhibition. However, these treatments may also prove to be more toxic to normal somatic cells expressing telomerase. The use of telomerase template antagonists would require a lag phase prior to any detrimental effects on the cells and is unlikely to be a reasonable stand-alone strategy in patients with a large tumor burden. Telomerase template antagonists and other small molecule approaches to inhibit telomerase would be most effective in a situation of minimal residual disease or as an adjuvant treatment in combination with conventional therapies (Figures 1 and 3). Telomerase inhibitors will probably be used following standard therapies in which there is no clinical evidence of disease in order to treat possible micro metastases or in patients at high risk to develop cancer in a chemoprevention approach. In these situations, which would likely require prolonged treatment, it will be imperative that the drugs have a low toxicity profile and be easily administered by an oral or systemic “depo” route. At the present time, there is still a large body of basic research to pursue, but it is encouraging that formal preclinical investigation of telomerase inhibitors and hTERTp-based oncolytic viruses has begun, and there are already clinical studies underway with hTERT immunotherapy. While the vast majority of studies are encouraging, the ultimate utility of these and future drugs based on telomere biology will only be determined after completion of clinical trials and use in a broad range of cancer patients. However, it is reasonable to anticipate that some surprises may arise as important lessons from cell culture and animal models are validated in clinical trials.

Acknowledgments

Support is acknowledged from the Ellison Medical Foundation, Geron Corporation, CA74908, CA70907, and NCI Contract CN-0501763-63. We thank Calvin Harley for critical discussions and Kent Thompson for help with the figure illustrations.

References

Abdul-Ghani, R., Ohana, P., Matouk, I., Ayesh, S., Ayesh, B., Laster, M., Bibi, O., Giladi, H., Molnar-Kimber, K., Sughayer, M.A., et al. (2000). Use of transcriptional regulatory sequences of telomerase (hTER and hTERT) for selective killing of cancer cells. Mol. Ther. *2*, 539–544.

Aisner, D.L., Wright, W.E., and Shay, J.W. (2002). Telomerase regulation: not just flipping the switch. Curr. Opin. Genet. Dev. *12*, 80–85.

Blackburn, E.H. (2000). Telomere states and cell fates. Nature *408*, 53–56.

Bodnar, A.G., Chiu, C., Frolkis, M., Harley, C.B., Holt, S.E., Lichtsteiner, S., Morin, G.B., Ouellette, M., Shay, J.W., and Wright, W.E. (1998). Extension of life-span by introduction of telomerase into normal human cells. Science *279*, 349–352.

Boklan, J., Nanjangud, G., MacKenzie, K.L., May, C., Sadelain, M., and Moore, M.A. (2002). Limited proliferation and telomere dysfunction following telomerase inhibition in immortal murine fibroblasts. Cancer Res. *62*, 2104–2114.

Boultwood, J., Fidler, C., Kusec, R., Rack, K., Elliott, P.J.W., Atoyebi, O., Chapman, R., and Oscier, D.G. (1997). Telomere length in myelodysplastic syndromes. Am. J. Hematol. *56*, 266–271.

Brummendorf, T.H., Holyoake, T.L., Rufer, N., Barnett, M.J., Schulzer, M., Eaves, C.J., Eaves, A.C., and Lansdorp, P.M. (2000). Prognostic implications of differences in telomere length between normal and malignant cells from patients with chronic myeloid leukemia measured by flow cytometry. Blood *95*, 1883–1890.

Brummendorf, T.H., Maciejewski, J.P., Mak, J., Young, N.S., and Lansdorp, P.M. (2001). Telomere length in leukocyte subpopulations of patients with aplastic anemia. Blood *97*, 895–900.

Bryan, T.M., and Reddel, R.R. (1997). Telomere dynamics and telomerase activity in in vitro immortalised human cells. Eur. J. Cancer *33*, 767–773.

Bryan, T.M., Engelzou, A., Gupta, J., Bacchetti, S., and Reddel, R.R. (1995). Telomere elongation in immortal human cells without detectable telomerase activity. EMBO J. *14*, 4240–4248.

Bryan, T.M., Marusic, L., Bacchetti, S., Namba, M., and Reddel, R.R. (1997). The telomere lengthening mechanism in telomerase-negative immortal human cells does not involve the telomerase RNA subunit. Hum. Mol. Genet. *6*, 921–926.

Chin, L., Artandi, S.E., Shen, Q., Tam, A., Lee, S.L., Gottlieb, G.J., Greider, C.W., and DePinho, R.A. (1999). p53 deficiency rescues the adverse effects of telomere loss and cooperates with telomere dysfunction to accelerate carcinogenesis. Cell *97*, 527–538.

Collins, K. (2000). Mammalian telomeres and telomerase. Curr. Opin. Cell Biol. *12*, 378–383.

Collins, K., and Mitchell, J.R. (2002). Telomerase in the human organism. Oncogene *21*, 564–579.

Corey, D.R. (2000). Telomerase: an unusual target for cytotoxic agents. Chem. Res. Toxicol. *13*, 957–960.

Corey, D.R. (2002). Telomerase inhibition, oligonucleotides, and clinical trials. Oncogene *21*, 631–637.

Counter, C.M., Avilion, A.A., LeFeuvre, C.E., Stewart, N.G., Greider, C.W., Harley, C.B., and Bacchetti, S. (1992). Telomere shortening associated with chromosome instability is arrested in immortal cells which express telomerase activity. EMBO J. *11*, 1921–1929.

de Lange, T. (2002). Protection of mammalian telomeres. Oncogene *21*, 532–540.

de Lange, T., and Jacks, T. (1999). For better or worse? Telomerase inhibition and cancer. Cell *98*, 273–275.

Elayadi, A.N., and Corey, D.R. (2001). Applications of LNA and PNA oligomers to chemotherapy. Curr. Opin. Invest. New Drugs *2*, 558–561.

Fajkus, J., Simickova, M., and Malaska, J. (2002). Tiptoeing to chromosome tips: facts, promises and perils of today's human telomere biology. Philos. Trans. R. Soc. Lond. B Biol. Sci. *357*, 545–562.

Feng, J., Funk, W.D., Wang, S.S., Weinrich, S.L., Avilion, A.A., Chiu, C.-P., Adams, R.R., Chang, E., Allsopp, R.C., Yu, J.H., et al. (1995). The RNA component of human telomerase. Science *269*, 1236–1241.

Folini, M., Colella, G., Villa, R., Lualdi, S., Daidone, M.G., and Zaffaroni, N. (2000). Inhibition of telomerase activity by a hammerhead ribozyme targeting the RNA component of telomerase in human melanoma cells. J. Invest. Dermatol. *114*, 259–267.

Forsyth, N.R., Wright, W.E., and Shay, J.W. (2002). Telomerase and differentiation in multicellular organisms: turn it off, turn it on, and turn it off again. Differentiation *69*, 188–197.

Franco, S., Segura, I., Riese, H.H., and Blasco, M.A. (2002). Decreased B16F10 melanoma growth and impaired vascularization in telomerase-deficient mice with critically short telomeres. Cancer Res. *6*, 552–559.

Gomez, D.E., Tejera, A.M., and Olivera, O.A. (1998). Irreversible telomere shortening by 3′azido-2′,3′dideoxythymidine (AZT) treatment. Biochem. Biophys. Res. Commun. *246*, 107–110.

Gonzalez-Suarez, E., Samper, E., Flores, J.M., and Blasco, M.A. (2000). Telomerase-deficient mice with short telomeres are resistant to skin tumorigenesis. Nat. Genet. *26*, 114–117.

Gonzalez-Suarez, E., Samper, E., Ramirez, A., Flores, J.M., Martin-Caballero, J., Jorcano, J.L., and Blasco, M.A. (2001). Increased epidermal tumors and increased skin wound healing in transgenic mice overexpressing the catalytic subunit of telomerase, mTERT, in basal keratinocytes. EMBO J. *20*, 2619–2630.

Gowan, S.M., Harrison, J.R., Patterson, L., Valenti, M., Read, M.A., Neidle, S., and Kelland, L.R. (2002). A G-quadruplex-interactive potent small-molecule inhibitor of telomerase exhibiting in vitro and in vivo antitumor activity. Mol. Pharmacol. *61*, 1154–1162.

Goytisolo, F.A., and Blasco, M.A. (2002). Many ways to telomere dysfunction: in vivo studies using mouse models. Oncogene *21*, 584–591.

Granger, M.P., Wright, W.E., and Shay, J.W. (2002). Telomerase in cancer and aging. Crit. Rev. Oncol. Hematol. *4*, 29–40.

Greenberg, R.A., Chin, L., Femino, A., Lee, K.H., Gottlieb, G.J., Singer, R.H., Greider, C.W., and DePinho, R.A. (1999). Short dysfunctional telomeres impair tumorigenesis in the INK4a cancer-prone mouse. Cell *97*, 515–525.

Greider, C.W., and Blackburn, E.H. (1985). Identification of a specific telomere terminal transferase activity in *Tetrahymena* extracts. Cell *43*, 405–413.

Griffith, J.D., Comeau, L., Rosenfield, S., Stansel, R.M., Bianchi, A., Moss, H., and de Lange, T. (1999). Mammalian telomeres end in a large duplex loop. Cell *97*, 503–514.

Gryaznov, S., Pongracz, K., Matray, T., Schultz, R., Pruzan, R., Aimi, J., Chin, A., Harley, C., Shea-Herbert, B., Shay, J., et al. (2001). Telomerase inhibitors—oligonucleotide phosphoramidates as potential therapeutic agents. Nucleosides Nucleotides Nucleic Acids *20*, 401–410.

Gu, J., Kagawa, S., Takakura, M., Kyo, S., Inoue, M., Roth, J.A., and Fang, B. (2001). Tumor-specific transgene expression from the human telomerase reverse transcriptase promoter enables targeting of the therapeutic effects of the Bax gene to cancers. Cancer Res. *60*, 5359–5364.

Gu, J., Andreff, M., Roth, J.A., and Fang, B. (2002). hTERT promoter induces tumor-specific Bax gene expression and cell killing in syngenic mouse tumor model and prevents systemic toxicity. Gene Ther. *9*, 30–37.

Hackett, J.A., and Greider, C.W. (2002). Balancing instability: dual roles for telomerase and telomere dysfunction in tumorigenesis. Oncogene *21*, 619–626.

Hahn, W.C., Stewart, S.A., Brooks, M.W., York, S.G., Eaton, E., Kurachi, A., Beijersbergen, R.L., Knoll, J.H., Meyerson, M., and Weinberg, R.A. (1999). Inhibition of telomerase limits the growth of human cancer cells. Nat. Med. *5*, 1164–1170.

Hamilton, S.E., Simmons, C.G., Kathriya, I., and Corey, D.R. (1999). Cellular delivery of peptide nucleic acids and inhibition of human telomerase. Chem. Biol. *6*, 343–351.

Hanahan, D., and Weinberg, R.A. (2000). The hallmarks of cancer. Cell *100*, 57–70.

Harley, C.B. (1991). Telomere loss: mitotic clock or genetic time bomb? Mutat. Res. *256*, 271–282.

Harley, C.B. (2002). Telomerase is not an oncogene. Oncogene *21*, 494–502.

Harrington, L., and Robinson, M.O. (2002). Telomere dysfunction: multiple paths to the same end. Oncogene *21*, 592–597.

Helder, M.N., Wisman, G.B.A., and van der Zee, A.G.J. (2002). Telomerase and telomeres: from basic biology to cancer treatment. Cancer Invest. *20*, 82–101.

Henson, J.D., Neumann, A.A., Yeager, T.R., and Reddel, R.R. (2002). Alternative lengthening of telomeres in mammalian cells. Oncogene *21*, 598–610.

Herbert, B.-S., Pitts, A.E., Baker, S.I., Hamilton, S.E., Wright, W.E., Shay, J.W., and Corey, D.R. (1999). Inhibition of human telomerase in immortal human cells leads to progressive telomere shortening and cell death. Proc. Natl. Acad. Sci. USA *96*, 14276–14281.

Herbert, B.-S., Wright, A.C., Passons, C.M., Ali, I., Wright, W.E., Kopelovich, L., and Shay, J.W. (2001). Inhibition of the spontaneous immortalization of breast epithelial cells from individuals predisposed to breast cancer: effects of chemopreventive and anti-telomerase agents. J. Natl. Cancer Inst. *93*, 39–45.

Herbert, B.-S., Pongracz, K., Shay, J.W., and Gryaznov, S.M. (2002). Oligonucleotide N3′-P5′ phosphoramidates as efficient telomerase inhibitors. Oncogene *21*, 638–642.

Hodes, R. (2001). Molecular targeting of cancer: telomeres as targets. Proc. Natl. Acad. Sci. USA *98*, 7649–7651.

Holt, S.E., and Shay, J.W. (1999). Role of telomerase in cellular proliferation and cancer. J. Cell. Physiol. *180*, 10–18.

Holt, S.E., Aisner, D.L., Baur, J., Tesmer, V.M., Dy, M., Ouellette, M., Trager, J.B., Morin, G.B., Toft, D.O., Shay, J.W., et al. (1999). Functional requirement

of p23 and Hsp90 in telomerase complexes. Genes Dev. *13*, 817–826.

Kakuo, S., Asaoka, K., and Ide, T. (1999). Human is a unique species among primates in terms of telomere length. Biochem. Biophys. Res. Commun. *263*, 308–314.

Kelland, L.R. (2001). Telomerase: biology and phase I trials. Lancet Oncol. *2*, 95–102.

Kim, N.W., Pietyszek, M.A., Prowse, K.R., Harley, C.B., West, M.D., Ho, P.L.C., Coviello, G.M., Wright, W.E., Weinrich, S.L., and Shay, J.W. (1994). Specific association of human telomerase activity with immortal cells and cancer. Science *266*, 2011–2015.

Kim, M.M., Rivera, M.A., Botchkina, I.L., Shalaby, R., Thor, A.D., and Blackburn, E.H. (2001). A low threshold level of expression of mutant-template telomerase RNA inhibits human tumor cell proliferation. Proc. Natl. Acad. Sci. USA *98*, 7982–7987.

Koga, S., Hirohata, S., Kondo, Y., Komata, T., Takakura, M., Inoue, M., Kyo, S., and Kondo, S. (2000). A novel telomerase-specific gene therapy: gene transfer of caspase-8 utilizing the human telomerase catalytic subunit gene promoter. Hum. Gene Ther. *11*, 1397–1406.

Koga, S., Hirohata, S., Kondo, Y., Komata, T., Takakura, M., Inoue, M., Kyo, S., and Kondo, S. (2001). FADD gene therapy using the human telomerase catalytic subunit (hTERT) gene promoter to restrict induction of apoptosis to tumors in vitro and in vivo. Anticancer Res. *21*, 1937–1943.

Komata, T., Kondo, Y., Kanzawa, T., Hirohata, S., Koga, S., Sumiyoshi, H., Srinivasula, S.M., Barna, B.P., Germano, I.M., Takakura, M., et al. (2001). Treatment of malignant glioma cells with the transfer of constitutively active caspase-6 using the human telomerase catalytic subunit (human telomerase reverse transcriptase) gene promoter. Cancer Res. *61*, 5796–5802.

Lingner, J., and Cech, T.R. (1998). Telomerase and chromosome end maintenance. Curr. Opin. Genet. Dev. *8*, 226–232.

Lundblad, V. (2002). Telomere maintenance without telomerase. Oncogene *21*, 522–531.

Lundblad, V., and Blackburn, E.H. (1993). An alternative pathway for yeast telomere maintenance rescues est1$^-$ senescense. Cell *73*, 347–360.

Mahmud, N., Devine, S.M., Weller, K.P., Parmar, S., Sturgeon, C., Nelson, M.C., Hewett, T., and Hoffman, R. (2001). The relative quiescence of hematopoietic stem cells in nonhuman primates. Blood *97*, 3061–3068.

Majumdar, A.S., Hughes, D.E., Lichtsteiner, S.P., Wang, Z., Lebkowski, J.S., and Vasserot, A.P. (2001). The telomerase reverse transcriptase promoter drives efficacious tumor suicide gene therapy while preventing hepatotoxicity encountered with constitutive promoters. Gene Ther. *8*, 568–578.

Maser, R.S., and DePinho, R.A. (2002). Connecting chromosomes, crisis and cancer. Science *297*, 565–569.

Matthes, E., and Lehmann, C. (1999). Telomerase protein rather than its RNA is the target of phosphorothioate-modified oligonucleotides. Nucleic Acids Res. *27*, 1152–1158.

McEachern, M.J., Krauskopf, A., and Blackburn, E.H. (2000). Telomeres and their control. Annu. Rev. Genet. *34*, 331–358.

McKenzie, K.E., Umbricht, C.B., and Sukumar, S. (1999). Applications of telomerase research in the fight against cancer. Mol. Med. Today *5*, 114–122.

Minev, B., Hipp, J., Firat, H., Schmidt, J.D., Langlade-Demoyen, P., and Zanetti, M. (2000). Cytotoxic T cell immunity against telomerase reverse transcriptase in humans. Proc. Natl. Acad. Sci. USA *97*, 4796–4801.

Morales, C.P., Holt, S.E., Ouellette, M., Kaur, K.J., Ying, Y., Wilson, K.S., White, M.A., Wright, W.E., and Shay, J.W. (1999). Lack of cancer-associated changes in human fibroblasts after immortalization with telomerase. Nat. Genet. *21*, 115–118.

Morin, G.B. (1989). The human telomerase terminal transferase enzyme is a ribonucleoprotein that synthesizes TTAGGG repeats. Cell *59*, 521–529.

Murakami, J., Nagai, N., Shigemasa, K., and Ohama, K. (1998). Inhibition of telomerase activity and cell proliferation by a reverse transcriptase inhibitor in gynaecological cancer cell lines. Eur. J. Cancer *35*, 1027–1034.

Nair, S.K., Heiser, A., Boczkowski, D., Majumdar, A., Naoe, M., Lebkowski, J.S., Vieweg, J., and Gilboa, E. (2000). Induction of cytotoxic T lymphocyte responses and tumor immunity against unrelated tumors using telomerase reverse transcriptase RNA transfected dendritic cells. Nat. Med. *6*, 1011–1017.

Nakamura, T.M., Morin, G.B., Chapman, K.B., Weinrich, S.L., Andrews, W.H., Lingner, J., Harley, C.B., and Cech, T.R. (1997). Telomerase catalytic subunit homologs from fission yeast and humans. Science *277*, 955–959.

Neidle, S., and Parkinson, G. (2002). Telomere maintenance as a target for anticancer drug discovery. Nat. Rev. Drug Discov. *1*, 383–393.

Norton, J.C., Piatyszek, M.A., Wright, W.E., Shay, J.W., and Corey, D.R. (1996). Inhibition of human telomerase activity by peptide nucleic acids. Nat. Biotechnol. *14*, 615–619.

Nugent, C.I., and Lundblad, V. (1998). The telomerase reverse transcriptase: components and regulation. Genes Dev. *12*, 1073–1085.

Pathak, S., Multani, A.S., Furlong, C.L., and Sohn, S.H. (2002). Telomere dynamics, aneuploidy, stem cells, and cancer (review). Int. J. Oncol. *20*, 637–641.

Pitts, A.E., and Corey, D.R. (1998). Inhibition of human telomerase by 2'-O-methyl RNA oligonucleotides. Proc. Natl. Acad. Sci. USA *95*, 11549–11554.

Plumb, J.A., Bilsland, A., Kakani, R., Zhao, J., Glasspool, R.M., Knox, R.J., Evens, T.R.J., and Keith, W.N. (2001). Telomerase-specific suicide gene therapy vectors expressing bacterial nitroreductase sensitize human cancer cells to the pro-drug CB1954. Oncogene *20*, 7797–7803.

Ramirez, R.D., Morales, C.P., Herbert, B.S., Rohde, J.M., Passons, C., Shay, J.W., and Wright, W.E. (2001). Putative telomere-independent mechanisms of replicative aging reflect inadequate growth conditions. Genes Dev. *1*, 398–403.

Read, M.A., Wood, A.A., Harrison, J.R., Gowan, S., Kelland, S.R., Doganjh, H.S., and Neidle, S. (1999). Molecular modeling studies on G-quadruplex complexes of telomerase inhibitors: structure-activity relationships. J. Med. Chem. *42*, 4538–4546.

Riou, J.F., Guittat, L., Mailliet, P., Laoui, A., Renou, E., Petitgenet, O., Megnin-Chanet, F., Helene, C., and Mergny, J.L. (2002). Cell senescence and telomere shortening induced by a new series of specific G-quadruplex DNA ligands. Proc. Natl. Acad. Sci. USA *99*, 2672–2677.

Shawyer, L.K., Slamon, D., and Ullrich, A. (2002). Smart drugs: tyrosine kinase inhibitors in cancer therapy. Cancer Cell *1*, 117–121.

Shay, J.W., and Bacchetti, S. (1997). A survey of telomerase activity in human cancer. Eur. J. Cancer *5*, 787–791.

Shay, J.W., Pereira-Smith, O.M., and Wright, W.E. (1991a). A role for both Rb and p53 in the regulation of human cellular senescence. Exp. Cell Res. *196*, 33–39.

Shay, J.W., Wright, W.E., and Werbin, H. (1991b). Defining the molecular mechanisms of human cell immortalization. Biochim. Biophys. Acta *1072*, 1–7.

Sherr, C.J., and McCormick, F. (2002). The RB and p53 pathways in cancer. Cancer Cell *2*, 103–112.

Smogorzewska, A., and de Lange, T. (2002). Different telomere damage signaling pathways in human and mouse cells. EMBO J. *21*, 4338–4348.

Smogorzewska, A., van Steensel, B., Bianchi, A., Oelmann, S., Schaefer, M.R., Schnapp, G., and de Lange, T. (2000). Control of human telomere length by TRF1 and TRF2. Mol. Cell. Biol. *20*, 1659–1668.

Stewart, S.A., and Weinberg, R.A. (2002). Senescence: does it all happen at the ends? Oncogene *21*, 627–630.

Strahl, C., and Blackburn, E.H. (1996). Effects of reverse transcriptase inhibitors on telomere length and telomerase activity in two immortal human cell lines. Mol. Cell. Biol. *16*, 53–56.

Sun, D., Thompson, B., Cathers, B.E., Salazar, M., Kerwin, S.M., Trent, J.O., Jenkins, T.C., Neidle, S., and Hurley, L.M. (1997). Inhibition of human telomerase by a G-quadruplex-interactive compound. J. Med. Chem. *40*, 2113–2116.

Van Steensel, B., Smogorzewska, A., and de Lange, T. (1998). TRF2 protects human telomeres from end-to-end fusions. Cell *92*, 401–413.

Vonderheide, R.H. (2002). Telomerase as a universal tumor-associated anti-

gen for cancer immunotherapy. Oncogene *21*, 674–679.

Vonderheide, R.H., Schultze, J.L., Anderson, K.S., Maecker, B., Butler, M.O., Xia, Z., Kuroda, M.J., von Bergwelt-Baildon, M.S., Bedor, M.M., Hoar, K.M., et al. (2001). Equivalent induction of telomerase-specific cytotoxic T lymphocytes from tumor-bearing patients and healthy individuals. Cancer Res. *61*, 8366–8370.

Wantanabe, T., Kusynski, C., Ino, K., Heimann, D.G., Shepard, H.M., Yasui, Y., Maneval, D.C., and Talmadge, J.E. (1996). Gene transfer into human bone marrow hematopoietic cells mediated by adenovirus vectors. Blood *87*, 5032–5039.

White, L., Wright, W.E., and Shay, J.W. (2001). Telomerase inhibitors. Trends Biotechnol. *3*, 146–149.

Wright, W.E., and Shay, J.W. (2000). Telomere dynamics in cancer progression and prevention: fundamental differences in human and mouse telomere biology. Nat. Med. *6*, 849–851.

Wright, W.E., Pereira-Smith, O.M., and Shay, J.W. (1989). Reversible cellular senescence: a two-stage model for the immortalization of normal human diploid fibroblasts. Mol. Cell. Biol. *9*, 3088–3092.

Wright, W.E., Piatyszek, M.A., Rainey, W.E., Byrd, W., and Shay, J.W. (1996). Telomerase activity in human germline and embryonic tissues. Dev. Genet. *18*, 173–179.

Yokoyama, Y., Takahashi, Y., Shinohara, A., Lian, Z., Wan, X., Niwa, K., and Tamaya, T. (1998). Attenuation of telomerase activity by a hammerhead ribozyme targeting the template region of telomerase RNA in endometrial carcinoma cells. Cancer Res. *58*, 5406–5410.

Zhang, X., Mar, V., Zhou, W., Harrington, L., and Robinson, M.O. (1999). Telomere shortening and apoptosis in telomerase-inhibited human tumor cells. Genes Dev. *13*, 2388·2399.