Telomerase-directed molecular therapeutics

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The management of malignant disease remains one of the most challenging areas of modern medicine. The lifetime risk of developing cancer in the western world is estimated to be as high as 1 in 3. Traditionally, surgery, chemotherapy and radiotherapy have been the primary choice of treatment for patients with malignant tumours. Despite advances in the use and development of conventional cytotoxic agents, the cure rate remains disappointing in most patients with advanced disease of the common solid tumours. Consequently, the development of novel anti-cancer therapies is a high priority in cancer medicine. In recent years, a new generation of cancer therapies has emerged, based on a growing understanding of the molecular events that contribute to malignant transformation. A major difference between normal and cancer cells is the ability of cancer cells to multiply in an unrestricted and ungoverned fashion. In this context, there is considerable interest in elucidating

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the mechanisms that allow this unrestricted proliferation and that ultimately result in immortal cancer cells. It is now clear that the enzyme telomerase confers immortality on cells in most types of cancer. With the cancer cell reliant on telomerase for its survival, telomerase represents an extremely attractive mechanism-based target for the development of new cancer therapeutics.

A major goal in developing new molecular therapeutics is to identify targets that are differentially expressed between normal and cancer cells. The increased level and frequency of telomerase activity in cancers when compared with normal cells makes telomerase an extremely attractive target for anti-cancer strategies, particularly as it should be possible to use telomerase-based therapeutics over a broad range of malignancies (Refs 1, 2, 3). It is important to move away from considering telomerase as a single molecular target: telomeres, telomerase and the pathways regulating telomerase activity offer a wealth of targets for novel molecular therapeutics, and as our understanding of telomerase biology grows, so does the number of potential therapeutic strategies. A key issue over the next few years will be how to apply these strategies in the clinic. This review discusses the variety of telomerase-based molecular therapeutics and how these might successfully translate to the clinic.

The unique biology of telomeres and telomerase

Telomere structure and function

Telomeres are specialised structures at the end of chromosomes that consist of tandem nucleotide repeats; in humans, the repeated sequence is the 6 bp TTAGGG. Telomeres act as protective caps, preventing both degradation of the ends of the chromosomes and their recognition as double-strand breaks, which might otherwise result in aberrant recombination. As chromosomes are replicated during cell division, there is incomplete replication of the extreme 3' end of the lagging strand. This is known as the 'end replication problem' and results in the loss of approximately 50 bp of telomeric material with each division. It is postulated that this could act as a molecular counting mechanism, marking the number of cell divisions (Fig. 1) (Refs 4, 5).

Cell numbers are vigorously controlled within the body and, in human adults, only a few cell types are capable of continued division. Cultured cells in vitro can undergo only a limited number of cell divisions, known as the Hayflick limit, before entering a state of senescence where they remain metabolically active but have lost their replicative capacity (Refs 4, 6). Reduction in telomere length could provide the signal to cause growth arrest. Cultured cells can be induced to continue to divide beyond the Hayflick limit by expression of transforming oncogenes (Refs 4, 7). During this process of oncogenesis, their telomeres continue to shorten with each division and at a certain point cells enter a crisis where the majority will die (Fig. 1). Rare immortalised clones that emerge from crisis express the enzyme telomerase (Ref. 8).

Telomerase structure and function

Telomerase consists of an RNA component, hTR (also known as hTERC), which includes the template for synthesis of telomere DNA, and a protein catalytic component, hTERT, with reverse transcriptase activity and homology to viral reverse transcriptases. These two components are necessary and sufficient for telomerase activity in vitro, although several additional molecules might play a role in regulating in vivo activity (Fig. 2; Table 1) (Refs 9, 10).

Telomerase adds telomeric repeats on to the ends of chromosomes, thus maintaining their length despite continued cell division. It is postulated that, at crisis, critical telomere shortening results in end-to-end fusions and chromosome breakage–fusion cycles that cause marked chromosomal abnormalities and apoptosis (Ref. 5). Expression of telomerase would avoid this catastrophic series of events. This model is supported by the demonstration that forced expression of telomerase allows maintenance of telomere length and increases the replicative lifespan of some cell types without a crisis period. However, events additional to forced hTERT expression might be required to gain full telomerase activity and immortalisation.

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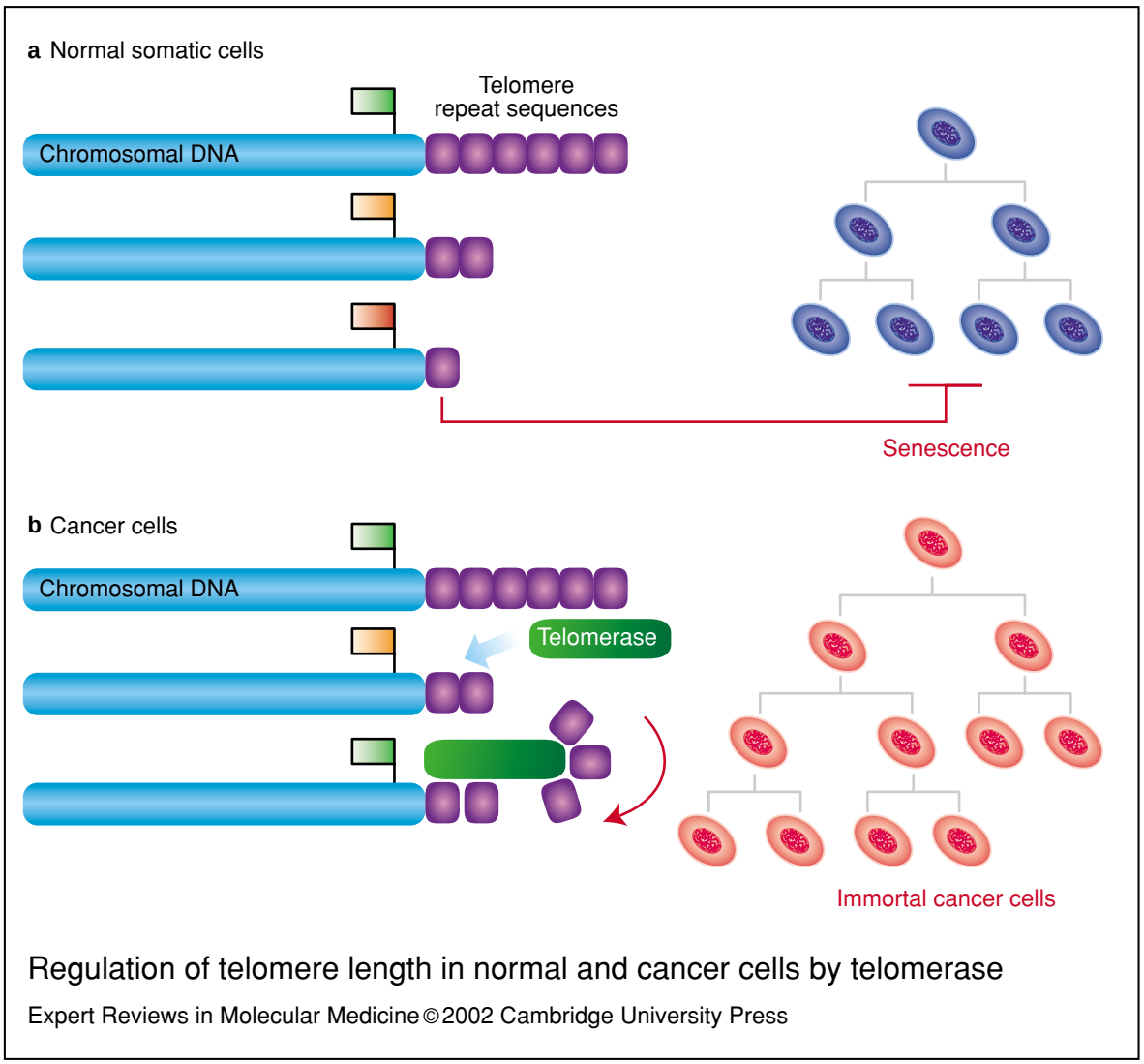

Figure 1. Regulation of telomere length in normal and cancer cells by telomerase. (a) In normal somatic cells telomerase is absent. Every time a normal cell divides, the telomeric repeat sequence (depicted as a purple bar) is lost from the end of the chromosome. Eventually, after many cell divisions, the gradual erosion of the telomere is sensed by the cell (an orange flag on the chromosome in the diagram depicts this event) and, when the telomeres reach a critically short length (red flag), a cell-signalling pathway initiates the senescence programme, resulting in a cessation of cellular proliferation. (b) In cancer cells, the expression of telomerase allows the senescence program to be bypassed (Refs 4, 7). Once activated, telomerase maintains telomeres at a length compatible with cell proliferation through the addition of telomere repeat sequences. Thus, the cancer cell becomes immortal **(fig001nkg)**.

Nevertheless, telomerase expression might be a critical step in the immortalisation process during oncogenesis (Refs 7, 11, 12, 13, 14, 15, 16, 17).

Telomerase expression in human cancer

In adult humans, most normal tissues have low or no detectable telomerase activity (Refs 3, 18, 19). Where telomerase activity has been demonstrated in normal tissue, this has generally been within cells that maintain their replicative capacity into adult life. These include male germ cells, activated lymphocytes, and stem cell populations such as haematopoietic progenitor cells, basal keratinocytes and those in the intestinal crypts (Refs 3, 18, 20, 21). By contrast, telomerase activity is found at high frequency across the whole spectrum of human cancers (Refs 3, 18, 19). However, the frequency of activation

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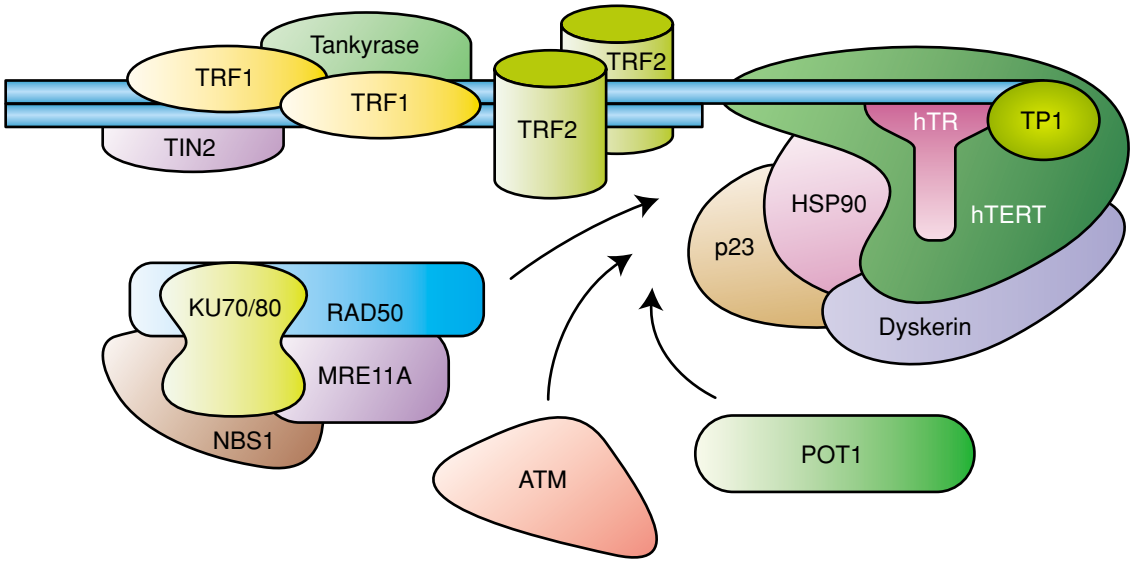

Schematic representation of the composition of telomeric complexes, telomerase and proteins implicated in cellular signalling from the telomere (telomere repair)

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Figure 2. Schematic representation of the composition of telomeric complexes, telomerase and proteins implicated in cellular signalling from the telomere (telomere repair). Telomerase comprises the RNA component hTR, which includes the template for telomere DNA synthesis, and the protein catalytic component hTERT. Additional molecules have been implicated in regulating the in vivo activity of hTR–hTERT and the maintenance of telomere structure. For example, the proteins TRF1, TRF2, tankyrase, TIN2, RAP1 (not shown) and POT1 are all involved in interacting with the telomere and might regulate the opening and closing of the free telomere end and access to the telomere by other protein complexes such as telomerase. A variety of proteins and ribonucleoproteins including HSP90, as well as DKC1, L22, P23 and GAR1 (not shown), might also assist telomerase assembly and facilitate interactions between telomerase and the telomere. Other proteins such as MRE11A, NBS1, KU70, KU80, DNAPK (not shown) and ATM might function in the detection of short telomeres and trigger DNA-damage response pathways or the repair of telomere sequences. Abbreviations and more information on each component can be found in Table 1 and the hyperlinks embedded therein **(fig002nkg)**.

can vary between cancer types, suggesting some tissue-specific regulation (Refs 22, 23).

Telomerase activity is assayed using a very sensitive polymerase chain reaction (PCR)-based assay known as the telomeric repeat amplification protocol (TRAP) (Ref. 24). It is not possible to interpret from TRAP studies based on whole-tissue lysates whether the increase in telomerase activity from normal, through pre-malignant, to invasive carcinoma is due to an increase in telomerase activity in all cells or whether it is due to an increase in the proportion of telomerase-expressing cells within the tissue sampled. However, a study of hTERT expression using in situ hybridisation found a low level of expression primarily in cells within the proliferative zone of the crypts, with a gradual increase in both the level of expression per cell and the proportion of hTERT-expressing cells during progression of colorectal cancer (Ref. 25). Hence, telomerase activation during carcinogenesis might not be the 'all-or-nothing' event observed in tissue culture models of immortalisation.

Given the apparent importance of telomere maintenance in the development of immortality, it is interesting to consider the tumours that are reported as having no detectable telomerase activity, particularly since this group will not be sensitive to telomerase-targeted therapeutics (Refs 26, 27). In some cell or tissue samples,

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Table 1. Human telomerase components, telomere proteins, and proteins involved in the repair of telomeric DNA (tab001nkg)

Telomerase components[a]	Description	Chromosome	Gene accession[b]	Protein accession
TERT (hTERT)	Telomerase reverse transcriptase	5p15.33	NM_003219	O14746
TERC (hTR)	Telomerase RNA component	3q26.3	HSU86046	
HSPCA (HSP90)	Heat shock 90 kDa protein 1, alpha	1q21.2-q22	NM_005348	NP_005339
P23	Telomerase-binding protein, p23	12	XM_006707	Q15185
TEP1 (TP1)	Telomerase-associated protein 1	14q11.2	NM_007110	XP_007488
SSB (La)	Sjogren syndrome antigen B (autoantigen La)	2p14-q14.3	NM_003142	NP_003133
RPL22 (L22)	Ribosomal protein L22	3q26	NM_000983	NP_000974
STAU	Staufen (*Drosophila* RNA-binding protein)	20q13.1	XM_016758	O95793
DKC1	Dyskeratosis congenita 1, dyskerin	Xq28	XM_053357	NP_001354
NOLA1 (GAR1)	Nucleolar protein family A, member 1 (H/ACA small nucleolar ribonucleoproteins)	4q	XM_054788	NP_127460
Telomere proteins				
TERF1 (TRF1)	Telomeric-repeat-binding factor (NIMA-interacting) 1	8q13	XM_016344	XP_016344
TERF2 (TRF2)	Telomeric-repeat-binding factor 2	16q22.1	XM_028687	XP_028687
TNKS (tankyrase)	Tankyrase, TRF1-interacting ankyrin-related (ADP-ribose)polymerase	8q	NM_003747	NP_003738
TNKS2 (TANK2)	Tankyrase, TRF1-interacting ankyrin-related (ADP-ribose)polymerase 2	10q23.3	NM_025235	NP_079511
TINF2 (TIN2)	TERF1 (TRF1)-interacting nuclear factor 2	14q12-14q21.3	XM_033252	XP_007309
RAP1	TRF2-interacting telomeric RAP1 protein	16	XM_033974	XP_033974
POT1	*Homo sapiens* cDNA FLJ11073, putative telomere-end-binding protein		AK001935	
WRN	Werner syndrome (control of genomic stability)	8p12-p11.2	NM_000553	Q14191
ADPRT (PARP)	ADP-ribosyltransferase [NAD^+; poly (ADP-ribose) polymerase]	1q41-q42	NM_001618	P09874
Telomere repair				
MRE11A	Meiotic recombination (*Saccharomyces cerevisiae*) 11 homologue A	11q21	XM_045811	P49959
NBS1	Nijmegen breakage syndrome 1 (nibrin)	8q21	NM_002485	NP_002476
RAD50	Rad50 (*S. cerevisiae*) homologue	5q31	NM_005732	XP_034865
G22P1 (KU70)	Thyroid autoantigen 70 kDa (Ku antigen)	22q13.2-q13.31	NM_001469	P12956
XRCC5 (KU80)	X-ray repair (double-strand-break rejoining; Ku autoantigen, 80 kDa)	2q35	M30938	P13010
PRKDC (DNAPK)	Protein kinase, DNA-activated, catalytic polypeptide (DNA-PKCS) (DNPK1)	8q11	NM_006904	P78527
ATM	Ataxia telangiectasia; involved in signal transduction, cell cycle control and DNA repair	11q22-q23	NM_000051	Q13315

[a] A diagrammatic representation of the possible relationships between some of these components can be seen in Figure 2. The official gene name is given in the first column with any common alternative name in parentheses. The gene names form a hyperlink to GeneCards™.

[b] The gene and protein accession numbers form a hyperlink to GenBank sequence information.

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inhibitors have been found that can interfere with the TRAP assay, giving false negatives. Appropriate handling of samples can avoid this (Ref. 28). Equally, accurate microdissection of tumour cells can increase the proportion of samples found to have telomerase activity (Ref. 29). It is also important to be aware of the potential for false positives resulting from lymphocytic infiltration of samples (Ref. 30). Nevertheless, it is clear that some tumours are truly telomerase-negative (Ref. 31). In some cases, it has been found that cells have developed an alternative mechanism of telomere lengthening (known as ALT) that is thought to involve recombination mechanisms (Ref. 27). Such cells are characterised by long and varied telomere lengths (Refs 27, 32, 33). If alternative mechanisms of telomere extension can be activated by telomerase inhibition, or are latent within telomerase-positive cells, it is conceivable that the use of telomerase inhibitors might select for the ALT phenotype. It is also possible that a subset of telomerase-negative tumours might in fact be mortal but have sufficient replicative capacity to reach significant clinical size (Refs 34, 35). Although admittedly a unique group, this would appear to be the case for so-called stage 4S neuroblastoma, a childhood tumour where a subset of patients present with widespread disease that does not subsequently progress. This group has been found to lack telomerase activity, whereas those with an aggressive phenotype are telomerase-positive (Refs 36, 37).

Finally, it should be remembered that although telomerase activity has been detected in all major human malignancies, it is not a prerequisite for tumourigenesis (Refs 7, 38, 39). Indeed, while it is clear that many tumour types, including small-cell lung cancer, are good candidates for anti-telomerase therapy, telomerase positivity of clinical samples derived from other cancer types such as non-small-cell lung cancers is variable (Refs 23, 40). A study of telomerase activity in small-cell versus non-small-cell lung cancers (Ref. 40) recorded a high level of TRAP positivity in 100% of samples from primary small-cell lung cancers and their metastases, but only 69.2–88.5% positivity, varying with histology, in primary non-small-cell samples, and only 50% positivity in metastases derived from non-small-cell lesions. The model proposed to explain this heterogeneity suggested that large solid tumours that display a variable frequency of telomerase positivity could contain a fraction of partially transformed cells that would be deemed to be mortal. Whether or not this might present a problem for telomerase-based therapies is unclear, as the growth of mortal subpopulations within a heterogeneous solid tumour, or the metastases derived from them, might be self-limiting, although it is also conceivable that spontaneous immortalising events might occur post-therapy.

In summary, although, for various reasons, some cancers do not express increased levels of telomerase, most cancers do show increased levels and activity of this ribonucleoprotein. Consequently, an increased understanding of its biology and regulation is of immense interest to the future development of anti-cancer strategies.

Regulation of telomerase occurs at multiple levels

The current picture of telomerase regulation is complex. Enzyme activity is likely to be controlled on several levels, with multiple pathways converging to modulate the functional activity of the holoenzyme (Fig. 2; Table 1). Although the story is far from complete, several regulatory pathways have already been implicated in the normal and aberrant activity of telomerase in human cells. This section briefly outlines the major mechanisms involved; these are discussed in more detail below, with reference to relevant therapeutic targeting opportunities emerging from our current understanding.

Extensive evidence from expression studies suggests that hTR and hTERT are regulated on a transcriptional level and that this regulation is a major deterministic factor governing the activation of telomerase activity in normal and cancer cells. Although much remains to be clarified, cloning of the promoter regions for the genes encoding hTR and hTERT has enabled the identification of several positive and negative regulators of telomerase transcription (Refs 41, 42, 43).

Other work has demonstrated that post-transcriptional mechanisms play a role in regulating telomerase activity. Kilian et al. (Ref. 44) identified several splice variants of the hTERT transcript that are expected to be inactive due to truncations or mutations in domains essential for catalytic activity. Of particular interest, one variant termed hTERTα contains a deletion in the conserved reverse transcriptase motif A and has been characterised as a dominant-negative

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inhibitor of hTERT activity (Refs 45, 46). Several studies have begun to examine more precisely the role that alternative splicing might play in the regulation of telomerase activity in various tissues (Refs 47, 48, 49, 50, 51, 52).

Telomerase activity can be reconstituted in vitro from its two essential subunits, hTR and hTERT (Refs 53, 54), although the enzyme exists in its active form at the telomere as a highly ordered multi-subunit complex. The structure of the holoenzyme therefore represents another level at which telomerase activity is likely to be regulated (Fig. 2; Table 1). Some of the constituent proteins that contribute to the complex have intrinsic regulatory functions, such as the poly (ADP-ribose)polymerase (PARP) domain of tankyrase, a protein identified as interacting with the telomeric-repeat-binding factor TRF1 at the telomere (Ref. 55). PARP activity is a major mechanism for post-translational regulation of nuclear proteins involved in a variety of cellular functions such as the DNA damage response (Ref. 5). It is now clear that these other post-translational signalling events acting directly on hTERT or on other proteins involved in the complex play a role in regulation of telomerase activity. The phosphorylation status of hTERT is also involved in modulation of the catalytic activity of telomerase: both protein phosphatase 2A and the c-Abl tyrosine kinase act as negative regulators of telomerase function, whereas protein kinase C (PKC) and the Akt protein kinase upregulate activity (Refs 56, 57, 58, 59, 60, 61, 62, 63).

The unique biology and function of telomerase, together with the complexity of its regulation, afford several potential targeting opportunities directed at various levels (Table 2). Many of the therapeutic strategies proposed to target telomerase in cancer have been tested extensively in vitro, and within available in vivo models, and it seems certain that some of these telomerase-based therapeutics will soon find their way into clinical trials. The sections below discuss several of the possible targets.

Potential telomerase-directed molecular therapeutics

Targeting transcriptional regulation: modulation of promoter activity by regulatory genes or drugs

An attractive approach for the development of novel anti-cancer therapies is to interfere with the transcription of genes involved in cancer, either by targeted expression or transduction of relevant regulatory molecules or by screening for promoter-interactive drugs (Table 2) (Ref. 64). It is therefore extremely important to understand the underlying mechanisms governing hTR and hTERT transcription. For diagrammatic representations overviewing what is currently known about the transcriptional regulation of hTR and hTERT, see the BioCarta pathways http://www.biocarta.com/pathfiles/tercPathway.asp (for hTR) and http://www.biocarta.com/pathfiles/tertPathway.asp (for hTERT).

Targeting promoter regions

The hTR promoter can be positively regulated by binding of the zinc finger transcription factor SP1 at several sites in the proximal promoter, whereas binding of the transcription factor SP3 represses promoter activity (Ref. 41). In addition, the retinoblastoma gene product, pRb, upregulates hTR promoter activity (Ref. 41) and binding of the NF-Y transcription factor to a CCAAT box in the promoter region is essential for activity (Ref. 41). Interestingly, transfection of a dominant-negative mutant of NF-YA inhibits hTR promoter activity, suggesting a possible strategy for development of transcriptional inhibitors directed against the specific activity of NF-Y at the hTR CCAAT box.

Studies of mechanisms governing hTERT transcription have similarly revealed several positive and negative regulatory factors. The c-Myc oncoprotein has been found to be a positive regulator of hTERT transcription (Ref. 43). The Myc–Max/Mad–Max network of transcription factors are intrinsically involved in the control of cell proliferation, cell-cycle progression and apoptosis, and this signalling network is often deregulated in cancer. Myc and Mad form individual heterodimers with Max and the resultant transcriptional regulators recognise the same DNA sequence and antagonise the effects of one another. It is likely that regulation of c-Myc-mediated transcription at the hTERT promoter is the result of competitive binding between these transcription factor complexes. However, it is becoming increasingly clear that full transcriptional activity of the hTERT promoter requires the SP1 transcription factor (Refs 65, 66, 67, 68, 69, 70, 71). Given that the hTR promoter is also regulated by SP1 (Ref. 41), there might be overlapping, although not necessarily co-ordinated, control mechanisms shared between hTR and hTERT.

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Table 2. Potential telomerase-based molecular therapeutics (tab002nkg)

Inhibition of telomerase/telomere function

Molecular target	Inhibitor	Potential examples/Comments
Telomerase gene transcription	Upstream signal transduction inhibitors Transcription factor inhibitors	Retinoids, tamoxifen NF-YA mutant
Telomerase transcripts	Oligonucleotides Ribozymes	Use of antisense hTR RNA Target hTR- and hTERT-specific sequences for ribozyme cleavage
Holoenzyme assembly and telomere interaction	Reverse transcriptase inhibitors Small-molecule inhibitors Inhibitors of telomere structure and capping	Azidothymidine (AZT); not specific for telomerase FJ5002 Potential for more-rapid action, but specificity for cancer cells uncertain

Telomerase-promoter-directed gene therapy

Promoter	Therapeutic	Comment
hTR or hTERC	Pro-drug activating systems (e.g. bacterial nitroreductase) Transporters of radiolabelled pharmaceuticals Apoptotic mediators (e.g. Bax, caspases) Toxin genes (e.g. diptheria toxin A)	Potential to cause immediate specific cancer cell death as a result of tumour-specific telomerase expression

Telomerase immunotherapy

Approach	Comment
Treatment of antigen-presenting cells with antigenic telomerase peptides to increase cytotoxic T lymphocytes specific for cancer cells expressing telomerase	Potential for broadly active anti-tumour immune response

The tumour suppressor protein p53 and the cell-cycle regulator E2F-1 negatively regulate hTERT transcription (Refs 69, 71, 72). In one recent study (Ref. 71), introduction of wild-type p53 into a cervical carcinoma cell line by means of a recombinant adenovirus was shown to downregulate telomerase activity, highlighting the potential of studies of transcriptional regulation to identify therapeutic opportunities. Although no obvious growth inhibition or apoptosis was observed as a result of p53 transfer in this study, the investigators did not analyse the growth-inhibitory effects of chronic suppression of telomerase activity under long-term culture conditions.

Targeting upstream signalling

An alternative approach to interfering with telomerase expression is the modulation of upstream signalling events that lead to transcriptional activation. For instance, it is now clear that regulation of telomerase activity can be mediated by differential actions of a variety of hormones. Retinoids have been reported to downregulate telomerase activity and induce differentiation of leukaemia cells, whereas estrogen and androgens are thought to upregulate this activity.

The effects of retinoids can be mediated by signalling through two related classes of receptor – retinoic acid receptors (RARs) and retinoic X receptors (RXRs) – in a pathway that appears to involve cyclic adenosine monophosphate (cAMP). A panel of promyelocytic leukaemia cell lines that express variants of the RAR-α subunit have been used to study the effects of retinoids on cellular differentiation and telomerase activity (Ref. 73). The parental cell line differentiates in the presence of retinoids, whereas two sub-lines do not. However, one of the sub-lines is competent to

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undergo maturation via RXR signalling in the presence of cAMP-elevating agents. Using these cells, Pendino and co-workers (Ref. 73) were able to dissect the various retinoid signalling pathways and to demonstrate downregulation of hTERT mRNA and of telomerase activity by RAR-dependent signalling in a pathway distinct from differentiation, suggesting that retinoids might be therapeutically useful even against maturation-resistant cells.

Misiti and co-workers (Ref. 74) identified binding of estrogen receptor α (ER-α) at a consensus sequence for the estrogen response element (ERE) at position –949 to –935 of the hTERT promoter. Further analysis showed that ER-α could activate de novo transcription of hTERT in the presence of estrogen, and thereby upregulate telomerase activity in telomerase-negative cells derived from estrogen-responsive tissues. Tamoxifen competes with estrogen binding at the ER-α and is one of the most active hormonal agents in the treatment of ER-α-expressing breast carcinoma. It has been shown that tamoxifen can downregulate telomerase activity and viability of a human breast carcinoma cell line (Ref. 75). Thus, targeting upstream signalling pathways that lead to promoter activation, such as those mediated by hormones, might prove to be an attractive approach for the treatment of specific disease types.

Post-transcriptional targeting: targeting hTR and hTERT RNA

The hTR component of telomerase has provided a target for antisense approaches based either on blocking the function of the mRNA template region, by base pairing to a complementary sequence, or by actively targeting the mRNA molecule for degradation, by the use of molecules such as hammerhead RNA enzymes (ribozymes) (Table 2) (Refs 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99). One problem with using RNA molecules as therapeutic agents is their instability when administered as drugs. For this reason, ideal antisense agents are administered either encoded within an expression construct such as a plasmid vector or a virus, or as chemically stabilised analogues of the active RNA. Several approaches have been developed to stabilise RNA, and some of the stabilised molecules have been applied to anti-telomerase studies (Ref. 97).

Telomeric antisense RNA

A retrovirus engineered to express a UUAGGG oligomer, complementary to the template sequence of hTR RNA, was demonstrated to potently inhibit TRAP activity, as well as decrease telomere length and viability, in two human kidney carcinoma cell lines (Ref. 91). One way in which oligonucleotides can be stabilised is by phosphorothioate modification of the phosphodiester backbone. Phosphorothioate oligodeoxynucleotides (PS-ODNs) directed against the hTR RNA component demonstrated potent inhibitory effects on telomerase activity (Ref. 71). However, the precise mechanism of action of PS-ODNs has been proposed to be independent of sequence; indeed, evidence was presented in one study (Ref. 76) that PS-ODNs directed against the template region of hTR elicited their effects in a sequence-independent manner. Moreover, because PS modification of the backbone of a telomeric sequence primer enhances telomerase activity, it was suggested that inhibitory effects could be attributed to interaction with the primer-binding site of hTERT rather than the hTR template region.

Hammerhead ribozymes

Another approach to targeting RNA is the use of hammerhead ribozymes. Hammerhead ribozymes, named because of their shape, are short catalytic sequences of RNA 40–50 bases in length, consisting of a catalytic domain with ribonuclease activity against trinucleotide sequences, preferentially GUC, flanked on either side by specific complementary sequences that direct the ribozyme to its target RNA, thereby promoting cleavage of the target (Ref. 100).

Several potential sequences for ribozyme cleavage exist in hTR and, although not all of the specific sites have been evaluated, of most interest is a target trinucleotide inside the template region at position +44 to +46. Several groups have developed ribozymes directed against this sequence. The ability of these molecules to downregulate telomerase activity has been demonstrated in two endometrial carcinoma cell lines (Refs 98, 99), two melanoma cell lines (Ref. 92), three surgical specimens of melanoma (Ref. 92), and extracts of two human hepatocellular carcinoma cell lines (Ref. 94). However, conclusions from these studies regarding the influence of hTR inhibition on cell proliferation and regulation of telomere

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length were unclear. Indeed, in the study of Folini et al. (Ref. 92), no significant effects on cell proliferation and telomere length were observed after 20 population doublings in stable clones of malignant melanoma cells with reduced telomerase activity. By contrast, Yokoyama et al. (Ref. 98) reported a reduction in telomere length and slowed cell division in all stable endometrial cell lines tested. Kanazawa et al. did not evaluate the effects of their ribozyme in vivo (Ref. 94).

More recently, Yokayama et al. (Ref. 99) reported that the 5' untranslated region of hTERT mRNA also presents a suitable sequence for targeting of ribozymes. Several ribozymes directed against sequences throughout the hTERT mRNA were developed, but most failed to demonstrate anti-telomerase activity, possibly because of secondary structural elements of the hTERT message. However, ribozyme directed against the 5' end was able to cleave hTERT mRNA and downregulate telomerase activity. Another hTERT-directed ribozyme, targeted to the T motif (which might be involved in the hTR–hTERT interaction) of hTERT mRNA, was able to cleave hTERT mRNA, downregulate telomerase activity, reduce telomere length and induce apoptosis in human breast carcinoma cell lines when delivered in an adenoviral vector (Ref. 95). Moreover, stable clones harbouring the anti-hTERT ribozyme showed an enhanced apoptotic response to the chemotherapeutic agents doxorubicin and etoposide, which act at least in part by inhibition of topoisomerase activity, suggesting that anti-telomerase strategies might be useful as part of a combinatorial anti-cancer approach.

2,5-A oligonucleotides

Specific RNA sequences can also be actively targeted for degradation by the use of 5'-phosphorylated 2'-5'-linked oligoadenylate (2,5-A) oligonucleotides. Incorporation of this moiety into antisense oligonucleotides can actively target RNAseL to specific RNA sequences. This approach has been successfully used against the hTR component to downregulate telomerase activity and induce apoptosis of malignant glioma cells, both in culture and in subcutaneous and intra-cranial xenograft models (Refs 78, 84, 101). However, it is worth noting that, in these studies, massive apoptosis occurred in the target cell populations within 4–5 days post-treatment, a time period that is inconsistent with effects dependent on telomere shortening but more consistent with effects associated with telomere uncapping (discussed below).

Targeting telomerase holoenzyme structure and function

Among the most convincing evidence that telomerase inhibitors could be used to genuine therapeutic effect are the results of studies in which dominant-negative hTERT mutants were introduced into human cancer cells (Refs 102, 103, 104, 105). The investigators were able to show telomere shortening and, with continued passage, cells underwent senescence and apoptotic cell death. The advantage of dominant-negative mutants over reverse transcriptase inhibitors clearly lies in the specificity of inhibition. However, in order to adapt such an approach to a therapeutic environment, an appropriate delivery system is necessary. Indeed, a more feasible approach would be to screen for small-molecule inhibitors of telomerase that mimic the hTERT dominant-negative reagents (Table 2). Nevertheless, the hTERT dominant-negative studies serve as a valuable proof of principle for the notion that telomerase inhibition is indeed a valid therapeutic approach.

Non-specific reverse transcriptase inhibitors

Several investigators have examined strategies based on the use of reverse transcriptase inhibitors to downregulate the functional activity of telomerase (Table 2) (Refs 106, 107). One of the best-characterised reverse transcriptase inhibitors currently in use in a therapeutic setting is azidothymidine (AZT) (Refs 106, 107), a potent inhibitor of human immunodeficiency virus 1 (HIV-1)-encoded reverse transcriptase and, therefore, one of the major drugs prescribed for the management of HIV infection. Although AZT is not specifically targeted to telomerase, and the activated analogue is a general inhibitor of polymerase activity, several studies have examined the effects of AZT on telomerase activity in cancer cell lines and have shown an inhibitory effect, although the therapeutic value is uncertain. AZT was able to inhibit cloning efficiency in four human breast cancer cell lines and could inhibit telomerase activity in a dose-dependent fashion (Ref. 107). However, another study into the effects of AZT on human lymphocytes concluded that the telomerase-inhibitory effects of AZT were

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not cytotoxic but merely led to a transient suppression of cellular growth that could be reversed by removal of the drug (Ref. 108).

Post-translational modification

It is now clear that post-translational modification of constituents of the telomerase complex plays an important role in modification of enzyme activity. Upregulation of enzyme activity through phosphorylation by PKC isoforms α and ζ has been described in breast and nasopharyngeal cancer cells, respectively, whereas both c-Abl tyrosine kinase, which is a major player in the DNA damage response, and protein phosphatase 2A have been reported to downregulate activity (Refs 56, 57, 58, 60, 62, 109). Specific inhibition of PKC, using compounds such as bisindolmaleimide or antisense mRNA against specific PKC isoforms, also downregulates telomerase activity (Refs 57, 58). Although it is acknowledged that the actions of kinases such as PKC regulate multiple complex signalling pathways, this might represent another level at which drugs could be developed to target telomerase activity (Table 2).

Small-molecule inhibitors

The rhodacyanine FJ5002 has been identified as a telomerase inhibitor following screening for compounds with pharmacological properties resembling those of the moderate telomerase inhibitor berberine (Ref. 110). FJ5002 was found to be considerably more potent than berberine as an inhibitor of telomerase activity. Furthermore, it was demonstrated to act in the fashion expected of a 'classical' telomerase inhibitor in that continued passage of cells in the presence of FJ5002 led to replication-dependent shortening of telomeres with a concurrent increase in aneuploid metaphases and apoptotic cells. Thus FJ5002 might be one of the first genuine anti-telomerase agents and a useful lead compound for further drug development programmes aimed at targeting telomerase.

However, a major advance in the development of novel chemical compounds that selectively inhibit telomerase has recently been made by the Boehringer Ingelheim pharmaceutical company in collaboration with its academic partners (Ref. 111). Small-molecule inhibitors of telomerase were identified by screening against nuclear extracts containing telomerase. These lead compounds were shown to exhibit all the hallmarks desired of a true telomerase inhibitor: namely, they had no effect on short-term cell viability or proliferation, and cancer cells expressing telomerase showed a reduction in telomere length on exposure to the drugs. Inhibition of cell proliferation was observed only after a lag period and, importantly, only in telomerase-positive cells. In addition, inhibition was independent of the p53 status of the cells, suggesting that these inhibitors might have wide use in human tumours. Interestingly, inhibition of tumourigenic potential was also seen in vivo using xenografts in nude mice and oral administration of the drug – the latter a key point in the successful transfer of this therapeutic to the treatment of human tumours. This study also made an exhaustive analysis of gene expression patterns using microarray technology in the inhibitor-treated cells and identified specific patterns of gene expression associated with cellular senescence. This analysis of gene expression patterns alone might be of considerable value in determining the mechanisms by which telomerase inhibitors activate the signal transduction pathways resulting in senescence, and might therefore facilitate the rational development of drugs targeting this pathway.

Telomere-directed molecular therapeutics

The essential, protective role performed by the telomere, in protecting the chromosome ends from being recognised as DNA damage or from undergoing aberrant fusions, is believed to be mediated by a cap of proteins associated with the telomere (Refs 112, 113). Thus, another way in which telomerase-positive cells could be targeted in a manner that is specific, but not dependent on telomere shortening, is by targeting the cap, the removal of which would initiate DNA damage pathways (Table 2).

In a recent study (Ref. 112) in breast and prostate cancer cell lines and in a xenograft model, it was demonstrated that ectopic expression of template-mutated telomerase RNAs, designed to have sequences not recognised by telomere-binding proteins, led to decreased DNA synthesis and cell proliferation without either telomere shortening or inhibition of the endogenous telomerase activity. Strategies targeted at telomeric uncapping might represent an effective way to target cancer cells more rapidly than by the 'classical' effects of inhibition of telomerase

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activity (Ref. 96). However, it might be difficult to maintain the specificity for cancer cells, and unacceptable toxicity to normal tissues might result if the telomeric structure of normal cells is also disrupted.

The identification of drugs that act by disruption of the telomere structure is of considerable interest (Table 2). At the very terminus of the telomere there is a short single-stranded overhang of guanine-rich sequence (Ref. 5). In vitro, this sequence can produce a structure known as the G-quadruplex. It has been suggested that these structures, should they exist in vivo, might be involved in several cellular processes, including telomere capping and termination of telomere elongation by telomerase (Refs 114, 115, 116). Therefore, compounds that interact with G-quadruplex structures, such as substituted acridines, might represent a new class of specific telomerase-targeting agents. Several groups have applied molecular modelling approaches to the design of compounds that interact with these sequences and have been able to demonstrate inhibition of telomerase activity (115, 116). Once again, however, there might be a danger in such an approach if the telomeric structure of normal cells is also disrupted.

Gene therapy directed by telomerase promoters

Ultimately, the therapeutic strategies discussed above all aim to inhibit telomerase activity. However, it is probable that pharmacological inhibition of telomerase activity will not be immediately cytotoxic to cancer cells – a phenomenon known as 'phenotypic lag'. The classic action of telomerase inhibitors will be to cause erosion of telomeric sequence over several cell divisions until critically short telomeres signal senescence and cell death to the cancer cell. Thus, on administration, the cancer volume might initially continue to increase prior to the desired therapeutic reduction in tumour volume (see below). An alternative strategy is to exploit the tumour-specific expression of the genes encoding telomerase in order to target cytotoxic molecules to cancer cells and cause immediate cell killing (Table 2). An essential characteristic of any promoter-driven therapeutic strategy must be its ability to target cancer cells while leaving normal cells relatively unaffected. From this point of view, the hTR and hTERT promoters are excellent candidates for achieving tumour-specific expression of therapeutic molecules, and the initial data from telomerase-promoter-directed gene therapy systems that have been reported show considerable promise, as summarised below (Ref. 117).

Gene therapy strategies

Majumdar and co-workers (Ref. 118) described an expression system comprising the hTERT promoter and the herpes simplex thymidine kinase (HSTK) system that sensitised tumour cells derived from osteosarcoma, pancreatic cancer, medulloblastoma and fibrosarcoma to the effects of the pro-drug gancyclovir. In this study, three normal human fibroblast cell lines, as well as normal retinal pigmented epithelial cells, were unaffected. Moreover, in vivo transduction with adenoviral vectors containing the expression construct resulted in decreased tumour volumes as well as prolonged survival in mice bearing osteosarcoma-derived xenografts, with no increase in the liver enzymes or histopathology that would be associated with an unwanted cytotoxic effect on the normal liver.

The transcriptional regulatory sequences of both the hTR and hTERT promoters have been used to drive expression of the bacterial nitroreductase gene (Ref. 119), which converts the pro-drug CB1954 to a cytotoxic form. This study demonstrated clear differentials in the activity of both promoters between normal and cancer cell lines, resulting in efficient cell killing in tumour cell lines derived from cervical, ovarian, lung and colon cancers. Moreover, sensitisation to the pro-drug CB1954 was retained in vivo in xenografts of the cell lines. In this model, a single administration of the drug could significantly reduce tumour volumes in cervical and small-cell lung cancer cells. However, a subset of telomerase-positive cancer cell lines with low promoter activity were not sensitised, suggesting a limiting dependence on high promoter activity.

Several studies have examined the effects of restricting the expression of apoptotic mediators to tumour cells using hTERT promoter sequences. Genes for Bax (Ref. 120), caspase-8 (Ref. 121), a novel form of caspase-6 that has been engineered to be constitutively active (Ref. 122), and Fas-associated protein with death domain (FADD) (Ref. 123) have been variously expressed. These studies have demonstrated the induction of

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apoptosis in tumour cell lines derived from malignant glioma, malignant melanoma, breast and lung cancer; by contrast, expression and apoptosis were not detected in normal human cell lines. Efficient cell killing has also been demonstrated using both hTR and hTERT promoters to drive expression of the diptheria toxin A gene (Ref. 124).

One of the most efficient ways to kill cancer cells is with radiation. However, the clinical use of radiation to treat cancer patients is limited by its toxicity to normal tissue. Boyd and co-workers have successfully used the hTR promoter to drive expression of the gene encoding the noradrenaline transporter (NAT) in glioma cells (Ref. 125). Expression of the NAT gene induces active uptake of the radiopharmaceutical [^{131}I]-metaiodobenzylguanidine (MIBG) and results in efficient glioma cell killing through radiation-induced damage.

Thus, an efficacious and selective anti-tumour effect through use of telomerase-based targeting has been described in a large number of cells derived from tissues of unrelated origin, and this effect has been preserved in vivo in xenograft models and in an adenoviral model of delivery. Moreover, as telomerase-based targeting potentially provides us with two promoters of differing strengths and tissue specificity, and since several therapeutic transgene systems have already been described, there is the potential for the development of different combinations of promoter–transgene constructs for use in different situations. However, the optimisation of gene therapy approaches will probably require both transcriptional restriction and efficient gene delivery strategies.

Telomerase immunotherapy

Enhancement of the anti-tumour immune response has recently aroused considerable interest as a therapeutic approach and has been applied to telomerase (Refs 126, 127, 128, 129, 130, 131, 132, 133). One way in which immune responses can be specifically targeted to particular antigens is by the ex vivo manipulation of autologous antigen-presenting cells (APCs) such as macrophages or dendritic cells.

Antigenic peptide sequences are processed by the APC proteasome and are transported to the endoplasmic reticulum where they can interact with the products of major histocompatibility (MHC) alleles such as the MHC class I human leukocyte antigen (HLA)-2. Subsequent budding of the Golgi and transport to the cell surface results in the presentation of antigen–MHC complexes for interaction with the T-cell receptor (TCR) of CD8$^+$ populations of cytotoxic T lymphocytes (CTLs) (see http://www-ermm.cbcu.cam.ac.uk/smc/swf001smc.htm for an animation of this process). It has been demonstrated repeatedly that peptides that interact with MHC molecules can be introduced into APCs, either by direct transduction with protein or mRNA molecules or by expression from DNA vaccines, and thereby enrich specific CTL populations in vitro and in vivo (Ref. 133). Moreover, it has recently been shown that several peptide sequences of the hTERT protein match consensus sequences for interaction with HLA-2 and can be naturally processed (Ref. 127). This suggests that there might be an endogenous and pre-existing anti-TERT immune response that could be enhanced by ex vivo manipulation of autologous APCs to generate a broadly active anti-tumour immune response. Indeed, transduction of peripheral blood mononuclear cells from a prostate cancer patient (Ref. 127) with HLA-2-interactive sequences from hTERT resulted in an enriched population of anti-hTERT specific CTLs. These CTLs were able to lyse HLA-2$^+$hTERT$^+$ tumour cell lines derived from ovarian cancer, malignant melanoma and multiple myeloma, in addition to freshly isolated primary tumour cells from patients presenting with acute myeloid leukaemia and non-Hodgkin's lymphoma, whereas normal blood cells from the same patients were not affected.

Clinical perspectives: translating telomerase therapeutics into clinical trials

The early clinical evaluation of telomerase-based therapeutics presents specific challenges and requires the design of appropriate models for pre-clinical evaluation. The efficacy of telomerase inhibitors and gene therapy approaches will depend on the presence of telomerase enzyme activity and gene expression, respectively, and it is therefore essential that telomerase-based strategies are tested in appropriate patient populations – that is, patients with telomerase-positive tumours. Although this eligibility criterion will increase the patient population that will need to be screened, it will increase the likelihood of observing a significant effect from

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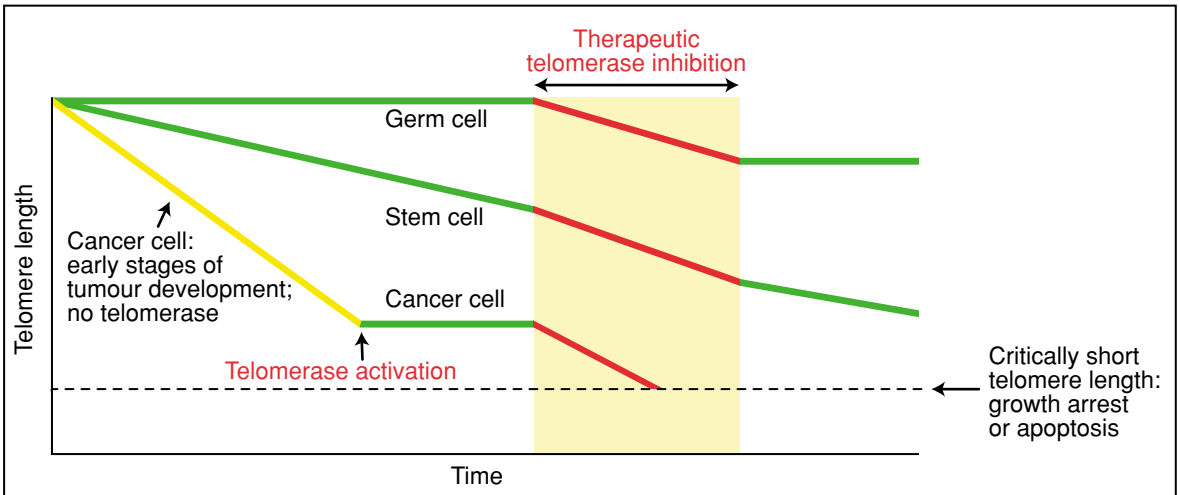
the experimental therapy in early studies, and in turn could potentially decrease the number of patients required in Phase III studies to demonstrate a significant difference compared with the control patient population.

Conventional drug development involves the assessment of toxicity and pharmacokinetics (Phase I), the demonstration of activity (Phase II) and, finally, comparison with existing standard practice (Phase III). With conventional cytotoxic drugs, the maximally tolerated dose generally equates with the maximally effective dose. However, this is not necessarily the case with molecular therapies. Consequently, it is appropriate to evaluate pharmacodynamic end-points such as telomerase inhibition, telomere shortening or induction of markers of senescence within tumour biopsy tissues (Refs 1, 134) in Phase I trials, even though assessment of toxicity, as determined by standard criteria, remains the primary end-point within these studies.

Traditionally, the conventional end-point in demonstrating activity of cytotoxic chemotherapy agents in Phase II studies has been objective tumour response as determined by a reduction in tumour dimensions, usually on radiological assessments. However, if our assumptions regarding classical telomerase inhibitors are correct, it is anticipated that a cytotoxic effect will occur only after several population doublings, once telomeres have shortened to a critical length (Figs 3 and 4). In patients with measurable, advanced disease, which make up the usual Phase II study population, the tumour will undergo only a limited number of cell doublings before the disease progresses radiologically. Consequently, it is possible that if the conventional criterion of objective tumour reduction is applied as an end-point of efficacy in these Phase II studies, the activity of telomerase inhibitors might be inappropriately underestimated. Selecting patients with short telomere lengths for entry into studies remains an intriguing notion, but this approach has significant practical difficulties that preclude it at present. Thus, demonstration of a desired biological effect in Phase II studies should justify proceeding to Phase III studies in optimal patient populations.



The potential effects of telomerase inhibition over time on telomere length and proliferative capacity in cancer cells compared with nomal human germ cells and stem cells

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Figure 3. The potential effects of telomerase inhibition over time on telomere length and proliferative capacity in cancer cells compared with normal human germ cells and stem cells. Both human germ cells and stem cells are considered to be telomerase-positive or competent to express telomerase, and so inhibition of telomerase should affect telomere length in these cells. However, tumour cells frequently have shorter telomeres than telomerase-competent normal cells and would therefore be expected to reach a critically short telomere length, leading to growth arrest or apoptosis, at an earlier stage **(fig003nkg)**.

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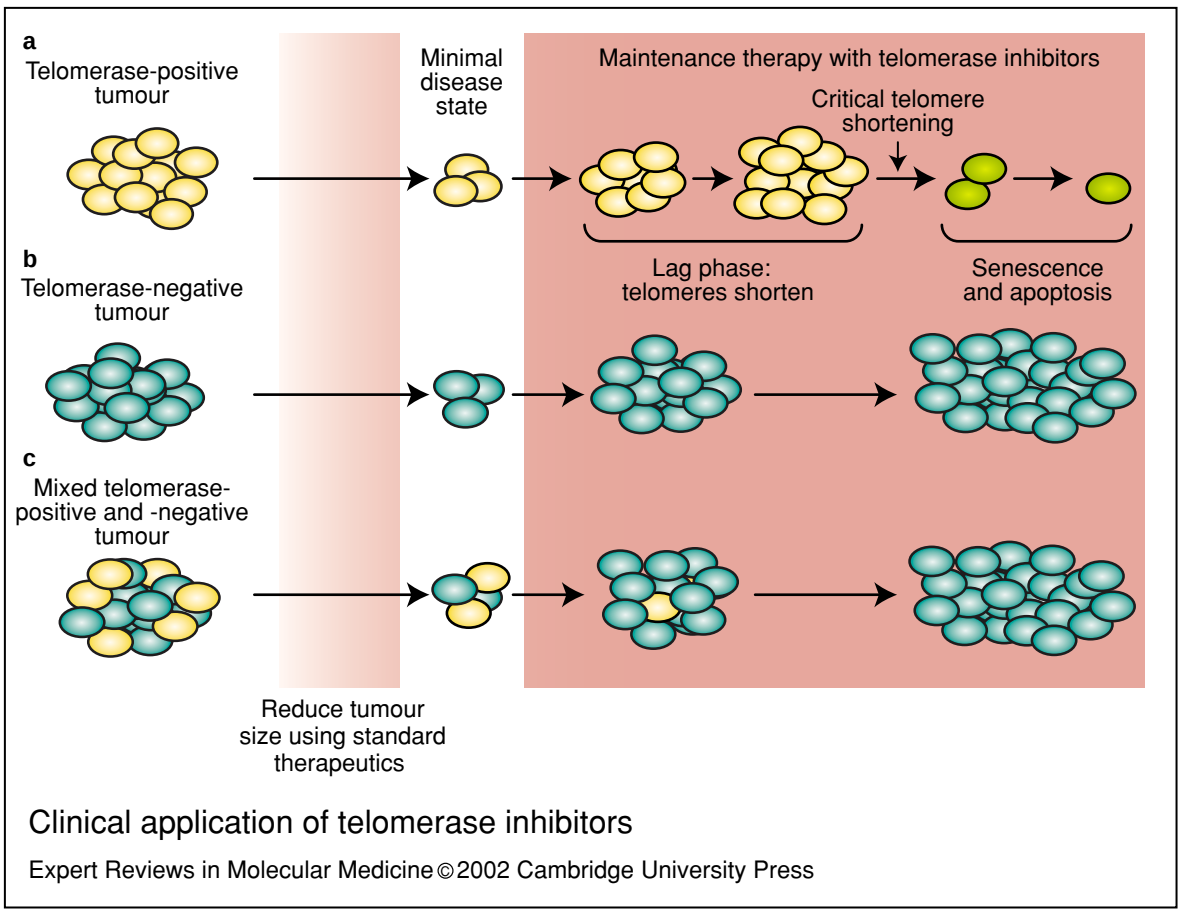

Figure 4. Clinical application of telomerase inhibitors. The classic action of telomerase inhibitors will be to cause erosion of telomeric sequence over several cell divisions until critically short telomeres signal senescence and cell death to the cancer cell (a). Thus, on administration, the cancer volume might initially continue to increase prior to the desired therapeutic reduction in tumour volume (this is known as the lag phase). Therefore, telomerase inhibitors are likely to have their greatest clinical impact in minimal disease states, for example as maintenance therapy after tumour debulking by chemotherapy, as this would allow time for telomerase agents to have effect. Telomerase inhibitors will work only on telomerase-positive cells. Thus, telomerase-negative tumours (either mortal tumours or tumours that maintain their telomeres by alternative mechanisms to telomerase – i.e. ALT mechanisms) will be resistant to telomerase inhibitors (b). There are now numerous assays to test for telomerase activity, enabling tumours to be tested for suitability for telomerase therapy. However, these assays might fail to detect mixed populations of telomerase-positive and telomerase-negative cells in the tumour population (c). In this situation, although the tumour might be predicted to respond to telomerase inhibitors, only the telomerase-positive fraction of tumour cells will be targeted, leaving the ALT cells to repopulate the tumour mass **(fig004nkg)**.

It is anticipated that classical telomerase inhibitors will ultimately have their greatest clinical impact in 'minimal disease states'. With a small tumour bulk, or with micrometastatic disease, the number of cell doublings that need to occur before the telomeres are eroded to a critical length is unlikely to have an adverse clinical effect. In this situation, progression-free or relapse-free survival can be used as a measure of efficacy. However, evaluating progression-free or relapse-free survival in non-randomised Phase II trials in minimal disease states does not give any useful information, as Phase II populations from different trials cannot meaningfully be compared with each other or with historical controls. Consequently, once again, convincing evidence of biological activity will be required in Phase II studies in advanced disease to justify proceeding to randomised Phase III trials in patients with minimal disease states, which will

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have significant implications in terms of both patient numbers and resources. Furthermore, the development of robust surrogate assays is paramount, since serial biopsies are not readily available in most tumour types.

In which patient population might it be appropriate to investigate telomerase inhibitors within Phase III trials? Small-cell lung cancer might provide an excellent target with which to study telomerase inhibitors (Ref. 23). In most cases, small-cell lung cancer is highly sensitive to chemotherapy, with good tumour debulking, but the disease almost invariably relapses in 6–12 months and responses to second-line treatment are poor and of short duration. Furthermore, small-cell lung cancer shows high levels and frequency of telomerase activity and gene expression. Consequently, in a Phase III trial, patients with small-cell lung cancer who had responded to chemotherapy would be randomised to a telomerase inhibitor or placebo, with time to progression as the primary end-point of efficacy. Similarly, patients with telomerase-positive tumours who have undergone potentially curative surgery (e.g. for gastro–oesophageal cancer) could be randomised into an adjuvant Phase III study of telomerase inhibitor versus placebo, with relapse-free and overall survival as end-points of efficacy.

In contrast to telomerase inhibitors, gene therapy strategies that use telomerase gene promoters to target cancer cells with therapeutic genes should result in objective tumour responses without a time lag (Ref. 119). Therefore, the early clinical development of this approach will include the conventional end-points of toxicity, pharmacokinetics and tumour response as an indication of activity, but will also require the identification of appropriate patients on the basis of expression of genes for the telomerase components in tumour tissue (Refs 23, 134, 135). In general, the introduction of telomerase gene therapy approaches into the clinic might therefore be more straightforward than that of drugs that inhibit telomerase, as telomerase gene therapy most closely resembles existing therapeutic approaches and does not pose any major new issues in clinical trial design.

Therefore, molecular profiling of tumours with respect to telomerase activity, telomerase component gene expression, telomere length and markers of senescence will be an integral part of both identifying appropriate patients for telomerase therapeutic trials as well as monitoring the response and outcome of these therapeutic strategies.

Concluding remarks

The science of telomerase gene regulation and its therapeutic applications is evolving rapidly. The simple concept that telomerase is either on or off in cells is gradually being replaced by a rather more complex picture of regulated expression and a continuous range of activity. Thus, a detailed understanding of telomerase regulation will be required for any therapeutic applications.

Although telomerase-directed therapies are an extremely exciting prospect, the complexity of telomerase biology suggests that they might not be universally applicable. Furthermore, because of the problem of phenotypic lag of telomerase-based therapeutics, many tumours might become lethal before the inhibitor is effective. It will therefore be necessary to evaluate the use of telomerase inhibitors in combination with other conventional treatment modalities such as tumour debulking (using radiotherapy, chemotherapy or surgery) in order to allow time for telomerase agents to have effect (Fig. 4).

By contrast, it is also possible that telomerase inhibitors might sensitise cells to conventional anti-cancer agents. For instance, Kondo et al. (Ref. 84) reported that inhibition of telomerase activity by stable expression of an anti-hTR RNA increased the sensitivity of human malignant glioma cells to cisplatin-induced apoptosis in vitro and decreased overall viability, and Ludwig et al. (Ref. 95) showed that an anti-hTERT ribozyme could enhance the apoptotic effect of topoisomerase inhibitors.

These studies and treatment schedules are encouraging. However, the progression of telomerase inhibitors to the clinic will require the development of new tissue culture and animal models for pre-clinical testing of efficacy and toxicity. Indeed, current mouse models might be inappropriate for pre-clinical studies of telomerase therapeutics because of the marked differences in telomere biology and telomerase regulation between humans and mice (Refs 41, 104, 136).

Finally, it is worth noting that telomerase therapeutics offer a large number of diverse opportunities. Telomerase expression and activity can vary greatly even among cancers, yet it is clear that many cancers have high levels of telomerase

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and are totally dependent on this activity for their long-term survival. A major challenge of the future will be in combining telomerase inhibitors with conventional therapeutics in order to bring telomerase therapeutics successfully to the clinic.

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Further reading, resources and contacts

Research news

There are now several web services that bring together topical information and up-to-date summaries of research news. Many have search facilities, e-mail alert systems or personalisation of information.

BioMedNet: http://reviews.bmn.com/?subject=cancerbiology

The Scientist: http://www.the-scientist.com/

TheScientific World: http://thescientificworld.com/

BioMed Central: http://www.biomedcentral.com/

Cancer and cancer therapy

The National Cancer Institute website contains information on cancer, resources for scientists and news items.

http://www.nci.nih.gov/index.html

Medscape provides healthcare information and digital data for healthcare professionals and consumers.

http://www.medscape.com/Home/Topics/oncology/oncology.html

OncoLink presents cancer-related information for cancer patients, families, healthcare professionals and the general public.

http://www.oncolink.com/

The Cancer Genetics*Web* is primarily intended for health professionals and researchers. It provides comprehensive links to reliable information about genes, their associated proteins, and genetic mutations associated with cancer and related disorders. Each gene page includes links to major genetic databases and where possible links to other related websites, abstracts, external searches, and summary information.

http://www.cancerindex.org/geneweb/index.htm

Medicine OnLine provides medical information and education in oncology and HIV/AIDS, Medline® literature searches, daily medical news, cancer discussion groups, and reports from medical meetings, for healthcare professionals, patients, and other interested consumers.

http://www.meds.com/

Genes and cell signalling resources

BioCarta is an interactive web-based resource for life scientists. The site provides access to existing life science research and integrates new data as they emerge. Information falls into four categories: gene function, proteomic pathways, ePosters and research reagents.

http://www.biocarta.com/

GeneCards™ is a database of human genes, their products and their involvement in diseases. It offers concise information about the functions of all human genes.

http://bioinformatics.weizmann.ac.il/cards/

The US National Center for Biotechnology Information (NCBI) creates public databases, conducts research in computational biology, develops software tools for analysing genome data, and disseminates biomedical information. The site includes GenBank, a public resource for DNA and protein sequences.

http://www.ncbi.nlm.nih.gov/

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Companies producing telomerase/telomere products

Intergen: kits for telomerase activity measurements in cell and tissue extracts.

http://www.intergenco.com/index_flash.html

Dako: kits for in situ detection of telomere sequences.

http://www.dako.com/default.htm

BD Biosciences Clontech: telomerase-immortalised cell lines.

http://www.clontech.com/index.shtml

BD Biosciences Pharmingen: kits for telomere and telomerase detection.

http://www.bdbiosciences.com/pharmingen/

Roche Applied Science: kits for telomerase detection, telomere detection and telomerase gene expression.

http://www.biochem.roche.com/

Geron: involved in drug discovery, regenerative medicine and oncology.

http://www.geron.com/

Telomerase websites

The Shay/Wright laboratory's website covers the concepts of telomeres and telomerase, and includes informative animations.

http://www.swmed.edu/home_pages/cellbio/shay-wright/intro/sw_intro.html

Animation of telomerase activity created by Donald Slish at SUNY, Plattsburgh:

http://faculty.plattsburgh.edu/donald.slish/Telomerase.html

Animations of telomerase activity and telomerase detection methods from the Goertz laboratory, University of Stuttgart:

http://www.uni-stuttgart.de/bio/zoologie/teloweb_e.htm

Authors' laboratory homepage

The Cancer Research UK Laboratories in Glasgow, UK, houses nearly 20 research groups working towards understanding cancer growth and its treatment.

http://www.beatson.gla.ac.uk/

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