ELSEVIER

European Journal of Cancer 41 (2005) 971–979

European Journal of Cancer

www.ejconline.com

Review

Overcoming the immortality of tumour cells by telomere and telomerase based cancer therapeutics – current status and future prospects

Lloyd R. Kelland *

Antisoma Research Laboratories, St. Georges Hospital Medical School, Cranmer Terrace, London SW17 OQS, UK

Received 4 November 2004; accepted 24 November 2004
Available online 1 April 2005

Abstract

A key property of malignant tumours is their immortality or limitless replicative potential. Cell replication is associated with the maintenance of telomeres and in the great majority of cases, through the reactivation of the reverse transcriptase telomerase. Targeting the telomere/telomerase machinery offers a novel and potentially broad-spectrum anticancer therapeutic strategy since telomerase is constitutively overexpressed in the vast majority of human cancers. Telomeres are also critically short in most tumours compared to normal tissues. Strategies that exploit these differences include the direct targeting of components of telomerase: the protein component hTERT or RNA component hTR. Examples of such agents include the small molecule hTERT inhibitor BIBR1532 and GRN163L, a thio-phosphoramidate oligonucleotide targeting the template region of hTR as a "template antagonist". Anti-tumour effects have been observed in both cell lines and, especially for GRN163L, in xenografted human tumours in mice. Effects, however, are largely dependent upon initial telomere length, which can result in a substantial lag before antitumour activity is observed in tumours possessing relatively long telomeres. An alternative approach is to target the telomere itself (Telomere Targeting Agents, TTAs). Several classes of small molecules have been described that induce the G-rich single-stranded overhang of telomeric DNA to fold into 4-stranded G-quadruplex structures. Such folding is incompatible with telomerase function and may induce rapid telomere uncapping. These molecules have shown potent telomerase inhibition in nanomolar concentrations *in vitro* and the rapid induction of senescence in cancer cells. The trisubstituted acridine based TTA, BRACO19, has demonstrated single agent activity against human tumour xenografts with anti-tumour effects apparent from only 7 days of treatment. In the near future, it is expected that lead examples from both the direct telomerase targeted agents (e.g., GRN163L) and from the distinct class of those targeting telomeres (e.g., AS1410 based on BRACO19) will enter Phase I clinical trial where clinical benefit from this class of novel drugs will be determined.

1. Introduction

Five years ago, a commentary was provided on telomerase as an anti-cancer target [1]. At that time, the key observation from the Shay laboratory [2] describing the specific association of telomerase activity with immortal cells and cancer was 5 years old and had been substantiated by numerous other studies using cancer cell lines and clinical biopsy material. Much has been learned since, both at the level of cell and molecular biology and in the context of cancer therapeutics. Telomerase-based therapeutics includes: using gene promoters of the various components of telomerase for gene-therapy strategies (e.g., to activate prodrugs) and using telomerase peptides, proteins or RNA as vaccines for immunotherapy. This review will focus on therapeutic attempts to target telomerase activity with emphasis on those that

* Tel.: +44 208 772 4393; fax: +44 208 767 1809.
E-mail address: lloyd@antisoma.com.

0959-8049/$ - see front matter © 2005 Elsevier Ltd. All rights reserved.
doi:10.1016/j.ejca.2004.11.024

act on the telomeres themselves since this approach has recently produced small molecule leads with biological properties that support clinical testing.

2. Rationale for targeting telomerase/telomere maintenance mechanisms

Ideal cancer targets are those that (a) are specifically expressed on or in tumours and are not present in any normal cells/tissues and (b) where the target is critically involved in maintaining the malignant phenotype. How well does telomerase/telomeres fulfil these criteria?

One of the key properties of tumours is deregulated proliferation. This is achieved through self-sufficiency in growth signals, insensitivity to anti-growth signals and resistance to apoptosis [3] and many targets for therapeutic intervention have arisen from our increased knowledge of the underlying mechanisms involved in these acquired properties. However, this deregulation is not sufficient on its own to ensure continued tumour growth. Normal human cells in culture possess a finite replicative potential through the induction of senescence (M1) and then crisis (M2). This 'counting' mechanism for cell divisions is widely believed to occur *via* telomere attrition. Telomeres are specialised, complex, structures that cap the ends of eukaryotic chromosomes and comprise a repetitive sequence of DNA (TTAGGG in mammals) that varies among different mammals (see below) and various associated proteins (see [4]). Some of these associated proteins, such as the TTAGGG repeat binding factors 1 and 2 (TRFs), appear to be involved in controlling telomere length. There is also evidence for a guanine-rich telomere extension or overhang of a few hundred bases which, when exposed, induces cellular senescence [5]. However, under normal circumstances this region is protected (capped) by proteins such as protection of telomeres 1 (Pot1) [6], and by telomerase (see below) and is folded back into a loop motif [7]. Due to the inability of DNA polymerases to replicate the ends of linear molecules, on each cell division 50–200 bases of telomeric DNA is lost. Tumours, in order to achieve immortality, need to maintain telomere length and integrity. In the vast majority of cases (approx 90%), this is achieved through the reactivation of a reverse transcriptase mechanism, where telomerase [2] adds TTAGGG units to telomeres [8]. The genes encoding the two major components of human telomerase, the RNA portion hTR (or hTERC) and the reverse transcriptase hTERT, were cloned in the mid 1990s [9,10]. A role for telomerase in contributing to tumorigenesis has been demonstrated by cultured normal human cells being transformed by ectopic expression of the *ras* oncogene, simian virus 40 small and large T antigens and hTERT [11], although, in some cases, the role of hTERT in this context may not depend on its ability to maintain telomere length [12]. In addition, the lifespan of several normal human cell types can be extended by ectopic expression of hTERT [13].

On rare occasions, tumours maintain telomeres (at particularly long lengths, typically >17 kb) by a non-telomerase-based mechanism, known as alternative lengthening of telomeres (ALT). This is characterised by high rates of telomeric exchange [14], is proposed to occur by means of homologous recombination [15] and, at least theoretically, could provide a mechanism of resistance to telomerase inhibitors (see below). Although comparatively rare (<10% overall) some tumour types such as osteosarcomas [16] and gliomas [17] appear to use this mechanism more often than many epithelial derived tumours.

Since the original observation concerning telomerase expression in cancer and in normal tissue where it was limited to germline tissues [2], telomerase expression in some normal cells (e.g., haematopoietic stem cells [18] and keratinocytes [19]) has now been subsequently shown. Recently one report concluded that transient telomerase (hTERT) expression during S-phase maintains telomere structure in normal human fibroblasts [20]. However, due to large differences in constitutive telomerase expression between tumours and most adult somatic tissues, there is still a potential window of selectivity provided by the fact that tumours generally maintain their telomeres at critically short lengths (2–6 kb; Table 1). Moreover, there is considerable evidence that telomere shortening is an early alteration in human tumorigenesis and is seen in precancerous lesions in the prostate [21], pancreas [22] and other epithelial tissues, including breast [23]. One could hypothesise that tumours, in contrast to normal tissues (including telomerase positive stem and germ cells), are more critically

Table 1
Telomere lengths in various species

		Telomere length (kb)	Reference
Mouse	*Mus musculus*	40–100	[78]
	Mus spretus	5–15	[79]
Rat		20–100	[80]
Dog		12–23	[81]
Sheep		~20	Personal communication
Primate	Cynomolgus (PBMCs)	14–15	[82]
Human	Adult somatic cells	7–12	[83]
	Germ cells, stem cells	9–13	[84]
	Cancer cell lines (NCI 60 cell line panel)	2–6	[85]
	Colon cancer biopsies	5.7 (median)	[86]
	Adjacent normal colon	6.8 (median)	[86]
	Myeloma	5.6 (median)	[87]
	Healthy donors (plasma cells)	10.6 (median)	[87]

dependent on telomerase to prevent telomere length reduction/dysfunction/uncapping with resulting senescence/apoptosis. Hence, overall, it could be that a therapeutic window exists in Man for drugs that inhibit telomerase and/or interfere with the telomerase/telomere machinery. Table 1 also shows that telomeres in mice (*Mus musculus*) and rats are substantially longer than in humans. This may have implications in terms of the predictive interpretation of telomerase biology in mTR (mouse RNA component) knock-out mice (where phenotypic changes were delayed until the seventh generation [24]) and for toxicology studies of telomerase inhibitors in mice and rats.

2.1. Genetic evidence supporting targeting hTR, hTERT or telomeres

From the above described cell and molecular biological information, it is apparent that the telomerase machinery offers an attractive novel strategy to target cancer. Many possible approaches are feasible (Fig. 1), several of which are now being pursued (see below).

2.1.1. Targeting hTR, hTERT

There is a variety of particularly compelling supportive data for telomerase representing a good cancer therapeutic target. For example, two independent studies have shown that transfection of dominant-negative mutants of hTERT into human cancer cell lines inhibited the growth of these cells. The onset of inhibition correlated with the initial telomere length of each cell line [25,26]. These findings highlighted the concern that a prolonged lag period was seen in therapeutic response for tumours possessing relatively long telomeres (e.g. no change in growth was seen in 36M cells of 5–7 kb telomere length until approximately day 35) [25]. Most, but not all, studies using antisense strategies to either hTR or hTERT revealed similar findings with the onset of biological effects dependent on initial telomere length (e.g., using peptide nucleic acids) [27]. However, some recent studies, e.g., using ribozymes directed to the catalytic subunit of hTERT [28] or antisense oligodeoxynucleotides to hTERT mRNA [29] have shown more rapid growth inhibition, independent of telomere shortening, and possibly relating to telomere uncapping.

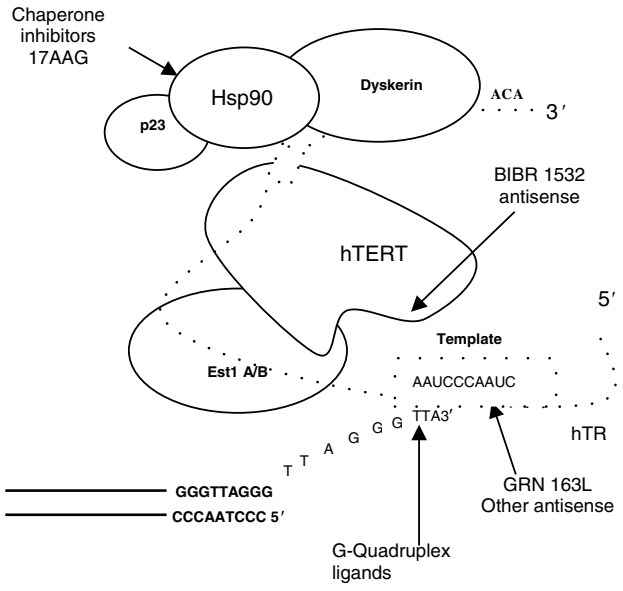

Fig. 1. Targeting the telomerase complex.

2.1.2. Targeting telomeres

As shown above, telomeres are shorter in telomerase-positive tumours than in corresponding normal tissues and especially in relation to stem cells (Table 1 for summary). Genetic proof of principle for targeting telomeres has arisen in recent years, in both yeast and human cells. These studies show that expression of mutant template telomerase RNA in human cells (by transfection) rapidly decreased cell viability and increased apoptosis [30–32]. These rapid effects occurred independent of p53 status and initial telomere length, did not require telomere shortening and only occurred in telomerase positive cells. Recently these observations have been extended using lentiviral delivery where tumorigenesis of human cancer cell lines containing mutant template hTR was suppressed in mice [33]. These rapid effects are proposed to occur *via* telomere uncapping leading to activation of DNA damage response genes, such as the cell cycle inhibitor *p21* [33].

In conclusion, genetic proof of principle has been demonstrated with respect to targeting and inhibiting telomerase and/or telomere maintenance. As well as these approaches, it may also be possible to induce antitumour effects *via* indirect mechanisms that regulate telomerase expression. These include inhibitors of chaperones such as HSP90 (e.g., 17-allylamino, 17-demethoxygeldanamycin; Fig. 1) [34], inhibitors of hypoxia-inducible factor 1 HIF1α [35], inhibitors of the nuclear factor κB pathway [36] or modulators of the pRb/E2F1 pathway [37].

3. Targeting telomerase (hTERT, hTR) directly or targeting the telomere?

3.1. Inhibitors of hTERT or hTR

Targeting the active site of hTERT has been reported using some small molecules including nucleoside analogues such as AZT (3′-azido-2′,3′-dideoxythymidine) [38] but lack of selectivity for telomerase compared to other polymerases has limited this approach. The most extensively studied non-nucleoside molecule is BIBR1532 (Fig. 2), which inhibits telomerase *in vitro* with a half-maximal inhibitory concentration (IC_{50}) of

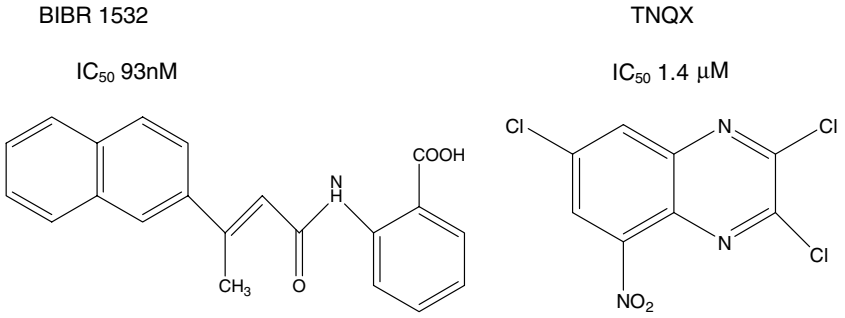

Fig. 2. Small molecule inhibitors of telomerase.

93 nM [39]. BIBR1532 is a mixed-type non-competitive inhibitor of telomerase with a proposed binding site distinct from the sites for deoxyribonucleotides or the DNA primer [40].

However, the cellular and *in vivo* (rodent) pharmacological properties of BIBR1532 have highlighted a major drawback of directly targeting hTERT or hTR. Studies have shown phenotypic lag between enzyme inhibition and cellular growth arrest, apoptosis and tumour growth delay. Cell-based data with the compound are in accordance with the genetic (dominant negative hTERT) experiments described above in that cell proliferation was slowed only after an extensive lag-phase during which time telomeres progressively shortened to critical lengths (e.g., after 120 days of exposure of NCI-H460 non-small cell lung cancer cells) [39]. As expected, no change in growth was seen in a telomerase negative cell line (normal human lung fibroblasts) or in an osteosarcoma cell line (SAOS-2), which maintains telomere length by the ALT mechanism. Because of the long lag period required for telomere shortening, *in vivo* antitumour effects were only demonstrable by pre-exposing cells (HT1080 fibrosarcoma) *in vitro* for over 100 days thereby reducing telomere length to only 1.6 kb prior to implanting in nude mice. The times taken for tumours to form after subcutaneous injection of 1.5 million cells were 4.53 days for control cells of telomere length of approx 4 kb and 16.5 days for control cells where telomere length had been reduced *in vitro* to 1.6 kb. Treatment of another group of mice with 100 mg/kg/day BIBR1532 given orally from the day of implantation of cells (where telomere length had been reduced *in vitro* to 1.6 kb) resulted in a significant reduction in the number of animals bearing tumours of >1000 mm^3 on day 43 [39]. Hence, despite potent direct inhibition of telomerase by BIBR1532 in cell-free assays, its *in vivo* antitumour efficacy is marginal and probably insufficient to merit clinical testing. More recently a second molecule, 2,3,7-trichloro-5-nitroquinoxaline (TNQX) (Fig. 2) with similar mixed-type non-competitive inhibition (IC_{50} of 1.4 μM) properties to BIBR1532 has been described [41]. Again, cellular effects (growth inhibition, induction of senescence) required extensive periods of agent exposure.

A second general strategy to achieve direct targeting of telomerase (hTERT or hTR) is through various antisense-based molecules. The most advanced is GRN163L (Geron Corporation): a 13-mer thio-phosphoramidate oligonucleotide targeted to the template region of hTR as a 'template antagonist' rather than working through the classical antisense mechanism of RNase-H activation. GRN163 (the original molecule without a lipid carrier) inhibited telomerase activity in cells at nanomolar concentrations where uptake was facilitated by lipid carriers such as Lipofectamine [42,43]. As with BIBR1532, antitumour effects were largely dependent upon telomere length. Firstly, little effect on growth was seen in U266 multiple myeloma cells (long telomeres, 9 kb) after 56 days of cell culture in the presence of GRN163, whereas growth inhibition was apparent from 28 days in MM.1S multiple myeloma cells (short telomeres, 2.5 kb) [44]. Secondly, intratumoural administration of GRN163 inhibited the growth of multiple myeloma and non-Hodgkin lymphoma xenografts established from cell lines with short telomeres (2.7 kb) but had less effect on xenografts with long telomeres (11 kb) [45]. Thirdly, daily intraperitoneal injection of GRN163 for 3 weeks to mice bearing CAG myeloma xenografts (telomeres of 2.7 kb) also reduced tumour volumes [45] and similar effects were also observed using DU145 prostate cancer xenografts (telomere length of 2.8 kb) with long-term intraperitoneal administration (14–19 mg/kg/day *via* minipumps) [43]. Finally, antitumour activity has also recently been demonstrated in U-251 human glioblastoma xenografts following intratumoural administration of GRN163 by either repeat injection or *via* osmotic minipumps [46]. GRN163L (containing a palmitoyl lipid conjugated covalently to an aminoglycerol thiophosphate linker) is currently undergoing late-stage preclinical development, including regulatory toxicology and safety pharmacology in non-human primates, in preparation for Phase I clinical evaluation.

3.2. Targeting the telomere (telomere targeting agents, TTAs)

As stated above, telomeres are shorter in tumours than in normal cells and are essentially repetitive sequences of duplex DNA plus proteins that cap the ends of chromosomes [47]. An inherent design challenge to telomere targeting is to avoid therapeutic agents from interacting non-specifically with other regions of the genome, which would likely cause non-selective cytotoxic effects. However, since the early 1990's, a potential means to selectively target telomeric DNA was identified which exploits the ability of G-rich sequences of DNA, like telomeres, to fold into 4-stranded (quadruplex) intramolecular structures [48]. Since the first demonstration of telomerase inhibition by a G-quadruplex interactive compound (a 2,6-diamidoanthraquinone) in 1997 [49] a number of other pharmacophores have been described (cationic porphyrins such as TMPyP4 [50], trisubstituted acridines such as BRACO19 [51]; the natural product telomestatin also known as SOT-095 [52]; dibenzophenanthrolines [53]; ethidium derivatives [54]; triazines such as 12459 [55]; pentacyclic acridines such as RHPS4 [56]; fluoroquinophenoxazines such as QQ58 [57], see Fig. 3). Many first generation G-quadruplex ligands suffered from a relative lack of selectivity for 4- *versus* 2-stranded DNA resulting in similar potencies for inhibiting telomerase *in vitro* and for producing non-specific acute cytotoxicity [58]. Later molecules (Fig. 3) have improved potency for telomerase inhibition such as BRACO19 aided by the resolved crystal structure of parallel quadruplexes from human telomeric DNA [59]. Further impetus to the development of G-quadruplex ligands has been provided by the demonstration of quadruplex structures *in vivo*, in a ciliate using antibodies [60] and from human cells using a fluorescent probe [61].

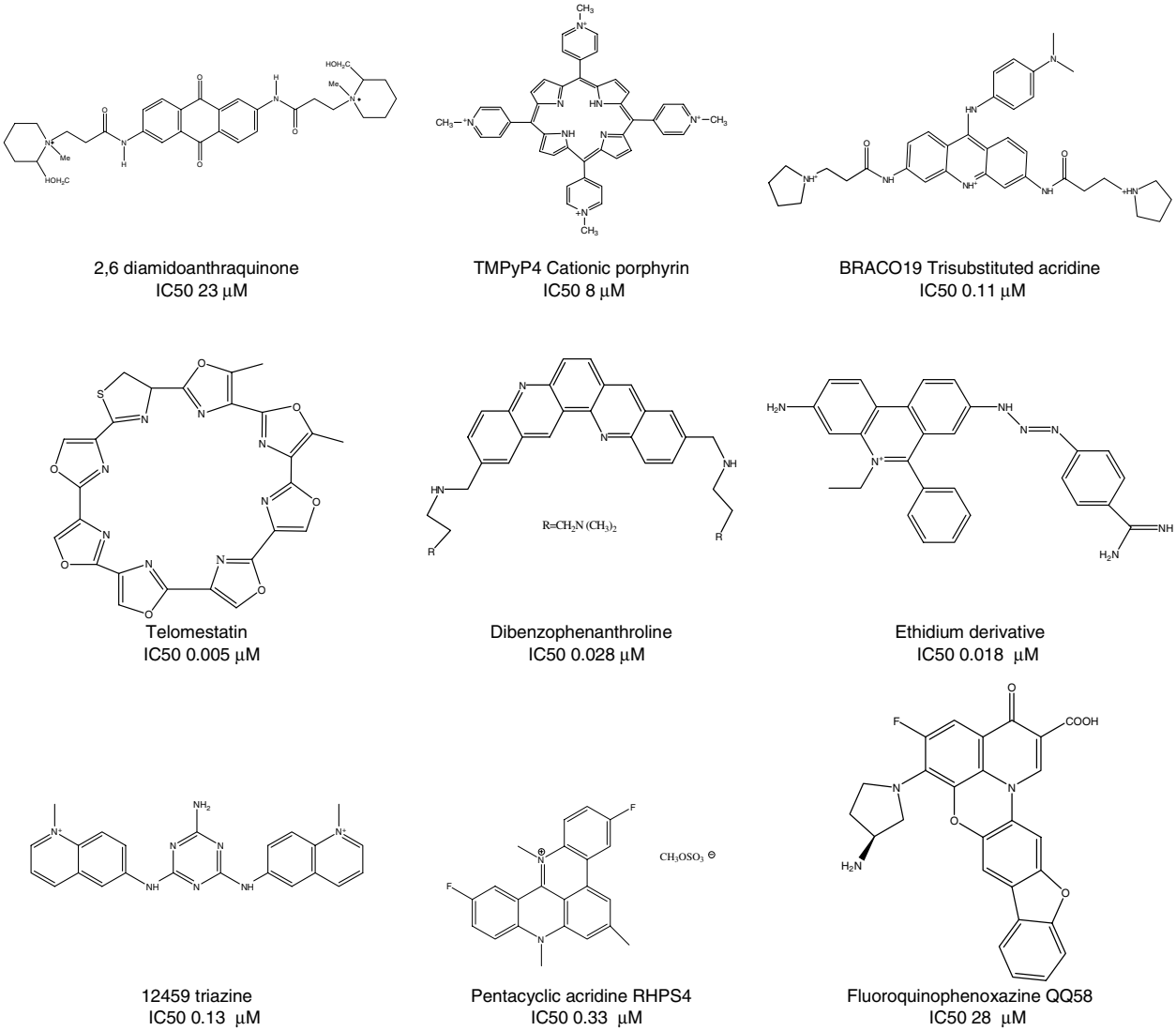

Fig. 3. Telomere targeting agents (TTAs). Values are for potency in cell free assays to inhibit telomerase.

There is now increasing evidence that most G-quadruplex ligands confer their biological activity through interaction with the telomeric G-rich single-strand overhang and thereby affecting telomere capping state and leading to relatively rapid senescence and/or apoptosis [62,5]. As shown genetically by TRF2 overexpression, senescence may be induced by altered telomere state rather than telomere loss [63]. For example, the TTA BRACO-19 has been shown to induce extensive end-to-end chromosomal fusions in DU145 human prostate cancer cells and senescence from 7 days post exposure to non-acute cytotoxic concentrations [64]. Senescence was associated with an increase in the cyclin-dependent kinase inhibitor p21 (as described above for cells transfected with mutant template hTR, [33]) [64]. This is consistent with a senescence pathway being triggered by telomere shortening or loss of integrity [65] and activation of a DNA damage response pathway from dysfunctional telomeres [66]. The cellular effects of RHPS4 in some human melanoma cell lines were associated with telomere dysfunction (telomeric fusions, polynucleated cells and telophase bridges) [67] while direct evidence for binding to the single-strand overhang has recently been reported for telomestatin [68]. Resistance to senescence induction and telomere shortening by the triazine-based G-quadruplex ligand 12459 in JFA2 cells (derived from the A549 human non-small cell lung cancer line) was associated with increased telomerase activity and increased telomere length [69]. In some cases however, as with the porphyrin TMPyP4, interaction with intermolecular rather than intramolecular G-quadruplex structures appears to be favoured [70]. Intermolecular TMPyP4 G-quadruplex structures could also be responsible for the reported repression of *c-myc* oncogene transcription mediated through interaction with a G-quadruplex structure formed in the c-*myc* promoter region [71].

While the cell-free telomerase inhibition and cellular (growth inhibition, senescence) properties of many G-quadruplex ligands have been reported, thus far, little *in vivo* efficacy data has been reported. A notable exception is with the trisubstituted acridine, BRACO19 (Anti-

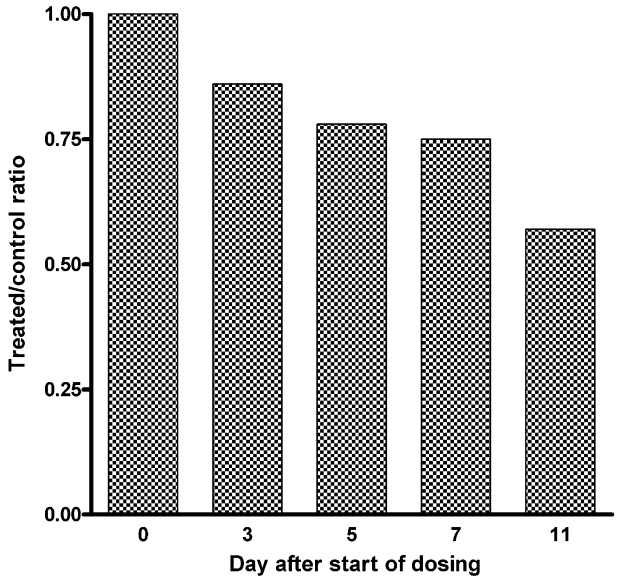

Fig. 4. Single-agent antitumour activity of BRACO19 against DU-145 prostate cancer xenografts. Dosing was 2 mg/kg, intraperitoneally on days 1 to 5 and 8 to 12 following establishment of tumours.

soma). Initial results showed a significant increase in antitumour efficacy when used in combination following dosing with paclitaxel in a human vulval carcinoma xenograft (A431) [72]. Moreover, recently, significant single-agent growth inhibition of 96% in comparison to controls has been demonstrated with BRACO19 against a uterus carcinoma xenograft model (UXF1138LX [73] and against DU-145 prostate cancer xenografts (Fig. 4), both using a schedule of daily intraperitoneal dosing at 2 mg/kg for 2–3 cycles of 5 daily doses. Notably, in both experiments and in contrast to antitumour studies described above with the 'direct' hTERT inhibitor BIBR1532, antitumour effects were manifest within 7–10 days of the initiation of treatment. In addition, the porphyrin TmPyP4 has demonstrated *in vivo* efficacy against a human prostate cancer xenograft within 15 days after starting treatment [74]. These data hold considerable promise with respect to the clinical potential of G-quadruplex ligands (summarised in Table 2).

Table 2
Potential of telomere targeting agents using G-quadruplex ligands

Positives
- Potential for broad-spectrum antitumour activity, especially in tumours possessing relatively short telomeres or requiring telomerase to maintain telomere capping status
- Onset of antitumour effect is generally more rapid (days) than observed using direct hTERT inhibitors such as BIBR1532
- Potential for synergistic combination with radiotherapy and cytotoxic cancer drugs, especially those inducing a DNA damage response pathway

Potential drawbacks
- Normal tissue toxicity due to quadruplex formation in other regions of the genome and presence of telomerase in stem cells and germ cells – but selectivity for tumours maybe achievable since cancer cells have shorter telomeres than in stem cells and proliferate more
- Possibility, not seen to date, of rapid resistance to G-quadruplex ligand *via* the ALT pathway

4. Conclusions and future directions

There is now a plethora of data, both genetic and using small molecules that establish preclinical proof of principle for targeting telomerase/telomere maintenance in a cancer therapeutic context. However, the underlying biology is complex with respect to determining the inter-relationships between telomere length, capping status and telomerase activity in tumours *versus* normal tissues and the predictive utility of evaluating efficacy and toxicology in the mouse (where telomeres are abnormally long compared to humans).

In general terms, direct targeting of telomerase usually results in a lag between enzyme inhibition and reduced tumour cell proliferation, the length of the lag being dependent upon initial telomere length and has recently been modelled with respect to cancer treatment by telomerase inhibitors [75]. For clinical application, this approach may require the pre-selection of patients with tumours possessing relatively short telomeres or use in preventative or post surgery/cytotoxic chemotherapy settings but haemopoietic stem cell repopulation may be an issue. By contrast, telomere targeting agents (TTAs), such as the trisubstituted acridine BRACO19, have shown significant single agent antitumour activity *in vivo* with effects manifest within a few days of treatment initiation. These agents appear to exploit the critical necessity of telomeres, especially in tumours where they are relatively short, to be retained in a capped state by telomerase and other telomere-associated proteins. Targeting or disrupting this machinery has been demonstrated (using both genetic and small molecule G-quadruplex interactive agents) to induce rapid cellular senescence and apoptosis. Whether using a 'direct' hTR or hTERT inhibitor or the TTA class it is probable, post early clinical evaluation, that these agents will be used in combination with established cytotoxics, radiotherapy or other moleculary-targeted agents. Interestingly, telomere dysfunction (in cells from mTR knock-out mice) has been shown to confer hypersensitivity to some cancer drugs, especially doxorubicin that induce DNA double-strand breaks [76].

Small molecules have now been described which show antitumour activity in preclinical models at non-toxic doses and thus may themselves (or pharmaceutically optimised analogues) be considered as preclinical development candidates. However, some major areas of concern to be resolved, prior to Phase I and especially Phase II trials, are (1) optimal scheduling regimes (2) predictive markers of tumours/patients most likely to respond to this class of agent and pharmacodynamic markers of response and (3) the selection of relevant species to conduct regulatory toxicology and safety pharmacology studies. While, TTAs might provide broad-spectrum utility across a wide spectrum of cancer types (both solid tumours and haematological malignancies) it is probable that the greatest effects will be seen in tumours possessing relatively short telomeres and/or in those which are critically dependent upon the maintenance of capped telomeres. Pre-selection of patients with such tumours would be desirable and may be necessary (e.g., using measures of telomere length in biopsy material and through the development of sensitive assays of capping status). In addition, markers (ideally surrogates using peripheral blood or *via* non-invasive imaging using PET) for providing a rapid readout of responding patients would also be useful. Appropriate preclinical human tumour xenograft experiments could prove useful in this context as described for other classes of agents (e.g., HSP90 inhibitors; [77]) as well as for resolving scheduling issues. As described above (Table 1), mice have relatively long telomeres and different telomerase biology (reflected by the fact that unlike human samples, mouse cells can sometimes spontaneously immortalise in cell culture). This places possible doubt on the predictive clinical utility of findings from regulatory toxicology and safety pharmacology studies conducted in mice. Of note however, is that dogs possess telomeres of a similar length to those present in humans (Table 1).

It is anticipated that the above issues will soon be resolved and that the first telomerase inhibitors (e.g., GRN163L, Geron Corporation) and TTAs (based on BRACO19 (such AS1410) Antisoma) will enter Phase I trials. This could mark the beginning of an era where new classes of anticancer drugs are targeted against the unlimited proliferative capacity of tumour cells, a feature that was first described over 40 years ago.

Conflict of interest statement

LK is a full-time employee of Antisoma.

Acknowledgements

Thanks to Prof. Stephen Neidle (now at the School of Pharmacy, University of London) and colleagues for many years of fruitful collaboration and discussion. The xenograft data for BRACO19 shown in Fig. 4, was produced by colleagues at Antisoma (Colin Green, David Griffiths-Johnson).

References

1. Neidle S, Kelland LR. Telomerase as an anti-cancer target: current status and future prospects. *Anti-Cancer Drug Des* 1999, **14**, 341–347.
2. Kim NW, Piatyszek MA, Prowse KR, *et al*. Specific association of human telomerase activity with immortal cells and cancer. *Science* 1994, **266**, 2011–2015.
3. Hanahan D, Weinberg RA. The hallmarks of cancer. *Cell* 2000, **100**, 57–70.

4. Smogorzewska A, de Lange T. Regulation of telomerase by telomeric proteins. *Annu Rev Biochem* 2004, **73**, 177–208.
5. Li GZ, Eller MS, Firoozabadi R, *et al*. Evidence that exposure of the telomere 3′ overhang sequence induces senescence. *PNAS* 2003, **100**, 527–531.
6. Baumann P, Cech TR. Pot1, the putative telomere end-binding protein in fission yeast and humans. *Science* 2001, **292**, 1171–1175.
7. Griffith JD, Comeau L, Rosenfield S, *et al*. Mammalian telomeres end in a large duplex loop. *Cell* 1999, **97**, 503–514.
8. Morin GB. The human telomere terminal transferase enzyme is a ribonucleoprotein that synthesizes TTAGGG repeats. *Cell* 1989, **59**, 521–529.
9. Feng J, Funk WD, Wang S-S, *et al*. The RNA component of human telomerase. *Science* 1995, **269**, 1236–1240.
10. Nakamura TM, Morin GB, Chapman KB, *et al*. Telomerase catalytic subunit homologs from fission yeast and human. *Science* 1997, **277**, 955–959.
11. Hahn WC, Counter CM, Lundberg AS, *et al*. Creation of human tumour cells with defined genetic elements. *Nature* 1999, **400**, 464–468.
12. Stewart SA, Hahn WC, O'Connor BF, *et al*. Telomerase contributes to tumorigenesis by a telomere length-independent mechanism. *Proc Natl Acad Sci* 2002, **99**, 12606–12611.
13. Bodnar AG, Ouellette M, Frolkis M, *et al*. Extension of life-span by introduction of telomerase into normal human cells. *Science* 1998, **279**, 349–352.
14. Londono-Vallejo JA, Der-Sarkissian H, Cazes L, *et al*. Alternative lengthening of telomeres is characterized by high rates of telomeric exchange. *Cancer Res* 2004, **64**, 2324–2327.
15. Dunham MA, Neumann AA, Fasching CL, *et al*. Telomere maintenance by recombination in human cells. *Nat Genet* 2000, **26**, 447–450.
16. Ulaner GA, Hoffman AR, Otero J, *et al*. Divergent patterns of telomere maintenance mechanisms among human sarcomas: sharply contrasting prevalence of the alternative lengthening of telomeres mechanism in Ewing's sarcomas and osteosarcomas. *Gene Chromosome Canc* 2004, **41**, 155–162.
17. Hakin-Smith V, Jellinek DA, Levy D, *et al*. Alternative lengthening of telomeres and survival in patients with glioblastoma multiforme. *Lancet* 2003, **361**, 836–838.
18. Broccoli D, Young JW, Lange de T. Telomerase activity in normal and malignant hematopoietic cells. *Proc Natl Acad Sci* 1995, **92**, 9082–9086.
19. Harle-Bachor C, Boukamp P. Telomerase activity in the regenerative basal layer of the epidermis in human skin and in immortal and carcinoma-derived skin keratinocytes. *Proc Natl Acad Sci* 1996, **93**, 6476–6481.
20. Masutomi K, Yu EY, Khurts S, *et al*. Telomerase maintains telomere structure in normal human cells. *Cell* 2003, **114**, 241–253.
21. Meeker AK, Hicks JL, Platz EA, *et al*. Telomere shortening is an early somatic DNA alteration in human prostate tumorigenesis. *Cancer Res* 2002, **62**, 6405–6409.
22. van Heek NT, Meeker AK, Kern SE, *et al*. Telomere shortening is nearly universal in pancreatic intraepithelial neoplasia. *Am J Pathol* 2002, **161**, 1541–1547.
23. Meeker AK, Hicks JL, Gabrielson E, *et al*. Telomere shortening occurs in subsets of normal breast epithelium as well as *in situ* and invasive carcinoma. *Am J Pathol* 2004, **164**, 925–935.
24. Lee HW, Blasco MA, Gottlieb GJ, *et al*. Essential role of mouse telomerase in highly proliferative organs. *Nat Med* 1998, **392**, 569–574.
25. Hahn WC, Stewart SA, Brooks MW, *et al*. Inhibition of telomerase limits the growth of human cancer cells. *Nat Med* 1999, **5**, 1164–1170.
26. Zhang X, Mar V, Zhou W, *et al*. Telomere shortening and apoptosis in telomerase-inhibited human tumor cells. *Gene Develop* 1999, **13**, 2388–2399.
27. Herbert BS, Pitts AE, Baker SI, *et al*. Inhibition of human telomerase in immortal human cells leads to progressive telomere shortening and cell death. *PNAS* 1999, **96**, 14276–14281.
28. Saretzki G, Ludwig A, von Zglinicki T, *et al*. Ribozyme-mediated telomerase inhibition induces immediate cell loss but not telomere shortening in ovarian cancer cells. *Cancer Gene Therap* 2001, **8**, 827–834.
29. Kraemer K, Fuessel S, Schmidt U, *et al*. Antisense-mediated hTERT inhibition specifically reduces the growth of human bladder cancer cells. *Clin Cancer Res* 2003, **9**, 3794–3800.
30. Marusic L, Anton M, Tidy A, *et al*. Reprogramming of telomerase by expression of mutant telomerase RNA template in human cells leads to altered telomeres that correlate with reduced cell viability. *Mol Cell Biol* 1997, **17**, 6394–6401.
31. Guiducci C, Cerone MA, Bacchetti S. Expression of mutant telomerase in immortal telomerase-negative human cells results in cell cycle deregulation, nuclear and chromosomal abnormalities and rapid loss of viability. *Oncogene* 2001, **20**, 714–725.
32. Kim MM, Rivera MA, Botchkina IN, *et al*. A low threshold level of expression of mutant-template telomerase RNA inhibits human tumor cell proliferation. *PNAS* 2001, **98**, 7982–7987.
33. Li S, Rosenberg JE, Donjacour AA, *et al*. Rapid inhibition of cancer cell growth induced by lentiviral delivery and expression of mutant-template telomerase RNA and anti-telomerase short-interfering RNA. *Cancer Res* 2004, **64**, 4833–4840.
34. Holt SE, Aisner DL, Baur J, *et al*. Functional requirement of p23 and HSP90 in telomerase complexes. *Gene Develop* 1999, **13**, 817–826.
35. Nishi H, Nakada T, Kyo S, *et al*. Hypoxia-inducible factor 1 mediates upregulation of telomerase (hTERT). *Mol Cell Biol* 2004, **24**, 6076–6083.
36. Akiyama M, Hideshima T, Hayashi T, *et al*. Nuclear factor kappaB p65 mediates tumor necrosis factor alpha-induced nuclear translocation of telomerase reverse transcriptase protein. *Cancer Res* 2003, **63**, 18–21.
37. Nguyen DC, Crowe DL. Intact functional domains of the retinoblastoma gene product (pRb) are required for downregulation of telomerase activity. *Biochim Biophys Acta* 1999, **1445**, 207–215.
38. Strahl C, Blackburn EH. The effects of nucleoside analogs on telomerase and telomeres in tetrahymena. *Nucleic Acids Res* 1994, **22**, 893–900.
39. Damm K, Hemmann U, Garin-Chesa P, *et al*. A highly selective telomerase inhibitor limiting human cancer cell proliferation. *EMBO J* 2001, **20**, 6958–6968.
40. Pascolo E, Wenz C, Lingner J, *et al*. Mechanism of human telomerase inhibition by BIBR1532, a synthetic, non-nucleosidic drug candidate. *J Biol Chem* 2002, **277**, 15566–15572.
41. Kim JH, Kim JH, Lee GE, *et al*. Identification of a quinoxaline derivative that is a potent telomerase inhibitor leading to cellular senescence of human cancer cells. *Biochem Soc* 2003, **373**, 523–529.
42. Herbert BS, Pongracz K, Shay JW, *et al*. Oligonucleotide N3′ → P5′ phosphoramidates as efficient telomerase inhibitors. *Oncogene* 2002, **21**, 638–642.
43. Asai A, Oshima Y, Yamamoto Y, *et al*. A novel telomerase template antagonist (GRN163) as a potential anticancer agent. *Cancer Res* 2003, **63**, 3931–3939.
44. Akiyama M, Hideshima T, Shammas MA, *et al*. Effects of oligonucleotide N3′–P5′ thio-phosphoramidate (GRN163) targeting telomerase RNA in human multiple myeloma cells. *Cancer Res* 2003, **63**, 6187–6194.
45. Wang ES, Wu K, Chin AC, *et al*. Telomerase inhibition with an oligonucleotide telomerase template antagonist: *in vitro* and *in vivo* studies in multiple myeloma and lymphoma. *Blood* 2004, **103**, 258–266.
46. Ozawa T, Gryaznov SM, Hu LJ, *et al*. Antitumor effects of specific telomerase inhibitor GRN163 in human glioblastoma xenografts. *Neuro-oncology* 2004, **6**, 218–226.

47. Blackburn EH. Switching and signaling at the telomere. *Cell* 2001, **106**, 661–673.
48. Zahler AM, Williamson JR, Cech TR, *et al*. Inhibition of telomerase by G-quartet DNA structures. *Nature* 1991, **350**, 718–720.
49. Sun D, Thompson B, Cathers BE, *et al*. Inhibition of human telomerase by a G-quadruplex-interactive compound. *J Med Chem* 1997, **40**, 2113–2116.
50. Wheelhouse RT, Sun D, Han H, *et al*. Cationic porphyrins as telomerase inhibitors: the interaction of tetra-(*N*-methyl-4-pyridyl)porphine with quadruplex DNA. *J Am Chem Soc* 1998, **120**, 3261–3262.
51. Read MA, Harrison JR, Romagnoli B, *et al*. Structure-based design of selective and potent G quadruplex-mediated telomerase inhibitors. *PNAS* 2001, **98**, 4844–4849.
52. Shin-ya K, Wierzba K, Matsuo K-I, *et al*. Telomestatin, a novel telomerase inhibitor from *Streptomyces anulatus*. *J Am Chem Soc* 2001, **123**, 1262–1263.
53. Mergny JL, Lacroix L, Teulado-Fichou M-P, *et al*. Telomerase inhibitors based on quadruplex ligands selected by a fluorescence assay. *Proc Natl Acad Sci* 2001, **98**, 3062–3067.
54. Koeppel F, Riou JF, Laoui A, *et al*. Ethidium derivatives bind to G-quartets, inhibit telomerase and act as fluorescent probes for quadruplexes. *Nucleic Acids Res* 2001, **29**, 1087–1096.
55. Riou JF, Guittat L, Mailliet P, *et al*. Cell senescence and telomere shortening induced by a new series of specific G-quadruplex DNA ligands. *PNAS* 2002, **5**, 2672–2677.
56. Gowan SM, Heald R, Stevens MFG, *et al*. Potent inhibition of telomerase by small-molecule pentacyclic acridines capable of interacting with G-quadruplexes. *Mol Pharmacol* 2001, **60**, 981–988.
57. Duan W, Rangan A, Vankayalapati H, *et al*. Design and synthesis of fluoroquinophenoxazines that interact with human telomeric G-quadruplexes and their biological effects. *Mol Cancer Therap* 2001, **1**, 103–120.
58. Harrison RJ, Gowan SM, Kelland LR, *et al*. Human telomerase inhibition by substituted acridine derivatives. *Bioorg Med Chem Lett* 1999, **9**, 2463–2468.
59. Parkinson GN, Lee MPH, Neidle S. Crystal structure of parallel quadruplexes from human telomeric DNA. *Nature* 2002, **417**, 876–880.
60. Schaffitzel C, Berger I, Postberg J, *et al*. *In vitro* generated antibodies specific for telomeric guanine-quadruplex DNA react with *Stylonychia lemnae* macronuclei. *PNAS* 2001, **98**, 8572–8577.
61. Chang CC, Kuo IC, Ling I-F, *et al*. Detection of quadruplex DNA structures in human telomeres by a fluorescent carbazole derivative. *Anal Chem* 2004, **76**, 4490–4494.
62. Stewart SA, Ben-Porath I, Carey VJ, *et al*. Erosion of the telomeric single-strand overhang at replicative senescence. *Nat Genet* 2003, **4**, 492–496.
63. Karlseder J, Smogorzewska A, de Lange T. Senescence induced by altered telomere state, not telomere loss. *Science* 2002, **295**, 2446–2449.
64. Incles CM, Schultes CM, Kempski H, *et al*. A G-quadruplex telomere maintenance inhibitor produces p16-associated senescence and chromosomal fusions in human prostate cancer cells. *Mol Cancer Therap* 2004, **3**, 1201–1206.
65. Herbig U, Jobling WA, Chen BPC, *et al*. Telomere shortening triggers senescence of human cells through a pathway involving ATM, p53 and p21CIP1, but not p16INK4a. *Mol Cell* 2004, **14**, 501–513.
66. d'Adda dl Fagagna F, Reaper PM, Clay-Farrace L, *et al*. A DNA damage checkpoint response in telomere-initiated senescence. *Nature* 2003, **426**, 194–198.
67. Leonetti C, Amodei S, D'Angelo C, *et al*. Biological activity of the G-quadruplex ligand RHPS4 is associated with telomere capping alteration. *Mol Pharmacol* 2004, **66**, 1138–1146.
68. Gomez D, Paterski R, Lemarteleur T, *et al*. Interaction of telomestatin with the telomeric single-strand overhang. *J Biol Chem* 2004, **279**, 41487–41494.
69. Gomez D, Aouali N, Renaud A, *et al*. Resistance to senescence induction and telomere shortening by a G-quadruplex ligand inhibitor of telomerase. *Cancer Res* 2003, **63**, 6149–6153.
70. Kim MY, Gleason-Guzman M, Izbicka E, *et al*. The different biological effects of telomestatin and TMPyP4 can be attributed to their selectivity for interaction with intramolecular or intermolecular G-quadruplex structures. *Cancer Res* 2003, **63**, 3247–3256.
71. Siddiqui-Jain A, Grand CL, Bearss DJ, *et al*. Direct evidence for a G-quadruplex in a promoter region and its targeting with a small molecule to repress c-MYC transcription. *Proc Natl Acad Sci* 2002, **99**, 11593–11598.
72. Gowan SM, Harrison JR, Patterson L, *et al*. A G-quadruplex-interactive potent small-molecule inhibitor of telomerase exhibiting *in vitro* and *in vivo* antitumor activity. *Mol Pharmacol* 2002, **61**, 1154–1162.
73. Burger AM, Dai F, Schultes CM, *et al*. The b-Quadruplex-interactive molecule BRACO-19 inhibits tumor growth, consistent with telomere targeting and interference with telomerase function. *Cancer Res* 2005, **65**, 1489–1496.
74. Grand CL, Han H, Munoz RB, *et al*. The cationic porphyrin TMPyP4 down-regulates c-MYC and human telomerase transeriphase expression and inhibits tumor growth *in vivo*. *Mol Cancer Therap* 2002, **1**, 565–573.
75. Sidorov IA, Hirsch KS, Harley CB, *et al*. Cancer treatment by telomerase inhibitors: predictions by a kinetic model. *Math Biosci* 2003, **181**, 209–221.
76. Lee KH, Rudolph KL, Ju YJ, *et al*. Telomere dysfunction alters the chemotherapeutic profile of transformed cells. *PNAS* 2001, **98**, 3381–3386.
77. Kelland LR. "Of mice and men": values and liabilities of the athymic nude mouse model in anticancer drug development. *Eur J Cancer* 2004, **40**, 827–836.
78. Kipling D, Cooke HJ. Hypervariable ultra-long telomeres in mice. *Nature* 1990, **347**, 400–402.
79. Coviello-McLaughlin GM, Prowse KR. Telomere length regulation during postnatal development and ageing in *Mus spretus*. *Nucleic Acids Res* 1997, **25**, 3051–3058.
80. Makarov VL, Lejnine S, Bedoyan J, *et al*. Nucleosomal organization of telomere-specific chromatin in rat. *Cell* 1993, **73**, 775–787.
81. Nasir L, Devlin P, McKevitt T, *et al*. Telomere lengths and telomerase activity in dog tissues: a potential model system to study human telomere and telomerase biology. *Neoplasia* 2001, **3**, 351–359.
82. Lee WW, Nam KH, Terao K, *et al*. Age-related telomere length dynamics in peripheral blood mononuclear cells of healthy cynomolgus monkeys measured by Flow FISH. *Immunology* 2002, **105**, 458–465.
83. Moyzis RK, Buckingham JM, Cram LS, *et al*. A highly conserved repetitive DNA sequence (TTAGGG)n, present at the telomeres of human chromosomes. *Proc Natl Acad Sci* 1988, **85**, 6622–6626.
84. Vaziri H, Dragowska W, Allsopp RC, *et al*. Evidence for a mitotic clock in human hematopoietic stem cells: loss of telomeric DNA with age. *Proc Natl Acad Sci* 1994, **91**, 9857–9860.
85. Chu CT, Piatyszek MA, Wong SSY, *et al*. Telomerase activity and telomere lengths in the NCI cell panel of human cancer cell lines. *Proc Am Assoc Cancer Res* 2000, **41**, #3405.
86. Gertler R, Rosenberg R, Stricker D, *et al*. Telomere length and human telomerase reverse transcriptase expression as markers for progression and prognosis of colorectal carcinoma. *J Clin Oncol* 2004, **10**, 1807–1814.
87. Wu KD, Orme LM, Shaughnessy Jr J, *et al*. Telomerase and telomere length in multiple myeloma: correlations with disease heterogeneity, cytogenetic status, and overall survival. *Blood* 2003, **101**, 4982–4989.