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Telomerase and telomere dynamics in ageing and cancer: current status and future directions

F. Sampedro Camarena, G. Cano Serral and F. Sampedro Santaló

Unidad de Investigación Oncológica. Hospital de la Santa Cruz y San Pablo. Barcelona, Spain

Abstract This review will focus on the clinical utilities of telomerase for human cancer diagnosis and prognosis. Much attention has been focused on control of telomerase activity in early and late stage tumours. Telomerase stabilisation may be required for cells to escape replicative senescence and to proliferate indefinitely. Because of a very strong association between telomerase and malignancy, both clinicians and pathologists expect this molecule to be a useful diagnostic and prognostic marker and a new therapeutic target. These data have greatly inspired the development of various strategies to target telomere and telomerase for cancer therapy. Finally, evidence is now emerging that G-quadruplex ligands produce rapid senescence and selective cell death. A summary of recent experimental works with new small molecules as potential inhibitors of telomerase is presented.

Key words Telomerase • Telomere • Immortality • Ageing • Senescence • G-quadruplex • Targeted anticancer therapy

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Telomerase is an enzyme that prevents critical telomere shortening during cell division, allowing cells to bypass replication senescence [1, 2]. Telomeres are tracts of repetitive DNA (TTAGGG/AATCCC for human telomeres) that protect chromosomes from degradation and loss of essential genes and allow the cell to distinguish between double-strand breaks and natural chromosome ends. They are crucial for the preservation of genome integrity.

Telomeres progressively shorten in most human cells with increased age and telomere length in almost all middle-aged human tissues is approximately half that of the newborn length [3]. Telomerase is activated in more than 85% of malignant tumours. In contrast, telomerase activity is usually not detectable in normal somatic tissues except for some self-renewing tissues with high regenerative potential. These observations suggest that telomerase is a new marker of cancer and raise the possibility that anti-telomerase compounds may provide a new generation of cancer therapeutics. Maintenance of telomeres by telomerase is critical for the continuing proliferation of most advanced cancer cells.

The human telomerase, which is composed of two subunits including telomerase RNA template (hTR) and telomerase transcriptase protein (hTERT), functions as a reverse transcriptase enzyme in the process of telomere synthesis [4]. Today, the main question asked by experimental and medical oncologists is “will telomerase be a therapeutic target for the third millennium?” The response is yes, because telomerase inhibitors may display a good selectivity for tumour cells with minimal toxicity for normal tissues and cells. Evidence is presented to link telomere dynamics in stem cells and ageing with cancer initiation, metastasis and resistance therapies. An interesting question regarding whether loss of telomere functionality is exploited to programme organismal ageing, or to create a normal barrier to present cancer, needs an urgent answer. Also, the outstanding question that remains to be determined is if inhibiting telomerase specifically will cause cancer-cell death, and only clinical trials will ultimately determine this.

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F. Sampedro Camarena (✉)
Unidad de Investigación Oncológica
Hospital de la Santa Cruz y San Pablo
C/ Sant Antoni M.ª Claret, 167
ES-08025 Barcelona, Spain

Telomerase as a tumour marker

The telomere–telomerase hypothesis of ageing and cancer is based on the thinking that most human tumours

Table 1 Telomerase activity in normal and immortal cells

Tissue of origin	Cell type	Telomerase activity, no. positive/no. tested	% Positive
Skin	Tumour	8/8	100
Skin	Normal	0/5	0
Breast	Tumour	22/22	100
Breast	Normal	0/8	0
Lung	Tumour	18/18	100
Lung	Normal	0/3	0
Pancreas	Tumour	3/3	100
Ovary	Tumour	5/5	100
Kidney	Tumour	8/8	100
Colon	Tumour	7/7	100
Blood	Tumour	9/9	100
Prostate	Tumour	2/2	100
Prostate	Normal	0/2	0

Type of malignancy	No. samples positive/no. tested	% Positive
Acute myeloid leukaemia	47/64	73
Basal cell carcinoma	73/77	95
Bladder carcinoma	172/185	92
Breast carcinoma (ductal and lobular)	300/339	88
Cervical carcinoma	16/16	100
Chronic myeloid leukaemia		
Chronic	30/42	71
Blast	21/21	100
Colorectal carcinoma	123/138	89
Gastric carcinoma	72/85	85
Head and neck squamous cell carcinoma	112/120	86
Hepatocellular carcinoma	149/173	86
Lymphoma		
Low grade	12/14	86
High grade	16/16	100
Melanoma	6/7	85
Neuroblastoma	99/105	94
Non-small-cell lung carcinoma	98/125	78
Ovarian carcinoma	21/23	91
Pancreatic carcinoma	41/43	95
Prostate carcinoma	52/58	90
Renal carcinoma	95/115	83
Retinoblastoma	17/34	50
Small-cell lung carcinoma	15/15	100
Squamous cell carcinoma	15/18	83

Pathology	No. positive/no. tested	Telomerase positive (%)
Normal	1/196	0.5
Preinvasive (benign)	125/410	30
Malignant	1734/2031	85
Adjacent to malignant	77/660	11

have telomerase activity, whereas normal somatic cells do not. Telomeres that protect chromosomes at both ends are shortened with each somatic cell division through replication-dependent sequence loss at DNA termini. The chromosomes with shortened telomeres tend to become unstable, leading to cell death. Telomeric association of chromosomes is an early manifestation of programmed cell death [5]. Synthesis of DNA at chromosome ends by telomerase may be necessary for indefinite proliferation of human cells, thus, telomerase appears to be stringently repressed in normal human somatic tissues but reactivated in cancer, where immortal cells are likely required to maintain tumour growth [6]. In extracts of human cells and tissues, telomerase activity was identified in immortal cell lines but was not detected in somatic cells derived from different tissues and individuals by a modification of the telomeric repeat amplification protocol, the TRAP assay (Table 1).

Telomerase reactivation in a large proportion of human cancers has led to the proposal of telomerase as a cancer marker [6–9]. Telomerase has been frequently

described as an ideal cancer target because it is activated in most tumour cells that concurrently have short telomeres and is not expressed in normal somatic cells (Table 1). There are increasing numbers of studies indicating the utility of telomerase assays in the early detection of cancer [10, 11]. With the knowledge that telomerase activation occurs so frequently in cancer, there is the possibility that telomerase detection could be put to use in screening. Currently, there is increasing interest in identifying molecular markers that could ultimately replace the older anatomically or cytologically oriented methods for the early detection of cancer. For example, in breast and lung cancer, telomerase activity appears very early, even in preneoplasia of smokers or former smokers, whereas in others, such as pancreatic and colon cancer, it appears in 90–95% of early stage but not in preneoplasia.

Another area of interest is monitoring patients for residual or recurring of disease using telomerase as a marker [12]. As can be seen in Table 1, telomerase activity is detected in adjacent, presumably cleaved regions obtained during biopsy. In these instances, occult micrometastases of the primary tumour can invade the surrounding normal tissue and small amounts of tumour may remain undetected by pathologists. In these cases, recurrence of cancer could be monitored by fine needle aspiration of cancer patients' tissues to assess the progression of the tumour due to micrometastatic events using telomerase detection.

Telomerase and telomere dynamics and ageing

Telomerase is essential for the indefinite growth capacity of tumour cells because of its capacity to stabilise telomeres. Telomeres in most human cells shorten during ageing *in vivo* as well, suggesting that telomere length could be a biomarker of ageing and age-related morbidity and mortality. The challenge is to maintain the length of telomeres. Telomeric loss is the internal clock of ageing. Telomerase is able to add telomeric sequences to the outer-most ends of the telomeres and thereby stabilise or even elongate the telomeres [13]. Each time that a cell divides, telomeres become progressively shorter and it is thought that this shortening is one of the critical features of cellular ageing. When telomeres reach a critically short length, normal cells irreversibly arrest proliferation and acquire a characteristic enlarged morphology and a variety of altered functions. This response has been termed replicative or cellular senescence. Thus, despite protecting from cancer in young adults, cellular senescence, driven in part by telomere dysfunction, may promote cancer progression in aged organisms [14].

Control of ageing involves both genetic programmes and environmental factors. The environmental factors include accumulation of methodic hazards and regulation of the metabolic processes of hormones and growth factors, while genetic programmes account for up to 25% of the variation in human lifespan. Identifying factors that enhance or limit telomerase enzymatic activity and telomere maintenance are important for understanding basic aspects of telomere biology [15–17].

Telomeres, genomic instability, replicative senescence and tumorigenesis

The idea is that telomere shortening may suppress the growth of tumour originated from the model of primary human culture cells. It has been proposed that the senescence that occurs in human culture might parallel a similar barrier to growth as a result of short telomeres in tumorigenesis [18]. Parallels have also been drawn between the increased genetic instability just prior to crisis and telomerase activation and the presence of genetic instability followed by telomerase activation in human cancer [19]. Maintenance of telomere length is a common feature of tumours late in tumorigenesis (Fig. 1). Even in those tumours without detectable telomerase activity, telomere lengthening occurs through an alternative mechanism [20].

It is not clear whether telomere dysfunction inhibits tumour growth before or after the initiation of genetic instability [21]. Telomerase activation seems to occur late in tumorigenesis and may contribute to the growth potential of the tumour after genetic instability has been established (Fig. 1). What is clear is that telomerase is active in immortal cell lines *in vitro* and also in tumour cells *in vivo* [22]. Cell cultures are an excellent *in vitro* model to study the shortening of telomeres and their consequences (Fig. 2). In contrast to normal cells, tumour cells generally have short telomeres lengths and show no net loss of average telomere length with successive cell divisions, suggesting that telomere stability might be required for cells to escape from replicative senescence and proliferate indefinitely. Most, but not necessarily all, malignant tumours might need to become immortal to sustain their growth and telomerase activity could therefore be a rate-limiting step required for the continuing proliferation of advanced cancers [23, 24]. As can be seen in Fig. 2, M1 (senescence) and M2 (crisis) pathways are initiated by telomere shortening. If M1 is bypassed or abrogated, cells enter an extended period of proliferation and telomeres continue to shorten in the period between M1 and M2. When cells bypass M1, telomeres continue to shorten until a critical stage leading to mitotic catastrophe where these cells die or, rarely, reactivate telomerase, leading to an immortalised cell line [25].

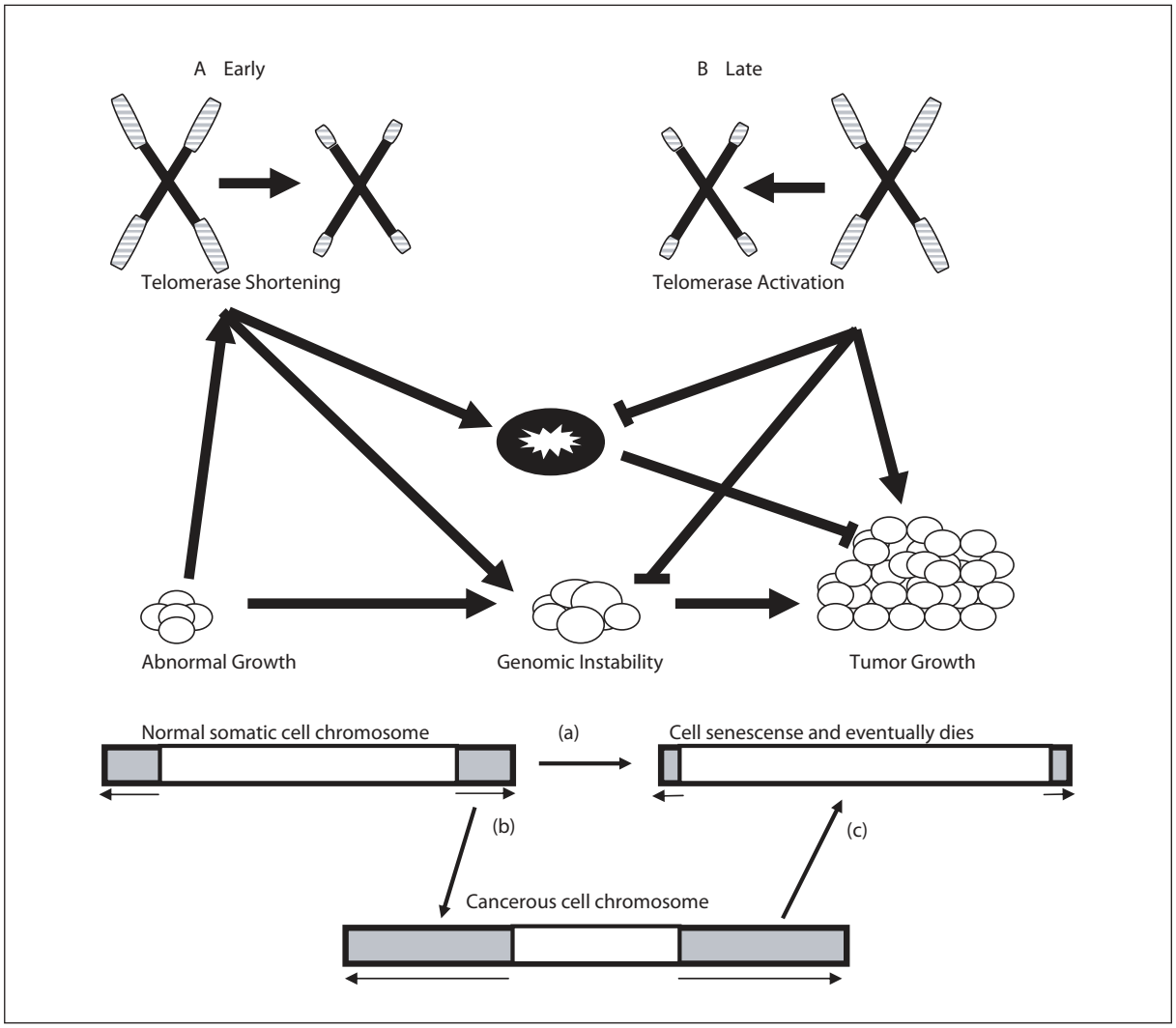


Fig. 1 Telomeres, genomic instability and tumorigenesis

Clinical utility of telomerase as a diagnostic and prognostic marker

Telomerase activity measurements have two clinical utilities for cancer diagnosis. The first one is the early detection of cancer cells for diagnosis of malignant tumours whose telomerase activity is up-regulated in early stages and the second is as a prognostic indicator in tumours whose telomerase is activated according to tumour progression [26]. The main telomerase methods currently being evaluated for cancer diagnosis are: (a) fine needle aspiration (FNA), (b) secretion, washing or brushing samples and (c) biopsy samples (Table 2).

How can we control telomerase activity?

Several strategies have been proposed to inhibit telomerase activity in cells: anti-sense technologies against hTER and hTERT, ribozymes against hTER, anti-oestrogens, progesterone, vitamin D, retinoic acid, G-quartet interactions, telomere targeting agents, interaction with other proteins involved in the regulation of telomerase and telomeres etc. Also, several indications point towards a role for telomerase/telomeres as targets for cancer chemotherapy: (a) telomerase is required for maintenance of shortened telomeres and (b) telomere length is decreased in tumours as compared with normal tissue. In addition, several studies have shown that telomerase activity exists between early and late stage tumours. Thus, if advanced tumours have lower telomere lengths and higher telomerase activity which may stabilise these shortened telomeres, there may be a good argument for development of inhibitors of telomerase as tumours could be selectively sensitive to these agents.

An ideal telomerase inhibitor would possess the following properties: (a) high specificity for telomerase, (b) minimal toxicity, (c) oral bio-availability, (d) low cy-

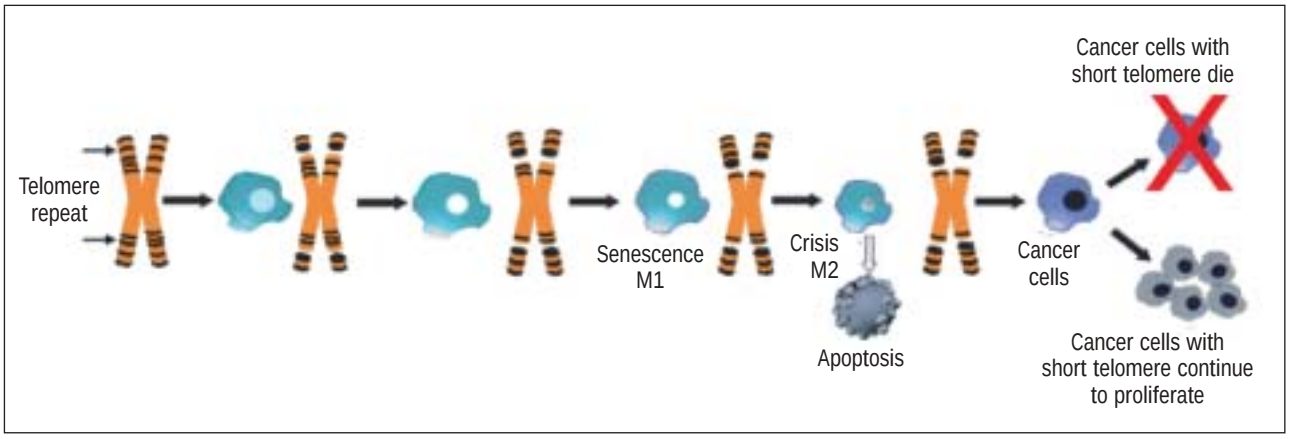


Fig. 2 The two-stage M1/M2 model of senescence, apoptosis and cancer. M1 (senescence) and M2 (Crisis) pathways are initiated by telomere shortening

totoxic potential alone or in combination with other drugs and (e) unaffected by multi-drug resistance and other mechanisms of resistance. Taking into account these properties, two distinct parts of the telomerase, the RNA template (hTER) and the reverse transcriptase (hTERT), have been investigated so far for the discovery of direct telomerase inhibitors. This approach is mainly driven by nucleic acid chemistry. Recently, another group, the telomere targeting agents, has been the target of many studies regarding their influence on G-quartet structures and telomeric DNA [67].

The aim of this review is to impartially present the current status (actual state) of the new inhibitors of telomerase and their clinical applications in combination with chemotherapy.

According to the paradigm currently proposed, it can be predicted that telomerase inhibition will not affect a tumour until its telomeres reach a critical size for entering senescence. This means that during anti-telomerase therapy the tumour cells will continue to grow, undergoing 20–30 divisions until the telomeres reach a critical size, leading to tumour senescence [67].

Telomerase inhibitors and their potential clinical applications

The anti-tumour compounds cisplatin, carboplatin and oxaliplatin are the most extensively used anti-tumour drugs in the world [68]. During the last two decades our group has synthesised more than 100 new platinum complexes from cisplatin and carboplatin to platinum(II) substituted pyrrolidine complexes. In this area, the Hospital de San Pablo was a pioneer in introducing the carboplatin synthesised in our laboratory in the treatment of advanced head and neck and bladder cancer [69, 70]. Now, our challenge is to synthesise small molecules as inhibitors of telomerase.

Telomerase activity is inhibited in a dose- and time-dependent manner with the treatment with these platinum complexes. Also, growth inhibition and cell cycle accumulation in G2/M phase are found to be correlated with telomerase inhibition and this may be cell-type specific. These facts suggest that telomerase inhibition and telomere shortening may contribute to possible mechanisms in efficiency of the treatment of some types of cancers [71]. As it has been widely demonstrated that platinum-based drugs, like cisplatin, carboplatin and oxaliplatin bind preferentially to guanine in N7 position, and that telomerase assemblage includes a RNA portion rich in guanine, new platinum complexes have been synthesised with the aim of selecting carrier ligands able to inhibit telomerase enzyme [72–74]. Certain *cis*-Pt(II) complexes have recently been shown to be effective inhibitors of telomerase in both cell-free and *in vitro* assays, most likely by targeting the nucleobases of the RNA component of the enzyme [75].

There are now thousands of publications supporting the association between tumorigenesis and activation of telomerase. Only in the last 10 years has targeting telomerase for potential therapeutics begun to emerge. Recent studies have suggested that the G-quadruplex structures could be stabilised by specific ligands in a new approach to cancer treatments consisting in inhibition of telomerase that is involved in telomerase maintenance and cell immortality. A G-quadruplex agent is able to impair telomerase function in a tumour cell, thus providing a basis for the development of new anticancer agents. The telomeric overhangs are an important target for the biological effect of inhibitors of telomerase and telomere agents. Interference with telomerase and telomere maintenance is emerging as an attractive target for anticancer therapies. Ligand-induced stabilisation of G-quadruplex formation by the telomeric DNA simple-stranded 3' overhang inhibits telomerase from catalysing telomeric DNA synthesis and from capping telomeric ends.

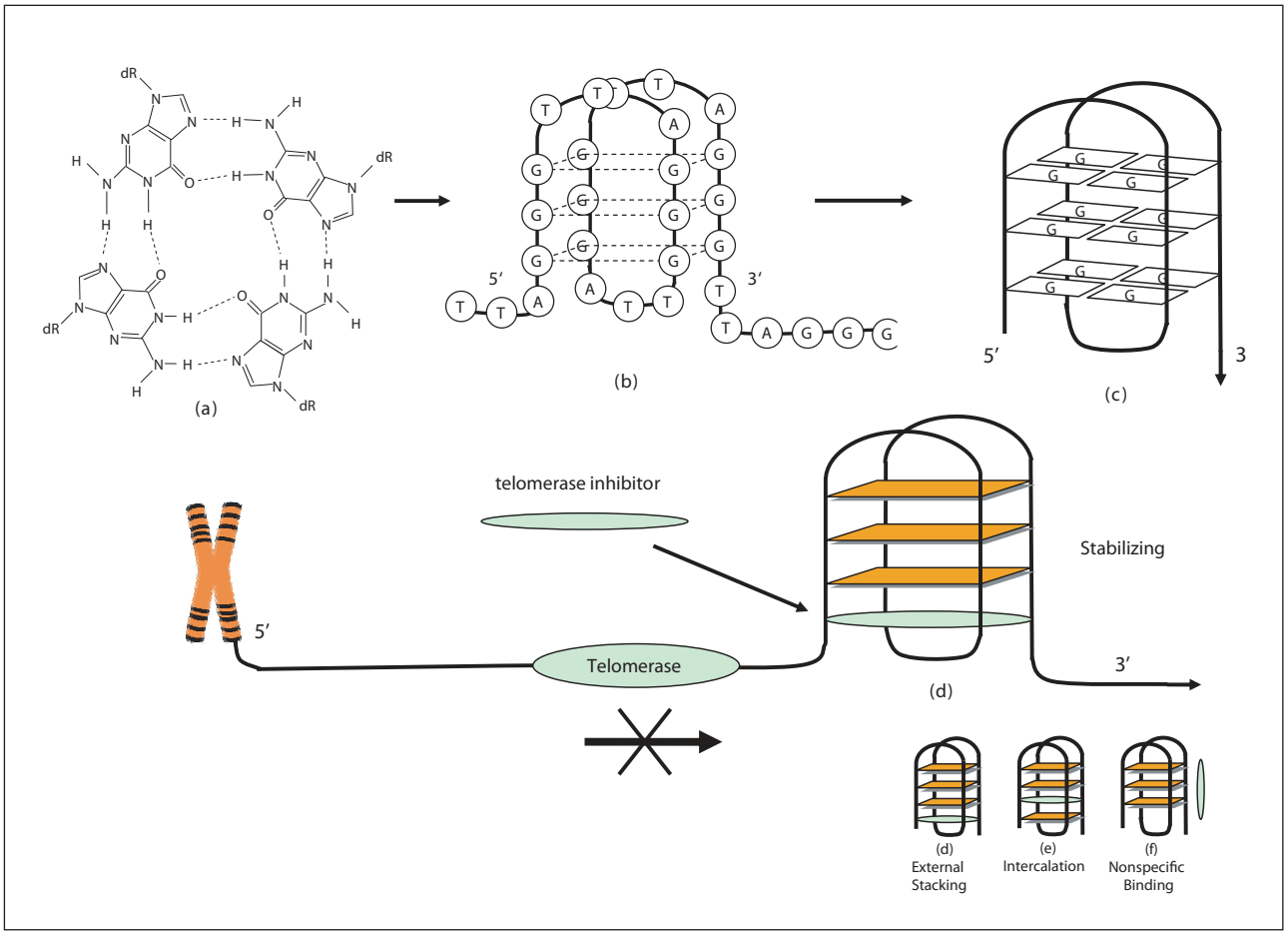


Fig. 3 Structure of a G-quartet (a) and a schematic representation of a G-quadruplex (b) and (c). Possible binding site of the inhibitors of telomerase (d) external stacking (e) and intercalation (f) non-specific binding

The anticancer effect is produced by inhibiting the capping and catalytic functions of telomerase.

New small molecules as inhibitors of telomerase

Several classes of small molecules have been described that stabilise the folding of the G-rich telomere strand in G-quadruplex structures (Fig. 3). Ligands that stabilise the telomeric G-rich telomere simple-stranded DNA overhang into the G-quadruplex can be considered as potential antitumour agents that block telomere replication [76, 77]. Here we review the current status of some of these molecules and their possible clinical applications (Table 3).

1. *Triazine derivatives*. The triazine derivative 12459 is a potent G-quadruplex interacting agent that inhibits telomerase activity and induces time- and dose-dependent telomere shortening, senescence-like growth arrest and apoptosis in the human A549 tumour cell line [78–80].
2. *Trisubstituted acridines*. The trisubstituted acridines are potent telomerase inhibitors that have shown little immediate cytotoxicity, and there also exists a strong relationship between G-quadruplex stabilisation and telomerase inhibition and optimal 3,6- and 9-substituent side-chain lengths for maximal activity. Braco 19- is a trisubstituted anilino-acridine that has been shown during short incubation times to produce chromosome end fusion and senescence [79, 81].
3. *BIBR-1532*. BIBR-1532 is a small-molecule inhibitor of telomerase in drug-resistant leukaemia and breast cancer cells and their parenteral counterparts when treated in combination with chemotherapeutics. This may be a valid strategy for the treatment of both drug-sensitive and drug-resistant cancers [82, 83].
4. *Telomestatin*. Telomestatin is a natural product found to inhibit telomerase activity in vitro, most likely by highly specific interactions with a particular form of intramolecular G-quadruplex stabilising cations such as K^+. *In vivo* testing has also demonstrated that it causes G-strand over-

Table 2 Clinical utility of telomerase as a diagnostic and prognostic marker

(A) Telomerase as a diagnostic marker		
Site	Type of samples	Cancer positive (%)
Head and neck	Biopsy	25/26 (96)
Breast	FNA	210/265 (79)
Thyroid	FNA	57/69 (68)
Stomach	Biopsy	23/29 (79)
Colon	Biopsy	110/126 (87)
Liver	FNA/biopsy	53/61 (87)
Pancreas	Pancreatic juice	48/59 (81)
Pancreas	FNA	18/18 (100)
Uterus	Biopsy	28/29 (97)
Lung	Biopsy	32/42 (76)
Lung	BAL/washing	115/176 (65)
(B) Telomerase as a prognostic marker		
Site	Histology	References
Breast	Invasive duct cell carcinoma	[27–30]
Breast	Node-positive breast cancer	[31, 32]
Thyroid	Papillary carcinoma	[33–36]
Stomach	Gastric cancer (adenocarcinoma)	[37–39]
Colon	Colorectal cancer (adenocarcinoma)	[40–42]
Liver	Hepatocellular carcinoma	[43–46]
Pancreas	Endocrine tumours	[47–49]
Uterus	Endometrial carcinoma	[50–53]
Uterus	Cervical intraepithelial neoplasia	[54–56]
Lung	Stage I–III NSCLC	[57, 58]
Lung	Stage I NSCLC	[59–62]
Skin	Melanoma	[63–66]

hang reduction. This high specificity could explain why telomestatin only exerts its effects on telomerase-positive cells [84–86].

5. *UCS1025A.* This compound is a new telomerase inhibitor that has been shown to possess antiproliferative activity against human cancer cell lines by direct inhibition of telomerase [87].
6. *Peptide nucleic acids (PNAs).* The advantages of PNAs are that they target the telomeric G-rich strand, and their efficacy in reversing the immortality of transformed human fibroblast in laboratory situations. The experiments resulted in increased cell death rate by apoptosis. Further, a combination of these anti-telomere PNA inhibitors with PNAs that additionally blocked telomerase activity resulted in an early complete inhibition of colony growth, induction of apoptosis and reduction in telomere length [88].
7. *Cyclo(n)pyrroles*. Cyclo(n)pyrroles are a new class of aromatic expanded porphyrins that are structurally similar to telomestatin. These compounds are capable of binding to G-quadruplexes and interacting with the intramolecular G-quadruplex. At this time the role that these analogues may have to play as telomerase inhibitors is unknown [89].
8. *Dictyodendrin derivatives*. These polycyclic alkaloids telomerase inhibitors of marine origin are now in study as possible potent anticancer agents [90].
9. *Berberine derivatives.* Berberine is an antibiotic alkaloid originating from Chinese herbal medicine, and its anti-bacterial activity has been demonstrated against many species. More recently, berberine was shown to inhibit telomere elongation by binding to G-quadruplex DNA. This compound is selective in inducing intermolecular G-quadruplex with respect to intramolecular G-quadruplex [91].

Given our knowledge that many cancers cells have shorter telomeres, they may be especially susceptible to telomerase, such as the small molecules mentioned here.

Conclusive remarks and future directions

There is, at the preclinical level, a multitude of strategies targeting telomerase and telomeres. Additional phase I trials with new chemicals compounds and early phase II are eagerly awaited. The choice of candidate

Table 3 Small molecules targeting telomeres and telomerase

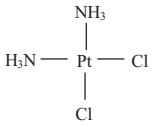
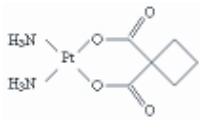
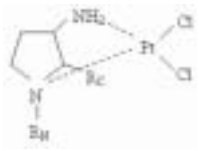
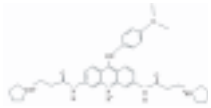
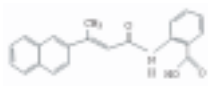
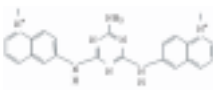
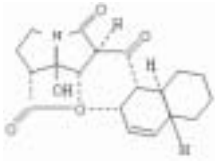
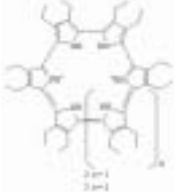
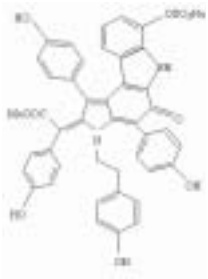
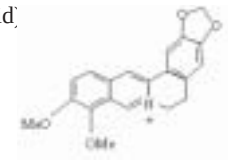
Class of inhibitor	Example of compound	Molecular structure	Mechanism of action
Combination therapy	Cisplatin		
	Carboplatin		Cis-Pt(II) complexes are effective inhibitors of telomerase by targeting the nucleobases of the RNA component of the enzyme
	Oxaliplatin		
Under study	Platinum aminopyrrolidine derivatives		Under study
Active site inhibitor	BRACO-19 (trisubstituted anilino-acridine)		Chromosome and fusion, senescence, and inhibition of catalytic and capping functions of telomerase
Direct damage to the telomerase structure	BIBR-1532 (non-nucleoside analogue)		Binds non-covalently in the reverse transcriptase active side of hTERT
Telomere capping alterations	Triazine derivative 12459		Inhibition of telomerase activity, senescence and apoptosis
Telomerase inhibitor (interference with telomeric G-overhang)	Telomestatin		Inhibition of telomerase activity by highly specific interactions with a particular form of intramolecular G-quadruplex
Telomerase inhibition	UCS1025A		This compound is a potent inhibitor of telomerase but the possible mechanism of action is under study
G-quadruplex binding (interaction)	Cyclo(n)pyrroles (aromatic porphyrins)		Binds to G-quadruplex through an intramolecular form

Table 3 Small molecules targeting telomeres and telomerase (Continuation)

Class of inhibitor	Example of compound	Molecular structure	Mechanism of action
G-quadruplex binding	Dictyodendrin E (polycyclic alkaloid)		This polycyclic alkaloid is a possible potent anticancer agent and the mechanism of action is under study
Telomere elongation inhibitor	Berberine (antibiotic alkaloid)		This compound is selective in inducing intermolecular G-quadruplexes instead of intramolecular G-quadruplexes

telomere/telomerase-based drugs to be tested in clinical trials would also depend on further results and their possibilities for combination with conventional treatments in cancer chemotherapy.

Experimental and medical oncologists are in agreement that the increase in the ageing world population implies the appearance of new diseases and new types of tumours that at younger ages are practically unobserved. Current knowledge about the mechanisms of telomerase action and that of telomeres, along with the development of new molecules, that, acting on the telomerase, inhibit the tumoral growth without affecting healthy cells, should facilitate better diagnoses, prognoses and treatments of cancer and at the same time obtain a better quality of life for treated patients.

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