Clinical implications of antitelomeric drugs with respect to the nontelomeric functions of telomerase in cancer

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Abstract: Telomerase is responsible for maintaining the length of telomeres at the ends of chromosomes. Although most somatic cells do not exhibit telomerase activity, it is reactivated in approximately 85% of cancers. This simple and attractive phenomenon steers the development of anticancer drugs targeting telomeres and telomerase. Recent studies have been revealing extratelomeric roles of telomerase in normal tissues, affecting processes that are critical for survival and aging of organisms. In this review, we will discuss the current therapeutic strategies targeting telomeres and telomerase and evaluate their potential advantages and risks with respect to nontelomeric functions.

Keywords: telomerase, telomere, TERT, TERC, telomerase inhibitors

Introduction
Telomeres are located at the end of chromosomes (TTAGGG in humans) and vary in length across species (eg, 5–15 kb in humans, ~48 kb in mice). During every cell division, telomeres are shortened by 50–200 bp due to the end replication problem.\(^1,2\) Therefore, successive replication leads to progressive shortening of telomeres in most somatic cells. Telomerase is a ribonucleoprotein complex comprising two main components: the enzymatic protein subunit telomerase reverse transcriptase (TERT) and a noncoding telomerase RNA component (TERC).\(^3\) Telomerase elongates telomeres using TERC as a template, thereby maintaining telomere length during cell division. Most somatic cells, however, do not exhibit telomerase activity, and their telomeres shorten with successive rounds of cell division, resulting in critically short telomeres and leading to cellular senescence and apoptosis.

Considering the immortal and proliferative characteristics of cancer cells, short telomeres can have clinical utility in inducing deleterious responses against cancer cells, such as senescence and apoptosis. Furthermore, due to the prevalent reactivation of telomerase in cancer cells,\(^4\) these cells can be eliminated by stimulating immune responses specific for TERT in patients. These findings are now being actively applied to generate anticancer drugs (Figure 1 and Table 1). Recent advances in this field indicate that telomerase regulates a diverse array of physiological functions other than telomere elongation and alternative spliced variants of telomerase without reverse transcriptase domain are present in normal tissues.\(^5\)–\(^16\) By considering these new discoveries, this review will provide a reinterpretation of the current cancer treatments targeting telomeres and telomerase.
Drugs targeting telomeres

In normal cells, short telomeres and telomere dysfunction cause DNA damage responses, resulting in cellular senescence and death through various signals including master regulators of DNA damage such as protein kinases ataxia telangiectasia mutated (ATM) and ATM and Rad3-related (ATR).17,18 Likewise, oligonucleotides mimicking the 3' overhang of telomere sequences (T-oligo) cause DNA damage-like responses by inducing a telomere shortening signal, which is similar to the phenotype of functional loss of telomeric repeat-binding factor 2, a telomeric DNA-binding protein.19 T-oligo activates ATM, p53, transcription factor E2F1, and p95/NBS1 protein (synthesis phase regulator)20–24 rather than suppressing telomerase activity. T-oligo induces apoptosis in melanoma, breast carcinoma, lymphoma, prostate cancer, and fibrosarcoma cell lines but not in normal cells.19,20,25 Histone deacetylase inhibitors promote the effect of T-oligo,26 suggesting a functional contribution of histone deacetylase to telomeres and/or telomere damage signaling.27 As T-oligo does not require telomerase reactivation, it can efficiently target almost all types of cancer cells including

### Table 1 Drugs targeting telomeres and telomerase

<table>
<thead>
<tr>
<th>Name</th>
<th>Target</th>
<th>Mechanism</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>GRN163(L)</td>
<td>TERC</td>
<td>TERC binding</td>
<td>41</td>
</tr>
<tr>
<td>T-oligo</td>
<td>Telomere</td>
<td>Mimics dysfunctional telomere</td>
<td>19</td>
</tr>
<tr>
<td>DN-hTERT</td>
<td>TERT</td>
<td>TERT degradation</td>
<td>77,78</td>
</tr>
<tr>
<td>BIBR1532</td>
<td>TERT</td>
<td>Enzyme inhibitor of TERT</td>
<td>47</td>
</tr>
<tr>
<td>BRACO19</td>
<td>Telomere</td>
<td>G-quadruplex formation</td>
<td>32,79</td>
</tr>
<tr>
<td>RHPS4</td>
<td>Telomere</td>
<td>G-quadruplex formation</td>
<td>80</td>
</tr>
<tr>
<td>Telomestatin</td>
<td>Telomere</td>
<td>G-quadruplex formation</td>
<td>81</td>
</tr>
<tr>
<td>I540</td>
<td>HLA-A</td>
<td>Induction of immune response</td>
<td>58</td>
</tr>
<tr>
<td>Vx-001</td>
<td>HLA-A</td>
<td>Induction of immune response</td>
<td>60,62</td>
</tr>
<tr>
<td>GV1001</td>
<td>Multiple HLA classes</td>
<td>Vaccination/modified TERT mRNA</td>
<td>68</td>
</tr>
<tr>
<td>GRNVAC1</td>
<td>Dendritic cell</td>
<td>Vaccination/modified TERT mRNA</td>
<td></td>
</tr>
</tbody>
</table>

**Abbreviations:** DN-hTERT, dominant negative form of human telomerase; HLA-A, human leukocyte antigen A; mRNA, messenger RNA; T-oligo, 3' overhang of telomere sequences; TERC, telomerase RNA component; TERT, telomerase reverse transcriptase.
telomerase-negative cancer cells that maintain their telomeres through alternative lengthening of telomeres.28,29

As the folding of a telomere to G-quadruplex (G4) prevents telomerase-induced telomere elongation,30,31 ligands that induce and stabilize G4 structures can be used as effective drugs against telomerase-positive cancer cells. Among G4-forming small molecules (Table 1), the well-investigated G4 ligand BRACO19 effectively promotes the formation of G4 structures at the end of telomeres.32 BRACO19 exhibits low cytotoxicity and efficiently inhibits telomere elongation, resulting in end-to-end chromosomal fusion.33 It also induces tumor regression and shows remarkable antitumor activity in vivo.34 However, BRACO19 is not currently available for therapeutic trials due to membrane impermeability. In addition, it can be secreted from the cell by ATP-binding cassette transporter superfamily.35 Therefore, modifications that increase its membrane permeability and prevent the release of T-oligo might improve the clinical efficacy of BRACO19.

Drugs that mimic or accelerate telomere shortening may exert toxic side effects in highly proliferative tissues. As evidenced by phenotypes of Terc knockout mice in late (G5) generations, short telomeres result in increased programmed cell death (apoptosis) and decreased proliferation in reproductive tissues, spleen, and bone marrow.36 Cardiac abnormalities similar to human dilated cardiomyopathy and impaired wound healing are also observed in these mice.31,32 Furthermore, short telomeres are carcinogenic, with chromosome end-to-end fusion and aneuploidy apparent in late generations.36 As humans have much shorter telomeres than mice, prolonged treatment with antitelomere drugs may be harmful to normal tissues.

**Drugs targeting telomerase**

The first generation of Terc and Tert knockout mice are developmentally normal, with short telomere-associated phenotypes observed only in later generations.30 As knockdown of Tert or Terc significantly reduces tumor growth,37,38 telomerase-targeting drugs may be an alternative to drugs that target telomeres.

For the elongation of telomere length by telomerase, key regions of TERC must be exposed to the surface of telomerase. GRN163 and imetelstat as its lipid (palmitate)-conjugated form (GRN163L) contain a short (13-mer) oligonucleotide with N3′→P5′ thio-phosphoramidate that binds to the template region of TERC.39 GRN163L is water soluble, shows high thermal and acid stability, and is resistant to several nucleases.40–42 It exerts potent inhibitory effects on telomerase activity in cancer cells.41 As GRN163L can penetrate plasma membranes through its lipid conjugation, it does not require additional vehicles for its delivery.43 Through intranasal or systemic treatment, both GRN163 and GRN163L can bypass the blood–brain barrier and preferentially affect brain tumor cells with minimum toxicity to normal brain tissue.44,45 These studies provide the basis for using both GRN163 and GRN163L as potent drugs against brain tumors, and both have already reached clinical trial stages. Notably, administration of GRN163L reduced the features of cancer stem cells that show multidrug resistance, self-renewal capacity, differentiation, and high metastatic potential.46 As MST312, another type of telomerase inhibitor, also exhibits similar effects on cancer stem cells,46 telomerase inhibitors may be a promising candidate targeting cancer stem cells as well.

Small molecule inhibitors against telomerase are likely good candidates for cancer therapy. BIBR1532, a mixed-type, nonnucleosidic inhibitor, is one of the molecules that most potently inhibit telomerase activity.47 In germ cell tumor cell lines, simultaneous treatment of BIBR1532 with cisplatin for 300 population doublings reduces telomere length from 18.5 kb to 8.9 kb.48 However, BIBR1532 does not increase sensitivity to cisplatin, and more prolonged treatment is required to induce the telomere shortening crisis (1.5–4 kb).49

Expression of the dominant negative form of telomerase (DN-TERT) causes telomere shortening, apoptosis, and regression of tumor formation.50–52 DN-human TERT (DN-hTERT) forms heterodimers with wildtype hTERT that are exported to the cytosol.53 As cytosolic hTERT is ubiquitinated by several E3 ubiquitin ligases including MKRN1, CHIP, and HDM2,54,55 DN-hTERT causes degradation of wild-type hTERT protein.55

Telomerase inhibitors only inhibit the enzymatic activity of telomerase, therefore, strategies to avoid telomere-independent antiapoptotic functions of TERT should be considered. Furthermore, because DN-TERT exerts similar antiapoptotic activity as hTERT, it may promote the survival of cancer cells, especially when wildtype hTERT is depleted.10

**Immunotherapy for TERT-expressing tumors**

As previously noted, telomerase is frequently activated in cancers. As telomerase-expressing cancer cells may present epitopes of hTERT through human leukocyte antigen, these cells can be eliminated by stimulating the immune system with specific vaccines derived from hTERT.
Anticancer strategies targeting telomeres and telomerase may be effective, they can also be severely influenced by certain genetic environments. Mutations in p53 are frequently found (approximately 50%) in human breast and colorectal adenocarcinomas, and inhibition of TERT leads to cell cycle arrest, senescence, and apoptosis in a p53-dependent manner. In a p53-negative cell line, expression of DN-hTERT does not cause apoptosis. Restoration of p53, however, sensitizes cancer cells to DN-hTERT, implying that p53 mutant cancers might be resistant to antitelomerase drugs. A recent study revealed that reexpression of TERT in Tert knockout mice causes aggressive tumor formation and bone metastasis, probably through loss of SMAD4. This finding demonstrates the risk of virtually imperfect cancer treatment with antitelomerase drugs, which may possibly lead to more aggressive cancers when the patients discontinue treatment. Thus, monitoring SMAD4 mutation status may be helpful for antitelomerase drug therapy. Based on the patient’s genotype, additional chemotherapeutics could be coprescribed with telomerase inhibitors. Thus, the clinical application of drugs targeting telomeres and telomerase should be accompanied with, or preceded by, genetic monitoring of the patients.

In spite of their promise, more detailed analyses are required to confirm the safety of anticancer drugs targeting telomeres and telomerase. Prior to clinical trials, the potential risks of the treatments should be evaluated at the level of the entire organism, as accumulating evidence indicates that telomerase has functions other than regulating telomere elongation. In addition, as exemplified by p53 mutations, genetic modifiers affecting clinical outcomes should be prescreened. Moreover, the creation and use of novel mouse models in preclinical studies is essential for the development of anticancer drug strategies that are both effective and safe.

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Disclosure
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