

NOVEL ANTICANCER THERAPEUTICS TARGETING TELOMERASE

(SUPPLEMENT)

Maria Ruden, M.S.^{a b*} and Neelu Puri, Ph.D.^{a^}

^a *Department of Biomedical Sciences, University of Illinois College of Medicine at Rockford, Rockford, Illinois 61107-1822, USA;*

^b *Presently at: Department of Medicine, University of Illinois at Chicago, Chicago, IL 60607-4067, USA*

Author address:

Maria Ruden, MS: mruden2@uic.edu
University of Illinois at Chicago
Department of Medicine
900 S Ashland Ave., Suite 3306 (M/C 767)
Chicago, IL 60607-4067
Tel.: 312-413-4575 Fax: 815-516-8439

Address correspondence to:

Neelu Puri, Ph.D.: neelupur@uic.edu
University of Illinois College of Medicine at Rockford
Department of Biomedical Sciences
1601 Parkview Avenue
Rockford, Illinois 61107
Tel: 815-395-5678 Fax: 815-395-5666 Email: neelupur@uic.edu

*^ Both authors contributed equally.

NOVEL ANTICANCER THERAPEUTICS TARGETING TELOMERASE

(SUPPLEMENT)

ANTISENSE OLIGONUCLEOTIDES (AS-ODNs) - TARGETING hTER

GRN163L - Preclinical Studies

GRN163L has been tested in several preclinical studies on multiple tumor models *in vitro* and *in vivo*, resulting in a successful inhibition of TA¹⁻³. Gellert et al. have shown that *in vitro* treatment of MDA-MB-231 and MDA-MB-435 breast cancer cell lines with GRN163L led to inhibition of TA, resulting in decreased proliferation, increased senescence and apoptosis, reduced tumorigenicity and diminished tumor invasive potential, possibly due to inhibition of cell migration and prevention of growth at distant sites^{1,4}. The latter indicates that GRN163L may be a prospective drug candidate for treatment of patients after surgical removal of tumors^{1,5}. Studies have found that the disruption of colony formation after treatment with GRN163L was reversible, since cells had regained the ability to form colonies after being taken off the drug treatment. This indicates that GRN163L may be causing an acute DNA damage response⁵. A study of GRN163L in multiple types of breast cancer cell lines, including ER⁺, ER⁻, HER2⁺, BRCA1 mutant breast cancer cell lines *in vitro* have shown a differential effect which may be dependent on the genetic composition of these cell lines¹. In addition, GRN163L administered intranasally into human intracerebral glioblastoma xenografts could successfully cross the blood-brain barrier (BBB), leading to inhibition of tumor cell growth without imposing the cytotoxic effects on normal human brain cells⁶.

Studies have also shown that GRN163L can sensitize cells with critically shortened telomeres to radiation, chemotherapy, as well as hyperthermia mediated IR treatment. This may be linked with a decrease in HSP70, which is associated with the telomerase complex and could be responsible for resistance to IR (44; 49; 52). Using GRN163L in combination with IR therapy may require lower doses of the IR, which would be less toxic on normal cells. Several preclinical studies have shown that GRN163L can sensitize cancer cells to certain cytotoxic drugs or synergistically enhance the effect of some chemotherapeutics on cancer cells^{2,7-9}. This may be due to a significant telomere shortening as a result of GRN163L treatment, ultimately resulting in DNA damage responses and enhanced cytotoxic drug responses. It has been shown that pre-treating or treating cells with GRN163L can sensitize cancer cells to cisplatin and synergistically enhance the response to paclitaxel, leading to the impairment of the DNA repair pathways and inhibition of cellular invasion *in vitro* and tumor growth *in vivo*^{2,7}. These studies indicate that GRN163L may have great potential in preventing tumor cell proliferation and metastasis².

Another novel finding about GRN163L is its ability to inhibit tumor initiating cells (TICs) and cancer stem cells (CSCs) in breast, pancreatic, prostate, glioblastoma (GBM) and multiple myeloma (MM) cell lines *in vitro* and, in xenograft models, *in vivo*^{3,10-12}. In breast and prostate cancer cell lines, GRN163L reduced the growth of bulk tumor cells and CSC subpopulations, suggesting that the depletion of CSCs may be an alternative mechanism for the reduction of tumorigenicity^{3,10}. Because CSCs are responsible for maintaining tumor progression and metastases, the ability of GRN163L to target TICs and CSCs may be of great importance, especially for treatment of highly metastatic and clonogenic cancers like prostate and MM^{3,10,11}.

The potential negative effect of GRN163L is that it can inhibit normal proliferative stem cells, which also contain telomerase, and this may further lead to a decline in their regenerative capacity¹². However, *in vitro* study with bone-marrow-derived rat mesenchymal stem cells and *in vivo* study with TICs in GBM tumors, this effect has been found to be reversible^{12,13}. In conclusion, the results of multiple preclinical studies have shown that GRN163L has a wide biodistribution and using it clinically could achieve highly efficacious results^{14,15}.

TELOMERASE BASED IMMUNOTHERAPIES

GV1001 - Clinical Trials

The first successful phase I/II clinical trial with GV1001 conducted in 48 patients with non-resectable histologically confirmed pancreatic adenocarcinoma has shown 63% overall immune response rate for 38 evaluable patients, after eight injections of GV1001 with GM-CSF, administered over a 10-week period. Interestingly, the median survival time for GV1001 (8.6 month) was higher than the median survival time for Gemcitabine (4.8-5.6 months), which is a commonly used treatment for non-resectable pancreatic adenocarcinoma¹⁶. This indicates that treatment with GV1001 as a single-regimen therapy may be a more effective method for non-resectable pancreatic adenocarcinoma, producing fewer side effects and adverse events (AEs) compare to Gemcitabine.

A phase I/II clinical study conducted in 25 stage IV metastatic melanoma patients has shown 81% immune response rate in 17 out of 21 patients treated and a good clinical response with some patients developing stable disease (SD) and 1 of the 25 patients, exhibiting a partial response (PR), evidenced by shrinkage or disappearance of multiple lung metastases, indicating that GV1001 could possibly be a successful treatment for advanced stages of melanomas. The immune response was seen after only 6 vaccinations with a combination of GV1001, temozolomide and GM-CSF, over a period of three months. The vaccine did not produce delayed-type hypersensitivity (DTH) reactions and CD4+ T cells displayed a Th1 cytokine profile^{17,18}.

A phase I/II clinical trial conducted in patients with advanced stages of NSCLC (IIIB and IV) has shown a positive immune response in 13 out of 24 patients after only about 3 to 9 weeks of treatment¹⁹. The immune response to high doses of the vaccine could not be completely assessed, because patients who were in advanced stages of NSCLC developed brain metastases (a common problem in patients with advanced NSCLC) and had to be removed from the study. The median survival time for NSCLC patients in this trial was 8.5 months, with 8 alive at 12, 6 at 18, and 4 at 30 to 45 months after beginning treatment²⁰. Based on the results of this trial, it is suspected that better study results would have been obtained in patients with less advanced stages of NSCLC.

TELOMERASE BASED GENE THERAPIES

Gene Therapy Approach Overview

Gene therapy is defined by an introduction of exogenous nucleic acids into host cells via delivery of suicide genes or viral vectors²¹. Adenoviruses driven with hTERT or hTER promoters can be used for targeting of telomerase positive cancer cells without affecting normal human cells (106). One of the most prominent advantages of hTERT/hTER promoter driven gene therapy compared to other anticancer treatments is its tumor specificity and no toxicity on normal human cells. The oncolytic virus therapy and suicide gene therapy are two of the most studied approaches that are reviewed below²²
²³.

Oncolytic Virus Based Therapy

Oncolytic virus therapy is a rapidly growing method for selective targeting of tumor cells with especially designed oncolytic adenoviruses. The adenovirus-mediated tumor cell targeting is accomplished with a hTERT promoter that selectively drives viral transgene expression and may serve as a central control mechanism for transcription of several adenoviral genes, such as E1A and E1B, which encode adenoviral proteins and are critical for viral replication^{22,24,25}. This approach is also referred to as a hTERT/hTER promoter driven tumor-specific replication competent adenoviral gene therapy (hTERT/hTERp-TRAD), since hTERT promoter controls the replication of the lytic virus within specific telomerase positive tumor cells only, leading to viral replication and tumor cell lysis^{22,25-29}. One of the advantages of this therapy is that the oncolytic virus will replicate within the targeted cells until all tumor cells are eliminated^{30,31}.

Telomelysin (OB-301)

Telomelysin® (OB-301) (Oncolys BioPharma Inc., Tokyo, Japan) is one of the more successful therapeutics in this category. Telomelysin is composed of an attenuated TRAD-5 vector (Ad5), which has its E1 region replaced with the hTERT promoter driving E1A and E1B genes and resulting in better therapeutic efficacy in tumor cells and reduced toxicity in normal human cells^{29,32} (**Fig. 1S**). The attachment of IRES (internal ribosome entry site gene) to E1 and E2 gene complex may improve specificity and therapeutic index of (TI) the adenovirus, as seen in a study with hepatocellular carcinoma cells³². Compared to other adenoviruses, telomelysin is unique in its design as it contains a complete functional E3 viral region, which can bind and retain the MHC class-I molecules, decrease viral clearance and increase viral replication within specific tumors^{33,34}.

A preclinical study of telomelysin treatment of nu/nu mice *in vivo* has shown a significant tumor reduction and direct tumor cell lysis after just 14 days of treatment^{35,36}. A study of telomelysin treatment in NSCLC and colorectal carcinoma cell lines *in vitro* has shown a selective targeting of malignant cells in a dose-dependent manner without cytotoxic effect on normal human cells, indicating that viral replication is only occurring in hTERT-positive cells³⁶. This cytotoxic effect on tumor cells and not on normal cells was explained by viral replication occurring only in hTERT-positive cells and was confirmed by tumor cell ulceration, indicating necrotic cell death. *In vivo study* of telomelysin in xenograft orthotopic model of human colorectal tumors has shown a significant inhibition of lymph node tumors, which have metastasized into the regional draining lymphatic system, indicating that telomelysin treatment

could potentially be an effective approach for treatment or prevention of lymph node metastases³⁵. A more recent preclinical study showed that combination therapy of telomelysin and standard chemotherapies might be an effective treatment for telomelysin-resistant tumors. Pretreatment of telomelysin-resistant head and neck squamous cell carcinoma (HNSCC) cell lines with cisplatin, paclitaxel, or streptolysin O, has increased their virus infectivity and showed inhibition of early virus proliferation³⁷. Thus, telomelysin, like many other telomerase inhibiting agents, may be more effective when used in combination with other antitumor therapeutics³⁷.

Successful preclinical studies with telomelysin led to a phase I clinical trial, where safety and PD of telomelysin monotherapy were evaluated in 16 patients with various types of advanced solid tumors³⁴. The onset of the immune response was evident just 30 minutes after the treatment, followed by IL-6 production in 8 out of 9 patients, IL-10 in 7 out of 9, and IFN- γ in 2 patients, indicating stimulation of CD4+ response with activation of both Th1 and Th2 CTLs. This study has shown effective responses in most patients with only minor grade 1 and 2 toxicities at the intratumoral injection (IT) site in most of patients. Based on a RECIST evaluation criteria, one patient reached PR and seven DS after 56 days of telomelysin treatment and 56.7% reduction in tumor mass was seen in one patient. Overall, median survival for patients treated with telomelysin was 10 months (ranging from 1 to 21). Further studies with telomelysin may be required to fully assess its safety and its effect on various normal cells, including stem cells that express hTERT and to determine whether telomelysin therapy may be more effective in combination with other chemotherapy, IR, immunotherapy, surgery or molecular therapy³⁸.

Suicide Gene-Directed Enzyme Prodrug Therapy (GDEPT)

Suicide gene therapy, also called gene-directed enzyme prodrug therapy (GDEPT), uses the hTER/TERT promoter placed upstream of genes designed to kill cells when their products are expressed. Similar to oncolytic gene therapy, this approach targets tumor cells without negative effect on normal cells^{39,40}. In suicide gene therapy, the E1/E3 region of the adenoviral promoter is replaced with the hTERT/hTER sequence making the virus replication deficient⁴¹. The suicide gene therapy approach consists of three general steps. First, the targeting vector carrying an enzyme-encoding gene driven by hTERT or hTER is transfected into the cell, second step is the addition of the prodrug, and third is further activation of the prodrug to its toxic byproduct by the enzyme, resulting in release of toxins into the cytoplasm and killing of telomerase positive cells³⁹ (**Fig. 2S**).

Ad-hTER/hTERT-NTR/CB1954 - Suicide Gene Therapy

The Ad-hTER/hTERT-NTR/CB1954 is a well-studied telomerase-specific adenoviral suicide gene therapy vector expressing the nitroreductase (NTR) gene. It works by activating a prodrug CB1954, which is a weak alkylation agent, to its toxic form, resulting in a cross-linking of dividing and non-dividing tumor cells thus making it more effective compared to other suicide gene therapies⁴². It has been found that the hTER/NTR promoter activity is slightly stronger than the hTERT/NTR, and can successfully sensitize various cell lines *in vitro* and *in vivo* to CB1954 prodrug, leading to a bystander effect leading to destruction of infected cells and surrounding tumor cells⁴².

hTERTp-HRP/IAA - Suicide Gene Therapy

Another suicide gene therapy, which may have promising results in the near future as preclinical studies continue to show good outcomes, is the hTERT promoter (hTERTp)-driven horseradish peroxidase (HRP)/indole-3-acetic acid (IAA). The HRP is used to convert a non-cytotoxic IAA to cytotoxic metabolites, resulting in tumor cell death and initiating a bystander effect on surrounding cells. In a preclinical study with a combination of hTERTp-HRP/IAA and radiotherapy in laryngeal squamous cell carcinoma (LSCC) cells, a synergistic effect was noticed, which was evidenced by the upregulation of hTERT promoter activity, increased hTERT expression, and HRP expression, leading to more efficient liposome-mediated gene transfection in both radiosensitive and radioresistant cells⁴³. Another study has shown that combination therapy of cisplatin along with hTERTp-HRP/IAA GDEPT in human uterine cervical cancer (HeLa) cell lines significantly enhanced GDEPT therapy response⁴⁴. With future research, oncolytic and suicide gene therapies targeting telomerase may prove to be more effective than other anticancer therapies since their tumor specificity could prevent undesirable DLT's⁴¹.

Although suicide gene therapy or oncolytic vector hTERT/hTER promoter driven therapy have shown good results, they are not as effective as vaccines or oligonucleotide type of telomerase inhibitors and further research and must be tested on humans to prove their safety.

OTHER TELOMERASE INHIBITING STRATEGIES

BRACO-19 - a Trisubstituted Acridine G-quadruplex Ligand

BRACO-19, is a 3,6,9-trisubstituted acridine (9-[4-(N,N-dimethylamino)]-3,6-bis(3-pyrrolidinopropionamido)) compound, designed with computerized modeling using Molecular Dynamics Stimulation Methods to act as G-quadruplex stabilizer^{45,46}. BRACO19 may not be as potent a G-quadruplex ligand as telomestatin, since it induces senescence in only a limited number of cell lines and causes incomplete disruption of telomeric proteins⁴⁷. At sub-lethal doses and in short-term treatments of only 2 weeks, BRACO19 has shown high tumor selectivity and growth inhibition without long lag-times, resulting in a quiescent and non-replicative tumor cell phenotype in multiple types of tumors. In addition, treatment with BRACO19 resulted in a dose-dependent reduction in the overhang signal, evidenced by complete dissociation of POT1 and no effect on TRF2 association. These findings indicate that BRACO19 causes telomere uncapping and may lead to telomere shortening as a result of partial telomerase inhibition⁴⁸.

In a study of BRACO19 on early stage uterine carcinoma xenograft models, which have very short telomeres, almost complete inhibition of hTERT was observed after only a short period of treatment. In this study, it has been shown that BRACO19 was able to quickly localize to cell nuclei, without significant lag time, and resulted in quick inhibition of telomerase. This was suggested to be due to an ubiquitin-mediated proteolysis and a complete blocking of hTERT to telomeres⁴⁹. In addition, due to limited membrane permeability of BRACO19⁵⁰ in xenograft tumor models, BRACO19 has proven to be more effective when administered intraperitoneally (i.p.)

rather than by mouth (p.o.)⁴⁹. These data indicate that BRACO19 may be a successful single agent therapy for tumors with shorter telomeres.

RHPS4

RHPS4 is another promising and potent small molecule G-quadruplex stabilizing ligand, similar in structure to telomestatin but smaller in size and with less tumor selectivity. RHPS4, a 3,11-difluoro-6,8,13-trimethyl-8H-quinolo[4,3,2-kl]acridinium methosulfate, is a water soluble⁵¹ polycyclic fluorinated acridinium cation, which carries a net positive charge in its small acridinium rings. This gives it an enhanced affinity to G-quadruplex and ability to penetrate heavy tumor masses⁵²⁻⁵⁴. Similar to telomestatin, RHPS4 forms stacking interactions, by binding with high affinity, with G-quadruplex at the terminal G-tetrads, thus leading to telomere uncapping and telomerase inhibition^{51,55}. In a study of its efficacy and antineoplastic activity in various tumor xenograft models, RHPS4 has shown a significant tumor reduction, from 30%⁵¹ to 75%⁵⁶. RHPS4 has been well tolerated and had minimal cytotoxicity when used in lower treatment doses *in vitro* and *in vivo*, without any adverse effects on hematopoietic and bone marrow cell lines⁵³. Preclinical studies with the RHPS4 have shown that its antineoplastic activity and chemotherapeutic index (CI), which indicates a ratio between the minimal effective dose and the maximal tolerated dose of the chemotherapeutic drug were similar to or greater than that of common chemotherapeutic drugs⁵³. Studies in colorectal carcinoma cell lines have shown that RHPS4 can act as a successful G-quadruplex stabilizer by targeting both telomeres and telomerase and resulting in complete dissociation of hTERT from the nucleus⁵¹.

A combination therapy of RHPS4 with cisplatin in breast cancer cell lines⁵², camptothecin in melanoma and colorectal carcinoma⁵³, and taxol in uterus carcinoma⁵¹, has shown a synergistic effect. However, this effect was highly dependent on the ratio and the sequence of drug administration⁵⁷. In addition, studies have shown that when RHPS4 was combined with taxol, it induced DNA damage response, which resulted in enhanced effect on the anaphase bridging⁵¹, and when RHPS4 was combined with camptothecin, it showed blocking of the DNA damage repair response induced by camptothecin⁵³. This indicates that the synergistic effect of RHPS4 on certain chemotherapeutic drugs, such as taxol⁵¹ or camptothecin, may be due to the combined drugs targeting the replication of G-rich overhang of the telomere by recruiting different mechanisms of action⁵². On the contrary, a combination therapy of RHPS4 with other drugs, such as Adriamycin, gemcitabine and paclitaxel, has shown a slightly additive or an antagonistic effect, which could be because their action was sterically antagonized by the RHPS4.

TARGETING TELOMERE AND TELOMERASE-ASSOCIATED PROTEINS

Tankyrase Inhibitors

Considering that tankyrases are present in normal cells, it is important to know that PARP inhibitors do not induce telomere shortening in normal cells but in telomerase positive cells only⁵⁸. The resistance of cells with critically shortened telomeres to telomerase inhibitors could be related to a decrease in TRF1, which is directly correlated with an increase in PARP1⁵⁹. In addition, PARP1 activity might regulate other factors involved in tumor growth and development, angiogenesis and metastasis,

due to its ability to regulate transcription of proteins involved in tumorigenesis and angiogenesis ⁶⁰. It has been shown that when targeting critically shortened telomeres with a synthetic telomerase inhibitor MST-312, there was no effect on telomerase inhibition. However, when the same cells were targeted with the 3'-aminobenzamide (3AB), a PARP inhibitor, the function of tankyrase 1 was diminished, which resulted in inhibition of telomerase by MT-312 ⁵⁸. These findings suggest that PARP inhibitors may be able to reverse resistance against direct telomerase inhibitors, leading to accelerated telomere shortening and reduced treatment time ⁵⁹.

SUPPLEMENTAL FIGURE CAPTIONS:**Fig. 1S. Oncolytic virus-based cell therapy - telomelysin.**

Telomelysin® (OB-301) is oncolytic virus therapy vector, composed of an attenuated telomerase-specific replication-competent adenovirus-5 (TRAD) vector (Ad5) that uses hTERT promoter to control E1A and E1B genes⁶¹. Telomelysin contains a complete functional E3 viral region that allows it to bind and retain the MHC class-I molecules, decreases viral clearance and increases viral replication within specific tumors. Once the adenovirus-5 (Ad5) vector with the hTERT promoter is injected, it drives the virus to replicate and lyse in telomerase-positive tumor cells having no effect on normal cells^{33,34}. This viral replication causes an increase of uric acid, which serves as a danger signal and recruits dendritic cells, which in turn stimulate production of IL-10 and IFN- γ , leading to generation of CD4+ response and activates Th1 and Th2 CTLs³⁴.

Fig. 2S. Suicide gene vector therapy - Ad-hTERT/hTERp-NTR/CB1954.

Ad-hTER/hTERT-NTR/CB1954 is the telomerase-specific adenoviral suicide gene therapy with the hTER or hTERT driven vector expressing the nitroreductase (NTR) gene. First, the targeting vector with the enzyme-encoding gene is transfected into the tumor cell. Then the prodrug, such as CB1954 is added and activated to its toxic form. The activated form of CB1954, causes cross-linking of both dividing and non-dividing tumor cells. The suicide Ad-hTER/hTERT-NTR vector does not affect normal telomerase negative cells. However, once the activated prodrug releases its toxins into the cytoplasm, it leads to a bystander effect therefore infecting other tumor cells and successfully sensitizing various cell lines *in vitro* and *in vivo*^{41,42}.

REFERENCES:

1. Hochreiter AE, Xiao H, Goldblatt EM, Gryaznov SM, Miller KD, Badve S, et al. Telomerase template antagonist GRN163L disrupts telomere maintenance, tumor growth, and metastasis of breast cancer. *Clin Cancer Res* 2006;12:3184-92.
2. Goldblatt EM, Gentry ER, Fox MJ, Gryaznov SM, Shen C, Herbert BS. The telomerase template antagonist GRN163L alters MDA-MB-231 breast cancer cell morphology, inhibits growth, and augments the effects of paclitaxel. *Mol Cancer Ther* 2009;8:2027-35.
3. Brennan SK, Wang Q, Tressler R, Harley C, Go N, Bassett E, et al. Telomerase inhibition targets clonogenic multiple myeloma cells through telomere length-dependent and independent mechanisms. *PLoS One* 2010;5:e12487.
4. Jackson SR, Zhu CH, Paulson V, Watkins L, Dikmen ZG, Gryaznov SM, et al. Antiadhesive effects of GRN163L--an oligonucleotide N3'->P5' thio-phosphoramidate targeting telomerase. *Cancer Res* 2007;67:1121-9.
5. Gellert GC, Dikmen ZG, Wright WE, Gryaznov S, Shay JW. Effects of a novel telomerase inhibitor, GRN163L, in human breast cancer. *Breast Cancer Res Treat* 2006;96:73-81.
6. Hashizume R, Ozawa T, Gryaznov SM, Bollen AW, Lamborn KR, Frey WH, 2nd, et al. New therapeutic approach for brain tumors: Intranasal delivery of telomerase inhibitor GRN163. *Neuro Oncol* 2008;10:112-20.
7. Uziel O, Beery E, Dronichev V, Samocha K, Gryaznov S, Weiss L, et al. Telomere shortening sensitizes cancer cells to selected cytotoxic agents: in vitro and in vivo studies and putative mechanisms. *PLoS One* 2010;5:e9132.
8. Goldblatt EM, Erickson PA, Gentry ER, Gryaznov SM, Herbert BS. Lipid-conjugated telomerase template antagonists sensitize resistant HER2-positive breast cancer cells to trastuzumab. *Breast Cancer Res Treat* 2009;118:21-32.
9. Shammass MA, Qazi A, Batchu RB, Bertheau RC, Wong JY, Rao MY, et al. Telomere maintenance in laser capture microdissection-purified Barrett's adenocarcinoma cells and effect of telomerase inhibition in vivo. *Clin Cancer Res* 2008;14:4971-80.
10. Joseph I, Tressler R, Bassett E, Harley C, Buseman CM, Pattamatta P, et al. The telomerase inhibitor imetelstat depletes cancer stem cells in breast and pancreatic cancer cell lines. *Cancer Res* 2010;70:9494-504.
11. Marian CO, Wright WE, Shay JW. The effects of telomerase inhibition on prostate tumor-initiating cells. *Int J Cancer* 2010;127:321-31.
12. Marian CO, Cho SK, McEllin BM, Maher EA, Hatanpaa KJ, Madden CJ, et al. The telomerase antagonist, imetelstat, efficiently targets glioblastoma tumor-initiating cells leading to decreased proliferation and tumor growth. *Clin Cancer Res* 2010;16:154-63.
13. Tokcaer-Keskin Z, Dikmen ZG, Ayaloglu-Butun F, Gultekin S, Gryaznov SM, Akcali KC. The effect of telomerase template antagonist GRN163L on bone-marrow-derived rat mesenchymal stem cells is reversible and associated with altered expression of cyclin d1, cdk4 and cdk6. *Stem Cell Rev* 2010;6:224-33.
14. Tressler RJ, Chin AC, Gryaznov S, Harley CB. Preclinical development of GRN163L, a telomerase inhibitor for cancer therapy. *AACR Meeting Abstracts* 2005;2005:1022-d-1023.

15. Tressler RJ, Chin AC, Gryaznov SM, Harley CB. Preclinical efficacy, safety, and ADME of GRN163L, a novel telomerase inhibitor developed for the treatment of cancer. *J Clin Oncol (Meeting Abstracts)* 2005;23:3168.
16. Bernhardt SL, Gjertsen MK, Trachsel S, Moller M, Eriksen JA, Meo M, et al. Telomerase peptide vaccination of patients with non-resectable pancreatic cancer: A dose escalating phase I/II study. *Br J Cancer; J Clin Oncol* 2006;95:1474-82.
17. Aamdal S, Dueland S, Engebraaten O, Owre K, Dyrhaug M, Trachsel S, et al. A phase I/II study of telomerase peptide vaccination in combination with chemotherapy in patients with stage IV malignant melanoma. *J Clin Oncol (Meeting Abstracts)* 2006;24.
18. Trial With Telomerase Peptide Vaccine In Combination With Temozolomide in Patients With Advanced Malignant Melanoma. NCT01247623. <http://www.clinicaltrials.gov/ct2/results?term=NCT01247623>
19. Brunsvig P. F, Gjertsen M. K, Kvalheim G, Aamdal S, Markowski-Grimsrud... C. J, Sve I, et al. A phase I/II study of telomerase peptide vaccination of patients with non-small cell lung cancer. *ASCO Meeting Abstracts; J Clin Oncol* 2005;23:2580.
20. Brunsvig ,Paal, Aamdal ,Steinar, Gjertsen ,Marianne, Kvalheim ,Gunnar, Markowski-Grimsrud ,Carrie, Sve ,Ingunn, et al. Telomerase peptide vaccination: a phase I/II study in patients with non-small cell lung cancer. *Cancer Immunology, Immunotherapy* 2006:1553-64.
21. Wong HH, Lemoine NR. Biological approaches to therapy of pancreatic cancer. *Pancreatology* 2008;8:431-61.
22. Fujiwara T, Urata Y, Tanaka N. Therapeutic Targets and Drugs IV: Telomerase-Specific Gene and Vector-Based Therapies for Human Cancer. In: Hiyama K, editor. *Telomeres and Telomerase in Cancer*. : Springer; 2009. p. 293-312.
23. Shay JW. Telomerase as a Target for Cancer Therapeutics. In: Roth JA, editor. *Gene-based therapies for cancer, current cancer research*. New York; London: Springer; 2010. p. 231-49.
24. Gu J, Fang B. Telomerase promoter-driven cancer gene therapy. *Cancer Biol Ther* 2003;2:S64-70.
25. Irving J, Wang Z, Powell S, O'Sullivan C, Mok M, Murphy B, et al. Conditionally replicative adenovirus driven by the human telomerase promoter provides broad-spectrum antitumor activity without liver toxicity. *Cancer Gene Ther* 2004;11:174-85.
26. Keith W, Nicol, Bilslund A, E. Targeting Telomerase: Therapeutic Options for Cancer Treatment. In: Rudolph KL, editor. *Telomeres and Telomerase in Ageing, Disease, and Cancer*. Berlin Heidelberg: Springer-Verlag; 2008. p. 247-83.
27. Ouellette MM, Wright WE, Shay JW. Targeting Telomerase-Expressing Cancer Cells. *J Cell Mol Med* 2011.
28. Lanson NA, Jr, Friedlander PL, Schwarzenberger P, Kolls JK, Wang G. Replication of an adenoviral vector controlled by the human telomerase reverse transcriptase promoter causes tumor-selective tumor lysis. *Cancer Res* 2003;63:7936-41.
29. Kawashima T, Kagawa S, Kobayashi N, Shirakiya Y, Umeoka T, Teraishi F, et al. Telomerase-specific replication-selective virotherapy for human cancer. *Clin Cancer Res* 2004;10:285-92.
30. Glass M, Soling A, Messerle M. Tumor-specific activity of cellular regulatory elements is down-regulated upon insertion into the herpes simplex virus genome. *J Neurovirol* 2008;14:522-35.

31. Abou El Hassan MA, van der Meulen-Muileman I, Abbas S, Kruyt FA. Conditionally replicating adenoviruses kill tumor cells via a basic apoptotic machinery-independent mechanism that resembles necrosis-like programmed cell death. *J Virol* 2004;78:12243-51.
32. Fujiwara T. Targeted Oncolytic Adenovirus for Human Cancer Therapy: Gene-Based Therapies for Cancer. In: Roth JA, editor. *Gene-based therapies for cancer, current cancer research*. New York; London: Springer; 2010. p. 79-93.
33. Lichtenstein DL, Toth K, Doronin K, Tollefson AE, Wold WS. Functions and mechanisms of action of the adenovirus E3 proteins. *Int Rev Immunol* 2004;23:75-111.
34. Nemunaitis J, Tong AW, Nemunaitis M, Senzer N, Phadke AP, Bedell C, et al. A phase I study of telomerase-specific replication competent oncolytic adenovirus (telomelysin) for various solid tumors. *Mol Ther* 2010;18:429-34.
35. Fujiwara T, Shirakawa Y, Kagawa S. Telomerase-specific oncolytic virotherapy for human gastrointestinal cancer. *Expert Rev Anticancer Ther* 2011;11:525-32.
36. Kawashima T, Kagawa S, Kobayashi N, Shirakiya Y, Umeoka T, Teraishi F, et al. Telomerase-specific replication-selective virotherapy for human cancer. *Clin Cancer Res* 2004;10:285-92.
37. Sakakibara A, Tsukuda M, Kondo N, Ishiguro Y, Kimura M, Fujita K, et al. Examination of the optimal condition on the in vitro sensitivity to telomelysin in head and neck cancer cell lines. *Auris Nasus Larynx* 2011;38:589-99.
38. Kyo S, Takakura M, Fujiwara T, Inoue M. Understanding and exploiting hTERT promoter regulation for diagnosis and treatment of human cancers. *Cancer Sci* 2008;99:1528-38.
39. Tian X, Chen B, Liu X. Telomere and telomerase as targets for cancer therapy. *Appl Biochem Biotechnol* 2010;160:1460-72.
40. Harley CB. Telomerase and cancer therapeutics. *Nat Rev Cancer* 2008;8:167-79.
41. Bilsland AE, Anderson CJ, Fletcher-Monaghan AJ, McGregor F, Evans TR, Ganly I, et al. Selective ablation of human cancer cells by telomerase-specific adenoviral suicide gene therapy vectors expressing bacterial nitroreductase. *Oncogene* 2003;22:370-80.
42. Plumb JA, Bilsland A, Kakani R, Zhao J, Glasspool RM, Knox RJ, et al. Telomerase-specific suicide gene therapy vectors expressing bacterial nitroreductase sensitize human cancer cells to the pro-drug CB1954. *Oncogene* 2001;20:7797-803.
43. Liao Z, Huang C, Zhou F, Xiong J, Bao J, Zhang H, et al. Radiation enhances suicide gene therapy in radioresistant laryngeal squamous cell carcinoma via activation of a tumor-specific promoter. *Cancer Lett* 2009;283:20-8.
44. Zhang H, Liao ZK, Sun WJ, Huang C, Xiong J, Zhou FX, et al. Enhanced suicide gene therapy using a tumor-specific promoter in combination with cisplatin. *Mol Med Report* 2009;2:1017-22.
45. Gowan SM, Harrison JR, Patterson L, Valenti M, Read MA, Neidle S, et al. A G-quadruplex-interactive potent small-molecule inhibitor of telomerase exhibiting in vitro and in vivo antitumor activity. *Mol Pharmacol* 2002;61:1154-62.
46. Haider SM, Neidle S. A molecular model for drug binding to tandem repeats of telomeric G-quadruplexes. *Biochem Soc Trans* 2009;37:583-8.
47. Incles CM, Schultes CM, Kempfski H, Koehler H, Kelland LR, Neidle S. A G-quadruplex telomere targeting agent produces p16-associated senescence and chromosomal fusions in human prostate cancer cells. *Mol Cancer Ther* 2004;3:1201-6.

48. Gunaratnam M, Greciano O, Martins C, Reszka AP, Schultes CM, Morjani H, et al. Mechanism of acridine-based telomerase inhibition and telomere shortening. *Biochem Pharmacol* 2007;74:679-89.
49. Burger AM, Dai F, Schultes CM, Reszka AP, Moore MJ, Double JA, et al. The G-quadruplex-interactive molecule BRACO-19 inhibits tumor growth, consistent with telomere targeting and interference with telomerase function. *Cancer Res* 2005;65:1489-96.
50. Taetz S, Baldes C, Murdter TE, Kleideiter E, Piotrowska K, Bock U, et al. Biopharmaceutical characterization of the telomerase inhibitor BRACO19. *Pharm Res* 2006;23:1031-7.
51. Phatak P, Cookson JC, Dai F, Smith V, Gartenhaus RB, Stevens MF, et al. Telomere uncapping by the G-quadruplex ligand RHPS4 inhibits clonogenic tumour cell growth in vitro and in vivo consistent with a cancer stem cell targeting mechanism. *Br J Cancer* 2007;96:1223-33.
52. Cookson JC, Dai F, Smith V, Heald RA, Laughton CA, Stevens MF, et al. Pharmacodynamics of the G-quadruplex-stabilizing telomerase inhibitor 3,11-difluoro-6,8,13-trimethyl-8H-quino[4,3,2-kl]acridinium methosulfate (RHPS4) in vitro: activity in human tumor cells correlates with telomere length and can be enhanced, or antagonized, with cytotoxic agents. *Mol Pharmacol* 2005;68:1551-8.
53. Leonetti C, Scarsella M, Riggio G, Rizzo A, Salvati E, D'Incalci M, et al. G-quadruplex ligand RHPS4 potentiates the antitumor activity of camptothecins in preclinical models of solid tumors. *Clin Cancer Res* 2008;14:7284-91.
54. Linder J, Garner TP, Williams HE, Searle MS, Moody CJ. Telomestatin: formal total synthesis and cation-mediated interaction of its seco-derivatives with G-quadruplexes. *J Am Chem Soc* 2011;133:1044-51.
55. Garner TP, Williams HE, Gluszyk KI, Roe S, Oldham NJ, Stevens MF, et al. Selectivity of small molecule ligands for parallel and anti-parallel DNA G-quadruplex structures. *Org Biomol Chem* 2009;7:4194-200.
56. Salvati E, Leonetti C, Rizzo A, Scarsella M, Mottolese M, Galati R, et al. Telomere damage induced by the G-quadruplex ligand RHPS4 has an antitumor effect. *J Clin Invest* 2007;117:3236-47.
57. Gunaratnam M, Green C, Moreira JB, Moorhouse AD, Kelland LR, Moses JE, et al. G-quadruplex compounds and cis-platin act synergistically to inhibit cancer cell growth in vitro and in vivo. *Biochem Pharmacol* 2009;78:115-22.
58. Seimiya H, Muramatsu Y, Ohishi T, Tsuruo T. Tankyrase 1 as a target for telomere-directed molecular cancer therapeutics. *Cancer Cell* 2005;7:25-37.
59. Hiyama K. *Telomeres and Telomerase in Cancer*. : Springer; 2009.
60. Munoz-Gamez JA, Quiles-Perez R, Ruiz-Extremera A, Martin-Alvarez AB, Sanjuan-Nunez L, Carazo A, et al. Inhibition of poly (ADP-ribose) polymerase-1 enhances doxorubicin activity against liver cancer cells. *Cancer Lett* 2011;301:47-56.
61. Li Y, Yu DC, Chen Y, Amin P, Zhang H, Nguyen N, et al. A hepatocellular carcinoma-specific adenovirus variant, CV890, eliminates distant human liver tumors in combination with doxorubicin. *Cancer Res* 2001;61:6428-36.