Review Article

Telomeric DNA: New Target For Anticancer Drug Development

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ABSTRACT

Elizabeth Blackburn, Carol Greider, and Jack Szostak were awarded the 2009 Nobel Prize in physiology or Medicine for the discovery of how chromosomes are protected by telomeres and the enzyme telomerase. Since then, telomere and telomerase has gained attention of many researchers and has become hot-spot for the discovery of new anticancer agents. The following review presents a short account of the area, to introduce to the research community, the opportunities hidden behind and attract more and more research in the field thus enabling transformation of the still-infant approach into a successful clinical anticancer agent.

Keywords: Telomere, Telomerase, Anticancer, G-quadraplex, Immunotherapy, Virotherapy.

INTRODUCTION

Although the term "telo-mere" (telos meaning end and meros meaning part) and its role in replication and stability of chromosomes were known since 1930s, nothing much was known about its precise function, structure and mechanism of its activity.[1,2] In 1978, *Blackburn et al.* studied telomeres in *Tetrahymena*, a ciliated unicellular organism and discovered that the structures consisted of simple sequence of nucleotides-TTGGGG-repeated again and again (The base in T nucleotides is thymine; that in G nucleotides is guanine).[3] Similar studies conducted on mice, humans and other vertebrate species helped researchers to understand that they had species specific telomere makeup often rich in guanine and cytosine nucleotides and the number of repeated subunits in telomeres varied between species.[4,5]

Human telomeres consist of repeated TTAGGG units present at the end of each chromosome as a 3'-guanine rich single stranded overhang which cap and protect the DNA. The structures exist in a lariat-like structure as a t-loop formed due to invasion of the single-strand of the telomere into the preceding double stranded DNA.[6-9] The loop structure is stabilized by the association of telomere binding proteins (POT1, TRF1 and TRF2) which cap the chromosome ends. The whole protein complex is called shelterin complex.[10]

But the conundrum that remained unsolved all the while was that the number of subunits varied with the organism or between different cells of same organism and that the length shortened and lengthened repeatedly in some tissues. This led to the discovery of the enzyme called 'telomerase'.[11]

Role of Telomere and Telomerase in Cell Division

During cell division, the DNA replicates itself as does the cell. During this replication of DNA, complete genetic sequence is not copied. A small portion at the end (telomeres) is left uncopied each time the cell

multiplies.[12] In normal tissues which lack telomerase, the cell eventually reaches the stage where the telomere reaches the critical length (Hayflick limit)[13,14] and after which the critical genes start eroding. This loss of critical part of DNA triggers a response known as replicative senescence as a result of p53-mediated cell cycle arrest (also called M1 stage). In few cells, however, these checkpoints proteins of the p53 and p16/Rb pathways are inactivated and hence the cell continues to divide beyond the damaged telomeres and ultimately reach cell crisis stage (M2 stage), where even the resistant cells are destroyed. Out of these cells a few may escape both M1 and M2 stage and become cancer cells.[15] The mechanism of the maintenance of the telomeric length in the cancer cells requires the expression of the telomerase.[16] Another mechanism that is responsible for the maintenance of the telomeres apart from the telomerase activity is the ALT (Alternating Lengthening of Telomeres).[17]

It was only in 1984, again through experiments with *Tetrahymena* that the presence of enzyme telomerase was confirmed. The enzyme is responsible for maintaining the length of the telomeres so as to sustain the immortality of the unicellular organism.[6]

The telomerase is made up of two components, a telomerase reverse transcriptase catalytic subunit (TERT)[18] and a telomerase RNA template (TR or TERC), Figure 1.[19] The third component is the protein 'dyskerin' which is required for *in vitro* telomerase activity but the precise role of the protein for the *in vivo* activity is not known.[20] In humans, around 90% cancer cells have been shown to have activated telomerase while it is inactive in most somatic cells.[21] The rest 10% of the cancer cells are either mortal or have shown the presence of alternative pathway for the maintenance of the telomere length, ALT. The telomere is also shorter in cancer cells as compared to the normal cells.[22] This differential property of the telomeres and telomerase in cancer cells makes them promising targets for discovery of novel anticancer

therapy with favoring toxicity profiles and broad spectrum of activity.

How does the telomerase work?

The two components of the telomerase, the TERT and the TR, align together to form a complex and work in sync together for the elongation of the telomere component. The third component dyskerin is also present, possibly involved in complexing of the TR portion into the TERT. A small C-rich portion of the RNA serves as the template for the synthesis of the telomeres. The steps involved in the synthesis include: (i) binding of the 3′end of the telomere strand to the complementary portion of the template region. (ii) Elongation of the telomere by successive attachment of single nucleotide bases complementary to the RNA template. (iii) After the complementary strand has reached the other end of the template region, the telomeric strand translocates and shifts back to again make the template free for another round of elongation. This process of translocation and elongation continues until the telomere has elongated to sufficient length and the 3′end dissociates completely from the telomerase.[23]

Telomerase and Ageing

Although the connection between human telomeres and ageing is not clear yet, few scientist have been suggesting the successive erosion of the telomeres with every cell cycle to be associated with ageing and that telomerase can be used as an anti-ageing therapy. A study involving introduction of TERT gene into healthy mice has shown increase in lifespan.[24] However, the success of therapy would depend largely on the factor of controlling cancer development in such patients and the field has to be evaluated before concluding any such proposals.

Anticancer approach

The presence of telomerase in the majority of the cancer cells being responsible for the elongation of the telomere and hence providing the immortality to the cells makes the enzyme a potential drug target. If successful drugs could be developed which shuts off the enzyme in cancer cells, the cells would lose their immortality and undergo senescence like the normal cells and eventually be lost.

The various approaches to target telomerase activity include strategies attacking the different components required for the enzyme to function:

Inhibiting the telomerase

The telomerase activity is a result of integrated activities of its constituent components and associated proteins. Therefore targeting these components is an important way of limiting the telomere elongation by these enzymes. This class of inhibitors primarily includes inhibitors targeting the hTR and the hTERT components. Inhibitors which target the expression of telomerase have been accounted separately.

Direct inhibitors of the telomerase includes compounds which mimic the natural telomere or nucleotide substrates and acts as active site inhibitors and allosteric inhibitors which target the structural features of the TERT and the TR. Oligonucleotides such as 6-thio-2′-deoxyguanosine-5′-phosphate inhibit telomeric extension after its incorporation into DNA. The incorporation of faulty oligonucleotides occurs via competitive binding against the natural deoxyribonucleotide substrates that are required for the enzymatic function of hTERT.[25,26]

Oligomers such as 2′-O-MeRNA and peptide nucleic acid are the direct inhibitors of telomerase (11 base template region of telomerase RNA i.e. hTR) and confers improves selectivity and efficacy.[27] Another strategy is to alter the sugar phosphodiester backbone which leads to improved intracellular penetration and superior binding at the site and thus helps in better delivery of these agents to the targets. These forms of hTR inhibitors require lipid carriers for their delivery.[28] However, an agent GRN163L (Imetelstat) has been developed by Geron corporation.[29] Imetelstat is 5′-Palm-TAGGGTTAGACAA-3′a sequence complementary to the 13 nucleotide long region partially overlapping and extending by four nucleotides beyond the 5′-boundary of template region of hTR. The molecule binds to the telomerase RNA component sequence (hTR) in the active site region and has the advantage that it does not require a lipid carrier for its delivery as was the case with the precursor molecule GRN163[30] because of the intrinsic lipid palmitate moiety built within the molecule.[31]

Preclinical studies have been undertaken for Imetelstat for various types of cancers including that of liver, breast, multiple myeloma, melanoma, ovarian and combination with Melphalan, Velcade and Taxol and promising results with considerable safety margin have been observed.[32] For many of these indications phase I and Phase II studies have also been carried out or are in progress. Additionally, it has been demonstrated that telomerase inhibition can also sensitize cancer cells to radiation,[33] chemotherapy and targeted therapies.[34] Telomerase inhibitors therefore might be most useful in combination with other therapeutics, especially for drug-resistant tumors.[34] Moreover, combination therapies could mobilize cancer stem cells, accelerate their telomere loss, or even uncap telomeres in the presence of telomerase inhibitors, possibly leading to an earlier or more durable response in cancers.

Besides these nucleotide based telomerase targeting agents, a small molecule synthetic derivative BIBR1532 (2-[[E]-3-naphthalene-2-yl-but-2-enoylamino]-benzoic acid) was found to be a potent hTERT inhibitor. BIBR1532 is a non-competitive inhibitor of telomerase.[35] *In vitro* treatment of human cancer cell lines with BIBR1532 resulted in reduced telomerase length, inhibition of tumor growth and cell senescence. However, implantation of cancer cells pretreated with BIBR1532 in mice led to delayed tumor growth but no additional benefit was observed on further treatment.[36,37]

In addition to these inhibitors designed specifically to target telomerase components, studies employing inhibitors targeting reverse transcriptase activity of telomerase e.g. 3'-azido-3'-deoxythymidine (AZT) (traditional anti-HIV inhibitors) have demonstrated improved telomere shortening and apoptosis.[38,39]

Inhibiting the telomerase expression or biogenesis

This approach is based on the understanding of the biosynthesis of the telomerase in human cells, right from transcription to translation and post-translational modifications, followed by the incorporation of assembly proteins and its transport. Examples of this class include antisense oligonucleotides or siRNA (small interfering RNA) targeting the hTERT or hTR mRNA resulting in telomerase inhibition, telomere shortening and eventually cell death.[40,41]

Another approach is using ribozymes which are capable of cleaving target mRNAs at specific nucleotide sequence positions. These may be specific in targeting either of the hTR or the hTERT mRNAs resulting in inhibited telomerase activity, telomere shortening followed by apoptosis.[42,43]

Dominant negative hTERT is TERT component which is catalytically inactive and its application in tumor cells either alone[44,45] or in combination with other chemotherapeutic agents including Cisplatin, Docetaxel and Etoposide have shown encouraging results.[46]

Although the function of HSP90 proteins with respect to the activity of telomerase is not clear, it is evident from reports that inhibition of HSP90 protein in cancer cells resulted in inhibition of telomerase activity. There are both *in vitro*[47] and *in vivo* results[48] showing use of HSP90 inhibitors blocking telomere assembly and the activity of telomerase which resulted in telomere shortening and eventually cell death.

Immunotherapy

The immune response generated against the tumor associated antigens (TAA) is not as effective as anticancer therapy. Telomerase is considered as a promising target antigen for developing immunotherapy tools as these are present in all cancer cells and dictates the tumorigenicity in human cells. Human TERT-specific peptides presented on cancer cells as surface antigen via the human leukocyte antigen (HLA) class I pathway are used as specific antigens. These tumor cells then activate CD8+ cytotoxic T lymphocytes (CTLs) of the patient's immune system leading to killing of telomerase positive tumor cells.[49,50] Many such hTERT derived peptides have been presented and used for stimulation and amplification of the immune response. GV1001 is one such vaccine which reached phase III clinical trials recently. A phase III clinical trial in advanced metastatic pancreatic cancer comparing vaccine therapy with the standard therapy of Gemcitabine and Capecitabine was reported a failure and did not show a significant difference in overall survival between patients who received the vaccine and control patients treated with chemotherapy. However, the

clinical trials resulted in identification of two biomarkers in a group of patients with a higher survival response and anti-inflammatory effects of the therapy.[51] Another study presented at ASCO meeting held in Chicago suggested that simultaneous vaccination and chemotherapy was found effective for generating immune response as well as anti-inflammatory effects.[52]

Another telomere based vaccine, GRNVAC1, is an autologous dendritic cell vaccine which expresses the telomerase on their surface and induces an immune cell mediated response in tumor cells. Geron successfully completed Phase II clinical trial of GRNVAC1 in patients with acute myelogenous leukemia (AML).[53] Geron is also developing an allogenic telomerase vaccine candidate (GRNVAC2) based on dendritic cells derived from human embryonic stem cells.[54]

Gene Therapy/ Suicide Gene therapy

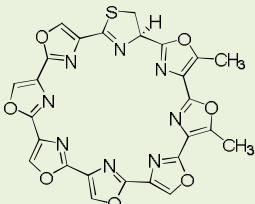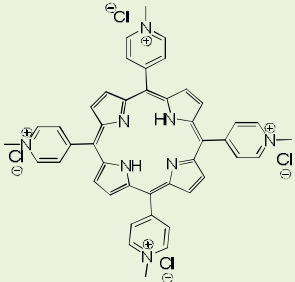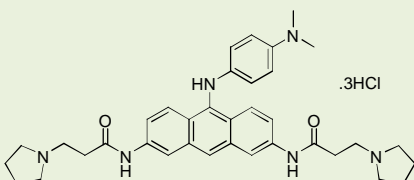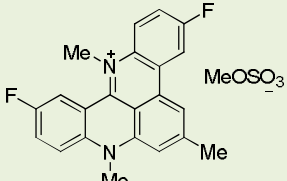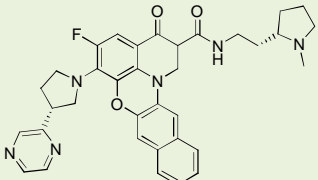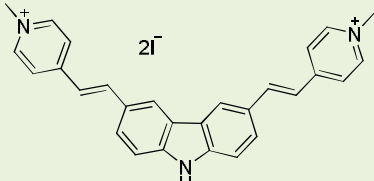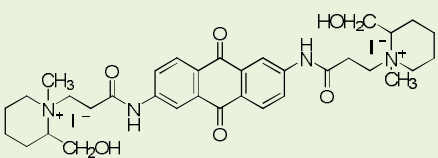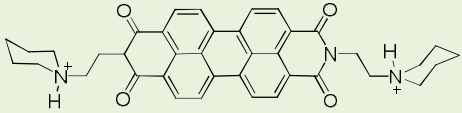Since RNA component of human telomerase is found ubiquitously in all tissues and hTERT component expression is restricted to most of the cancer cells, introducing genes which target TERT positive cells is also one of the approach exploited and being studied. The therapy involves introducing apoptotic suicide genes like TRAIL[55] and caspase-6[56] in a tumor cell whose expression is driven in by hTERT promoter and therefore leads to death of cancer cells selectively. An alternate way includes introduction of a prodrug of a toxin in conjunction with an enzyme for its metabolism in a tumor cell so that the toxin mediated apoptosis of tumor cell occurs.[57,58] Another method of anti-telomerase gene therapy includes introduction of hTERT gene promoter into the adenovirus which regulates the expression of viral proteins E1A and E1B required for viral replication.[59,60] Ability of viruses to act as carriers of genetic material and multiply and kill the host cells makes them promising candidates as vectors. Moreover, expression of the viral proteins in the modified adenovirus stimulates replication of oncolytic viral proteins in telomerase positive cells and makes the therapy selective as only tumor cells that express telomerase enzyme would activate this promoter.

Studies regarding the successful application of both of these therapies are ongoing. Telomelysin is an attenuated adenovirus in which hTERT promoter element drives expression of oncolytic E1 genes of the virus in cancer cell leading to selective cell death.[61] *In vitro* studies using Telomelysin, also known as OBP-301 virus, have demonstrated anticancer activity on oesophageal, gastric and colorectal cancer cell lines. *In vivo* studies performed on athymic mice carrying human colorectal xenografts have demonstrated significant tumor growth arrest and enhancement in survival. Telomelysin has also been studied to be effective in the metastatic tumors. In contrast to standard chemotherapy, oncolytic viruses can replicate in tumor cells, cause their cell lysis and eventually can reach distant cellular tumors. *In vitro* studies combining the oncolytic virus therapy with

ionizing radiations have shown that the combined use can sensitize the tumor cells mutually suggesting an effective cancer therapy.[62]

Onyx-015 was another example of the oncolytic-virus mediated approach where E1B 55-kDA gene was deleted, engineered to selectively replicate and lyse p53-deficient cancer cells.[63] The clinical trials showed promising results alone[64] or in combination with Cisplatin/5-fluorouracil in patients with recurrent head and neck cancer.[65] However, the later confirmation about the mechanism of its action that the replication of ONYX-015 was found irrelevant to the p53 status of the tumor cell line[66] and factors like multiplicity of infection, cell type, and cell cycle phase are more accurate predictors of ONYX-015 activity[67] brought down the interests of researchers in the development of ONYX-015 and the project was withdrawn.

TelomeScan developed by Oncolys based on the same principle was developed as a diagnostic tool to verify the progress of the tumor growth in head and neck cancer through the lymphatic vessels to the regional lymph nodes. TelomeScan is telomerase-specific and replication-selective GFP-expressing adenovirus for the detection of metastatic lymphatic nodes. The tool visualizes the cancerous lymph nodes by giving GFP flourescence signals which is expressed as a result of stimulation of replication of adenovirus in human cancer xenograft models.[68]

A few studies are being conducted to study the effects of suicide gene therapy/ oncolytic viral therapy with other therapies like chemotherapy, radiation therapy etc.

Stabilizing the telomeres

The open 3′-single stranded DNA overhang at the end of the chromosome is required for the telomerase binding and activity leading to elongation of telomeres. Often the G-rich 3′-overhangs of the chromosomes can form structures called G-quadraplexes. These are four-stranded structures formed by the folding of the single strand such that the four guanine bases come in same plane forming a tetrad and are held there by hydrogen bonds with a cation being posited in the central cavity. Many such tetrads stacked one upon the other formed by the repeated guanine nucleotides of the DNA strand forms the G-quadraplex (Figure 2).

These structures have been confirmed to be present at the telomeres *in vitro* and *in vivo*[69] and have been demonstrated to be important for inhibiting the activity of the telomerase and thus killing of the cancer cells. Recently, both NMR[70] and X-ray[71] analyses show the formation of parallel G4 structures from the telomere RNA in the presence of K^+. Regarding with DNA, although X-ray result shows that telomeric DNA forms a similar parallel G4 structure,[72] NMR analysis shows that telomeric DNA forms a nonparallel G4 structures in a K^+ solution.[73]

Figure 1: Conceptual diagram showing structure of (a) Telomerase and (b) Elongation of chromosomal telomere by telomerase. (Source: http://en.wikipedia.org/wiki/Telomerase)

Table 1: Few representative G-quadraplex stabilizing compounds

Compound	Chemical Class	References
Telomestatin	Macrocyclic natural compound isolated from actinomycete *Streptomyces anulatus*	Tauchi T, Shin-ya K, Sashida G, Sumi M, Okabe S, Ohyashiki JH, Ohyashiki K. Oncogene, 25, 2006,5719–5725.
TMPyP4	Porphyrin derivative	Grand CL, Han H, Munoz RM, Weitman S, Von Hoff DD, Hurley LH, Bearss DJ. Mol Cancer Ther, 1, 2002, 565–573; Wheelhouse RT, Sun D, Han H, Han FX and Hurley LH. J Am Chem Soc, 120, 2008, 3261-3262.
BRACO-19	Acridine derivative	Burger AM, Dai F, Schultes CM, Reszka AP, Moore MJ, Double JA, Neidle S. Cancer Res, 65(4), 2005, 1489-1496.
RHPS4	Polycyclic acridine derivative	Salvati E, Leonetti C, Rizzo A, Scarsella M, Mottolese M, Galati R, et al. J Clin Invest, 117(11), 2007, 3236-47.
CX-3543 (Quarfloxin)	Fluoroquinolone derivative	Drygin D, Siddiqui-Jain A, O'Brien S, Schwaebe M,Lin A, Bliesath J, Ho CB, Proffitt C, Trent K, Whitten JP, Lim JKC, Hoff DV, Anderes K and Rice WG. Cancer Res, 69, 2009, 7653–7661.
BMVC	Carbazole derivative	Huang F-C, Chang C-C, Lou P-J, Kuo I-C, Chien C-W, Chen C-T, Shieh F-Y, Chang T-C, and Lin J-J. Mol Cancer Res, 6, 2008, 955-964.
BSU-1051	Anthraquinone derivative	Sun DB, Thompson B, Cathers BE, Salazar M, Kerwin SM, Trent JO, Jenkins TC, Neidle S, Hurley LH. J Med Chem, 40, 1997, 2113–2116.
PIPER	Perylene derivative	Taka T, Huang L, Wongnoppavich A, Tam-Chang SW, Lee TR, Tuntiwechapikul W. Bioorg Med Chem, 21(4), 2013, 883-890.
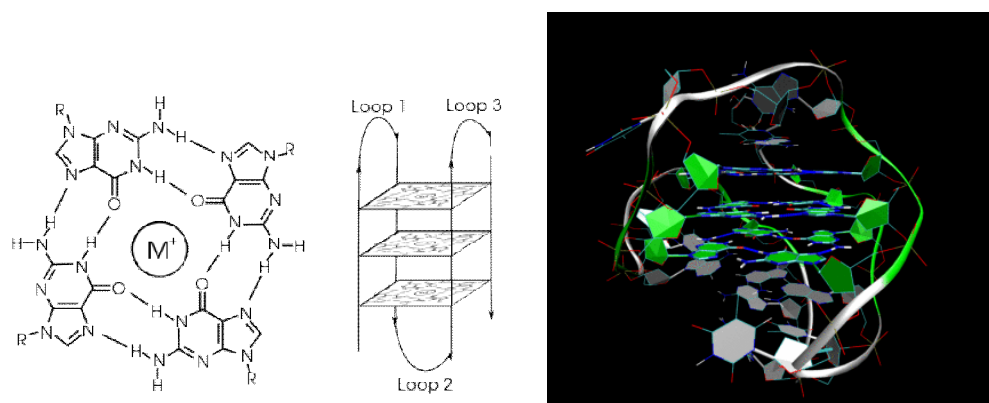
Figure 2: 3D Structure of the intramolecular human telomeric G-quadruplex in potassium solution (PDB ID 2HY9). The backbone is represented by a tube. The center of this structure contains three layers of G-tetrads. The hydrogen bonds in these layers are represented by blue dashed lines. (Source: http://en.wikipedia.org/wiki/G-quadruplex)

Table 2: Ongoing clinical trials telomere/telomerase targeting agents for use as anticancer therapy (www.clinicaltrials.gov)

Study	Status	Identifier
A Phase I Study of GRN163L in Combination With Paclitaxel and Carboplatin in Patients With Advanced or Metastatic Non-Small Cell Lung Cancer. (Sponsor: Geron Corporation)	Phase I Completed	NCT00510445
A Phase 1 Sequential Cohort, Dose Escalation Trial to Determine the Safety, Tolerability, and Maximum Tolerated Dose of GRN163L in Patients With Refractory or Relapsed Multiple Myeloma. (Sponsor: Geron Corporation)	Phase I Completed	NCT00594126
A Phase I Study of GRN163L in Combination With Bortezomib and Dexamethasone in Patients With Relapsed or Refractory Multiple Myeloma. (Sponsor: Geron Corporation)	Phase I Completed	NCT00718601
A Phase I, Sequential Cohort, Dose Escalation Trial to Determine the Safety, Tolerability, and MTD of GRN163L in Patients With Refractory or Relapsed Solid Tumor Malignancies. (Sponsor: Geron Corporation)	Phase I Active, not recruiting	NCT00310895
A Phase I, Sequential Cohort, Dose Escalation Trial to Determine the Safety, Tolerability, and Maximum Tolerated Dose of GRN163L in Patients With Chronic Lympho proliferative Disease. (Sponsor: Geron Corporation)	Phase I Active, not recruiting	NCT00124189
A Phase II Trial to Evaluate the Activity of Imetelstat (GRN163L) in Patients With Essential Thrombocythemia or Polycythemia Vera Who Require Cytoreduction and Have Failed or Are Intolerant to Previous Therapy, or Who Refuse Standard Therapy. (Sponsor: Geron Corporation)	Phase II Active, not recruiting	NCT01243073
A Phase II Trial to Determine the Effect of Imitelstat (GRN163L) on Patients With Previously Treated Multiple Myeloma. (Sponsor: Geron Corporation)	Phase II Active, not recruiting	NCT01242930
A Randomized Phase II Study of Imetelstat as Maintenance Therapy After Initial Induction Chemotherapy for Advance Non-small Cell Lung Cancer. The purpose of this is to evaluate the efficacy and safety of GRN163L as maintenance therapy for patients with advanced stage NSCLC who have not progressed after 4 cycles of platinum based therapy. (Sponsor: Geron Corporation)	Phase II Active, Not recruiting	NCT01137968
A Phase I/II Study of GRN163L in Combination With Paclitaxel and Bevacizumab in Patients With Locally Recurrent or Metastatic Breast Cancer. (Sponsor: Geron Corporation)	Phase I Completed	NCT00732056
A Randomized Phase II Study Of Imetelstat (GRN163L) In Combination With Paclitaxel (With Or Without Bevacizumab) in Patients With Locally Recurrent Or Metastatic Breast Cancer. (Sponsor: Geron Corporation)	Phase II Completed	NCT01256762
A Molecular Biology and Phase II Study of Imetelstat (GRN163L) in Children With Recurrent or Refractory Brain Tumors (Recurrent High-Grade Glioma, Ependymoma, Medulloblastoma/Primitive Neuroectodermal Tumor and Diffuse Intrinsic Pontine Glioma) (Sponsor: Pediatric Brain Tumor Consortium)	Phase II Recruiting	NCT01836549
A Pilot Open-Label Study of the Efficacy and Safety of Imetelstat (GRN163L) in Patients With DIPSS_plus Intermediate-2 or High Risk Primary Myelofibrosis (PMF), Post-Polycythemia Vera Myelofibrosis (Post-PV MF), or Post-Essential Thrombocythemia Myelofibrosis (Post-ET-MF). (Sponsor: Mayo Clinic)	Recruiting	NCT01731951
A Pilot Study of Imetelstat Given Intravenously on Day 1 and 8 of a 21 Day Schedule Alone and With Standard 13-Cis-Retinoic Acid in Children With Recurrent and/or Refractory Neuroblastoma. (Sponsor: NCIC Clinical Trials Group)	Phase I Not yet Recruiting	NCT01916187
A Phase 1 Study of Imetelstat, a Telomerase Inhibitor, in Children With Refractory or Recurrent Solid Tumors and Lymphomas (Sponsor: Children's Oncology Group)	Phase I Active, not recruiting	NCT01273090
A study to evaluate safety and biologic effects of giving GRN163L in combination with trastuzumab in patients diagnosed with HER2+ metastatic breast cancer that is resistant to therapy with trastuzumab. (Sponsor: Indiana University)	Phase I Active, not recruiting	NCT01265927
A Double-blind, Multicenter, Randomized Phase III Study of the Telomerase Vaccine, GV1001 Administered After Curative Intent Chemo-radiotherapy in Patients With Inoperable Stage III Non-small Cell Lung Cancer Compared to Best Supportive Care. (Sponsor: Kael-GemVax Co., Ltd.)	Phase III Not yet recruiting	NCT01579188

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A Phase II, Open-Label Trial Evaluating the Safety and Efficacy of GV1001 in Advanced Hepatocellular Carcinoma. (Sponsor: Pharmexa A/S)	Phase II Completed	NCT00444782
A Phase III Trial Comparing GV1001 and Gemcitabine in Sequential Combination to Gemcitabine Monotherapy in Advanced Un-Resectable Pancreatic Cancer. (Sponsor: Pharmexa A/S)	Phase III Terminated	NCT00358566
A Phase I, Combination, Immunologic Study of LTX-315 as Adjunct to GV1001 in Patients Following Curative Surgery for Carcinoma. (Sponsor: Lytix Biopharma AS)	Phase I Completed	NCT01223209
To examine the safety and efficacy of telomerase peptide vaccination (stimulation of the immune system) in patients with NSCLC after having been treated with conventional therapy with radiotherapy and docetaxel as a radiosensitizer. (Sponsor: Oslo University Hospital)	Phase II Completed	NCT00509457
Trial With Telomerase Peptide Vaccine In Combination With Temozolomide in Patients With Advanced Malignant Melanoma. (Sponsor: Oslo University Hospital)	Phase I,II Completed	NCT01247623
Exploratory Trial of Immunochemoradiotherapy for Locally Advanced Pancreatic Adenocarcinoma. (Radiation Therapy, Tadalafil, Sargramostim, Gemcitabine, and Telomerase Vaccine (GV1001) in Patients With Pancreatic Cancer). (Sponsor: Providence Health & Services)	Phase I Active, Not recruiting	NCT01342224
A Prospective, Phase III, Controlled, Multicentre, Randomised Clinical Trial Comparing Combination Gemcitabine and Capecitabine Therapy With Concurrent and Sequential Chemoimmunotherapy Using a Telomerase Vaccine in Locally Advanced and Metastatic Pancreatic Cancer [TELOVAC] (Sponsor: Royal Liverpool University Hospital)	PhaseIII Status Unknown	NCT00425360
Phase II Study of Active Immunotherapy With Mature, Human Telomerase Reverse Transcriptase Messenger RNA -Transfected, Autologous Dendritic Cells (DC) Administered In A Prime-Boost Format to Subjects With Metastatic Prostate Cancer. (Sponsor: University of Florida)	Phase I,II Withdrawn	NCT01153113
Immunization of HLA-A*0201 Patients With Metastatic Cancer Using a Peptide Epitope From the Telomerase Antigen (540-548 peptide vaccine). (Sponsor: National Cancer Institute)	Phase II Completed	NCT00021164
Telomerase: 540-548 peptide Vaccine Plus Montanide ISA-51 and Sargramostim in Treating Patients With Stage IV Breast Cancer. (Sponsor: University of Pennsylvania)	Phase I Unknown	NCT00079157
Phase I/II Clinical Trial Combining hTERT Tumor Vaccine & Autologous T Cells in Patients With Advanced Myeloma. (Sponsor:University of Pennsylvania)	Phase I,II Active, Not recruiting	NCT00834665
A Study of hTERT/Survivin Multi-peptide Vaccine With Daclizumab and Prevnar for Patients With Metastatic Breast Cancer. (Sponsor: Abramson Cancer Center of the Univ of Pennsylvania)	Phase I Completed	NCT00573495
Telomerase: 540-548 peptide vaccine and sargramostim (GM-CSF) in treating patients with sarcoma or brain tumor. (Sponsor: Dana-Farber Cancer Institute)	PhaseI Completed	NCT00069940
A Phase 2, Open-Label Evaluation of the Safety and Efficacy of CB-10-01, Transgenic Lymphocyte Immunization (TLI) Against Telomerase, as Adjuvant Therapy in Subjects With Stage III Melanoma. (Sponsor: Cosmo Bioscience)	Phase II Active, not recruiting	NCT00925314
A Study of Active Immunotherapy With GRNVAC1 in Patients With Acute Myelogenous Leukemia (AML). (Sponsor: Geron Corporation)	Phase II Active, not recruiting	NCT00510133
Vaccination With Autologous Dendritic Cells Pulsed With Tumor Antigens for Treatment of Patients With Malignant Melanoma. (Sponsor: Herlev Hospital)	Phase I,II Completed	NCT00197912
Vaccination With Autologous Dendritic Cells Pulsed With Tumor Antigens for Treatment of Patients With Renal Cell Carcinoma. (Sponsor: Inge Marie Svane)	PhaseI,II Completed	NCT00197860
Phase I Trial Of Cisplatin And KML-001 In Advanced Non-Small Cell Lung Cancer and Other Platinum Responsive Malignancies. (Sponsor: University of Maryland)	Phase I Recruiting	NCT01110226
A Phase 1, Evaluation of Transgenic Lymphocyte Immunization Vaccine in Subjects With Prostate Adenocarcinoma. (Sponsor: Cosmo Bioscience)	Phase I Completed	NCT00061035
A Phase I, Open, Randomized, Study to Investigate the Safety of Active Immunotherapy With Fully Mature, TERT-mRNA and Survivin - Peptide Double Loaded Dendritic Cells (DCs) in Subjects With Advanced Epithelial Ovarian Cancer, Enrolled in the Study Within Twelve Weeks After Completing Primary Therapy. (Sponsor: Life Research Technologies GmbH)	Phase I Active, not recruiting	NCT01456065
A Phase I/IIA Study of UV1/hTERT2012P Vaccination (a therapeutic synthetic peptide vaccine) in Patients With Hormone-sensitive Metastatic Prostate Cancer. (Sponsor: Oslo University Hospital)	Phase I,II Recruiting	NCT01784913
A Phase I/IIa Study of UV1-hTERT2012L Vaccination in Patients With Non Small Cell Lung Cancer. (Sponsor: Oslo University Hospital)	Phase I,II Recruiting	NCT01789099
Evaluation of Dendritic Cells Transfected With Survivin, hTERT and p53 mRNA as a Treatment for Patients With Metastatic Breast Cancer or Malignant Melanoma. (Sponsor: Herlev Hospital)	Phase I Recruiting	NCT00978913
Phase I/II Trial for Vaccine Therapy With Dendritic Cells - Transfected With hTERT-, Survivin- and Tumor Cell Derived mRNA + ex Vivo T Cell Expansion and Reinfusion in Patients With Metastatic Malignant Melanoma. (Sponsor: Oslo University Hospital)	Phase I,II Active, Not recruiting	NCT00961844
hase I/II Combination Immunotherapy After ASCT for Advanced Myeloma to Study HTERT Vaccination Followed by Adoptive Transfer of Vaccine-Primed Autologous T Cells (Sponsor: University of Maryland Greenebaum Cancer Center)	Phase I,II Completed	NCT00499577
A Study of hTERT/Survivin Multi-Peptide Vaccination With Basiliximab And Prevnar For Patients With Metastatic Breast Cancer. (Sponsor: Abramson Cancer Center of the University of Pennsylvania)	Recruiting	NCT01660529
Phase I/II Trial of Vaccine Therapy in Recurrent Platinum Sensitive Epithelial Ovarian Cancer Patients Using Autologous Dendritic Cells Loaded With Amplified Ovarian Cancer Stem Cell mRNA, hTERT and Survivin. (Sponsor: Oslo University Hospital)	Phase I,II Recruiting	NCT01334047

Trial of Vaccine Therapy in Curative Resected Prostate Cancer Patients Using Autologous Dendritic Cells Loaded With mRNA From Primary Prostate Cancer Tissue, hTERT and Survivin (Sponsor: Oslo University Hospital)	Recruiting	NCT01197625
Dendritic Cell Vaccination in Combination With Docetaxel for Patients With Cancer Prostate - a Randomized Phase II Study. (Sponsor: Inge Marie Svane)	PhaseII Recruiting	NCT01446731

Therefore, G-quadraplexes, recently, are been extensively explored as potential target for design of anticancer agents. In recent years, many small molecules agents that can stabilize the G-quadraplex structures have been studied for their anticancer activity. Studies in this area have led to identification of many lead compounds but none has been successful to reach into the clinics. The so-called classes of compounds identified as G-quadraplex stabilizing agents include derivatives of Anthraquinones and Flourinones, Acridine, Quindoline, Bisindole, Perylene, Porphyrine, Triazines, Berberine and Macrocyclics. The examplifying structures of these classes are given in Table 1.

The common structural feature of all the synthetic as well as those found from natural sources shown to have G-quadraplex stabilizing property is that the compounds possess planar chromophoric structures with a prevalence of electron deficiency at the core enhancing π-stacking interactions with guanine rich tetrads, inserting between these tetrads and stabilizing the G-quadraplex structures by stacking and electrostatic interactions. Building on these principles many groups have tried to rationally design inhibitors using modeling techniques like virtual ligand screening, molecular dynamics.

Out of all, Telomestatin, a macrocyclic natural product isolated from the actinomycete *Streptymyces anulatus* 3533-SV4[74] is currently the best known telomerase inhibitor which binds to G-quadraplex with an IC_{50} value of 5nM.[75] The compound also has 70-fold selectivity for intramolecular G-quadraplex than duplex DNA.[76] The role of telomestatin as telomerase inhibitor has been confirmed *in vivo* by gradual telomere shortening, growth arrest and apoptosis.[77] In addition, there exists another supporting mechanism for tumor growth arrest which occurs by direct uncapping of chromosome termini and therefore leads to proliferation defects irrespective of telomerase status of the cell in a shorter time frame as compared with telomere stabilizing mechanism.[78]

An account of all representative lead telomere/telomerase targeting agents is given in Table 2.

CONCLUSION

The telomerase and telomere offer impressive targets for the development of anticancer drug development. The use of the target to develop new therapeutic agents has application in a broad range of cancer therapy as many types of cells express the enzyme telomerase. However the therapy may lag behind due to a number of serious issues which may hold back the progress in this particular field for development of new cancer treatment therapies. One of these is the lag time in the onset of anticancer activity in regard to the reduction in number of cancer cells and the visibility of symptoms until the length of the telomeres reach the critical length and cell death occurs. This limitation of the therapy, however, can be easily overcome if it is combined with other therapies like chemotherapy, radiation etc. which work as de-bulking agents for reducing the cancer cell burden and the telomerase inhibitor providing the durable results. This methodology has also seen a number of applications with the emerging agents of the field in several clinical studies. Another worrying consideration may be the effect of these agents on general telomere structures in normal cells which may lead to toxicity concerns of these agents. A possible toxicity mechanism can be reduction of telomere length in normal cells leading to conditions which promote instability within cell and exacerbate the process of tumor growth. However, many of the studied agents have shown selectivity towards cancer cell lines for unknown reasons. These may be due to additional selectivity mechanisms and activities beyond the few studied. Thus, the area of telomerase inhibition in anticancer therapy is still in its infancy and there is much to be explored and studied before the area can give us a clinically successful candidate for the treatment of cancer.

REFERENCES

1. McClintock B. Cytological observations of deficiencies involving known genes, translocations and an inversion in *Zea mays*. Missouri Agricultural Experiment Station Research Bulletin, 163, 1931, 1–48.

2. Muller HJ. The remaking of chromosomes. Collecting Net 13, 1938, 181–198.

3. Blackburn EH, Gall JG. A tandemly repeated sequence at the termini of the extrachromosomal ribosomal RNA genes in *Tetrahymena*. J Mol Biol, 120, 1978, 33–53.

4. Moyzis RK. The Human Telomere. Scientific American, 265(2), 1991, 48-55.

5. Greider CW, Blackburn EH. Telomeres, Telomerase and Cancer. Scientific American, 274(2), 1996, 92-97.

6. Moyzis RK, Buckingham JM, Cram LS, Dani M, Deaven LL, Jones MD, Meyne J, Ratliff RL, Wu JR. A highly conserved repetitive DNA sequence, (TTAGGG)n, present at the telomeres of human chromosomes. Proc Nat Acad Sci, 85(18), 1988, 6622-6626.

7. de Lange T. Protection of mammalian telomeres. Oncogene, 21(4), 2002, 532-540.

8. Griffith JD, Comeau L, Rosenfield S, Stansel RM, Bianchi A, Moss H, de Lange T. Mammalian telomeres end in a large duplex loop. Cell, 97(4), 1999, 503-514.

9. Blackburn EH. Switching and signaling at the telomere. Cell, 106(6), 2001, 661-673.

10. de Lange T. Shelterin: the protein complex that shapes and safeguards human telomeres. Genes Dev, 19, 2005, 2100-2110.

11. Greider CW, Blackburn EH. Identification of a specific telomere terminal transferase activity in *Tetrahymena* extracts. Cell, 43, 1985, 405–413.

12. Harley CB, Futcher AB, Greider CW. Telomeres shorten during ageing of human fibroblasts. Nature, 345(6274), 1990, 458-460.

Int. J. Pharm. Sci. Rev. Res., 23(1), Nov – Dec 2013; n° 46, 243-252

ISSN 0976 – 044X

13. Hayflick L, Moorhead PS. The serial cultivation of human diploid cell strains. Exp Cell Res, 25, 1961, 585–621.

14. Shay JW, Wright WE. Hayflick, his limit, and cellular ageing. Nat Rev Mol Cell Biol, 1(1), 2000, 72-76.

15. Zou Y, Wright WE, Shay JW. Telomere Replication and Cellular Senescence. Toxicol Pathol, 32, 2004, 146.

16. Kim NW, Piatyszek MA, Prowse KR, Harley CB, West MD, Ho PL, Coviello GM, Wright WE, Weinrich SL, Shay JW. Specific association of human telomerase activity with immortal cells and cancer. Science, 266(5193), 1994, 2011-2015.

17. Reddel RR. Alternative lengthening of telomeres, telomerase, and cancer. Cancer Lett, 194(2), 2003, 155-162.

18. Harrington L, Zhou W, McPhail T, Oulton R, Yeung DS, Mar V, Bass MB, Robinson MO. Human telomerase contains evolutionarily conserved catalytic and structural subunits. Genes Dev, 11, 1997, 3109-3115.

19. Greider CW, Blackburn EH. A telomeric sequence in the RNA of Tetrahymena telomerase required for telomere repeat synthesis. Nature, 337, 1989, 331-337.

20. Cohen SB, Graham ME, Lovrecz GO, Bache N, Robinson PJ, Reddel RR. Protein composition of catalytically active human telomerase from immortal cells. Science, 315(5820), 2007, 1850-1853.

21. Shay JW, Wright WE. Telomerase: A target for cancer therapeutics. Cancer Cell, 2, 2002, 257-265.

22. Meyerson M. Role of Telomerase in Normal and Cancer Cells. J Clin Oncol, 18(13), 2000, 2626-2634.

23. de Boeck G, Forsyth RG, Praet M, Hogendoorn PC. Telomere-associated proteins: cross-talk between telomere maintenance and telomere-lengthening mechanisms. J Pathol, 217(3), 2009, 327-344.

24. de Jesus BB, Vera E, Schneeberger K, Tejera AM, Ayuso E, Bosch F, and Blasco MA. Telomerase gene therapy in adult and old mice delays aging and increases longevity without increasing cancer. EMBO Mol Med, 4(8), 2012, 691–704.

25. Fletcher TM, Cathers BE, Ravikumar KS, Mamiya BM, Kerwin SM. Inhibition of human telomerase by 7-deaza-2'-deoxyguanosine nucleoside triphosphate analogs: potent inhibition by 6-thio-7-deaza-2'-deoxyguanosine 5'-triphosphate. Bioorg Chem, 29(1), 2001, 36-55.

26. Rezler EM, Bearss DJ, Hurley LH. Telomeres and telomerases as drug targets. Curr Opin Pharmacol, 2(4), 2002, 415-423.

27. Core DR. Telomerase inhibition, oligonucleotides, and clinical trials. Oncogene, 21, 2002, 631-637.

28. Rezler EM, Bearss DJ, Hurley LH. Telomere inhibition and telomere disruption as processes for drug targeting. Annu Rev Pharmacol Toxicol, 43, 2003, 359-379.

29. Geron. Imetelstat: a first-in-class telomerase inhibitor. http://www.geron.com/imetelstat. Date last accessed: Aug 31, 2013.

30. Asai A, Oshima Y, Yamamoto Y, Uochi TA, Kusaka H, Akinaga S, Yamashita Y, Pongracz K, Pruzan R, Wunder E, Piatyszek M, Li S, Chin AC, Harley CB, Gryaznov S. A novel telomerase template antagonist (GRN163) as a potential anticancer agent. Cancer Res, 63(14), 2003, 3931-3939.

31. Herbert BS, Gellert GC, Hochreiter A, Pongracz K, Wright WE, Zielinska D, Chin AC, Harley CB, Shay JW, Gryaznov SM. Lipid modification of GRN163, an N3'-->P5' thio-phosphoramidate oligonucleotide, enhances the potency of telomerase inhibition. Oncogene, 24(33), 2005, 5262-5268.

32. Tressler RJ, Chin AC, Gryaznov SM and Harley CB. Preclinical efficacy, safety, and ADME of GRN163L, a novel telomerase inhibitor developed for the treatment of cancer. J Clin Oncol (Meeting Abstracts), 23(16), 2005, Suppl 3168.

33. Gomez-Millan J, Goldblatt EM, Gryaznov SM, Mendonca MS, Herbert BS. Specific telomere dysfunction induced by GRN163L increases radiation sensitivity in breast cancer cells. Int J Radiat Oncol Biol Phys, 67(3), 2007, 897-905.

34. Ward RJ, Autexier C. Pharmacological telomerase inhibition can sensitize drug-resistant and drug-sensitive cells to chemotherapeutic treatment. Mol Pharmacol, 68(3), 2005, 779-786.

35. Pascolo E, Wenz C, Lingner J, Hauel N, Priepke H, Kauffmann I, Garin-Chesa P, Rettig WJ, Damm K, Schnapp A. Mechanism of human telomerase inhibition by BIBR1532, a synthetic, non-nucleosidic drug candidate. J Biol Chem, 277(18), 2002, 15566-15572.

36. Damm K, Hemmann U, Garin-Chesa P, Hauel N, Kauffmann I, Priepke H, Niestroj C, Daiber C, Enenkel B, Guilliard B, Lauritsch I, Müller E, Pascolo E, Gabriele Sauter G, Pantic M, Martens UM, Wenz C, Lingner J, Kraut N, Rettig WJ, Schnapp A. A highly selective telomerase inhibitor limiting human cancer cell proliferation. EMBO J, 20, 2001, 6958-6968.

37. El-Daly H, Kull M, Zimmermann S, Pantic M, Waller CF, and Martens UM. Selective cytotoxicity and telomere damage in leukemia cells using the telomerase inhibitor BIBR1532. Blood, 105, 2005, 1742-1749.

38. Strahl C, Blackburn EH. Effects of Reverse Transcriptase Inhibitors on Telomere Length and Telomerase Activity in Two Immortalized Human Cell Lines. Mol Cell Biol, 16, 1996, 53-65.

39. Mo Y, Gan Y, Song SH, Johnston J, Xiao X, Wientjes MG and Jessie L-S. A Simultaneous Targeting of Telomeres and Telomerase as a Cancer Therapeutic Approach. Cancer Res, 63, 2003, 579-585.

40. Ashmawy AME-S, Khatab E, Anbar WBAE-A, Haroun BM, and Shaarawy SM. siRNA Mediated-hTERT Knockdown Impedes Proliferation of Mammalian Cancer MCF7 and HepG2 Cells. J Amer Sci, 7(3), 2011, 179-186.

41. Natarajan S, Chen Z, Wancewicz EV, Monia BP, Corey DR. Telomerase reverse transcriptase (hTERT) mRNA and telomerase RNA (hTR) as targets for downregulation of telomerase activity. Oligonucleotides, 14(4), 2004, 263-273.

42. Yeo M, Rha SY, Jeung HC, Hu SX, Yang SH, Kim YS, An SW, Chung HC. Attenuation of telomerase activity by hammerhead ribozyme targeting human telomerase RNA induces growth retardation and apoptosis in human breast tumor cells. Int J Cancer, 114, 2005, 484–489.

43. Song DP, Lin JS, Fu GL, Sun XM, Kong XJ, Li PY, Ma X. Hammerhead ribozyme against human telomerase catalytic subunit (hTERT) induced apoptosis of liver cancer cells. Zhonghua Gan Zang Bing Za Zhi (Chinese Journal of Hepatology), 12(10), 2004, 616-619.

44. Röth A, Vercauteren S, Sutherland HJ, Lansdorp PM. Telomerase is limiting the growth of acute myeloid leukemia cells. Leukemia, 17, 2003, 2410-2417.

45. Nakajima A, Tauchi T, Sashida G, Sumi M, Abe K, Yamamoto K, Ohyashiki JH, Ohyashiki K. Telomerase inhibition enhances apoptosis in human acute leukemia cells: possibility of antitelomerase therapy. Leukemia, 17, 2003, 560-567.

46. Sumi M, Tauchi T, Sashida G, Nakajima A, Gotoh A, Shin-Ya K, Ohyashiki JH, Ohyashiki K. A G-quadruplex-interactive agent, telomestatin (SOT-095), induces telomere shortening with apoptosis and enhances chemosensitivity in acute myeloid leukemia. Int J Oncol, 24, 2004, 1481–1487.

47. Villa R, Folini M, Porta CD, Valentini A, Pennati M, Daidone MG, Zaffaroni N. Inhibition of telomerase activity by geldanamycin and 17-allylamino, 17-demethoxygeldanamycin in human melanoma cells. Carcinogenesis, 24(5), 2003, 851-859.

48. Mitsiades CS, Mitsiades NS, McMullan CJ, Poulaki V, Kung AL, Davies FE, Morgan G, Akiyama M, Shringarpure R, Munshi NC, Richardson PG, Hideshima T, Chauhan D, Gu X, Bailey C, Joseph M, Libermann TA, Rosen NS, Anderson KC. Antimyeloma activity of heat shock protein-90 inhibition. Blood, 107(3), 2006, 1092-1100.

49. Liu J-P, Chen W, Schwarer AP, Li H. Telomerase in cancer immunotherapy. Biochim Biophys Acta, 1805, 2010, 35–42.

50. Vonderheide RH. Prospects and challenges of building a cancer vaccine targeting telomerase. Biochimie, 90, 2008, 173–180.

51. Genetic Engineering & Biotechnology News. Phase III Failure for TeloVac Pancreatic Cancer Vaccine.

http://www.genengnews.com/gen-news-highlights/phase-iii-failure-for-telovac-pancreatic-cancer-vaccine/81248436/ Date Last Accessed: Aug 31, 2013.

52. Genetic Engineering & Biotechnology News. Merck, BMS Immunotherapy Data Whets Clinical Oncology Appetite; More from ASCO http://genengnews.com/gen-news-highlights/merck-bms-immunotherapy-data-wets-clinical-oncology-appetite-more-from-asco/81248435/ Date Last Accessed: Aug 31, 2013.

53. MPR. Phase 2 study of GRNVAC1 for the vaccination of acute myelogenous leukemia (AML). http://www.empr.com/phase-2-study-of-grnvac1-for-the-vaccination-of-acute-myelogenous-leukemia-aml/article/192358/#. Date Last Accessed: Aug 31, 2013.

54. Nishimoto KP, Tseng S-Y, Lebkowski JS, Reddy A. Modification of human embryonic stem cell-derived dendritic cells with mRNA for efficient antigen presentation and enhanced potency. Regenerative Medicine, 6(3), 2011, 303-318.

55. Katz MH, Spivack DE, Takimoto S, Fang B, Burton DW, Moossa AR, Hoffman RM, Bouvet M. Gene therapy of pancreatic cancer with green fluorescent protein and tumor necrosis factor-related apoptosis-inducing ligand fusion gene expression driven by a human telomerase reverse transcriptase promoter. Ann Surg Oncol, 10, 2003, 762–772.

56. Komata T, Kondo Y, Kanzawa T, Hirohata S, Koga S, Sumiyoshi H, Srinivasula SM, Barna BP, Germano IM, Takakura M, Inoue M, Alnemri ES, Shay JW, Kyo S, Kondo S. Treatment of malignant glioma cells with the transfer of constitutively active caspase-6 using the human telomerase catalytic subunit (human telomerase reverse transcriptase) gene promoter. Cancer Res, 61, 2001, 5796–5802.

57. Plumb JA, Bilsland A, Kakani R, Zhao J, Glasspool RM, Knox RJ, Evans TR, Keith WN. Telomerase-specific suicide gene therapy vectors expressing bacterial nitroreductase sensitize human cancer cells to the pro-drug CB1954. Oncogene, 20, 2001, 7797–7803.

58. Schepelmann S, Ogilvie LM, Hedley D, Friedlos F, Martin J, Scanlon I, Chen P, Marais R, Springer CJ. Suicide gene therapy of human colon carcinoma xenografts using an armed oncolytic adenovirus expressing carboxypeptidase G2. Cancer Res, 67, 2007, 4949–4955.

59. Kawashima T, Kagawa S, Kobayashi N, Shirakiya Y, Umeoka T, Teraishi F, Taki M, Kyo S, Tanaka N, Fujiwara T. Telomerase-specific replication-selective virotherapy for human cancer. Clin Cancer Res, 10, 2004, 285–292.

60. 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, 63, 2003, 7936–7941.

61. Kyo S, Takakura M, Fujiwara T, Inoue M. Understanding and exploiting hTERT promoter regulation for diagnosis and treatment of human cancers. Cancer Sci, 99(8), 2008, 1528-1538.

62. Fujiwara T. A novel molecular therapy using bioengineered adenovirus for human gastrointestinal cancer. Acta Med Okayama, 65(3), 2011, 151-162.

63. Bischoff JR, Kirn DH, Williams A, Heise C, Horn S, Muna M, Ng L, Nye JA, Sampson-Johannes A, Fattaey A, McCormick F. An adenovirus mutant that replicates selectively in p53-deficient human tumour cells. Science, 274, 1996, 373-376.

64. Nemunaitis J, Khuri F, Ganly I, Arseneau J, Posner M, Vokes E, Kuhn J, McCarty T, Landers S, Blackburn A, Romel L, Randlev B, Kaye S, Kirn D. Phase II Trial of Intratumoral Administration of ONYX-015, a Replication-Selective Adenovirus, in Patients With Refractory Head and Neck Cancer. J Clin Oncol, 19, 2001, 289-298.

65. Khuri FR, Nemunaitis J, Ganly I, Arseneau J, Tannock IF, Romel L, Gore M, Ironside J, MacDougall RH, Heise C, Randlev B, Gillenwater AM, Bruso P, Kaye SB, Hong WK, Kirn DH. A controlled trial of intratumoral ONYX-015, a selectively-replicating adenovirus, in combination with cisplatin and 5-fluorouracil in patients with recurrent head and neck cancer. Nat Med, 6, 2000, 879-885.

66. Goodrum FD, Ornelles DA. p53 Status Does Not Determine Outcome of E1B 55-Kilodalton Mutant Adenovirus Lytic Infection. J Virol, 72, 1998, 9479-9490.

67. O'Shea CC, Johnson L, Bagus B, Choi S, Nicholas C, Shen A, Boyle L, Pandey K, Soria C, Kunich J, Shen Y, Habets G, Ginzinger D, McCormick F. Late viral RNA export, rather than p53 inactivation, determines ONYX-015 tumor selectivity. Cancer cell, 6, 2004, 611-623.

68. Kurihara Y, Watanabe Y, Onimatsu H, Kojima T, Shirota T, Hatori M, Liu D, Kyo S, Mizuguchi H, Urata Y, Shintani S, Fujiwara T. Telomerase-specific virotheranostics for human head and neck cancer. Clin Cancer Res, 15, 2009, 2335-2343.

69. Paeschke K, Simonsson T, Postberg J, Rhodes D, Lipps HJ. Telomere end-binding proteins control the formation of G-quadruplex DNA structure in vivo. Nature Structural & Molecular Biology, 12(10), 2005, 847-854.

70. Martadinata H, Phan AT. Structure of propeller-type parallel-stranded RNA G-quadruplexes, formed by human telomeric RNA sequences in K+ solution. J Am Chem Soc, 131(7), 2009, 2570-2578.

71. Collie GW, Haider SM, Neidle S, Parkinson GN. A crystallographic and modelling study of a human telomeric RNA (TERRA) quadruplex. Nucleic Acids Res, 38, 2010, 5569 – 5580.

72. Parkinson GN, Lee MP, Neidle S. Crystal structure of parallel quadruplexes from human telomeric DNA. Nature, 417(6891), 876-880.

73. Ambrus A, Chen D, Dai J, Bialis T, Jones RA, Yang D. Human telomeric sequence forms a hybrid-type intramolecular G-quadruplex structure with mixed parallel/antiparallel strands in potassium solution. Nucleic Acid Research, 34 (9), 2006, 2723-2735.

74. Shin-ya K, Wierzba K, Matsuo K, Ohtani T, Yamada Y, Furihata K, Hayakawa Y, Seto H. Telomestatin, a novel telomerase inhibitor from Streptomyces anulatus. J Am Chem Soc, 123, 2001, 1262-1263.

75. Kim M-Y, Vankayalapati H, Shin-ya K, Wierzba K, and Hurley LH. Telomestatin, a Potent Telomerase Inhibitor That Interacts Quite Specifically with the Human Telomeric Intramolecular G-Quadruplex. J Am Chem Soc, 124, 2002, 2098-2099.

76. Rezler EM, Seenisamy J, Bashyam S, Kim M-Y, White E, Wilson WD, Hurley LH. Telomestatin and Diseleno Sapphyrin Bind Selectively to Two Different Forms of the Human Telomeric G-Quadruplex Structure. J Am Chem Soc, 127, 2005, 9439-9447.

77. Shammas MA, Reis RJS, Li C, Koley H, Hurley LH, Anderson KC, Munshi NC. Telomerase Inhibition and Cell Growth Arrest After Telomestatin Treatment in Multiple Myeloma. Clin Cancer Res, 10, 2004, 770-776.

78. Tahara H, Shin-ya K, Seimiya H, Yamada H, Tsuruo T, Ide T. G-Quadruplex stabilization by telomestatin induces TRF2 protein dissociation from telomeres and anaphase bridge formation accompanied by loss of the 3' telomeric overhang in cancer cells. Oncogene, 25, 2006, 1955-1966.