Title: NOVEL ANTICANCER THERAPEUTICS TARGETING TELOMERASE
Abbreviated Title: Novel Anticancer Therapeutics Targeting Telomerase

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**ABSTRACT (250 words)**

Telomeres are protective caps at the ends of human chromosomes. Telomeres shorten with each successive cell division in normal human cells whereas in tumors they are continuously elongated by human telomerase reverse transcriptase (hTERT). Telomerase is overexpressed in 80%-95% of cancers and is present in very low levels or is almost undetectable in normal cells. Because telomerase plays a pivotal role in cancer cells growth it may serve as an ideal target for anticancer therapeutics. Inhibition of telomerase may lead to a decrease of telomere length resulting in cell senescence and apoptosis in telomerase positive tumors. Several strategies of telomerase inhibition are reviewed, including small molecule inhibitors, antisense oligonucleotides, immunotherapies and gene therapies, targeting the hTERT or the ribonucleoprotein subunit hTER. G-quadruplex stabilizers, tankyrase and HSP90 inhibitors targeting telomere and telomerase assembly, and T-oligo approach are also covered. Based on this review the most promising current telomerase targeting therapeutics are the antisense oligonucleotide inhibitor GRN163L and immunotherapies that use either dendritic cells (GRVAC1), hTERT peptide (GV1001) or cryptic peptides (Vx-001). Most of these agents are undergoing or have completed several phase I and II clinical trials in patients with various tumors, and have shown good immune response rates, evidenced with reduction in tumor cell growth, increased overall disease survival, disease stabilization in advanced staged tumors and complete/partial responses. Most therapeutics proved to be more effective when used in combination with standard therapies, resulting in concomitant telomere shortening, tumor mass shrinkage, and preventing tumor relapse and resistance to single agent therapy.

**KEYWORDS**
hTERT; hTER; telomerase inhibitor; telomere; cancer therapy; oligonucleotide; G-quadruplex; GV1001; GRN163L; GRNVAC1

## INTRODUCTION

Telomeres are specialized structures at the ends of human chromosomes that were discovered in 1985 by Elizabeth Blackburn and Carol Greider {{24 Blackburn,E.H. 2005;}}. Telomeres are composed of 1000-2000 non-coding tandem repeats of TTAGGG nucleotide DNA sequences and serve as protective "caps" at the ends of chromosomes protecting them from DNA degradation and unwanted repair {{24 Blackburn,E.H. 2005;13 Blackburn,E.H. 2006;14 Harley,C.B. 2008;1 Tian,X. 2010;}}. In normal human cells telomeres shorten with each successive cell division, and upon reaching critical lengths they elicit DNA-damage responses resulting in cell senescence and apoptosis. In contrast, the malignant cells are capable of bypassing normal cell cycle check points resulting in uncontrolled growth and proliferation **(Fig. 1)**. Therefore, it is believed that cancer cells with critically short telomeres and chromosomal aberrations may respond to DNA-damage signals by eliciting responses that may lead to activation of telomerase {{1 Tian,X. 2010;23 Rankin,A.M. 2008;}}.

Telomerase is a human ribonucleoprotein reverse transcriptase (hTERT) composed of two main subunits, the catalytic protein hTERT and a ribonucleoprotein template hTER {{1 Tian,X. 2010;24 Blackburn,E.H. 2005;138 Bisoffi,M. 2006;85 Dikmen,Z.G. 2008;}}. Telomerase synthesizes telomeric DNA by continually adding single stranded TTAGGG sequences onto the single stranded 3' end of telomere in a 5' to 3' direction {{1 Tian,X. 2010;24 Blackburn,E.H. 2005;224 Artandi,S.E. 2010;}}. Telomerase consists of 451 nucleotides but only the 11-base region, consisting of nucleotides 46 through 56 (5' - CUAACCCUAAC - 3'), serves as the template for telomere synthesis {{24 Blackburn,E.H. 2005;37 Meyerson,M. 2000;222 Corey,D.R. 2000;}} **(Fig. 2)**.

The increase of telomerase activity is often directly correlated with uncontrolled growth of cells, which is a known hallmark of cancer {{19 Shay,J.W. 2008;246 Shay,J.W. 2010;}}. Telomerase, and specifically its catalytic subunit hTERT is overactive in 85%-90% of most cancers {{35 Gellert,Ginelle C. 2005;138 Bisoffi,M. 2006;}}. In normal cells telomerase is present in embryonic, male germline and some adult stem cells and is present in very low levels in most somatic cells {{1 Tian,X. 2010;23 Rankin,A.M. 2008;24 Blackburn,E.H. 2005; 26 Kelland,L.R. 2005;32 Ulaner,G.A. 2004;}}. Therefore, telomerase upregulation is considered to be a critical step in cell tumorigenesis and telomerase has become a widely accepted tumor marker and a popular target for anticancer therapeutics. Another advantage to telomerase targeting therapies is that telomeres in cancer cells have been found to be much shorter (5kb) compared to normal cells (10-20kb) {{26 Kelland,L.R. 2005;97 Phatak,P. 2007;}}, thus increasing therapeutic cytotoxicity on cancer cells while exhibiting minimal impact on normal cells {{222 Corey,D.R. 2000;}}.

Under normal aging conditions, telomeres become shorter with each cell division and loose their G-rich nucleotide sequences {{10 Riethman,Harold 2008;}}. To maintain proper activity of telomeres, required for protection of chromosomal integrity, a 150-250 nucleotides long single-stranded G-rich 3' overhang forms one of the two higher order structures, a T-loop or a G-quadruplex complex, which can help to maintain proper activity of telomeres {{10 Riethman,Harold 2008;291 Ou,T.M. 2008;290

Xu,Y. 2011;}} **(Fig. 3)**. Shelterin complex is a set of specialized proteins that are responsible for maintaining the T-loop structure by capping the telomeres and aiding in telomere-telomerase assembly {{1 Tian,X. 2010;24 Blackburn,E.H. 2005;23 Rankin,A.M. 2008;138 Bisoffi,M. 2006;}} **(Fig. 3)**. The T-loop structure is also protected from exposure to extracellular DNA damage or repair mechanisms by multiple copies of POT1 (protection of telomerase) protein, an important ssDNA binding protein in humans {{29 Zaug,A.J. 2005;276 Neidle,S. 2010;290 Xu,Y. 2011;291 Ou,T.M. 2008;}}. Therefore, targeting telomere-associated proteins can compromise the T-loop structure leading to significant telomere shortening and premature cell death. A G-quadruplex is another higher order structure, formed by stacking of guanosine (G) tetrads by incorporating a 16-nucleotide d(GGGTTAGGGTTAGGGT) and a 6-nucleotide d(TAGGGT) sequence of telomeric 3' overhang, folded via hydrogen-bonding {{290 Xu,Y. 2011;291 Ou,T.M. 2008;}} **(Fig. 3)**. The G-quadruplex protects the telomeric 3'-overhang from being accessed by telomerase, thereby regulating its catalytic activity {{290 Xu,Y. 2011;291 Ou,T.M. 2008;}}. By stabilizing the G-quadruplex with small molecule ligands such as BRACO19, RHPS4 and telomestatin, the telomeric 3' overhang can be locked in place thus blocking telomerase from accessing telomeres.

Telomerase targeting strategies can be assigned to two general categories. One is directly targeting telomerase by inhibiting the activity of its catalytic subunit the hTERT or targeting the RNA template of telomerase the hTER, leading to inhibition of telomerase activity (TA), telomere shortening and inhibition of cell proliferation. Another approach, is indirectly targeting telomerase with G-quadruplex stabilizers or targeting telomere and telomerase associated proteins, such as Tankyrases or HSP90, thus blocking telomerase access to telomeres or inhibiting binding of telomerase associated proteins, which could consequently lead to telomere uncapping resulting in cell apoptosis {{14 Harley,C.B. 2008;19 Shay,J.W. 2008;}}. One of the most recent approaches of telomere targeting is using T-oligo, which can induce DNA damage responses leading to tumor cell apoptosis or inhibition of cell proliferation {{1 Tian,X. 2010;4 Burger,A.M. 2007;23 Rankin,A.M. 2008;26 Kelland,L.R. 2005;}}. The most novel therapeutics reviewed here are small molecule inhibitors, antisense oligonucleotides, immunotherapies, gene therapies, G-quadruplex stabilizers, telomere and telomerase associated protein inhibitors, and T-oligo **(Table I).**

## ANTISENSE OLIGONUCLEOTIDES (AS-ODNs) - TARGETING hTER

### Advantages of Antisense Oligonucleotide Inhibitors

The antisense oligonucleotides (AS-ODNs) approach for targeting telomerase was first derived using AS-ODNs to block the translation of mRNA with a sequence complementary to sense RNA. It was initially used as an anticancer treatment in 1995 {{95 Cunningham,A.P. 2006;97 Phatak,P. 2007;}}. One of the advantages of this approach is that AS-ODNs are easy to identify among large libraries of drug candidates and they do not promote multidrug resistance (MDR) {{14 Harley,C.B. 2008;130 Roth,A. 2010;}}. AS-ODNs can be used to target the catalytic component of telomerase (hTERT) or the RNA template (hTER). AS-ODNs targeting the RNA component of telomerase are composed of short single-stranded DNA (ss-DNA) sequences that inhibit TA by complementary binding to the RNA template {{97 Phatak,P. 2007;}}. AS-ODNs have been intensively studied and their structure has been modified and significantly improved over the past decade. One of the most

successful AS-ODNs used to target hTER, the GRN163L (Imetelstat®) (Geron Corporation, Menlo Park, CA), is reviewed below.

**GRN163L - an AS-ODN Telomerase Inhibitor**

GRN163, the first most promising oligonucleotide and the first-generation lead compound targeting hTER is a N3'-P5'-thio-phosphoramidate, which requires a lipid carrier molecule and a lipid-based transfection agent to effectively traverse cell and tissue membranes {{23 Rankin,A.M. 2008;130 Roth,A. 2010;}}. While GRN163 showed good results in inhibiting telomerase, the lack of a lipid carrier has diminished its potential due to limited uptake. To solve this problem, a lipid-modified version, the GRN163L, has been developed {{130 Roth,A. 2010;}}. GRN163L is a 13-mer oligonucleotide N3'-P5'-thio-phosphoramidate with a covalently bound lipophilic palmitoyl (C16) group attached to its 5'-thio-phosphate {{85 Dikmen,Z.G. 2008;}}. GRN163L causes inhibition of TA by acting as a direct telomerase RNA template antagonist. GRN163L does not act like a normal antisense oligonucleotide, but with its 5'-Palmitoyl-TAGGGTTAGACAA-3' oligonucleotide chain, complementary to the hTER region of telomerase, it partially overlaps the hTER template by binding with high specificity and affinity at its active site, leading to complete inhibition of the enzyme **(Fig. 2)** {{85 Dikmen,Z.G. 2008;130 Roth,A. 2010;}}.

Studies have found that GRN163L may be slightly less potent than the GRN163 in a cell-free telomere repeat amplification protocol (TRAP) assay, possibly due to some interference of the lipid moiety with the telomerase template {{21 Baker,N. 2008;}}. However, in a study with 13 solid and hematologic tumor cell lines, GRN163L has shown an $IC_{50}$ range between 0.8 and 6.5 mg/mL, with the average $T_{1/2}$ being 2.9-5.3 hrs for 5-15 mg/kg IV administered drug {{136 Tressler,Robert J. 2006;}}. This indicates that GRN163L has better cell and tissue penetration, as evidenced by its low $IC_{50}$ values, and greater biodistribution in normal and cancer cell lines, possibly due to the fact that it does not require a lipid carrier and can traverse membranes more effectively than GRN163 {{130 Roth,A. 2010;21 Baker,N. 2008;}}. In addition, *in vivo* studies with human hepatoma tumor xenograft mouse model have shown that when administered parenterally GRN163L has a greater uptake than GRN163 {{130 Roth,A. 2010;}}.

**GRN163L - Preclinical Studies (Table II)**

*For discussion see supplemental section*

**GRN163L - Clinical Trials**

Currently, GRN163L is undergoing six stage I and stage I/II clinical trials targeting patients with chronic lymphoproliferative diseases (CLD), refractory and relapsed solid tumor malignancies, refractory and relapsed MM, locally recurrent or metastatic breast cancer (MBC), advanced and metastatic non-small cell lung cancer (NSCLC). More information on these trials can be found in **Table III**. To determine the maximum tolerated dose (MTD) including dose limiting toxicities (DLT), the doses of GRN163L were escalated continually in each cohort study based on 3 or 4 weeks cycles until MTD was reached {{130 Roth,A. 2010;}}.

In the phase I study of GRN163L in combination with paclitaxel and bevacizumab in patients with locally recurrent or metastatic breast cancer, a total of 14 patients were treated, 3 with *de novo* MBC and 6 with prior (neo) adjuvant taxane therapy. Due to a dose escalation design, the majority of patients (78.6%) experienced dose reductions/delays with GRN163L and/or paclitaxel during later treatment cycles. The objective response rate (ORR) (measurable response) was 38.5% and currently alternative dosing schedules are being tested {{102 Kozloff,M. 2010;}}. In a phase I clinical trial in patients with relapsed or refractory MM and phase I trial in patients with advanced solid tumors, MTD's were determined with most prominent DLTs being thrombocytopenia and active prolonged thromboplastin time (aPTT) in the first trial and thrombocytopenia and hypersensitivity reactions in the second trial, which have appeared only in later treatment cycles {{134 Chanan-Khan,Asher Alban 2008;146 Ratain,Mark J. ;}}. The preliminary clinical trial results showed good tolerability to GRN163L despite some toxicities, which hare common to the oligonucleotide class of drugs and minor adverse effects {{130 Roth,A. 2010;146 Ratain,Mark J. ;}}.

The successful preliminary results of phase I clinical trials have allowed GRN163L to be moved to four phase II clinical trials for which patients with NSCLC, locally recurrent or metastatic breast cancer, previously treated MM and patients with essential thrombocythemia (ET) are currently being recruited. The phase II clinical trial on patients with ET is designed to conduct a dose reduction study with patients who require cytoreduction and were intolerant to previous therapies. In addition, since preclinical studies with GRN163L showed that it can restore sensitivity of HER2+ breast cancer cell lines to trastuzumab {{88 Goldblatt,E.M. 2009;}}, patients with HER2+ breast cancers are currently being recruited into a phase I clinical trial.

## SMALL MOLECULE INHIBITORS - TARGETING hTERT

### BIBR1532

Currently, there have been only a few successful hTERT inhibitors developed. BIBR1532 *(2-[E)-3-naphtalen-2-yl-but-2-enylylamino]-benzoic acid)* is one of the most promising hTERT specific active site inhibitors to date {{21 Baker,N. 2008;}}. BIBR1532 is a non-nucleotidic small molecule synthetic compound that inhibits telomerase by non-competitively binding to the active site of hTERT {{34 Pascolo,E. 2002;133 El-Daly,H. 2005;1 Tian,X. 2010;}}. BIBR1532 does not block the basic template copying steps, but specifically impairs DNA substrate elongation upon template copying by reducing the number of TTAGGG repeats **(Fig. 2)**. Therefore, it is suspected that BIBR1532 may have an influence on the translocation of the enzyme DNA substrate complex or may lead to dissociation of the enzyme from the DNA substrate during template copying {{34 Pascolo,E. 2002;}}. Results from multiple studies on BIBR1532 have shown a dose-dependent inhibition of telomerase with higher concentrations of BIBR1532, and have not shown any significant effects on normal human cells {{73 Chen,H. 2009;66 Parsch,D. 2008;}}.

Preclinical studies conducted in several human cancer cell lines have shown that treatment with BIBR1532 can repress TA and lead to tumor cell growth arrest without causing acute cytotoxicity {{66 Parsch,D. 2008;140 Damm,K. 2001;}}. In DU145 (prostate cancer), MDA-MB-231 (breast cancer), HT1080 (fibrosarcoma cancer) and

HTI-H430 (lung cancer) cell lines, treatment with BIBR1532 has shown significant telomere shortening, however it was preceded by a long lag time {{140 Damm,K. 2001;}}. In the study of BIBR1532 in chondrosarcoma cancer cell lines and germ cell tumors (GCTs), prolonged treatment with BIBR1532 led to significant telomere shortening and 80% decrease of TA in chondrosarcoma cell lines. However in GCTs, which normally have greater TA and longer telomere lengths, treatment with BIBR1532 did not completely inhibit tumor cell proliferation. In chondrosarcoma cell lines, where the growth of cells was inhibited in early passages, the growth of treated cells in the later passages was the same as that of untreated controls {{66 Parsch,D. 2008;64 Mueller,,Sandra 2007;}}. In the study of BIBR1532 in p53-negative BCR-ABL positive chronic myelogenous leukemia (CML) cell lines, which have critically short telomeres of approximately 3-5kb, BIBR1532 treatment caused significant telomere shortening without affect on their cell growth kinetics, cell morphology, rate of apoptosis or cell senescence {{149 Brassat,U. 2011;}}. Interestingly, it was found that treatment of T-PLL (T-cell prolymphocytic leukemia) cell lines, which also have short telomeres, led to noticeable changes in cell morphology indicating apoptotic cell death {{94 Roth,A. 2007;}}. Additionally, BIBR1532 can sensitize certain drug-resistant cell lines to other chemotherapies, as shown in a study with drug-resistant human promyelocytic leukemia (HL60-MX2) and breast cancer (MCF-7/Mln$^{R}$-melphalan and MCF-7/Adr$^{R}$-doxorubicin) cell lines and their drug-sensitive parental (WT) counterparts (HL60-WT and MCF7/WT), where BIBR1532 has effectively inhibited TA and caused progressive telomere shortening in all cell lines except MCF-7/Adr$^{R}$ doxorubicin resistant cell line {{25 Ward,R.J. 2005;}}.

## TELOMERASE BASED IMMUNOTHERAPIES

### Telomerase - a Universal Tumor Antigen

The use of an immunotherapy approach, which was designed to induce CD8+ cytotoxic T lymphocytes (CTL) response for hTERT antigens in malignant tumors, has shown better telomerase inhibition than other therapies {{190 Keith, W.,Nicol 2008;}}. Since telomerase is present in most cancers, its peptides are universal telomerase-associated antigens (TAAs). They are capable of producing strong immune response (IR) by eliciting both CD4+ and CD8+, T-cell responses and stimulating hTERT peptide-specific CTL activity, potentially leading to tumor cell lysis {{19 Shay,J.W. 2008;160 Beatty,G.L. 2008;194 Vonderheide,R.H. 2008;}}. Preclinical studies with hTERT peptides have led to successful progress in the development of telomerase-targeting immunotherapies and several vaccines that are currently in phase III clinical trials **(Table IV)**. The two commonly used approaches of anticancer immunotherapies are the dendritic cell (DC) approach and the hTERT peptide vaccine approach. The most promising vaccines, GV-1001, GRNVAC1 and Vx-001 are further reviewed {{14 Harley,C.B. 2008;}}.

### GV1001 - hTERT Peptide-Based Vaccine

GV1001 (KAEL-GemVax Co., Ltd., Gangnam-gu Seoul, Republic of Korea) is a 16 amino acid MHC class II-restricted hTERT peptide vaccine, which consists of amino acids 611-626 (EARPALLTSRLRFIPK) of the hTERT active site ({{157 Kyte,J.A. 2009;161 Middleton,G. 2008;159 Nava-Parada,P. 2007;}}. GV1001 is used in conjunction with an adjuvant, such as granulocyte-monocyte colony-stimulating

factor (GM-CSF) or Toll-like receptor-7 (TLR7) agonist (imiquimod) {{155 Shaw,V.E. 2010;159 Nava-Parada,P. 2007;}}. This may prevent the rapid degradation and elimination of anticancer vaccine peptides before recognition by the appropriate antigen presenting cells (APCs), which may occur due to a self-tolerance to self-peptides {{159 Nava-Parada,P. 2007;195 Wong,S.B. 2008;}}. GV1001 is administered as an MHC class-II peptide, which is endogenously processed to yield a MHC class-I peptide producing both CD4+ and CD8+ responses, thus leading to a robust CTL signaling cascade and a maximum IR **(Fig. 4)** (93; 94). The activity of CD4+ T cells at the tumor site leads to a secretion of IFN-γ and IL-2, further stimulating CD8+ CTLs and natural killer cells (NKs), which may help to increase the infiltration and the retention of CD8+ T-cells into the tumor sites leading to upregulation and re-expression of MHC class-I molecules {{161 Middleton,G. 2008;155 Shaw,V.E. 2010;}}. This may have therapeutic advantage for treatment of advanced cancers that are associated with a progressive loss of MHC class-I molecules {{14 Harley,C.B. 2008;210 Seliger,B. 2008;155 Shaw,V.E. 2010;}}.

A preclinical study with GV1001 in patients with hTERT positive B-cell chronic lymphocytic leukemia (B-CLL), of which 75% have overexpressed hTERT, has shown that these cells contain naturally occurring telomerase-specific T cells, which can mediate the IR to hTERT peptide 611-626 (GV1001) leading to lysis of autologous leukemic cells (92). The hTERT positive B-CLL patients treated with GV1001 peptide-loaded DCs showed positive CD4+ and CD8+ T-cell responses, without a negative effect on normal cells or autoimmunity {{162 Kokhaei,P. 2007;}}. This study has revealed that GV1001 may be an effective method for treatment of patients with B-CLL and may be moved for testing in clinical trials.

**GV1001 - Clinical Trials**

GV1001 has successfully completed several phase I and II clinical trials, conducted in patients with advanced stage melanoma, NSCLC, hepatocellular carcinoma (HCC) and multiple trials in patients with pancreatic cancer {{157 Kyte,J.A. 2009;163 Kyte,J.A. 2009;215 Anonymous 2010;170 Buanes,T. 2008;152 Bernhardt,S.L. 2006;171 Bernhardt S.,L. 2005;167 Choudhury,Aniruddha 2007;156 Greten,T.F. 2010;166 Buanes,T. 2009;216 Anonymous 2010;185 Aamdal,S. 2006;}} (See **Table IV** for details**)**. A new large randomized phase III clinical trial (TeloVac) is currently recruiting patients with locally advanced or metastatic pancreatic adenocarcinoma in multiple centers around the UK, with a primary aim to compare standard therapy to GV1001 and to measure the length of survival for a primary outcome measure {{216 Anonymous 2010;}}. Results and information on additional clinical trials with GV1001 may be viewed in **Table IV.**

*For additional information on GV1001 clinical trials, see supplemental section.*

**Vx-001 - Cryptic Peptide-Based Vaccine**

Studies have shown that tumor non-specific self-antigens could prevent a self-tolerance problem often caused by self-antigens {{211 Menez-Jamet,J. 2009;}}. The HLA-I molecules consist of dominant and cryptic peptides {{175 Mavroudis,D. 2006;}}. The dominant peptides have strong affinity for HLA-I alleles, they are abundant on the cell surface and are strongly immunogenic, whereas cryptic peptides

are not as abundant on the cell surface, have weak HLA-I affinity and are weakly immunogenic or completely lack immunogenicity. However, unlike dominant peptides, cryptic peptides do not undergo massive clonal deletion due to their poor expression and do not induce immune tolerance. Therefore, they may be better suited to be used as a peptide antitumor vaccine therapy. In addition, using tumor non-specific antigens may be a better choice for anticancer vaccines since they are not dependant on adjuvants or the efficacy of delivery {{211 Menez-Jamet,J. 2009;175 Mavroudis,D. 2006;}}.

The new peptide-based anticancer therapy vaccine, Vx-001 (Vaxon Biotech, Paris, France), consists of a low affinity cryptic peptide $hTERT_{572}$ (RLFFYRKSV) and its optimized version, the $hTERT_{572Y(1)}$ (YLFFYRKSV) which has the first amino-acid residue replaced with a modified tyrosine (Y1) residue {{227 Tourdot,S. 2000;}}. This sequence enhances the peptide's affinity for HLA-I molecules and may circumvent the self-tolerance problem {{211 Menez-Jamet,J. 2009;227 Tourdot,S. 2000;}}. In addition, it leads to enhanced immunogenicity of the cryptic peptide when presented by HLA-A*0201 molecules (present in 40-45% of population) without changing antigen's specificity {{175 Mavroudis,D. 2006;}}.

Vx-001 has shown good antitumor efficacy evidenced by inhibition of tumor growth *in vivo* in HHD transgenic mice and in phase I and II clinical trials in patients with various types of tumors **(Table IV)** {{211 Menez-Jamet,J. 2009;175 Mavroudis,D. 2006;}}. Vx-001 has completed a large phase I/II clinical trial in 116 patients with different types of advanced stage cancers, including patients with NSCLC, breast cancer, melanoma and cholangiocarcinoma. Vx-001 has shown strong immune response rates in cancer cells, produced long-lasting disease stabilization and prolonged overall survival {{60 Bolonaki,I. 2007;175 Mavroudis,D. 2006;181 Vetsika,E.K. 2008;182 Kotsakis,A. 2008;184 Kosmatopoulos,K. 2006;}}. The vaccine was well tolerated, did not induce autoimmunity and resulted only in minor toxicities, and showed a positive correlation between immune response and clinical response in patients with NSCLC {{211 Menez-Jamet,J. 2009;}}. VX-001 is now scheduled for testing in a phase III clinical trial in NSCLC patients {{211 Menez-Jamet,J. 2009;}}.

**GRNVAC1/GRNVAC2 - Dendritic Cell (DC) Based Immunotherapy**

Dendritic cells (DC) are the most efficient APCs that are capable of producing strong immune response and can be used for tumors for which potent T-cell epitopes have not yet been identified {{158 Huo,L.F. 2006;}}. Because they are nonbiased with respect to MHC allele restriction they can encode epitopes for multiple types of HLA alleles, which may eliminate the need for alleles testing {{155 Shaw,V.E. 2010;191 Su,Z. 2005;}}. GRNVAC1 (Geron Corporation, Menlo Park, CA) is an autologous dendritic cell vaccine capable of stimulating both CD4+ and CD8+ immune responses {{217 Su,Z. 2002;}}. GRNVAC1 consists of immature DCs transfected *ex vivo* with a complete mRNA sequence encoding hTERT and a portion of the lysosomal-associated membrane protein (LAMP-1) **(Fig. 4)** {{191 Su,Z. 2005;189 DiPersio,John F. 2009;221 Srivastava,R. 2006;}}. LAMP-1 directs the hTERT to a lysosome making it easily degradable into peptides and leading to a stronger immune response {{191 Su,Z. 2005;}}.

GRNVAC1 was tested in a randomized phase I clinical trial in 20 patients with metastatic prostate cancer and in a more recent phase II clinical trial in 21 patients with acute myelogenous (myeloid) leukemia (AML) **(Table IV)**. A phase I clinical trial in patients with a metastatic prostate cancer compared the effect of hTERT mRNA-transfected DC vaccine to that of LAMP-hTERT mRNA-transfected DC vaccine (GNVAC1) {{191 Su,Z. 2005;}}. After three or six weekly injections, vaccinated patients have shown good tolerance with only mild side effects and no signs of autoimmunity. In this trial the LAMP-hTERT group of patients experienced more robust immune responses compared to a group with non-modified hTERT due to a stronger hTERT specific CD4+ and CD8+ T-cell responses {{191 Su,Z. 2005;}}. The results of treatment showed 95% immune response rate, which was evidenced by a reduction in prostate-specific antigen (PSA) in circulating tumor cells {{191 Su,Z. 2005;160 Beatty,G.L. 2008;}} and consistent hTERT-specific T cell responses providing a good baseline for future clinical trials. A phase II clinical trial of GRNVAC1 in AML patients showed 55% immune response rate, with 19 out of 21 patients in clinical remission (CR) and two in early relapse {{188 Khoury,H.Jean 2010;189 DiPersio,John F. 2009;}}. This trial has shown that prolonged vaccination of patients with up to 32 administrations of GRNVAC1 was well tolerated and produced no toxicities in all but one patient. In addition the results of this trial have shown that GRNVAC1 may be more beneficial for high-risk AML patients as proven by increased DFS compared to low or intermediate groups **(Table IV)** {{189 DiPersio,John F. 2009;}}. Recently developed GRNVAC2 is designed using the same dendritic cell approach except that the DCs are being derived from human embryonic stem cells (hESCs) instead of leukapheresis of each individual patient, and is thus a better vaccine delivery system {{59 Brower,V. 2010;}}.

## TELOMERASE BASED GENE THERAPIES

### Oncolytic and Suicide Gene Therapies

Telomelysin (OB-301) (Oncolys BioPharma Inc., Tokyo, Japan), Ad-hTER/hTERT-NTR/CB1954 and hTERTp-HRP/IAA

*Supplemental section*

## OTHER TELOMERASE INHIBITING STRATEGIES

### G-quadruplex Stabilizers

G-quadruplex stabilizers are potent ligands that indirectly target telomerase resulting in inhibition of its catalytic activity. G-quadruplex ligands stabilize or promote G-quadruplex structure by preventing G-quadruplex from unwinding and opening the telomeric ends to telomerase thus locking the single stranded telomeric substrate within the T-loop. G-quadruplex ligands may also trigger telomere uncapping by causing dissociation of telomere-associated proteins {{14 Harley,C.B. 2008;290 Xu,Y. 2011;}}. Most of the G-quadruplex stabilizing ligands contain a polycyclic heteroaromatic structure. BRACO-19, RHPS4 and Telomestatin are three of the most commonly studied G-quadruplex binding ligands. They inhibit telomerase by activating DNA damage responses similar to those activated in response to dsDNA breaks {{276 Neidle,S. 2010;}}.

### Telomestatin - a Natural Macrocyclic Compound

Telomestatin has been one of the most potent small molecule G-quadruplex stabilizers to date, with high selectivity for tumor cells and with minimal effects on normal cells. Telomestatin is a natural product with a complex structure composed of a large array of polyoxazole rings that form macrocyclic linkages (Telomestatin-R) {{294 Shin-ya,K. 2001;300 Doi,T. 2006;}}. Studies have shown that telomestatin may promote formation and stabilization of G-quadruplex structure by binding to G-quadruplex guanosines at both termini and sandwiching the G-quadruplex in a 2:1 complex {{307 Kim,M.Y. 2002;}}. Telomestatin may also cause earlier than expected telomere shortening in various tumors, indicating that factors other than telomerase inhibition may be contributing to this effect. Telomestatin induces activation of apoptosis in acute leukemia cells as evidenced by activation of p38 MAP kinase, caspase-3 and poly-(ADP-ribose) polymerase (PARP) {{131 Tauchi,T. 2006;}}. Telomestatin has also shown inhibition of telomerase activity and tumor cell proliferation at low $IC_{50}$ values in various tumor cell lines, including myeloma, neuroblastoma, myeloid leukemia, breast, pancreatic, cervical and some other cancer cell lines {{131 Tauchi,T. 2006;132 Sumi,M. 2004;295 Tahara,H. 2006;296 Gomez,D. 2006;301 Binz,N. 2005;}}. In addition, telomestatin was found to either significantly down regulate important telomeric proteins, such as POT1 and TRF2, or result in complete dissociation of TRF2, as seen in HT1080 fibrosarcoma cell lines {{296 Gomez,D. 2006;}}, thus leading to uncapping of telomeres with shortening of both single and double stranded telomeric sequences {{296 Gomez,D. 2006;295 Tahara,H. 2006;}}. A major limitation of telomestatin is the difficulty of its synthesis, making it unfeasible for large-scale production {{273 Monchaud,D. 2010;}}. A recent study has reported a development of a new (S)-stereoisomer of telomestatin-(R), which has shown four-fold greater telomerase inhibiting activity than the (R)-stereoisomer {{299 Doi,T. 2011;}}. However, future research is necessary to discover a better method for telomestatin synthesis to enable its application for patient use.

**BRACO-19 - a Trisubstituted Acridine G-quadruplex Ligand**

*Supplemental section*

**RHPS4 - a Polycyclic Acridinium G-quadruplex Ligand**

*Supplemental section*

**TARGETING TELOMERE AND TELOMERASE-ASSOCIATED PROTEINS**

**Tankyrase Inhibitors**

Studies have shown that inhibition of tankyrases may lead to inhibition of residual telomerase activity, which is often seen in drug resistant tumors, and could potentially sensitize cells that became resistant to telomerase inhibitors {{288 Seimiya,H. 2005;226 Hiyama,Keiko 2009;354 Munoz-Gamez,J.A. 2011;}}. Tankyrase 1 and 2 (TNKS1 and TNKS2) belong to the family of telomerase-specific a poly (ADP-ribose) polymerases (PARPs) {{320 Cook,B.D. 2002;316 van Steensel,B. 1998;}}. TNKS1 is activated by binding to DNA breaks and takes part in DNA base excision repair {{354 Munoz-Gamez,J.A. 2011;}}. TNKS1 can modify telomere structure by exposing telomeric DNA to telomerase {{315 Smith,S. 1998;}}. The telomeric DNA

binding protein TRF1 is a negative regulator of telomere length, which blocks telomerase access to telomeres {{316 van Steensel,B. 1998;}}. Removing TRF1 from telomeres allows telomerase access to telomeric DNA, leading to telomere elongation in telomerase positive cells {{317 Hsiao,S.J. 2008;}}. TNKS1 causes poly (ADP-ribosyl)ation of TRF1 protein (PARsylation), reducing TRF1's binding to telomeric DNA, leading to ubiquination {{352 Chang,W. 2003;}} and a complete release of TRF1 from the DNA strand {{315 Smith,S. 1998;317 Hsiao,S.J. 2008;}}. TNKS1 may directly contribute to telomere elongation by acting as a positive regulator of telomere length and causing the dissociation of TRF1 from telomeric DNA. Therefore targeting tankyrases with PARP inhibitors may be a new novel anticancer therapy approach {{315 Smith,S. 1998;320 Cook,B.D. 2002;}}.

*See supplemental section for additional information.*

**Inhibition of HSP90**

Heat shock protein 90 (HSP90) is a molecular chaperone that exists in association with a co-chaperone p23 and is responsible for folding and regulation of its client proteins. The HSP90-p23 chaperone complex is required for maturation and activation of telomerase *in vitro* and *in vivo* {{322 Holt,S.E. 1999;327 Kim,R.H. 2008;}}. Inhibition of HSP90 modulates signaling events in tumors, suppresses angiogenesis and tumorigenicity by regulating tumorigenic proteins {{51 Koga,F. 2009;}}. In a study with telomerase positive oral cancer cell lines it was found that HSP90-hTERT association was required for hTERT promoter activity in cancer cell lines and inhibition of HSP90 could lead to a proteasome-dependent degradation of hTERT {{327 Kim,R.H. 2008;324 Mizuno,H. 2007;}}.

Geldanamycin (GA) (INVIVOGEN, San Diego, California) is one example of current HSP90 inhibitors, which inhibits telomerase by blocking the ATP-dependent binding of HSP90 to p23, resulting in the disruption of HSP90-p23 complex {{322 Holt,S.E. 1999;}}. The 17-AAG and 17-DAMG are the analogs of GA and are currently in the phase I and phase II clinical trials {{50 Powers,M.V. 2006;}}. A recent study showed that curcumin, which is a natural compound derived from turmeric {{330 Kawamori,T. 1999;}}, can cause time- and dose-dependent inhibition of nuclear localization of hTERT in the H1299 NSCLC cell line via proteasome-mediated degradation causing dissociation of p23 from hTERT complex. However, it does not have effect on HSP90-hTERT binding, indicating that HSP90-p23 complex is required for telomerase activity {{283 Lee,J.H. 2010;}}. While it is still unclear how HSP90-p23 mediates nuclear translocation of hTERT from its nonfunctional state in the cytoplasm to a biologically functional state in the nucleus, inhibition of nuclear translocation of telomerase with curcumin or other HSP90-p23 inhibitors may be a promising approach in regulating telomerase translocation during tumorigenic development {{283 Lee,J.H. 2010;}}.

**T-oligo**

T-oligo is another novel anticancer agent, composed of a single stranded 11-base oligonucleotide sequence (GTTAGGGTTAG) that is homologous to the sequence of a single stranded telomeric overhang {{264 Yaar,M. 2007;331 Eller,M.S. 2002;}}. Studies showed that treating various tumor cell lines with T-oligo for a short time

could activate potent DNA damage responses in tumor cell lines, mediated through the ATM kinase and its effector proteins such as p53, p57, p95/Nbs1, and E2F1 {{264 Yaar,M. 2007;331 Eller,M.S. 2002;268 Puri,N. 2004;265 Longe,H.O. 2009;267 Gnanasekar,M. 2009;}}. The responses were similar to DNA damage responses activated by uncapping of telomeres and those caused by a knockdown of TRF2, leading to activation of DNA damage signals and apoptosis of cancer cells {{331 Eller,M.S. 2002;264 Yaar,M. 2007;316 van Steensel,B. 1998;}}. However, treating normal cells with T-oligo would lead to only a transient cell cycle arrest, since normal cells unlike cancer cells undergo cell cycle check points (14; 166). Other studies showed that treatment with T-oligo can cause inhibition of angiogenesis in melanoma cell lines, resulting from a decrease in VEGF receptor signal {{269 Coleman,C. 2010;}} and a reduction of oxidative damage to cells {{266 Lee,M.S. 2009;}}. Current studies on T-oligo show very promising results and future research is required to assess T-oligos' full potential.

## DISCUSSION AND CONCLUSION

Among various telomerase inhibitors reviewed, the AS-ODN inhibitor GRN163L, hTERT and DC based vaccines GV1001 and GRNVAC1 respectively, may be potential new treatment strategies. Treatment of various tumor cells lines with GRN163L *in vitro* and *in vivo* has shown not only inhibition of TA or tumor cell proliferation, but also inhibition of tumor metastasis, indicating its potential for treatment of metastatic cancers. MTDs and DLTs were successfully determined in phase I and II clinical trials for GRN163L in patients with CLD, solid tumor malignancies, MM and breast cancer, with no major cytotoxicities seen, indicating GRN163L can be moved for testing in phase III clinical trials.

While BIBR1532 may not be as promising as GRN163L, studies have shown that it is highly selective telomere length-dependent inhibitor, which may not be an ideal single therapy agent for most cancers but may be a good choice of treatment for cancers with short telomeres {{66 Parsch,D. 2008;}}, for which studies should be further explored. In addition BIBR1532 may be used as a combination therapy with standard of care or traditional therapy to sensitize certain drug-resistant cell lines to other chemotherapies {{66 Parsch,D. 2008;25 Ward,R.J. 2005;}}.

GV1001 and GRNVAC1 are both promising telomerase targeting vaccines, capable of stimulating CD4+ and CD8+ responses in telomerase positive tumors, showing minimal effects on normal cells, low cytotoxicity and no autoimmunity. Clinical trials with GV1001 in patients with pancreatic adenocarcinoma, stage IV metastatic melanoma, and advanced stages of NSCLC and HCC, have shown good overall immune response rates, after only a short treatment times, ranging from 39% to 95% depending on tumor type, stage and dosage **(Table IV)**. Larger and long-term studies may be required to further determine long-term toxicity of GV1001. Because patients with advanced stages of cancers have poor survival or may develop disease metastases, it is suggested that studying GV1001 in patients with less advanced stages may increase overall survival rates {{151 Brunsvig,,Paal 2006;159 Nava-Parada,P. 2007;}}.

Compared to GV1001, GRNVAC1 vaccine has shown better efficacy in clinical trials, with stronger hTERT specific CD4+ T-cell responses, leading to IL-2 secretion and

stimulating CTL-mediated tumor cell lysis. It has had good immune responses in patients with metastatic prostate tumors and AML, showing greater DFS in high-risk AML patients. If difficulties with DC vaccine production, such as DCs derivation and maturation could be easily overcome in the future trials, DC vaccines may become successful methods of immunogenic telomerase targeting.

Although, small molecule inhibitors or immunotherapies may be effective methods of telomerase inhibition, there is always a concern that cells may gain resistance to direct telomerase inhibitors after excessive telomere shortening {{288 Seimiya,H. 2005;}}. Thus, studying G-quadruplex stabilizers, tankyrase enzyme inhibitors and T-oligo may help to solve this problem.

While most of the telomerase targeting therapeutics showed success in preclinical or clinical studies, a combination therapy of telomerase inhibitors and standard of care or traditional therapy may be the most effective way to target telomerase positive tumors. This may help to overcome common issues with standard treatments, such as tumor relapse or recurrence and a long lag time, common for many telomerase targeting monotherapies. Combination therapy may increase drug efficacy causing critical telomerase shortening, antitumor mass shrinkage, and may require lower drug doses thus reducing cytotoxicity on normal cells.

**Conflict of Interest Statement:**
No conflict of interest.

## FIGURE CAPTIONS

**Fig. 1. Telomeres shortening in normal vs. cancer cells**

**Fig. 2. Targeting telomerase with small molecules and AS-ODNs.**
Telomeres are composed of 1,000 to 2,000 oligonucleotides of non-coding G-rich nucleotide sequences, $(TTAGGG)_n$, that form a 3'-overhang on the 3' end of chromosomes {{37 Meyerson,M. 2000;224 Artandi,S.E. 2010;225 Batista,L.F. 2009;}}. The RNA template of telomerase (hTER) consists of a total of 451 nucleotides of which nucleotides 46 through 56 (5'-CUAACCCUAAC-3') serve as a template for adding new telomeric repeats {{222 Corey,D.R. 2000;225 Batista,L.F. 2009;}}. GRN163L is a 13-mer oligonucleotide that inhibits telomerase by acting as a direct telomerase RNA template antagonist binding with high specificity and affinity at the active site of hTER, leading to a complete inhibition of the enzyme {{23 Rankin,A.M. 2008;130 Roth,A. 2010;}}. GRN163L is currently in 12 phase I/II clinical trials **(Table III).** BIBR1532 *(2-[E)-3-naphtalen-2-yl-but-2-enylylamino]-benzoic acid)* is non-competitive non-nucleosidic mixed type hTERT active site inhibitors {{21 Baker,N. 2008;}}. BIBR1532 is a small molecule telomerase inhibitor, which impairs the DNA substrate elongation upon template copying by reducing the number of TTAGGG repeats {{34 Pascolo,E. 2002;}}.

**Fig. 3. T-loop and G-quadruplex.**
T-loop and G-quadruplex are higher order structures of telomerase required for proper maintenance of telomeres and protection of chromosome integrity. The T-loop consists of 150-250 nucleotides long single-stranded G-rich 3' overhang {{10 Riethman,Harold 2008;291 Ou,T.M. 2008;}}. A D-loop is a 3'-stranded region, which serves as the primer for telomerase elongation of telomeres {{290 Xu,Y. 2011;}}. The 6 proteins, located on the chromosomal terminus of telomeres, form a Shelterin complex and are required for capping of telomeric ends and regulation of telomere length {{1 Tian,X. 2010;24 Blackburn,E.H. 2005;23 Rankin,A.M. 2008;138 Bisoffi,M. 2006;}}. The POT1 protein binds to the single stranded region of telomere and forms a heterodimer with the TPP1 protein, which links it with the dsDNA of telomere and the rest of the Shelterin complex proteins {{290 Xu,Y. 2011;}}. TRF1/2 bind the double stranded region of telomere. TRF1/2 are important regulators of telomere length, whereas TRF1 serves as a negative regulator of telomeres by blocking telomerase access to telomeres, TRF2 serves a protective function by preventing telomere uncapping {{1 Tian,X. 2010;}}. The model of G-quadruplex depicted here is one of a few possible versions of G-quadruplexes {{290 Xu,Y. 2011;}}.

**Fig. 4. Telomerase peptide-based and DC immunotherapies.**
The immunotherapy approach has proven to be a convenient and a successful method for anticancer therapeutics targeting telomerase. Studies have shown that hTERT, which is normally a self-antigen molecule, is capable of producing immunogenic response by stimulating hTERT peptide-specific CTLs via MHC presentation leading to tumor cell lysis {{1 Tian,X. 2010;14 Harley,C.B. 2008;19 Shay,J.W. 2008;160

Beatty,G.L. 2008;}}. GV1001 is MHC class II-restricted hTERT peptide-based vaccine that has strong affinity for multiple alleles of HLA class-II alleles {{157 Kyte,J.A. 2009;161 Middleton,G. 2008;}}. It is injected i.d. as an MHC class-II peptide following after the injection of an adjuvant. It is endogenously processed by APCs to shorter 9-10 mer MHC class-I peptide, which is further presented by APCs as a CD8+ epitope, thus stimulating CD4+ and CD8+ responses ({{157 Kyte,J.A. 2009;155 Shaw,V.E. 2010;159 Nava-Parada,P. 2007;}}). CD4+ T cells may generate antitumor response that is independent of CD8+ response, leading to a recruitment and activation of macrophages and eosinophils and producing tumor-destroying free radicals {{195 Wong,S.B. 2008;155 Shaw,V.E. 2010;}}. GRNVAC1 is a promising DC vaccine, which stimulates the CD4+ T cells and leads to secretion of cytokines IL-2, IL-5, IFN-γ, as well as some IL-4, indicating a mixed type Th1/Th2 inhibition {{191 Su,Z. 2005;}}. The CD4+ T-cell response may also lead to CTL-mediated killing of hTERT expressing tumor cells. The CD8+ T cells isolated from the LAMP-hTERT group of subjects also showed the ability to secrete IL-2. These results suggest that LAMP-enhanced CD8+ and CD4+ responses may lead to the development of central memory T cells {{191 Su,Z. 2005;}}.

**Table I**
Strategies of targeting telomeres and telomerase.

| THERAPEUTIC APPROACH | TARGET | MECHANISM | INHIBITORS |
|---|---|---|---|
| Small Molecule Inhibitors | hTERT | Direct enzyme inhibition via active site binding | BIBR1532 |
| Antisense Oligonucleotides (AS-ODN)s) | hTER | Targeting RNA template of telomerase | GRN163L |
| Immunotherapy | hTERT | Using hTERT peptides to elicit CD4+ and CD8+ immune responses | GV1001, GRNVAC1/2, Vx-001 |
| Gene Therapy | hTERT | hTERT promoter driven tumor cell lysis; hTERT promoter activated enzyme prodrug therapy inducing tumor cell lysis | Telomelysin<br>Ad-hTERT-NTR/CB1954<br>hTERTp-HRP/IAA |
| G-quadruplex Stabilizers | G-quadruplex | Blocking telomerase access to telomeres; uncapping of telomeres; mimicking of ssDNA overhang exposure | BRACO19<br>RHPS4<br>Telomestatin |
| Telomere and Telomerase Associated Proteins | HSP90 | Disruption of telomerase assembly resulting in a nonfunctional telomerase | Geldanamycin (GA)<br>Curcumin |
| | Tankyrase | Preventing dissociation of TRF1 leading to blocking of telomerase | PARP inhibitors |
| T-oligo Approach | G-rich overhang | Inducing DNA damage responses by mimicking telomeric DNA overhang exposure | N/A |

**Table II**
GRN163L in preclinical studies

| Tumor Type | Effect of Treatment |
|---|---|
| Multiple Myeloma (MM) | Effective inhibition of TA in MM and CSCs; induction of cell death due to apoptosis and significant reduction in tumor cells growth; inhibition of clonogenicity and expression of CSC markers; enhanced myeloma cell death in combination treatment with HSP90 inhibitor 17AGG {{144 Brennan,S.K. 2010;75 Shammas,M.A. 2008;}}. |
| Lung Cancer | Dose- and sequence-dependent inhibition of TA; rapid alteration of cell morphology and reduction in cellular attachment and surface spreading; reduction of colony formation and prevention of cell metastases *in vitro* and *in vivo* {{90 Jackson,S.R. 2007;79 Dikmen,Z.G. 2005;}}. |
| Breast Cancer | Inhibition of TA; induction of DNA damage response; sensitization to radiation and chemo-therapies; synergistic effect with cisplatinum, trastuzumab ($HER2^+$ breast cancer cell line), paclitaxel; inhibition of cell migration; reduction of tumor growth, clonogenicity, cells invasion and metastases; restoration of sensitivity in drug-resistant cells {{76 Uziel,O. 2010;86 Gomez-Millan,J. 2007;93 Gellert,G.C. 2006;61 Hochreiter,A.E. 2006;88 Goldblatt,E.M. 2009;87 Goldblatt,E.M. 2009;}}. |
| Bladder Cancer | Significant inhibition of TA; morphological alterations in tumor cells; growth arrest in G0/G1 phase {{85 Dikmen,Z.G. 2008;}}. |
| Glioblastoma (GBM) | Dose-dependent inhibition of TA; reduction in cell proliferation and apoptosis in GBM TICs; ability to cross BBB; preferential accumulation in intracerebral tumors; successful inhibition of tumor growth in intracerebral human tumor xenograft model; enhanced cell survival and activation of DNA damage response pathways in combination with IR and temozolomide treatment {{77 Hashizume,R. 2008;82 Marian,C.O. 2010;}}. |
| Prostate Cancer | Inhibition of TA in prostate TICs; reduction of TICs, TIC markers and their capacity of self-renewal {{92 Marian,C.O. 2010;}}. |
| Hepatoma (Liver Cancer) | Dose-dependent inhibition of TA and tumor cell growth *in vitro* and *in vivo*; reduction of cell proliferation and tumor growth; increased apoptosis and sensitivity due to doxorubicin treatment {{28 Djojosubroto,M.W. 2005;}}. |
| Pancreatic Cancer | Inhibition of TA in bulk tumors and TIC subpopulations; reduction in tumor engraftment concomitant with reduction in TIC numbers; inhibition of cell growth and apoptosis with prolonged treatment; reduced tumorigenicity *in vivo* {{141 Joseph,I. 2010;}}. |
| Barrett's Adenocarcinoma | Inhibition of TA; induction of senescence or apoptosis; enhanced inhibition of cell proliferation with combination of doxorubicin and ritonavir; significant reduction in tumor size {{147 Shammas,M.A. 2008;}}. |

TA - telomerase activity; CSCs - cancer stem cells; TICs - tumor-initiating cells; BBB - blood brain barrier

**Table IV**

Vaccines in clinical trials

| Vaccine Formulation | Condition | Phase, (n) Status | Immune Response | Outcomes / Ref. |
|---|---|---|---|---|
| GV1001, temozolomide (T), GM-CSF | Melanoma (stage IV) | I/II, (n=25) completed | 17/21 (81%) | *Safety*: no major toxicity. *Clinical*: 6/25 - SD; 10/25 - PD; 1/25 - PR {{185 Aamdal,S. 2006;}} |
| GV1001, I540 peptide | Melanoma (IIB-IV) | (n=16) completed | No results yet available | No data available {{157 Kyte,J.A. 2009;}} |
| GV1001, I540 peptide, GM-CSF | NSCLC (stage IIB, IIIB, IV) | I/II, (n=26) completed | GV1001: 13/24 (54%); I540:2/24 (8%) | *Safety*: no major toxicity. *Clinical*: 1 - CR; 3 - SD; 1 - PD; med. OS 8.5m, 8pts. - 12m., 6pts. - 18m. {{169 Brunsvig P.,F. 2005;}} |
| GV1001, cyclophosphamide (C), GM-CSF | HCC | II, (n=40) completed | No immune responses detected | Safety: no major toxicity. *Clinical*: 17/24 - SD; med. TTP - 1.9m.; med. TTSP - 11.7m; med. PFS - 1.9m; med. OS - 11.7m. {{156 Greten,T.F. 2010;}} |
| GV1001, imiquimod (I) | Pancreatic | I, (n=14) completed | 6/13 (46%) | *Safety:* well tolerated; no major toxicity or serious AEs reported {{171 Bernhardt S.,L. 2005;}} |
| GV1001, gemcitabine (G), GM-CSF | Pancreatic | II, (n=12) completed | 7/12 (56%) | *Safety:* no major toxicity. *Clinical*: induction of IFNγ, IL-6, IL-13 {{167 Choudhury,Aniruddha 2007;}} |
| GV1001, imiquimod, GM-CSF, 3Gyx10, C | Pancreatic | II, (n=39) completed | (1n=15) - 39% (2n=10) - 50% (3n=14) - 64% | *Safety:* 221 AEs reported, mostly - mild to moderate; GV1001 was well tolerated in all treatment regimens {{170 Buanes,T. 2008;}} |
| GV1001, GM-CSF | Pancreatic | I/II (n=48) completed | 24/38 (63%) | *Safety:* no major toxicity/AEs. *Clinical:* med. survival for 560μg of GV1001 - 8.6m {{152 Bernhardt,S.L. 2006;}} |
| GV1001, GM-CSF, gemcitabine (G) | Pancreatic | III, (365/520) terminated | N/A | *Safety:* grade 3-4 AEs. *Survival:* OS 7.3-5.9m.; PFS 3.7-1.9m.; as of 8/2008 238 deaths were recorded {{166 Buanes,T. 2009;}} |
| GV1001, GM-CSF, G, capecitabine | Pancreatic | III, (n=1100) recruiting | N/A (recruiting) | No results available {{216 Anonymous 2010;}} |
| GV1001, LTX-315 | Carcinoma | I, (n=20) recruiting | N/A | No results available {{215 Anonymous 2010;}} |
| GRNVAC1 | Prostate | I, (n=20) completed | 19/20 (95%) | *Safety*: no major toxicity. *Clinical:* increase in PSAdt and molecular clearance of circulating micrometastases {{191 Su,Z. 2005;}} |
| GRNVAC1 | AML | II, (n=21) completed | 55% | *Safety:* no major toxicity. CR*-19/10pts. DFS: 79% - low risk group, 75% - Intermediate risk, 81% - high risk {{188 Khoury,H.Jean 2010;189 DiPersio,John F. 2009;}} |
| Vx-001 | Advanced cancers (various) | I/II, (n=116) completed | Positive immune response (>3yr) | *Safety:* no major toxicity. *Clinical:* 1pts. with BC - CR, 2pts. - PR, 34pts. - SD. Prolonged survival & positive correlation between immune and clinical responses {{211 Menez-Jamet,J. 2009;}} |
| Vx-001 | NSCLC | I/II, (n=22) | 16/21 (76%) - early; 10/11 (91%) - late | *Safety:* no major toxicity. *Clinical:* 8/32pts. (36%) - SD; med. OS - 30.6m. {{60 Bolonaki,I. 2007;183 Kosmatopoulos,K. 2007;}} |
| Vx-001 | Advanced cancers | I, (n=19) | 13/14 (93%) - early | *Safety:* no major toxicity; no DLT at 2-6mg doses. *Clinical:* 4/19 (21%) - SD; no CR or PR; med. OS-15.2m {{175 Mavroudis,D. 2006;}} |
| Vx-001 | NSCLC (7pts - IIIB, 6pts - IV) | I/II, (n=13) | 7/7 (100%) | *Safety:* grade I/II toxicity. *Clinical:* 3/10pts. (30%) - SD; med. TTP - 8.1m. (IIIB), 2.3m. (IV) {{184 Kosmatopoulos,K. 2006;}} |

| | | | | |
|---|---|---|---|---|
| Vx-001 | Advanced solid tumors | I/II (n=71) | 29/56 (52%) - early IR; 25/30 (83%) - late IR | *Safety:* grade I toxicity. *Clinical*: 3pts. (4.2%) objective response; 22/71 (31%) - SD; med. OS - 23.5m. (early) {{184 Kosmatopoulos,K. 2006;}} |
| Vx-001 | Various cancers | I/II, (n=97) | 31% - before, 58% - early IR, 79% - late IR | *Immune response:* 24/78 (31%), 46/79 (58%), 37/47 (79%). Inverse correlation between IL-10 Tc and IFN$\gamma$ {{181 Vetsika,E.K. 2008;}} |

SD - stable disease; CR - complete response; PR - partial response; OS - overall survival; TTSP - time to symptomatic progression TTP - time to progression; AEs - adverse events; PFS - progression free survival; PSAdt - Prostate Specific Antigen doubling time; DFS - disease free survival; PD - disease progression; DTL - dose limiting toxicities.

**Table III**
GRN163L in clinical trials

| Phase/ Identifier | Status/ Start -End* | Condition | Drug interventions | Outcome measures | Current results/Ref. |
|---|---|---|---|---|---|
| I NCT00124189 | Ongoing 7/05-3/10 | CLD | GRN163L | Safety, tolerability, DLT, MTD, PK, PD | Trial ongoing, no study results yet available |
| I NCT00310895 | Ongoing 3/06-9/10 | Solid Tumor Malignancies | GRN163L | Safety, DLT, MTD, PK, disease response | 31 treated; 4 remain on study; MTD in range 9.4-11.7mg/kg/d1,d8 of 21d cy; DLT - thrombocytopenia, myelosuppression and hypersensitivity reactions {{100 Ratain,M.J. 2008;}} |
| I NCT00594126 | Ongoing 11/07-3/10 | Multiple Myeloma | GRN163L | Safety, MTD, PK, PD, efficacy | DLT - thrombocytopenia & aPTT; MTD in range of 4.8-7.2mg/kg/2hr IV/t.i.w. {{134 Chanan-Khan,Asher Alban 2008;}} |
| I NCT00510445 | Ongoing 6/07-8/10 | Non-Small Cell Lung Cancer | GRN163L, paclitaxel (P), carboplatin (C) | Safety, MTD, PK, efficacy | Trial ongoing, no study results yet available |
| I NCT00718601 | Ongoing 7/08-12/10 | Multiple Myeloma | GRN163L, bortezomib, dexamethasone | MTD, Safety, PK, efficacy | Trial ongoing, no study results yet available |
| I/II NCT00732056 | Ongoing 7/08/12/10 | Recurrent or Metastatic Brest Cancer | GRN163L, paclitaxel (P), bevacizumab (B) | Safety, MTD, efficacy, PK, efficacy | 14 treated; 2 remain on study; dose delays of GRN163L and/ or P in later cycles; ORR 38.5% {{102 Kozloff,M. 2010;}}. |
| II NCT01137968 | Recruiting 5/10-2/12 | Non-Small Cell Lung Cancer | GRN163L, bevacizumab | PFS, ORR, time to all-cause mortality, safety, tolerability | Trial ongoing, no study results yet available |
| II NCT01256762 | Recruiting 11/10-2/13 | Recurrent or Metastatic Breast Cancer | GRN163L, paclitaxel with or without bevacizumab | PFS, ORR, clinical benefit rate, safety, tolerability | Trial ongoing, no study results yet available |
| II NCT01243073 | Recruiting 12/10-1/13 | Essential Thrombocythemia | GRN163L, standard of care | Hematologic response, safety, tolerability, number of patients with hematological toxicities, non-heme grade 3 and 4 AEs and hemorrhagic events | Trial ongoing, no study results yet available |
| II NCT01242930 | Recruiting 12/10-2/13 | Multiple Myeloma | GRN163L, standard of care | PFS, ORR, safety, tolerability, number of patients with hematological toxicities | Trial ongoing, no study results yet available |
| I NCT01265927 | Recruiting 1/11-1/14 | HER2+ Breast Cancer | GRN163L, trastuzumab | DLT, PK, ORR, PFS, safety and biologic effects of GRN163L in combination with trastuzumab | Trial ongoing, no study results yet available |

| | | | | | |
|---|---|---|---|---|---|
| I<br>NCT01273090 | Recruiting<br>5/11-11/14 | Solid Tumors or Lymphoma | GRN163L | MTD, toxicities, PK, biologic effects, effect on telomeres and telomerase | Trial ongoing, no study results yet available |

* Study end dates are estimated.

DLT - dose limiting toxicity; MTD - maximum tolerated dose; PK - pharmacokinetics; PD - pharmacodynamics; PFS - progression free survival; ORR - objective response rate; AEs - adverse events; aPTT - active thromboplastin time; t.i.w. - three times a week